CHARACTERIZING THE EFFICACY OF NOVEL NITROGEN STABILIZER PRODUCTS AT REDUCING FERTILIZER NITROGEN LOSSES

By

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ABSTRACT

Sithabile Edith Nyathi, M.Sc., The University of Manitoba, June 2024. Characterizing The Efficacy of Novel Nitrogen Stabilizer Products at Reducing Fertilizer Nitrogen Losses

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Urea-based fertilizers (urea and urea ammonium nitrate (UAN)) are susceptible to nitrate (NO_3^-) leaching, ammonia (NH_3^-) volatilization, and nitrous oxide (N_2O) emission losses, resulting in low fertilizer nitrogen (N) efficiency and presenting a risk to human and environmental health. Nitrogen stabilizer products containing the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) and the nitrification inhibitor 3,4-dimethyl pyrazole phosphate (DMPP) can enhance wheat yield and optimize fertilizer efficiency while mitigating N losses. However, their high price hinders wider adoption by farmers. This study examined the efficacy of potentially cost-effective, double inhibitor (DI) formulations in decreasing N losses and improving crop N efficiency. Specifically, the study assessed the impacts of ARMU Advanced (ARMU-Adv) formulations (i.e., NBPT:DMPP ratios of 1:1 and 1:0.5) at mitigating N losses and improving wheat biomass yield and N efficiency. A no-inhibitor and a no-fertilizer (control) treatment were included for comparison. Results showed that the ARMU-Adv formulations were effective at reducing NH_3 volatilization and N_2O emission and improving shoot N uptake and apparent N recovery (ANR) relative to untreated fertilizers. Overall, the ARMU-Adv formulations were more effective with urea than with UAN in reducing NH_3 volatilization and enhancing shoot N uptake and ANR, whereas inhibitor-treated UAN showed greater efficacy in mitigating N_2O losses relative to urea. These findings demonstrate the potential of ARMU-Adv formulations to reduce N losses and enhance yield and N efficiency in wheat-based cropping systems.

Key words: double inhibitor, urease inhibitor, nitrification inhibitor, NBPT, DMPP, ammonia volatilization, nitrous oxide, nitrate, wheat, urea, urea ammonium nitrate

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FOREWORD

This thesis was prepared in manuscript style and followed the thesis standards of the Department of Soil Science at the University of Manitoba. The thesis consists of 3 chapters, the first of which is the literature review (Chapter 1). Chapter 2 was written as an individual research manuscript. Chapter 3 is the overall synthesis of the thesis. The research reported in Chapter 2 examined the efficacy of urea and urea ammonium nitrate (UAN) fertilizers treated with two formulations (i.e., NBPT:DMPP ratios of 1:1 and 1:0.5) of a novel double-inhibitor, ARMU-Advanced, on ammonia (NH_3) volatilization, nitrate (NO_3^-) leaching, nitrous oxide (N_2O) emission and crop (wheat) response. The overall synthesis (Chapter 3) includes the implications of the study and recommendations for future research.

CONTRIBUTION OF AUTHORS

The authors' contributions for Chapter 2 are as follows:

- Sithabile Nyathi: Analyzed data, interpreted findings, authored the initial manuscript draft
- Francis Zvomuya: Oversaw project administration and supervision, secured funding, and reviewed and edited manuscript drafts
- Theresa Adesanya: Gathered data, supervised the project, and reviewed and edited manuscript drafts
- Xiaopeng Gao: Advisory committee, reviewed manuscript drafts
- Inoka Amarakoon: Advisory committee, reviewed manuscript drafts

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1. LITERATURE REVIEW

1.1 Introduction

Nitrogen (N) is an essential element for organisms and plays a vital role in their growth. In agricultural systems, N is one of the most crucial crop-limiting macronutrients, essential for maximizing crop yields and ensuring food security for the rapidly increasing global population (Malhi et al., 2001). Despite its abundance as nitrogen gas (N_2) in the atmosphere, plants cannot directly utilize N_2 due to its unreactive nature (Ohyama et al., 2010). The N_2 must undergo various transformations before it can be utilized by crops for growth (Galloway et al., 2003). Prior to the extensive adoption of synthetic fertilizers, biological nitrogen fixation (BNF) was the predominant natural mechanism for converting N_2 into forms (ammonium (NH_4^+) and nitrate (NO_3^-)) that plants could utilize (Grant et al., 2001; Malhi et al., 2001). The transition from dependence on BNF to synthetic N fertilizers has been driven by the inability of BNF to provide sufficient N to meet the food needs of a growing population (Ladha et al., 2022). For more than a century, synthetic fertilizers have consistently supported global agriculture, leading to the concurrent expansion of both the global population and global wealth (Smil et al., 2001). Therefore, synthetic fertilizers will continue to be utilized to meet the substantial N demands of agricultural production (Smil et al., 1999).

