STABILIZATION OF NITROGEN FERTILIZERS USING NITRIFICATION INHIBITORS IN MANITOBA

by

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A Thesis Submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

Department of Soil Science
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Winnipeg

ABSTRACT

Afzal, Muhammad Junaid. M.Sc., The University of Manitoba, August 2024. Stabilization of Nitrogen Fertilizers Using Nitrification Inhibitors in Manitoba. Supervisor; Dr. Mario Tenuta

Nitrogen (N) fertilization is an effective tool in sustaining crop production and the economic viability of farming systems. Depending on the soil, management practices, and climate, a significant portion of the applied N to crops is lost to the environment in the form of ammonia (NH_3) volatilization, nitrate (NO_3^-) leaching, nitrous oxide (N_2O) emissions, and dinitrogen gas (N_2) losses. Nitrification inhibitors (NIs) can mitigate these losses by stabilizing N as NH_4^+. While early fall urea application with NIs has been studied, the effectiveness of NIs applied late in fall and their persistence into spring remains uncertain. This study involved three field experiments conducted in southern Manitoba from 2020 to 2022 to assess whether applying nitrapyrin or pronitridine in late fall with anhydrous ammonia (AA, 82-0-0) at 80% of the recommended N rate could effectively delay nitrification into spring and influence the yield and N uptake of spring-sown crops compared to AA applied at 80% and 100% of the recommended N rate without NIs. Results indicated that late fall-applied AA with the NIs, nitrapyrin, or pronitridine, did not significantly ($p > 0.05$) delay nitrification, resulting in no significant NH_4^+ stabilization. However, trends suggested slight reductions in NO_3^- appearance on band locations and movement between band locations. There were no significant differences in agronomic yield and crop N uptake among the treatments. A laboratory study was also conducted using urea ammonium nitrate (UAN, 28-0-0) to compare the effectiveness of different NIs across varying soil textures. In this laboratory experiment, nitrapyrin significantly ($p < 0.05$) inhibited nitrification in sand but had limited effectiveness in loam and clay soils. Dicyandiamide (DCD) slightly reduced nitrification ($p < 0.05$) only in the sand on days 14 and 21 of the experiment. However, 3,4-dimethylpyrazole phosphate (DMPP) and pronitridine, at the concentrations used, were not effective ($p < 0.05$) in reducing nitrification in the soils examined. Overall, the results underscore the limited effectiveness of NIs in spring from late fall-applied AA due to delayed banding operations until the soil has cooled and the variable effectiveness among nitrification inhibitor types in soils.

FOREWORD

This thesis was developed following the manuscript guidelines established by the Department of Soil Science, University of Manitoba. Chapters 2 and 3 are earmarked for future submission to relevant academic journals for publication. The core aspects of this thesis, including data collection, analytical results, and its written content, were diligently undertaken by Muhammad Junaid Afzal under the advisement of Dr. Mario Tenuta. The execution and management of the field trials were significantly supported by Dr. Mario Tenuta and John Heard, whose suggestions were invaluable. The thesis drafts were revised with the assistance of Dr. Mario Tenuta and Dr. Xiaopeng Gao, whose insights and feedback were instrumental in refining the content.

ACKNOWLEDGEMENTS

“Whoever does not thank people has not thanked Allah”, Prophet Muhammad, peace be upon Him (Source: Sunan Abī Dāwūd 4811).

With my deepest respect, I first want to thank my supervisor Dr. Mario Tenuta for his support and guidance throughout the project. He consistently encouraged me to hone my skills as a critical observer and a future researcher. I am grateful to have had John Heard on my committee; it was with him that I first stepped onto Canadian farms. He taught me to be more practical in the field[i]. I extend my thanks to Dr. Xiaopeng Gao for his invaluable suggestions and directions that significantly contributed to the success of my research work and thesis write-up. Thank you to Rida Sabirova and Dr. Mikhail Maslov for their help and support in the laboratory study. I am equally grateful to our laboratory technicians, Brad Sparling, Emma Unruh, and Zhe Song, for always being friendly and helpful. A bundle of thanks also goes to the entire team of technicians in Soil Science, namely Rob Ellis, Trevor Fraser, and Fernando Esposito, for their unwavering dedication and support. I also wish to thank our farmers, Edward Wolmann, Steph Comte, and Landon Friesen, for hosting the three farm-scale research trials. I appreciate the assistance of Tammy Jones from Corteva and Myron Kroeker from Rosenort Agro for their advice and product contributions. Special thanks to Jordan Karpinchick of Tone Ag-Consulting for assistance with the site geospatial treatments layout. My gratitude extends to Norman Chabbert of Nutrien Ag Solutions and Steve Barron of Double Diamond for their cooperation and support. Ray Dowbenko, my friend, has always been there for me, providing virtual support and guidance. I would also like to acknowledge the invaluable contribution of all the summer students who helped in soil sampling and processing. I'm thankful for my dear friends Mubashir, Hammad, Usman, Saad, Abdul Moiz, Syed Shehryar, Sarib Jadoon, Muhammad Rameez, Sakshi, Ehsan Zarrinabadi, and Ravneet who welcomed me to Winnipeg and have been such wonderful companions. Lastly, I dedicate my work to my mother, sisters, brother, and especially my father, who as a progressive farmer, has always tried to compare his practical knowledge and skills with my academic understanding. Your support means the world to me.

Table of Contents

ABSTRACT ii
FOREWORD iv
ACKNOWLEDGEMENTS v
LIST OF TABLES 14
LIST OF FIGURES 15
LIST OF ABBREVIATIONS 17

1. INTRODUCTION 19
1.1 Nitrogen (N) and N Use Efficiency 19
1.2 Anhydrous Ammonia 20
1.3 Urea Ammonium Nitrate (UAN) 14
1.4 Nitrification as a Major Pathway for N Losses 14
1.5 Implications of N Fertilizers for Agronomy and Environment 15
1.5.1 Nitrate Leaching 15
1.5.2 Nitrous Oxide Emissions 16
1.6 Nitrification Inhibitors 17
1.7 Previous Field and Laboratory Experiments on Nitrification Inhibitors 18
1.8 Objectives 20
1.9 Hypotheses 20
1.10 Structure of Thesis 22
1.11 Contributions 23
1.12 References 24

2. FIELD STUDY: NITRIFICATION INHIBITION OF LATE FALL-APPLIED ANHYDROUS AMMONIA USING NITRAPYRIN AND PRONITRIDINE IN MANITOBA 36
2.1 Abstract 36
2.2 Introduction 38
2.3 Materials and Methods 42
2.3.1 Experimental Sites Description 42
2.3.2 Experimental Layout 42
2.3.3 Soils Sampling and Preparation for Extractable Nitrogen 45
2.3.4 Weather Data and Agronomic Management 46
2.3.5 Agronomic Yield 48
2.3.6 Total Nitrogen Uptake 48
2.3.7 Statistical Analysis 49
2.4 Results 50
2.4.1 Weather Conditions 50
2.4.2 Inorganic Soil Nitrogen Dynamics at Each Site 53
2.4.3 Agronomic Yield 62
2.4.4 Total Nitrogen Uptake 64
2.5 Discussion 65
2.5.1 Inorganic Soil Nitrogen 65
2.5.2 Yield and Nitrogen Uptake 66
2.7 References 70

3. EFFECT OF NITRIFICATION INHIBITORS ON NITROGEN TRANSFORMATIONS IN SOILS OF VARYING TEXTURE—AN INCUBATION STUDY 79
3.1 Abstract 79
3.2 Introduction 81
3.3 Material and Methods 86
3.3.1 Experimental Setup 86
3.3.2 Soil Collection and Preparation 86
3.3.3 Soil Incubation 89
3.3.4 Sampling for Extractable Soil Nitrogen (NH_4^+ /NO_3^-) 90
3.3.5 Statistical Analysis 91
3.4. Results 92
3.4.1. Clay Soil 92
3.4.2 Loam Soil 93
3.4.3. Sand Soil 96
3.5. Discussion 98
3.5.1. Effect of Soil Type 98
3.5.2. Effect of Nitrification Inhibitors 98
3.6. Conclusions 100
3.7. References 101

4. SYNTHESIS 107
4.1 Project Accomplishments 107
4.2 Surprising and Interesting Observations 109
4.3 Challenges and Improvements 110
4.4 Future Work 112
4.5 Recommendations for Growers and Policy Makers 112
4.6 References 114

APPENDICES 115
Appendix A: Supplementary Material for Chapter 2 115
Appendix B: Supplementary Material for Chapter 3 121

LIST OF TABLES

Table 2.1 Average soil physico-chemical properties (0-30 cm) at the study sites. 44

Table 2.2 Information on nitrogen application, soil types, and sampling events for the study. ... 47

Table 2.3 Average monthly air temperature and precipitation for Manitoba Agrometeorology stations near three field sites (2020-2022). The long-term (1981-2010) normal for the stations is also given. 52

Table 3.1 Average physicochemical properties of the three soils used in the laboratory incubation study (0-25 cm). 88

Table 3.2 Average percentage reduction in nitrate appearance in UAN-treated with different nitrification inhibitors across three soils over 28 days of incubation at 20°C and 65% water-filled pore space (WFPS). 95

LIST OF FIGURES

Figure 2.1 Effect of nitrification inhibitors on soil (0-30 cm) ammonium (NH_4^+), nitrate (NO_3^-), and total extractable nitrogen ($NH_4^+ + NO_3^-$) concentrations in the anhydrous ammonia (AA) banded and between the banded rows (15 cm away from the bands) on different sampling times at Sperling (2020-2021). Means with different letters within a sampling time are significantly different ($p < 0.05$) according to Tukey's multiple comparison procedure. Error bars indicate standard errors of the means (n=4). Control: Without nitrogen and nitrification inhibitor; 157N: 157 kg N ha^{-1} added as AA; 123N: 123 kg N ha^{-1} added as AA; Nitrapyrin (123N): 123 kg N ha^{-1} added as AA treated with N-Serve; Pronitridine (123N): 123 kg N ha^{-1} added as AA treated with Centuro. (Late Fall = Nov-07, Early Spring = Apr 27, Late Spring = May 12). 54

Figure 2.2 Effect of nitrification inhibitors on soil (0-30 cm) ammonium (NH_4^+), nitrate (NO_3^-), and total extractable nitrogen ($NH_4^+ + NO_3^-$) concentrations in the anhydrous ammonia (AA) banded and between the banded rows (15 cm away from the bands) on different sampling times at Notre Dame (2021-2022). Means with different letters within a sampling time are significantly different ($p < 0.05$) according to Tukey's multiple comparison procedure. Error bars indicate standard errors of the means (n=4). Control: Without nitrogen and nitrification inhibitor; 135N: 135 kg N ha^{-1} added as AA; 108N: 108 kg N ha^{-1} added as AA; Nitrapyrin (108N): 108 kg N ha^{-1} added as AA treated with N-Serve; Pronitridine (108N): 108 kg N ha^{-1} added as AA treated with Centuro. (Late Fall = Nov-03, Early Spring = May 18, Late Spring = Jun 02). 58

Figure 2.3 Effect of nitrification inhibitors (nitrapyrin and pronitridine) on soil (0-30 cm) ammonium (NH_4^+), nitrate (NO_3^-), and total extractable nitrogen ($NH_4^+ + NO_3^-$) concentrations in the anhydrous ammonia (AA) banded and between the banded rows (19 cm away from the bands) on different sampling times at Manitou (2021-2022). Means with different letters within a sampling time are significantly different ($p < 0.05$) according to Tukey's multiple comparison procedure. Error bars indicate standard errors of the means (n=4). Control: Without nitrogen and nitrification inhibitor; 146N: 146 kg N ha^{-1} added as AA; 117N: 117 kg N ha^{-1} added as AA; Nitrapyrin (117N): 117 kg N ha^{-1} added as AA treated with N-Serve; Pronitridine (117N): 117 kg N ha^{-1} added as AA treated with Centuro. (Late Fall = Nov-09, Early Spring = May 19, Late Spring = Jun 03)................ 60

Figure 2.4 Effect of nitrification inhibitors on the average grain yield and total N uptake of wheat and canola at Notre Dame and Manitou, respectively. Means with different letters within each graph are significantly different ($p < 0.05$) according to Tukey's multiple comparison procedure. Error bars indicate standard errors of the means (n=4). For all measurements, the sample size (n=4) except for the nitrogen treatments in canola yield, where n=2. All plots were combined for wheat and canola yield, except control plots for canola, which were manually harvested before combining for yield estimation. Control: Without nitrogen and nitrification inhibitor; Control: Without nitrogen and nitrification inhibitor; 135N: 135 kg N ha-1 added as anhydrous ammonia (AA); 108N: 108 kg N ha^{-1} added as AA; Nitrapyrin (108N): 108 kg N ha^{-1} added as AA treated with N-Serve; Pronitridine (108N): 108 kg N ha^{-1} added as AA treated with Centuro. 146N: 146 kg N ha^{-1} added as AA; 117N: 117 kg N ha^{-1} added as AA; Nitrapyrin (117N): 117 kg N ha^{-1} added as AA treated with N-Serve; Pronitridine (117N): 117 kg N ha^{-1} added as AA treated with Centuro. 63

Figure 3.1 Effect of nitrification inhibitors on ammonium (NH_4^+) and nitrate NO_3^-) concentrations in clay, loam, and sand soil (400 g in microcosms) during 28 days of incubation at 20°C and 65% water-filled pore space (WFPS). Asterisks within each soil type indicate significant differences ($p < 0.05$) between nitrogen treatments (excluding controls) for the respective days. Control: Without nitrogen and any nitrification inhibitor; UAN: 60 mg kg^{-1} dry soil N added as Urea ammonium nitrate (UAN); Pronitridine: Centuro + UAN; DCD: Drive-N + UAN; DMPP: ARM U advanced + UAN; Nitrapyrin: eNtrench + UAN. Error bars indicate the standard errors of the means ($n = 4$). ARM U advanced, utilized in the lab study, was provided by Active AgriScience for research, containing exclusively DMPP as its active ingredient. 94

LIST OF ABBREVIATIONS

AA	anhydrous ammonia
C	carbon
cm	centimeter
CO_2	carbon dioxide
°C	degree Celsius
CEC	cation exchange capacity
DCD	dicyandiamide
DMPP	3,4-Dimethylpyrazole phosphate
GHG	greenhouse gases
GMC	gravimetric moisture content
KCl	potassium chloride
kg	kilogram
km	kilometre
L	litre
Mg	megagram
mg	milligram
mL	millilitre
mm	millimetre
N	nitrogen
N_2O	nitrous oxide
NH_4^+	ammonium
NH_3	ammonia
NIs	nitrification inhibitors
NO_2^-	nitrite
NO_3^-	nitrate
NUE	nitrogen use efficiency

SOM	soil organic matter
TOC	total organic carbon
TN	total nitrogen
UAN	urea ammonium nitrate
WFPS	water-filled pore space

1. INTRODUCTION

1.1 Nitrogen (N) and N Use Efficiency

Nitrogen (N) is a primary nutrient required by plants in a relatively large amount for their physiological functioning and life cycle (Bibi et al., 2016). Nitrogen is of prime importance in crop production, where a deficiency can limit crop yield and protein content (Xin et al., 2014; Azimi et al., 2021). Therefore, N should no longer be a limiting factor in producing good yield and quality (Chattha et al., 2022). However, adding N fertilizers is a significant input cost in maintaining crop productivity and soil fertility status because of the high prices of fertilizers (Anas et al., 2020). Nitrogen fertilizers constitute a significant portion of the total energy input in agricultural systems. Nonetheless, a substantial portion of the N from applied fertilizers is typically lost due to susceptibility to nitrification and subsequent losses through leaching and denitrification (Arora et al., 2013). This reveals a significant inefficiency in N use in modern high-production agricultural systems.

The primary loss pathways include nitrate (NO_3^-) leaching, ammonia (NH_3) emissions, and emissions of nitrous oxide (N_2O) and dinitrogen gas (N_2) (Delgado, 2002; Qiao et al., 2015). Management practices collaboratively contribute to optimizing the efficiency of applied N fertilizers (Snyder et al., 2009). This efficiency hinges on choosing the right source, rate, timing, and placement method for the N fertilizers (Chattha et al., 2022; Tenuta et al., 2023). The significant losses from nitrification and its associated processes are NO_3^--leaching and nitrous oxide (N_2O) emissions (Subbarao et al., 2006; Heil et al., 2016). Nitrate (NO_3^-), a highly oxidized form of N and six times more mobile than NH_4^+, is susceptible to reduction reactions and leaching (Bibi et al., 2016). The fate of NO_3^- largely depends on soil conditions; especially under saturated conditions, it can either be denitrified, releasing N_2O or N_2 gases (Follett and Delgado, 2002).

In the Canadian prairie provinces of Manitoba, Alberta, and Saskatchewan, around 75% of the total Canadian N fertilizer application occurs with anhydrous ammonia (AA, 82-0-0), urea (46-0-0), and urea ammonium nitrate (UAN, 28-0-0) as the most common N sources (Mezbahuddin et al., 2020). Late fall-applied AA has been reported as the second most common source and timing among growers between the 2014 and 2019 growing seasons in western Canada (Machado et al., 2020). Late fall application of AA remains popular among Manitoba farmers due to its cost-effectiveness, convenient timing, equipment availability, and favourable soil conditions (Tiessen et al., 2005). It is recommended to apply AA in late fall as soil temperatures drop below 10°C, with a mid-November regulatory deadline in Manitoba, while avoiding poorly or excessively drained soils (Tenuta et al., 2016). Unlike other common N fertilizers, AA initially inhibits nitrifying bacteria, making it preferable for fall application (Sawyer, 2020). However, early AA application risks nitrification losses since the soil is still above 10°C while delaying the application risks the inability to complete the fall application (Swoboda, 2019). Hence, farmers may consider stabilization of fall AA as an effective option (Kim et al., 2012; Schmidt et al., 2020). Nearly 34-46% of farms in Manitoba utilize AA as a fall N source annually (MB Soil Fertility, 2023). Given these practices, understanding the N transformational losses linked to the application of AA is paramount.

1.2 Anhydrous Ammonia

Anhydrous ammonia, the most concentrated N fertilizer, is often considered an economically viable option, particularly for fall N application in western Canada (Griesheim et al., 2019). Anhydrous ammonia is a gas under ambient conditions; it is converted to a liquid state when subjected to refrigeration and pressurization, and it requires special equipment for transportation, storage, and application (Nelson and Singh, 2019). Upon soil injection, AA disperses, typically extending to a diameter of roughly 15 cm, depending on application rate, soil moisture, and texture (Hanna et al., 2005; Sawyer, 2020). It gets dissolved immediately in soil moisture, forming ammonium hydroxide (NH_4OH), which subsequently dissociates into NH_4^+ and the hydroxyl (OH^-) ions (Overdahl and Rehm, 1990; Shafreen et al., 2021). The NH_4^+ is either held within the soil by negatively charged clay and the soil organic matter particles or oxidizes to NO_3^- via nitrification (Shafreen et al., 2021). Depending on soil pH and cation exchange capacity (CEC), NH_4^+ can dissociate, emitting ammonia, particularly under alkaline conditions (Kim et al., 2012; Lasisi et al., 2017). To mitigate AA emissions during application, a depth of 10-15 cm is recommended for AA banding (Erwiha et al., 2020). The combined effects of AA banding and the cooler temperatures of late fall serve as natural suppressants for soil nitrifying microbes, thus inducing a transient delay in nitrification (Norton and Ouyang, 2019). To prolong this delay, ranging from days to several weeks, and to enhance nitrogen use efficiency (NUE), it is advised to stabilize fall-applied AA in the NH_4^+ form using nitrification inhibitors (NIs) (Parkin and Hatfield, 2010; Kim et al., 2012; Schmidt et al., 2020).

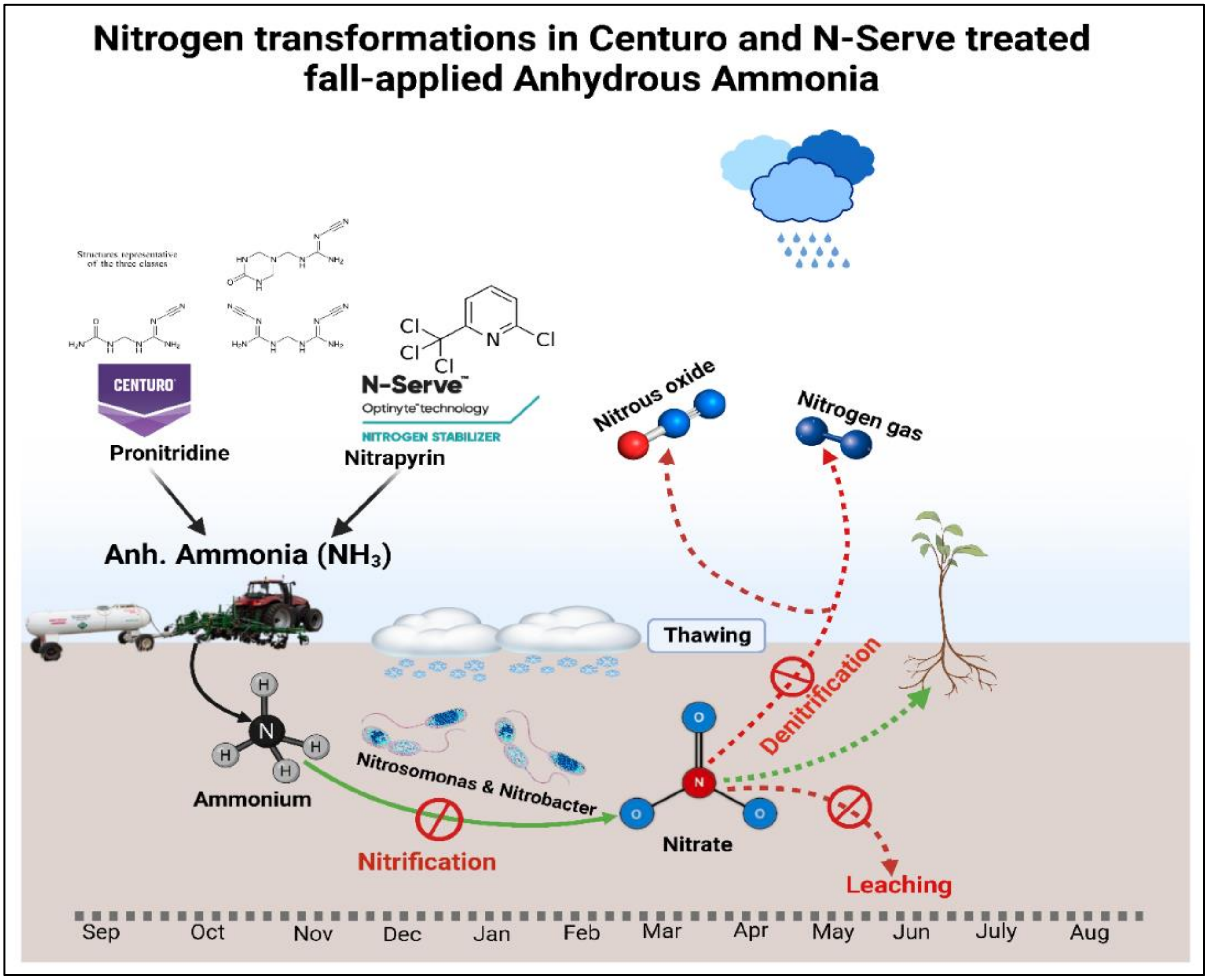


Fig. 1A Nitrogen dynamics post-fall application of AA, highlighting N-Serve and Centuro action points for delaying nitrification and preventing subsequent leaching and denitrification losses. (Created by Muhammad Junaid Afzal)

1.3 Urea Ammonium Nitrate (UAN)

Urea ammonium nitrate is a liquid fertilizer composed of urea, ammonium, and nitrate (Ren et al., 2021). UAN is preferred over urea as an N source, particularly for in-season applications, due to reduced volatilization losses, especially when dribble banded (Griesheim et al., 2023). However, deciding the application timing based on the N source is crucial to mitigate potential N losses before plant assimilation (Yadav et al., 2017). While surface application of UAN increases susceptibility to NH_3 volatilization losses (Mendes Bastos, 2015), injecting UAN 5-10 cm deep into the soil lowers its volatilization potential compared to urea (Woodley et al., 2018). A portion of UAN, existing in the NO_3^- form, is predisposed to losses via leaching and denitrification soon after application. However, to mitigate the potential risks of NO_3^- losses from the NH_4^+ portion of UAN, incorporating NIs stabilizes the NH_4^+ fraction, synchronizing N-availability with plant uptake and averting NO_3^- losses (Lasisi et al., 2021; Li et al., 2023).

1.4 Nitrification as a Major Pathway for N Losses

All applied N fertilizers eventually get converted to NO_3^- form which is prone to leaching and denitrification losses (Norton and Ouyang, 2019). In nitrification, NH_4^+ (an immobile form of N) is oxidized first to nitrite (NO_2^-) and then to NO_3^- by autotrophic bacteria called nitrifiers (Sahrawat, 2008).

$$2NH_4^+ + 3O_2 \longrightarrow 2NO_2 + 2H_2O + 4H^+ \qquad \text{(nitrifiers)}$$

$$2NO_2 + O_2 \longrightarrow 2\,NO_3^- \qquad \text{(nitrifiers)}$$

A significant amount of applied N can be lost via nitrification and related processes from managed ecosystems (Subbarao et al., 2006). Furthermore, while nitrification is widely accepted as a major contributor to N_2O emissions from soil, it has been comparatively less investigated than denitrification (Heil et al., 2016). Several interrelated factors, including soil texture, structure, aeration status, moisture content, cation exchange capacity (CEC), temperature, pH, organic matter content, and substrate (NH_4^+) availability, influence the population of nitrifying microorganisms and thus nitrification (Subbarao et al., 2006).

The N requirement and uptake by plants follow a sigmoid function with a minimal N demand during the germination period, followed by a substantial N requirement during the vegetative and reproductive growth stages, and finally, a small N requirement at the stage of reproductive maturity (Delgado et al., 2011; Lasisi, 2016). Most crops can utilize NH_4^+ and NO_3^-, while some prefer NO_3^- over NH_4^+ (Li et al., 2013; Lasisi, 2016). However, crops well-adapted to anaerobic and acidic soil conditions tend to favour NH_4^+ over NO_3^- (Subbarao et al., 2006; McKane et al., 2013). The form of N taken up by crops depends on several factors, including plant species, cultivar, growth stage, soil aeration status, and NH_4^+/NO_3^- tolerance level of the plants (Li et al., 2013). Suppressing the nitrification process to synchronize N availability with plant uptake offers a great potential to improve NUE and agronomic efficiency while reducing environmental risks (Touchton et al., 1979; Subbarao et al., 2006; Sahrawat, 2008; Li et al., 2023).

1.5 Implications of N Fertilizers for Agronomy and Environment

1.5.1 Nitrate Leaching

Nitrate, being a soluble and negatively charged molecule, cannot be held by the negatively charged soil and organic matter particles and is susceptible to leaching (Zhaohui et al., 2012; Rosenstock et al., 2014; Cicek et al., 2015). Fall N applications are typically more prone to leaching losses with less N recovery than spring applications (Schwager et al., 2016; Lasisi et al., 2021). In cold agricultural regions, the fate of fall-applied N remains uncertain and may depend on N source, soil texture, and regional and interannual variations during winter (Chantigny et al., 2019). Van Es et al. (2006) reported that in the temperate and humid climates of the Midwest United States, fall N applications can increase the potential for NO_3^--leaching, consequently raising the likelihood of N_2O emissions. In contrast, the continental climate of western Canada, characterized by long and cold winters, poses comparatively less risk of nitrification losses. However, a significant amount of N can still be lost through leaching and denitrification, especially during soil thawing and rewetting (Zhaohui, 2012). Thus, to minimize NO_3^- leaching, it is crucial to stabilize or effectively manage applied N, preventing the build-up of NO_3^- levels during precipitation and thawing events.

1.5.2 Nitrous Oxide Emissions

Canada's agriculture sector shares almost 71% of the total national N_2O emissions, impacting global GHG emissions (Environment and Climate Change Canada, 2021). Soil N fertilization is the primary source of these agricultural N_2O emissions, accounting for 60% of Canada's agricultural emissions (Maraseni and Qu, 2016). Generally, fall N applications have higher risks of N losses in the form of N_2O emissions particularly during spring thaw (Tenuta et al., 2016). In the Canadian prairies, emissions from denitrification during spring thaw are one of the major concerns (Rochette et al., 2018; Tenuta et al., 2019; Pelster et al., 2022). Laboratory and field studies have reported significant N_2O emissions following soil thawing events (Tenuta and Sparling, 2011; Hung et al., 2020). Increased N_2O emissions during spring and post-thaw can be attributed to enhanced microbial activity resulting from elevated moisture and temperature levels, greater nutrient availability, and the release of N_2O trapped in deeper soil layers during winter (Tenuta et al., 2016; Chen et al., 2021). With the ever-increasing use of synthetic N fertilizers, N_2O emissions will likely increase unless N use for agricultural food production is managed more effectively (Reay et al., 2012; Burton, 2018; Environment and Climate Change Canada, 2021).

1.6 Nitrification Inhibitors

Nitrification inhibition is a strategy to delay nitrification, reduce N losses, increase NUE, extend N availability over time, and mitigate N_2O emissions (Akiyama et al., 2010; Meng et al., 2021). Nitrification inhibitors delay the oxidation of NH_4^+ by selectively inhibiting the ammonia-oxidizing bacteria, keeping N in the less mobile NH_4^+ form, increasing overall N stabilization and presence in the root zone, and providing plants with greater opportunities for N uptake (Tiessen et al., 2005; Halvorson et al., 2014; Gao et al., 2015; De Laporte et al., 2021). Nitrification inhibitors are considered the best option to overcome N losses, particularly for soils with high moisture content, anaerobic conditions, frequent rainfall events, and soil thaw periods (Tenuta and Sparling, 2011; Zhou et al., 2020).

