

**FREEZE-THAW CYCLE, SOIL MOISTURE, AND THAWING TEMPERATURE
EFFECTS ON NITROGEN DYNAMICS IN A BLACK CHERNOZEMIC SANDY LOAM**

By

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ABSTRACT

Roger Kpankpari, M.Sc., The University of Manitoba, February 2025. Freeze-thaw cycle, soil moisture, and thawing temperature effects on nitrogen dynamics in a Black Chernozemic sandy loam

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Many farmers in the Canadian Prairies apply urea during the fall before freezing with the aim to reduce field operational tasks in the spring considering the short growing season and the risk of excess soil moisture delaying field operation and also leverage the lower fertilizer prices in the fall. Farmers are advised to apply fertilizers at a temperature of 5°C or lower to reduce nitrogen (N) losses. This recommendation, however, does not take into account the interaction between temperature and moisture and how that impacts N- dynamics in the soil and the potential impact on N₂O. The objective of this study was to evaluate the effects of soil freezing and thawing cycle, moisture content (32%, 50%, or 100% water-filled pore space, WFPS) and thawing temperature (4, 8, 12, or 16°C) on N₂O emissions from a sandy loam soil, treated with stabilized urea-based fertilizer (SuperU). The results showed that moisture significantly affected N₂O emissions under all temperatures, with the highest emissions recorded for 100% WFPS at 16°C. SuperU was not effective in reducing cumulative N₂O emissions at 100% WFPS. There was a significant positive correlation between volumetric heat capacity and N₂O flux at higher thawing temperatures. The results demonstrate the potential of N₂O reduction when urea is applied in bands of 5 cm deep at a temperature of 5°C or lower with soil moisture at or below 50% WFPS. However, spring conditions present a high risk of N losses.

Key words: nitrous oxide, freeze-thaw cycle, SuperU, urea, water-filled pore space, temperature, nitrate, ammonium, volumetric heat capacity

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FOREWORD

The thesis was written in a manuscript style in accordance with the guidelines set by the Department of Soil Science, University of Manitoba. It consists of three chapters. Chapter 1 presents pertinent literature to support the research, including an overview of freezing and thawing effects on nutrient management in the Canadian prairies, N dynamics under freeze-thaw cycles, effects of freeze-thaw cycles on nitrous oxide emissions, management strategies for nitrogen losses, and research needs to advance our understanding of nitrogen dynamics in the prairies. Chapter 2 addresses the research objective, which focused on the effects of freezing and thawing cycles, moisture and temperature variability on N loss through nitrous oxide (N₂O) emissions from urea and SuperU in sandy loam soil. Chapter 3 presents the overall synthesis of the thesis highlighting the implications from the results and recommendations to further advance our knowledge on freeze-thaw cycles, moisture, and temperature effects on N-losses in soil and agronomic systems.

CONTRIBUTION OF AUTHORS

The authors' contributions to Chapter 2 are outlined below:

- Roger Kpankpari: conceptualization, investigation, formal analysis, data curation, writing original manuscript draft
- Afua Mante: conceptualization, supervision, fund acquisition, review and editing of manuscript drafts
- Francis Zvomuya: Advisory committee, review and editing of manuscript draft
- Henrique Carvalho: Advisory committee, review of manuscript draft

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1. INTRODUCTION

1.1 Overview of freezing and thawing effects on nutrient management in the Canadian prairies

About 55% of the land mass in the northern hemisphere is characterized by seasonal freeze-thaw cycles (FTCs) (Zhang et al., 2003; Climate Atlas, 2019). The frequencies of the FTC are diurnal and annual, both of which are defined by the time it takes to complete a cycle (Matsuoka et al., 1998). Diurnal FTC occurs when the cycle is completed within a day (Ho and Gough, 2006) while annual FTC occurs when the freezing in winter is followed by thawing anytime in the summer (GLISA, 2024). In the Canadian prairies for instance, based on a 30-year data (1979-2005), an average of 59.7 daily FTCs were recorded in Winnipeg, 71.8 in Saskatoon, and 78.1 in Regina (Climate Atlas, 2019).

It is critical to note that due to the higher heat capacity on the ground than the air (Zheng et al., 1993), there is a temperature lag between the atmosphere and the soil (Zhang et al., 2005), which distinguishes atmospheric FTC, where freezing and thawing occur in the air from soil FTC, where freezing and thawing occur in the soil. The air temperature recorded at a particular time may not be the same in the soil. For example, if the air temperature recorded is 0°C at a particular time, the soil temperature may be comparatively warmer, especially in winter. Conversely, in summer, the soil tends to be cooler than the air (Costa, 2006; Zhang et al., 2005). This phenomenon is due to the higher heat capacity of soil compared to air, which causes it to experience smaller temperature changes when exposed to heating or cooling. Additional factor to this effect is the presence of snow cover acting as insulation, thereby preventing direct contact between cold air from the atmosphere and the soil, leading to relatively warmer soil conditions (Durán et al., 2016).

The opposite is true when the soil eventually freezes and begins to thaw as the air temperature rises.

It has been well demonstrated in previous studies that soil FTCs (SFTC) have impact on soil structural and hydraulic dynamics (Liu et al., 2021; Leuther and Schlüter, 2021; Oztas and Fayetorbay, 2003). For example, Liu et al. (2021) showed that soil is made more porous when exposed to higher FTC frequency (1 to 20) because smaller pores are interconnected to form larger ones. In another study, Leuther and Schlüter (2021) demonstrated that increasing the number of FTCs from 2 to 5 caused a gradual but significant decrease in mean pore sizes, porosity, and the connectivity of macropores in packed soil columns.

The understanding of the dynamics of soil structure and hydraulics under SFTC is critical to advancing agronomic management for sustainable crop production in temperate regions. In recent years, the use of N-fertilizer in replenishing soil N has come under scrutiny with regards to N-losses, especially nitrous oxide (N_2O) emissions, and their interaction with climate change (Tenuta and Sparling, 2011; Ejack and Whalen, 2021; Pelster et al., 2019; Congreves et al., 2018). We are seeing increase in studies on N-losses under FTC in temperate regions possibly due to the report issued by the Intergovernmental Panel on Climate Change (IPCC) in 1997 about the source of N_2O in cold regions. The report indicated that large emissions of N_2O occur in cold regions because N_2O is produced and trapped under ice and is released into the atmosphere during thawing (IPCC, 1997). N_2O is about 265 times more effective at warming the climate than CO_2 (Stocker, 2014) and has a residence time of 109 years (Prather et al., 2015). Considering the severity of N_2O on climate change, it is imperative that we advance our understanding on N-fertilizer application and management in temperate regions to ensure sustainable crop production and healthy environment.

Over the last three decades, there have been significant work done on N management in Canada. However, only a small portion (42) focused on SFTC interaction with N-management with 9% in Manitoba, 2% in Saskatchewan, 12% in Alberta, 10% in British Columbia, 7% in New Brunswick, 36% in Ontario, and 24% in Quebec. In these studies, 33 were in the field and 9 in the laboratory. As presented in Table (Table 1.2), it can be observed that the levels of emissions reported were dependent on several factors, including treatment of soil sample, N-fertilizer source, soil textural class, water content, freezing temperature, thawing temperature, freezing duration, thawing duration, and frequency of freezing and thawing.

The short growing season in Canada has significant influence on agronomic planning. Recent study by Amiro et al. (2017) demonstrated that many farmers in the Canadian prairies apply their N fertilizers in the fall before freezing. This choice for fall time application is driven by cheaper fertilizer cost, ease spread of workload and reduction in tillage operations in the spring (Tiessen et al., 2005). Fall time N-fertilizer application is also favored due to the perception that once the soils are frozen, the N remains stable and will be available in the subsequent growing season (Lasisi et al., 2020). In Manitoba, Tiessen et al. (2003) and Wood et al. (2023) demonstrated in their studies that fall N fertilizer application leads to lower N recovery than in spring N fertilizer application as most of the N is lost through ammonia volatilization (Lasisi et al., 2021) or N₂O emissions during thawing events (Wood et al., 2023; Tenuta et al., 2016). Tenuta et al. (2016) reported a higher thaw N₂O emissions in late fall applied anhydrous ammonia in clay textured soil.

To reduce the N losses from fall N-fertilizer application, however, it is recommended in a previous study to delay application until late fall when the soil temperature is at or below 5°C to reduce nitrification (Tenuta et al., 2016). In addition to the timing of application, N inhibitors have been shown to reduce N losses. For instance, in Manitoba, Wood et al. (2023) reported on the

efficacy of SuperU in reducing N₂O emissions by 37–57%. In another study conducted in Manitoba, Nyathi (2024) demonstrated that ARMU-Advanced formulations, when applied to urea ammonium nitrate (UAN), reduced N₂O emissions by 50% compared to untreated UAN in sandy loam soils.

From previous studies, it can be observed that SFTCs indeed interact with N-management in cold regions. As demonstrated by the number of studies (Table 1.1, Table 1.2) in Canada, it can be deduced that SFTC and its impact on N-dynamics is an emerging research area. Currently, there is a limitation in our understanding of how the wide range of moisture, temperature, and frequencies of SFTC that characterize our climate from fall through spring impact N dynamics. This is not surprising as there are limitations with field studies such as lack of monitoring equipment to capture the moisture, thermal, and N dynamics from fall through spring as well as the wet conditions in the spring, which limits personnel access to the field to sample gas (Wood et al., 2023). With these limitations, important processes can be missed during the thawing period, leading to poor estimation of N₂O emissions (Wagner-Riddle et al., 2017). We can advance the knowledge from previous studies by resorting to laboratory studies. Laboratory studies are ideal for mimicking thaw emissions and enable us to capture all the critical processes of freezing and thawing events. In this study, a laboratory experiment was set up to study the effects of freezing and thawing cycle, moisture, and temperature variability on N-dynamics in soil samples (sandy loam) treated with urea-based fertilizer with and without double inhibitors. The freezing and thawing events were mimicked in a freezing and thawing chamber (UTEST, UTD-1442).

Table 1.1 Some previous studies on nitrogen dynamics under freezing and thawing conditions in the Canadian prairies.

Authors	Objective of study	Findings	Study type	Location
Cui and Wang (2019)	Enhancing the performance of the existing DNDC model to better estimate soil temperature and greenhouse gases in temperate regions by improving sub models like the snow cover, rain-snow partitioning and soil freeze-thaw cycle.	Modified DNDC model better predicted CO ₂ (R ² increased from 0.23 to 0.35) and N ₂ O (R ² increased from 0.12 to 0.36).	Field	Alberta
Elliott (2013)	The study aimed to identify plant and soil factors that affect nutrient release by conducting a snowmelt simulation study on various residue types and their interaction with soil during thawing of the snow.	Nutrient release (total N, total dissolved phosphorus, dissolved organic carbon) during snowmelt was greatest for residues that were actively growing than stubble.	Laboratory	Saskatchewan
Tenuta and Sparling (2011)	The effects of soil compaction and depth on the emissions of N ₂ O.	Thaw emissions of N ₂ O reduced as bulk density increased from 1.1, 1.2 to 1.25 Mg m ⁻³ . Also, thaw emissions were lower with depth (0-10 cm, 10 -30 cm, and 30-60 cm).	Laboratory	Manitoba
Malhi and Nyborg (1986)	The effects of N fertilizer source, application timing and placement method on the yield of barley N recovery.	Banding increased N recovery compared to incorporation and topdressing. Recovery of N in barley was highest in the spring than with fall application.	Field	Alberta
Wood et al. (2023)	To evaluate the effects of enhanced efficiency fertilizers (EFF) and time of fertilizer application on the emissions of N ₂ O, protein amount, and yield of spring wheat evaluated in three growing seasons.	Fall application resulted in larger emissions, with thaw emissions ranging from 33 to 67%. SuperU was effective in reducing emissions by 37 to 57%.	Field	Manitoba

Nyborg and Malhi (1992)	To evaluate how the size of fertilizer pellets and the depth of placement affect barley yield when applying urea during both fall and spring seasons.	Spring-incorporated commercial urea produced higher barley yield compared to fall application, which produced 792 kg ha ⁻¹ lower yield and 15 kg ha ⁻¹ less N. Larger N pellets were less efficient compared to commercial urea.	Field	Alberta
Tenuta et al. (2016)	To assess the effect of time of fertilizer application, that is fall versus spring pre-plant of anhydrous ammonia on the emissions of N ₂ O from a cropped agricultural field.	Large emission of N ₂ O during thawing was recorded for fall application of anhydrous ammonia than for the early growing and spring pre-plant application.	Field	Manitoba
Tiessen et al. (2005)	Examine how the timing of application, location within the landscape, and the use of a urease- and nitrification-inhibited formulation of urea influence the efficacy of fall-banded nitrogen fertilizer.	Yield was significantly higher with late fall and spring applications than with early fall and midfall applications. Also, there were reported agronomic benefits with the use of nitrogen inhibitors compared to without.	Field	Manitoba
Wagner-Riddle et al. (2017)	Sampling of N ₂ O emissions at half-hourly resolution in agricultural fields in Manitoba for over 9 years and 14 years in Ontario, Canada.	It was found that the crop lands that have seasonally frozen soils produce 1.07 ± 0.59 Tg N ₂ O emissions.	Field	Manitoba and Ontario
Lin et al. (2023)	To measure N ₂ O emission factors in the second year of successive manure applications and evaluate any enduring impacts on the following spring thaw after discontinuing the manure-NIs injections.	The yearly N ₂ O emissions varied significantly across all manure treatments, ranging from 1.3 to 15.8 kg N ₂ O-N ha ⁻¹ .	Field	Alberta

1.2 Nitrogen dynamics under freeze-thaw cycles

1.2.1 Nitrogen fixation in the soil

Nitrogen is a major component of crop yield as it aids in photosynthesis and protein buildup in plants (Mosaic Crop Nutrition, 2023). Sources of soil N supply include biological N fixation, atmospheric deposition, and the application of N fertilizers and livestock manures (Van Drecht et al., 2003). The predominant biological agents responsible for fixing N in agricultural soils are rhizobia bacteria, which live symbiotically with plant roots (Herridge et al., 2008). It is globally estimated that approximately 50 – 70 Tg of N is fixed annually in the soil through biological processes in agricultural ecosystems (Herridge et al., 2008). Atmospheric N is deposited in the soil through rainfall events (Liu et al., 2006) or by lightning during thunderstorms (Schumann and Huntrieser, 2007). Livestock manure has been demonstrated to be a significant source of N, with its application increasing from 21.4 Tg N per year in 1860 to 131.0 Tg N per year in 2014 (Zhang et al., 2017). Biological and atmospheric N deposition are not sufficient to meet crop requirements (Rosa and Gabrielli, 2023), which necessitates the need for the application of synthetic N fertilizer, which accounts for 85% of the N fertilizer used for crop production globally (Zhang et al., 2021). Synthetic N is produced by the Haber–Bosch process, which converts unreactive atmospheric N₂ into reactive forms (Zhang et al., 2021).

Nitrogen presents us with the benefits of quality yield (Blumenthal et al., 2008) and is a fundamental component of nucleic acids, including DNA (Brady and Weil, 2016; New Zealand Government, 2025). Notwithstanding these benefits, N losses to the environment is critical to climate change due to the potency of N₂O in warming the climate (Smith et al., 2012) and quality of water resources due to potential leaching of nitrate (NO₃⁻) to groundwater and eutrophication resulting from nutrient-loaded discharge into receiving surface waters (Rosa and Gabrielli, 2023).

It should however be noted that the losses and their impact on the environment is dependent on N transformation processes (including mineralization, nitrification, and denitrification) (Hayatsu et al., 2008) to produce N_2O , and factors that influence these transformation processes.

1.2.2 Nitrogen mineralization

Once nitrogen (N) becomes available in the soil, it undergoes mineralization, transforming into ammonium (NH_4^+) (Fu et al., 2019; Schimel and Bennett, 2004b). Ammonium can be converted to nitrates (NO_3^-) through nitrification, both of which can be taken up by actively growing plants (Girkin and Cooper, 2023). Identified factors that influence the accumulation of NH_4^+ include a) nitrification b) microbial consumption or immobilization of NH_4^+ , c) ammonia volatilization, d) mineralization rate of organic N in the soil (Ramakrishnan, 2015). Other identified factors such as precipitation, total nitrogen, microbial biomass, soil pH (Li et al., 2019) and freeze thaw cycles (FTCs) (DeLuca et al., 1992; Durán et al., 2016) affect the rate of N mineralization (N_{min}). Li et al. (2019) conducted a global meta-analysis and found that microbial biomass was the dominant factor in determining changes in N_{min} , rather than soil chemical and physical properties. In the same study, they observed no significant effect of mean annual temperature on N_{min} on a global scale. However, much remains to be explored in temperate regions regarding how N_{min} is affected under continental climates. In the temperate regions, FTCs have been demonstrated to have effects on N_{min} . For example, DeLuca et al. (1992) demonstrated that frequent freezing and thawing increased N_{min} rates compared to unfrozen soils. Additionally, the presence or absence of snow cover affects the rate of mineralization. For instance, a climate change study in the United States demonstrated that multiple FTCs in soil without snow cover led to decreased N_{min} due to the mortality of soil microorganisms when the soil freezes without insulation (Durán et al., 2016).

Conversely, snow cover acts as an insulating layer, protecting soil microorganisms from extreme cold, thereby supporting microbial activity and promoting N_{\min} (Durán et al., 2016).

1.2.3 Nitrification

Nitrification is a biological process that involves the oxidation of ammonium (NH_4^+), resulting in the production of nitrites (NO_2^-) and nitrates (NO_3^-) (Ramakrishnan, 2015). Factors that influence nitrifier abundance include moisture, substrates, temperature and FTCs. Németh et al. (2014) demonstrated an increase in nitrifier abundance during thawing. This observation was attributed to substrate availability during thawing, which are used by the microbes. Soil moisture plays an important role in nitrification; and because it is an aerobic process, the percentage of soil pores filled with water affect the activity of microbes. Braker and Conrad (2011) found that nitrification dominates in soils with less than 60% WFPS. Németh et al. (2014) found an increase in the population of ammonia oxidizers at 48% WFPS. Nitrification significantly depends on the activity of ammonia-oxidizing bacteria (AOB), which initiate ammonia oxidation (Gong et al., 2013). Another group of microorganisms, ammonia-oxidizing archaea (AOA), also contributes to soil nitrification, though AOB dominate at 60% WFPS soil moisture (Gong et al., 2013). AOB and AOA convert NH_4^+ to NO_2^- , while nitrite-oxidizing bacteria (NOB) transform NO_2^- into NO_3^- (Ward, 2011). Ammonium is first oxidized to nitrite (NO_2^-) and then to nitrate (NO_3^-), which can be lost through leaching in more porous soils or reduced to nitrous oxide (N_2O) through denitrification.