In Canada, urea-based fertilizers are a vital to agriculture and account for over 50% of the sector's synthetic N fertilizer use (Fertilizer Canada, 2023). Urea-based fertilizers, such as granular urea (46% N) and liquid urea ammonium nitrate (UAN) (28-32% N) are widely utilized N sources due to their high efficiency when properly managed (Grant et al., 2001; Malhi et al., 2001). Despite the higher price of UAN in comparison to urea, UAN fertilizer is the preferred N fertilizer in Canada, the United States, and other countries because it integrates various forms of N, consequently enhancing efficacy and regulating soil N levels (Millar et al., 2010). Fifty percent of UAN N is in the ammonium nitrate form, ranging in N concentration from 28–32% by weight (Wang et al., 2020). As a result, UAN is less susceptible to NH_3 volatilization losses relative to urea, given that it contains only 50% urea-N, which can undergo hydrolysis (Peng et al., 2015).

Additionally, UAN has other beneficial attributes, such as uniform distribution and easy mixing (Mosaic Crop Nutrition, 2024). Various studies have emphasized the superiority of UAN in delivering optimal crop yield, increasing N availability in the soil and reducing excess soil N (Gagnon and Ziad et al., 2010; Fu et al., 2019; Sundaram et al., 2019; Ren et al., 2021). Nevertheless, urea remains the dominant N source, accounting for more than 50% of N fertilizers used in agriculture globally (Bremner et al., 2007; Suter et al., 2016). While UAN may present advantages in terms of efficiency and soil management, urea remains attractive to farmers due to its cost-effectiveness, high N content, ease of application, and adaptability to various soil types and farming practices (Glibert et al., 2006; Smith et al., 2007). It is noteworthy that proper application techniques, timing, and environmental conditions may greatly impact the effectiveness of both urea and UAN fertilizers (Sistani et al., 2011, 2014; Venterea et al., 2011).

While urea-based fertilizers offer several advantages, a considerable proportion of N is lost via various pathways, including ammonia (NH_3) volatilization, nitrous oxide (N_2O) emission, and NO_3^- leaching, when applied to the soil (Rawluk et al., 2001; Malhi et al., 2001). These losses not only affect crop production but also have substantial environmental consequences, contributing to water pollution and global climate change, which incur considerable costs to society (Mosier et al., 2001; Galloway et al., 2003; Bolan et al., 2004; Ravishankara et al., 2009). Ammonium nitrate is an alternate N fertilizer option that has been shown to effectively decrease NH_3 emissions relative to urea as it supplies N in a readily available form for plants, obviating the need for transformation processes necessary to render urea N bioavailable (Chambers and Dampney, 2009; Ti et al., 2019; Li et al., 2020). However, ammonium nitrate use in agriculture is now restricted for security reasons arising from its misuse in making explosives (Buczkowski et al., 2014; Kavický et al., 2014). Urea-based fertilizers therefore remain the dominant sources of N in agriculture.

1.2 Nitrogen Loss Pathways from Urea-Based Fertilizers

1.2.1 Ammonia Volatilization

Ammonia volatilization is a significant pathway of urea-based fertilizer N loss to the atmosphere. The volatilization process occurs when urea-based fertilizers are applied to the soil and undergo urea hydrolysis to NH_4^+ and CO_2, catalyzed by the enzyme urease (Mazzei et al., 2019). In addition to increasing the concentration of NH_4^+, which may then be taken up by plants (Silva et al., 2017), hydrolysis may also result in an episodic increase in soil pH in the vicinity of urea granules (Rochette et al. 2009). The increase in pH enhances the conversion of NH_4^+ to NH_3, leading to increased volatilization losses (Caicedo et al., 2000; Bouwman et al., 2002a). This process generally takes place within 3-7 d following urea application to the soil and may persist for several days (Jiang et al., 2023). Nevertheless, under dry conditions, the process may be delayed for up to 12 d until significant precipitation is received, highlighting the role of moisture in facilitating urea hydrolysis and subsequent NH_3 volatilization (Woodley et al., 2020).

Other factors that accelerate NH_3 volatilization include high pH, low cation exchange capacity (CEC), high wind intensity, high temperature, high soil N concentration and low soil organic matter (SOM) (Zhenghu and Honglang, 2000; Mikkelsen et al., 2009; Ma et al., 2010). When urea-based fertilizers are surface-applied under hot and humid conditions, up to 40% of the N applied might be lost as NH_3 (Cantarella et al., 2018). Results from several previous studies indicate negligible NH_3 volatilization under low temperature (e.g., Anderson et al., 2003; Fageria and Baligar, 2005; Hussain et al., 2021). However, significant NH_3 volatilization can still occur under low temperatures as a result of the extended duration of volatilization in colder conditions (Drury et al., 2017; Perin et al., 2020). The range of factors influencing the variability in NH_3 volatilization complicates its management and the implementation of effective mitigation techniques.