Common products available to farmers as NIs include the trade names, N-Serve and eNtrench, containing nitrapyrin [2-chloro-6-(trichloromethyl) pyridine]; Drive-N with dicyandiamide (DCD); and the recently available Centuro with pronitridine (Subbarao et al., 2006; Singh and Nelson, 2019; Zhou et al., 2020). Despite offering numerous benefits, there are some cons associated with each inhibitor. For example, N-Serve is highly volatile, corrosive to equipment, and cannot be mixed and stored in AA beyond three weeks (Wolt, 2004; Tiessen et al., 2005) and DCD being highly water soluble is prone to leaching beyond the root zone (Akiyama et al., 2010; Callaghan et al., 2010). However, DCD can be adsorbed onto soil colloids due to its positive charge, which makes its effectiveness dependent on soil properties (Marsden et al., 2016). While Pronitridine is a novel NI, recently developed with benefits of easier handling due to its non-corrosive and non-hazardous formula, higher stability, and claimed to be more efficient, especially with fall-applied AA (Vetsch et al., 2014; Singh and Nelson, 2019).

1.7 Previous Field and Laboratory Experiments on Nitrification Inhibitors

Several studies have investigated the stabilization of N using common NIs, reporting significant delays in nitrification, increased NUE, and enhanced long-term mineral N availability to crop plants during the growing season (Degenhardt et al., 2016; Habibullah et al., 2018; Pawlick et al., 2019). Among these NIs, nitrapyrin has undergone extensive examination with fall-applied AA and spring-applied UAN, demonstrating effectiveness (Touchton et al., 1978; Gomes and Loynachan, 1984; Randall and Vetsch, 2003; Griesheim et al., 2019). However, some studies have observed partial nitrification control with nitrapyrin, indicating possible efficacy variations based on site-specific conditions (Hughes and Welch, 1970; Hendrickson et al., 1978; Hergert and Wiese, 1980), with inconsistencies in crop yield responses also noted (Touchton and Boswell, 1983; Blackmer and Sanchez, 1988; Bailey, 1990; Wolt, 2004).

Moreover, pronitridine offers advantages over nitrapyrin, being non-corrosive to metals in storage vessels and application equipment, and purportedly stable longer in storage, with claimed higher efficiency with fall AA (Habibullah et al., 2018; Singh and Nelson, 2019). However, its efficacy in western Canadian climatic conditions, especially in Manitoba, remains untested. Late fall application of AA is common in Manitoba, typically when the soil temperature drops to 10°C, aiming to limit microbial activity and N conversion to inorganic forms prone to losses (Tenuta et al., 2016; MB Soil Fertility, 2023). Adding nitrapyrin or pronitridine to late-fall AA applications may complement the benefits by reducing N losses and maintaining N in the NH_4^+ form during winter until planting. Limited literature exists on nitrapyrin with fall AA in Manitoba; small plot studies indicate inaccuracies in replicating farm-scale NI recommendations (Wood, 2018). However, no field research has directly compared nitrapyrin and pronitridine in the region. Given the tendency for soil to cool rapidly post-application of AA in late fall by mid-October to early November, evaluating the effectiveness of NIs after prolonged frozen conditions is crucial.

Since ammonium nitrate (NH_4NO_3) production ceased in western Canada in 2005, side-dressing or in-crop application of urea and UAN has become prevalent (Karamanos et al., 2013). UAN is favored for in-season applications due to its low NH_4^+ content and versatility, allowing for either surface broadcasting or, preferably, surface dribbling or in-soil injection (McKenzie et al., 2006; Owens et al., 2023). The urea fraction in UAN hydrolyzes to NH_4^+, then converts to NO_3^-, which plants absorb or lose via leaching or denitrification (Pawlick et al., 2019). Nitrate is most plants' preferred form, but its release must synchronize with crop demand (Lasisi et al., 2020). To delay NO_3^- accumulation and enhance NUE, stabilizing the NH_4^+ portion of UAN with NIs is advised (Vaio, 2006; Meng et al., 2021). Soil texture significantly affects NIs' efficacy by influencing soil pore space, aeration, microbial diversity, and community structure (Fortuna et al., 2012; Xia et al., 2020). Numerous studies have underscored the effectiveness of NIs in delaying nitrification, albeit with varying results across diverse soil types (Wolt, 2000; Barth et al., 2001; Fisk et al., 2015; McGeough et al., 2016; Elrys et al., 2020). Given the development and marketing of various products as NIs, testing, and comparing their effects on nitrification in different soil textures is crucial.

To address these gaps, three field research studies were conducted in Southern Manitoba, comparing the impacts of late fall-applied AA treated with nitrapyrin or pronitridine (at 80% of the recommended N rate) in contrast to untreated AA applied at both full (100%) and reduced rates (80% of the recommended N). Furthermore, a laboratory study on the comparative efficacy of four different NIs (pronitridine, DCD, DMPP, and nitrapyrin) in sand, clay, and loam soils of Manitoba was conducted.

1.8 Objectives

The overall objective of this study was to evaluate the effectiveness of different NIs in delaying the nitrification of N fertilizer sources. Specific objectives were:

(i) To quantify and compare whether applications of nitrapyrin and pronitridine can delay nitrification in late fall-applied AA from the time of application until late spring under field conditions (Chapter 2),

(ii) To compare the potential of late fall-applied nitrapyrin and pronitridine with AA in improving crop yield and aboveground mass N-uptake (Chapter 2),

(iii) To determine whether the use of nitrapyrin and pronitridine with late fall-applied AA allows for reduced N rates while maintaining optimal yields (Chapter 2), and

(iv) To compare the effectiveness of four different NIs—pronitridine, DCD, DMPP, and nitrapyrin—in delaying nitrification in UAN under controlled temperature and moisture conditions in three contrasting soils in the laboratory (Chapter 3).

1.9 Hypotheses

It was hypothesized that:

(i) Use of nitrapyrin and pronitridine will delay nitrification, maintaining more N in NH_4^+ form and inhibiting the buildup of soil NO_3^- in the weeks before soil freeze-up,

(ii) The NIs will remain effective after winter into spring to slow nitrification and appearance of NO_3^- first on bands and then between band positions,

(iii) Overall, delaying nitrification and inhibiting soil NO_3^- buildup with the NIs will increase available N to crops during the growing season compared to AA without NIs, leading to improved crop yield and N uptake,

(iv) Using nitrapyrin and pronitridine in late fall-applied AA at 80% recommended N-rate would yield better agronomic returns and N uptake compared to untreated AA at the same rate, potentially mitigating the need for the extra N (10-20%) that most farmers include with fall application, and

(v) The effectiveness of NIs, pronitridine, DCD, DMPP, and nitrapyrin will vary depending on the product and soil type.

1.10 Structure of Thesis

This thesis was written in a format with two manuscript-format research chapters following the thesis guidelines of the Department of Soil Science, University of Manitoba. Chapter 1 provides a general introduction. Chapter 2 focuses on the field research studies conducted at three different sites in Southern Manitoba, investigating the effect of NIs (nitrapyrin and pronitridine) on stabilization of late fall-AA at a reduced rate of 80% of the recommended N, compared to AA applied at 80% and 100% of recommended N rates without NIs. This chapter discusses the relative concentrations of NH_4^+ and NO_3^- on and between the AA-banded rows, along with agronomic measurements, including yield and total N uptake of wheat and canola crops at two sites. Chapter 3 presents the data from a laboratory incubation study comparing the efficacies of four different NIs (pronitridine, DCD, DMPP, and nitrapyrin) in delaying the nitrification rate for over four weeks in sand, clay, and loam-textured Manitoba soils. Chapter 4 is a comprehensive overview, presenting a synthesis of the project's achievements, notable findings, encountered challenges, improvements made, prospective research directions, recommendations to the growers and policymakers, and similar research conducted in North America.

1.11 Contributions

I led field research during the 2021 and 2022 crop years and the incubation study between 2022 and 2023. The project commenced with the first study at Sperling in the fall of 2020, followed by two studies at Notre Dame and Manitou in 2021. Our collaborating growers, Edward Wollmann, Steph Comte, and Landon Friesen graciously hosted these field studies. The field setups were meticulously designed and implemented with Dr. Mario Tenuta and John Heard. While the farmers managed the fertilizing, seeding, and harvesting operations utilizing their equipment, I supervised the AA band markings during application and oversaw the soil and harvest sampling events. My responsibilities also included liaising with farmers, technicians, and summer students. I undertook comprehensive data collection, organization, and calculations for the research presented here. I conducted statistical analyses with the assistance of Dr. Mario Tenuta. Furthermore, I presented our findings at field tours, conferences, and seminars. Rida Sabirova assisted me in setting up the laboratory incubation study, and she took charge of gas sampling and conducting flux calculations.

1.12 References

Akiyama, H., Yan, X., Yagi, K. 2010. Evaluation of the effectiveness of enhanced-efficiency fertilizers as mitigation options for N_2O and NO emissions from agricultural soils: Meta-analysis. Global Change Biology. 16(6), 1837–1846. https://doi.org/10.1111/J.1365-2486.2009.02031.X

Anas, M., Liao, F., Verma, K. K., Sarwar, M. A., Mahmood, A., Chen, Z. L., Li, Q., Zeng, X. P., Liu, Y., and Li, Y. R. 2020. Fate of nitrogen in agriculture and environment: agronomic, eco-physiological and molecular approaches to improve nitrogen use efficiency. Biological Research, 53(1), 1-20. https://doi.org/10.1186/S40659-020-00312-4

Arora, K., Srivastava, A. 2013. Nitrogen losses due to nitrification: plant-based remedial prospects. Int. J. Bioassays, 2(7), 984-991.

Azimi, S., Kaur, T., Gandhi, T. K. 2021. A deep learning approach to measure stress levels in plants due to Nitrogen deficiency. Measurement, 173, 263–2241. https://doi.org/10.1016/j.measurement.2020.108650

Bailey, L.D. 1990. The effects of 2-chloro-6-(trichloromethyl)-pyridine ('N-Serve') and fertilizers on productivity and quality of Canadian oilseed rape. Canadian Journal of Plant Science, 70, 979–986. https://doi.org/10.4141/cjps90-120

Barth, G., Von Tucher, S., Schmidhalter, U. 2001. Influence of soil parameters on the effect of 3, 4-dimethylpyrazole-phosphate as a nitrification inhibitor. Biology and Fertility of Soils, 34, 98–102. https://doi.org/10.1007/s003740100382

Bibi, S., Saifullah, S., Naeem, A., Dahlawi, S. 2016. Environmental impacts of nitrogen use in agriculture, nitrate leaching, and mitigation strategies. In: Soil Science: Hakeem, K., Akhtar, J., Sabir, M. (eds) Soil Science: Agricultural and Environmental Prospectives. Springer, Cham. Agricultural and Environmental Prospectives. (pp. 131–157). https://doi.org/10.1007/978-3-319-34451-5_6

Blackmer, A. M., Sanchez, C. A. 1988. Response of corn to nitrogen-15-labeled anhydrous ammonia with and without nitrapyrin in Iowa. Agronomy Journal, 80, 95–102. https://doi.org/10.2134/agronj1988.00021962008000010022x

Bremer et al., 2024. Evaluation of urease and nitrification inhibitors in western Canada. Crops and Soils Magazine, 57(2), 38–42. https://doi.org/10.1002/crso.20346.

Burton, D. 2018. A review of the recent scientific literature documenting the impact of 4R management on N_2O emissions relevant to a Canadian context. https://fertilizercanada.ca/wp-content/uploads/2018/08/NERP-Science-Review-Paper-.pdf

Chantigny, M. H., Bittman, S., Larney, F. J., Lapen, D., Hunt, D. E., Goyer, C., Angers, D. A., Chantigny, M., Angers, D., Bittman, S., Hunt, D., Larney, F., Lapen, D., Goyer, C., Thomas, B. W. 2019. A multi-region study reveals high overwinter loss of fall-applied reactive nitrogen in cold and frozen soils. Canadian Journal of Soil Science https://doi.org/1Table139/cjss-2018-0151

Chattha, M. S., Ali, Q., Haroon, M., Afzal, M. J., Javed, T., Hussain, S., Mahmood, T., Solanki, M. K., Umar, A., Abbas, W., Nasar, S., Schwartz-Lazaro, L. M., Zhou, L. 2022. Enhancement of nitrogen use efficiency through agronomic and molecular-based approaches in cotton. Frontiers in Plant Science, 13, 994306. https://doi.org/10.3389/fpls.2022.994306

Chen, Z., Li, Y., Xu, Y., Lam, S. K., Xia, L., Zhang, N., Castellano, M. J., Ding, W. 2021. Spring thaw pulses decrease annual N2O emissions reductions by nitrification inhibitors from a seasonally frozen cropland. Geoderma, 403, 115310. https://doi.org/10.1016/j.geoderma.2021.115310

Cicek, H., Martens, J. R. T., Bamford, K. C., Entz, M. H. 2015. Late-season catch crops to reduce nitrate leaching risk after grazed green manures but release N slower than wheat demand. Agriculture, Ecosystems and Environment, 202, 31–41. https://doi.org/10.1016/j.agee.2014.12.007

De Laporte, A., Banger, K., Weersink, A., Wagner-Riddle, C., Grant, B., Smith, W. 2021. Economic and environmental nitrate leaching consequences of 4R nitrogen management practices including use of inhibitors for corn production in Ontario. Journal of Environmental Management, 300, 113739. https://doi.org/10.1016/j.jenvman.2021.113739

Degenhardt, R. F., Juras, L. T., Smith, L. R. A., MacRae, A. W., Ashigh, J., McGregor, W. R. 2016. Application of Nitrapyrin with Banded Urea, Urea Ammonium Nitrate, and Ammonia Delays Nitrification and Reduces Nitrogen Loss in Canadian Soils. Crop, Forage and Turfgrass Management, 2(1), 1–11. https://doi.org/10.2134/CFTM2016.03.0027

Delgado, J. A. 2002. Quantifying the loss mechanisms of nitrogen. Journal of Soil and Water

Conservation, 57(6), 389–398. https://www.jswconline.org/content/57/6/389.short

Delgado, J. A., Follett, R. F. 2011. Advances in nitrogen management for water quality. Journal of Soil and Water Conservation, 66(1), 25A-26A. https://doi-org.uml.idm.oclc.org/10.2489/jswc.66.1.25A

Elrys, A. S., Raza, S., Elnahal, A. S. M., Na, M., Ahmed, M., Zhou, J., Chen, Z. 2020. Do soil property variations affect dicyandiamide efficiency in inhibiting nitrification and minimizing carbon dioxide emissions? Ecotoxicology and Environmental Safety, 202, 110875. https://doi.org/10.1016/J.ECOENV.2020.110875

Environment and Climate Change Canada. 2021. Accessed August 13, 2021. https://www.canada.ca/en/environment-climate-change.html

Erwiha, G. M., Ham, J., Sukor, A., Wickham, A., Davis, J. G. 2020. Organic fertilizer source and application method impact ammonia volatilization. Communications in Soil Science and Plant Analysis, 51(11), 1469–1482. https://doi.org/10.1080/00103624.2020.1784919

Fageria, N. K., Baligar, V. C. 2005. Enhancing nitrogen use efficiency in crop plants. Advances in Agronomy, 88, 97-185. https://doi.org/10.1016/S0065-2113(05)88004-6

Fisk, L., Maccarone, L. D., Barton, L., Murphy, D. V. 2015. Nitrapyrin decreased nitrification of nitrogen released from soil organic matter but not amoA gene abundance at high soil temperatures. Soil Biology and Biochemistry, 88, 214-223. https://doi.org/10.1016/j.soilbio.2015.05.029

Follett, R. F., Delgado, J. A. 2002. Nitrogen fate and transport in agricultural systems. Journal of Soil and Water Conservation, 57(6), 402–408. https://www-jswconline-org.uml.idm.oclc.org/content/57/6/402

Fortuna, A. M., Wayne Honeycutt, C., Griffin, T. S., Larkin, R. P., Wayne, C., Mark, J., Richard, J. 2012. Links among nitrification, nitrifier communities, and edaphic properties in contrasting soils receiving dairy slurry. Journal of Environmental Quality, 41(1), 262–272. https://doi.org/10.2134/jeq2011.0202

Gao, X., Asgedom, H., Tenuta, M., and Flaten, D. N. 2015. Enhanced efficiency urea sources and placement effects on nitrous oxide emissions. Agronomy Journal, 107(1), 265–277. https://doi.org/10.2134/agronj14.0213

Gomes, S. L., Loynachan, T. E. 1984. Nitrification of anhydrous ammonia related to nitrapyrin and time-temperature interactions. Agronomy Journal, 76(1), 9–12.

https://doi.org/10.2134/agronj1984.00021962007600010003x

Goos, R. J., Johnson, B. E. 1999. Performance of two nitrification inhibitors over a winter with exceptionally heavy snowfall. Agronomy Journal, 91(6), 1046–1049. https://doi.org/10.2134/agronj1999.9161046x

Griesheim, K. L., Mulvaney, R. L., Smith, T. J., Henning, S. W., and Hertzberger, A. J. 2019. Nitrogen-15 evaluation of fall-applied anhydrous ammonia: I. Efficiency of nitrogen uptake by corn. Soil Science Society of America Journal, 83(6), 1809–1818. https://doi.org/10.2136/sssaj2019.04.0098

Griesheim, K., Mulvaney, R., Smith, T., Hertzberger, A. J. 2023. Isotopic evaluation of in-season Y drop applications for corn production. Soil Science Society of America Journal, 87(2), 260–277. https://doi.org/10.1002/SAJ2.20488

Habibullah, H., Nelson, K. A., Motavalli, P. P. 2018. Management of nitrapyrin and pronitridine nitrification inhibitors with urea ammonium nitrate for winter wheat production. Agronomy, 8(10). https://doi.org/10.3390/agronomy8100204

Halvorson, A. D., Snyder, C. S., Blaylock, A. D., Del Grosso, S. J. 2014. Enhanced-efficiency nitrogen fertilizers: Potential role in nitrous oxide emission mitigation. Agronomy Journal, 106(2), 715–722. https://doi.org/10.2134/AGRONJ2013.0081

Hanna, H. M., Boyd, P. M., Baker, J. L., Colvin, T. S. 2005. Anhydrous ammonia application losses using single-disc and knife fertilizer injectors. Applied Engineering in Agriculture, 21(4), 573–578. https://doi.org/10.13031/2013.18564

Hanson, R. G., Maledy, S. R., & Jentes, C. E. (1987). Effect of anhydrous ammonia with and without nitrapyrin applied to fall and spring on corn yield. Communications in soil science and plant analysis, 18(4), 387–403. https://doi.org/10.1080/00103628709367828

Heil, J., Vereecken, H., Brüggemann, N. 2016. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. European Journal of Soil Science, 67(1), 23–39. https://doi.org/10.1111/ejss.12306

Hendrickson, L. L., Walsh, L. M., Keeney, D. R. 1978. Effectiveness of nitrapyrin in controlling nitrification of fall and spring-applied anhydrous ammonia. Agronomy Journal, 70(5), 704–708. https://doi.org/10.2134/agronj1978.00021962007000050003xa

Hergert, G. W., Wiese, R. A. 1980. Performance of nitrification inhibitors in the Midwest. In: Meisinger, J. J., Randall, G.W. Vitosh, M. L. (eds), Nitrification inhibitors—potentials and limitations. American Society of Agronomy and Soil Science Society of America. (pp. 89–105). https://doi.org/10.2134/asaspecpub38.c7

Huber, D. M., Warren, H. L., Nelson, D. W., Tsai, C. Y., Shaner, G. E. 1980. Response of winter wheat to inhibiting nitrification of fall-applied nitrogen. Agronomy Journal, 72(4), 632–637. https://doi.org/10.2134/agronj1980.00021962007200040015x

Hughes, T. D., Welch, L. F. 1970. 2-chloro-6-(Trichloromethyl) pyridine as a nitrification inhibitor for anhydrous ammonia applied in different seasons. Agronomy Journal, 62(6), 821–824. https://doi.org/10.2134/agronj1970.00021962006200060044x

Hung, C.-Y., Ejack, L., Whalen, J. K. 2020. Fall-applied manure with cover crops did not increase nitrous oxide emissions during spring freeze-thaw periods. https://doi.org/10.1016/j.apsoil.2020.103786

Karamanos, R. E., Stevenson, F. C. 2013. Nitrogen fertilizer products and timing alternatives exist for forage production in the Peace region of Alberta. Canadian Journal of Plant Science, 93(2), 151–160. https://doi.org/10.4141/CJPS2012-150

Kim, D. G., Saggar, S., Roudier, P. 2012. The effect of nitrification inhibitors on soil ammonia emissions in nitrogen managed soils: a meta-analysis. Nutrient Cycling in Agroecosystems, 93, 51–64. https://doi.org/10.1007/s10705-012-9498-9

Kuang, W., Gao, X., Tenuta, M., Zeng, F. 2021. A global meta-analysis of nitrous oxide emission from drip-irrigated cropping system. Global Change Biology, 27(14), 3244–3256. https://doi.org/10.1111/GCB.15636

Lasisi, A. A. 2016. Ammonium-N persistence and root nitrogen content of annual crops and perennial forage grasses following pig manure application. c, 96. http://mspace.lib.umanitoba.ca/bitstream/handle/1993/31721/Lasisi_Ahmed.pdf

Lasisi, A. A., Akinremi, O. O., and Kumaragamage, D. 2021. Efficiency of fall versus spring-applied urea-based fertilizers treated with urease and nitrification inhibitors II. Crop yield and nitrogen use efficiency. Soil Science Society of America Journal, 85(2), 299–313. https://doi.org/10.1002/saj2.20126

Lasisi, A. A., Akinremi, O. O., Tenuta, M., Cattani, D. 2017. Short-term persistence of ammonium-N following pig manure application is greater with perennial forage grasses than annual crops. Canadian Journal of Soil Science, 97(4), 769–782. https://doi.org/10.1139/cjss-2017-0040

Lasisi, A. A., Akinremi, O. O., Zhang, Q., and Kumaragamage, D. 2020. Efficiency of fall versus spring-applied urea-based fertilizers treated with urease and nitrification inhibitors I. Ammonia volatilization and mitigation by NBPT. Soil Science Society of America Journal, 84(3), 949–962. https://doi.org/10.1002/SAJ2.20062

Li, S. X., Wang, Z. H., Stewart, B. A. 2013. Responses of crop plants to ammonium and nitrate N. Advances in agronomy, 118, 205-397. https://doi.org/10.1016/B978-0-12-405942-9.00005-0

Li, X., Zhang, X., Wang, S., Hou, W., Yan, L. 2023. The combined use of liquid fertilizer and urease/nitrification inhibitors on maize yield, nitrogen loss, and utilization in the Mollisol region. Plants, 12(7), 1486. https://doi.org/10.3390/plants12071486

Liu, S. L., Varsa, E. C., Kapusta, G., Mburu, D. N. 1984. Effect of etridiazol and nitrapyrin treated N fertilizers on soil mineral N status and wheat yields. Agronomy Journal, 76(2), 265–270. https://doi.org/10.2134/agronj1984.00021962007600020022x

Machado, P. V. F., Tenuta, M., Bruulsema, T. W., Liang, C., MacDonald, J. D., Reid, D. K., Wagner-Riddle, C. The Canadian fertilizer use survey 2015-2020: Nitrogen baselines, trends, and opportunities. In ASA, CSSA, SSSA International Annual Meeting. ASA-CSSA-SSSA. https://scisoc.confex.com/scisoc/2021am/prelim.cgi/Paper/138035

Marsden, K. A., Marín-Martínez, A. J., Vallejo, A., Hill, P. W., Jones, D. L., Chadwick, D. R. 2016. The mobility of nitrification inhibitors under simulated ruminant urine deposition and rainfall: a comparison between DCD and DMPP. Biology and Fertility of Soils, 52, 491-503. https://doi.org/10.1007/s00374-016-1092-x

MB Soil Fertility. 2023. Fall ammonia applications on dry soils. Accessed January 10, 2024. https://www.gov.mb.ca/agriculture/crops/seasonal-reports/pubs/fall-nh3-application-dry-soil.pdf

McGeough, K. L., Watson, C. J., Müller, C., Laughlin, R. J., Chadwick, D. R. 2016. Evidence that the efficacy of the nitrification inhibitor dicyandiamide (DCD) is affected by soil properties in UK soils. Soil Biology and Biochemistry, 94(3), 222–232. https://doi.org/10.1016/j.soilbio.2015.11.017

McKane, R. B., Johnson, L. C., Shaver, G. R., Nadelhoffer, K. J., Rastetter, E. B., Fry, B., Murray, G. 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. Nature, 415(6867), 68–71. https://doi.org/10.1038/415068a

McKenzie, R. H., Bremer, E., Grant, C. A., Johnston, A. M., DeMulder, J., and Middleton, A. B. 2006. In-crop application effect of nitrogen fertilizer on grain protein concentration of spring wheat in the Canadian prairies. Canadian Journal of Soil Science, 86(3), 565–572. https://doi.org/10.4141/S05-026

Mendes Bastos, L. 2015. N fertilizer source and placement impacts nitrous oxide losses, grain yield, and N use efficiency in no-till corn. (Doctoral dissertation, Kansas State University). https://krex.k-state.edu/handle/2097/18797

Meng, Y., Wang, J. J., Wei, Z., Dodla, S. K., Fultz, L. M., Gaston, L. A., Xiao, R., Park, J.-H., Scaglia, G. 2021. Nitrification inhibitors reduce nitrogen losses and improve soil health in subtropical pastureland. https://doi.org/10.1016/j.geoderma.2021.114947

Mezbahuddin, S., Spiess, D., Hildebrand, D., Kryzanowski, L., Itenfisu, D., Goddard, T., Iqbal, J., Grant, R. 2020. Assessing Effects of agronomic nitrogen management on crop nitrogen use and nitrogen losses in the western Canadian prairies. Frontiers in Sustainable Food Systems, 4, 512292. https://doi.org/10.3389/FSUFS.2020.512292

Narayan Maraseni, T., Qu, J. 2016. An international comparison of agricultural nitrous oxide emissions. https://doi.org/10.1016/j.jclepro.2016.07.035

Nelson, K. A. 2018. Pronitridine nitrification inhibitor with urea ammonium nitrate for corn. Journal of Agricultural Science, 10(6), 16. https://doi.org/10.5539/jas.v10n6p16

Nelson, K. A., and Singh, G. 2019. Comparison of applicator knives for fall and spring strip-till-applied anhydrous ammonia. Crop, Forage and Turfgrass Management, 5(1), 190038. https://doi.org/10.2134/CFTM2019.05.0038

Norton, J., and Ouyang, Y. 2019. Controls and adaptive management of nitrification in agricultural soils. Frontiers in Microbiology, 10, 1931. https://doi.org/10.3389/fmicb.2019.01931

O’Callaghan, M., Gerard, E. M., Carter, P. E., Lardner, R., Sarathchandra, U., Burch, G., Ghani, A., Bell, N. 2010. Effect of the nitrification inhibitor dicyandiamide (DCD) on microbial communities in a pasture soil amended with bovine urine. Soil Biology and Biochemistry, 42(9), 1425–1436. https://doi.org/10.1016/J.SOILBIO.2010.05.003

Overdahl, C. J., Rehm, G. W. 1990. Using anhydrous ammonia in Minnesota. Accessed December 20, 2023. https://conservancy.umn.edu/bitstream/handle/11299/93928/1/3073.pdf

Owens, J. L., Wang, Z., Thomas, B. W., Hao, X., Coles, K., Rahmani, E., Karimi, R., Gill, K., Beres, B. L. 2023. Winter wheat responses to enhanced efficiency liquid nitrogen fertilizers in the Canadian Prairies. Canadian Journal of Plant Science. https://doi.org/10.1139/CJPS-2022-0208

Parkin, T. B., Hatfield, J. L. 2010. Influence of nitrapyrin on N_2O losses from soil receiving fall-applied anhydrous ammonia. Agriculture, Ecosystems & Environment, 136(1–2), 81–86. https://doi.org/10.1016/j.agee.2009.11.014

Pawlick, A. A., Wagner-Riddle, C., Parkin, G. W., Berg, A. A. 2019. Assessment of nitrification and urease inhibitors on nitrate leaching in corn (Zea mays L.). Canadian Journal of Soil Science, 99(1), 80–91. https://doi.org/10.1139/CJSS-2018-0110

Pelster, D. E., Thiagarajan, A., Liang, C., Chantigny, M. H., Wagner-Riddle, C., Congreves, K., Lemke, R., Glenn, A., Tenuta, M., Hernandez-Ramirez, G., Derek Hunt, S., Owens, J., MacDonald, D. 2022. Ratio of non-growing season to growing season N_2O emissions in Canadian croplands: an update to national inventory methodology. Canadian Journal of Soil Science, 103(2), 344–352. https://doi.org/10.1139/cjss-2022-0101

Qiao, C., Liu, L., Hu, S., Compton, J. E., Greaver, T. L., Li, Q. 2015. How inhibiting nitrification affects the nitrogen cycle and reduces environmental impacts of anthropogenic nitrogen input. Global Change Biology, 21(3), 1249–1257. https://doi.org/10.1111/GCB.12802

Randall, G. W., Vetsch, J. A. 2003. Nitrate losses in subsurface drainage from a corn–soybean rotation as affected by fall and spring application of nitrogen and nitrapyrin. Journal of Environmental Quality, 34(2), 590–597. https://doi.org/10.2134/JEQ2005.0590

Reay, D. S., Davidson, E. A., Smith, K. A., Smith, P., Melillo, J. M., Dentener, F., Crutzen, P. J. 2012. Global agriculture and nitrous oxide emissions. Nature Climate Change 2012 2:6, 2(6), 410–416. https://doi.org/10.1038/nclimate1458

Ren, B., Guo, Y., Liu, P., Zhao, B., Zhang, J. 2021. Effects of urea-ammonium nitrate solution on yield, N_2O emission, and nitrogen efficiency of summer maize under integration of water and fertilizer. Frontiers in Plant Science, 12, 700331. https://doi.org/10.3389/fpls.2021.700331

Rochette, P., Liang, C., Pelster, D., Bergeron, O., Lemke, R., Kroebel, R., MacDonald, D., Yan, W. Flemming, C. 2018. Soil nitrous oxide emissions from agricultural soils in Canada: Exploring relationships with soil, crop and climatic variables. Agriculture, Ecosystems & Environment 254, 69–81. https://doi.org/10.1016/j.agee.2017.10.021

Rosenstock, T., T. S., Liptzin, D., Dzurella, K., Fryjoff-Hung, A., Hollander, A., Jensen, V., Harter, T. 2014. Agriculture's contribution to nitrate contamination of Californian groundwater (1945–2005). Journal of Environmental Quality, 43(3), 895–907. https://doi.org/10.2134/jeq2013.10.0411

Sahrawat, K. L. 2008. Factors affecting nitrification in soils. Communications in Soil Science and Plant Analysis, 39(9–10), 1436–1446. https://doi.org/10.1080/00103620802004235