1.2.4 Denitrification

Denitrification follows nitrification under limited oxygen conditions, reducing nitrate NO_3^- to N_2O and N_2O to dinitrogen (N_2) (Hayatsu et al., 2008). This process is mediated by the enzymes

nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos) (Hayatsu et al., 2008). The Nar enzyme first reduces NO_3^- to NO_2^- , which Nir then converts to nitric oxide (NO). Nitric oxide reductase further reduces nitric oxide to N_2O , and finally, Nos reduces N_2O to N_2 (Philippot and Højberg, 1999; Jung et al., 2011). Factors that affect the abundance of denitrifier communities include soil moisture, NO_3^- concentration, carbon (C), and FTCs. Nitrate is a key determinant of denitrification (Xiong et al., 2017), which is a function of soil moisture (Lin et al., 2023). A study conducted on a wetland in China revealed that NO_3^- increased the abundance of denitrifiers, which in turn increased denitrification rate (Xiong et al., 2017). Tenuta and Sparling (2011) observed higher NO_3^- concentrations near the soil surface, which resulted in elevated N_2O emissions when soils were frozen for 2.5 d at -20°C , and thawed at 4°C for the same duration, and then incubated at 15°C .

The main driver for NO_3^- release into the soil N pool is moisture (Oztas and Fayetorbay, 2003). Oztas and Fayetorbay (2003) found that increased soil moisture correlates with higher NO_3^- levels, and Fu et al. (2019) demonstrated that initial soil moisture content (15%, 20%, and 25% vol) impacts NO_3^- release during FTCs. Under saturated conditions, denitrification dominates due to the anaerobic environment (Wertz et al., 2016). A soil column study in Quebec demonstrated that higher soil moisture (33% vol) content facilitates denitrification, resulting in significant N_2O emissions ranging from 0.2 to 1.3 $\text{kg ha}^{-1} \text{d}^{-1}$ when sandy loam was subjected to 12 hours of freezing and 24 hours of thawing (Chen, 1995). Similarly, Singurindy et al. (2009) found that the optimal moisture content for initiating denitrification in clay loam soil ranged from 40% to 70% WFPS in manure-treated fields.

Aside moisture, the availability of NO_3^- for denitrification depends on soil cation exchange capacity (CEC) and macroaggregate disintegration during FTCs (Kettunen and Saarnio, 2013; Fu

et al., 2019; Oztas and Fayetorbay, 2003). Soils with higher CEC immobilize NH_4^+ on the soil surface, reducing its availability for nitrification and subsequent denitrification (Kettunen and Saarnio, 2013). Freeze-thaw cycles (FTCs) disintegrate macroaggregates, which lead to releasing NO_3^- into the soil N pool for denitrification (Fu et al., 2019; Christensen and Christensen, 1991).

Denitrification is also influenced by soil depth (Wagner-Riddle et al., 2008; Bhowmik et al. 2017; Tenuta and Sparling, 2011). Tenuta and Sparling (2011) compared emissions at three soil layers, 0-10 cm, 10-30 cm, and 30-60 cm and found that emissions decreased with depth. The reduction was attributed to lower levels of organic carbon, which serves as a substrate for denitrification, and a decrease in N_2O -producing bacteria at deeper depths. Wagner-Riddle et al. (2008) reported that N_2O emission at the soil surface (0-5 cm) was 1.5 to 5 times higher than at deeper layers (12-17 cm), where N_2O is converted to N_2 before it can escape into the atmosphere.

Based on the discussion in the sections above, it is evident that FTCs play a key role in N dynamics in temperate regions experiencing freezing and thawing. It is important to further investigate how these factors affect fall fertilizer efficiency. The subsequent section discusses past studies on the effects of FTCs on N in the prairies, some management practices and summary of research opportunities for future FTC studies.

1.3 Effects of FTCs on N_2O dynamics

A systematic literature search on the effects of FTCs on N_2O was done in Scopus and Web of Science using the search string (“freeze-thaw” OR “freeze-thaw cycl*” OR “freezing and thawing” OR “soil column stud*” OR “fall and spring” AND N_2O OR “nitrous oxide”) in 2024. The search retrieved 42 studies that were conducted in Canada. In the prairies (Table 1.1), ten (10) studies were identified for the prairies (Alberta, Manitoba, and Saskatchewan). Out of these publications, two (2) were laboratory studies, one conducted on the emissions of N_2O in packed soil cores in

Manitoba (Tenuta and Sparling 2011), and the other was a snowmelt simulation study conducted in Saskatchewan to understand N, phosphorus (P), and carbon (C) release from different residue types (Elliott 2013). In this study, however, no N₂O emissions were measured. Some factors that affect N₂O emissions under FTCs are discussed below.

1.3.1 Freezing and thawing temperatures

In Manitoba, a laboratory experiment was conducted to evaluate the effect of soil conditions on N₂O emissions under FTCs. Three different layers (0–10 cm, 10–30 cm, and 30–60 cm) of soil were sampled using a spade and packed at three bulk densities (1.1, 1.2, and 1.25 Mg/m³). The soils were subjected to two Freezing and thawing cycles at -20°C and 4°C, respectively (Tenuta and Sparling, 2011). In the same study, during the first thawing period at 80% WFPS, average N₂O emissions were 0.3% in soils sampled at deeper layers (10–30 cm and 30–60 cm), with the highest emissions observed in those sampled at shallow layers (0–10 cm). These higher emissions were attributed to carbon substrate availability in packed soils sampled from the shallow layer, supporting denitrifying bacteria activity and N₂O production. A field study by Tenuta et al. (2016) observed higher N₂O emissions at temperatures of 10 – 20 °C compared to 5 °C during an experiment examining the impact of anhydrous ammonia on emissions in Manitoba in a clay-textured soil. Another field experiment conducted in Alberta in 1992 on the efficiency of fall applied N reported activity of denitrification at temperatures ranging from 2 to 12°C in a loam-textured soil. Chen (1995) reported N₂O emissions during thawing when undisturbed soil cores (6 cm dia by 10 cm ht) were collected in spring and subjected to three FTCs with freezing and thawing at -15°C and 10°C, respectively in southern Quebec. The study found that frozen soils produced 60% more N₂O emissions compared to unfrozen soils. Another study conducted in Quebec found that N₂O emissions were six to nine times higher in packed soils frozen at -4°C than in unfrozen

soils at 4°C (Ejack and Whalen, 2021). In the study, the packed soils were frozen for 7 d at -4°C and thawed for 9-h period at 4°C.

Overall, the studies evaluated have shown that thawing temperatures have significant effects on N₂O emissions, even with brief exposure to higher temperatures (10 and 25°C for 24 h and 3.5 h, respectively) leading to notable emissions (Van Bochove et al., 2000a). In contrast, Tenuta and Sparling (2011) reported negligible emissions at a thawing temperature of 4°C after freezing their soil sample at -20°C while another study (Ejack and Whalen, 2021) observed significant emissions at the same thawing temperature. In both studies, N was not limiting, so the possible explanation is the difference in freezing temperature and the soil quantity. Tenuta and Sparling (2011) used a smaller quantity of soil (115g) (Table 1.2), which was exposed to extreme freezing temperature (-20°C) for 2.5 d. This was likely responsible for microbial mortality due to freezing from the sides of the container (Henry, 2007). In comparison, Ejack and Whalen (2021) used a larger quantity (285 g) of soil, which was exposed to a milder freezing temperature (-4°C) for 7 d. Consequently, it is possible that microbial populations adapted more effectively to the changing temperature conditions.

1.3.2 Freezing and thawing rate and intensity

The duration and intensity of freezing and thawing affect N₂O emissions in soils (Table 1.2). Libby et al. (2020) found that soils exposed to 185 freezing degree days (FDD) emitted more N₂O (17.7 mg N₂O-N m⁻² d⁻¹) compared to soils frozen at 50 FDD, which emitted 7.7 mg N₂O-N m⁻² d⁻¹. This observation was attributed to substrates release into the C and N pools in soils frozen for longer duration, which supports microbial activity for N₂O production during thawing (Oztas and Fayetorbay, 2003; Fu et al., 2019). In another study, Pelster et al. (2019) evaluated the effects of freezing intensity and rate on N₂O and CO₂ emissions in silt clay and sandy loam soils. Two

freezing rates, rapid ($0.5\text{ }^{\circ}\text{C h}^{-1}$) and slow ($0.017\text{ }^{\circ}\text{C h}^{-1}$), were compared at temperatures of -1°C and -3°C for durations of 24 or 48 FDD. The study showed that N_2O emissions were higher in soils exposed to rapid freezing at both temperatures (-1°C and -3°C) for short durations compared to those subjected to slow freezing for the same period. The higher emissions during shorter durations were attributed to substrate release at thaw, whereas the lower emissions in soils exposed to longer durations were ascribed to the mortality of denitrifier communities or the degradation of enzymes responsible for denitrification. Contrary to Pelster et al. (2019), who observed the highest N_2O emissions in soils that experienced rapid freezing rates for short durations at -3°C , Libby et al. (2020) reported higher emissions ($17.7\text{ mg N}_2\text{O-N m}^{-2}\text{ d}^{-1}$) with longer freezing durations at -9°C . The difference in findings may be due to variations in thawing and freezing durations and intensities. The freezing intensity used by Libby et al. (2020) was higher (-9°C) than the one used by Pelster et al. (2019) (-3°C), which might have caused microbial cell lysis and aggregate disruption to release substrates for microbial use.

It has been demonstrated that freezing rate and duration directly affect microbial survival (King et al., 2021). In Ontario, King et al. (2021) compared the effects of faster and slower freezing rates on the sources of substrates for N_2O emissions in loamy sand and silt loam soils. The study found that silt loam soils frozen for a longer duration (21 d) at a slower rate produced 1.8 times more N_2O emissions than those frozen for a shorter duration (6 d) at a faster rate. These higher emissions were attributed to the survival of microbes at lower temperatures, which remained active in the denitrification process.

Table 1.2 Freezing and thawing temperatures, duration, frequency, water filled pore space, soil depth, study type and soil type from laboratory studies on the effects of FTC on N₂O.

Authors	Study type	Soil	Freezing (°C)		Thawing (°C)		FTC Freq.	Moisture	Bulk density	Soil depth (cm)	Soil ^s	Mass of soil (g)
			Tmp	Duration	Tmp	Duration						
Tenuta and Sparling (2011)	Laboratory	Sandy loam	-20	2.5 d	+4	2.5 d	2	80% WFPS	1.1,1.2, 1.25 Mgm ⁻³	0-10, 10-30, 30-60	H	115
Libby et al. (2020)	Laboratory	Sandy loam	-9	42, 125, 208 FDD	NA	NA	3	80 mls	1.20 gcm ⁻³	0-15	I	NA
Hung and Whalen (2020)	Laboratory	Sandy loam	-20	2 h	+4	96 h	1	64% WFPS	1.1 gcm ⁻³	0-10	H	2376
King et al. (2021)	Laboratory	Silt loam, Loam sand	-2.5	6, 21 d	+4	6 d	1	-28 hPa	NA	0-10	I	NA
Pelster et al. (2013)	laboratory	Silt clay, Sandy loam	-3, -7	5 d	+1	5 d	8	40%, 55% vol	0.96 gcm ⁻³	0-20	H	200

Van Bochove et al. (2000b)	Laboratory	Silt clay	-12, - 20	20 to 24 h	25	3, 3.5 h	1	NA	NA	0 - 5	H, I	NA
Ejack and Whalen (2021)	Laboratory	Sandy loam	-4	7 d	4	9 h	3	80% WFPS	1110 kg m ⁻³	0 - 10	H	280, 285
Chen (1995)	Laboratory	Sandy loam	-15	12 h	10	24 h	3	33% vol	NA	0-10	I	NA

Soil^s represents homogenized soils (H) and intact soils (I), FTC is freeze-thaw cycles, NA stands for not available, FDD stands for freeze degree days, Tmp stands for temperature, Freq. stands frequency and WFPS stands for water-filled pore space.

1.3.3 Frequency of freezing and Thawing

The frequency of freeze-thaw cycles (FTCs) affects N₂O emissions. In Manitoba, Tenuta and Sparling (2011) applied two FTCs in an experiment investigating soil conditions that affect N₂O in sandy loam soil. In the study, it was found that higher N₂O emissions was recorded at the first FTC period compared to the second one at 80% WFPS. In the study, packed soils were exposed to freezing of -20°C for 2.5 d and thawed at 4°C for the same period. Other experiments have employed three frequencies of FTCs in evaluating the dynamics of N₂O under freezing and thawing conditions (Chen 1995; Ejack and Whalen 2021; Pelster et al. 2019) or two FTCs (Koponen and Martikainen, 2004) or one FTC (Hung and Whalen, 2020; King et al., 2021; Van Bochove et al., 2000). In a study by Koponen and Martikainen (2004), two FTCs were applied to intact or packed soil cores at a moisture content range of 56 to 85% WFPS and then frozen at -1.5°C for 5 d and thawed at 4°C for 193 h. The study reported about 58% and 89% more N₂O emissions in intact and packed cores respectively at the first FTC than the second FTC. This observation was attributed to the release of most of the organic and inorganic substrates at the first FTC for higher microbial activity, which reduces with subsequent FTCs as substrates deplete. Ejack and Whalen (2021) observed higher N₂O emissions at the first FTC when packed cores of sandy loam soils were exposed to three FTCs. In the study, soil cores were frozen at -4°C for 7 d and thawed at 4°C for 9 h at 80% WFPS. Chen (1995) reported otherwise when undisturbed cores were taken in sandy loam soils and exposed to three FTCs. The soils were frozen at -15°C for 12 h and thawed at 10°C for 24 h. The study demonstrated that increasing the frequencies of FTCs from two cycles to three cycles results in increased N₂O fluxes compared to one (1) cycle. The observation was attributed to release of substrate with increasing frequency of FTC.

One consistent finding with most of previous studies is that higher N₂O emissions occur during the first FTC (Koponen and Martikainen 2004; Ejack and Whalen, 2021). These emissions are attributed to substrate availability during thawing (Chen, 1995; Edwards and Killham, 1986). King et al. (2021) found that FTCs do not necessarily make substrates available for N₂O emission, suggesting instead that the increase in emissions is due to a delay in soil microbial death. The study showed that under freezing conditions, microbes adapt by producing osmolytes, which help them survive in the soil and produce N₂O even under lower temperatures.

1.3.4 Timing of N fertilizer application

In prairie agriculture, it is recommended that farmers apply N fertilizers in mid fall (early October) or late fall (from mid-October to November 10), particularly when temperatures drop to or below 5 °C (Tenuta et al., 2016; The Manitoba Water Protection Act., 2008). This recommendation is based on the premise that nitrogen (N) remains stable at lower temperatures, reducing N transformation and losses as has been established in section 1.2 (Lasisi et al., 2021). Some studies have observed significantly higher N₂O emissions from late fall-applied N than spring. For instance, in Alberta, Hao et al. (2001) recorded higher N₂O fluxes (63 g N ha⁻¹ d⁻¹) from N fertilizer applied at a rate of 100 kg ha⁻¹ in the fall (October 30) compared to spring applications in a Dark Brown Chernozemic soil. This was attributed to the presence of N and optimal moisture levels (70% WFPS) during thawing, which facilitated the denitrification process, leading to N₂O production. Another field study in Alberta found that fertilizer recovery for fall-applied N decreased compared to spring applications due to N losses in loam soil (Malhi and Nyborg 1992).

In Manitoba, Tiessen et al. (2005) investigated the efficiency of fall-banded, inhibitor-treated urea in clay soils across three sites. The study used a double inhibitor formulation (urease plus nitrification inhibitors) to treat urea, which was banded at a depth of 7.5 cm during early fall

(September 15), mid-fall (September 30), or late fall (October 15). Soil temperatures at the time of application ranged from 5.6°C to 12.5°C, with gravimetric moisture levels ranging from 25% to 44%. The study found that the inhibitors were ineffective in maintaining N in the NH_4^+ form. Two explanations were proposed for this outcome: first, the efficacy of the inhibitors may not have persisted from fall to the growing season; second, the field conditions did not promote N loss to a degree that would necessitate the inhibitors' action in reducing such losses. In Alberta, Lin et al. (2023) investigated the efficacy of two nitrification inhibitors (NIs), 3,4-dimethylpyrazole phosphate (DMPP) and nitrapyrin, in reducing N_2O emissions from swine and dairy liquid manure applied to barley crop fields. The manure was injected in the fall to depths of 12.7 cm and 15.2 cm at soil moisture levels ranging from 48% to 57% WFPS and a temperature of 10°C. The study reported that NIs (DMPP) significantly reduced spring-thaw N_2O emissions by 52% compared to untreated manure at about 80% WFPS.

These findings highlight the importance of timing N application and considering soil conditions, particularly moisture content during and after fall fertilizer application, which can affect the efficacy of N fertilizers and inhibitors.

1.4 Management strategies for nitrogen losses

To mitigate nitrogen (N) losses and improve N use efficiency (NUE), the 4R Nutrient Stewardship framework is widely recommended in the prairies. This framework aims to reduce nutrient losses, improve NUE, and enhance crop yield, quality, and economic returns (Grant and Flaten, 2019). The 4R framework emphasizes the application of nutrients from the right source, at the right rate, at the right time, and in the right place (Liu et al., 2022).

Currently, the Department of Soil Science at the University of Manitoba hosts a research program on nutrient management led by the Natural Sciences and Engineering Research Council

of Canada (NSERC) Industrial Research Chair (IRC) in 4R Nutrient Stewardship. This program aims to advance our understanding of how this beneficial management practice contributes to reducing greenhouse gas emissions in Manitoba.

Recent studies have demonstrated the effectiveness of adopting the 4R nutrient framework in reducing N losses, particularly nitrous oxide (N₂O) (Gao et al., 2017; Tenuta et al., 2023; Wood et al., 2023). For example, Gao et al. (2017) evaluated the 4R framework in an irrigated potato field in Manitoba. The study found that integrating all components of the framework, including source, rate, placement, and timing, significantly reduced N₂O emissions. Similarly, another study in Manitoba highlighted the effectiveness of the 4R practices—source, rate, and placement—in reducing N₂O emissions (Tenuta et al., 2023). This study compared urea and SuperU as nitrogen sources, applied through shallow banding (2.0–2.5 cm), deep banding (4.0–5.0 cm), and broadcasting with incorporation. The study showed that SuperU reduced emissions by 46% compared to untreated urea, while shallow banding significantly increased N₂O emissions across all sites. It was also observed that band placement was less effective than broadcast incorporation at one of the sites. This was attributed to high soil moisture levels, which created anoxic conditions that favored denitrification, in contrast to the broadcast incorporation method.