1.2.2 Nitrate Leaching

Nitrate leaching is a major route of N loss in agriculture and reduces the efficiency of urea and UAN N (Rawluk et al., 2001; Dawar et al., 2011). Leaching occurs when dissolved soil NO_3^- from fertilizers and other sources moves with percolating water down the soil profile (Banning et al., 2015; Bijay-Singh and Craswell, 2021). Urea-based fertilizers contain NH_4^+, which can be converted to NO_3^- through nitrification (Acharya et al., 2022). Nitrification is a two-step process wherein nitrifying bacteria such as Nitrosomonas and Nitrobacter facilitate the transformation of NH_4^+ to NO_3^- (Subbarao et al., 2012). During the first stage, Nitrosomonas bacteria transform NH_4^+ to nitrite (NO_2^-), which is then converted to NO_3^- by the nitrite-oxidizing microbe, Nitrobacter, in the second stage (Norton et al., 2008).

This two-step process is crucial for plants as they acquire most of their N in the NO_3^- form, which is an efficient energy source and promotes a well-balanced absorption of other essential nutrients (Clément et al., 2020). However, the high solubility of NO_3^- makes it susceptible to leaching (Addiscott et al., 1996). Nevertheless, it is noteworthy that nitrification has an impact on various aspects of N management in agricultural systems. Specifically, nitrification slows down urea hydrolysis and the subsequent loss of NH_3 into the atmosphere. This is because nitrification can lead to a decrease in soil pH, which reduces NH_3 volatilization

(Cantarella et al., 2018). The pH increase further interferes with Nitrobacter activity, resulting in the build-up of NO_2^- in the vicinity of urea granules (Monaghan and Barraclough, 1992). The accumulation of NO_2^- may subsequently inhibit seed germination and enhance N_2O emission (Bremner and Krogmeier, 1988; Firestone and Davidson, 1989).

Factors such as soil type, climate, and management practices have been shown to influence NO_3^- leaching (Di and Cameron, 2002). Intensive agronomic techniques such as the overapplication of urea-based fertilizers, combined with periods of heavy precipitation, can also accelerate NO_3^- leaching (Arregui and Quemada, 2006; Velthof et al., 2014). Coarse-textured soils with high permeability and minimal CEC are particularly susceptible to NO_3^- leaching losses (Acharya et al., 2022) whereas soils with higher proportions of silt, clay, and organic matter demonstrate greater retention of NO_3^- (Gaines and Gaines, 1994). Potato production is especially susceptible to NO_3^- leaching losses due to the requisite coarse textured soils, high N inputs, and high water requirement of potatoes, which necessitates supplemental irrigation (Zvomuya et al., 2003). Controlling NO_3^- leaching is therefore complex due to the multitude of influencing factors involved. The use of urease inhibitors, such as DCD and DMPP, has been suggested as a viable approach to reduce such losses (Zaman et al., 2009; Abalos et al., 2014).

1.2.3 Nitrous Oxide Emissions

Nitrification and denitrification are major pathways for the production of N_2O (Park et al., 2012). Denitrification is typically the primary contributor to N_2O emissions under anaerobic conditions (Wrage et al., 2001; Azam et al., 2002). Denitrification is a biological process in which denitrifying bacteria utilize NO_3^- as an electron acceptor under anaerobic conditions resulting in the conversion of NO_3^- to gaseous N forms, such as N_2O and N_2 (Mosier and Klemedtsson, 1994; Wrage et al., 2001). Nitrification and denitrification are driven by various factors, most notably moisture, which regulates soil O_2 concentrations (Gödde and Conrad, 2000; Ding et al., 2011). Therefore, an efficient strategy to lower N_2O losses should inhibit both processes, for example, through the use of urease and nitrification inhibitors (Di and Cameron, 2002).

Nitrogen fertilizers that undergo alkaline hydrolysis, such as urea, produce more N_2O through denitrification in saturated soil than those that undergo acidic hydrolysis, such as ammonium nitrate (Mulvaney et al., 1997). This is likely due to the increase in soil pH caused by alkaline fertilizers, which might enhance denitrification under wet conditions (Bollag et al. 1970). Soils with low pH (Mulvaney et al., 1997), low aeration (Mosier and Klemedtsson, 1994), and high soluble C and organic matter contents (Tenuta and Sparling, 2011) are the most prone to high denitrification losses. Denitrification is also an essential step in NO_3^- removal from the soil, which would otherwise accumulate and potentially be discharged in surface and groundwater, thereby causing water pollution and health risks (Seitzinger et al., 1988).