Sawyer, J. 2020. Fall 2020 nitrogen considerations. Corn and Soybean Digest. Accessed March 26, 2024. https://uml.idm.oclc.org/login?url=https://www.proquest.com/trade-journals/fall-2020-nitrogen-considerations/docview/2441296550/se-2

Schmidt, R., Wang, X. B., Garbeva, P., Yergeau, É. 2020. Nitrapyrin has far-reaching effects on the soil microbial community structure, composition, diversity, and functions. bioRxiv. https://doi.org/10.1101/2020.07.21.205765

Schwager, E. A., VanderZaag, A. C., Wagner-Riddle, C., Crolla, A., Kinsley, C., Gregorich, E. 2016. Field nitrogen losses induced by application timing of digestate from dairy manure biogas production. Journal of Environmental Quality, 45(6), 1829–1837. https://doi.org/10.2134/JEQ2016.04.0148

Shafreen, M., Vishwakarma, K., Shrivastava, N., Kumar, N. 2021. Physiology and Distribution of Nitrogen in Soils. 3–31. https://doi.org/10.1007/978-3-030-71206-8_1

Singh, G., Nelson, K. A. 2019. Pronitridine and nitrapyrin with anhydrous ammonia for corn. Journal of Agricultural Science, 11(4), 13. https://doi.org/10.5539/jas.v11n4p13

Snyder, C. S., Bruulsema, T. W., Jensen, T. L., Fixen, P. E. 2009. Review of greenhouse gas emissions from crop production systems and fertilizer management effects. Agriculture, Ecosystems and Environment, 133(3-4), 247–266. https://doi.org/10.1016/j.agee.2009.04.021

Stehouwer, R. C., Johnson, J. W. 1990. Urea and anhydrous ammonia management for conventional tillage corn production. Journal of Production Agriculture, 3(4), 507–513. https://doi.org/10.2134/jpa1990.0507

Subbarao, G., Ishikawa, T., Ito, O., Nakahara, K., Wang, H. Y., Berry, W. L. 2006. A bioluminescence assay to detect nitrification inhibitors released from plant roots: a case study with Brachiaria humidicola. Plant and Soil, 288, 101–112. https://doi.org/10.1007/s11104-006-9094-3

Subbarao, G., Ito, O., Sahrawat, K., Berry, W., Nakahara, K., Ishikawa, T., Watanabe, T., Suenaga, K., Rondon, M., Rao, I. 2006. Scope and strategies for regulation of nitrification in agricultural systems — challenges and opportunities. Critical Reviews in Plant Sciences, 25(4), 303–335. https://doi.org/10.1080/07352680600794232

Swezey, A. W., Turner, G. O. 1962. Crop experiments on the effect of 2-chloro-6-(trichloromethyl)-pyridine for control of nitrification of ammonium and urea fertilizers. Agronomy Journal, 532–534. https://doi.org/10.2134/agronj1962.00021962005400060020x

Swoboda, R. 2019. Get the most from the fall N application. Farm Industry News. Accessed March 26, 2024. https://uml.idm.oclc.org/login?url=https://www.proquest.com/trade-journals/get-most-fall-n-application/docview/2312471488/se-2

Tenuta, M., Amiro, B.D., Gao, X., Wagner-Riddle, C., Gervais, M. 2019. Agricultural management practices and environmental drivers of nitrous oxide emissions over a decade for an annual and an annual-perennial crop rotation. Agricultural and Forest Meteorology, 276, 107636. https://doi.org/10.1016/j.agrformet.2019.107636

Tenuta, M., Gao, X., Flaten, D. N., Amiro, B. D. 2016. Lower nitrous oxide emissions from anhydrous ammonia application prior to soil freezing in late fall than spring pre-plant application. Journal of Environmental Quality, 45(4), 1133–1143. https://doi.org/10.2134/jeq2015.03.0159

Tenuta, M., Gao, X., Tiessen, K. H., Baron, K., Sparling, B. 2023. Placement and nitrogen source effects on N_2O emissions for canola production in Manitoba. Agronomy Journal, 115(5), 2369–2383. https://doi.org/10.1002/agj2.21408

Tenuta, M., Sparling, B. 2011. A laboratory study of soil conditions affecting emissions of nitrous oxide from packed cores subjected to freezing and thawing. Canadian Journal of Soil Science, 91(2), 223–233. https://doi.org/10.4141/cjss09051

Tiessen, K. H. D., Flaten, D. N., Bullock, P. R., Grant, C. A., Karamanos, R. E., Burton, D. L., Entz, M. H. 2008. Interactive effects of landscape position and time of application on the response of spring wheat to fall-banded urea. Agronomy Journal, 100(3), 557–563. https://doi.org/10.2134/agronj2006.0225

Tiessen, K. H. D., Flaten, D. N., Grant, C. A., Karamanos, R. E., Entz, M. H. 2005. Efficiency of fall-banded urea for spring wheat production in Manitoba: Influence of application date, landscape position, and fertilizer additives. Canadian Journal of Soil Science, 85(5), 649–

666. https://doi.org/10.4141/S05-017

Touchton, J. T., Boswell, F. C. 1983. Performance of nitrification inhibitors in the southeast. In: Meisinger, J. J., Randall, G.W. Vitosh, M. L. (eds), Nitrification inhibitors—potentials and limitations. American Society of Agronomy and Soil Science Society of America. (pp. 63–74). https://doi.org/10.2134/asaspecpub38.c5

Touchton, J. T., Hoeft, R. G., Welch, L. F. 1978. Effect of nitrapyrin on nitrification of fall and spring-applied anhydrous ammonia. Agronomy Journal, 70(5), 805–810. https://doi.org/10.2134/agronj1978.00021962007000050026x

Touchton, J. T., Hoeft, R. G., Welch, L. F., Mulvaney, D. L., Oldham, M. G., Zajicek, F. E. 1979. N uptake and corn yield as affected by applications of nitrapyrin with anhydrous ammonia1. Agronomy Journal, 71(2), 238–242. https://doi.org/10.2134/agronj1979.00021962007100020006x

Vaio, N. 2006. Ammonia volatilization and N-uptake from urea, urea ammonium nitrate (UAN), and Nitamin® (urea-polymer) applied to tall fescue in Georgia. https://getd.libs.uga.edu/pdfs/vaio_nicolas_200612_ms.pdf

Van Es, H. M., Sogbedji, J. M., Schindelbeck, R. R. 2006. Effect of manure application timing, crop, and soil type on nitrate leaching. Journal of Environmental Quality, 35(2), 670–679. https://doi.org/10.2134/JEQ2005.0143

Vetsch, J. A., Schwab, G. J. 2014, November. Corn grain yield as affected by the nitrification inhibitor KAS771G77. In Proceedings of the ASA, CSSA, SSSA International Annual Meeting, Long Beach, CA, USA (pp. 2–5). https://scisoc.confex.com/scisoc/2014am/videogateway.cgi/id/21059?recordingid=21059

Wolt, J. D. 2000. Nitrapyrin behavior in soils and environmental considerations. Journal of Environmental Quality, 29(2), 367–379. https://doi.org/10.2134/JEQ2000.00472425002900020002X

Wolt, J. D. 2004. A meta-evaluation of nitrapyrin agronomic and environmental effectiveness with emphasis on corn production in the Midwestern USA. Nutrient Cycling in Agroecosystems 2004 69:1, 69(1), 23–41. https://doi.org/10.1023/B:FRES.0000025287.52565.99

Wood, M. D. 2018. Right source and right time: reducing nitrous oxide emissions with enhanced efficiency nitrogen fertilizers (Master's thesis, University of Manitoba).

Woodley, A. L., Drury, C. F., Yang, X. M., Reynolds, W. D., Calder, W., Oloya, T. O. 2018.

Streaming urea ammonium nitrate with or without enhanced efficiency products impacted corn yields, ammonia, and nitrous oxide emissions. Agronomy Journal, 110(2), 444–454. https://doi.org/10.2134/AGRONJ2017.07.0406

Xia, Q., Rufty, T., Shi, W. 2020. Soil microbial diversity and composition: Links to soil texture and associated properties. Soil Biology and Biochemistry, 149, 107953. https://doi.org/10.1016/j.soilbio.2020.107953

Xin, C., Qing-Wei, Y., Jia-lin, S., Shuang, X., Fu-Chun, X., Ya-jun, C. 2014. Research progress on nitrogen use and plant growth. Journal of Northeast Agricultural University, 21(2), 68–74. https://doi.org/10.1016/S1006-8104(14)60036-2

Yadav, M. R., Kumar, R., Parihar, C. M., Yadav, R. K., Jat, S. L., Ram, H., Jat, M. L. 2017. Strategies for improving nitrogen use efficiency: A review. Agricultural Reviews, 38(1), 29–40. https://doi.org/10.18805/ag.v0iOF.7306

Zhaohui, L., Xiaozong, S., Lihua, J., Haitao, L., Yu, X., Xinhao, G., Yuwen, S. 2012. Strategies for managing soil nitrogen to prevent nitrate-N leaching in intensive agriculture systems. In: Hernandez-Soriano, M. C. (eds), Soil Health and Land Use Management. In Tech. China. (pp. 133–154). https://www.intechopen.com/chapters/25275

Zhou, X., Wang, S., Ma, S., Zheng, X., Wang, Z., Lu, C. 2020. Effects of commonly used nitrification inhibitors-dicyandiamide (DCD), 3, 4-dimethylpyrazole phosphate (DMPP), and nitrapyrin-on soil nitrogen dynamics and nitrifiers in three typical paddy soils. Geoderma, 380, 114637. https://doi.org/10.1016/j.geoderma.2020.114637

2. FIELD STUDY: NITRIFICATION INHIBITION OF LATE FALL-APPLIED ANHYDROUS AMMONIA USING NITRAPYRIN AND PRONITRIDINE IN MANITOBA

2.1 Abstract

Late fall application of anhydrous ammonia (AA, 82-0-0) is a common practice in Manitoba. However, it is susceptible to the nitrification process and subsequent losses through leaching and denitrification, and emissions of N_2O during the thaw by the time of the next growing season. Consequently, farmers often apply 10-20% higher nitrogen (N) rates in the fall than in spring to compensate for these N losses. Three field research trials were conducted in Southern Manitoba on poorly-drained clay (Osborne) and moderately to well-drained loam soils (Firdale and Manitou) to investigate the effects of nitrification inhibitors (NIs), nitrapyrin (N-Serve), and pronitridine (Centuro), in combination with AA, in slowing down nitrification, maintaining optimal yields and N uptake from spring-sown crops. Nitrogen was applied in late fall as AA at 80% of the recommended N rate (based on soil test and target yield) with and without NIs. Additionally, treatments without N addition (as a control) and with 100% of the recommended N rate were included. After AA application and before planting for all sites, the soil was sampled (0-30 cm, both on and between the AA bands) in late fall, early, and late spring. Results indicated that nitrapyrin and pronitridine-treated AA did not significantly delay the nitrification compared to AA without NIs at any site. However, at Sperling, nitrate (NO_3^-) tended to be recovered more on bands coupled with a tendency for lower NO_3^- between bands with NIs at the late spring sampling, which might have been caused by delayed nitrification and lack of moisture for diffusion. This suggests that delayed nitrification may have limited the diffusion of NO_3^- from bands by planting time. At Notre Dame and Manitou, there was a tendency for lower NO_3^- on bands with NIs at the early

spring sampling and for both sites with nitrapyrin at the late spring sampling. At Manitou, NO_3^- concentration between bands tended to be lower for NIs, indicating a potential reduction in NO_3^- appearance on bands and diffusion between bands by planting. However, among the AA treatments, no significant differences were observed in grain yield and N uptake of spring wheat and canola from well-drained loam soils. Overall, these findings do not provide substantial evidence to support the recommendation of using NIs with late fall AA applications in Manitoba.

2.2 Introduction

In western Canadian provinces, especially in Manitoba, fall application of nitrogen (N) as anhydrous ammonia (AA, 82-0-0) is a common practice due to reduced fall workloads, lower fertilizer cost, increased seedbed preservation, and generally drier soil conditions for field activities, compared to spring (Tiessen et al., 2005; Tenuta et al., 2016). A survey conducted by Amiro et al. (2017) in Manitoba showed that two-thirds of the respondents (growers and agronomists) prefer fall N application. Machado et al. (2020) reported fall AA banding as 2nd most common practice among the growers of Western Canada, analyzing the dataset of the Fertilizer Use Survey conducted between 2014 and 2019 across Canada. Due to fall AA application's popularity and potential benefits, research has investigated the processes responsible for soil N transformations and losses from fall AA applications (Tiessen et al., 2006; Hatfield et al., 2014; Tenuta et al., 2016). However, the efficiency of fall-applied AA is still a big concern owing to nitrification and subsequent losses of N which influence the farm profitability and overall nitrogen use efficiency (NUE; Sun et al., 2016).

It is recommended to apply AA in late fall as soil temperatures drop to 10°C, with a mid-November deadline in Manitoba, while avoiding poorly or excessively drained soils (Tenuta et al., 2016; MB Soil Fertility, 2023). Undoubtedly, in temperate regions, soil freezing has a repressive effect on microbial activity and overwinter N transformation rates. However, fall AA is still considered prone to different microbial transformations (Kyveryga et al., 2004; Chantigny et al., 2019). Different mechanisms drive N losses depending on the soil and environmental factors (Dunmola et al., 2010). Ammonia volatilization can be a major contributor to N losses, mainly in alkaline soils (Silva et al., 2017), especially when AA is shallow banded and in coarse-textured soils that do not provide a good seal (Rochette et al., 2013; Lasisi et al., 2020). Overall, nitrification and subsequent denitrification can significantly contribute to high risks of over-winter and early-spring N losses in the form of nitrate (NO_3^-) leaching and nitrous oxide (N_2O), and dinitrogen gas (N_2) emissions (Malhi et al., 2001; Risk et al., 2014; Reynolds et al., 2016; Smith et al., 2019).

Nitrate, being six times more mobile than ammonium (NH_4^+) is more prone to leaching losses, especially in coarse-textured soils, where seasonal rainfall tends to be higher (Jayasundara et al., 2007; NikièmaPaligwende et al., 2013). While NH_4^+ does not readily leach and is typically adsorbed to the surface of negatively charged soil or organic matter particles, where it remains available for plant uptake and is less susceptible to losses compared to NO_3^- (Huber et al., 1980; Di and Cameron, 2016). Consequently, to minimize N losses from fall applications, it is essential to retain N in the NH_4^+ form in the soil from winter through late spring (Huber et al., 1980; Subbarao et al., 2006; Dromantienė et al., 2020). Among the different recommended options for stabilizing fall AA, the use of nitrification inhibitors (NIs) offers great potential by delaying nitrification, reducing N losses, and improving crop productivity, especially in soils with higher risks of N losses via leaching and denitrification (Abalos et al., 2014; Cahalan et al., 2015; Degenhardt et al., 2016; Ren et al., 2021).

Extensive research has investigated the impact of using nitrapyrin with fall-applied AA to reduce N losses and boost crop yields, particularly in the Midwest United States and some Canadian prairie provinces (Burzaco et al., 2014; Degenhardt et al., 2016; Habibullah et al., 2018). Studies have reported reduced nitrification by applying nitrapyrin with fall AA (Touchton et al., 1978; Liu et al., 1984; Gomes and Loynachan, 1984; Randall and Vetsch, 2003; Griesheim et al., 2019). Studies in Illinois and south-central Wisconsin have observed partial nitrification control extending into early and late spring with nitrapyrin-treated fall AA (Hughes and Welch, 1970; Hendrickson et al., 1978). Furthermore, nitrapyrin has been observed to effectively delay nitrification, particularly in irrigated sand and poorly drained heavy clays, compared to well-drained fine-textured soils (Hergert and Wiese, 1980; Touchton and Boswell, 1983). Some studies suggest that the effectiveness of nitrapyrin may vary depending on the site-specific conditions (Touchton et al., 1978; Hergert and Wiese, 1980).

Despite nitrapyrin's documented ability to inhibit nitrification, inconsistent responses in crop yields have been observed (Touchton and Boswell, 1983; Blackmer and Sanchez, 1988; Bailey, 1990). While some studies reported increased crop yields and N uptake with nitrapyrin-treated fall AA (Swezey and Turner, 1962; Hanson et al., 1987; Stehouwer and Johnson, 1990; Randall and Vetsch, 2003; Randall and Vetsch, 2005), others observed minimal impact (Touchton et al., 1979; Huber et al., 1980; Wolt, 2004; Hu et al., 2014), suggesting varying conditions and excess N fertilizer application as factors influencing nitrapyrin's effect on crop yield. Moreover, they speculated that a higher N fertilizer application rate might explain their experiments' lack of positive responses. In eastern North Dakota, significantly increased yield, and N uptake efficiency were observed for spring wheat with nitrapyrin-treated fall AA. However, extreme winter conditions during the experiment year may have influenced these results (Goos and Johnson, 1999). Limited research in Manitoba has evaluated the beneficial impact of nitrapyrin in delaying nitrification and reducing N_2O emissions with late fall-applied AA (Wood, 2018). However, these small-plot studies encountered challenges in accurately applying low nitrapyrin rates to replicate farm-scale recommendations, potentially influencing outcomes due to difficulties in metering small application rates. To date, no field-scale investigations using farmers' equipment have been conducted.

The main problem associated with nitrapyrin is that its carrier is a mix of benzene, xylene, and cumene, which makes the product (N-Serve) corrosive to equipment, and it cannot be mixed and stored in AA tanks for over three weeks. Many farmers use the sidekick unit to meter nitrapyrin into the AA stream as it leaves the tank, which enables variable rate application to high-risk areas. The corrosion of tanks by nitrapyrin is often quoted as a marketing slogan for alternative products. Therefore, there has been interest in using pronitridine (Centuro) a novel NI, recently introduced by Koch Fertilizers Inc. in 2018. Pronitridine offers advantages over nitrapyrin, being non-corrosive to metals, and easily storable without compromising effectiveness (Habibullah et al., 2018; Sfiligoj, 2019). While preliminary research in Missouri indicates the potential benefits of pronitridine in corn and wheat production (Habibullah et al., 2018; Nelson, 2018; Singh and Nelson, 2019), its efficacy remains untested in western Canadian climatic conditions, particularly in Manitoba, where late fall AA application is common. Given the tendency for soil to cool rapidly after late fall AA application by mid-October to early November, examining the effectiveness of nitrapyrin and pronitridine after prolonged frozen conditions is crucial.

Therefore, this field research aimed to quantify and compare the potential of nitrapyrin and pronitridine in delaying the nitrification of late fall-applied AA until spring. Additionally, the goal was to determine whether using NIs can eliminate the need for extra N application, thereby maintaining optimal yields and N-uptake from spring-sown crops. We hypothesized that using nitrapyrin or pronitridine would delay nitrification, retain more NH_4^+ within the AA bands, reduce NO_3^- accumulation on bands, and delay its subsequent movement from one to between bands. This delay in nitrification until planting could improve mineral N availability, leading to increased crop yield and N uptake compared to AA applied without NIs, potentially reducing the need for extra N rates during fall application.

2.3 Materials and Methods

2.3.1 Experimental Sites Description

Three commercial scale field experiments were conducted during 2020-22 in southern Manitoba at the three different sites of Sperling (NE-4-6-2W1), Notre Dame (NW-30-6-9W1), and Manitou (NE-10-4-9W1). The soil was classified as poorly-drained clayey lacustrine (Osborne) for Sperling, moderately well-drained Orthic Dark Gray Chernozem (Firdale) for Notre Dame, and loamy till well-drained Orthic Black Chernozem (Manitou) for Manitou in the Canadian Soil Classification System. Before establishing the study, soil from all three sites was sampled (0-30 cm deep) and sent to Farmers Edge Lab for analysis. Soils were analyzed for physicochemical properties including pH, electrical conductivity (EC), NO_3^--N, Olsen-P, potassium (K), sulphate (SO_4^{-2}-S), total organic carbon (TOC), and texture (Table 2.1).

2.3.2 Experimental Layout

The study at the Sperling site was initiated in the fall of 2020, while at Notre Dame and Manitou in the following year of 2021. The treatment setup at each site followed a randomized complete block design comprising four replications. The treatments included control (no fertilizer), 80% of the recommended N (based on soil test and target yield) applied as AA with and without nitrapyrin and pronitridine, and 100% of recommended N without any NI. Inhibitors, nitrapyrin, and pronitridine were applied as N-Serve (Corteva Agriscience) and Centuro (Koch Agronomic Services, LLC.) at the rate of 2.35 L ha^{-1} and 21 L 1,000 kg^{-1} of AA, respectively. Anhydrous ammonia was banded (15 cm deep) during late fall using farmers' AA applicator in the fields of Sperling, Notre Dame, and Manitou on Oct 18, 2020, Oct 25, and 30, 2021, respectively, following the recommendation of soil temperature below 10°C. The AA bands were marked with flags. The spacing between the injection ports of the AA applicators was 30 cm for the Sperling and Notre Dame sites and 38 cm for the applicator used at the Manitou site. For phosphate and sulphur application, S15 MicroEssentials® (13-33-0 15S) was seed-banded at a variable rate according to the map recommendation at the Manitou site. However, we do not have exact information for the other two sites. All the field operations were performed using commercial equipment in cooperation with growers. Treatment strips covered 0.35 ha at Sperling and Notre Dame, and 0.81 ha at Manitou. Smaller control strips, without AA application, minimized economic impact.

Table 2.1 Average physico-chemical properties of soil (0-30 cm) at the study sites.

Site	**pH (H_2O)**	**EC**	**NO_3^--N**	**Olsen-P**	**K**	**SO_4^{-2}-S**	**CEC**	**SOM[a]**	**Clay**	**Sand**	**Silt**
		dS m^{-1}	——mg kg^{-1}——				Cmol$^+$ kg^{-1}	——g kg^{-1}——			
Sperling (2020-21)	8.1	1.3	12.9	7.1	317.5	451.7	50.9	40	695	95	210
Notre Dame (2021-22)	6.4	0.2	7.9	29.0	265.0	11.8	23.7	45	220	300	480
Manitou (2021-22)	6.2	0.3	19.3	18.3	230.0	14.4	24.9	73	260	315	425

[a] SOM, soil organic matter

2.3.3 Soils Sampling and Preparation for Extractable Nitrogen

After the late fall application of AA, to get a clear picture of the delay in nitrification by the NIs, soil from all three sites was sampled (0-30 cm) on and between the AA banded rows. Truck-mounted hydraulic Giddings were used at the Sperling site, while the soil was sampled manually using soil probes at the other two sites of Notre Dame and Manitou. For the on-band sampling, the soil was sampled right at the flagged marks for the AA bands, and to sample the soil between the bands, half of the AA applicators' injection ports spacing was used: 15 cm away from the bands at Sperling and Notre Dame, and 19 cm away from the bands at Manitou. Each composite sample combined 12 cores (diameter 2.5 cm). Soil from Sperling was sampled for the late fall of 2020 and early and late spring of 2021, while Notre Dame and Manitou sites were sampled during the 2021-22 season at the same intervals (exact sampling dates provided in Table 2.2). All the soil samples from the fields were brought to the Soil Science Shed building and were stored deep-frozen at -20°C to avoid microbial activity and N transformations until further processing.

For soil preparation, soil samples were taken out from the freezer and were kept at 4°C overnight to de-freeze and then the soil was mixed and chopped using a knife into fine-sized particles for extractions and further analyses. Soil gravimetric moisture content (GMC) was determined by oven drying 15 g of fresh soil samples for 24 hours at 105°C. After oven drying, samples were allowed to cool and reweighed to determine moisture loss, and GMC was determined using the difference methods for fresh and oven-dried samples as follows:

$$\mathrm{GWC} = \frac{[(\text{tin weight } + \text{ fresh soil weight}) - (\text{tin weight } + \text{ dry soil weight})]}{(\text{dry soil weight})}$$

For extractable NH_4^+ and NO_3^- analysis from the soil, 5 g of moist soil for each sample was weighed and transferred to a 50 mL centrifuge tube. Following a 1:5 extraction ratio, 25 mL of 2 M potassium chloride (KCl) solution was added using a dispenser pump. Then the tubes were placed onto a reciprocating shaker set at 150 rpm for 60 minutes. After shaking, tubes were centrifuged in an IEC Centrifuge at 3000 rpm for 1 minute and 30 seconds, and 15 mL of the clear supernatant from the centrifuge tubes was pipetted out and transferred to marked scintillation vials using an autopipette. Extractable concentrations of NH_4^+ and NO_3^- were determined using a segmented flow analyzer (Technicon AAII).

2.3.4 Weather Data and Agronomic Management

Average monthly air temperature and precipitation data were obtained from the Manitoba Agrometeorology Department on request for the three weather stations installed approximately within twenty km of each field site (Environment Canada, 2024). For the Sperling site, data from Brunkild; for the Notre Dame site, data from Treherne; and for the Manitou site, data from the Manitou weather station was used. While these stations were recently installed by the Manitoba Agrometeorology Department, long-term annual mean air temperature and precipitation data from 1981 to 2010 were obtained for the Carman station, which is nearest to all three field sites. Detailed agronomic site-specific management, including field locations, respective soil series, soil classes, AA application dates, full and reduced N rates, soil temperature at the time of N application, and sampling dates, are given in Table 2.2.

Table 2.2 Information on nitrogen application, soil types, and soil sampling events for the study.

Site	**Treatment strips (m x m)**	**Field Location**	**Soil Series**	**Soil Classification**	**Application Date**	**Full N (kg ha^{-1})**	**Reduced N (kg ha^{-1})**	**Soil Temp °C (5 cm) at application**	**Sampling dates**		
									1st	**2nd**	**3rd**
Sperling (2020-21)	13 x 274	NE-4-6-2W1	Osborne (80%)-Scanterbury (20%)[a]	Clayey Lacustrine Black-Chernozem	18-Oct-20	157	123	8	Nov-07-20	Apr-27-21	May-12-21
Notre Dame (2021-22)	13 x 274	NE-30-6-9W1	Firdale (100%)[a]	Loamy Lacustrine Dark Gray-Chernozem	25-Oct-21	135	108	6	Nov-03-21	May-18-22	Jun-02-22
Manitou (2021-22)	18 x 450	NE-10-4-9W1	Manitou (100%)[a]	Loamy Till Black-Chernozem	30-Oct-21	146	117	6	Nov-09-21	May-19-22	Jun-03-22

[a] Percentage values indicate the land area occupied by different soil series at each site.

2.3.5 Agronomic Yield

The harvest was done using commercial combine harvesters for the wheat and canola at Notre Dame and Manitou, respectively. Yield data were obtained by combining 0.32 ha of each treatment strip for wheat and 0.45 ha for canola. For wheat yield, data were obtained by weighing grains using a grain cart with a fitted weighing scale. The canola yield numbers for the treatment strips were obtained using a weigh wagon after combining two replicates. Control plots for the canola were randomly sampled with manual harvest using sickles, and yield was averaged for the four replicates. At the Manitou site, we encountered several challenges in collecting yield data. The control plots were too small for combining, and two of the four replicates were accidentally harvested by the farmer's assistant a day earlier than scheduled while squaring off the edges.

2.3.6 Total Nitrogen Uptake

Biomass samples for N uptake for wheat and canola crops were collected by manually harvesting using sickles. Wheat biomass was sampled at the late milk to ripening stage, while canola biomass was sampled at the medium to end of pod formation. The hand harvest involved selecting five distinct samples per plot by randomly choosing 2 m from five separate rows within each treatment strip, covering an area of 2.5 m^2 for wheat and 2.3 m^2 for canola. The above-ground biomass samples were dried for over 72 hours at 35°C. The biomass samples were weighed for dry weight (g) after drying. For wheat, the grains were threshed using a WINTERSTEIGER Classic combine (WINTERSTEIGER, Ried im Innkreis, Austria), and the threshed grains were weighed. Meanwhile, the canola biomass samples were chipped. Subsamples of wheat straw and grains, as well as canola chipped biomass, were ground to pass through a 2 mm sieve (Thomas Wiley Laboratory Mill Model 4 Grinder, Thomas Scientific, Swedesboro, NJ, US) and analyzed for %

total N using the modified Dumas method of combustion (Muñoz-Huerta et al., 2013). Total N uptake (kg N ha^{-1}) was calculated as the product of biomass and the %N concentration in the whole plant (straw + grains), averaging the values from the five samples for one replicate.

2.3.7 Statistical Analysis

Analysis of variance (ANOVA) was conducted using the PROC GLIMMIX in SAS 9.4 (SAS Institute, 2013) to determine the effects of different treatments within each sampling time on soil extractable N (NH_4^+, NO_3^-, and Total N) both on and between the AA bands. The treatment effects on agronomic yield and plant N uptake were also determined. A lognormal distribution was used for all parameters not conforming to a normal distribution. The model statement modelled the treatment as a fixed effect, while the block was treated as a random effect. Heterogeneous variance was addressed through adjustments made using the Satterthwaite method. Treatment means were compared using the Tukey multiple comparison procedure with a significance level set at $\alpha = 0.05$.

2.4 Results

2.4.1 Weather Conditions

The soil temperature at a depth of 5 cm reached 0°C by December 24th, 16th, and 6th at Sperling, Notre Dame, and Manitou, respectively, corresponding to 66, 51, and 36 days after the AA application at these sites. While the average monthly air temperature did not vary much between the sites in 2022, notable differences were observed compared to 2021 (Table 2.3). Specifically, in 2021, at Sperling, the air temperature was 5°C warmer in December, January, and March, and 3°C warmer in June and July, but 3°C cooler in October and 5°C cooler in February than the 30-year normal air temperature. Comparing the average monthly air temperature of 2022 at Notre Dame and Manitou with 30-year normal values, most months were comparable, except for October, February, and April. At both sites, October 2022 was 3°C warmer, February 2022 was 6°C warmer, and April 2022 was 6°C cooler than the 30-year normal monthly average air temperature (Table 2.3).