Another nutrient management strategy involves the use of nitrogen inhibitors, particularly urease and nitrification inhibitors (Trenkel, 2010; Lin et al., 2023). Research suggests that combining the 4R framework with enhanced efficiency fertilizers could further reduce N losses and improve environmental sustainability. For example, a meta-analysis by Abalos et al. (2014) demonstrated the effectiveness of inhibitors to improve NUE while simultaneously increasing crop yields. The 4R strategy is most effective when integrated with other management practices that enhance NUE (The Fertilizer Institute, 2017). Applying the 4R framework alongside inhibitor-

treated fertilizers offers a promising approach to increasing NUE and reducing N losses to the environment. While inhibitors have shown potential to improve NUE and reduce emissions, there is limited information on their performance under varying temperatures and moisture conditions in Manitoba. The specific types of inhibitors and their mechanisms of action are discussed in the following sections.

1.4.1 Types of nitrogen inhibitors

Nitrification inhibitors (NIs) and urease inhibitors (UIs) are commonly used with nitrogen (N) fertilizers to reduce N losses (Trenkel, 2010). NIs delay the conversion of NH_4^+ to NO_3^- , while UIs slow the transformation of applied N into NH_3 gas (Trenkel, 2010). Nitrification, the process that converts NH_4^+ to nitrites and then NO_3^- , can lead to significant N losses. Nitrification inhibitors such as DMPP and DCD slow down the process of nitrification by inhibiting the activity of ammonia monooxygenase. This helps to retain more N in the NH_4^+ form rather than the NO_3^- form, thereby enhancing N use efficiency (NUE) (Castellano-Hinojosa et al., 2020; Liu et al., 2013) and reducing N losses to the environment (Manitoba Agriculture, 2023).

Urease Inhibitors, like N-(n-butyl) thiophosphoric triamide (NBPT), are often used with urea-based fertilizers to inhibit urease activity, reducing NH_3 volatilization (Trenkel, 2010; Liu et al., 2019). In some cases, both urease and nitrification inhibitors are applied together, known as double inhibitors (Koch Agronomic Services, 2019). Some examples of double inhibitors include SuperU, which has both UIs and NIs already incorporated in the urea granule by the manufacturer. There are also liquid formulations of double inhibitors sold under trade names like Agrotain Plus, ARM U Advanced, and Active Stabilizer Plus (ASP), which are applied separately to the urea granules. Inhibitors have been demonstrated to enhance NUE and reduce N losses, especially N_2O emissions (Abalos et al., 2014; Castellano-Hinojosa et al., 2020; Tenuta et al., 2023). For example,

a study in Manitoba demonstrated that SuperU can effectively reduce N₂O emissions and enhance NUE, with emissions reduced by 37% to 57% (Wood et al., 2023; Tenuta et al., 2023). Another field study revealed that combining NBPT with a NI did not enhance overall inhibitor efficiency; instead, the NI diminished the effectiveness of NBPT (Lasisi et al., 2019). In this study, NBPT and DMPP were used to coat urea, forming a double inhibitor. The variability in findings may be attributed to environmental factors such as temperature and moisture levels, which can influence inhibitor efficacy (Abalos et al., 2014). Additionally, differences in product characteristics may play a role, as SuperU contains DCD and NBPT, whereas Lasisi et al. (2019) used a combination of DMPP and NBPT. SuperU is a dual N inhibitor composed of 45.5% urea treated with 0.85% (8500 ppm) dicyandiamide (DCD) and 0.06% (600 ppm) N-(n-butyl) thiophosphoric triamide (NBPT) (Koch Agronomic Services, 2019). The NBPT in SuperU inhibits urease enzyme activity, reducing ammonia volatilization, while DCD delays the bacterial conversion of NH₄⁺ to NO₃⁻, thereby reducing denitrification and nitrate leaching (Koch Agronomic Services, 2019).

1.4.2 Challenges associated with the use of N Inhibitors

In Canada's pursuit of net-zero emissions by 2050 (Government of Canada, 2020), identifying and understanding the factors controlling N₂O emissions is crucial for implementing effective management strategies. Nitrogen inhibitors are a promising approach to reducing N₂O emissions from the environment (Wood et al., 2023) as discussed in section 1.5.1.

One of the main challenges with using inhibitors is the additional cost to farmers. Tenuta et al., (2023) noted that although farmers are willing to use inhibitors, their widespread adoption is limited by the high costs. For example, SuperU is about 42% more expensive than conventional urea (Robin, 2019), significantly increasing farmers' production costs. Considering the high cost associated with inhibitors, it is imperative that we evaluate their efficacy under the wide range of

moisture and temperature conditions experienced from fall through spring to ensure their economic benefits in addition to the environmental benefits. The effectiveness of inhibitors under freezing and varying thawing conditions typical of the Canadian prairies is yet to be fully assessed.

1.5 Research needs to advance our understanding of N dynamics in Canadian prairie soils

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

Most freeze-thaw cycle (FTC) studies report significant N₂O emissions during the first FTC. However, in field studies, especially during excessively wet springs, field access to sample emissions during the first thaw can be limited, potentially missing key emission processes. In contrast, laboratory studies allow continuous sampling of N₂O emissions during thawing and can guide field research by defining hypotheses to test and parameters to measure. Despite the importance of understanding N inhibitors under prairie climates, there is inadequate information on their effects under FTCs in the prairies. This study will examine the effects of varying thawing temperatures and moisture levels on the efficacy of double inhibitors in reducing N₂O emissions in a laboratory setting.

b. Variable moisture content and temperature effects on N₂O emissions under FTCs

The literature review revealed that the few FTC studies in the prairies on N losses have primarily focused on direct N₂O emissions under a single thaw temperature or moisture level (typically at field capacity). No studies have mimicked the effects of fall fertilizer application under varying moisture levels on spring-thaw emissions. This is particularly important because fall moisture conditions influence those in the spring, potentially impacting spring-thaw emissions.

c. Testing realistic soil temperature on lab FTC studies

Using temperatures that reflect actual field conditions reduces the risk of exposing soils to unrealistic temperatures (Henry, 2007). Only a few studies have reported the use of historical soil temperature data to establish treatment temperatures (eg., Fouli et al., 2013). Future laboratory studies could use historical weather data from a local station to set freezing and thawing temperatures, ensuring the soils are exposed to conditions that mirror those at the research site. In so doing, stronger inferences could be drawn from the study results.

d. Establishing the volume of soils for FTC impact on N₂O emissions in lab studies

Some previous studies, such as the one by Tenuta and Sparling (2011), exposed small quantities of soil to extreme temperatures, resulting in abnormally high freezing rates that did not reflect natural field conditions (Henry, 2007). These intense freezing rates tend to kill microbes. It is, therefore, not surprising that Tenuta and Sparling (2011) recorded negligible N₂O emissions when 115 g of soil was frozen at -20°C and thawed at 4°C. Based on the literature reviewed, a higher quantity of soil should be used in FTC laboratory studies, particularly when freezing temperatures of -20°C or lower are applied (Hung and Whalen 2020). The freeze-thaw chamber (UTEST, UTD-1442) used in this study features an automated system that allows for precise adjustments to the rate of freezing or thawing, a function often absent in incubators utilized in previous studies, which typically rely on default and frequently undefined freezing rate settings.

e. Expanding FTC studies on a wide range of agricultural soils

Only a few FTC studies have focused on clay soils (Van Bochove et al. 2000b; Pelster et al. 2013; King et al. 2021), likely due to the challenges of working with clay soils in the laboratory. Most studies have concentrated on sandy loam soils, resulting in limited information on how FTCs influence N₂O emissions in clay soils.

1.6 Research Objectives

The literature reviewed revealed research opportunities that should be pursued to advance our knowledge of FTC effects on N- dynamics. In the present study, a laboratory experiment was set up to study the effects of freezing and thawing cycle, moisture, and temperature variability on N-dynamics in sandy loam treated with urea and SuperU.

The study aimed to evaluate the effects of three moisture levels (32%, 50%, and 100% WFPS) and four temperatures levels (4°C, 8°C, 12°C, and 16°C) on cumulative N₂O thaw-emissions, N₂O flux, and mineral N (NH₄⁺ and NO₃⁻) concentration after subjecting soil samples to one freeze-thaw cycle after freezing at -20°C.

1.7 Thesis outline

The thesis was written in manuscript style in accordance with the guidelines set by the Department of Soil Science, University of Manitoba. It consists of three chapters: Chapter 1 presents pertinent literature to support the research, including an overview of freezing and thawing effects on nutrient management in the Canadian prairies, N dynamics under freeze-thaw cycles, effects of freeze-thaw cycles on N₂O emissions, management strategies for N losses, and research needs to advance our understanding of N dynamics in the prairies. Chapter 2 addresses the research objective, which focused on the effects of freezing and thawing cycle, moisture and temperature variability on N loss through N₂O emissions from a urea-based fertilizer treated with and without double inhibitors

in a sandy loam soil. Chapter 3 presents the overall synthesis of the thesis, highlighting the implications of the results and recommendations to further advance our knowledge of FTCs, moisture, and temperature effects on N- losses in soil and agronomic systems.

Chapter 2: Freeze-Thaw Cycle, Soil Moisture, and Thawing Temperature effects on Nitrogen Dynamics in a Black Chernozemic Sandy Loam

1.8 References

- Abalos, D., S. Jeffery, A. Sanz-Cobena, G. Guardia, and A. Vallejo. 2014. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agriculture, Ecosystems & Environment* 189: 136–144. doi: 10.1016/j.agee.2014.03.036.
- Amiro, B., M. Tenuta, K. Hanis-Gervais, X. Gao, D. Flaten, et al. 2017. Agronomists' Views on the Potential to Adopt Beneficial Greenhouse Gas Nitrogen Management Practices Through Fertilizer Management. *Can. J. Soil. Sci.: CJSS-2017-0062*. doi: 10.1139/CJSS-2017-0062.
- Braker, G., and R. Conrad. 2011. Diversity, Structure, and Size of N₂O-Producing Microbial Communities in Soils—What Matters for Their Functioning? *Advances in Applied Microbiology*. Elsevier. p. 33–70
- Castellano-Hinojosa, A., J. Gonzalez-Lopez, A. Vallejo, and E. Bedmar. 2020. Effect of urease and nitrification inhibitors on ammonia volatilization and abundance of N-cycling genes in an agricultural soil. *Journal Of Plant Nutrition And Soil Science* 183(1): 99–109. doi: 10.1002/jpln.201900038.
- Chen, Y. 1995. Nitrous oxide emission from an agricultural soil subjected to different freeze-thaw cycles. *Agriculture, Ecosystems & Environment* 55(2): 123–128. doi: 10.1016/0167-8809(95)00611-U.
- Christensen, S., and B.T. Christensen. 1991. Organic matter available for denitrification in different soil fractions: effect of freeze/thaw cycles and straw disposal. *Eur. J. Soil Sci.* 42(4): 637–647. doi: 10.1111/j.1365-2389.1991.tb00110.x.
- Climate Atlas. 2019. Freeze-Thaw Cycles | Canada | Climate Atlas of Canada. https://climateatlas.ca/map/canada/freezethaw_2030_85 (accessed 6 January 2024).
- Congreves, K.A., C. Wagner-Riddle, B.C. Si, and T.J. Clough. 2018. Nitrous oxide emissions and biogeochemical responses to soil freezing-thawing and drying-wetting. *Soil Biology and Biochemistry* 117: 5–15. doi: 10.1016/j.soilbio.2017.10.040.

- Costa, V.A.F. 2006. Thermodynamic analysis of building heating or cooling using the soil as heat reservoir. *International Journal of Heat and Mass Transfer* 49(21–22): 4152–4160. doi: 10.1016/j.ijheatmasstransfer.2006.03.029.
- Cui, G., and J. Wang. 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. doi: 10.1016/j.scitotenv.2019.06.054.
- DeLuca, T.H., D.R. Keeney, and G.W. McCarty. 1992. Effect of freeze-thaw events on mineralization of soil nitrogen. *Biol Fertil Soils* 14(2): 116–120. doi: 10.1007/BF00336260.
- Durán, J., J.L. Morse, P.M. Groffman, J.L. Campbell, L.M. Christenson, et al. 2016. Climate change decreases nitrogen pools and mineralization rates in northern hardwood forests (D.P.C. Peters, editor). *Ecosphere* 7(3). doi: 10.1002/ecs2.1251.
- Ejack, L., and J. Whalen. 2021. Freeze-thaw cycles release nitrous oxide produced in frozen agricultural soils. *Biology and Fertility of Soils* 57(3): 389–398. doi: 10.1007/s00374-020-01537-x.
- Elliott, J. 2013. Evaluating the potential contribution of vegetation as a nutrient source in snowmelt runoff. *Can. J. Soil. Sci.* 93(4): 435–443. doi: 10.4141/cjss2012-050.
- Fouli, Y., B.J. Cade-Menun, and H.W. Cutforth. 2013. Freeze-thaw cycles and soil water content effects on infiltration rate of three Saskatchewan soils. *Can. J. Soil Sci.* 93(4): 485–496. doi: 10.4141/CJSS2012-060.
- Fu, Q., J. Yan, H. Li, T. Li, R. Hou, et al. 2019. Effects of biochar amendment on nitrogen mineralization in black soil with different moisture contents under freeze-thaw cycles. *Geoderma* 353: 459–467. doi: 10.1016/j.geoderma.2019.07.027.
- Girkin, N.T., and H.V. Cooper. 2023. Nitrogen and ammonia in soils. In: Goss, M.J. and Oliver, M., editors, *Encyclopedia of Soils in the Environment (Second Edition)*. Academic Press, Oxford. p. 142–151
- Gong, P., L. Zhang, Z. Wu, Z. Chen, and L. Chen. 2013. Responses of Ammonia-Oxidizing Bacteria and Archaea in Two Agricultural Soils to Nitrification Inhibitors DCD and DMPP: A Pot Experiment. *Pedosphere* 23(6): 729–739.
- Hao, X., C. Chang, J.M. Carefoot, H.H. Janzen, and B.H. Ellert. Nitrous oxide emissions from an irrigated soil as affected by fertilizer and straw management.
- Hayatsu, M., K. Tago, and M. Saito. 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. doi: 10.1111/j.1747-0765.2007.00195.x.
- Heard, J. Enhanced Efficiency Additives for Nitrogen - How they Work. Province of Manitoba - Agriculture. <https://www.gov.mb.ca/agriculture/> (accessed 19 January 2024).

- Heard, J., and D. Flaten. 2020. Fertilization Considerations after an Unusually Wet Fall: Challenges and Opportunities.
- Henry, H.A.L. 2007. Soil freeze–thaw cycle experiments: Trends, methodological weaknesses and suggested improvements. *Soil Biology and Biochemistry* 39(5): 977–986. doi: 10.1016/j.soilbio.2006.11.017.
- Herridge, D.F., M.B. Peoples, and R.M. Boddey. 2008. Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil* 311(1–2): 1–18. doi: 10.1007/s11104-008-9668-3.
- Hung, C.-Y., and J.K. Whalen. 2020. Biophysical controls on nitrous oxide emissions following rain-induced thawing of frozen soil microcosms by simulated rainfall. *Soil Biol. Biochem.* 149. doi: 10.1016/j.soilbio.2020.107960.
- IPCC. 1997. The Regional Impacts of Climate Change: An Assessment of Vulnerability — IPCC. <https://www.ipcc.ch/report/the-regional-impacts-of-climate-change-an-assessment-of-vulnerability/> (accessed 30 August 2023).
- Jung, J., J. Yeom, J. Kim, J. Han, H.S. Lim, et al. 2011. Change in gene abundance in the nitrogen biogeochemical cycle with temperature and nitrogen addition in Antarctic soils. *Research in Microbiology* 162(10): 1018–1026. doi: 10.1016/j.resmic.2011.07.007.
- King, A., F. Rezanezhad, and C. Wagner-Riddle. 2021. Evidence for microbial rather than aggregate origin of substrates fueling freeze-thaw induced N₂O emissions. *SOIL BIOLOGY & BIOCHEMISTRY* 160. doi: 10.1016/j.soilbio.2021.108352.
- Koch Agronomic Services. 2019. How SuperU Works.
- Lasisi, A.A., O.O. Akinremi, and D. Kumaragamage. 2019. Efficacy of a new *N*-(*n*-butyl) thiophosphoric triamide formulation in reducing ammonia volatilization from urea-based fertilizers (N. Lupwayi, editor). *Can. J. Soil. Sci.* 99(4): 395–405. doi: 10.1139/cjss-2018-0072.
- Lasisi, A., O. Akinremi, and D. Kumaragamage. 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. doi: 10.1002/saj2.20126.
- Lasisi, A., O. Akinremi, Q. Zhang, and D. Kumaragamage. 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. doi: 10.1002/saj2.20062.
- Leuther, F., and S. Schlüter. 2021. Impact of freeze–thaw cycles on soil structure and soil hydraulic properties. *SOIL* 7(1): 179–191. doi: 10.5194/soil-7-179-2021.

- Li, Z., D. Tian, B. Wang, J. Wang, S. Wang, et al. 2019. Microbes drive global soil nitrogen mineralization and availability. *Global Change Biology* 25(3): 1078–1088. doi: 10.1111/gcb.14557.
- Libby, M.D., A.C. Vander Zaag, E.G. Gregorich, and C. Wagner-Riddle. 2020. An improved laboratory method shows that freezing intensity increases n₂o emissions. *Can. J. Soil Sci.* 100(2): 136–149. doi: 10.1139/cjss-2019-0073.
- Lin, S., G. Hernandez-Ramirez, L. Kryzanowski, G. Lohstraeter, and T. Wallace. 2023. Adding inhibitors to manure injections can mitigate nitrous oxide emissions from barley croplands. *Nutr Cycl Agroecosyst* 126(1): 81–100. doi: 10.1007/s10705-023-10277-x.
- Liu, X., X. Ju, Y. Zhang, C. He, J. Kopsch, et al. 2006. Nitrogen deposition in agroecosystems in the Beijing area. *Agriculture, Ecosystems & Environment* 113(1–4): 370–377. doi: 10.1016/j.agee.2005.11.002.
- Liu, B., R. Ma, and H. Fan. 2021. Evaluation of the impact of freeze-thaw cycles on pore structure characteristics of black soil using X-ray computed tomography. *Soil Tillage Res.* 206. doi: 10.1016/j.still.2020.104810.
- Liu, C., K. Wang, and X. Zheng. 2013. Effects of nitrification inhibitors (DCD and DMPP) on nitrous oxide emission, crop yield and nitrogen uptake in a wheat-maize cropping system. *Biogeochemistry: Greenhouse Gases.*
- Liu, X., M. Zhang, Z. Li, C. Zhang, C. Wan, et al. 2019. Inhibition of urease activity by humic acid extracted from sludge fermentation liquid. *Bioresource Technology* 290: 121767. doi: 10.1016/j.biortech.2019.121767.
- Malhi, S.S., and M. Nyborg. 1992. Recovery of nitrogen by spring barley from ammonium nitrate, urea and sulphur-coated urea as affected by time and method of application. *Fertilizer Research* 32(1): 19–25. doi: 10.1007/BF01054390.
- Manitoba Agriculture. 2023. A Fall Fertilization Refresher. Province of Manitoba - Agriculture. <https://www.gov.mb.ca/agriculture/> (accessed 7 June 2023).
- Matsuoka, N., K. Hirakawa, T. Watanabe, W. Haeberli, and F. Keller. 1998. THE ROLE OF Diurnal, Annual and Millennial Freeze-Thaw Cycles in Controlling Alpine Slope Instability.
- Mosaic Crop Nutrition. 2023. Nitrogen | Key Nutrients. Mosaic Crop Nutrition. <https://www.cropnutrition.com/nutrient-management/nitrogen/> (accessed 17 January 2024).
- Németh, D.D., C. Wagner-Riddle, and K.E. Dunfield. 2014. Abundance and gene expression in nitrifier and denitrifier communities associated with a field scale spring thaw N₂O flux event. *Soil Biology and Biochemistry* 73: 1–9. doi: 10.1016/j.soilbio.2014.02.007.