Therefore, to achieve sustainable N management, it is important to maintain a balance between reducing NO_3^- leaching and preventing N_2O emission from denitrification. Nitrification inhibitors (NIs), such as DMPP, can play a crucial role in maintaining this equilibrium by temporarily suppressing the microbial process of nitrification, thereby decreasing the production of N_2O while still allowing denitrification to occur (Zerulla et al., 2001). Application of commonly used NIs (DMPP and DCD), or the urease inhibitor NBPT or a combination of the two inhibitor types may be an effective strategy to increase N efficiency and crop yields (Abalos et al., 2014).

1.3 Implications of Nitrogen Loss from Urea-Based Fertilizers

1.3.1 Agronomic Implications

Spring wheat (*Triticum aestivum* L.) is an intensively grown crop in the Prairie provinces of Canada, contributing to the country's status as the sixth largest producer and third largest exporter of wheat in the world in 2019 (FAOSTAT, 2019). The cultivation of spring wheat and other cereal crops relies on applied fertilizer N to improve the nutritional value of the crop and enhance crop yield. However, the efficiency of urea N is often low due to various factors and N loss processes such as NH_3, NO_2^- and NO_3^-, thereby reducing the amount of N available for plant uptake, presenting an economic concern to farmers (Li et al., 2015; Tian et al., 2015). For instance, the efficiency of N recovery by cereal crops typically falls below 50%, suggesting that a significant fraction of applied N is lost or immobilized (Raun et al., 2002; Subbarao et al., 2006; Johnston and Bruulsema, 2014). Unlike spring wheat, crops like sugarcane are less dependent on fertilizer N because of their prolonged growth period, leading to reduced response to N fertilizers, and instead primarily utilizing mineralized N (Abalos et al., 2014).

Nitrogen losses, and therefore fertilizer N efficiency, vary with crop type and other factors such as fertilizer type and rate, and environmental conditions (Cai et al., 2002; Galloway et al., 2003; Congreves et al., 2021). The addition of N inhibitors to urea-based fertilizers has been shown to aid the synchronization of N release from applied fertilizer and crop uptake, thereby improving fertilizer N efficiency (Sigurdarson et al., 2018). Enhancement of crop N efficiency could translate to cost savings on fertilizers, reduced N losses, and enhanced crop yields (Baligar et al., 2001).

It is noteworthy that crops may take up substantial amounts of N from fertilizer but not effectively utilize it for growth and protein production in some cases (Gao et al., 2012). A crop’s ability to efficiently utilize N for its growth is evaluated based on N efficiency indices. There are various NUE indices, some of which have been partitioned into either fertilizer, plant, or soil-based indices (Congreves et al., 2021). This study examined indices such as apparent N recovery efficiency (ANR), agronomic efficiency (AE), partial factor productivity (PFP), fertilizer contribution rate (FCR), and plant-based indices such as nutrient efficiency ratio (NER) and physiological efficiency (PE). Fertilizer-based N efficiency indices measure the amount of fertilizer used in relation to other factors influencing a plant's growth and development.

Compared with fertilizer-based indices, plant-based indices are more insightful due to their emphasis on plant tissue N contribution to crop yield (Congreves et al., 2021). For example, the plant-based indices, NER and PE, provide a better understanding of how efficiently plants utilize N to improve yield and minimize fertilizer costs (Baligar et al., 2007; Congreves et al., 2021). By comparison, AE evaluates the effectiveness of N fertilizer in increasing crop growth, thus, providing a more precise measure of the fertilizer's efficiency. Another fertilizer index, ANR, is the ratio of yield to fertilizer N applied, and is a measure of plant N utilization. FCR quantifies the proportion of fertilizer N utilized by plants through the analysis of the proportion of labeled fertilizer added that is either absorbed by the plant or lost, while PFP is another helpful metric for assessing the efficiency of a cropping system's nutrient management (Moll et al., 1982; Yadav et al., 2003; Dobermann et al., 2007; Congreves et al., 2021). Assessing N indices is therefore crucial for the sustainability of agricultural systems.

1.3.2 Environmental Implications

Nitrogen losses from urea-based fertilizers also pose a substantial threat to the global environment. Nitrous oxide emissions from agricultural soils due to application of synthetic N fertilizers account for 70% of global N_2O emissions (Bouwman et al., 1995; Thomson et al., 2012; Tenuta et al., 2019). Nitrous oxide is a highly potent greenhouse gas (roughly 300 times than the potency of carbon dioxide (CO_2)) contributing to depletion of the ozone layer (Ravishankara et al., 2009). Agricultural soils contribute nearly half of the NH_3 that is volatilized globally, mostly from the use of synthetic N fertilizers (Sommer et al., 2004; Cameron et al., 2013). The process of NH_3 volatilization can further result in the release of N_2O (Ussiri and Lal, 2012). Ammonia can react with sulfuric acid and nitric acid in the surrounding environment, resulting in the formation of particulate matter ($PM_{2.5}$). Inhalation of $PM_{2.5}$ can lead to respiratory complications and has been linked to more than 2 million premature deaths globally (Anderson et al., 2003; Bryce et al., 2005).