At the Sperling site, spring 2021 was notably dry, with available moisture mainly attributed to snowmelt. However, spring 2022 presented a stark difference with sticky-wet soil conditions due to the March snowmelt and heavy rainfall in April and May. Continuous rain in April 2022 delayed the early spring sampling to mid-May at both Notre Dame and Manitou sites (Table 2.3). There was a significant variation in cumulative monthly precipitation between 2021 and 2022. Notable differences were also observed between the 2022 sites, Notre Dame, and Manitou. In 2021, Sperling received the least amount of rainfall, with the summer being counted among the driest in MB's history. When compared to the 30-year average precipitation, Sperling had a particularly dry fall in 2020, receiving 50% less rain in September and October. From March through late summer in July of 2021, the precipitation levels did not exceed 25% of the 30-year average. In contrast, in 2022, Notre Dame and Manitou received substantially more rainfall in April, May, and July, with precipitation levels 60-70% higher than the 30-year average. In terms of total precipitation from March to August, Sperling received 165 mm in 2021. Meanwhile, Notre Dame and Manitou recorded 415 mm and 410 mm, respectively, in 2022, compared to the 30-year average of 340 mm.

Table 2.3 Average monthly air temperature and precipitation for Manitoba Agrometeorology stations near three field sites (2020-2022). The long-term (1981-2010) normal for the stations is also given.

	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Average Air Temperature (°C)												
Sperling (2020-21)	12.2	1.9	-3.6	-8.0	-10.4	-17.5	-0.6	3.1	10.9	19.8	21.8	18.7
Notre Dame (2021-22)	15.6	8.0	-2.7	-13.3	-17.3	-18.4	-6.9	-1.6	10.3	17.0	19.3	18.6
Manitou (2021-22)	15.3	7.7	-2.9	-13.7	-17.5	-18.6	-7.3	-1.8	10.3	16.9	19.2	18.7
Normal (1981-2010)[a]	12.4	4.9	-4.6	-13.2	-15.9	-12.6	-5.7	4.0	10.9	16.3	18.8	18.0
Total Precipitation (mm)												
Sperling (2020-21)	13.0	19.3	2.1	12.4	0.9	0.9	4.1	3.4	23.8	47.7	10.9	73.7
Notre Dame (2021-22)	36.1	80.7	14.0	7.5	9.1	11.1	5.9	85.8	113.2	49.2	123.1	33.8
Manitou (2021-22)	32.9	80.3	24.3	17.1	16.4	31.7	12.0	103.5	95.9	55.0	112.5	27.8
Normal (1981-2010)[a]	47.0	42.0	28.1	25.5	21.9	18.4	25.7	28.1	63.6	88.2	68.0	65.0

[a] Long-term annual data from 1981-2010 were sourced from the Carman weather station, which is the closest to the respective sites.

2.4.2 Inorganic Soil Nitrogen Dynamics at Each Site

2.4.2.1 Sperling (2020-2021)

The applied AA predominantly existed in the NH_4^+ form during the late fall sampling, with subsequent NH_4^+ concentration decline, and the progressive accumulation of soil NO_3^- on and between the AA bands in the early and late spring samplings across all three sites. At Sperling, the NH_4^+ recovery across all three samplings, showed no significant ($p < 0.05$) differences among AA treatments (Fig. 2.1). Nonetheless, slight differences were observed in the late fall and early spring samplings, with pronitridine-treated AA tended to show slightly higher NH_4^+ retention (58.4 and 28.9 mg kg^{-1}) compared to AA applied without NIs (50.1 and 26.8 mg kg^{-1}). In late spring, both nitrapyrin and pronitridine-treated AA treatments tended to retain more NH_4^+ (9.2 and 8.7 mg kg^{-1}) compared to AA without NIs (2.9 mg kg^{-1}). Meanwhile, NH_4^+ retention in the AA was applied at 100% of the recommended N rate, slightly higher than that of NI treatments (11.7 mg kg^{-1}). However, NH_4^+ retention by the treatments receiving AA either with or without NIs remained significantly ($p < 0.05$) higher than controls throughout all samplings, except for the late spring sampling, where the NH_4^+ retention by the AA applied at 80% of the recommended N rate with and without pronitridine did not differ significantly ($p < 0.05$) from the control (Fig. 2.1). Most of the applied AA was recovered as NH_4^+ or NO_3^- in AA-banded rows or as NO_3^- between bands in early and late spring. The NH_4^+ concentration between bands was minimal, with no consistent trends or significant differences, except for the tendency by nitrapyrin-treated AA in late fall sampling at the Sperling (7.3 mg kg^{-1}).

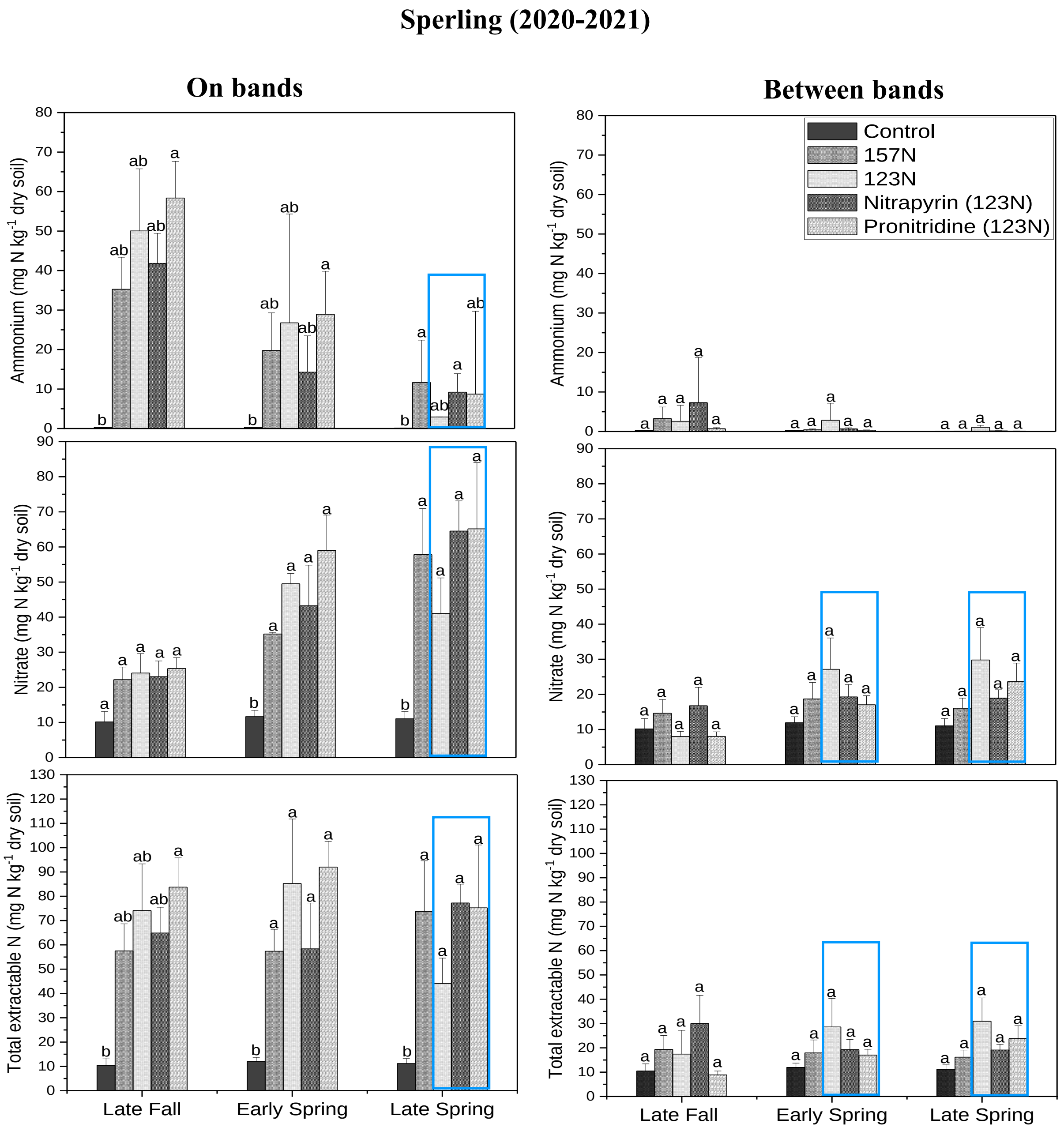


Figure 2.1 Effect of nitrification inhibitors on soil (0-30 cm) ammonium (NH_4^+), nitrate (NO_3^-), and total extractable nitrogen ($NH_4^+ + NO_3^-$) concentrations in the anhydrous ammonia (AA) banded and between the banded rows (15 cm away from the bands) on different sampling times at Sperling (2020-2021). According to Tukey's multiple comparison procedure, means with different letters within a sampling time are significantly different ($p < 0.05$). Error bars indicate standard errors of the means (n=4). Control: Without nitrogen and nitrification inhibitor; 157N: 157 kg N ha^{-1} added as AA; 123N: 123 kg N ha^{-1} added as AA; Nitrapyrin (123N): 123 kg N ha^{-1} added as AA treated with N-Serve; Pronitridine (123N): 123 kg N ha^{-1} added as AA treated with Centuro. (Late Fall = Nov-07, Early Spring = Apr 27, Late Spring = May 12). Blue annotations indicate a tendency of slightly higher NH_4^+, NO_3^-, and total extractable N recovery on bands and slightly lower NO_3^- and total extractable N between bands, with NIs at different samplings.

At Sperling, NO_3^- accumulation on and between the bands did not differ significantly ($p < 0.05$) between the AA treatments throughout all samplings, except for the NO_3^- on bands from the controls in early and late spring (Fig. 2.1). However, some interesting trends were observed in the early and late spring samplings, with NO_3^- tended to be recovered more on bands with pronitridine-treated AA in early and late spring (59.1 and 65.2 mg kg^{-1}), and with nitrapyrin-treated AA in late spring (65.1 mg kg^{-1}), compared to AA applied at 80% of the recommended N (41.1 mg kg^{-1}) and 100% of the recommended N without NIs (57.8 mg kg^{-1}). In early and late spring, at Sperling, the NO_3^- accumulation between the bands for AA treated with nitrapyrin (19.3 and 18.9 mg kg^{-1}) and pronitridine (17.1 and 23.7 mg kg^{-1}) was slightly lower than for the AA applied without NIs (27.2 and 29.8 mg kg^{-1}). The NO_3^- accumulation between bands for the AA applied at 100% of the recommended N without NIs was equivalent to nitrapyrin-treated AA in early and late spring but was slightly lower than AA at 80% of the recommended N without NIs, and pronitridine-treated AA in late spring. Despite being consistently higher for all AA treatments than controls, NO_3^- accumulation between bands in early and late spring samplings was not significant ($p < 0.05$) (Fig. 2.1).

For the on bands' total extractable N, trends aligned with NH_4^+ in late fall sampling and NO_3^- concentration on bands in early and late spring, with total extractable N between bands mainly due to NO_3^- movement. In late spring, nitrapyrin and pronitridine-treated AA and AA applied at 100% of the recommended N rate tended to show slightly higher extractable N on bands than at 80% of the recommended N without NIs. The AA applied at 80% of the recommended N without NIs, tended to show slightly higher total extractable N between bands compared to AA applied with NIs, and without NIs at 100% of the recommended rate of N (Fig. 2.1).

2.4.2.2 Notre Dame (2021-2022)

Like Sperling, at Notre Dame, no significant delay in nitrification was observed, as NH_4^+ recovery on bands did not differ significantly ($p < 0.05$) among various AA treatments on all samplings. However, in late spring, nitrapyrin-treated AA tended to show slightly higher NH_4^+ retention (11.4 mg kg^{-1}) compared to AA without NIs (5.5 mg kg^{-1}). In comparison, this tendency by nitrapyrin was lower than NH_4^+ retention by AA applied at 100% of the recommended rate of N (13.4 mg kg^{-1}). Pronitridine was not observed to have any impact on NH_4^+ retention compared to AA without NIs (Fig. 2.2). However, NH_4^+ retention for the treatments receiving AA either with or without NIs remained significantly ($p < 0.05$) higher than the controls, throughout all samplings, except for the late spring sampling, where the NH_4^+ retention by the AA applied at 80% of the recommended N rate with and without pronitridine did not differ significantly ($p < 0.05$) from the control. There were no consistent trends or significant differences in NH_4^+ concentration between bands. However, nitrapyrin-treated AA showed a slightly greater tendency of AA leakage as NH_4^+ concentration between bands in late fall at Notre Dame (11.9 mg kg^{-1}).

As for NO_3^- accumulation on bands, there was no difference between N treatments and the control in late fall. However, some significant differences emerged during early and late spring. In early spring, nitrapyrin and pronitridine-treated AA tended to show slightly lower NO_3- accumulation on bands (14.4 mg kg^{-1} and 17.8 mg kg^{-1}, respectively) compared to AA applied at 80% of the recommended N rate without NIs (21.9 mg kg^{-1}). Furthermore, the NO_3^-accumulation on bands using both NIs was significantly ($p < 0.05$) lower than AA applied at 100% of the recommended N rate without NIs (26.4 mg kg^{-1}). Similar trends were observed in late spring sampling, with nitrapyrin and pronitridine-treated AA showing a tendency to reduce NO_3^- accumulation on bands (10.3 mg kg^{-1} and 13.2 mg kg^{-1}, respectively) compared to AA applied at

80% (14.9 mg kg^{-1}) and 100% of the recommended rates of N (20.3 mg kg^{-1}) without NIs. Overall, in early and late spring samplings, NO_3^- accumulation on bands for the treatments receiving AA, either with or without NIs, was significantly ($p < 0.05$) higher than controls, except for the late spring sampling, where NO_3^- accumulation by nitrapyrin and pronitridine-treated AA did not differ significantly ($p < 0.05$) from control (Fig. 2.2).

The disappearance of NO_3^- from AA bands increased over time for the AA treatments; however, no significant accumulation of NO_3^- between the bands was observed compared to the controls (Fig. 2.1). In late spring, NO_3^- accumulation between the bands for AA applied at the 100% of the recommended N rate (12.3 mg kg^{-1}) was slightly greater than for all other AA treatments (7.7 mg kg^{-1}). Total extractable N remained significantly ($p < 0.05$) higher on bands for AA treatments than controls, with an overall decreasing trend from late fall to late spring. However, no significant shift of total extractable N was observed on and between the bands throughout the samplings (Fig. 2.2).

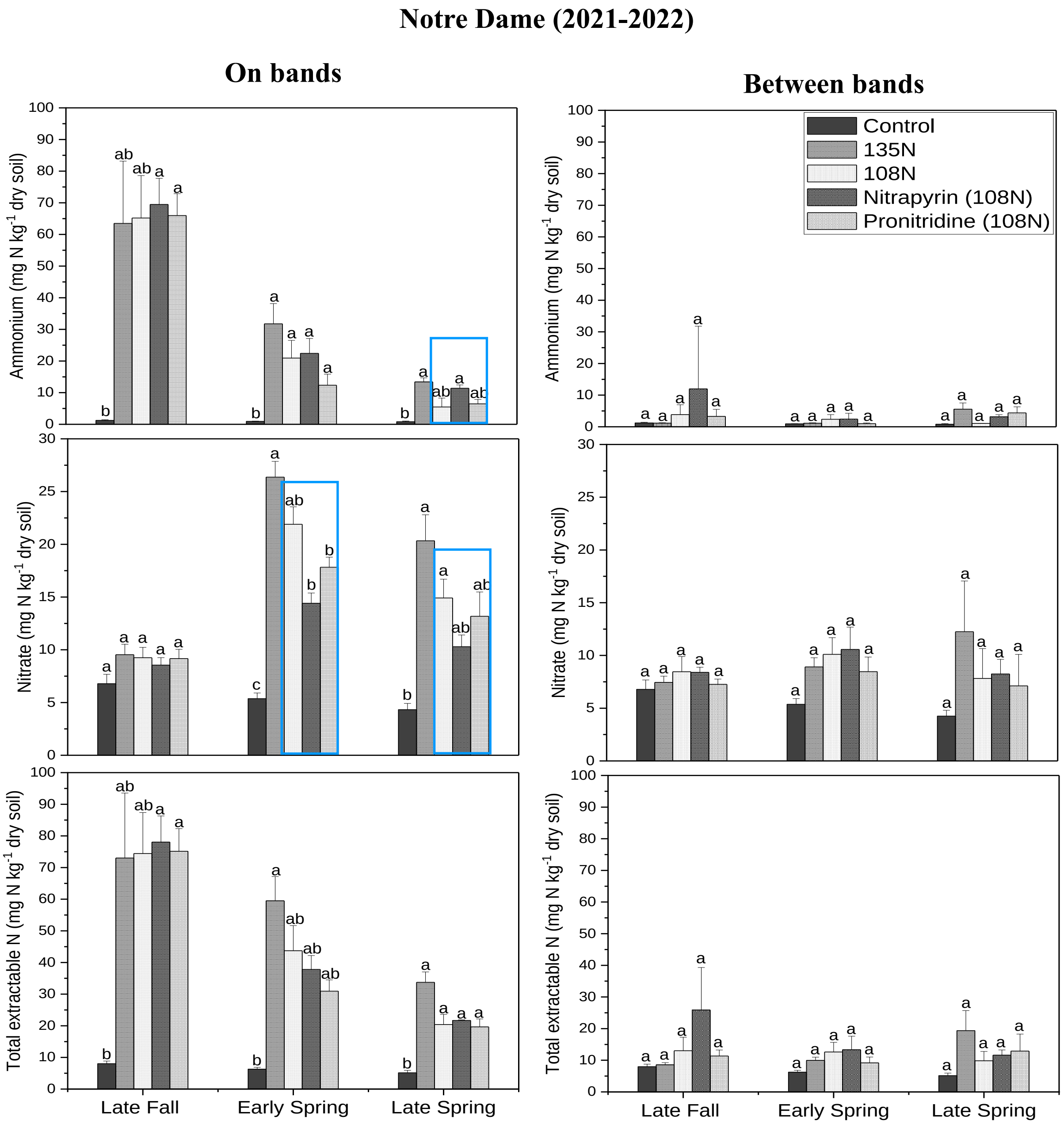


Figure 2.2 Effect of nitrification inhibitors on soil (0-30 cm) ammonium (NH_4^+), nitrate (NO_3^-), and total extractable nitrogen (NH_4^+ + NO_3^-) concentrations in the anhydrous ammonia (AA) banded and between the banded rows (15 cm away from the bands) on different sampling times at Notre Dame (2021-2022). According to Tukey's multiple comparison procedure, means with different letters within a sampling time are significantly different ($p < 0.05$). Error bars indicate standard errors of the means (n=4). Error bars indicate standard errors of the means (n=4). Control: Without nitrogen and nitrification inhibitor; 135N: 135 kg N ha^{-1} added as AA; 108N: 108 kg N ha^{-1} added as AA; Nitrapyrin (108N): 108 kg N ha^{-1} added as AA treated with N-Serve; Pronitridine (108N): 108 kg N ha^{-1} added as AA treated with Centuro. (Late Fall = Nov-03, Early Spring = May 18, Late Spring = Jun 02). Blue annotations indicate a tendency for slightly higher NH_4^+ and lower NO_3^- recovery on bands, with NIs at different samplings.

2.4.2.3 Manitou (2021-2022)

Like the other two sites, no significant delay in nitrification was observed at Manitou, as NH_4^+ recovery on bands did not differ significantly ($p < 0.05$) among all N treatments throughout all samplings. However, some notable differences were observed in late fall and late spring samplings. In late fall, nitrapyrin- and pronitridine-treated AA tended to show lower NH_4^+ recovery compared to AA applied at 100% and 80% of recommended N rates without NIs (Fig. 2.3). NH_4^+ recovery for AA treatments at the 100% and 80% of the recommended N without NIs (204.4 and 188.8 mg kg^{-1}, respectively) tended to show exceeding the targeted N application rates (146 and 117 kg N ha^{-1}, respectively). Meanwhile, in late spring, nitrapyrin-treated AA tended to show higher NH_4^+ retention (63.7 mg kg^{-1}) than all other AA treatments (24.8 mg kg^{-1}). Overall, all treatments that received AA either with or without NIs, retained significantly ($p < 0.05$) higher NH_4^+ on bands compared to the controls in late fall and early spring. In contrast, in late spring sampling, only nitrapyrin-treated AA retained significantly ($p < 0.05$) higher NH_4^+ than control. No consistent or significant trends were observed for NH_4^+ concentration between bands.

Regarding NO_3^- accumulation on bands, no significant differences among the AA treatments were observed, while some interesting trends emerged within each sampling frame. In late fall, NO_3^- accumulation on bands for pronitridine-treated AA (7.5 mg kg^{-1}) was significantly ($p < 0.05$) lower than the control (15.5 mg kg^{-1}). In early spring sampling, nitrapyrin and pronitridine-treated AA tended to show slightly reduced NO_3^- accumulation on bands (19.6 mg kg^{-1} and 20.2 mg kg^{-1}, respectively) compared to AA applied at 80% and 100% of the recommended rate of N (29.8 mg kg^{-1} and 30.1 mg kg^{-1}, respectively). Similar trends were observed in late spring sampling, while the reduction in NO_3^- accumulation on bands with pronitridine-treated AA was minimal (33.2 mg kg^{-1}). However, in late spring sampling, nitrapyrin tended to show slightly reduced NO_3^- accumulation on bands (24.7 mg kg^{-1}) compared to AA applied at 80% and 100% of the recommended N without NIs (37.9 mg kg^{-1} and 43.3 mg kg^{-1}, respectively). Overall, NO_3^- accumulation on bands for the treatments receiving AA was significantly ($p < 0.05$) greater than control in late spring sampling. While in early spring sampling, the NO_3^- accumulation in control (13.3 mg kg^{-1}) was slightly lower than in NIs treatments (20.2 mg kg^{-1}). However, it was significantly ($p < 0.05$) lower than AA applied at 100% of the recommended rate of N without NIs (30.1 mg kg^{-1}).

The movement of NO_3^- accumulation between bands for the AA treatments, compared to the control, was minimal in late fall and early spring. However, in late spring, nitrapyrin- and pronitridine-treated AA tended to show slightly reduced NO_3^- accumulation between the bands (8.5 mg kg^{-1} and 11.1 mg kg^{-1}, respectively) compared to AA applied at 80% and 100% of the recommended N rate without NIs (22.4 mg kg^{-1} and 20.6 mg kg^{-1}, respectively) (Fig. 2.3). Total extractable N levels for AA treatments remained significantly ($p < 0.05$) higher on bands compared to controls throughout the samplings, with an overall decreasing trend from late fall to late spring. However, between the bands, no significant shift of total extractable N was observed for the AA treatments compared to controls throughout the samplings. In late spring, nitrapyrin- and pronitridine-treated AA tended to reduce total soil inorganic N compared to AA applied without NIs (Fig. 2.3).

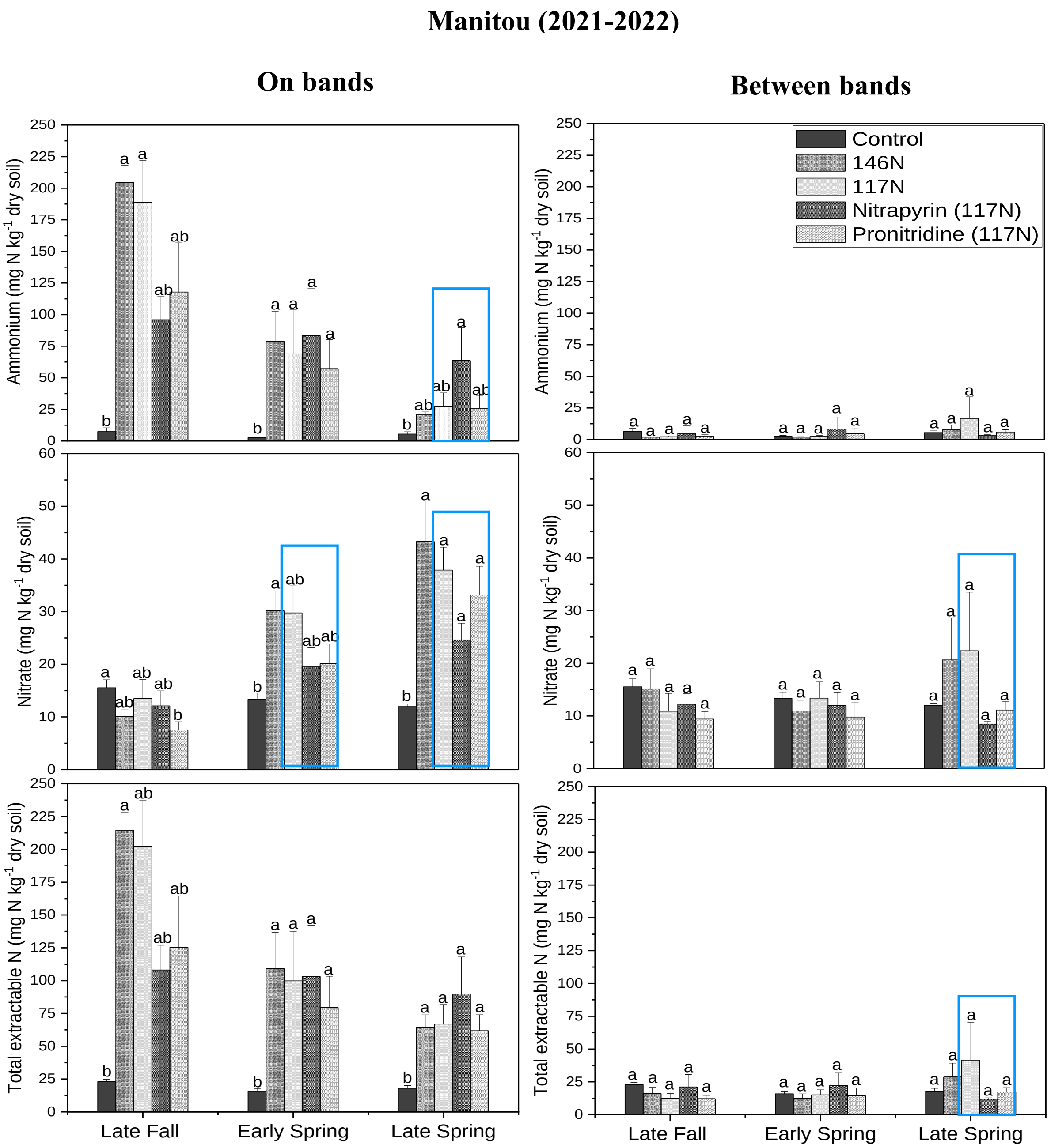


Figure 2.3 Effect of nitrification inhibitors (nitrapyrin and pronitridine) on soil (0-30 cm) ammonium (NH_4^+), nitrate (NO_3^-), and total extractable nitrogen (NH_4^+ + NO_3^-) concentrations in the anhydrous ammonia (AA) banded and between the banded rows (19 cm away from the bands) on different sampling times at Manitou (2021-2022). According to Tukey's multiple comparison procedure, means with different letters within a sampling time are significantly different ($p < 0.05$). Error bars indicate standard errors of the means (n=4). Control: Without nitrogen and nitrification inhibitor; 146N: 146 kg N ha^{-1} added as AA; 117N: 117 kg N ha^{-1} added as AA; Nitrapyrin (117N): 117 kg N ha^{-1} added as AA treated with N-Serve; Pronitridine (117N): 117 kg N ha^{-1} added as AA treated with Centuro. (Late Fall = Nov-09, Early Spring = May 19, Late Spring = Jun 03). Blue annotations indicate slightly higher NH_4^+ on bands, slightly lower NO_3^- recovery on and between bands, and slightly lower total extractable N between bands, with NIs at different samplings.

2.4.3 Agronomic Yield

In the summer of 2021, Sperling experienced extreme yield loss in the canola crop due to drought conditions, leading to the crop remaining unharvested. The crop insurance yield estimate was even lower than 0.34 Mg ha^{-1}. Moving to 2022 at Notre Dame, the wheat grain yield from the control plots (3.86 Mg ha^{-1}) was significantly ($p < 0.005$) lower than all other treatments that received fall AA, with or without NIs (Fig. 2.4). Application of AA at 80% of the recommended N resulted in a non-significant decrease in wheat grain yield of 0.23 Mg ha^{-1}, compared to AA applied at 100% of the recommended N rate with yield of 5.99 Mg ha^{-1}. However, nitrapyrin- and pronitridine-treated AA tended to show slightly increased wheat grain yields of 0.10 and 0.08 Mg ha^{-1}, respectively, compared to AA applied at 80% of the recommended N rate without NIs. Notre Dame produced higher wheat yields than both the 5-year average wheat grain yield of 3.99 Mg ha^{-1} and the 2022 average wheat yield of 4.84 Mg ha^{-1} in the same region, as reported by the Manitoba Agricultural Services Corporation (MASC, 2024). At Manitou, we had only two replicates, compromising the statistical power. Overall, no notable differences in canola grain yield were observed among the different AA treatments. However, the canola yield from the control strips with no AA application was lower (1.39 Mg ha^{-1}) (Fig 2.4). Strips receiving nitrapyrin-treated AA had the highest canola grain yield of 2.8 Mg ha^{-1}. Manitou produced higher canola yields compared to the Manitoba 5-year average canola grain yield of 2.33 Mg ha^{-1}; however, it was comparable to the average canola yield in this region for 2022, which was 2.7 Mg ha^{-1}, as reported by the Manitoba Agricultural Services Corporation (MASC, 2024).