- Ni, K., H. Kage, and A. Pacholski. 2018. Effects of novel nitrification and urease inhibitors (DCD/TZ and 2-NPT) on N₂O emissions from surface applied urea: An incubation study. *Atmospheric Environment* 175: 75–82. doi: 10.1016/j.atmosenv.2017.12.002.
- Nyborg, M., and S.S. Malhi. 1986. Comparison of Fall and Spring Application of Nitrogen Fertilizers in Northern and Central Alberta. *Can. J. Soil. Sci.* 66(2): 225–236. doi: 10.4141/cjss86-025.
- Oztaş, T., and F. Fayetorbay. 2003. Effect of freezing and thawing processes on soil aggregate stability. *Catena* 52(1): 1–8. doi: 10.1016/S0341-8162(02)00177-7.
- Pelster, D., M. Chantigny, P. Rochette, D. Angers, J. Laganier, et al. 2013. Crop residue incorporation alters soil nitrous oxide emissions during freeze-thaw cycles. *Canadian Journal of Soil Science* 93(4): 415–425. doi: 10.4141/CJSS2012-043.
- Pelster, D.E., M.H. Chantigny, P. Rochette, N. Bertrand, D.A. Angers, et al. 2019. Rates and intensity of freeze–thaw cycles affect nitrous oxide and carbon dioxide emissions from agricultural soils. *Can. J. Soil Sci.* 99(4): 472–484. doi: 10.1139/cjss-2019-0058.
- Philippot, L., and O. Højberg. 1999. Dissimilatory nitrate reductases in bacteria. *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression* 1446(1–2): 1–23. doi: 10.1016/S0167-4781(99)00072-X.
- Ramakrishnan Vv, G.A. 2015. Nitrogen Sources and Cycling in the Ecosystem and its Role in Air, Water and Soil Pollution: A Critical Review. *J Pollut Eff Cont* 03(02). doi: 10.4172/2375-4397.1000136.
- Robin, B. 2019. New study finds risks in broadcasting urea. *The Western Producer*. <https://www.producer.com/crops/new-study-finds-risks-in-broadcasting-urea/> (accessed 27 February 2025).
- Rosa, L., and P. Gabrielli. 2023. Energy and food security implications of transitioning synthetic nitrogen fertilizers to net-zero emissions. *Environ. Res. Lett.* 18(1): 014008. doi: 10.1088/1748-9326/aca815.
- Schimel, J.P., and J. Bennett. 2004. Nitrogen Mineralization: Challenges of a Changing Paradigm. *Ecology* 85(3): 591–602. doi: 10.1890/03-8002.
- Schumann, U., and H. Huntrieser. 2007. The global lightning-induced nitrogen oxides source. *Atmos. Chem. Phys.*
- Smith, K., K. Dobbie, R. Thorman, C. Watson, D. Chadwick, et al. 2012. The effect of N fertilizer forms on nitrous oxide emissions from UK arable land and grassland. *Nutrient Cycling in Agroecosystems* 93(2): 127–149. doi: 10.1007/s10705-012-9505-1.
- Tenuta, M., X. Gao, D.N. Flaten, and B.D. Amiro. 2016. Lower Nitrous Oxide Emissions from Anhydrous Ammonia Application Prior to Soil Freezing in Late Fall Than Spring Pre-Plant Application. *J. Environ. Qual.* 45(4): 1133–1143. doi: 10.2134/jeq2015.03.0159.

- Tenuta, M., X. Gao, K.H.D. Tiessen, K. Baron, and B. Sparling. 2023. Placement and nitrogen source effects on N₂O emissions for canola production in Manitoba. *Agronomy Journal*: agj2.21408. doi: 10.1002/agj2.21408.
- Tenuta, M., and B. Sparling. 2011. A laboratory study of soil conditions affecting emissions of nitrous oxide from packed cores subjected to freezing and thawing. *Can. J. Soil Sci.* 91(2): 223–233. doi: 10.4141/cjss09051.
- Tian, H., X. Xu, C. Lu, M. Liu, W. Ren, et al. 2011. Net exchanges of CO₂, CH₄, and N₂O between China's terrestrial ecosystems and the atmosphere and their contributions to global climate warming. *J. Geophys. Res.* 116(G2): G02011. doi: 10.1029/2010JG001393.
- Tiessen, K.H.D., D.N. Flaten, C.A. Grant, R.E. Karamanos, and M.H. Entz. 2005. Efficiency of fall-banded urea for spring wheat production in Manitoba: Influence of application date, landscape position and fertilizer additives. *Can. J. Soil. Sci.* 85(5): 649–666. doi: 10.4141/S05-017.
- Trenkel, M.E. 2010. *Slow- and controlled-release and stabilized fertilizers: an option for enhancing nutrient use efficiency in agriculture*. 2nd ed. IFA, International fertilizer industry association, Paris.
- Van Bochove, E., D. Prevost, and F. Pelletier. 2000a. Effects of freeze-Thaw and soil structure on nitrous oxide produced in a clay soil. *Soil Sci. Soc. Am. J.* 64(5): 1638–1643. doi: 10.2136/sssaj2000.6451638x.
- Van Bochove, E., D. Prévost, and F. Pelletier. 2000b. Effects of Freeze-Thaw and Soil Structure on Nitrous Oxide Produced in a Clay Soil. *Soil Sci. Soc. Am. J.* 64(5): 1638–1643. doi: 10.2136/sssaj2000.6451638x.
- Van Drecht, G., A.F. Bouwman, J.M. Knoop, A.H.W. Beusen, and C.R. Meinardi. 2003. Global modeling of the fate of nitrogen from point and nonpoint sources in soils, groundwater, and surface water. *Global Biogeochemical Cycles* 17(4): 2003GB002060. doi: 10.1029/2003GB002060.
- Wagner-Riddle, C., K.A. Congreves, D. Abalos, A.A. Berg, S.E. Brown, et al. 2017. Globally important nitrous oxide emissions from croplands induced by freeze–thaw cycles. *Nature Geosci* 10(4): 279–283. doi: 10.1038/ngeo2907.
- Ward, B.B. 2011. Measurement and Distribution of Nitrification Rates in the Oceans. *Methods in Enzymology*. Elsevier. p. 307–323
- Wood, M.D., X. Gao, K.H.D. Tiessen, M. Tenuta, and D.N. Flaten. 2023. Enhanced efficiency urea fertilizers and timing effects on N₂O emissions from spring wheat production in Manitoba. *Agronomy Journal*: agj2.21448. doi: 10.1002/agj2.21448.
- Zhang, T., R.L. Armstrong, and J. Smith. 2003. Investigation of the near-surface soil freeze-thaw cycle in the contiguous United States: Algorithm development and validation. *J. Geophys. Res. D Atmos.* 108(22): GCP21-1. doi: 10.1029/2003jd003530.

- Zhang, Y., W. Chen, S.L. Smith, D.W. Riseborough, and J. Cihlar. 2005. Soil temperature in Canada during the twentieth century: Complex responses to atmospheric climate change. *J. Geophys. Res.* 110(D3): 2004JD004910. doi: 10.1029/2004JD004910.
- Zhang, B., H. Tian, C. Lu, S.R.S. Dangal, J. Yang, et al. 2017. Global manure nitrogen production and application in cropland during 1860–2014: a 5 arcmin gridded global dataset for Earth system modeling. *Earth Syst. Sci. Data* 9(2): 667–678. doi: 10.5194/essd-9-667-2017.
- Zhang, X., T. Zou, L. Lassaletta, N.D. Mueller, F.N. Tubiello, et al. 2021. Quantification of global and national nitrogen budgets for crop production. *Nat Food* 2(7): 529–540. doi: 10.1038/s43016-021-00318-5.
- Zheng, D., E. Hunt, and S. Running. 1993. A daily soil temperature model based on air temperature and precipitation for continental applications. *Clim. Res.* 2: 183–191. doi: 10.3354/cr002183.

2. FREEZE-THAW CYCLE, SOIL MOISTURE, AND THAWING TEMPERATURE EFFECTS ON NITROGEN DYNAMICS IN A BLACK CHERNOZEMIC SANDY LOAM

2.1 Abstract

In the Canadian prairies, many farmers apply urea during the fall before freezing. This practice is motivated by lower fertilizer prices in the fall and the need to reduce field operational tasks in the spring considering the short growing season and the risk of excess soil moisture delaying field operations. This practice is also perceived to have a low environmental impact considering the lower temperatures characterizing the fall through spring and how that curtails the nitrogen (N) transformation processes hence leading to reduced N₂O emissions. In the prairies, farmers are advised to apply fertilizers in the fall at a temperature of 5°C or lower to reduce N losses. However, considering the freezing and thawing events resulting in varying soil moisture and temperature that characterize the prairies, the risk of N losses through N₂O emissions is high even when fertilizers are treated with inhibitors. Management of N fertilizer and losses is critical to our efforts towards mitigating and adapting to climate change impacts, which necessitates advancing our knowledge on N dynamics under Canadian prairie conditions. Therefore, this study evaluated the effects of freezing and thawing cycle, soil moisture (32%, 50%, or 100% WFPS) and thawing temperatures (4, 8, 12, or 16°C) on N₂O emissions from a Black Chernozemic sandy loam soil treated with a stabilized urea-based fertilizer. The soil samples were homogenized and packed to a bulk density of 1.2 g cm⁻³, followed by band-application of N fertilizers (SuperU and Urea). Soil moisture content significantly affected cumulative N₂O emissions, with 100% WFPS producing the highest emissions (582 µg kg⁻¹) at 16°C, compared to 32% and 50% WFPS across all temperature levels. Hourly N₂O flux began at 10 h, with 100% WFPS recording the highest flux (10.3 µg kg⁻¹ h⁻¹) at 60 h. In contrast, N₂O fluxes at 32% and 50% WFPS showed no significant

differences across the sampling times. Volumetric heat capacity (VHC) was the primary property affecting soil heating during thawing. There was a significant positive correlation between VHC and N₂O flux at higher temperatures, with correlation coefficients (r) of -0.14, 0.36, 0.17, and 0.44 for 4, 8, 12, and 16°C, respectively. SuperU was not effective in reducing emissions relative to urea. This study demonstrates that, in addition to temperature guidelines for fall fertilization, establishing a safe application threshold for fall soil moisture could help reduce N₂O emissions. The results suggest that farmers in the prairies could reduce N₂O emissions by applying banded urea in fall at 5 cm depth when soil moisture is at or below 50% WFPS. However, spring conditions pose a high risk of N losses.

2.2 Introduction

The agricultural sector in Canada consumes a significant amount of fertilizers (4.6 MMT), with approximately 60% being nitrogen (N)-based (Fertilizer Canada, 2020; Statista, 2023). This highlights N as the most applied plant nutrient in the country due to its critical role in yield and protein synthesis in crops (Fertilizer Canada, 2020). Common N-based fertilizers produced in Canada include ammonia, urea ammonium nitrate, ammonium sulfate and urea (Government of Canada, 2018). Among them, urea is the most used, accounting for over 50% of all N-based fertilizers (Statista, 2023). Farmers prefer urea because of its high N content, which makes it cost-effective per unit of N applied (Glibert et al., 2006). Additionally, it is safe to store, easy to transport in its granulated form, and simple to apply (Glibert et al., 2006). These advantages make it a desirable option for many farmers in the Canadian prairies (Tiessen et al., 2005). Notwithstanding its advantages, urea is prone to losses in the form of ammonia volatilization and nitrous oxide (N₂O) emissions. N₂O is a potent stratospheric gas that depletes the ozone layer, warms the climate

about 265 times faster than CO₂ (Stocker, 2014), and has a residence time of 109 years (Prather et al., 2015).

Two main processes that lead to N₂O production include nitrification and denitrification, which are influenced by environmental factors such as moisture and temperature (Grundmann et al., 1995; Braker et al., 2010). Nitrification begins with *Nitrosomonas* oxidizing ammonium into nitrite (NO₂⁻), followed by *Nitrobacter* converting NO₂⁻ into nitrate (NO₃⁻) (Trenkel, 2010). Where not taken up by plants, NO₃⁻ can subsequently be denitrified to N₂O (Hayatsu et al., 2008) or leached to pollute ground-water (Smolders et al., 2010).

To reduce the rate of these processes, N inhibitors (urease and nitrification inhibitors) are applied with urea-based fertilizers to decrease microbial activity responsible for N transformation (Trenkel, 2010). While urease inhibitors (UIs) like N-(n-butyl) thiophosphoric triamide (NBPT) reduce urea hydrolysis, nitrification inhibitors (NIs), such as dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) reduce nitrification (Abalos et al., 2014). Manufacturers often coat urea granules with both UIs and NIs (double inhibitors), as seen in products like SuperU (Koch Agronomic Services, 2019), or offer liquid formulations like Agrotain Plus, Active Stabilizer™ Plus (ASP), and ARM U™ Advanced for treating urea-based fertilizers before application. Nitrogen inhibitors have been demonstrated to improve N use efficiency under the 4R nutrient stewardship. In the 4R nutrient stewardship, farmers are advised to apply N from the right source, at the right rate, time, and place (Gao et al., 2017; Johnston and Bruulsema, 2014). Tenuta et al. (2023) found that using SuperU as the N source under the 4R nutrient stewardship reduced N₂O emissions at a 100% fertilizer application rate.

For many farmers in Manitoba, the time of fertilizer application is in the fall before freezing (Amiro et al., 2017). Farmers prefer fall application because it reduces spring tillage operations,

spreads out the workload, and takes advantage of lower fertilizer costs (Amiro et al., 2017; Tiessen et al., 2003). Farmers are advised to apply fertilizers when the soil temperature is 5°C or lower to reduce N losses (Tenuta et al., 2016). This recommendation is based on the assumption that lower temperatures reduce microbial activity responsible for N transformation (Lasisi et al., 2020).

However, the wide variability of soil moisture conditions and temperature from fall through spring still presents risks of N losses. The ideal soil moisture content for application remains unclear, as soil moisture in fall subsequently affects moisture in the spring (Heard and Flaten, 2020). This, in turn, could influence spring-thaw N₂O emissions from fall-applied N, which account for 30-50% of annual emissions (Wood et al., 2023). These emissions are attributed to increased moisture and elevated temperatures during thawing (Congreves et al., 2018). As soil thaws, substrates bound to soil minerals are released into the soil's carbon and N pools for microbial use (Van Bochove et al., 2000) - a process influenced by moisture and temperature (Lin et al., 2023). Despite the effects of moisture and temperature on N₂O emissions from fall-applied N application, much remains unexplored about their combined effects in the prairies, which can be better understood in controlled experiments. Laboratory studies are essential for advancing our understanding of N₂O emissions, as they allow for precise control of variables that are difficult to isolate in field settings. While field studies provide insights into real-world conditions, their outcomes are often influenced by environmental variability, making it challenging to identify specific factors driving N₂O emissions. In our environment, field studies face challenges such as snowmelt during spring thaw, which creates wet conditions and limits field access for gas sampling. This can lead to inadequate capturing of gas episodes during thawing, potentially underestimating thaw-related N₂O emissions by 17 – 18% (Wagner-Riddle et al., 2017). Laboratory experiments, in contrast, offer controlled environments to investigate the complex

interactions between variables under study to advance our understanding of N dynamics, which is critical to our efforts towards mitigating and adapting to climate change impacts. Therefore, the objective of this study was to evaluate the effects of freezing and thawing cycles, soil moisture (32%, 50%, or 100% WFPS) and thawing temperatures (4, 8, 12, or 16°C) on N₂O emissions from a sandy loam soil treated with stabilized urea-based fertilizer. We hypothesized that 1) higher temperature and moisture will increase N₂O emissions during thawing with those treated with double inhibitor (DI) producing lower emissions compared to untreated fertilizers, 2) N₂O flux will increase at higher moisture content and temperature during the thawing period, and 3) higher thawing temperature and moisture levels will increase mineral N concentration in the soil treated with and without DI, with those treated with DIs retaining more ammonium.

2.3 Materials and Methods

2.3.1 Study site description

Soil samples were collected (0 – 15 cm) from the Ian N. Morrison Research Farm (Figure 2.1), Carman, Manitoba (latitude 49°30'04.6"N, longitude 98°01'42.1"W) in fall 2023 to conduct the laboratory experiment. The experiment was undertaken at the Soil Physical Processes Laboratory in the Department of Soil Science, University of Manitoba. The sampling site has a gentle slope, and the soil is Orthic Black Chernozem, Denham series, and has a sandy loam texture (Agriculture and Agri-Food, 2013). The site is characterized by seasonal freezing and thawing cycles (real-time in-situ soil monitoring for agriculture (RISMA, 2023)) and receives 545 mm of precipitation annually with 100.3 cm in the form of snow and 445 mm in the form of rainfall. The site has an annual minimum temperature of -21.2°C, which occurs in January, and a maximum temperature

of 25.3°C in July. Prior to soil sampling, the site had been used to cultivate soybean in 2020, oats in 2021, canola in 2022, and spring wheat in 2023.

2.3.1.1 Historical moisture and temperature data for the study site

Based on historical weather data (2013 – 2022) from RISMA at the study site, the top 5 cm of soil freezes to a minimum temperature range of -19.3°C to -21.2°C, with an average initial spring-thaw temperature of 3.8°C (RISMA, 2023). As a result, a freezing temperature of -20°C was used in the study. The thawing temperatures (4°C, 8°C, 12°C or 16°C) used in the study were representative of spring conditions at the study site (RISMA, 2023). The volumetric moisture content at the site from October 15 to November 10 (the recommended time for fall fertilizer application in Manitoba) ranges from 0.22 to 0.37 m³/m³. While climate decisions are typically based on at least 30 years of data, this study used 9 years of soil temperature data from RISMA because it provides accurate measurements through probes installed in representative agricultural systems (RISMA, 2023). Historical data was limited, as RISMA was established in 2010, with most site data available only after 2013 (RISMA, 2023).