Ammonia may also react with other atmospheric acids, leading to the formation of ammonium salts such as ammonium sulfate, which may result in the formation of acid rain (Saggar et al., 2004; Pozzer et al., 2017). The deposition of these compounds onto the earth's surface as acid rain can result in soil acidification (Behera et al. 2013; Sigurdarson et al., 2018). Nitrate leaching poses a risk to human health and the environment by contaminating groundwater and drinking water sources (Di and Cameron, 2002a). Nitrate enters rivers or lakes and enriches water bodies, leading to eutrophication, which may cause algal blooms and lead to the loss of aquatic life (Cameron et al., 2013). Nitrate leaching has also been linked to methemoglobinemia or “blue baby syndrome” in infants, cancer and cardiovascular diseases (Di et al., 1999; Grizzetti et al., 2011). Effective fertilizer management strategies aimed at reducing N losses from urea-based fertilizers are therefore crucial to reduce adverse environmental impacts while simultaneously optimizing crop NUE (Snyder et al., 2009).

1.4 Mitigation of Nitrogen Losses from Urea-Based Fertilizers

1.4.1 4R Nutrient Stewardship

Management approaches aimed at reducing N losses from urea-based fertilizers are crucial in protecting the environment while optimizing crop NUE. An example is the 4R Nutrient Stewardship approach, which emphasizes the application of the right fertilizer source, at the right rate, at the right time, and using the right placement (Snyder et al., 2009). Banding and broadcasting are commonly used fertilizer placement methods in Canada's northern Great Plains (Manitoba Soil Fertility Guide, 2007). Broadcast application of urea-based fertilizers can result in large N losses due to greater exposure of urea to ground surface conditions, which enhances urease activity and subsequent volatilization losses relative to sub-surface placement (Jiang et al., 2023). For example, Rochette et al. (2009a) observed 33% greater volatilization losses from surface-applied urea than from subsurface-banded urea under no-till. Byrne et al. (2020) reported that when banded urea is incorporated more than 7.5 cm into the soil, it reduces the loss of NH_3 by volatilization and improves N retention due to the reduced exposure to surface conditions that promote volatilization.

Adopting placement methods such as fertilizer incorporation or subsurface application may therefore help reduce N losses and improve N uptake efficiency (Sommer et al., 2004; Rochette et al., 2013; Pelster et al., 2019). Some studies, have shown that subsurface placement of urea in bands may not be an effective measure for minimizing NH_3 volatilization losses (Rochette et al., 2013). The increase in NH_3 loss from banding relative to surface application has been attributed to a localized increase in soil pH from 6.0 to 8.7, which likely enhanced the production of NH_3 in dry acidic soils (Rochette et al., 2009). Conversely, hydrolysis of surface-placed urea was inhibited by dry soil conditions. Such findings emphasize the importance of site-specific considerations to optimize fertilizer placement strategies. While soil incorporation of urea-based fertilizers is critical for enhancing N efficiency, incorporation is not always practical as some producers may prefer surface application of fertilizers due to financial, labour and time limitations (Amiro et al., 2017; Grant et al., 2019). In such cases, combining fertilizer incorporation with the application of urease- and nitrification inhibitors may be a more attractive strategy to minimize N loss and improve fertilizer N efficiency (Chambers and Dampney, 2009; Drury et al., 2017; Lasisi et al., 2019)

Fertilizer placement depth may impact the susceptibility to gaseous N loss. Although it is acknowledged that placing urea-based fertilizers below the soil surface often leads to reduced N_2O emissions compared to surface application (Grant et al., 2002), there are situations where this correlation can shift. Studies undertaken in some agricultural settings (e.g., Oleson et al., 2023; Tenuta et al., 2023) have shown greater N_2O emissions from fertilizer incorporation compared to surface application. For instance, Tenuta et al. (2023) demonstrated that shallow-banding urea N at depths less than the 8 cm recommended for deep-banding produced greater N_2O emissions than deep banded or surface/incorporated N. Additionally, deep banding has been shown to reduce N_2O emissions relative to surface application or shallow banding due to the longer upward diffusion distance for gaseous N (Wu et al., 2021). Fertilizer N incorporation into the soil can improve crop yields due to better N retention (Steusloff et al. 2019). Consistent with the results of Wu et al. (2021), Liu et al. (2006) reported that deeper injection of UAN at 10-15 cm reduced N_2O emissions compared to shallow injection at 5 cm. The lack of denitrifying bacteria in the deeper soil layer and a higher population of ammonia-oxidizing bacteria in the top layer may contribute to the slowdown of nitrification and denitrification, resulting in lower N_2O emissions (Mosier et al., 2005; Liu et al., 2006).