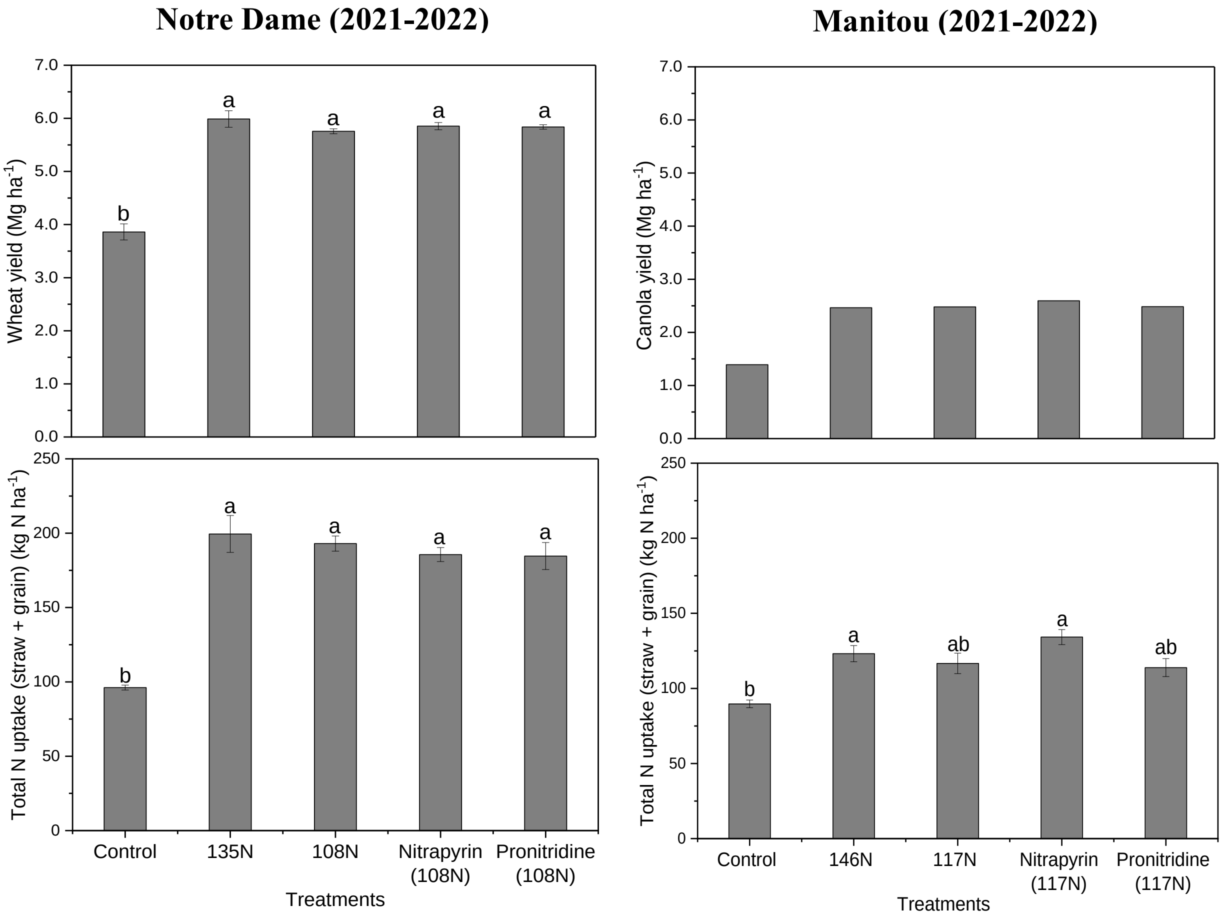


Figure 2.4 Effect of nitrification inhibitors on the average grain yield and total N uptake of wheat and canola at Notre Dame and Manitou, respectively. Means with different letters within each graph are significantly different ($p < 0.05$) according to Tukey's multiple comparison procedure. Error bars indicate standard errors of the means (n=4). For all measurements, the sample size (n=4) except for the nitrogen treatments in canola yield, where n=2. All plots were combined for wheat and canola yield, except control plots for canola, which were manually harvested before combining for yield estimation. Control: Without nitrogen and nitrification inhibitor; Control: Without nitrogen and nitrification inhibitor; 135N: 135 kg N ha^{-1} added as anhydrous ammonia (AA); 108N: 108 kg N ha^{-1} added as AA; Nitrapyrin (108N): 108 kg N ha^{-1} added as AA treated with N-Serve; Pronitridine (108N): 108 kg N ha^{-1} added as AA treated with Centuro. 146N: 146 kg N ha^{-1} added as AA; 117N: 117 kg N ha^{-1} added as AA; Nitrapyrin (117N): 117 kg N ha^{-1} added as AA treated with N-Serve; Pronitridine (117N): 117 kg N ha^{-1} added as AA treated with Centuro.

2.4.4 Total Nitrogen Uptake

At Notre Dame, similar to wheat grain yield, the aboveground N uptake by wheat plants (grain and straw) from control strips (96.18 kg N ha^{-1}) was significantly ($p < 0.05$) lower than from the strips that received AA either with or without NIs (Fig. 2.4). Total N uptake was highest for the wheat plants from the strips that received AA at 100% of the recommended N rate (199.47 kg N ha^{-1}), followed by a slight reduction in N uptake by wheat plants from the strips that received AA at 80% of the recommended N rate (193.01 kg N ha^{-1}) (Fig 2.4). However, nitrapyrin and pronitridine-treated AA did not impact the N uptake by wheat plants significantly ($p < 0.05$) compared to AA without NIs. Despite a tendency of a slight reduction in N uptake by wheat plants on using nitrapyrin- and pronitridine (185.62 and 184.63 kg N ha^{-1}, respectively) compared to AA applied without NIs.

At Manitou, N uptake by canola plants (grains + straw) from control strips (89.70 kg N ha^{-1}) was significantly ($p < 0.05$) lower than from the strips that received AA at 100% of recommended N rate (123.15 kg N ha^{-1}), and AA at 80% of the recommended N rate with nitrapyrin (134.19 kg N ha^{-1}) (Fig. 2.4). AA application at 80% of the recommended N rate tended to show a slight reduction in N uptake by canola plants (116.66 kg N ha^{-1}) compared to AA applied at 100% of the recommended rate of N. Nitrapyrin-treated AA tended to show a slight increase in N uptake by canola plants (134.19 kg N ha^{-1}) compared to AA applied at 80% and 100% of the recommended rate of N without NIs (123.15 kg N ha^{-1} and 116.66 kg N ha^{-1}). However, pronitridine-treated AA tended to show no impact on the total N uptake by canola plants compared to AA without NIs.

2.5 Discussion

2.5.1 Inorganic Soil Nitrogen

Higher NH_4^+ retention within the AA banded rows in the fall, with subsequent decline over time, and the progressive accumulation of soil NO_3^- on and between the AA bands with time across all three sites, is consistent with previous studies (Janke et al., 2020; Ahmed et al., 2023). This pattern is attributed to the nitrification activity of nitrifiers (Shi and Norton, 2000; Norton and Ouyang, 2019). In contrast to previous studies (Randall and Vetsch, 2005; Parkin and Hatfield, 2010; Degenhardt et al., 2016; Habibullah et al., 2018; Kaur et al., 2022), the NH_4^+ retention by NIs in our study was lesser than we expected, however nitrapyrin-treated AA tended to retain slightly higher NH_4^+ on bands in late spring sampling at Manitou, but not significant compared to all other AA treatments. The inconsistency and lack of significance in using NIs with fall AA with overall no impact on NH_4^+ retention. This could be due to the self-inhibitory effect of concentrated AA bands affecting soil microbial growth in the application zone, causing a transient delay in nitrification. Additionally, the low soil temperature (below 10°C) at the time of AA application can significantly suppress nitrification (Siripong et al., 2007; Rácz et al., 2022). Delayed banding operations of N fertilizers until the soil has cooled, which is considered best management practice, might have minimized the benefits of using NIs (Singh and Beauchamp, 1988; Norton and Ouyang, 2019).

Nitrate accumulation within the AA-banded rows, particularly during early and late spring, indicates nitrification activity (Angus et al., 2014). At Sperling, NO_3^- tended to be recovered more on bands coupled with a tendency for lower NO_3^- between bands with NIs at the late spring sampling. This suggests that AA applied without NIs may have been nitrified earlier, with NO_3^- subsequently moved towards between the bands due to moisture from freeze-thaw events, as spring

2021 was mostly dry. Reduced NO_3^- accumulation between the bands for nitrapyrin and pronitridine-treated AA suggests that delayed nitrification by NIs may have limited the diffusion of NO_3^- from bands by planting time (Degenhardt et al., 2016). However, the reduced NO_3^- accumulation between the bands for the AA applied at 100% of the recommended rate of N may be attributed to the concentrated bands' self-inhibition effect (Nyborg and Malhi, 1988; Wang et al., 1998). Conversely, in 2022, at both the Notre Dame and Manitou sites, both NIs, specifically nitrapyrin, tended to have lower NO_3^- levels on bands during the early and late spring samplings. However, NO_3^- concentration between bands tended to be lower for NIs, only in the late spring sampling at the Manitou site, indicating a potential reduction in NO_3^-diffusion from bands by planting. This could be due to the inhibition or delaying effect of NIs on nitrifiers' activity, as reported in previous studies (Abbasi et al., 2003; Giacometti et al., 2020). While at Notre Dame, the absence of notable variation in NO_3^- concentration between bands could be ascribed to variations in soil texture, influencing the lateral diffusivity of NO_3^- (Cameron et al., 2013). Overall, a reduction in total soil extractable N (NH_4^+ + NO_3^-) concentrations within the AA bands was observed across each site due to nitrification and the consequent movement of NO_3^- from on to between the bands. The apparent loss of over 60% of total extractable N from fall application to planting suggests reduced diffusion of NO_3^--N, potentially increasing mineral N availability closer to the injection bands. Future soil sampling should be conducted at a quarter distance from the AA injection bands to account for N movement.

2.5.2 Yield and Nitrogen Uptake

Nitrification inhibitors can outperform conventional N fertilizers in terms of yield increase, especially if soil conditions are conducive to high N losses via NO_3^--leaching or denitrification (Tiessen et al., 2008; Grant et al., 2016). However, the yield benefits of using NIs are minimal, especially if drought conditions persist, as they did in 2021 (Thapa et al., 2015). For instance, canola at the Sperling site suffered extreme yield loss due to insufficient moisture for plant N uptake from the soil through mass flow or diffusion (Matimati et al., 2014; Marschner et al., 2023).

The highest yields from both Notre Dame and Manitou sites, from fall-applied AA with and without NIs, confirm the high-yielding potential of 2022, which was characterized by frequent precipitation events throughout the growing season, favouring a consistent N supply (Gonzalez-Dugo et al., 2010; Grzebisz et al., 2013). The wheat and canola yields from both the Notre Dame and Manitou sites were 5.99 Mg ha^{-1} and 2.8 Mg ha^{-1}, respectively, which were higher than the average yields of 4.84 Mg ha^{-1} and 2.7 Mg ha^{-1} reported for wheat and canola in the same region in 2022 (MASC, 2024). Moreover, the statistical power in our field study at the Manitou site was compromised due to insufficient replicates.

At Notre Dame, the correlation between wheat yield and N uptake was inconsistent, where NIs tended to show a slight yield increment, coupled with a tendency to impact the N uptake by wheat plants negatively. This discrepancy could be attributed to the dilution effect stemming from crop yield, productivity, and the distribution of N within the crop (Lemaire et al., 2008). However, at Manitou, nitrapyrin-treated AA tended to show a more pronounced impact on N uptake by canola plants than all other AA treatments, while overall not significant. Our findings are consistent with those of Pittelkow et al. (2017) and Vetsch et al. (2019), who observed no increase in corn grain yield and N uptake using nitrapyrin with fall-AA in the Mid-west US. Similarly, Karamanos et al. (2014) found that fall AA, with or without nitrapyrin (N-Serve), did not affect wheat yield compared to non-stabilized AA.

The variability in crop yield response to the application of NIs can be attributed to frequency and amount of precipitation post-fertilizer use, complex environment and crop genotype management interactions influencing soil N dynamics and the effectiveness of the NI, and inadequate experimental sensitivity to prove the significance of smaller yield differences as previously reported (Randall and Vetsch, 2005; Burzaco et al., 2014). This emphasizes the importance of synchronizing peak N uptake periods with the efficacy window of NIs for optimizing crop yield and N uptake (Smiciklas and Moore, 2008; Degenhardt et al., 2016). Furthermore, our results confirm that the ineffectiveness of nitrapyrin with fall-applied AA in the previous study by Wood (2018) was not due to the inability to deliver small amounts of NI with a side-kick pump. We anticipated that applying nitrapyrin in the tank or as a sidekick would affect its effectiveness, but this did not happen.

2.6 Conclusions

Our study confirms that approximately 60-70% of the fall-applied AA undergoes complete nitrification by planting time. Despite applying nitrapyrin and pronitridine in commercial formulations of N-Serve and Centuro, respectively, with fall-applied AA, there was no significant increase in NH_4^+ retention across all three sites. Moreover, a slight delay in NO_3^- movement from on to between bands was observed at two of the three sites. However, this difference was not statistically significant compared to AA without NIs. No notable differences were observed in agronomic yield and crop N uptake among the various AA treatments, irrespective of the 20% reduction in N application rate and regardless of whether AA was applied with or without NIs. Our findings offer new evidence that both side-kick and in-tank applied NIs in late fall are not very effective. Overall, these findings do not provide substantial evidence to advocate for using NIs with late fall AA banding. Nevertheless, reducing late fall AA application rates is advised for potential cost savings and increased farm profitability, although the long-term sustainability of reduced N rates requires careful consideration. However, using NIs may give growers enough confidence to reduce their higher insurance rate of unprotected N.

2.7 References

Abalos, D., Jeffery, S., Sanz-Cobena, A., Guardia, G., Vallejo, A. 2014. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. Agriculture, Ecosystems & Environment, 189, 136–144. https://doi.org/10.1016/j.agee.2014.03.036

Abbasi, M. K., Shah, Z., Adams, W. A. 2003. Effect of the nitrification inhibitor nitrapyrin on the fate of nitrogen applied to a soil incubated under laboratory conditions. Journal of Plant Nutrition and Soil Science, 166(4), 513–518. https://doi.org/10.1002/JPLN.200320246

Ahmed, M., Yu, W. J., Lei, M., Raza, S., Elrys, A. S., Zhou, J. B. 2023. Effect of urease and nitrification inhibitors on nitrogen transformation and nitrogen use efficiency of rain-fed summer maize (Zea mays) at Loess Plateau of China. International Journal of Agriculture and Biology, 30(5), 317–328 ref. many https://doi.org/10.17957/IJAB/15.2090

Amiro, B., Tenuta, M., Hanis-Gervais, K., Gao, X., Flaten, D., Rawluk, C. 2017. Agronomists' views on the potential to adopt beneficial greenhouse gas nitrogen management practices through fertilizer management. Canadian Journal of Soil Science, 97(4), 801–804. https://doi.org/10.1139/cjss-2017-0062

Angus, J. F., Gupta, V. V. S. R., Pitson, G. D., Good, A. J. 2014. Effects of banded ammonia and urea fertilizer on soil properties and the growth and yield of wheat. Crop and Pasture Science, 65(4), 337–352. https://doi.org/10.1071/CP13337

Bailey, L.D. 1990. The effects of 2-chloro-6-(trichloromethyl)-pyridine ('N-Serve') and fertilizers on productivity and quality of Canadian oilseed rape. Canadian Journal of Plant Science, 70, 979–986. https://doi.org/10.4141/cjps90-120

Blackmer, A. M., Sanchez, C. A. 1988. Response of corn to nitrogen-15-labeled anhydrous ammonia with and without nitrapyrin in Iowa. Agronomy Journal, 80, 95–102. https://doi.org/10.2134/agronj1988.00021962008000010022x

Burzaco, J. P., Ciampitti, I. A., Vyn, T. J. 2014. Nitrapyrin impacts on maize yield and nitrogen use efficiency with spring-applied nitrogen: Field studies vs. meta-analysis comparison. Agronomy Journal, 106(2), 753–760. https://doi.org/10.2134/agronj2013.0043

Cahalan, E., Ernfors, M., Müller, C., Devaney, D., Laughlin, R. J., Watson, C. J., Hennessy, D., Grant, J., Khalil, M. I., McGeough, K. L., Richards, K. G. 2015. The effect of the nitrification inhibitor dicyandiamide (DCD) on nitrous oxide and methane emissions after cattle slurry application to Irish grassland. Agriculture, Ecosystems and Environment, 199, 339–349. https://doi.org/10.1016/j.agee.2014.09.008

Cameron, K. C., Di, H. J., Moir, J. L. 2013. Nitrogen losses from the soil/plant system: a review. https://doi.org/10.1111/aab.12014

Chantigny, M. H., Bittman, S., Larney, F. J., Lapen, D., Hunt, D. E., Goyer, C., Angers, D. A. 2019. A multi-region study reveals high overwinter loss of fall-applied reactive nitrogen in cold and frozen soils. Canadian Journal of Soil Science, 99, 126–135. https://doi.org/10.1139/cjss-2018-0151

Degenhardt, R. F., Juras, L. T., Smith, L. R. A., MacRae, A. W., Ashigh, J., and McGregor, W. R. 2016. Application of nitrapyrin with banded urea, urea ammonium nitrate, and ammonia delays nitrification and reduces nitrogen loss in Canadian soils. Crop, Forage and Turfgrass Management, 2(1), 1–11. https://doi.org/10.2134/cftm2016.03.0027

Di, H. J., Cameron, K. C. 2016. Inhibition of nitrification to mitigate nitrate leaching and nitrous oxide emissions in grazed grassland: a review. Journal of Soils and Sediments. 16, 1401–1420. https://doi.org/10.1007/s11368-016-1403-8

Dromantienė, R., Pranckietienė, I., Jodaugienė, D., Paulauskienė, A. 2020. The influence of various forms of nitrogen fertilization and meteorological factors on nitrogen compounds in soil under laboratory conditions. Agronomy, 10(12), 2011. https://doi.org/10.3390/agronomy10122011

Dunmola, A. S., Tenuta, M., Moulin, A. P., Yapa, P., Lobb, D. A. 2010. Pattern of greenhouse gas emission from a prairie pothole agricultural landscape in Manitoba, Canada. Canadian Journal of Soil Science, 90(2), 243–256. https://doi.org/10.4141/CJSS08053

Environment Canada, 2024. Canadian Climate Normals 1981–2010 Station Data. Government of Canada. URL: http://climate.weather.gc.ca/climate_normals/(last accessed January 16, 2024).https://climate.weather.gc.ca/climate_normals/results_1981_2010_e.html?stnID=3582andautofwd=1

Giacometti, C., Mazzon, M., Cavani, L., Ciavatta, C., Marzadori, C. 2020. A nitrification inhibitor, nitrapyrin, reduces potential nitrate leaching through soil columns treated with animal slurries and anaerobic digestate. Agronomy, 10(6), 865. https://doi.org/10.3390/agronomy10060865

Gomes, S. L., Loynachan, T. E. 1984. Nitrification of anhydrous ammonia related to nitrapyrin and time-temperature interactions. Agronomy Journal, 76(1), 9–12. https://doi.org/10.2134/agronj1984.00021962007600010003x

Gonzalez-Dugo, V., Durand, J. L., Gastal, F. 2010. Water deficit and nitrogen nutrition of crops. A review. Agronomy for Sustainable Development, 30(3), 529–544. https://doi.org/10.1051/agro/2009059

Goos, R. J., Johnson, B. E. 1999. Performance of two nitrification inhibitors over a winter with exceptionally heavy snowfall. Agronomy Journal, 91(6), 1046–1049. https://doi.org/10.2134/agronj1999.9161046x

Griesheim, K. L., Mulvaney, R. L., Smith, T. J., Henning, S. W., and Hertzberger, A. J. 2019. Nitrogen-15 evaluation of fall-applied anhydrous ammonia: I. Efficiency of nitrogen uptake by corn. Soil Science Society of America Journal, 83(6), 1809–1818. https://doi.org/10.2136/sssaj2019.04.0098

Grzebisz, W., Gransee, A., Szczepaniak, W., Diatta, J. 2013. The effects of potassium fertilization on water-use efficiency in crop plants. Journal of Plant Nutrition and Soil Science, 176(3), 355–374. https://doi.org/10.1002/JPLN.201200287

Habibullah, H., Nelson, K. A., Motavalli, P. P. 2018. Management of nitrapyrin and pronitridine nitrification inhibitors with urea ammonium nitrate for winter wheat production. Agronomy, 8(10). https://doi.org/10.3390/agronomy8100204

Hanson, R. G., Maledy, S. R., & Jentes, C. E. (1987). Effect of anhydrous ammonia with and without nitrapyrin applied to fall and spring on corn yield. Communications in soil science and plant analysis, 18(4), 387–403. https://doi.org/10.1080/00103628709367828

Hatfield, J. L., Parkin, T. B. 2014. Enhanced efficiency fertilizers: Effect on agronomic performance of corn in Iowa. Agronomy Journal, 106(2), 771–780. https://doi.org/10.2134/agronj2013.0104

Heil, J., Vereecken, H., Brüggemann, N. 2016. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. European Journal of Soil Science, 67(1), 23–39. https://doi.org/10.1111/ejss.12306

Hendrickson, L. L., Walsh, L. M., Keeney, D. R. 1978. Effectiveness of nitrapyrin in controlling nitrification of fall and spring-applied anhydrous ammonia. Agronomy Journal, 70(5), 704–708. https://doi.org/10.2134/agronj1978.00021962007000050003xa

Hergert, G. W., Wiese, R. A. 1980. Performance of nitrification inhibitors in the Midwest. In:

Meisinger, J. J., Randall, G.W. Vitosh, M. L. (eds), Nitrification inhibitors—potentials and limitations. American Society of Agronomy and Soil Science Society of America. (pp. 89–105). https://doi.org/10.2134/asaspecpub38.c7

Hu, Y., Schraml, M., Von Tucher, S., Li, F., Schmidhalter, U. 2014. Influence of nitrification inhibitors on yields of arable crops: A meta-analysis of recent studies in Germany. International Journal of Plant Production, 8(1), 33–50. https://www.academia.edu/download/50662851/IJPP-8-33-50-2014.pdf

Huber, D. M., Warren, H. L., Nelson, D. W., Tsai, C. Y., Shaner, G. E. 1980. Response of winter wheat to inhibiting nitrification of fall-applied nitrogen1. Agronomy Journal, 72(4), 632–637. https://doi.org/10.2134/agronj1980.00021962007200040015x

Hughes, T. D., Welch, L. F. 1970. 2-chloro-6-(Trichloromethyl) pyridine as a nitrification inhibitor for anhydrous ammonia applied in different seasons. Agronomy Journal, 62(6), 821–824. https://doi.org/10.2134/agronj1970.00021962006200060044x

Janke, C. K., Moody, P., Bell, M. J. 2020. Three-dimensional dynamics of nitrogen from banded enhanced efficiency fertilizers. Nutrient Cycling in Agroecosystems, 118, 227–247. https://doi.org/10.1007/s10705-020-10095-5

Jayasundara, S., Wagner-Riddle, C., Parkin, G., Von Bertoldi, P., Warland, J., Kay, B., Voroney, P. 2007. Minimizing nitrogen losses from a corn-soybean-winter wheat rotation with best management practices. Nutrient Cycling in Agroecosystems, 79(2), 141–159. https://doi.org/10.1007/S10705-007-9103-9

Karamanos, R. E., Hanson, K., Stevenson, F. C. 2014. Nitrogen form, time and rate of application, and nitrification inhibitor effects on crop production. Canadian Journal of Plant Science, 94(2), 425–432. https://doi.org/10.4141/CJPS2013-205

Kaur, G., Nelson, K. A. 2022. Nitrification inhibitors with anhydrous ammonia for corn production. Field Day Annual Report. Lee Greenley Jr. Memorial Research Farm, Northern Missouri Research, Extension, and Education Center. Accessed January 10, 2024. https://mospace.umsystem.edu/xmlui/bitstream/handle/10355/91761/sr0604.pdf?sequence=1#page=47

Kyveryga, P. M., Blackmer, A. M., Ellsworth, J. W., Isla, R. 2004. Soil pH effects on nitrification of fall-applied anhydrous ammonia. Soil Science Society of America Journal, 68(2), 545. https://doi.org/10.2136/sssaj2004.0545

Lasisi, A. A., Akinremi, O. O., Zhang, Q., Kumaragamage, D. 2020. Efficiency of fall versus spring-applied urea-based fertilizers treated with urease and nitrification inhibitors I. Ammonia volatilization and mitigation by NBPT. Soil Science Society of America Journal, 84(3), 949–962. https://doi.org/10.1002/SAJ2.20062

Lemaire, G., Jeuffroy, M. H., Gastal, F. 2008. Diagnosis tool for plant and crop N status in vegetative stage: Theory and practices for crop N management. European Journal of Agronomy, 28(4), 614–624. https://doi.org/10.1016/J.EJA.2008.01.005

Liu, S. L., Varsa, E. C., Kapusta, G., Mburu, D. N. 1984. Effect of etridiazol and nitrapyrin treated N fertilizers on soil mineral N status and wheat yields. Agronomy Journal, 76(2), 265–270. https://doi.org/10.2134/agronj1984.00021962007600020022x

Machado, P. V. F., Tenuta, M., Bruulsema, T. W., Liang, C., MacDonald, J. D., Reid, D. K., Wagner-Riddle, C. The Canadian fertilizer use survey 2015-2020: Nitrogen baselines, trends, and opportunities. In ASA, CSSA, SSSA International Annual Meeting. ASA-CSSA-SSSA.https://scisoc.confex.com/scisoc/2021am/prelim.cgi/Paper/138035

Malhi, S. S., Grant, C. A., Johnston, A. M., Gill, K. S. 2001. Nitrogen fertilization management for no-till cereal production in the Canadian Great Plains: a review. Soil and Tillage Research, 60(3–4), 101–122. https://doi.org/10.1016/S0167-1987(01)00176-3

Manitoba Markets. Estimates of Field Crop Production by Manitoba Agricultural Services Corporation. 2022. Accessed January 12, 2024. https://www.manitoba.ca/agriculture/markets-and-statistics/crop-statistics/pubs/estimates_of_field_crop_production-2022.pdf

Marschner, P., Rengel, Z. 2023. Nutrient availability in soils. In: Rengel, Z., Chamak, I., White, P. J. (eds), Marschner's Mineral Nutrition of Plants, Fourth Edition. Academic Press. (pp. 499–522). https://doi.org/10.1016/B978-0-12-819773-8.00003-4

Matimati, I., Verboom, G. A., Cramer, M. D. 2014. Nitrogen regulation of transpiration controls mass-flow acquisition of nutrients. Journal of Experimental Botany, 65(1), 159–168. https://doi.org/10.1093/JXB/ERT367

MB Soil Fertility. 2023. Fall ammonia applications on dry soils. Accessed January 10, 2024. https://www.gov.mb.ca/agriculture/crops/seasonal-reports/pubs/fall-nh3-application-dry-soil.pdf

Muñoz-Huerta, R. F., Guevara-Gonzalez, R. G., Contreras-Medina, L. M., Torres-Pacheco, I., Prado-Olivarez, J., Ocampo-Velazquez, R. V. 2013. A review of methods for sensing the nitrogen status in plants: advantages, disadvantages and recent advances. Sensors, 13(8), 10823-10843. https://doi.org/10.3390/s130810823

Nelson, K. A. 2018. Pronitridine nitrification inhibitor with urea ammonium nitrate for corn. Journal of Agricultural Science, 10(6), 16. https://doi.org/10.5539/jas.v10n6p16

NikièmaPaligwende, E., B., M., E., QiangH., O., A. 2013. Effects of liquid hog manure on soil available nitrogen status, nitrogen leaching losses and wheat yield on a sandy loam soil of western Canada. https://Doi.Org/10.4141/Cjss2012-070, 93(5), 573–584. https://doi.org/10.4141/CJSS2012-070

Norton, J., Ouyang, Y. 2019. Controls and adaptive management of nitrification in agricultural soils. Frontiers in Microbiology, 10(AUG). https://doi.org/10.3389/FMICB.2019.01931/FULL

Nyborg, M., Malhi, S. S. 1988. Band application reduces soil acidification by nitrogen fertilizers. Communications in Soil Science and Plant Analysis, 19(7–12), 819–829. https://doi.org/10.1080/00103628809367978

Parkin, T. B., Hatfield, J. L. 2010. Influence of nitrapyrin on N_2O losses from soil receiving fall-applied anhydrous ammonia. Agriculture, Ecosystems & Environment, 136(1–2), 81–86. https://doi.org/10.1016/j.agee.2009.11.014

Pittelkow, C. M., Clover, M. W., Hoeft, R. G., Nafziger, E. D., Warren, J. J., Gonzini, L. C., Greer, K. D. 2017. Tile drainage nitrate losses and corn yield response to fall and spring nitrogen management. Journal of Environmental Quality, 46(5), 1057–1064. https://doi.org/10.2134/JEQ2017.03.0109

Rácz, D., Gila, B., Horváth, É., Illés, A., Széles, A. 2022. The efficiency of nitrogen stabilizer at different soil temperatures on the physiological development and productivity of maize (Zea mays L.). Agronomy Research 19(4), 1888–1900. https://doi.org/10.15159/AR.21.146

Randall, G. W., Vetsch, J. A. 2003. Nitrate losses in subsurface drainage from a corn–soybean rotation as affected by fall and spring application of nitrogen and nitrapyrin. Journal of Environmental Quality, 34(2), 590–597. https://doi.org/10.2134/JEQ2005.0590

Randall, G. W., Vetsch, J. A. 2005. Corn production on a subsurface-drained mollisol as affected by fall versus spring application of nitrogen and nitrapyrin. Agronomy Journal, 97(2), 472–

478. https://doi.org/10.2134/agronj2005.0472

Ren, B., Zhang, J., Dong, S., Liu, P., Zhao, B., Li, H. 2017. Nitrapyrin improves grain yield and nitrogen use efficiency of summer maize waterlogged in the field. Agronomy Journal, 109(1), 185–192. https://doi.org/10.2134/AGRONJ2016.06.0353

Reynolds, W. D., Drury, C. F., Parkin, G. W., Lauzon, J. D., Saso, J. K., Zhang, T., Reid, D. K. 2016. Solute dynamics and the Ontario nitrogen index: I. chloride leaching. Canadian Journal of Soil Science, 96(2), 105–121. https://doi.org/10.1139/cjss-2015-0069

Risk, N., Wagner-Riddle, C., Furon, A., Warland, J., Blodau, C. 2014. Comparison of simultaneous soil profile N_2O concentration and surface N_2O flux measurements overwinter and at spring thaw in an agricultural soil. Soil Science Society of America Journal, 78(1), 180–193. https://doi.org/10.2136/SSSAJ2013.06.0221

Rochette, P., Angers, D. A., Chantigny, M. H., Gasser, M.-O., MacDonald, J. D., Pelster, D. E., Bertrand, N. 2013. Ammonia volatilization and nitrogen retention: how deep to incorporate urea? Journal of Environmental Quality, 42(6), 1635–1642. https://doi.org/10.2134/JEQ2013.05.0192

SAS. 2013. The PLS Procedure. SAS/STAT® 13.1 User's Guide, 6248–6298.