Figure 2.1 Aerial view of the study site at Carman, where soils were sampled (red dotted lines show the specific field) (Source: Ian N. Morrison Research Farm).

2.3.2 Soil sample collection and preparation

It has been demonstrated that microbial biomass varies across seasons (Schadt et al., 2003). As a result, soils sampled during different seasons will not react the same way to the process of freezing and thawing (Henry, 2007). Therefore, to mimic the fall N application, soil samples were collected in the fall, November 2, 2023. Shovels were used to collect soil samples in the 0 - 15 cm layer of the soil profile into 20-liter plastic pails. Subsequently, the soils were air-dried for one week and sieved using a 3 by 7 mm screen and homogenized to ensure uniform physicochemical properties in all treatments. Afterwards, soil was transferred into the pails and stored at a temperature of 4°C (Ejack and Whalen, 2021) for 6 months until the start of the experiment. The baseline properties such as organic matter content, cation exchange capacity (CEC), pH, total nitrogen (N), nitrate (NO_3^- -N), ammonium (NH_4^+ -N) and soil texture were determined after soil was homogenized prior to the start of the experiment (Table 2.1).

2.3.3 Experimental design

The experimental design was a randomized complete block (RCBD) with a $4 \times 3 \times 3$ factorial treatment structure replicated three times (Figure 2.2). The soil was exposed to four thawing temperatures (4, 8, 12, and 16°C), three moisture levels (32%, 50%, and 100% WFPS) and two fertilizer sources (SuperU, Urea, and control (no fertilizer)). The three shelves in the freeze-thaw (FT) chamber (UTEST, UTD-1442) were treated as blocks (three in total) due to a temperature gradient across the shelves. Each experimental unit (jar with soil) was randomly assigned a position on the shelf in the FT chamber. Due to limited resources, one freeze-thaw (FT) chamber was available for the experiment, so each thawing temperature was tested at a time. For each thaw temperature, 27 units were assigned to the FT chamber, with nine units placed on each shelf (utilizing treatment combinations of three moisture levels by three fertilizer levels).

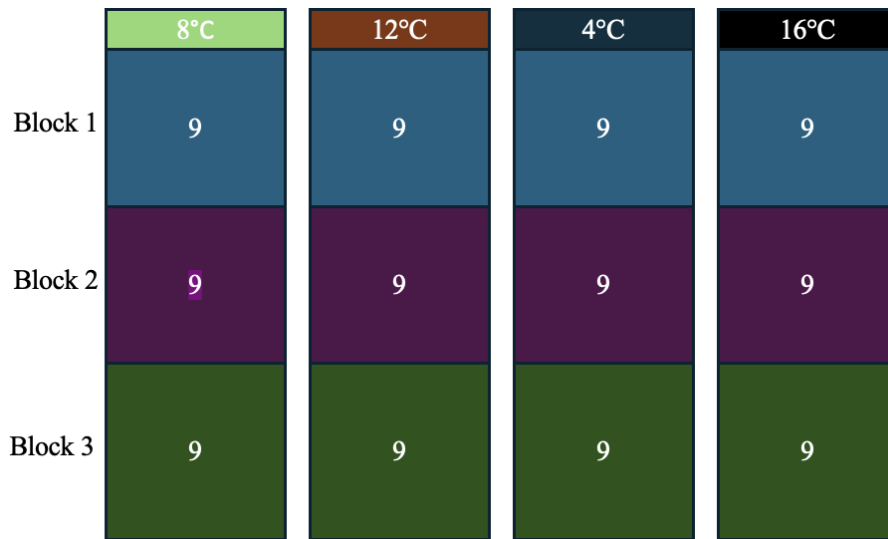


Figure 2.2 Treatment layout in the freeze-thaw chamber

2.4 Treatment application

2.4.1 Soil moisture treatment

Soil was weighed (1,292 g) into a 2 L freeze-tolerant glass jar to a bulk density of 1.2 g cm^{-3} at a height of 10 cm. After filling jars with air-dry soil, the moisture contents were adjusted to 32%, 50%, and 100% WFPS. Following this, the units were transferred into a refrigerator and allowed to equilibrate for 36 h at 4°C . Prior to equilibration, a preliminary test was conducted to inform on the length of equilibration by inserting moisture sensors (S-SMC-M005) near the bottom of the soil-filled jars and continuously monitoring the moisture levels until equilibrium was reached. The moisture level of 32% WFPS simulates fall seasons with dryer soil. The field moisture content determined for the site was 46% WFPS during sampling in the fall, hence the water content was adjusted to 50% WFPS. Application of 100% WFPS moisture level simulates seasons where soils are saturated due to excessive precipitation during the fall. To prevent excessive moisture loss caused by the inbuilt fan in the freeze-thaw (FT) chamber, the units were sealed with parafilm (PM-996, USA), and five holes (3 mm diameter) were made in them using a thin plastic to prevent accumulation of N_2O in the jars (Tenuta and Sparling, 2011). During the experiments, moisture levels were monitored by taking weight measurements at gas sampling intervals during the thawing process. There was no significant change in moisture throughout the study period, likely due to the low temperatures.

2.4.2 Fertilizer treatments

After soil-moisture equilibration, the fertilizers were applied in bands measuring 5 cm in depth and 10 cm in length. The fertilizers used were SuperU (double inhibitor) and urea. The application

rate was 128 kg/ha based on the soil test recommendation. Unfertilized jars were added and were treated as control.

2.4.3 Freezing and thawing temperatures

Following fertilizer application, all units were frozen at a temperature of -20°C for a duration of 48 h. Since freezing intensity affects microbial survival (Henry, 2007), the freeze-thaw chamber was adjusted to reach this temperature incrementally (for 12 h duration) with the aim of allowing microbes to gradually acclimatize to the changing temperature. For each batch, when the target freezing temperature was reached, the units were held for 48 h. During this time, the soil was considered to be exposed to winter conditions as it was frozen. The chosen freezing temperature represented the range of minimum recorded soil temperature at the study site. After freezing, the soils were thawed at either 4°C , 8°C , 12°C or 16°C for a duration of 60 h. During the thawing period, gas samples were collected at 0, 3, 6, 9, 10, 20, 30, 40, 50, and 60 h. The duration of exposure of soils to thawing temperatures was dependent on the activity of denitrifying bacteria, which reaches its peak performance after 50 hours in soils incubated at 4°C (Braker et al., 2010). After gas sampling, the experimental units were destructively sampled for soil analysis of mineral N (NO_3^- and NH_4^+). To do this, the jars were emptied, the soil thoroughly mixed and air-dried for mineral N determination, following the procedure outlined in Section 2.5.3.

2.5 Soil property measurements

2.5.1 Soil pH and cation exchange capacity

Soil pH and cation exchange capacity (CEC) affect N_2O emissions. Obia et al. (2015) observed a decrease in N_2O emissions as the soil pH increased from 4 to 6. This is due to the abundance of denitrifier strains at lower pH compared to higher pH (Richard et al., 2009). Additionally, soils

with higher CEC value means it has many exchangeable sites resulting in the reduction of NH_4^+ concentration in the soil solution (Kim et al., 2012). Soil pH and CEC were determined after soils were homogenized. With regards to pH, the measurement was done according to the method outlined by Thomas (1996). A 10 g was carefully transferred into a 50 mL beaker, followed by the addition of 10 mL of deionized water. The content was mechanically agitated for a duration of 15 minutes using a mechanical shaker. Following agitation, the mixture was allowed to remain undisturbed for a duration of 30 minutes. Subsequently, the electrodes of the pH metre (Thermo Scientific Orion Star A215) were inserted into the suspension to obtain pH readings.

The cation exchange capacity of the soils was determined following the protocol described by Tucker (1974). Ground soil was weighed (2 g) into a 30 mL centrifuge tube and 25 mL of 10% ethanol added and shaken for 30 minutes to remove soluble salts. Centrifuging was done at 10,000 rpm and decanted into a centrifuge tube. Following that, 20 mL of 1 M NH_4Cl in 60% aqueous ethanol buffered at pH of 8.5 with NH_3 was added and agitated for 30 minutes. The mixture was then centrifuged and decanted into 100 mL Flask. This was repeated three times and then once with 15 mL 0.05 NH_4Cl (for the purpose of reducing NH_4Cl concentration). In all, five separate extracts were obtained and mixed. The combined extracts were acidified with 2 mL 10 M HCl to the 100 mL mark in the flask for determining exchangeable Ca, Mg, K and Na. Residual NH_4Cl and adsorbed (NH_4^+) were displaced using 25 mL aliquots of 2 N nitrate solutions containing various cations. Each aliquot was analyzed in duplicate for chloride (Cl^-) using AgNO_3 potentiometric titration and ammonium (NH_4^+) using NaOH titration in a 25% acetone-water medium with formaldehyde. The cation exchange capacity (CEC) was derived from the difference between the Cl^- content and corrected NH_4^+ concentration.

2.5.2 Organic matter content

The organic matter in the soil samples was determined using the loss-on-ignition (LOI) method as described by Nelson and Sommers (1996). Crucibles were heated in a muffle furnace at 400°C for 2 h and then weighed empty. Three grams (3 g) of air-dried soil were measured into a tared crucible. The samples were oven-dried at 105°C for 24 h, cooled over CaCl₂, and their oven-dry weight was determined by subtracting the weight of the empty crucible. The contents were then transferred to a muffle furnace and heated at 400°C for 16 h. After ignition, the samples, representing the soil minerals, were weighed, and the weight of the empty crucible was subtracted. The LOI was calculated using the following equation:

$$\text{Organic matter content (\%)} = \frac{\text{Weight}_{105} - \text{Weight}_{400}}{(\text{Weight}_{105})} \times 100 \quad [1]$$

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.

2.5.3 Ammonium and nitrate

Ammonium (NH₄⁻-N) and nitrate (NO₃⁻-N) represent the forms of N available for plant uptake, and liable to losses. The extraction of NH₄⁺-N and NO₃⁻-N was done using a 2.0 M KCl solution (Carter and Gregorich 2008). Air-dry soil samples weighing 5 g was placed into a 50 mL Eppendorf tube. Subsequently, a 25 mL solution of KCl with a concentration of 2.0 M was added to the tube, maintaining a ratio of 1:5 between the mass of soil (g) and the volume of KCl solution (ml). The flasks were then sealed with stoppers and subjected to mechanical agitation at a rate of 60 strokes per minute for a duration of 30 minutes. The extract was filtered using Whatman No. 1 filter paper and mineral nitrogen (NH₄⁺-N and NO₃⁻-N) analyzed calorimetrically with a continuous flow analyzer (Skalar, SA 1100, Netherlands).

2.5.4 Total nitrogen

The total N was analyzed using the Kjeldahl method to determine the available N in the soil. Air-dry soil (0.2 g) was weighed into Kjeldahl digestion tubes, 4.4 mL of digestion mixture added. Digestion mixture was prepared following the procedure outlined by Thomas et al. (1967). The tubes were then transferred unto a digestion bloc and heated gently until froth subsides, then the temperature was gradually increased to 350 °C to digest for 1 h. Following digestion, the flasks were allowed to cool until warm and diluted to 50 mL with deionized water in a volumetric flask. The solution was transferred into vials and analysed using the Technicon Autoanalyzer.

2.5.5 Soil bulk density and water-filled pore space

The soil was packed to a bulk density of 1.2 g cm⁻³ representing the field conditions at the study site. The bulk density was then used to estimate for total porosity and WFPS. These equations are shown below.

The total porosity was calculated using the bulk density value in the formular shown below:

$$f = 1 - \frac{BD}{PD} \quad [2]$$

Where f is total porosity, BD is bulk density (g cm⁻³), and PD is particles density taken to be 2.65 g cm⁻³.

The WFPS was estimated following similar procedure by Fichtner et al. (2019). This was estimated from the formular:

$$WFPS = \frac{VMC}{f} \times 100 \quad [3]$$

where WFPS is water filled pore space (%), VMC is the volumetric soil water content (determined using gravimetric soil water content and bulk density) and f is porosity.

Table 2.1 Baseline properties of soil samples

Soil property	Estimate
Bulk density (g cm ⁻³)	1.2
Organic matter (%)	5.03
Soil Texture	
Sand (%)	66
Silt (%)	15
Clay (%)	19
CEC (cmolc kg ⁻¹)	21.2
pH (water)	5.2
NO ₃ ⁻ -N (mg kg ⁻¹)	38
NH ₄ ⁺ -N (mg kg ⁻¹)	4.5
Total N (g kg ⁻¹)	2.4

2.5.6 Volumetric heat capacity

The volumetric heat capacity of the soil was determined using De Vries approximation method as outlined by (Kluitenberg, 2018). The volumetric heat capacity was calculated from the formular below:

$$C = \rho b (C_m \Phi_m + C_o \Phi_o + C_w \theta_g) \quad [4]$$

Where C is the volumetric heat capacity (MJ m⁻³ K⁻¹), ρb is soil bulk density, subscripts m , o , w represents mineral material, organic matter, and water respectively. Additionally, C_m , C_o and C_w represent specific heat of mineral material, organic matter, and water respectively. The mass wetness is designated as θ_g , which was determined at the time of gas sampling. The organic matter fraction and mineral material fraction are given as Φ_o and Φ_m . Organic matter was determined (Φ_o) (Table 2.1) and then Φ_m was estimated using:

$$\Phi_m = 1 - \Phi_o \quad [5]$$

The soil bulk density (ρb) was consistent across all treatments, with a value of 1,200 kg/m³. The specific heat capacities of water (C_w) and ice used were 4.18 kJ kg⁻¹ K⁻¹ and 2.1 kJ kg⁻¹ K⁻¹,

respectively. The specific heat capacity of ice was used to calculate the volumetric heat capacity when the soil was frozen by modifying equation [4]. The average values used for C_m and C_o were $0.73 \text{ kJ kg}^{-1} \text{ K}^{-1}$ and $1.9 \text{ kJ kg}^{-1} \text{ K}^{-1}$ respectively (Kluitenberg, 2018).

2.5.7 Nitrous oxide emissions

Nitrous oxide was sampled from the headspace in the glass jar (Figure 2.3). Before sampling at any time, the gas in the headspace was evacuated by inserting (10 times repeatedly) a cylindrical plastic container (250 mL). The jars were then securely sealed using a lid equipped with a septum stopper and allowed for 2 h for gas to accumulate for sampling. During thawing at each temperature (4°C , 8°C , 12°C or 16°C), N_2O was sampled at 0, 3, 6, 10, 20, 30, 40, 50, and 60 h. During sampling, a disposable syringe (Becton, Dickson and Company, Franklin Lakes, USA) with a volume of 20 mL was inserted through the septum stopper to collect gas from the headspace. The gas obtained was transferred into a 12 mL evacuated exetainers (Labco Limited, Buckinghamshire, UK) for the purpose of analyzing N_2O concentration using a gas chromatograph (Varian CP-3800, Walnut Creek, USA). Following each sampling interval, the jar lids were replaced with parafilm (PM-996, USA) that have five evenly distributed holes made in them to reduce moisture loss and N_2O accumulation. Three blanks were added to the experimental setup for each temperature level. The blanks were exposed to the same temperature as soil samples, except they had no soil. The hourly N_2O flux was measured assuming a positive relationship between N_2O concentration in the jar's headspace and time (Velthof et al., 2003; Nyathi, 2024). The cumulative N_2O emissions were calculated by summing the fluxes for the sampling time intervals over the 60 h thawing period (Tenuta and E. G. Beauchamp, 2003; Nyathi, 2024).

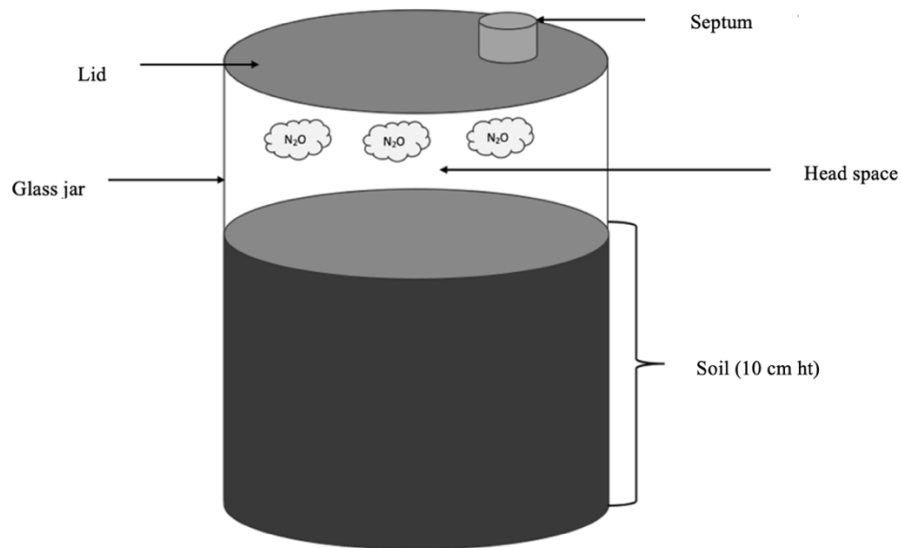


Figure 2.3 Schematic representation of a freeze-tolerant glass jar with soil that has been sealed with a lid for the purpose of sampling N₂O during thawing.

2.6 Data Analysis

The generalized linear mixed model procedure (PROC GLIMMIX) in SAS (version 9.4) was used to determine the treatment effects on N₂O flux over the 60-h period, cumulative N₂O emissions, and mineral N (residual NO₃⁻-N and NH₄⁺-N at the end of the experiment). A three-way ANOVA was performed for cumulative N₂O emissions, and a two-way for NO₃⁻-N and NH₄⁺-N concentrations. In the model statement for cumulative N₂O emission, moisture, temperature and fertilizer source were specified as fixed effects. For NO₃⁻-N and NH₄⁺-N, a separate ANOVA was conducted for each temperature level, with moisture and fertilizer source as fixed effects. A three-way ANOVA was performed for N₂O flux with moisture, temperature, and time as fixed effects. The cumulative N₂O emissions, NO₃⁻-N and NH₄⁺-N followed a normal distribution while the N₂O flux followed a gamma distribution. Normality of residuals was assessed using the residual panel. In the model for all analyses, the random variable was block nested within temperature. With regards to flux, which was a repeated measure, time was specified as the repeated measure factor.

Various covariance structures (UN, ANTE (1), CS, and SP(POW)) were compared, and the structure with the lowest Akaike Information Criterion (SP(POW)) was used for the repeated measures analysis. To assess the relationship between volumetric heat flux and N₂O flux, a Spearman rank correlation analysis was done. Treatment effects were assessed at $\alpha = 0.05$ using the Tukey multiple comparison procedure.