Deeper fertilizer placement may also lead to reduced NH_3 volatilization, especially in high pH soils, thereby further aiding in the mitigation of N_2O emissions (Garcia et al., 2019). Additional studies on NH_3 losses have shown that the efficacy of subsurface banding varies depending on depth, with deeper banding yielding lower volatilization losses (Li et al., 2009; Rochette et al., 2013a; Liu et al., 2020). Ammonia volatilization has been shown to be significantly lower for injected UAN than for urea following broadcast and broadcast followed by incorporation (broadcast-incorporation) into the soil (Woodley et al., 2020). A meta-analysis by Ti et al. (2019) showed that deep placement of N fertilizers several inches below the soil surface reduced NH_3 volatilization by up to 48% compared to surface application without incorporation. Lasisi et al. (2019) found that placing urea at a depth of at least 5 cm greatly reduced NH_3 emissions and therefore improved N retention in the soil.

Application of fertilizers at the right rate aims at supplying sufficient N to meet crop requirements while avoiding the over-application of fertilizers. Fertilizer application at high N rates can increase excess N susceptible to losses processes such as N_2O emission and NO_3^- leaching. (Jenkinson et al., 2001; Kim et al., 2013) To compensate for the N losses from fertilizers, some farmers frequently resort to overapplication of N fertilizers in an effort to optimize crop yields. This practice presents a risk to the environment and ultimately contributes to the reduction in fertilizer N efficiency (Subbarao et al., 2006; Jia et al., 2013). A study by Muller et al. (2023) showed that combining NI with a lower N fertilizer rate could be a cost-effective and environmentally viable approach for sustainable farming.

The “right time” component of the 4R nutrient stewardship emphasizes the importance of applying nutrients when demand by the crop is high, which ensures optimum uptake and utilization of the nutrient. This requires a careful consideration of various elements including the availability of labor, economic efficiency, and climatic conditions to attain optimal results in agricultural efficiency and long-term viability (Eagle et al., 2017). Canadian prairie farmers tend to prefer fall application of fertilizers as prices are lower in the fall (Amiro et al., 2017). Nevertheless, past studies in the northern Great Plains indicate that spring application of N fertilizer produces greater grain yields than fall application (Nyborg and Malhi, 1986). Additionally, applying fertilizer N in the fall leads to a greater NO_3^- leaching compared to spring application (Di et al., 1999). This is attributed to the fact that during the fall season, plant uptake of NO_3^- is lower as plants transition to dormancy or die off, resulting in high concentrations of soil NO_3^- susceptible to leaching (Jiang et al., 2023). Gao et al. (2013) reported that the application of N when crop uptake is restricted or N supply exceeds crop demand leads to higher concentrations of soil NH_4^+ and NO_3^- and subsequently increases N_2O emissions in irrigated potato fields in Manitoba.

1.4.2 Nitrogen Inhibitors

To achieve the goal of boosting food production while minimizing N losses, a combination of novel technological and economic advancements is essential (Davidson et al., 2016). Urease inhibitors (UI) reduce the release of NH_3 gas from urea by inhibiting the activity of the urease enzyme, which catalyzes the hydrolysis of urea into NH_3 and CO_2, therefore maintaining N in a stable and non-volatile form (NH_4^+) (Silva et al., 2017). There are a variety of UIs with varying active ingredients and properties, such as, N-(2-nitrophenyl) phosphoric triamide (2-NPT), N propyl thiosphosphoric triamide (NPPT) and N (n-butyl) thiophosphoric triamide (NBPT) (Awale and Chatterjee, 2017; Cantarella et al., 2018; Byrne et al., 2020).

NBPT is the most preferred active ingredient because of its versatile molecular interactions and inhibition longevity (Grant et al., 2014; Forrestal et al., 2016). This is an improvement from the past, where urea hydrolysis was inhibited using environmentally hazardous metals, such as mercury and boron (Modolo et al., 2018). Commercial products containing inhibitor active ingredients are available on the market. A recent example is ARM-U, which has a polymer formulation that incorporates 18% NBPT, which is a lower concentration than most commercially available inhibitors, making it one of the lower cost products on the market. Comparative analyses with well-established commercial products like Agrotain (30% NBPT) demonstrated that using ARMU in combination with urea-based fertilizers produced comparable efficacy in reducing NH_3 volatilization (Lasisi et al., 2019).