Sfiligoj, E. 2019. Koch reveals Centuro's plans. Accessed January 16, 2024. https://www.croplife.com/crop-inputs/koch-agronomic-services-reveals-centuro-plans/

Shi, W., Norton, J. M. 2000. Effect of long-term, biennial, fall-applied anhydrous ammonia and nitrapyrin on soil nitrification. Soil Science Society of America Journal, 64(1), 228–234. https://doi.org/10.2136/sssaj2000.641228x

Silva, A. G. B., Sequeira, C. H., Sermarini, R. A., Otto, R. 2017. Urease inhibitor NBPT on ammonia volatilization and crop productivity: A meta-analysis. Agronomy Journal, 109(1), 1–13. https://doi.org/10.2134/AGRONJ2016.04.0200

Singh, G., Nelson, K. A. 2019. Pronitridine and nitrapyrin with anhydrous ammonia for corn. Journal of Agricultural Science, 11(4), 13. https://doi.org/10.5539/jas.v11n4p13

Singh, Y., Beauchamp, E. G. 1988. Response of winter wheat to fall-applied large urea granules with dicyandiamide. Canadian Journal of Soil Science, 68(1), 133–142. https://doi.org/10.4141/CJSS88-012

Siripong, S., Rittmann, B. E. 2007. Diversity study of nitrifying bacteria in full-scale municipal wastewater treatment plants. Water Research, 41(5), 1110–1120.

https://doi.org/10.1016/j.watres.2006.11.050

Smiciklas, K. D., Moore, A. S. 2008. Tile drainage nitrate concentrations in response to fertilizer nitrogen application. Journal of Agronomy, 7(2), 163–169. https://doi.org/10.3923/JA.2008.163.169

Smith, W., Grant, B., Qi, Z., He, W., VanderZaag, A., Drury, C. F., Vergè, X., Balde, H., Gordon, R., and Helmers, M. J. 2019. Assessing the impacts of climate variability on fertilizer management decisions for reducing nitrogen losses from corn silage production. Journal of Environmental Quality, 48(4), 1006–1015. https://doi.org/10.2134/jeq2018.12.0433

Stehouwer, R. C., Johnson, J. W. 1990. Urea and anhydrous ammonia management for conventional tillage corn production. Journal of Production Agriculture, 3(4), 507–513. https://doi.org/10.2134/jpa1990.0507

Subbarao, G., Ito, O., Sahrawat, K., Berry, W., Nakahara, K., Ishikawa, T., Watanabe, T., Suenaga, K., Rondon, M., Rao, I. 2006. Scope and strategies for regulation of nitrification in agricultural systems - Challenges and opportunities. Critical Reviews in Plant Sciences, 25(4), 303–335. https://doi.org/10.1080/07352680600794232

Sun, L., Lu, Y., Yu, F., Kronzucker, H. J., Shi, W. 2016. Biological nitrification inhibition by rice root exudates and its relationship with nitrogen-use efficiency. New Phytologist, 212(3), 646–656. https://doi.org/10.1111/nph.14057

Swezey, A. W., Turner, G. O. 1962. Crop experiments on the effect of 2-chloro-6-(trichloromethyl)-pyridine for control of nitrification of ammonium and urea fertilizers. Agronomy Journal, 532–534. https://doi.org/10.2134/agronj1962.00021962005400060020x

Tenuta, M., Gao, X., Flaten, D. N., Amiro, B. D. 2016. Lower nitrous oxide emissions from anhydrous ammonia application prior to soil freezing in late fall than spring pre-plant application. Journal of Environmental Quality, 45(4), 1133–1143. https://doi.org/10.2134/jeq2015.03.0159

Tiessen, K. H. D., Flaten, D. N., Bullock, P. R., Burton, D. L., Grant, C. A., Karamanos, R. E. 2006. Transformation of fall-banded urea: Application date, landscape position, and fertilizer additive effects. Agronomy Journal, 98(6), 1460–1470. https://doi.org/10.2134/agronj2005.0304

Tiessen, K. H. D., Flaten, D. N., Grant, C. A., Karamanos, R. E., Entz, M. H. 2005. Efficiency of fall-banded urea for spring wheat production in Manitoba: Influence of application date, landscape position, and fertilizer additives. Canadian Journal of Soil Science, 85(5), 649–666. https://doi.org/10.4141/S05-017

Touchton, J. T., Boswell, F. C. 1980. Performance of nitrification inhibitors in the southeast. In: Meisinger, J. J., Randall, G.W. Vitosh, M. L. (eds), Nitrification inhibitors—potentials and limitations. American Society of Agronomy and Soil Science Society of America. (pp. 63–74). https://doi.org/10.2134/asaspecpub38.c5

Touchton, J. T., Hoeft, R. G., Welch, L. F. 1978. Effect of nitrapyrin on nitrification of fall and spring-applied anhydrous ammonia. Agronomy Journal, 70(5), 805–810. https://doi.org/10.2134/agronj1978.00021962007000050026x

Touchton, J. T., Hoeft, R. G., Welch, L. F., Mulvaney, D. L., Oldham, M. G., Zajicek, F. E. 1979. N uptake and corn yield as affected by applications of nitrapyrin with anhydrous ammonia1. Agronomy Journal, 71(2), 238–242. https://doi.org/10.2134/agronj1979.00021962007100020006x

Vetsch, J. A., Randall, G. W., Fernández, F. G. 2019. Nitrate loss in subsurface drainage from a corn–soybean rotation as affected by nitrogen rate and nitrapyrin. Journal of Environmental Quality, 48(4), 988–994. https://doi.org/10.2134/JEQ2018.11.0415

Wang, F., Bear, J., Shaviv, A. 1998. Modeling simultaneous release, diffusion, and nitrification of ammonium in the soil surrounding a granule or nest containing ammonium fertilizer. European Journal of Soil Science, 49(2), 351–364. https://doi.org/10.1046/J.1365-2389.1998.00158.X

Wolt, J. D. 2004. A meta-evaluation of nitrapyrin agronomic and environmental effectiveness with emphasis on corn production in the midwestern USA. Nutrient Cycling in Agroecosystems 2004 69:1, 69(1), 23–41. https://doi.org/10.1023/B:FRES.0000025287.52565.99

Wood, M. 2018. Right source and right time: reducing nitrous oxide emissions with enhanced efficiency nitrogen fertilizers (Master's thesis. University of Manitoba, Winnipeg)

3. EFFECT OF NITRIFICATION INHIBITORS ON NITROGEN TRANSFORMATIONS IN SOILS OF VARYING TEXTURE—AN INCUBATION STUDY

3.1 Abstract

Due to the ongoing escalation in the cost of nitrogen (N) fertilizers, farmers are increasingly interested in safeguarding their N investments. The use of nitrification inhibitors (NIs) has emerged as a potential strategy to enhance the efficiency of N fertilizers by delaying nitrification, thereby reducing nitrate (NO_3^-) leaching and nitrous oxide (N_2O) emissions. However, the effectiveness of a NI can vary significantly depending on soil type. To address this variability, we compared commonly available NIs: pronitridine (Centuro), DCD (Drive-N), DMPP (component of ARM-U advanced), and nitrapyrin (eNtrench) in a laboratory incubation study. This study examined clay, loam, and sand soils from Manitoba using 1L microcosms incubated at 20°C, maintaining a 65% water-filled pore space (WFPS). Each soil was subjected to six treatments: a control (no nitrogen), UAN alone (28-0-0, 60 mg N kg^{-1} dry soil), and UAN mixed with each of the four NIs at their recommended rates. Throughout the four-week incubation period, we periodically measured soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations on days 0, 1, 3, 7, 9, 14, 21, and 28. Overall, we observed rapid nitrification in loam compared to sand and clay soils. Among the inhibitors, nitrapyrin showed significant ($p < 0.05$) effectiveness in retaining higher NH_4^+ levels and reducing NO_3^- accumulation on days 14, 21, and 28 in sand soil, with moderate efficacy in clay soil and limited effectiveness in loamy soil. In contrast, DCD exhibited a slight reduction in nitrification only in sand soil on days 14 and 21 of the experiment. However, DMPP and pronitridine were less effective in delaying nitrification across all three soils. Observing the limited effectiveness of DMPP and pronitridine in the laboratory study indicates that injecting

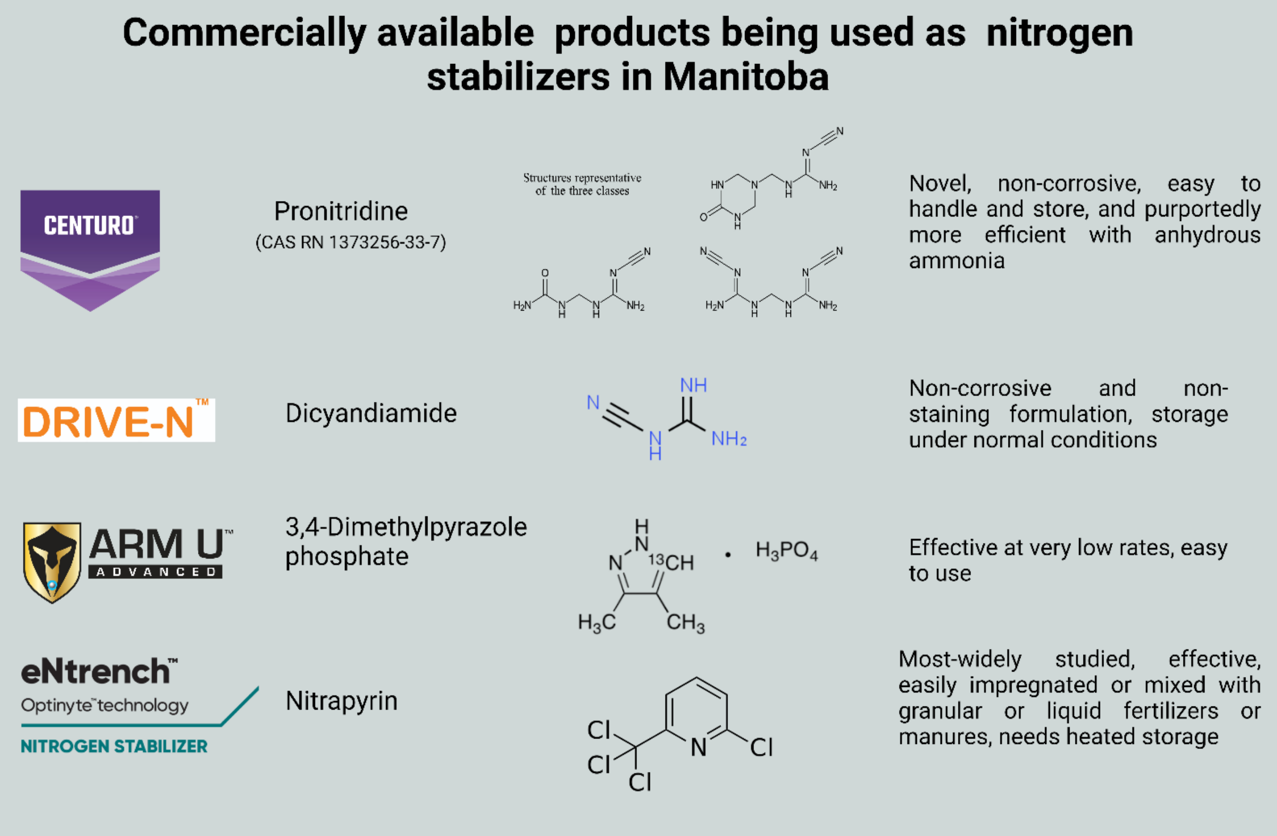
small amounts of the inhibitors with UAN by a syringe does not reproduce the effect of subsurface banding of the products in the field. Further laboratory studies are needed to examine the partitioning of the inhibitors from UAN upon syringe injection into the soil.

3.2 Introduction

Using nitrogen (N) fertilizers is vital for crop production; however, poor N recovery and use efficiency lead to economic and environmental challenges (Chattha et al., 2022). Urea Ammonium Nitrate (UAN) is a common N source in Canada for in-season applications because of its low ammonium (NH_4^+) content. It can be broadcast or injected into the soil (McKenzie et al., 2006). Being a liquid mixture of urea (50%) and ammonium nitrate (NH_4NO_3) (25% NH_4^+ and 25% NO_3^-) in water, with N concentrations ranging from 28% to 32%, UAN provides farmers with versatile nutrient management options, offering a balanced supply of quick-acting NO_3^-, longer-lasting NH_4^+, and sustained feeding from water-soluble organic N in urea (Owens et al., 2023). The urea fraction gets hydrolyzed to NH_4^+ and converted to nitrate (NO_3^-) in soil by microbes (Pawlick et al., 2019). The NH_4^+ fraction can either be directly absorbed by plants or nitrified by soil nitrifiers into NO_3^-. While NO_3^- being an anion, is weakly adsorbed to soil colloids, it tends to be absorbed by plants when needed. Alternatively, it may undergo leaching or denitrification, leading to N loss from farmlands (Lasisi et al., 2020). UAN has a lower volatilization potential than urea. However, its NH_4^+ fraction needs stabilization or slow oxidation to synchronize N-availability with plant uptake and avoid the risk of NO_3^- losses (Vaio, 2006).

Nitrification has been reported as a main process that impacts plant N availability and can lead to a significant amount of N losses (Beeckman et al., 2018; Ayiti and Babalola, 2022). Nitrification mainly supplies NO_3^-, most plants' preferred form of N for uptake and functioning. However, crop N demand varies depending on the plant’s growth stages (Angus, 2001) therefore, NO_3^- is needed in a gradual release and at the right time. Suppressing nitrification using NIs offers a great potential to increase NUE and agronomic efficiency, reducing environmental risks (Touchton et al., 1979; Subbarao et al., 2006; Sahrawat, 2008). Nitrification inhibitors delay the conversion of NH_4^+ to NO_3^- by inactivating the ammonia monooxygenase (AMO) enzyme, which oxidizes NH_4^+ to NO_3^-. Common NIs include nitrapyrin, dicyandiamide (DCD), and 3,4-dimethylpyrazole phosphate (DMPP), with commercial products being available as (eNtrench or N-Serve), (Drive-N), and (ARM-U_{adv}), respectively. At the same time, pronitridine (Centuro) is a novel NI, recently introduced by Koch Fertilizers, LLC (Singh and Nelson, 2019).

Among the different NIs, nitrapyrin, also known as 2-chloro-6-(trichloromethyl)-pyridine, was developed by Dow Chemical Company in 1962, is commonly used in North America, and injected into the soil with anhydrous ammonia (AA) due to its high vapour pressure (Goring, 1962; Wolt, 2000), its volatile nature makes it unsuitable as a coating for solid N fertilizers (Herr et al., 2020). Nitrapyrin is soluble in various organic solvents and AA and specifically inhibits the activity of the *Nitrosomonas* group. Nitrapyrin has been proven effective in delaying nitrification. However, its efficacy greatly varies under different soil and climatic conditions (Schmidt et al., 2020). Another compound identified as a NI in the early 1920s is DCD, it is water-soluble, non-volatile, and can be coated on fertilizers, animal manures, and slurries (Subbarao et al., 2006). DCD transforms into urea in soil and has specific bacteriostatic effects on *Nitrosomonas*. However, its water-soluble nature may leach from the soil root zone, reducing its efficacy (Vogeler et al., 2007). A newer inhibitor, DMPP, was recently developed by BASF in the 1990s in Germany; it is specific in its inhibitory action to ammonia-oxidizing bacteria (AOB) growth, requires lower application rates, and is immobile in the soil. However, it is costly (Zerulla et al., 2001; Subbarao et al., 2006). The most recent addition is pronitridine, which is available as Centuro and has garnered interest among the farmers due to its non-corrosive nature, making it easier to handle and store. It inhibits the activity of AMO enzymes by competitive and non-competitive enzymatic inhibition (Habibullah et al., 2018).



Commercially available products being used as nitrogen stabilizers in Manitoba

CENTURO	Pronitridine (CAS RN 1373256-33-7)	Novel, non-corrosive, easy to handle and store, and purportedly more efficient with anhydrous ammonia
DRIVE-N™	Dicyandiamide	Non-corrosive and non-staining formulation, storage under normal conditions
ARM U™ ADVANCED	3,4-Dimethylpyrazole phosphate	Effective at very low rates, easy to use
eNtrench™ Optinyte™ technology NITROGEN STABILIZER	Nitrapyrin	Most-widely studied, effective, easily impregnated or mixed with granular or liquid fertilizers or manures, needs heated storage

Fig. 3A Overview of commercially available products used in the laboratory experiment, including their active ingredients, chemical structures, and respective advantages and disadvantages.

It is well established that soil texture directly influences chemical and biological processes within soils, primarily by influencing the pore space, aeration status, microbial diversity, and community structure (Fortuna et al., 2012; Xia et al., 2020). Previous investigations have consistently reported higher nitrification rates in soils with higher clay content (Barth et al., 2019; Elrys et al., 2020), and concurrently, a positive correlation between nitrification and soil electrical conductivity (EC) has also been observed (Gao et al., 2021). The clay content, in particular, affects soil aeration status and redox potential, thus directly impacting nitrification (Yu et al., 2007; Benckiser et al., 2013). Furthermore, higher clay content could potentially increase the cation exchange capacity (CEC) and adsorption capacity for NH_4^+ and NIs (Yu et al., 2007; McGeough et al., 2016). Numerous studies have consistently reported a negative correlation between the efficacy of NIs and soil organic matter (SOM) and clay content (Pasda et al., 2001; Barth et al., 2008; McGeough et al., 2016).

Several studies have documented the considerable efficacy of NIs in delaying nitrification in sand soils compared to clay and organic soils (Wolt, 2000; Barth et al., 2001; Elrys et al., 2020). The scarce literature on the relative effectiveness of various NIs to soil textures reveals mixed findings, with some researchers reporting varied results on NI efficacy in diverse soil types (Gilsanz et al., 2016). Most studies have found NIs more efficient in light soils than in heavy and clay soils (Marsden et al., 2016; Volpi et al., 2017; Barth et al., 2019). Akiyama et al. (2010) reported that the effectiveness of NIs is relatively consistent across various soil types. Fisk et al. (2015) noted reduced efficacy of DMPP and DCD with increased SOM. With limited research available, the precise inhibitory effects of pronitridine remain to be fully elucidated. Preliminary studies from the University of Missouri suggest that pronitridine may benefit crop productivity by effectively delaying nitrification (Nelson, 2018). However, comprehensive evaluations are requisite to ascertain its comparative effectiveness. The objective of this study was to compare the efficacy of four different NIs, pronitridine, DCD, DMPP, and nitrapyrin in delaying nitrification in three contrasting textured soils commonly found in Manitoba. We hypothesized that different NIs would effectively delay the conversion of NH_4^+ to NO_3^-, and their effectiveness would vary due to their different chemical composition and persistence under a range of soils with different textures.

3.3 Material and Methods

3.3.1 Experimental Setup

This laboratory experiment was conducted in mason jars (1L volume) (16.5cm height x 8cm diameter). The experimental treatments, each having four replicates, were laid out in a completely randomized factorial design (6 x 3), considering six treatments as a treatment factor and three different soil types as a main factor. Soils with different types of texture (clay, loam, sand) were sampled (0-30 cm) using a flat spade from four different locations at each site, which represented four replicates (n=4). Each replicate was thoroughly mixed, air-dried under shade, and sieved through a 6.0 mm mesh to remove root and leaf residues. To determine soil bulk density, soil with a known mass was added to the jar and tapped within it to pack it comparably to the field level. Subsequently, water was added to the jar to reach the same volume as the soil. The bulk density of clay, loam, and sand was 1.2, 1.25, and 1.3 g cm^{-3}, while soil particle density was assumed to be 2.65 g cm^{-3}.

$$\text{Bulk density} = \left(\frac{\text{Dry mass of Soil}}{\text{Volume of Jar}}\right) \tag{i}$$

$$\text{Total soil porosity} = 1 - \left(\frac{\text{Soil bulk denisty}}{\text{Soil particle density}}\right) \tag{ii}$$

$$\text{WFPS (\%)} = \frac{100 \times \text{GMC} \times \text{Soil bulk density}}{\text{Total soil porosity}} \tag{iii}$$

$$\text{Required WFPS (\%)} = \text{Total req. WFPS (\%)} - \text{Current WFPS(\%)} \tag{iv}$$

3.3.2 Soil Collection and Preparation

Three different soil types were sourced from farm fields near the towns of Rosser (50°02'39.6"N 97°26'14.2"W), Forrest (50°09'20.3"N 99°56'28.0"W), and Roseisle (49°32'57.6"N 98°23'02.7"W). The soil was classified as clayey lacustrine gleysols (Osborne) at Rosser, loamy till black chernozem (Newdale) at Forrest, and sandy lacustrine (Almasippi) at Roseisle in the Canadian Soil Classification System. Soils (0-30 cm deep) were sampled using a flat spade from four locations at each site. Soil from each location from a site treated as a separate replicate (n=4) was thoroughly homogenized, air-dried under shade, and sieved through a 6.0 mm mesh to remove root and leaf residues. Each soil was analyzed for salient physicochemical properties, including pH, electrical conductivity (EC), NO_3^-, Olsen-P, potassium, sulphate, sodium (Na), cation exchange capacity (CEC), soil organic matter (SOM), and textural characteristics (determined using hydrometer procedure) given in Table 3.1.

Table 3.1 Average physicochemical properties of the three soils used in the laboratory incubation study (0-25 cm).

Soil type	**Soil Classification**	**pH (H_2O)**	**EC**	**NO_3^--N**	**Olsen-P**	**K**	**SO_4^{-2}-S**	**Na**	**CEC**	**SOM[a]**	**Clay**	**Sand**	**Silt**
			dS m^{-1}	————mg kg^{-1}————					cmol$^+$ kg^{-1}	————g kg^{-1}————			
Clay	Clayey Lacustrine	8.1	0.5	23.8	15.5	355	62.5	70.8	53.4	64	520	195	285
Loam	Loamy Till (Black Chernozem)	7.9	3.7	142.8	56.8	365	1300	782.5	70.1	88	240	350	410
Sand	Sandy Lacustrine	5.9	0.2	7.3	19.8	190	38.0	15.5	9.2	14	53	895	52

[a] SOM, soil organic matter

3.3.3 Soil Incubation

Each jar was filled with moist soil equivalent to 400 g of dry soil weight, and the jars were covered top with a perforated parafilm having two pokes. The soil aggregate's structure was maintained in the jars by gently tapping them over the soft surface. The current water-filled pore space (WFPS) of each soil replicate was determined, and the required amount of water was calculated to reach 65% of WFPS. After a week of pre-incubation at 20°C and 65% WFPS, urea-ammonium nitrate (UAN) was injected into the selective jars at the rate of 60 mg N kg^{-1} dry soil. After one week of pre-incubation at 20°C temperature maintaining 65% water-filled pore space (WFPS), UAN at 60 mg kg^{-1} dry soil equivalent to a rate of 120 kg N ha^{-1} was injected into the selective jars. Nitrification inhibitors, including pronitridine, DCD, DMPP, and nitrapyrin, were applied as Centuro (Koch Agronomic Services, LLC), Drive-N (Innovar Ag, USA), ARM-U advanced (Active AgriScience Inc.), and eNtrench (Dow AgroSciences Canada Inc.), respectively. The application rates were 6 L, 2 L, and 1.1 L per 1000 kg of UAN, and 1.75 L ha^{-1}, as specified on the product labels. The final volumes of all NIs per jar were calculated based on the amount of N applied as UAN per jar, using the soil mass, for the eNtrench rate calculation, the jar area was used instead. ARM U advanced, containing only DMPP, was provided by Active AgriScience for research. The active ingredients were 0.23 mg, 0.17 mg, 0.04 mg, and 0.79 mg per kg of dry soil for pronitridine, DCD, DMPP, and eNtrench, respectively.

Stock solutions were prepared, and the appropriate volumes of 28% UAN, pronitridine, DCD, DMPP, and nitrapyrin were added to five labeled bottles. All treatments, except nitrapyrin, were injected using a manual HPLC injection syringe with a final volume of 66 uL, directly into the centre of the soil both horizontally and vertically (4-6 cm deep). However, the nitrapyrin and UAN mixture was too dense for the HPLC syringe; therefore, it was diluted for a 1 mL injection using a mix of 6.6 mL nitrapyrin with UAN and 93.4 mL of DI water. For the nitrapyrin, a 1 mL syringe was used to inject 1 mL mix. Following the injections, all jars were incubated at 20°C, maintaining 65% WFPS by weighing the jars every 5th day to account for moisture loss, with deionized water added as necessary.

3.3.4 Sampling for Extractable Soil Nitrogen (NH_4^+ /NO_3^-)

Soil from all the jars was sampled, spanning seven setups of 72 jars each. After destructive sampling, the soil was analyzed for extractable N (NH_4^+ and NO_3^-) on various days: 1, 3, 7, 9, 14, 21, and 28 from the beginning of the experiment. Meanwhile, a separate setup consisting of four replicates of only control treatments (no nitrogen and UAN without NIs) across three soil types was extracted on day 0. The values from UAN control samples were assumed for NIs treatments for day 0. After collecting soil from each jar, it was thoroughly mixed and treated as an individual sample. These samples were then extracted using a 2M KCl solution, and the resulting extracts were analyzed for extractable N (NH_4^+ and NO_3^-) concentrations using an autoanalyzer (HQ - Skalar Analytical B.V., The Netherlands). The average percentage reduction in NO_3^- appearance (given in Table 3.2), represents the average percentage of nitrification inhibition (Bozal-Leorri et al., 2022) for each NI in a specific soil type on a particular day of incubation, where higher values indicate greater effectiveness, calculated as follows:

$$\text{Nitrification inhibition (\%)} = \left[1 - \left(\frac{\text{NINO3} - \text{ControlNO3}}{\text{UANNO3} - \text{ControlNO3}}\right)\right] \times 100 \qquad \text{(viii)}$$

Where, NINO3: Nitrate concentration in the treatment where a NI is applied to UAN.

Control NO3: Nitrate concentration in the control treatment where no N is added.

UANNO3: Nitrate concentration in the UAN treatment, without any NI.

3.3.5 Statistical Analysis

Data were analyzed using the generalized linear mixed model procedure (PROC GLIMMIX) of SAS version 9.4 (SAS Institute, 2013) to determine treatment effects on NH_4^+ retention and NO_3^- accumulation over time. Nitrification inhibitor treatment was modelled as the fixed effect, with replicates specified as a class statement. Analysis of variance (ANOVA) was used to test the treatment effects at each sampling day for each soil type. Percentage inhibition data were transformed to enhance suitability for robustness analysis, especially for meeting normality and constant variance assumptions. Tukey's multiple comparison procedure compared treatment means at $\alpha = 0.05$. Graphs were plotted using Origin Lab 2023, while statistical analyses were conducted using SAS.

3.4. Results

3.4.1. Clay Soil

Across all three types of soils—clay, loam, and sand—the patterns of decreasing NH_4^+ concentrations were approximately similar among all the UAN treatments without NIs. However, the decrease in NH_4^+ concentration was more rapid for loam soil in the first week than for clay and sand. In clay soil, NH_4^+ concentrations between UAN and NIs treatments remained similar over time with no significant differences ($p < 0.05$), except for the nitrapyrin treatment, which showed significantly higher NH_4^+ concentrations compared to UAN without NIs on days 14, 21, and 28. As expected, control treatments with no N showed significantly lower concentration of NH_4^+ until day 9 compared to treatments receiving UAN, either treated or untreated with NIs (Fig. 3.1). Conversely, to NH_4^+ concentration, NO_3^- concentration in all three types of soils increased over time. This increase was faster in loam soil, slower in sand soil, and moderate in clay soil. In clay soil, the increase in NO_3^- concentration was rapid for all treatments receiving UAN until day 9 from the start of incubation, with a slight decrease in NO_3^- concentration in all the NIs treatments (Fig. 3.1). However, no significant differences ($p < 0.05$) were observed between the treatments receiving UAN, either treated or untreated with NIs. In contrast, the NO_3^- concentrations in the control treatments remained significantly lower than all the other treatments receiving UAN, with or without NIs (Fig. 3.1). Among NIs, nitrapyrin exhibited an inhibition efficiency of approximately 15%, particularly on days 7, 9, 14, and 21 (Table 3.2). However, on day 28, its effectiveness in inhibiting NO_3^- accumulation seemed diminished. DMPP demonstrated 24% and 15% nitrification inhibition rates on days 7 and 9, respectively. DCD inhibited NO_3^--accumulation by 19%, 12%, 6%, and 6% on days 7, 9, 14, and 21, respectively. Pronitridine was the least effective in clay soil, with nitrification inhibition percentages ranging between 5% and 13%. Its effectiveness was observed on days 3, 7, 9, and 21, with a maximum inhibition of 13% on day 7.

3.4.2 Loam Soil

In loam soil, no significant differences ($p < 0.05$) in NH_4^+ concentrations were observed among the UAN treatments, either with or without NIs, with a significant decrease in NH_4^+ concentration during the first week of incubation (Fig. 3.1). However, on days 7 and 14, nitrapyrin was observed with significantly higher NH_4^+ concentrations compared to UAN treatment without NIs. Moreover, NH_4^+ concentrations in treatments receiving N as UAN were significantly ($p < 0.05$) higher than controls with no N until day 3. On day 7, there was no difference in NH_4^+ concentration between controls and the treatments receiving UAN, except for the pronitridine and nitrapyrin-treated UAN (Fig. 3.1). In loam soil, NO_3^- concentrations increased progressively during the incubation period with similar trends between UAN and NIs treatments (Fig 4e). However, no significant differences ($p < 0.05$) were observed among all the treatments, as the deviation in NO_3^- concentrations was too high within the replicates of loam soil. Overall, the nitrification inhibition by all tested NIs remained consistently below 10% for most of the incubation period. However, on day 7, greater but overall non-significant nitrification inhibition rates were observed, with percentages reaching 43%, 54%, 45%, and 38% for pronitridine, DCD, DMPP, and nitrapyrin, respectively (Table 3.2).