2.7 Results

2.7.1 Effects of moisture and temperature on N₂O flux during the thawing period

There was a significant moisture \times time interaction for N₂O flux (Table 2.2). There was no significant main effect of temperature on hourly N₂O flux (Table 2.2). There was no difference in N₂O flux between soil with lower moisture (32% and 50% WFPS) and saturated soil (100% WFPS) during the initial thawing period (0-10 h) (Figure 2.4). From 0 to 10 h, N₂O flux was similar for all moisture levels. However, as time elapsed, treatments with 100% WFPS produced significantly higher N₂O flux compared to 32% and 50% WFPS (Figure 2.4). At 20 h, treatments with 100% WFPS produced 78% more N₂O emission than treatments with 50% WFPS. There was a gradual but significant increase in flux at 100% WFPS from 30 to 60 h, which reached the maximum at 60 h (10.28 $\mu\text{g kg}^{-1} \text{h}^{-1}$). Overall, N₂O flux started at 10 h during thawing in treatments with 100% WFPS, and moisture was the dominant contributor to this effect.

Table 2.2 Effect of soil moisture, temperature and fertilizer source on hourly nitrous oxide flux and cumulative emissions.

Effect	N ₂ O flux ($\mu\text{g kg}^{-1} \text{h}^{-1}$)	Cumulative N ₂ O ($\mu\text{g kg}^{-1}$)
Moisture		
32% WFPS	0.4	18.9
50% WFPS	0.4	24.6
100% WFPS	1.8	290.5
Temperature		
4 °C	0.5	2.5
8 °C	0.8	87.4
12 °C	0.7	140.1
16 °C	0.8	290.5
Fertilizer Source		
Control		77.6
Urea		113.04
SuperU		143.3
Sampling time (h)		
0	0.3	
3	0.5	
6	0.4	
10	0.5	
20	0.7	
30	0.9	
40	1.1	
50	1.1	
60	1.2	
		<i>p-value</i>
Moisture	<0.0001	<0.0001
Temperature	<0.81	0.003
Source		0.02
Temperature × Moisture	<0.93	<0.0001
Source × Moisture		0.01
Source × Temperature		0.3
Source × Temperature × Moisture		0.6
Time	<0.004	
Time × Temperature	1	
Moisture × Time	<0.003	
Moisture × Time × Temperature	1	

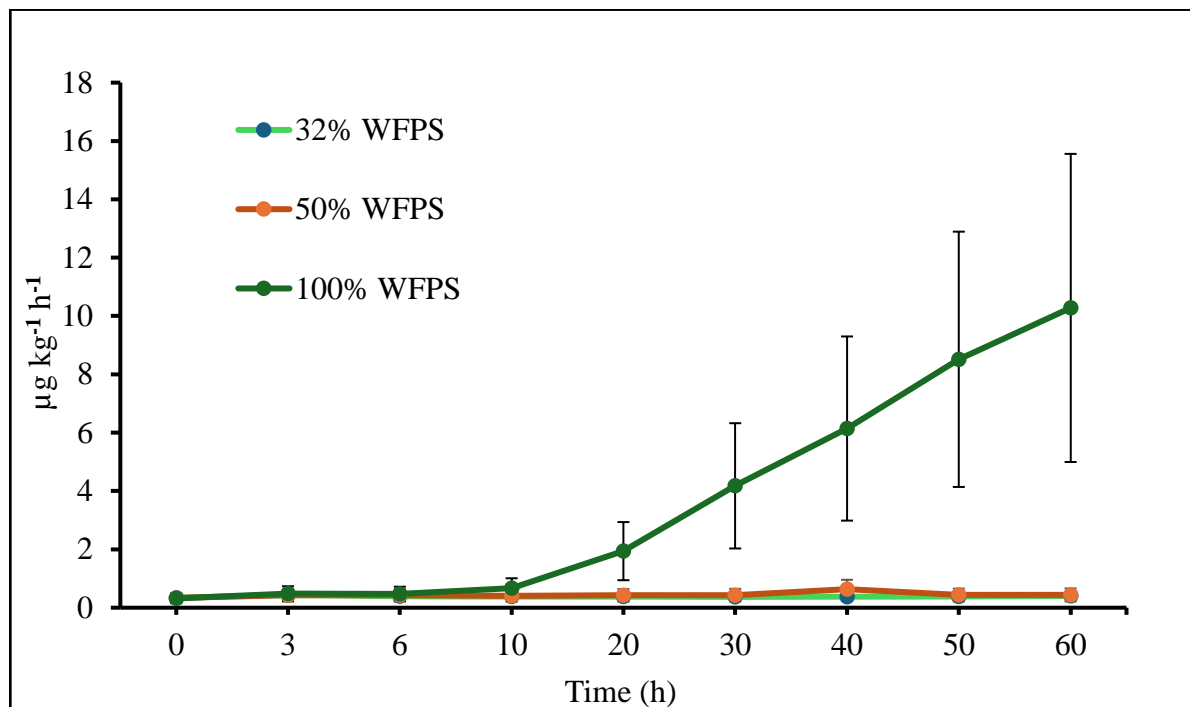


Figure 2.4 Effect of moisture (WFPS) and thawing duration (time) on N₂O flux. Error bars represent the standard errors of the mean.

2.7.2 Correlation between volumetric heat capacity and nitrous oxide flux

The property that influences soil heating process is its heat capacity, which could affect N₂O emissions. A correlation analysis was performed to determine the relationship between soil volumetric heat capacity (VHC) and N₂O flux (Table 2.3). There was a poor correlation between VHC and N₂O flux at 4°C ($r = -0.14$, $p = 0.03$). There was a significant positive correlation between VHC and N₂O flux at 8°C ($r = 0.36$, $p = <0.0001$), at 12°C ($r = 0.17$, $p = <0.0001$), and 16°C ($r = 0.44$, $p = <0.0001$). Overall, the correlation was stronger at the higher thawing temperatures compared to 4°C.

Table 2.3 Correlation analysis between volumetric heat capacity and N₂O flux at 4, 8, 12 and 16°C.

	VHC(MJ/m ³ /K)	Flux (μg kg ⁻¹ h ⁻¹) (<i>r</i>)	<i>p-value</i>
VHC (MJ/m³/K)			
4°C	1	-0.14	0.03
8°C	1	0.36	<0.0001
12°C	1	0.17	<0.0001
16°C	1	0.44	<0.0001

‡ *r* is the Pearson correlation coefficient.

2.7.3 Cumulative N₂O emissions

The cumulative N₂O emissions were analyzed across moisture levels (32%, 50%, and 100% WFPS), temperatures (4°C, 8°C, 12°C, and 16°C), and fertilizer sources (urea and SuperU) at the end of the 60-h thawing period. There was no significant moisture × temperature × fertilizer source (FS) interaction in the cumulative N₂O emission (Table 2.2). There was a significant moisture × temperature interaction in the cumulative N₂O emission. Moisture content significantly affected

cumulative emissions across all temperatures, with the cumulative N₂O emissions increasing with increasing temperature (Figure 2.5). Treatments exposed to 100% WFPS produced N₂O emissions, which were significantly higher than 32% and 50% WFPS by 96.3% and 92.6%, respectively at 16°C. The highest cumulative N₂O emissions was observed at 100% WFPS (582 µg kg⁻¹) at 16°C. At 100% WFPS, treatments exposed to 8°C, 12°C or 16°C produced significantly higher emissions of 97%, 98% and 99%, respectively than those at 4°C (Figure 2.5). At 4°C, a temperature within the recommended range for fall fertilizer application, cumulative emissions in 100%, 50% and 32% WFPS were not significantly different from each other. Although treatments at 12°C emitted 46% cumulative N₂O more than 8°C, the difference was not statistically significant. Similarly, no significant difference was observed between 32% and 50% WFPS across all temperatures.

There was a significant moisture × FS interaction on cumulative N₂O emissions (Table 2.2). Fertilizer sources significantly affected cumulative N₂O emissions, which increased with increasing moisture (Figure 2.6). At 32% and 50% WFPS, cumulative N₂O emissions were significantly lower than at 100% WFPS across all FS (urea and SuperU) but did not differ significantly between 32% and 50% WFPS. At 100% WFPS, SuperU produced significantly higher cumulative N₂O emissions than at 32% and 50% WFPS, by 95% and 91%, respectively. Similarly, urea at 100% WFPS produced significantly higher emissions than at 32% and 50% WFPS, by 94% and 93%, respectively. The control treatment (with no fertilizer applied) followed similar trend where significantly higher emissions were observed at 100% than at 32% and 50% WFPS by 90%. Although urea emitted 25% less N₂O than SuperU at 100% WFPS, the difference was not statistically significant.

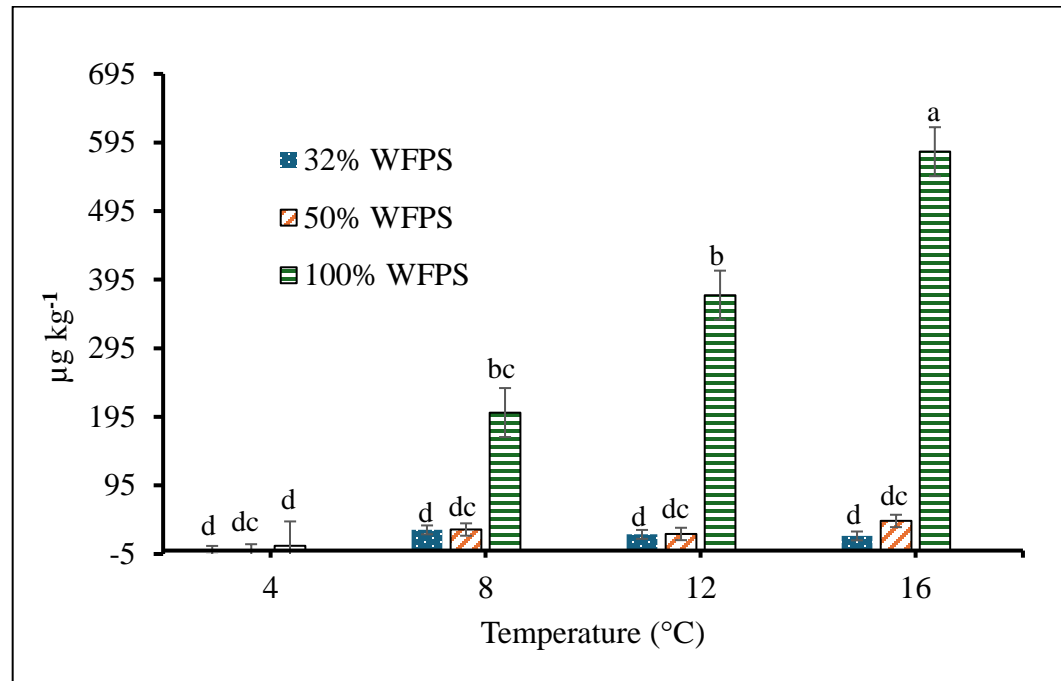


Figure 2.5 Effects of temperature and moisture (WFPS) on cumulative N₂O emissions at the end of 60 h thawing period. Error bars represent the standard errors of the mean. Bars with different letters are significantly different at $\alpha = 0.05$.

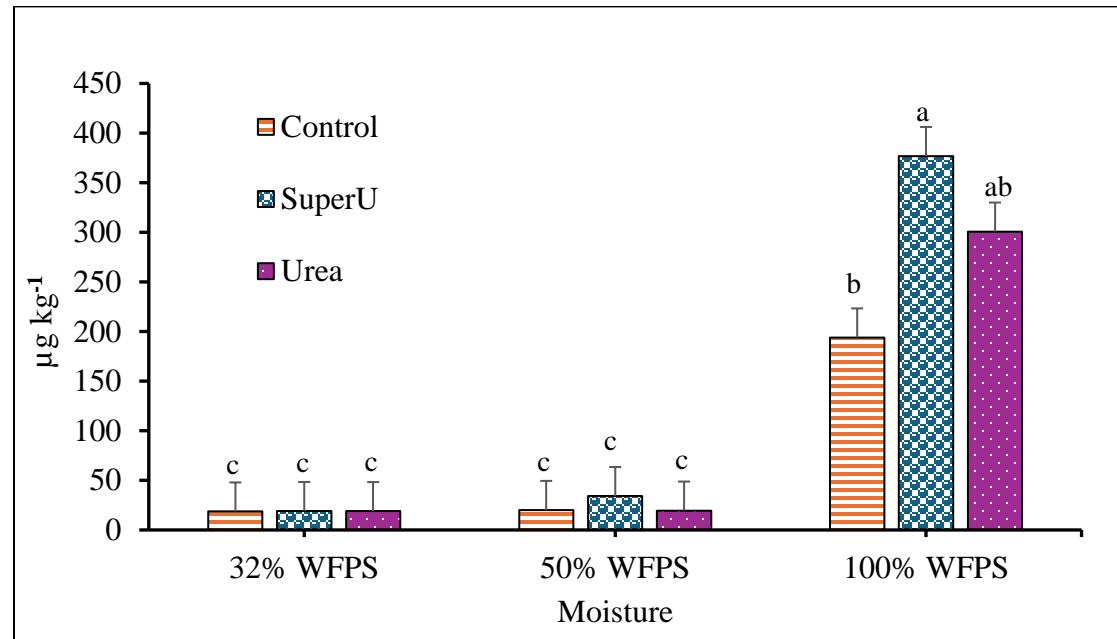


Figure 2.6 Effects of fertilizer source and moisture on cumulative N₂O emissions at the end of 60 h thawing period. Error bars represent the standard errors of the mean. Bars with different letters are significantly different at $\alpha = 0.05$.

2.7.4 Mineral nitrogen concentration after freeze-thaw cycle

Farmers in the prairies are advised to treat fertilizers with nitrogen (N) inhibitors before fall application with the aim of reducing urea hydrolysis and the conversion of ammonium ($\text{NH}_4^+\text{-N}$) to nitrate ($\text{NO}_3^-\text{-N}$) (Trenkel, 2010). As a result, N inhibitor-treated fertilizer will reduce $\text{NO}_3^-\text{-N}$ concentration in the soils while retaining more N in the $\text{NH}_4^+\text{-N}$ form (Krol et al., 2020). To examine this effect, the impact of moisture (32%, 50% and 100% WFPS) and FS (Urea and SuperU) on mineral N ($\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$) under varying thawing temperatures (4°C, 8°C, 12°C or 16°C) was evaluated at the end of the experiment. There was a moisture \times FS interaction at 4°C and 8°C (Table 2.4). Across all treatments, $\text{NO}_3^-\text{-N}$ concentration was higher than $\text{NH}_4^+\text{-N}$ (Table 2.4). Moisture significantly reduced $\text{NO}_3^-\text{-N}$ at 100% compared to 32% and 50% WFPS under all temperatures (Table 2.4). There was no significant difference in $\text{NO}_3^-\text{-N}$ concentration between 50% WFPS and 32% WFPS under all temperature levels. At 4°C and 8°C, there was a significant interaction between FS and moisture, with urea and SuperU producing significantly lower $\text{NO}_3^-\text{-N}$ concentration at 100% WFPS compared to 50% and 32% WFPS (Figure 2.7). As temperature was increased from 4°C to 8°C, the $\text{NO}_3^-\text{-N}$ concentration increased by 29% for SuperU and 47% for Urea at 100% WFPS. Only moisture significantly affected $\text{NO}_3^-\text{-N}$ concentration at 12°C and 16°C (Table 2.4). For both temperatures at 100% WFPS, significantly less $\text{NO}_3^-\text{-N}$ was observed compared to 32% and 50% WFPS. Temperature rise from 12°C to 16°C increased $\text{NO}_3^-\text{-N}$ by 25% in treatments at 100% WFPS.

There was a significant effect of FS on $\text{NH}_4^+\text{-N}$ across all the thaw temperatures (Table 2.4). At all temperatures, $\text{NH}_4^+\text{-N}$ concentration was not significantly different between urea and Super, except at 12°C. At 8°C, SuperU retained 6.3% more $\text{NH}_4^+\text{-N}$ compared to 12°C, with the

lowest retention observed at 16°C (9.0879 mg kg⁻¹) (Table 2.4). The overall efficacy of SuperU in reducing NO₃⁻-N was not significantly different from untreated urea.

Table 2.4 Effects of fertilizer source and moisture on mineral nitrogen concentration following freezing and thawing at 4°C, 8°C, 12°C and 16°C

Effect	4°C		8°C		12°C		16°C	
	NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)
Moisture								
32% WFPS	12.0	7.7ab	12.7	8.2a	13a	10.8a	13.8a	7.5a
50% WFPS	12.1	9.4a	13.4	8.3a	14a	9.9a	14.7a	8.3a
100% WFPS	6.5	5.3b	4.6	7.5a	5.8b	5.8b	7.7b	5.4a
Fertilizer source (FS)								
Control	11.7	3.4b	8.9	3.5b	9.6b	4.7c	10.7b	2.2b
SuperU	9.4	8.8a	10.3	10.1a	11.2a	9.5b	13a	9.1a
Urea	9.4	10a	11.4	10.4a	12a	12.3a	13a	10a
<i>p-value</i>								
Moisture	0.0004	0.007	<0.0001	0.9	<0.0001	0.001	0.0001	0.09
FS	0.15	<0.0001	0.001	0.001	0.02	<0.0001	0.2	<0.0001
FS × Moisture	0.01	0.07	0.01	1	0.2	0.1	0.5	0.9

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

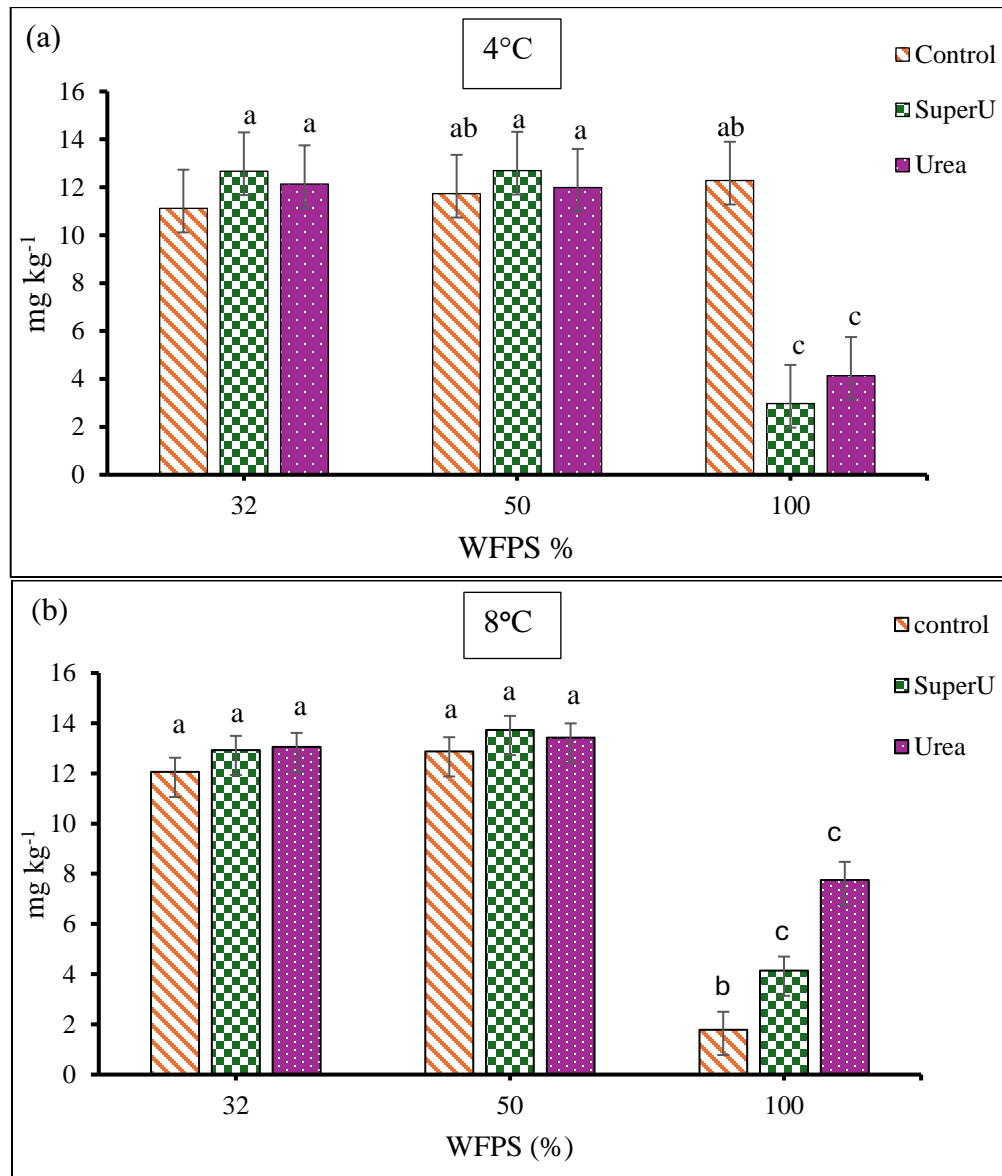


Figure 2.7 Effects of fertilizer source and soil moisture on NO_3^- -N at the end of the 60-h thawing period at 4°C (a) and 8°C (b). Error bars represent standard errors of the mean. Error bars with different letters are statistically different at $\alpha = 0.05$.