Nitrification inhibitors suppress the activity of ammonia-oxidizing bacteria, thereby delaying the conversion of NH_4^+ to NO_2^- and NO_3^- (Di and Cameron, 2012; Silva et al., 2017). This results in the retention of N in a plant-accessible, steady form that is less prone to loss processes such as denitrification and NO_3^- leaching (Trenkel et., 2010; Grant et al., 2013; Rajkovich et al., 2017; Drury et al., 2017). Examples of nitrification inhibitors include 3-4 dimethyl 1H pyrazole phosphate (DMPP), 4,5-dimethylpyrazole-1-yl succinic acid (DMPSA), nitrapyrin, and dicyandiamide (DCD) (Ruser and Schulz, 2015). DMPP and DCD can effectively decrease N loss from fertilizers by inhibiting the nitrification process (Di and Cameron, 2012; Weiske et al., 2001). Nevertheless, although the combined use of NBPT and DCD has demonstrated significant efficacy in mitigating N losses, its wider adoption is restricted by its high cost and application rates as well as the environmental implications linked to DCD. DMPP has been shown to have superior efficacy at low doses and a longer-lasting inhibitory effect compared to DCD (Zerulla et al., 2001; Weiske et al., 2001; Guardia et al., 2018).

Inhibitor products (double inhibitors) that contain both urease and nitrification inhibitors are commercially available and serve to reduce urea hydrolysis, inhibit nitrification

(Ding et., 2011; Soares et al., 2012; Zaman et al., 2013, Tenuta et al., 2023) and improve crop yields (Feng et al., 2016; Folina et al., 2021). The use of UIs and NIs in conjunction with late-fall fertilizer N application is often recommended (Tiessen et al., 2006) to take advantage of reduced fertilizer prices in late fall (Ruser and Schulz, 2015). An example of a notable DI formulation currently on the market is Super-U, comprising of 45.5% urea N with 0.06% NBPT and 0.85 % DCD, evenly spread and pre-mixed. The inhibitory effect of NIs may cause the build-up of NH_4^+ in the soil, in which may enhance NH_3 volatilization losses (Di et al., 2007; Chen et al., 2008; Kim et al., 2012; Frame et al., 2017). The increase in NH_3 volatilization resulting from the addition of DI has been attributed to accelerated urea hydrolysis in the presence of higher nitrification inhibitor application rates (Lasisi et al., 2020).

Research has shown that the performance of DIs can vary depending on several factors, such as soil type, environmental conditions, and management techniques, which add complexity to their potential (Mosier et al. 2002). Moreover, the effectiveness of such N inhibitors has been shown to vary across different crops. For instance, N_2O emissions have been shown to be more pronounced under maize compared to wheat or rice (Gardiner et al., 2022). Furthermore, the possible trade-offs between reducing N_2O emissions and other environmental consequences, including NH_3 volatilization, emphasize the need for comprehensive management strategies to reduce greenhouse gas emissions in agriculture (Lam et al., 2017). Understanding the various factors (e.g., climate, soil type, and management practices) influencing the efficacy of DI is crucial for formulating site-specific strategies to optimise the benefits of DIs while mitigating undesirable effects. Zaman et al. (2009) found the efficacy of DIs to vary with moisture content following application. The efficacy of DIs in improving NUE and crop yields has been found to be highly variable (Abalos et al. 2014). The variation in the effectiveness of DIs in agricultural systems has been attributed to a limited understanding of their interactions with edaphic, environmental and management factors (Mosier et al. 2002).

Variables such as fertilizer placement, fertilizer source, temperature and N fertilizer application rates can potentially influence N losses (Nelson et al., 2008; Snyder et al., 2009). This highlights the need for further in-depth investigation into the effects of DIs on N losses and NUE under variable soil conditions, N fertilizers, and fertilizer placement methods (Sanz-Cobena et al., 2012; Frame et al., 2017). Inhibitors are most effective under conditions where the risk of N loss is high (Li et al., 2018). For example, Lasisi et al. (2020a) found that NBPT effectively reduced NH_3 losses from urea by up to 53% in high pH soils, but only 18% in low pH soils. Higher efficacy of NBPT was observed with urea relative to UAN due to the greater susceptibility of urea to N losses in agricultural soils (Lasisi et al., 2020b).

Given the high cost of DIs, which hinders their extensive use, this research aimed to explore potentially cost-effective alternatives which will encourage greater adoption by farmers who are keen to implement N loss mitigation strategies towards sustainable agriculture without incurring significant financial costs. Relatively new DIs, such as Active Stabilizer Plus (ASP) (12% NBPT and 2% DMPP) and ARMU Advanced (ARMU-Adv) (30% NBPT and 15% DMPP), could be more attractive alternatives for farmers than most established commercial inhibitors such as Super-U.

1.5 Research Objectives

To our knowledge, there is limited information on the agronomic effectiveness of ARMU-Adv and its efficacy in reducing N losses in agricultural systems. This research examined the efficacy of the DI ARMU-Adv, applied at lower concentrations than most commercial products, in reducing N losses and improving wheat yield. The study evaluated the effects of different ARMU-Adv formulations (i.e., NBPT:DMPP ratios) when combined with urea and UAN fertilizers. The objectives of the study were to (i) determine the efficacy of ARMU-Adv formulations (NBPT: DMPP ratios of 1:0.5 and 1:1) in reducing N_2O emissions, NH_3 volatilization and NO_3^- leaching, and improving spring wheat biomass yield, N efficiency indices, and soil residual N; (ii) determine the interaction between ARMU-Adv and fertilizer source (urea versus UAN) on the above measurements (Chapter 2).