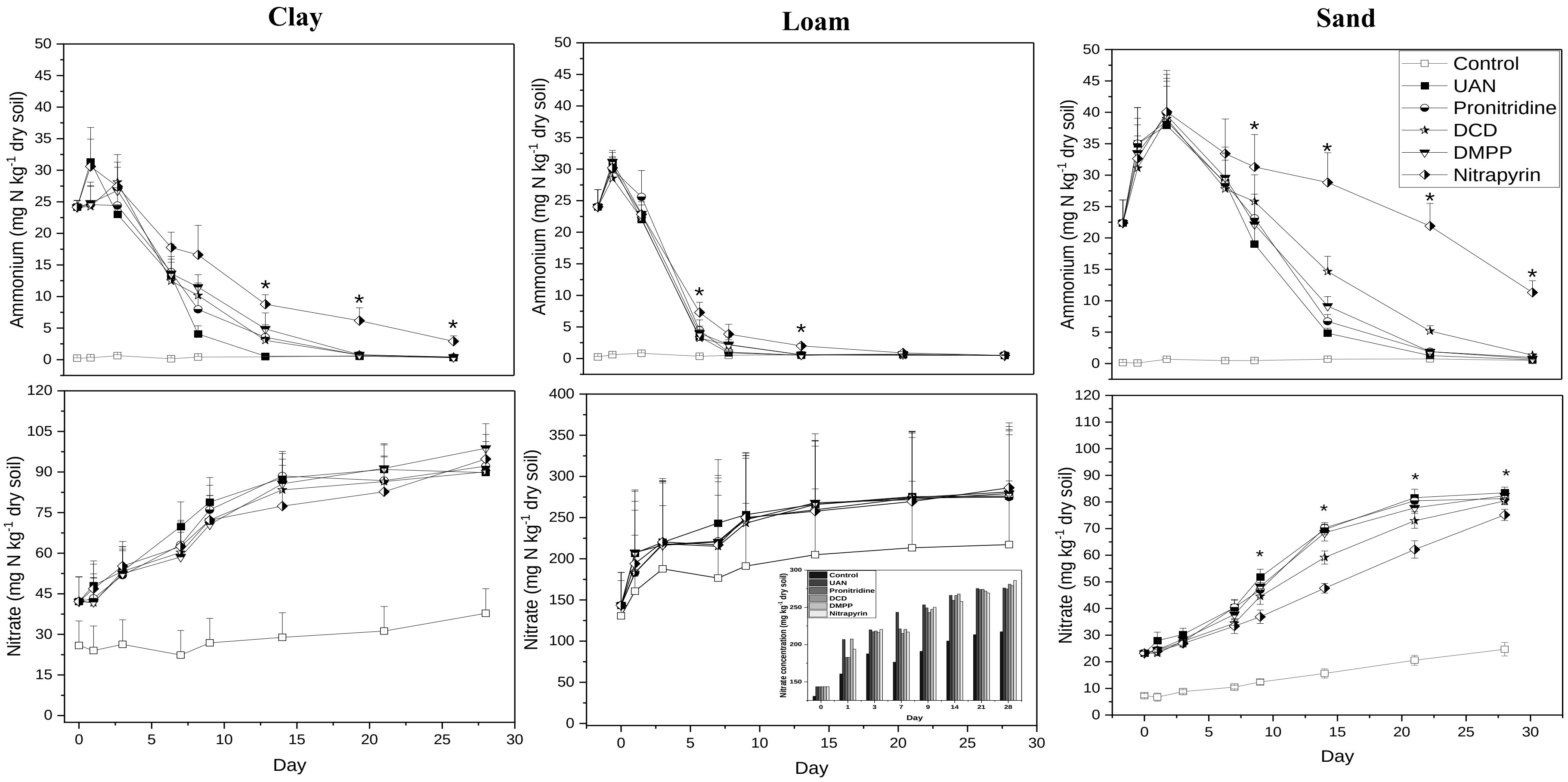


Figure 3.1 Effect of nitrification inhibitors on ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in clay, loam, and sand soil (400 g in microcosms) during 28 days of incubation at 20°C and 65% water-filled pore space (WFPS). Asterisks within each soil type indicate significant differences ($p < 0.05$) between nitrogen treatments (excluding controls) for the respective days. Control: Without nitrogen and any nitrification inhibitor; UAN: 60 mg kg^{-1} dry soil N added as Urea ammonium nitrate (UAN); Pronitridine: Centuro + UAN; DCD: Drive-N + UAN; DMPP: ARM U advanced + UAN; Nitrapyrin: eNtrench + UAN. Error bars indicate the standard errors of the means (n = 4). ARM U advanced, utilized in the lab study, was provided by Active AgriScience for research, containing exclusively DMPP as its active ingredient. The inset bar graph shows mean nitrate concentrations (n=4) for different treatments over time.

Table 3.2 Average percentage reduction in nitrate appearance in UAN-treated with different nitrification inhibitors across three soils over 28 days of incubation at 20°C and 65% water-filled pore space (WFPS).

Soil Type	**Nitrification Inhibitors**	**Nitrification Inhibition (%)**					
		Days					
		3	**7**	**9**	**14**	**21**	**28**
Clay	Pronitridine	10.5 ± 9.6a	13.7 ± 8.3a	4.9 ± 13.0a	-1.9 ± 10.4a	6.6 ± 4.3a	-6.0 ± 6.6a
	DCD	0.3 ± 6.7a	19.5 ± 5.4a	12.7 ± 5.9a	6.6 ± 9.2a	6.8 ± 5.0a	-2.8 ± 9.7a
	DMPP	6.6 ± 5.7a	24.5 ± 5.1a	15.5 ± 7.2a	3.1 ± 4.5a	-0.8 ± 3.0a	-19.7 ± 9.4a
	Nitrapyrin	-8.2 ± 3.4a	15.2 ± 9.1a	12.8 ± 3.6a	17.5 ± 2.5a	13.5 ± 4.3a	-12.4 ± 11.2a
Loam	Pronitridine	7.9 ± 10.2a	43.2 ± 18.4a	6.8 ± 2.9a	7.8 ± 16.7a	2.5 ± 4.9a	1.0 ± 20.4a
	DCD	4.6 ± 11.1a	54.1 ± 25.5a	15.3 ± 10.2a	0.6 ± 1.8a	1.1 ± 5.2a	-9.4 ± 4.8a
	DMPP	10.3 ± 7.0a	45.5 ± 25.0a	8.0 ± 13.8a	-5.2 ± 12.4a	4.9 ± 8.4a	-6.9 ± 10.6a
	Nitrapyrin	-2.2 ± 12.4a	38.4 ± 28.4a	5.4 ± 2.3a	12.9 ± 5.5a	8.6 ± 6.8a	-17.9 ± 6.3a
Sand	Pronitridine	12.0 ± 4.76a	-0.1 ± 1.1b	12.1 ± 3.0b	-1.3 ± 0.6c	1.6 ± 3.7b	3.7 ± 0.4b
	DCD	11.0 ± 5.38a	18.5 ± 5.6ab	18.6 ± 1.3b	19.5 ± 1.1b	14.1 ± 1.8b	5.3 ± 2.0b
	DMPP	8.2 ± 3.47a	8.0 ± 2.3ab	9.0 ± 2.4b	2.3 ± 2.2c	6.0 ± 3.3b	1.8 ± 0.8b
	Nitrapyrin	14.9 ± 4.32a	23.3 ± 4.0a	37.5 ± 4.3a	40.7 ± 0.8a	31.9 ± 1.8a	14.1 ± 0.4a

Mean ± standard errors (n=4). Means within a column followed by the same letters are not significantly different at $\alpha = 0.05$ according to the Tukey multiple comparison procedure for each soil type.

3.4.3. Sand Soil

The decrease in NH_4^+ concentration over time was slower in sand soil than in loam and clay soils. However, the NI treatments, particularly nitrapyrin, exhibited higher NH_4^+ concentrations than UAN, without any NI throughout the experiment. Overall, the decrease in NH_4^+ concentration in sand soil was faster in UAN treatments applied without NIs. At the same time, it was slower with pronitridine and DMPP, and slowest with nitrapyrin and DCD (Fig. 3.1). Significant differences ($p < 0.05$) in NH_4^+ concentration between UAN and NIs treatments were noted on day 09, 14, 21, and 28. The NH_4^+ concentration in nitrapyrin-treated UAN was significantly ($p < 0.05$) higher than all other treatments receiving UAN, either with or without NIs, on days 09, 14, 21, and 28. Meanwhile, nitrapyrin, followed by DCD-treated UAN, was observed with higher NH_4^+ concentrations on days 14 and 21 compared to UAN alone and treated with DMPP and pronitridine. However, pronitridine and DMPP did not retain more NH_4^+ than untreated UAN throughout the incubation period. The NH_4^+ concentration in controls remained significantly ($p < 0.05$) lower than all the treatments receiving UAN, either treated or untreated, for the first two weeks of incubation. On day 21, DCD and nitrapyrin-treated still held more NH_4^+ than the control (Fig. 3.1). Overall, all the NIs treatments were observed with lower NO_3^- concentrations compared to UAN without NIs in sand soil (Fig. 3.1). However, nitrapyrin-treated UAN maintained significantly ($p < 0.05$) lower NO_3^- concentrations on days 9, 14, 21, and 28, exhibiting inhibition efficiency rates of 37%, 40%, 31% and 14%, respectively (Table 3.2). Nitrapyrin, followed by DCD-treated UAN, showed significantly ($p < 0.05$) lower NO_3^- concentrations compared to all other NI treatments on day 14, with reductions in NO_3^- appearance by 19%. However, NO_3^- concentrations in pronitridine and DMPP-treated UAN did not significantly ($p < 0.05$) differ from untreated UAN. Pronitridine notably inhibited nitrification on days 3 and 9 with 12% inhibition efficiency, while DMPP induced slight reductions in NO_3^- levels on days 3, 7, 9, and 21. Overall, NO_3^- concentrations in control treatments remained significantly ($p < 0.05$) lower than all other UAN treatments, with or without NIs.

3.5. Discussion

3.5.1. Effect of Soil Type

Our results indicated that loam soil exhibited a high nitrification rate right after the UAN application, clay soil exhibited a moderate nitrification rate, and sand soil exhibited a low nitrification rate. The soil organic matter and EC levels of these three soils exhibited a decreasing trend, similar to the nitrification rate. The sand, slightly acidic soil showed a slow nitrification rate, consistent with previous studies that found a decrease in nitrification rate with decreasing soil pH (De Boer and Kowalchuk, 2001; Zebarth et al., 2015). Similarly, Ahmed et al. (2023) reported higher nitrification rates in organic soils compared to clay and sandy soils, primarily due to the high content of potentially mineralizable native SOC, and soil organic matter favours soil respiration (Thomsen et al., 2003; Guo et al., 2021). Nitrification has been reported to positively correlate with soil organic carbon (SOC) content, as it promotes the population of nitrifying bacteria (Cébron et al., 2003; Han et al., 2017). Soils with higher microbial activity, particularly a higher population of nitrifiers, are likely to experience more rapid nitrification (Isobe et al., 2008; Waqas et al., 2021). Thus, the higher SOC in loam soil could be one plausible explanation for the observed rapid nitrification in this soil. The slow nitrification in sand soil could be attributed to the slightly acidic to neutral soil pH (Lu et al., 2018; Cui et al., 2021).

3.5.2. Effect of Nitrification Inhibitors

Among the NIs, only nitrapyrin and DCD significantly delayed nitrification at certain sampling days; however, this delaying effect did not show a consistent trend across different soil textures. Nitrapyrin appeared to be the most effective in delaying nitrification, particularly in sand and clay soil, followed by DCD, while pronitridine and DMPP were the least effective in all soil types. The overall reduced efficiency of NIs observed in our study during the initial two weeks of incubation, in contrast to previous studies, may be attributed to the elevated incubation temperature (20°C) setup (Zerulla et al., 2001). The observed ineffectiveness of DMPP might be due to a poorly formulated product, while the rates of pronitridine may need adjustment based on soil conditions. Our findings on the efficacy of DCD are consistent with those of previous studies that reported the efficacy of DCD to be soil-specific (Ernfors et al., 2014; Guo et al., 2021), in contrast to Wakelin et al. (2014), who reported the efficacy of DCD to be not soil-specific. The highest efficacy of nitrapyrin and DCD in sand soil may be explained by the lower decomposition of these NIs under lower SOM than in clay and loam soils (Gilsanz et al., 2016; Guo et al., 2021). However, the reduced effectiveness in clay and especially in loam soil could be attributed to the adsorption of these NIs on clay and silt particles or rapid decomposition under high SOM content (Pasda et al., 2001; Barth et al., 2008; McGeough et al., 2016; Elrys et al., 2020). In our study, the preparation of stock solutions differed, with micro-litre solutions replicating field applications for pronitridine, DCD, and DMPP, while nitrapyrin, too dense for the HPLC syringe, was diluted for a 1 mL injection using a mix of 6.75 mL eNtrench (nitrapyrin) with UAN and 93.25 mL of DI water. This difference in solution methodology may account for the better effectiveness of nitrapyrin compared to other NIs.

3.6. Conclusions

In conclusion, this laboratory experiment unveiled varying effectiveness of different NIs across sand, clay, and loam soils. Nitrapyrin proved most effective, maintaining higher NH_4^+ levels and reducing NO_3^- accumulation in sand soil over 28 days. It showed moderate efficacy in clay soil, retaining higher NH_4^+ levels on specific days, but had limited effectiveness in loam soil. DCD showed moderate effectiveness, particularly in sand soil, while DMPP and pronitridine exhibited lower inhibitory effects overall. However, this laboratory study could not reproduce the subsurface banding results of UAN with NIs, notably due to significant variability within loam soil replicates. This underscores the need for further research to validate these findings and investigate the dynamics of inhibitor partitioning from UAN upon soil injection. Despite limitations, this experiment highlights the potential of nitrapyrin and DCD as effective tools for managing nitrification in agricultural soils.

3.7. References

Ahmed, M., Yu, W., Lei, M., Raza, S., Elrys, A., Zhou, J. 2023. Effect of urease and nitrification inhibitors on nitrogen transformation and nitrogen use efficiency of rain-fed summer maize (Zea mays) at Loess Plateau of China. International Journal of Agriculture and Biology, 30(5), 317–328. https://doi.org/10.17957/IJAB/15.2090

Akiyama, H., Yan, X., Yagi, K. 2010. Evaluation of the effectiveness of enhanced-efficiency fertilizers as mitigation options for N_2O and NO emissions from agricultural soils: Meta-analysis. Global Change Biology, 16(6), 1837–1846. https://doi.org/10.1111/J.1365-2486.2009.02031.X

Angus, J. F. 2001. Nitrogen supply and demand in Australian agriculture. Australian Journal of Experimental Agriculture, 41(3), 277–288. https://doi.org/10.1071/EA00141

Ayiti, O. E., Babalola, O. O. 2022. Factors influencing soil nitrification process and the effect on environment and health. Frontiers in Sustainable Food Systems, 6, 821994. https://doi.org/10.3389/FSUFS.2022.821994/BIBTEX

Barth, G., Von Tucher, S., Schmidhalter, U. 2001. Influence of soil parameters on the effect of 3, 4-dimethylpyrazole-phosphate as a nitrification inhibitor. Biology and Fertility of Soils, 34, 98–102. https://doi.org/10.1007/s003740100382

Barth, G., Von Tucher, S., Schmidhalter, U. 2008. Effectiveness of 3, 4-dimethylpyrazole phosphate as nitrification inhibitor in the soil as influenced by inhibitor concentration, application form, and soil matric potential. Pedosphere, 18(3), 378–385. https://doi.org/10.1016/S1002-0160(08)60028-4

Barth, G., von Tucher, S., Schmidhalter, U., Otto, R., Motavalli, P., Ferraz-Almeida, R., Meinl Schmiedt Sattolo, T., Cantarella, H., Vitti, G. C. 2019. Performance of nitrification inhibitors with different nitrogen fertilizers and soil textures. Journal of Plant Nutrition and Soil Science, 182(5), 694–700. https://doi.org/10.1002/JPLN.201800594

Beeckman, F., Motte, H., Beeckman, T. 2018. Nitrification in agricultural soils: impact, actors, and mitigation. Current Opinion in Biotechnology, 50, 166–173. https://doi.org/10.1016/j.copbio.2018.01.014

Benckiser, G., Christ, E., Herbert, T., Weiske, A., Blome, J., Hardt, M. 2013. The nitrification inhibitor 3,4-dimethylpyrazole-phosphate (DMPP)-quantification and effects on soil metabolism. Plant and Soil, 371(1–2), 257–266. https://doi.org/10.1007/s11104-013-1664-

6

Bozal-Leorri, A., Corrochano-Monsalve, M., Vega-Mas, I., Aparicio-Tejo, P. M., González-Murua, C., Marino, D. 2022. Evidences towards deciphering the mode of action of dimethylpyrazole-based nitrification inhibitors in soil and pure cultures of *Nitrosomonas europaea*. Chemical and Biological Technologies in Agriculture, 9(1), 56. https://doi.org/10.1186/s40538-022-00321-3

Cébron, A., Berthe, T., Garnier, J. (2003). Nitrification and nitrifying bacteria in the lower Seine River and estuary (France). Applied and Environmental Microbiology, 69(12), 7091–7100. https://doi.org/10.1128/AEM.69.12.7091–7100.2003

Chattha, M. S., Ali, Q., Haroon, M., Afzal, M. J., Javed, T., Hussain, S., Mahmood, T., Solanki, M. K., Umar, A., Abbas, W., Nasar, S., Schwartz-Lazaro, L. M., Zhou, L. 2022. Enhancement of nitrogen use efficiency through agronomic and molecular-based approaches in cotton. Frontiers in Plant Science, 13, 1–24. https://doi.org/10.3389/fpls.2022.994306

Cui, L., Li, D., Wu, Z., Xue, Y., Xiao, F., Zhang, L., Song, Y., Li, Y., Zheng, Y., Zhang, J., Cui, Y. 2021. Effects of nitrification inhibitors on soil nitrification and ammonia volatilization in three soils with different pH. Agronomy, 11(8), 1–13. https://doi.org/10.3390/agronomy11081674

De Boer, W., Kowalchuk, G. A. 2001. Nitrification in acid soils: micro-organisms and mechanisms. Soil Biology and Biochemistry, 33(7-8), 853–866. https://doi.org/10.1016/S0038-0717(00)00247-9

Elrys, A. S., Raza, S., Elnahal, A. S. M., Na, M., Ahmed, M., Zhou, J., Chen, Z. 2020. Do soil property variations affect dicyandiamide efficiency in inhibiting nitrification and minimizing carbon dioxide emissions? Ecotoxicology and Environmental Safety, 202, 110875. https://doi.org/10.1016/J.ECOENV.2020.110875

Ernfors, M., Brennan, F. P., Richards, K. G., McGeough, K. L., Griffiths, B. S., Laughlin, R. J., Watson, C. J., Philippot, L., Grant, J., Minet, E. P., Moynihan, E., Müller, C. 2014. The nitrification inhibitor dicyandiamide increases mineralization-immobilization turnover in slurry-amended grassland soil. Journal of Agricultural Science, 152(S1), 137–S149. https://doi.org/10.1017/S0021859613000907

Fisk, L., Maccarone, L. D., Barton, L., Murphy, D. V. 2015. Nitrapyrin decreased nitrification of nitrogen released from soil organic matter but not amoA gene abundance at high soil temperatures. Soil Biology and Biochemistry, 88, 214-223. https://doi.org/10.1016/j.soilbio.2015.05.029

Fortuna, A. M., Wayne Honeycutt, C., Griffin, T. S., Larkin, R. P., Wayne, C., Mark, J., Richard, J. 2012. Links among nitrification, nitrifier communities, and edaphic properties in contrasting soils receiving dairy slurry. Journal of Environmental Quality, 41(1), 262–272. https://doi.org/10.2134/jeq2011.0202

Gao, J., Luo, J., Lindsey, S., Shi, Y., Sun, Z., Wei, Z., Wang, L. 2021. Benefits and risks for the environment and crop production with application of nitrification inhibitors in China. Journal of Soil Science and Plant Nutrition, 21(1), 497–512. https://doi.org/10.1007/S42729-020-00378-9

Gilsanz, C., Báez, D., Misselbrook, T. H., Dhanoa, M. S., Cárdenas, L. M. 2016. Development of emission factors and efficiency of two nitrification inhibitors, DCD and DMPP. Agriculture, Ecosystems and Environment, 216, 1–8. https://doi.org/10.1016/J.AGEE.2015.09.030

Goring, C. A. 1962. Control of nitrification by 2-chloro-6-(trichloro-methyl) pyridine. Soil Science, 93(3), 211–218. https://journals.lww.com/soilsci/citation/1962/03000/control_of_nitrification_by.10.aspx

Guo, Y., Naeem, A., and Mühling, K. H. 2021. Comparative effectiveness of four nitrification inhibitors for mitigating carbon dioxide and nitrous oxide Emissions from three different textured soils. Nitrogen, 2(2), 155–166. https://doi.org/10.3390/nitrogen2020011

Habibullah, H., Nelson, K. A., Motavalli, P. P. 2018. Management of nitrapyrin and pronitridine nitrification inhibitors with urea ammonium nitrate for winter wheat production. Agronomy, 8(10). https://doi.org/10.3390/agronomy8100204

Han, J., Shi, J., Zeng, L., Xu, J., Wu, L. 2017. Impacts of continuous excessive fertilization on soil potential nitrification activity and nitrifying microbial community dynamics in a greenhouse system. Journal of Soils and Sediments, 17(2), 471–480. https://doi.org/10.1007/S11368-016-1525-Z/FIGURES/4

Herr, C., Mannheim, T., Müller, T., Ruser, R. 2020. Effect of nitrification inhibitors on N_2O emissions after cattle slurry application. Agronomy, 10(8), 1174. https://doi.org/10.3390/agronomy10081174

Isobe, K., Koba, K., Otsuka, S., Senoo, K. 2011. Nitrification and nitrifying microbial communities in forest soils. Journal of Forest Research, 16(5), 351–362. https://doi.org/10.1007/s10310-011-0266-5

Lasisi, A. A., Akinremi, O. O., Zhang, Q., and Kumaragamage, D. 2020. Efficiency of fall versus spring-applied urea-based fertilizers treated with urease and nitrification inhibitors I. Ammonia volatilization and mitigation by NBPT. Soil Science Society of America Journal, 84(3), 949–962. https://doi.org/10.1002/SAJ2.20062

Lu, Y., Zhang, X., Jiang, J., Kronzucker, H. J., Shen, W., and Shi, W. 2018. Effects of the biological nitrification inhibitor 1, 9-decanediol on nitrification and ammonia oxidizers in three agricultural soils. Elsevier. https://doi.org/10.1016/j.soilbio.2018.11.008

Marsden, K. A., Marín-Martínez, A. J., Vallejo, A., Hill, P. W., Jones, D. L., Chadwick, D. R. 2016. The mobility of nitrification inhibitors under simulated ruminant urine deposition and rainfall: a comparison between DCD and DMPP. Biology and Fertility of Soils, 52(4), 491–503. https://doi.org/10.1007/S00374-016-1092-X

McGeough, K. L., Watson, C. J., Müller, C., Laughlin, R. J., Chadwick, D. R. 2016. Evidence that the efficacy of the nitrification inhibitor dicyandiamide (DCD) is affected by soil properties in UK soils. Soil Biology and Biochemistry, 94(3), 222–232. https://doi.org/10.1016/j.soilbio.2015.11.017

McKenzie, R. H., Bremer, E., Grant, C. A., Johnston, A. M., DeMulder, J., and Middleton, A. B. 2006. In-crop application effect of nitrogen fertilizer on grain protein concentration of spring wheat in the Canadian prairies. Canadian Journal of Soil Science, 86(3), 565–572. https://doi.org/10.4141/S05-026

Nelson, K. A. 2018. Pronitridine nitrification inhibitor with urea ammonium nitrate for corn. Journal of Agricultural Science, 10(6), 16. https://doi.org/10.5539/jas.v10n6p16

Owens, J. L., Wang, Z., Thomas, B. W., Hao, X., Coles, K., Rahmani, E., Karimi, R., Gill, K., Beres, B. L. 2023. Winter wheat responses to enhanced efficiency liquid nitrogen fertilizers in the Canadian Prairies. Canadian Journal of Plant Science. https://doi.org/10.1139/CJPS-2022-0208

Pasda, G., Hähndel, R., Zerulla, W. 2001. Effect of fertilizers with the new nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops. Biology and Fertility of Soils, 34(2), 85–97.

https://doi.org/10.1007/S003740100381/METRICS

Pawlick, A. A., Wagner-Riddle, C., Parkin, G. W., Berg, A. A. 2019. Assessment of nitrification and urease inhibitors on nitrate leaching in corn (Zea mays L.). Canadian Journal of Soil Science, 99(1), 80–91. https://doi.org/10.1139/CJSS-2018-0110

Sahrawat, K. L. 2008. Factors affecting nitrification in soils. Communications in Soil Science and Plant Analysis, 39(9–10), 1436–1446. https://doi.org/10.1080/00103620802004235

Schmidt, R., Wang, X. B., Garbeva, P., Yergeau, É. 2020. Nitrapyrin has far-reaching effects on the soil microbial community structure, composition, diversity, and functions. BioRxiv. https://doi.org/10.1101/2020.07.21.205765

Singh, G., Nelson, K. A. 2019. Pronitridine and nitrapyrin with anhydrous ammonia for corn. Journal of Agricultural Science, 11(4), 13. https://doi.org/10.5539/jas.v11n4p13

Subbarao, G., Ito, O., Sahrawat, K., Berry, W., Nakahara, K., Ishikawa, T., Watanabe, T., Suenaga, K., Rondon, M., Rao, I. 2006. Scope and strategies for regulation of nitrification in agricultural systems - challenges and opportunities. Critical Reviews in Plant Sciences, 25(4), 303–335. https://doi.org/10.1080/07352680600794232

Thomsen, I. K., Schjønning, P., Olesen, J. E., Christensen, B. T. 2003. C and N turnover in structurally intact soils of different textures. Soil Biology and Biochemistry, 35(6), 765–774. https://doi.org/10.1016/S0038-0717(03)00093-2

Touchton, J. T., Hoeft, R. G., Welch, L. F., Mulvaney, D. L., Oldham, M. G., Zajicek, F. E. 1979. N uptake and corn yield as affected by applications of nitrapyrin with anhydrous ammonia 1. Agronomy Journal, 71(2), 238–242. https://doi.org/10.2134/agronj1979.00021962007100020006x

Vaio, N. 2006. Ammonia volatilization and N-uptake from urea, urea ammonium nitrate (UAN), and Nitamin® (urea-polymer) applied to tall fescue in Georgia. https://getd.libs.uga.edu/pdfs/vaio_nicolas_200612_ms.pdf

Vogeler, I., Blard, A., Bolan, N. 2007. Modeling DCD effect on nitrate leaching under controlled conditions. Soil Research, 45(4), 310-317. https://doi.org/10.1071/SR06177

Volpi, I., Laville, P., Bonari, E., di Nasso, N. N. o., Bosco, S. 2017. Improving the management of mineral fertilizers for nitrous oxide mitigation: The effect of nitrogen fertilizer type, urease, and nitrification inhibitors in two different textured soils. Geoderma, 307, 181–188. https://doi.org/10.1016/J.GEODERMA.2017.08.018

Wakelin, S., Williams, E., O'Sullivan, C. A., Cameron, K. C., Di, H. J., Cave, V., O'Callaghan, M. 2014. Predicting the efficacy of the nitrification inhibitor dicyandiamide in pastoral soils. Plant and Soil, 381(1–2), 35–43. https://doi.org/10.1007/S11104-014-2107-8

Waqas, M. A., Ashraf, M. N., Ahmed, W., Wang, B., Sardar, M. F., Ma, P., Li, Runan., Wan, Yunfan., Kuzyakov, Y. 2021. Long-term warming and elevated CO2 increase ammonia-oxidizing microbial communities and accelerate nitrification in paddy soil. Applied Soil Ecology, 166, 104063.

Waqas, M., Ashraf, M. N., Ahmed, W., Wang, B., Sardar, M. F., Ma, P., Kuzyakov, Y. 2021. Long-term warming and elevated CO_2 increase ammonia-oxidizing microbial communities and accelerate nitrification in paddy soil. Applied Soil Ecology, 166, 104063. https://doi.org/10.1016/j.apsoil.2021.104063

Wolt, J. D. 2000. Nitrapyrin behavior in soils and environmental considerations. Journal of Environmental Quality, 29(2), 367–379. https://doi.org/10.2134/jeq2000.00472425002900020002x

Xia, Q., Rufty, T., Shi, W. 2020. Soil microbial diversity and composition: Links to soil texture and associated properties. Soil Biology and Biochemistry, 149, 107953. https://doi.org/10.1016/j.soilbio.2020.107953

Yu, Q., Chen, Y., Ye, X., Zhang, Q., Zhang, Z., Tian, P. 2007. Evaluation of nitrification inhibitor 3, 4-dimethylpyrazole phosphate on nitrogen leaching in undisturbed soil columns. Chemosphere, 67(5), 872–878. https://doi.org/10.1016/j.chemosphere.2006.11.016

Zebarth, B. J., Forge, T. A., Goyer, C., Brin, L. D. 2015. Effect of soil acidification on nitrification in soil. Canadian Journal of Soil Science, 95(4), 359–363. https://doi.org/10.4141/cjss-2015-040

Zerulla, W., Barth, T., Dressel, J., Erhardt, K., Horchler von Locquenghien, K., Pasda, G., Rädle, M., Wissemeier, A. 2001. 3,4-Dimethylpyrazole phosphate (DMPP) - A new nitrification inhibitor for agriculture and horticulture. An introduction. Biology and Fertility of Soils, 34(2), 79–84. https://doi.org/10.1007/s003740100380

4. SYNTHESIS

4.1 Project Accomplishments

The imperative to enhance global food production drives the continual application of essential plant nutrients through fertilizers, with nitrogen (N) playing a pivotal role, frequently applied to soil to optimize crop growth (Dimkpa et al., 2023). Anhydrous ammonia (AA) and urea ammonium nitrate (UAN) are common N sources, with AA applied in the late fall and UAN widely used for in-season applications across Canada. While the late fall application of AA in north-western Canadian provinces provides the benefits of reduced workloads and lower fertilizer costs, its susceptibility to losses prompts farmers to apply 10-20% more N to compensate. It is advised to apply AA in late fall in Manitoba when soil temperature reaches 10°C. While soil freezing suppresses microbial activity, late fall AA is still susceptible to nitrification and associated losses till early spring. Overwinter and early-spring losses, particularly nitrate (NO_3^-) leaching and nitrous oxide (N_2O) emissions impact N use efficiency, prompting farmers to stabilize fall AA for effectiveness. UAN, extensively used for in-season applications, offers versatile nutrient management with readily available NO_3^-, longer-lasting NH_4^+, and sustained feeding from urea. Although UAN has a lower volatilization potential than urea, stabilizing its NH_4^+ fraction is essential to synchronize N-availability with plant uptake for increased efficiency. Emphasizing the importance of stabilizing N in NH_4^+ form and a gradual release of NO_3^- based on crop needs highlights the necessity of precise nutrient management. One recommended approach to stabilize N is using nitrification inhibitors (NIs), which delay nitrification, reducing N losses through leaching or denitrification. Extensive research supports the significant impact of NIs in reducing N losses, though their effectiveness may vary depending on the site and year.