2.8 Discussion

2.8.1 N₂O flux during thawing

The temperature effect on hourly N₂O flux was not significantly different from each other, which may be attributed to the high volumetric heat capacity (VHC) of the soil, particularly at 100% WFPS. Soils at 32% and 50% WFPS had lower VHC and therefore responded more rapidly to heating than at 100% WFPS (Hillel, 2004), but insufficient moisture might have limited denitrification, leading to lower emissions (Pelster et al., 2019). Soil with higher VHC tend to warm up or cool down more slowly compared to soils with lower VHC due to high specific heat capacity of water (Kluitenberg, 2018). Alnefaie and Abu-Hamdeh (2020) demonstrated that increasing moisture content in loam soil from 0.1 to 0.2 kg kg⁻¹ increased the volumetric heat capacity from 1.55 to 3.35 MJ⁻¹ m⁻³ °C⁻¹. In this present study, the use of relatively low temperatures (4°C, 8°C, 12°C, and 16°C), resulted in a slow heating process over the thawing period. At the initial thawing period, the VHC of the soil was low because the soil was frozen (from 0 - 10 h) but increased after phase change from ice to liquid. During thawing, the applied heat could have been absorbed to convert the ice to liquid, but did not raise the temperature of the soil due to the latent heat transfer of the ice (Wagner-Riddle et al., 2010). When ice is exposed to heat source, the energy is primarily utilized to convert ice into liquid water, rather than raising its temperature (Wagner-Riddle et al., 2010). This could be the plausible reason the applied temperatures had no significant effect on N₂O flux. Nevertheless, significant N₂O flux was observed after phase change from ice to liquid at 10 h and increased gradually to 60 h (10.28 µg kg⁻¹ h⁻¹) at 100% WFPS. The emissions observed after phase change could be attributed to dominance of denitrification in 100% WFPS than 32% and 50% WFPS (Tan et al., 2018; Wang et al., 2022). Qin et al. (2020) reported higher N₂O flux at 100% WFPS compared to 50% WFPS,

attributing the increase to enhanced denitrification rate under saturated conditions. Bhowmik et al. (2017) investigated the effects of water-filled pore space (WFPS) levels (40%, 60%, and 80%) on N₂O emissions in loam soils in the USA. The study reported the highest emissions at 80% WFPS (1182 µg kg⁻¹ dry soil), compared to 579 µg kg⁻¹ and 176 µg kg⁻¹ dry soil at 60% and 40% WFPS, respectively. This increase in emissions was attributed to enhanced denitrification resulting from reduced oxygen availability in soils with higher WFPS during thawing.

Our findings agree in part with those of Wagner-Riddle et al. (2010), who conducted a study in Ontario, Canada, investigating the timing of water phase changes and their relationship with surface N₂O fluxes. Using heat pulse probes (HPP), the study measured VHC from fall to spring in conventional tillage (CT) and no-tillage (NT) plots with silt loam soil. The findings indicated that N₂O fluxes were linked to the phase change from ice to liquid, as detected by the HPP at the soil surface (0–5 cm). In CT plots, higher emissions were observed following immediate phase changes at temperatures above 0°C. However, fluxes declined after the phase change was complete when temperatures exceeded 5°C. The study also noted more frequent N₂O flux events in CT than NT plots, which was ascribed to intense freezing in the CT plots, which increased the availability of carbon (C) and N substrates for denitrification. One consistent finding for both studies was the N₂O emissions at the phase change from ice to liquid, but Wagner-Riddle et al. (2010) observed smaller flux after complete phase change while our study showed a gradual increase in N₂O flux. One of the reasons for the difference in results may be the influence of moisture, which plays a critical role in determining volumetric heat capacity (Kluitenberg, 2018). In our study, the applied moisture levels did not significantly change over the thawing period. As a result, the higher moisture content, particularly at 100% WFPS, increased the VHC of the soil after phase change from ice to liquid. In contrast, the field study conducted by Wagner-Riddle et

al. (2010) may have experienced variable moisture conditions, potentially contributing to reduced N₂O emissions following phase change. Our findings indicate a positive correlation between VHC and N₂O flux (Table 2.3). Soils with higher heat capacity possibly retained heat for extended periods, which influenced microbial activity and subsequent N₂O flux. This is further supported by the stronger correlation coefficient (r) observed as the temperature increased from 4°C to 16°C (Table 2.3).

2.8.2 Source of N₂O production in thawing soil

There are two main schools of thought regarding the source of spring-thaw emissions observed in freeze-thaw studies. The first suggests that emissions result from the trapping of N₂O under ice during freezing, with the gas being released as surface ice melts. Studies that support this claim asserts that there are unfrozen zones in the soil where microbial activities are enabled although soil may be frozen (Ejack & Whalen, 2021; Li et al., 2000; Teepe et al., 2001). The N₂O produced is trapped under ice and released when the ice thaws. The second states that N₂O production is biological *de novo* or ‘newly’ produced during thawing, as substrates become available to enhance microbial activity (Wagner-Riddle et al., 2008; King et al., 2021). In our study, the gradual increase in N₂O production over time, particularly at 100% WFPS, suggests that emissions were primarily due to biological *de novo* or ‘newly’ produced N₂O (King et al., 2021). If N₂O emissions had been caused by the physical release of trapped N₂O beneath ice, as reported in other studies (Ejack & Whalen, 2021; Li et al., 2000; Teepe et al., 2001), a sharp spike in N₂O flux would have been expected around 10 h, coinciding with the melting of surface ice at 100% WFPS. This observation is consistent with Wagner-Riddle et al. (2008), who attributed N₂O emissions during thawing to biological *de novo* production rather than physical release. Conversely, Ejack and Whalen (2021) observed a different mechanism, where N₂O emissions were predominantly caused by physical

trapping in unfrozen zones beneath the ice. In their study, 50% of the total N₂O flux was released within the first 3 h of a 9 h thawing period, indicated by a sharp spike at the onset of thawing. They attributed this to N₂O trapped in unfrozen zones, which was emitted as the ice on the soil surface thawed.

2.8.3 Cumulative N₂O emissions after freezing and thawing

The present study demonstrates that cumulative N₂O emissions under freezing and thawing conditions is impacted variably by soil moisture under different thawing temperatures. The lower emissions observed in the 32% and 50% than 100% WFPS under all temperatures could be attributed to the dominance of nitrification, a process that releases N₂O as by-product (Singh and Verma, 2007), but in lower levels (Huang et al., 2014) compared to denitrification. In the soil with lower WFPS (32% and 50%), majority of the pores were occupied with oxygen, which tends to reduce the activity of denitrifying organisms (Guo et al. 2014). This is consistent with other previous studies, which have demonstrated that nitrification dominates in soils with less than 60% WFPS (Pelster et al., 2019; Huang et al., 2014). On the other hand, the higher emissions observed in soil exposed to 100% WFPS is due to 1) denitrification as a result of anaerobic conditions (Wertz et al., 2016; Guo et al., 2014), 2) substrate abundance for enhanced microbial activity (Chen, 1995; Feng et al., 2007) and 3) increase in NO₃⁻-N concentration during thawing (Oztas and Fayetorbay, 2003; Fu et al., 2019; Lin et al., 2023). Our findings align with those of Guo et al. (2014), who reported that cumulative N₂O emissions were 7.4 times higher in nearly saturated soil (90% WFPS) compared to 75% WFPS, primarily due to enhanced denitrification.

Although some freeze-thaw studies have attributed N₂O emissions to substrate release or NO₃⁻-N concentration due to aggregate disintegration (Fu et al., 2019; Tenuta and Sparling, 2011; Christensen and Christensen, 1991; Chen, 1995), our study shows that both processes depend on

soil moisture and possibly temperature. This is supported by the fact that higher NO_3^- -N concentration was observed in soil with 32% and 50% than 100% WFPS at the end of the experiment (Table 2.4), indicating that NO_3^- -N was available in soil with lower moisture (32% and 50%), but probably in lower quantities to support denitrification. Additionally, organic carbon (C) was not a limiting factor. Although carbon levels were not measured at the end of the experiment, the soil was rich in organic matter (Table 2.1). The available substrates (NO_3^- -N) were likely insufficient to affect denitrification, as higher NO_3^- -N levels are known to increase the abundance of denitrifiers (Xiong et al., 2017). Given the positive relationship between soil moisture and NO_3^- -N (Lin et al., 2023a), a possible explanation for the reduced emissions is that moisture contents (32% and 50% WFPS) were inadequate to stimulate denitrification (Tan et al., 2018). In contrast, soil at 100% WFPS released higher concentrations of NO_3^- -N into the soil N pool, facilitating N_2O production by denitrification (Wang et al., 2022). Furthermore, our study suggests that temperature likely influenced cumulative N_2O emissions, as emissions varied across different thawing temperatures (Figure 2.5). This is supported by other studies, which have shown that denitrification increases with increasing temperatures (Li et al., 2022; Braker et al., 2010; Tan et al., 2018).

In this study, SuperU was not effective in reducing cumulative N_2O emissions compared to Urea (Figure 2.7). A field study conducted by Asgedom et al. (2014) in Manitoba found that SuperU was ineffective in reducing N_2O emissions in clay soil. They attributed this to high organic C content of the soil, which reduced the efficacy of NBPT and DCD in SuperU. When DCD is applied in soils with high C content, the inhibitor tends to adsorb to the hydrophobic domains of the soil organic matter, thereby reducing its potency (Zhang et al., 2004). The soil used for our study was a Black Chernozem, known for its high organic matter content (Table 2.1). Hence, the DCD in SuperU could have been adsorbed onto the organic matter, rendering it ineffective in

reducing N₂O emissions (Asgedom et al., 2014). Another possible explanation is the microbial degradation of inhibitors due to high moisture content (Figure 2.6) (Puttanna and Gowda, 1999). During thawing, substrates are released into the soil C and N pools, enhancing microbial activity and potentially accelerating the degradation of inhibitors in SuperU (Xiong et al., 2017; Huang and Hall, 2017). The increased solute diffusion and microbial activity could have reduced the potency of the inhibitors (NBPT and DCD) in the SuperU.

2.8.4 Mineral nitrogen after freezing and thawing

Contrary to what was expected, this study showed the enhanced efficiency urea-based (SuperU) fertilizer was neither effective in reducing NO₃⁻-N (Table 2.4, Figure 2.7) nor retaining more N in the NH₄⁺-N form compared to urea after freezing and thawing. The ineffectiveness of SuperU to reduce significant NO₃⁻-N compared to urea could be attributed to microbial degradation of the inhibitor especially in the saturated soil (100% WFPS) (Lin et al., 2020). This is attributed to the fact that at 100% WFPS more substrates were released, which increased microbial activity and abundance (Xiong et al., 2017). The fertilizer treatments at 100% WFPS had lower NO₃⁻-N under all temperatures because most was reduced to N₂O by denitrification (Huang et al., 2014). Lin et al. (2020) found that nitrification inhibitor was not effective in reducing N₂O in soil with higher WFPS when it increased from 60% to 80%. They attributed their observation to the degradation of nitrification inhibitor by microbes. The highest NO₃⁻-N concentration observed in 32% and 50% WFPS was an indication of nitrification due to prevalent aerobic conditions (Hu et al., 2015; Burton and Beauchamp, 1994). Pelster et al. (2019) reported similar findings in sandy loam soils where they found higher concentration of NO₃⁻-N than NH₄⁺-N at the end of the thawing period. Overall, there was an increase in the NO₃⁻-N under higher thawing temperature than lower ones (Table 2.4). This suggests that temperature could possibly have effects on NO₃⁻-N release.

2.9 Conclusion

The findings indicate a significant positive relationship between volumetric heat capacity (VHC) and N₂O flux. This study further shows that VHC increases during thawing, and it is the main property that controls heating of the soil. Hourly N₂O flux measurements revealed that N₂O is ‘newly’ produced, further confirming that microbial activity is reduced in frozen soils and becomes active only during thawing. Also, this study demonstrated that soil moisture prior to fall fertilizer application, significantly affects cumulative thaw N₂O emissions, with emissions being highest at saturation (100% WFPS) compared to 32% and 50% WFPS across all temperatures (4°C, 8°C, 12°C, and 16°C). At 32% and 50% WFPS, both cumulative N₂O emissions and flux were low, likely due to higher aerobic conditions favoring nitrification. The double inhibitor (SuperU) was ineffective in reducing cumulative emissions compared to urea across all temperature treatments. These results suggest that farmers in the prairies could reduce N₂O emissions by applying banded urea at 5 cm depth when soil moisture is at or below 50% WFPS. However, spring conditions present a high risk of N losses as shown in the 100% WFPS treatments. Overall, our study identified soil moisture as the primary driver of denitrification, emphasizing the need to establish a recommended soil moisture threshold for fall fertilizer applications to minimize N₂O emissions and nutrient losses.

2.9.1 References

- Abalos, D., S. Jeffery, A. Sanz-Cobena, G. Guardia, and A. Vallejo. 2014. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agriculture, Ecosystems & Environment* 189: 136–144. doi: 10.1016/j.agee.2014.03.036.
- Akoglu, H. 2018. User's guide to correlation coefficients. *Turkish Journal of Emergency Medicine* 18(3): 91–93. doi: 10.1016/j.tjem.2018.08.001.
- Alnefaie, A.K., and H.N. Abu-Hamdeh. 2020. Specific Heat and Volumetric Heat Capacity of Some Saudian Soils as affected by Moisture and Density. *International Journal of Materials* 7. doi: 10.46300/91018.2020.7.8.
- Amiro, B., M. Tenuta, K. Hanis-Gervais, X. Gao, D. Flaten, et al. 2017. Agronomists' Views on the Potential to Adopt Beneficial Greenhouse Gas Nitrogen Management Practices Through Fertilizer Management. *Can. J. Soil. Sci.: CJSS-2017-0062*. doi: 10.1139/CJSS-2017-0062.
- Asgedom, H., M. Tenuta, D.N. Flaten, X. Gao, and E. Kebreab. 2014. Nitrous Oxide Emissions from a Clay Soil Receiving Granular Urea Formulations and Dairy Manure. *Agronomy Journal* 106(2): 732–744. doi: 10.2134/agronj2013.0096.
- Bhowmik, A., A.-M. Fortuna, L.J. Cihacek, S. Rahman, M.S. Borhan, et al. 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. doi: 10.1016/j.soilbio.2017.04.015.
- Bi, J., Y. Pan, S. Yang, R. Li, and Z. Wu. 2024. Predicting the volumetric heat capacity of freezing soils using the soil freezing characteristic curve. *Journal of Hydrology* 645: 132151. doi: 10.1016/j.jhydrol.2024.132151.
- Braker, G., J. Schwarz, and R. Conrad. 2010. Influence of temperature on the composition and activity of denitrifying soil communities: Temperature effects on soil denitrifier communities. *FEMS Microbiology Ecology*: no-no. doi: 10.1111/j.1574-6941.2010.00884.x.
- Burton, D.L., and E.G. Beauchamp. 1994. Profile nitrous oxide and carbon dioxide concentrations in a soil subject to freezing. *SOIL SCI. SOC. AM. J.* 58(1): 115–122. doi: 10.2136/sssaj1994.03615995005800010016x.
- Carter, M.R., and E.G. Gregorich, editors. 2008. *Soil sampling and methods of analysis*. 2nd ed. Canadian Society of Soil Science ; CRC Press, [Pinawa, Manitoba] : Boca Raton, FL.
- Chen, Y. 1995. Nitrous oxide emission from an agricultural soil subjected to different freeze-thaw cycles. *Agriculture, Ecosystems & Environment* 55(2): 123–128. doi: 10.1016/0167-8809(95)00611-U.