This research aimed to assess the impact of different NIs on delaying nitrification under field and laboratory conditions. A relatively new NI, pronitridine (Centuro, Koch Agronomic Services LLC, KS), was tested in comparison with the widely tested nitrapyrin (N-Serve, Corteva Agriscience) in delaying nitrification of late fall-applied AA until spring across three field sites. Limited literature exists on nitrapyrin with fall AA in Manitoba; small plot studies indicated inaccuracies in replicating farm-scale NI recommendations (Bailey, 1990; Wood, 2018). However, no field research has directly compared nitrapyrin and pronitridine in the region. Given the rapid soil cooling following AA application in late fall, evaluating NI effectiveness after prolonged frozen conditions becomes imperative. The primary objective of this study was to compare the efficacy of these NIs and determine whether their application could eliminate the need for additional N application during the late fall while ensuring optimal yields and N uptake in spring-sown crops.

This study successfully captured the relative concentrations of NH_4^+ and NO_3^-, especially during late fall before soil freezes, early spring during thaw, and late spring before planting. It also assessed the yield and N uptake from the spring wheat and canola at two sites. With three site years of sampling under changing soil and climatic conditions, this experiment comprehensively investigated the effect of tested NIs across southern Manitoba on well-drained clay and loam soils. Furthermore, this study highlighted the contrasting climatic conditions between the 2021 and 2022 growing seasons, with 2021 experiencing drought and 2022 having above-normal precipitation. On averaging treatment effects across sites, nitrapyrin and pronitridine tended to show a slight reduction in NO_3^- accumulation between the bands compared to late fall-applied AA at the same rate without NIs. However, the impact of these NIs on delaying nitrification in late fall-banded AA across all three field sites was not significant. Furthermore, no significant differences were observed in agronomic yield and N uptake among the AA treatments, whether applied at 100% or 80% of the recommended N rate, without or with NIs. Our findings do not strongly support the recommendation of using NIs with late fall AA banding in well-drained loam and clay soils.

The study reported in Chapter 4 compared the efficacy of four different NIs—pronitridine (Centuro), DCD (Drive-N), DMPP (ARM-U advanced), and nitrapyrin (eNtrench)—in delaying nitrification in UAN under laboratory conditions in three contrasting soils. This study found nitrapyrin effective in sand soil, retaining higher NH_4^+ levels and reducing NO_3^- accumulation significantly on days 9, 14, 21, and 28. DCD slightly reduced nitrification, mainly in sand soil, on days 14 and 21. DMPP and pronitridine were less effective across three soil types. Overall, in clay, nitrapyrin inhibited nitrification by approximately 15% from days 7 to 21, showing reduced effectiveness by day 28. DCD reduced NO_3^- accumulation by 19% to 6% from days 7 to 21, while pronitridine had limited effectiveness, ranging from 5% to 13%. However, DMPP demonstrated only 24% and 15% nitrification inhibition rates on days 7 and 9, respectively. Loam soil exhibited greater but not significant nitrification inhibition by all NIs on day 7, with inhibition rates reaching 43%, 54%, 45%, and 38% for pronitridine, DCD, DMPP, and nitrapyrin, respectively. In sand, nitrapyrin was notably effective, with inhibition efficiencies ranging from 15% to 40%, while DCD inhibited nitrification from 11% to 20%. Pronitridine and DMPP showed relatively less effectiveness, with inhibition rates ranging between 5% and 10%, exhibiting slight effects on specific days. These results underscore the necessity for further laboratory investigations to validate these results and comprehend the behaviour of inhibitors upon soil injection.

4.2 Surprising and Interesting Observations

The field study showed no significant variations in grain yield and N uptake among different AA treatments. However, N uptake by wheat plants at the Notre Dame site tended to be slightly lower with pronitridine compared to the same rate of N applied as AA without any NIs treatment. This finding contradicts previous work (Gao et al., 2015; Cui et al., 2022), suggesting that NIs may primarily delay nitrification. However, their impact on increasing the efficiency of N use by the crops depends on the synchrony between N availability and plant uptake requirements. Significant variations in precipitation between 2021 and 2022 led to interesting and somewhat opposing trends in NO_3^- movement from on to between the bands across both years. At the droughty Sperling site, NO_3^- tended to be recovered more on bands during late spring sampling with NIs. This, coupled with a tendency for lower NO_3^- between bands during late spring sampling at Sperling, indicates delayed nitrification, possibly limited NO_3^- diffusion from bands by planting time or a lack of water for movement. However, in 2022, at Notre Dame and Manitou, there was a tendency for lower NO_3^- concentrations on bands with both NIs at the early spring sampling and for both sites with N-Serve at the late spring sampling. At Manitou, NO_3^- concentration between bands tended to be lower for NIs, indicating a potential reduction in NO_3^- diffusion from bands by planting. Nevertheless, the retention of NH_4^+, crop yield, and N uptake using NIs were not significantly different from AA applied without NIs. In the laboratory study, clay and loam soils exhibited substantial variation in their NO_3^- levels within replicates. The NIs, DMPP, and pronitridine did not appear to delay nitrification in UAN across all soil types effectively. The observed ineffectiveness of DMPP might be attributed to a poorly formulated product, while the rates of pronitridine may require adjustment based on soil conditions.

4.3 Challenges and Improvements

Sampling on and between the AA bands technique offers advantages, as it can detect small differences in NO_3^- concentrations. However, marking the AA bands proved challenging, especially at the Manitou site, due to good sealing. Sampling between bands generally 15 cm away from the AA-banded rows can increase the likelihood of errors. Therefore, future field trials should adopt a standardized protocol for delineating the AA bands and utilize wooden blocks with pointed holes for precise sampling on and between band positions. Furthermore, sampling from research strips at the farm scale requires more samples per strip to accommodate field variability, which entails additional labour resources. One potential approach to address the substantial variability within the farm locations is identifying critical variability zones to avoid executing treatment strips in these areas, strategically allocating replicates, or planning sampling while considering the variable zones. One interesting observation in our results is that the deviation or standard errors of means become much smaller each year in late spring, at the end of the growing season. This suggests that the team may become well-trained by the end of the growing season. We encountered several challenges in the field study, particularly our interactions with farmers. Farmers are typically occupied with wrapping up their harvest operations and AA applications before the onset of snowfall in the fall. Consequently, field research activities rely heavily on the cooperation of farmers. Additionally, when mixing AA with N-Serve and Centuro, it is essential to conduct rate and equipment testing before implementing commercial plot fertilization in consultation with the farmers. Therefore, arranging a meeting with the farmer before the trial would be beneficial when planning future commercial field trails. Additionally, I conducted two detailed research trials in the laboratory study, each involving over 500 microcosms. However, we faced challenges in achieving the emission factor for nitrous oxide (N_2O). Moreover, handling NIs at the micro level was tricky, which may have influenced their claimed efficiency in our study. Therefore, it is suggested that fertilizer and NIs solutions should be prepared and mixed in bulk to mitigate the issue of reduced active ingredient concentration and minimize random error. This approach will help prevent underestimating the research efficiency of the product.

4.4 Future Work

Recent studies in Illinois have suggested applying half of the recommended N rate in the fall, with the remaining half applied during spring applications (Nafziger, 2022). Considering economic evaluations, reducing the late fall AA application rate and applying the remainder during seeding or in-season is plausible. This reduction in fall AA application rates can optimize NUE compared to other N options. Checking N levels before planting enhances farm N management, enabling adjustments in spring applications. Overall, suppose similar yields and protein levels can be maintained with lower application rates, in conjunction with reduced NIs' prices. In that case, the economic feasibility of consistent NIs usage within the agricultural community may be justified. Furthermore, it is suggested that tests be conducted on a commercial scale to evaluate the impact of NIs on N_2O emissions, especially in soils prone to N_2O emissions (Adhikari et al., 2021). Additionally, future research should focus on comparing the potential of different NIs in reducing N losses through various pathways and their direct role in improving agricultural commodities' yield and protein content under varying soil and climatic conditions. The ineffectiveness of NIs in our laboratory experiment suggests that further studies are needed to examine the partitioning of the inhibitors from UAN upon syringe injection into the soil.

4.5 Recommendations for Growers and Policy Makers

Our findings do not provide substantial evidence to support the recommendation of using NIs with late fall AA banding in poorly-drained clay and moderately well-drained loam soils. Based on three site years of data, farmers should delay AA application until the soil temperature falls below 10°C. However, farmers aiming to extend the fall N application period from late October to mid-September or early October may consider incorporating NIs as an additional safeguard for optimizing their N investments. Additionally, efforts should be made to reduce the cost of NIs and incentivize growers to use them more frequently. Therefore, policymakers must consider subsidizing growers to reduce the overall cost of adopting NIs. Another viable option is to offer carbon credits to growers who incorporate NIs into their farm management practices. These supportive measures should hold particular significance for federal policymakers, given Canada's commitment to the Paris Climate Agreement, which aims to reduce greenhouse gas emissions across all major industry sectors. Furthermore, it is worth mentioning that adopting reduced rates of AA as late-fall N applications on well-drained soils can prove to be cost-effective for growers. Nevertheless, sustained application of reduced N rates in the long run necessitates careful consideration of farm profitability.

4.6 References

Adhikari, K. P., Chibuike, G., Saggar, S., Simon, P. L., Luo, J., de Klein, C. A. 2021. Management and implications of using nitrification inhibitors to reduce nitrous oxide emissions from urine patches on grazed pasture soils–A review. Science of The Total Environment, 791, 148099.

Bailey, L.D. 1990. The effects of 2-chloro-6-(trichloromethyl)-pyridine ('N-Serve') and fertilizers on productivity and quality of Canadian oilseed rape. Canadian Journal of Plant Science, 70, 979–986. https://doi.org/10.4141/cjps90-120

Cui, L., Li, D., Wu, Z., Xue, Y., Xiao, F., Gong, P., Zheng, Y. 2022. Effects of combined nitrification inhibitors on soil nitrification, maize yield, and nitrogen use efficiency in three agricultural soils. Public Library of Science One, 17(8), e0272935. https://doi.org/10.1371/journal.pone.0272935

Dimkpa, C., Adzawla, W., Pandey, R., Atakora, W. K., Kouame, A. K., Jemo, M., Bindraban, P. S. 2023. Fertilizers for food and nutrition security in sub-Saharan Africa: An overview of soil health implications. Frontiers in Soil Science, 3. https://doi.org/10.3389/fsoil.2023.1123931

Gao, X., Asgedom, H., Tenuta, M., and Flaten, D. N. 2015. Enhanced efficiency urea sources and placement effects on nitrous oxide emissions. Agronomy Journal, 107(1), 265–277. https://doi.org/10.2134/agronj14.0213

Nafziger, E. 2022. Fall Nitrogen. Department of Crop Sciences, University of Illinois. The Bulletin. October 19, 2022. Accessed February 20, 2024. https://farmdoc.illinois.edu/field-crop-production/fall-nitrogen.html

APPENDICES

Appendix A: Supplementary Material for Chapter 2

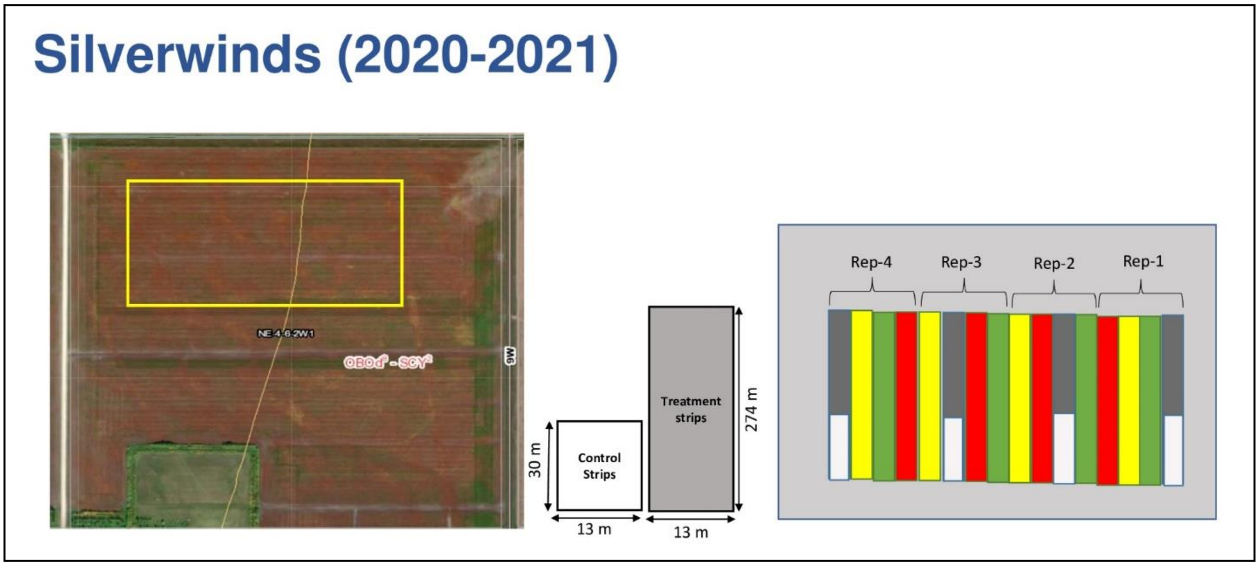


Fig. A-1 Layout and site map of the Silverwinds site treatment zones. White indicates control areas, dark grey represents AA applied at 100% of the recommended N rate, green signifies AA applied at 80% of the recommended N rate, yellow depicts AA applied at 80% of the recommended N rate with nitrapyrin, and red indicates AA applied at 80% of the recommended N rate with pronitridine.

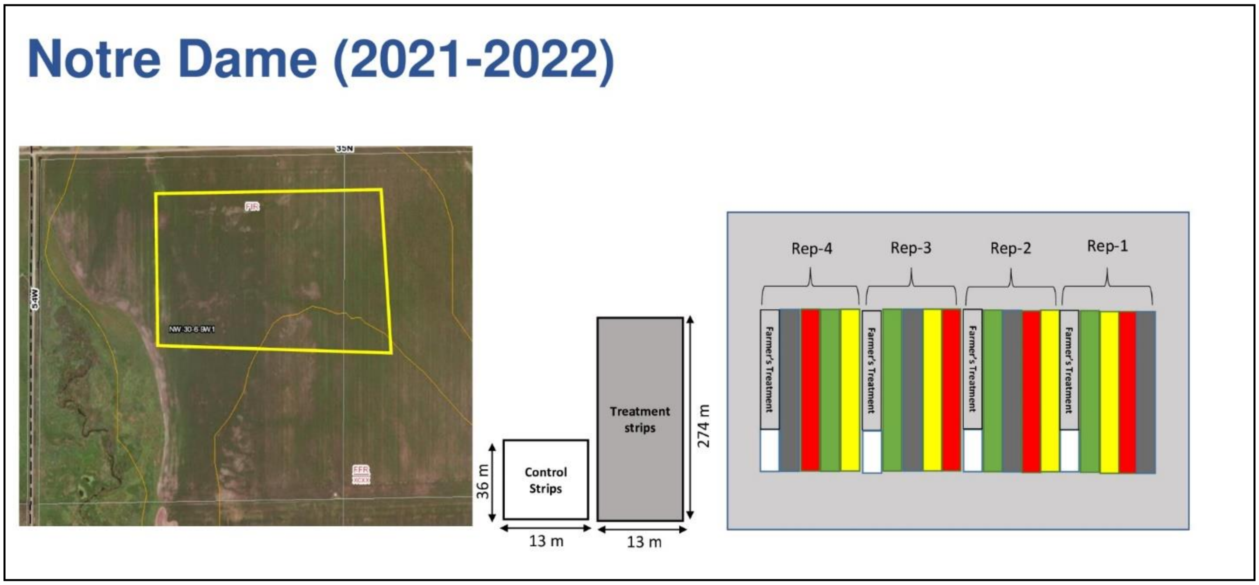


Fig. A-2 Layout and site map of the Notre Dame site treatment zones. White indicates control areas, dark grey represents AA applied at 100% of the recommended N rate, green signifies AA applied at 80% of the recommended N rate, yellow depicts AA applied at 80% of the recommended N rate with nitrapyrin, and red indicates AA applied at 80% of the recommended N rate with pronitridine. Farmer's Treatment was AA applied at 100% of the recommended N rate with nitrapyrin (not part of the study).

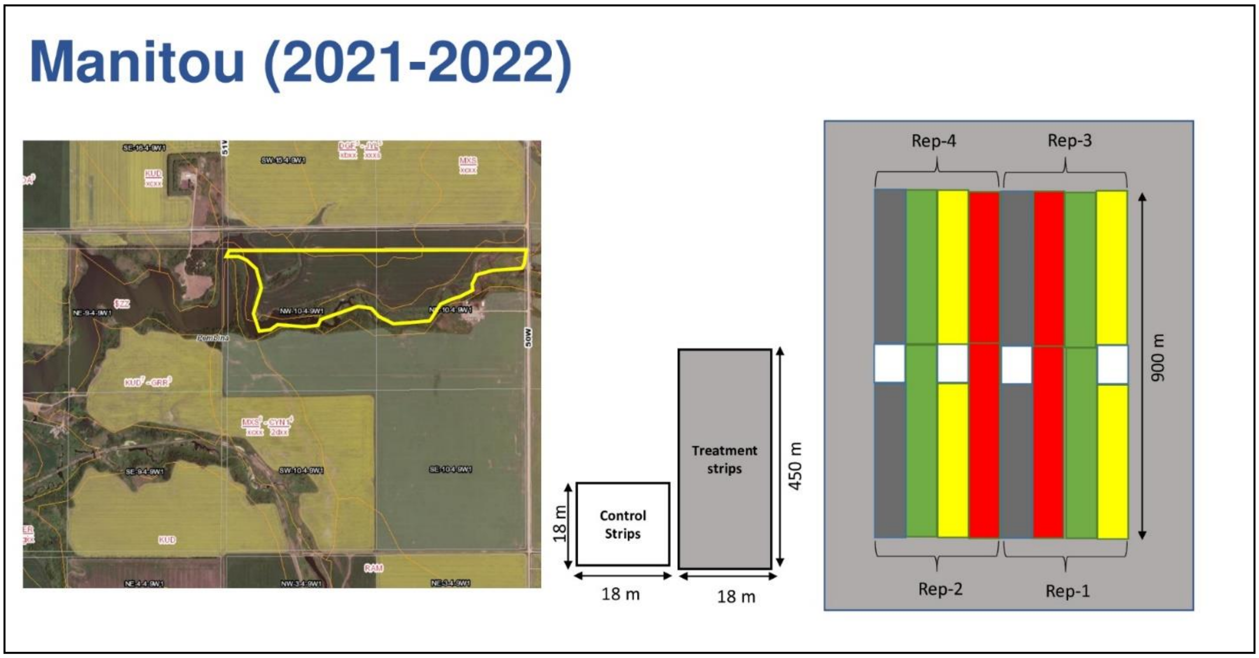


Fig. A-3 Layout and site map of the Manitou site treatment zones. White indicates control areas, dark grey represents AA applied at 100% of the recommended N rate, green signifies AA applied at 80% of the recommended N rate, yellow depicts AA applied at 80% of the recommended N rate with nitrapyrin, and red indicates AA applied at 80% of the recommended N rate with pronitridine.

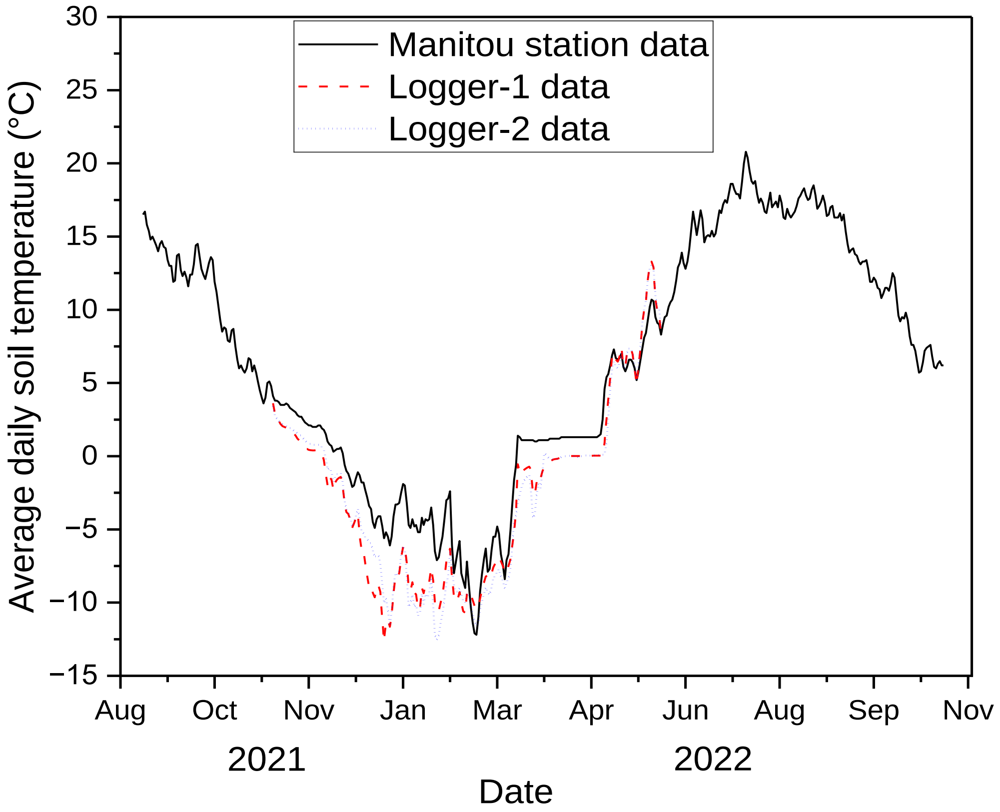


Fig. A-4 Comparison of average daily soil temperatures measured by two HOBO loggers buried (15 cm) at the Manitou site on the first sampling date with Ag meteorology data for the respective season (2021-2022). Minimal differences were observed, but weather station data was used for consistency due to its continuous records from the day of AA application onward.

Table A-5 ANOVA p-values for extractable nitrogen at each sampling time, and for crop yield and nitrogen uptake.

		Late Fall						**Early Spring**						**Late Spring**							
Effect		**On bands**			**Off bands**			**On bands**			**Off bands**			**On bands**			**Off bands**				
Site	**Trt**	NH_4^+	NO_3^-	**Total N**	NH_4^+	NO_3^-	**Total N**	NH_4^+	NO_3^-	**Total N**	NH_4^+	NO_3^-	**Total N**	NH_4^+	NO_3^-	**Total N**	NH_4^+	NO_3^-	**Total N**	**Yield**	**N uptake**
Silverwinds (2020-21)	**Trt**	**0.0126**	0.0597	**0.0059**	0.0824	0.3457	0.2948	**0.0023**	**0.0015**	**<0.0001**	0.4021	0.2545	0.2666	**0.0011**	**0.0018**	**0.0003**	**0.0208**	0.1165	0.1066	…….	…….
Notre Dame (2021-22)	**Trt**	**0.0048**	0.3224	**0.0045**	0.3835	0.4517	0.2365	**<0.0001**	**<0.0001**	**0.0053**	0.5252	**0.0484**	0.0626	**0.0029**	**0.0035**	**<0.0001**	0.0600	0.0808	0.0972	<0.0001	<0.0001
Manitou (2021-22)	**Trt**	**0.0026**	0.0596	**0.0032**	0.1771	0.1435	0.1306	**0.0003**	**0.0226**	**0.0012**	0.6816	0.721	0.8269	**0.0203**	**0.0008**	**0.0009**	0.4138	0.0715	0.1066	…….	0.7591

Table A-6 Details on crop history, types of crops sown, and the application of fungicides and herbicides at each respective site.

Site	**Previous Crop**	**Targeted Crop**	**Crop Variety**	**Sowing date**	**Herbicide**	**Fungicide**	**Harvest date**	**Target Yield (Mg ha^{-1})**
Silverwinds (2020-21)	Oats	Canola	L357	Jun-17-21	Liberty, Centurion	------------	-----------	3.36
Notre Dame (2021-22)	Soybean	Wheat	Starbuck	May-28-22	0.2 L ha^{-1} Axil Extreme, 0.08 L ha^{-1} MCPA[a]	Miravis.	Sep-13-22	5.71
Manitou (2021-22)	Wheat	Canola	Invigor-233PC	June-17-22	Liberty	Nexicor, Interlock	Oct-10-22	3.36

[a] 2-methyl-4-chlorophenoxyacetic acid

[b] All fungicides, herbicides, and insecticides were applied at their recommended rates

Appendix B: Supplementary Material for Chapter 3

Table B-1 ANOVA p-values for the impact of different nitrogen treatments (excluding controls) on extractable nitrogen concentrations at different sampling days for each soil type.

		Sampling Day															
Effect		0		1		3		7		9		14		21		28	
		NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
Clay	Trt	1.000	1.000	0.6476	0.5176	0.6069	0.8967	0.4651	0.6443	0.1629	0.2829	0.0484*	0.1788	0.0131*	0.2799	0.0070*	0.1194
Loam	Trt	1.000	1.000	0.4663	0.4863	0.9637	0.4353	0.0255*	0.7306	0.1369	0.4723	<.0001*	0.2607	0.0703	0.9863	<.0601	0.5043
Sand	Trt	1.000	1.000	0.7669	0.5835	0.7332	0.5669	0.1685	0.1251	0.0065*	0.0245*	0.0013*	0.0004*	0.0090*	0.0040*	0.0028*	0.1368

* Asterisks within each soil type indicate significant differences ($p < 0.05$) between nitrogen treatments (excluding controls) for the respective days.

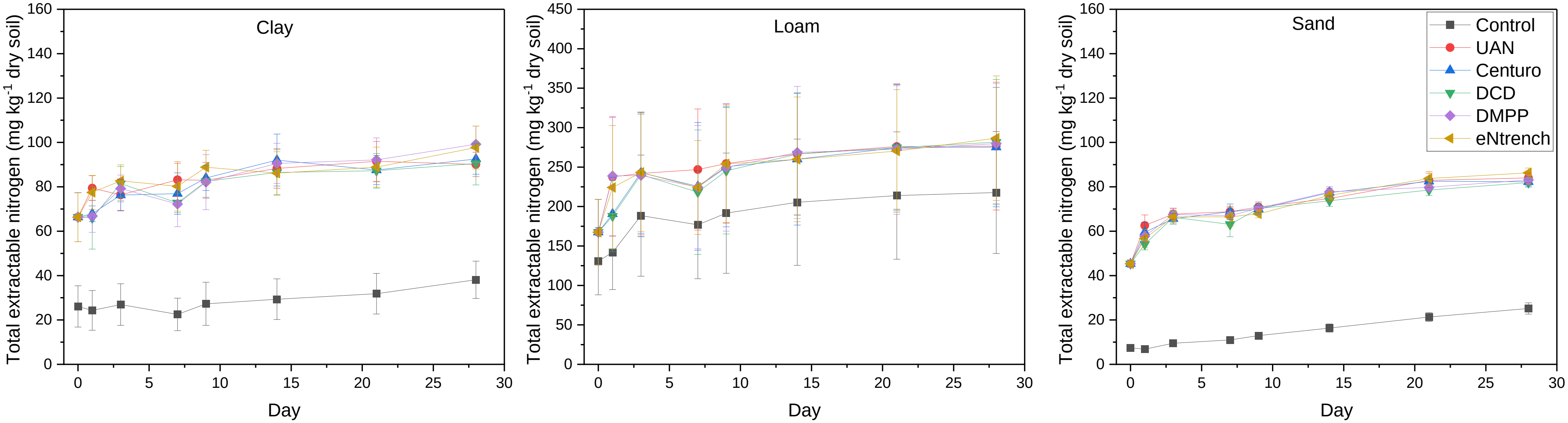


Fig. B-2 Effect of nitrification inhibitors on total extractable nitrogen (ammonium + nitrate) concentrations in clay, loam, and sand soil (400 g in microcosms) during 28 days of incubation at 20 °C and 65% water-filled pore space (WFPS). Control: Without nitrogen and any nitrification inhibitor; UAN: 60 mg kg^{-1} dry soil N added as Urea ammonium nitrate (UAN); Pronitridine: Centuro + UAN; DCD: Drive-N + UAN; DMPP: ARM U advanced + UAN; Nitrapyrin: eNtrench + UAN. Error bars indicate the standard errors of the means (n = 4). ARM U advanced, utilized in the lab study, was custom-ordered for research purposes, containing exclusively DMPP as its active ingredient.

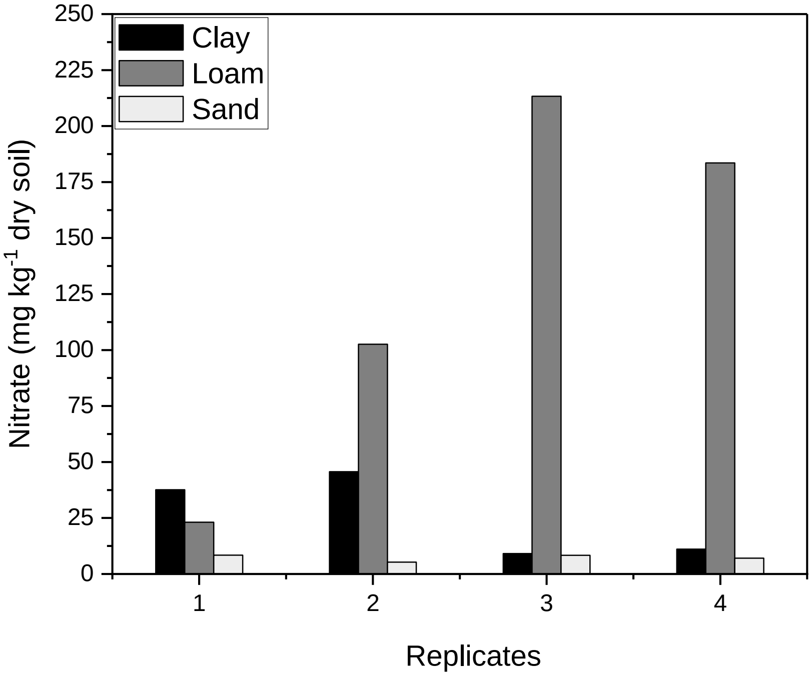


Fig. B-3 Variations in nitrate (NO_3^-) levels observed in control replicates of three soils used for the laboratory experiment.