- Christensen, S., and B.T. Christensen. 1991. Organic matter available for denitrification in different soil fractions: effect of freeze/thaw cycles and straw disposal. *Eur. J. Soil Sci.* 42(4): 637–647. doi: 10.1111/j.1365-2389.1991.tb00110.x.
- Congreves, K.A., C. Wagner-Riddle, B.C. Si, and T.J. Clough. 2018. Nitrous oxide emissions and biogeochemical responses to soil freezing-thawing and drying-wetting. *Soil Biology and Biochemistry* 117: 5–15. doi: 10.1016/j.soilbio.2017.10.040.
- Ejack, L., and J. Whalen. 2021. Freeze-thaw cycles release nitrous oxide produced in frozen agricultural soils. *Biology and Fertility of Soils* 57(3): 389–398. doi: 10.1007/s00374-020-01537-x.
- Feng, X., L.L. Nielsen, and M.J. Simpson. 2007. Responses of soil organic matter and microorganisms to freeze–thaw cycles. *Soil Biology and Biochemistry* 39(8): 2027–2037. doi: 10.1016/j.soilbio.2007.03.003.
- Fichtner, T., N. Goersmeyer, and C. Stefan. 2019. Influence of Soil Pore System Properties on the Degradation Rates of Organic Substances during Soil Aquifer Treatment (SAT). *Applied Sciences* 9(3): 496. doi: 10.3390/app9030496.
- Fu, Q., J. Yan, H. Li, T. Li, R. Hou, et al. 2019. Effects of biochar amendment on nitrogen mineralization in black soil with different moisture contents under freeze-thaw cycles. *Geoderma* 353: 459–467. doi: 10.1016/j.geoderma.2019.07.027.
- Gao, X., S. Parsonage, M. Tenuta, K. Baron, K. Hanis-Gervais, et al. 2017. Nitrogen Fertilizer Management Practices to Reduce N₂O Emissions from Irrigated Processing Potato in Manitoba. *Am. J. Potato Res.* 94(4): 390–402. doi: 10.1007/s12230-017-9574-4.
- Glibert, P.M., J. Harrison, C. Heil, and S. Seitzinger. 2006. Escalating Worldwide use of Urea – A Global Change Contributing to Coastal Eutrophication. *Biogeochemistry* 77(3): 441–463. doi: 10.1007/s10533-005-3070-5.
- Government of Canada, S.C. 2018. Canadian fertilizer production, by product type and fertilizer year, cumulative data. <https://www150.statcan.gc.ca/t1/tb11/en/tv.action?pid=3210003701> (accessed 26 October 2023).
- Grundmann, G.L., P. Renault, L. Rosso, and R. Bardin. 1995. Differential Effects of Soil Water Content and Temperature on Nitrification and Aeration. *Soil Science Soc of Amer J* 59(5): 1342–1349. doi: 10.2136/sssaj1995.03615995005900050021x.
- Guo, X., C.F. Drury, X. Yang, W. Daniel Reynolds, and R. Fan. 2014. The Extent of Soil Drying and Rewetting Affects Nitrous Oxide Emissions, Denitrification, and Nitrogen Mineralization. *Soil Science Society of America Journal* 78(1): 194–204. doi: 10.2136/sssaj2013.06.0219.
- Hayatsu, M., K. Tago, and M. Saito. 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. doi: 10.1111/j.1747-0765.2007.00195.x.

- Heard, J., and D. Flaten. 2020. Fertilization Considerations after an Unusually Wet Fall: Challenges and Opportunities.
- Henry, H.A.L. 2007. Soil freeze–thaw cycle experiments: Trends, methodological weaknesses and suggested improvements. *Soil Biology and Biochemistry* 39(5): 977–986. doi: 10.1016/j.soilbio.2006.11.017.
- Hu, H.-W., D. Chen, and J.-Z. He. 2015. Microbial regulation of terrestrial nitrous oxide formation: understanding the biological pathways for prediction of emission rates (J.R. Van Der Meer, editor). *FEMS Microbiology Reviews* 39(5): 729–749. doi: 10.1093/femsre/fuv021.
- Huang, T., B. Gao, X.-K. Hu, X. Lu, R. Well, et al. 2014. Ammonia-oxidation as an engine to generate nitrous oxide in an intensively managed calcareous Fluvo-aquic soil. *Sci Rep* 4(1): 3950. doi: 10.1038/srep03950.
- Huang, W., and S.J. Hall. 2017. Elevated moisture stimulates carbon loss from mineral soils by releasing protected organic matter. *Nat Commun* 8(1): 1774. doi: 10.1038/s41467-017-01998-z.
- Johnston, A.M., and T.W. Bruulsema. 2014. 4R Nutrient Stewardship for Improved Nutrient Use Efficiency. *Procedia Engineering* 83: 365–370. doi: 10.1016/j.proeng.2014.09.029.
- Kim, D.-G., S. Saggar, and P. Roudier. 2012. The effect of nitrification inhibitors on soil ammonia emissions in nitrogen managed soils: a meta-analysis. *Nutr Cycl Agroecosyst* 93(1): 51–64. doi: 10.1007/s10705-012-9498-9.
- King, A., F. Rezanezhad, and C. Wagner-Riddle. 2021. Evidence for microbial rather than aggregate origin of substrates fueling freeze-thaw induced N₂O emissions. *Soil Biology & Biochemistry* 160. doi: 10.1016/j.soilbio.2021.108352.
- Kluitenberg, G.J. 2018. 5.2 Heat Capacity and Specific Heat. In: Dane, J.H. and Clarke Topp, G., editors, *SSSA Book Series*. Soil Science Society of America, Madison, WI, USA. p. 1201–1208
- Koch Agronomic Services. 2019. How SuperU Works.
- Krol, D.J., P.J. Forrester, D. Wall, G.J. Lanigan, J. Sanz-Gomez, et al. 2020. Nitrogen fertilisers with urease inhibitors reduce nitrous oxide and ammonia losses, while retaining yield in temperate grassland. *Science of The Total Environment* 725: 138329. doi: 10.1016/j.scitotenv.2020.138329.
- Lasisi, A., O. Akinremi, Q. Zhang, and D. Kumaragamage. 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. doi: 10.1002/saj2.20062.

- Li, C., J. Aber, F. Stange, K. Butterbach-Bahl, and H. Papen. 2000. A process-oriented model of N₂ O and NO emissions from forest soils: 1. Model development. *J. Geophys. Res.* 105(D4): 4369–4384. doi: 10.1029/1999JD900949.
- Li, Z., Z. Tang, Z. Song, W. Chen, D. Tian, et al. 2022. Variations and controlling factors of soil denitrification rate. *Global Change Biology* 28(6): 2133–2145. doi: 10.1111/gcb.16066.
- Lin, S., G. Hernandez-Ramirez, L. Kryzanowski, G. Lohstraeter, and T. Wallace. 2023a. Adding inhibitors to manure injections can mitigate nitrous oxide emissions from barley croplands. *Nutr Cycl Agroecosyst* 126(1): 81–100. doi: 10.1007/s10705-023-10277-x.
- Lin, S., G. Hernandez-Ramirez, L. Kryzanowski, G. Lohstraeter, and T. Wallace. 2023b. Adding inhibitors to manure injections can mitigate nitrous oxide emissions from barley croplands. *Nutr Cycl Agroecosyst* 126(1): 81–100. doi: 10.1007/s10705-023-10277-x.
- Obia, A., G. Cornelissen, J. Mulder, and P. Dörsch. 2015. Effect of Soil pH Increase by Biochar on NO, N₂O and N₂ Production during Denitrification in Acid Soils (R.M. Lehman, editor). *PLoS ONE* 10(9): e0138781. doi: 10.1371/journal.pone.0138781.
- Oztas, T., and F. Fayetorbay. 2003. Effect of freezing and thawing processes on soil aggregate stability. *Catena* 52(1): 1–8. doi: 10.1016/S0341-8162(02)00177-7.
- Pelster, D., M. Chantigny, P. Rochette, N. Bertrand, D. Angers, et al. 2019. Rates and intensity of freeze-thaw cycles affect nitrous oxide and carbon dioxide emissions from agricultural soils. *Canadian Journal of Soil Science* 99(4): 472–484. doi: 10.1139/cjss-2019-0058.
- Qin, H., X. Xing, Y. Tang, B. Zhu, X. Wei, et al. 2020. Soil moisture and activity of nitrite- and nitrous oxide-reducing microbes enhanced nitrous oxide emissions in fallow paddy soils. *Biol Fertil Soils* 56(1): 53–67. doi: 10.1007/s00374-019-01403-5.
- RISMA. 2023. Data - Living Labs Time-Series WebPortal. https://agrifood.aquaticinformatics.net/Data/DataSet/Export/Location/RISMA_MB2/DataSet/Soil%20temperature/Soil%20temperature%200%20to%205%20cm%20depth%20sensor%201/Interval/Monthly/Calendar/CALENDARYEAR/2024/11 (accessed 29 November 2024).
- Schadt, C.W., A.P. Martin, D.A. Lipson, and S.K. Schmidt. 2003. Seasonal Dynamics of Previously Unknown Fungal Lineages in Tundra Soils. *Science* 301(5638): 1359–1361. doi: 10.1126/science.1086940.
- Singh, S.N., and A. Verma. 2007. Environmental Review: The Potential of Nitrification Inhibitors to Manage the Pollution Effect of Nitrogen Fertilizers in Agricultural and Other Soils: A Review. *Environmental Practice* 9(4): 266–279. doi: 10.1017/S1466046607070482.
- Smolders, A.J.P., E.C.H.E.T. Lucassen, R. Bobbink, J.G.M. Roelofs, and L.P.M. Lamers. 2010. How nitrate leaching from agricultural lands provokes phosphate eutrophication in groundwater fed wetlands: the sulphur bridge. *Biogeochemistry* 98(1–3): 1–7. doi: 10.1007/s10533-009-9387-8.

- Statista. 2023. Canada: fertilizer consumption by nutrient. Statista. <https://www.statista.com/statistics/1330033/canada-fertilizer-consumption-by-nutrient/> (accessed 22 January 2024).
- Tan, X., D. Shao, and W. Gu. 2018. Effects of temperature and soil moisture on gross nitrification and denitrification rates of a Chinese lowland paddy field soil. *Paddy Water Environ* 16(4): 687–698. doi: 10.1007/s10333-018-0660-0.
- Teepe, R., R. Brumme, and F. Beese. 2001. Nitrous oxide emissions from soil during freezing and thawing periods. *Soil Biol. Biochem.* 33(9): 1269–1275. doi: 10.1016/S0038-0717(01)00084-0.
- Tenuta and E. G. Beauchamp, M. 2003. Nitrous oxide production from granular nitrogen fertilizers applied to a silt loam soil. *Can. J. Soil. Sci.* 83(5): 521–532. doi: 10.4141/S02-062.
- Tenuta, M., X. Gao, K.H.D. Tiessen, K. Baron, and B. Sparling. 2023. Placement and nitrogen source effects on N₂O emissions for canola production in Manitoba. *Agronomy Journal: agj2.21408*. doi: 10.1002/agj2.21408.
- Tenuta, M., and B. Sparling. 2011. A laboratory study of soil conditions affecting emissions of nitrous oxide from packed cores subjected to freezing and thawing. *Can. J. Soil Sci.* 91(2): 223–233. doi: 10.4141/cjss09051.
- Thomas, R.L., R.W. Sheard, and J.R. Moyer. 1967. Comparison of Conventional and Automated Procedures for Nitrogen, Phosphorus, and Potassium Analysis of Plant Material Using a Single Digestion¹. *Agronomy Journal* 59(3): 240–243. doi: 10.2134/agronj1967.00021962005900030010x.
- Tiessen, K.H.D., D.N. Flaten, C.A. Grant, R.E. Karamanos, and M.H. Entz. 2005. Efficiency of fall-banded urea for spring wheat production in Manitoba: Influence of application date, landscape position and fertilizer additives. *Can. J. Soil. Sci.* 85(5): 649–666. doi: 10.4141/S05-017.
- Trenkel, M.E. 2010. *Slow- and controlled-release and stabilized fertilizers: an option for enhancing nutrient use efficiency in agriculture*. 2nd ed. IFA, International fertilizer industry association, Paris.
- Tucker, B.M. 1974. Displacement of Ammonium Ions for Cation Exchange Capacity Measurements. *Journal of Soil Science* 25(3): 333–337. doi: 10.1111/j.1365-2389.1974.tb01129.x.
- Van Bochove, E., D. Prévost, and F. Pelletier. 2000. Effects of Freeze-Thaw and Soil Structure on Nitrous Oxide Produced in a Clay Soil. *Soil Sci. Soc. Am. J.* 64(5): 1638–1643. doi: 10.2136/sssaj2000.6451638x.
- Velthof, G.L., P.J. Kuikman, and O. Oenema. 2003. Nitrous oxide emission from animal manures applied to soil under controlled conditions. *Biol Fertl Soils* 37(4): 221–230. doi: 10.1007/s00374-003-0589-2.

- Wagner-Riddle, C., Q.C. Hu, E. Van Bochove, and S. Jayasundara. 2008. Linking nitrous oxide flux during spring thaw to nitrate denitrification in the soil profile. *Soil Sci. Soc. Am. J.* 72(4): 908–916. doi: 10.2136/sssaj2007.0353.
- Wagner-Riddle, C., J. Rapai, J. Warland, and A. Furon. 2010. Nitrous oxide fluxes related to soil freeze and thaw periods identified using heat pulse probes. *Can. J. Soil. Sci.* 90(3): 409–418. doi: 10.4141/CJSS09016.
- Wang, L., D.-C. Hao, S. Fan, H. Xie, X. Bao, et al. 2022. N₂O Emission and Nitrification/Denitrification Bacterial Communities in Upland Black Soil under Combined Effects of Early and Immediate Moisture. *Agriculture* 12(3): 330. doi: 10.3390/agriculture12030330.
- Weil. Ray R. Brady, N.C., and R.R. Weil. 2016. *The nature and properties of soils*. Fifteenth edition. Pearson, Columbus.
- Wertz, S., C. Goyer, B. Zebarth, E. Tatti, D. Burton, et al. 2016. The amplitude of soil freeze-thaw cycles influences temporal dynamics of N₂O emissions and denitrifier transcriptional activity and community composition. *Biology and Fertility of Soils* 52(8): 1149–1162. doi: 10.1007/s00374-016-1146-0.
- Xiong, Z., L. Guo, Q. Zhang, G. Liu, and W. Liu. 2017. Edaphic Conditions Regulate Denitrification Directly and Indirectly by Altering Denitrifier Abundance in Wetlands along the Han River, China. *Environ. Sci. Technol.* 51(10): 5483–5491. doi: 10.1021/acs.est.6b06521.

3. OVERALL SYNTHESIS

3.1 Research findings

This study evaluated the effects of moisture and temperature variability on N₂O flux, cumulative N₂O emissions, and mineral N in sandy loam soil treated with a urea-based fertilizer, both with and without double inhibitors. The moisture levels tested were 32%, 50%, and 100% WFPS, while the temperature levels were 4, 8, 12, and 16°C. Fertilizer sources included urea and SuperU. All samples were frozen for 48 h at -20°C and then thawed for 60 h at 4°C, 8°C, 12°C or 16°C.

The findings indicated that moisture and temperature significantly influenced cumulative N₂O emissions, with higher emissions observed at 100% WFPS across all temperatures. There was no significant emission at 32% and 50% WFPS at any temperature. SuperU did not effectively reduce cumulative emissions compared to urea at 100% WFPS, suggesting that the efficacy of the inhibitor may be compromised under certain soil moisture conditions. Regarding hourly N₂O flux, temperature had no significant main effect. However, a positive correlation was observed between volumetric heat capacity and N₂O flux.

The source of N₂O flux was identified as “newly” produced, influenced by substrate release during thawing, a process facilitated by moisture. This highlights the importance of adopting the 4R nutrient stewardship framework to enhance nitrogen (N) use efficiency while minimizing residual nitrate levels, which serves as substrates for microbes (Xiong et al., 2017). This is important because, as observed in the control treatment of our study, residual nitrates can be a significant source of N₂O emissions depending on soil moisture conditions. Overall, moisture was identified as the dominant factor driving denitrification. This study demonstrates that applying fall fertilizer at a depth of 5 cm in soils with 50% WFPS or less has the potential to mitigate N₂O emissions.

3.2 Implications of the study

This study was designed to further our understanding on how N₂O losses occur, as well as the moisture and temperature levels that facilitate these processes. Our Findings indicate that the dominant process of N₂O production is denitrification, which is a function of soil moisture and temperature. In this study, moisture contents of 32% and 50% WFPS are safe levels recommended for application of fall fertilizer. This knowledge is important for farmers to make informed decisions considering the moisture levels in their soils prior to applying fall N.

As part of contributing to policymaking locally, our findings contribute towards national objectives aimed at achieving a 40-45% reduction in emissions below 2005 levels by the year 2030, as well as the ultimate goal of reaching net-zero emissions by 2050 (Government of Canada, 2023). The findings from this study contribute to the consensus of other studies aimed at lowering N₂O emissions and enhancing NUE for sustainable crop production. Regarding global policies, this study indirectly contributes to the three pillars of the United Nations Sustainable Development Goals (UN-SDGs), namely social, environmental, and economic development (Mangukiya and Sklarew, 2023), by addressing local environmental and agricultural challenges. In terms of social development, the findings support efforts to reduce hunger (SDG 2) by enhancing the nutrient use efficiency of N fertilizers. Furthermore, the study contributes to environmental sustainability by promoting responsible food production (SDG 12) and helping mitigate climate change (SDG 13) through strategies aimed at reducing N₂O emissions.

Findings from this research are valuable for fertilizer and inhibitor manufacturers, providing insights into the performance of their products in prairie climates. Industries can utilize this information to enhance product development and optimize performance, contributing to

economic growth (SDG 8) for both farmers and the agricultural sector while supporting innovation and infrastructure development (SDG 9).

3.3 Recommendations for future studies on freeze-thaw cycle

Based on our findings, SuperU was ineffective in reducing N₂O emissions in higher moisture content, highlighting the need to investigate the efficacy of other inhibitors with different active ingredients under these conditions. Future laboratory studies should investigate the effects of moisture and temperature dynamics across a wider range of soil types, especially in clay-textured soils, as adsorptive forces may affect N losses differently. Additionally, future studies should consider the use of intact soil cores (King et al., 2021), as undisturbed pore geometry of the intact soil may respond differently to freezing and thawing compared to homogenized soils.

3.4 References

- Government of Canada, L.S. 2023. Consolidated federal laws of Canada, Canadian Net-Zero Emissions Accountability Act. <https://laws-lois.justice.gc.ca/eng/acts/c-19.3/fulltext.html> (accessed 4 March 2025).
- King, A., F. Rezanezhad, and C. Wagner-Riddle. 2021. Evidence for microbial rather than aggregate origin of substrates fueling freeze-thaw induced N₂O emissions. *Soil Biology & Biochemistry* 160. doi: 10.1016/j.soilbio.2021.108352.
- Mangukiya, R.D., and D.M. Sklarew. 2023. Analyzing three pillars of sustainable development goals at sub-national scales within the USA. *World Development Sustainability* 2: 100058. doi: 10.1016/j.wds.2023.100058.
- Xiong, Z., L. Guo, Q. Zhang, G. Liu, and W. Liu. 2017. Edaphic Conditions Regulate Denitrification Directly and Indirectly by Altering Denitrifier Abundance in Wetlands along the Han River, China. *Environ. Sci. Technol.* 51(10): 5483–5491. doi: 10.1021/acs.est.6b06521.