1.6 Thesis Outline

The thesis was written in manuscript style and adhered to the thesis standards of the Department of Soil Science, University of Manitoba. The thesis comprises three chapters, including the literature review (Chapter 1), the research chapter (Chapter 2), and the overall synthesis of the research project (Chapter 3). Chapter 2 was prepared as a research manuscript:

Chapter 2: Efficacy of Novel Urease and Nitrification Double-Inhibitor Ratios at Mitigating Nitrogen Losses from Urea and UAN Fertilizers

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2. EFFICACY OF NOVEL UREASE AND NITRIFICATION DOUBLE-INHIBITOR FORMULATIONS IN MITIGATING NITROGEN LOSSES FROM UREA-BASED NITROGEN FERTILIZERS

2.1 Abstract

Double-inhibitors (DIs) containing both nitrification inhibitors (NIs) and urease inhibitors (UIs) have recently emerged as potentially-effective technologies to improve fertilizer efficiency and economic benefit from urea-based fertilizers. However, their excessive costs have limited their use. This research evaluated the efficacy of novel and potentially cost-effective DI formulations in minimizing N losses and improving crop wheat yields from urea and urea ammonium nitrate (UAN) applications. Specifically, we investigated the effectiveness of ARMU Advanced (ARMU-Adv) containing the urease inhibitor N-(n-Butyl) thiophosphoric triamide (NBPT) and the nitrification inhibitor 3,4-Dimethylpyrazole phosphate (DMPP) at NBPT:DMPP ratios of 1:1 (ARMU-Adv$_{1:1}$) and 1:0.5 (ARMU-Adv$_{1:0.5}$). We tested factorial combinations of inhibitor treatment (0, ARMU-Adv$_{1:1}$, ARMU-Adv$_{1:0.5}$) and N fertilizer (urea, UAN) plus an unfertilized control, resulting in the treatments: untreated (no inhibitor) urea (UR$_0$), untreated UAN (UAN$_0$), urea treated with ARMU-Adv$_{1:1}$ (UR-ARMU$_{1:1}$) and ARMU-Adv$_{1:0.5}$ (UR-ARMU$_{1:0.5}$), and UAN treated with ARMU-Adv$_{1:1}$ (UAN-ARMU$_{1:1}$) and ARMU-Adv$_{1:0.5}$ (UAN-ARMU$_{1:0.5}$). An unfertilized control (0 N) was included for comparison. Fertilizers were surface-applied to supply 10 g N m^{-2}. Wheat yield, NO_3^- leaching, NH_3 volatilization and N_2O emissions were measured over durations of 55 d, 49 d, 28 d and 14 d, respectively. Results indicated that the application of urea and UAN treated with either ARMU-Adv formulation significantly reduced NH_3 volatilization relative to untreated fertilizers. Cumulative N_2O losses were significantly lower for the UAN-ARMU$_{1:1}$ treatment than UAN$_0$. Inhibitor formulations had no significant effect on NO_3^- leaching regardless of N fertilizer. The ARMU-Adv$_{1:0.5}$ treatment significantly improved shoot N uptake and apparent fertilizer N recovery (ANR) relative to untreated fertilizers. Overall, ARMU-Adv was more effective with urea relative to UAN in reducing cumulative NH_3 and in improving shoot biomass yield and ANR, whereas UAN demonstrated greater efficacy in reducing N_2O losses. Our findings indicate that the use of ARMU-Adv formulations has the potential to minimize N losses associated with urea-based fertilizers, thereby mitigating adverse environmental impacts.

2.2 Introduction

Canada is a major exporter of wheat due to advances in plant genetics and agronomic practices and increased utilization of nitrogen (N) fertilizers (Campbell et al., 2006; Asgedom et al., 2014; Collier et al., 2022). In Canada's Prairie provinces, spring wheat production is intensively managed primarily due to its high protein content. Spring wheat cultivation relies heavily on the application of N fertilizers, mainly urea-based fertilizers, to enhance crop yield and quality. However, urea-based fertilizers (urea and urea ammonium nitrate (UAN)) are susceptible to N losses via ammonia (NH_3) volatilization, nitrous oxide (N_2O) emission, and nitrate (NO_3^-) leaching in agricultural fields. These losses significantly reduce the amount of N available for plant uptake and plant growth and development (Bouwman et al., 2002a). A meta-analysis of data from over 800 studies indicates that only about 50% of the fertilizer N applied to cereal crops is utilized by the crops (Chien et al., 2009). It is important to note that, in some cases, crops can take up significant amounts of N from the added fertilizer but fail to utilize it for growth and protein synthesis (Gao et al., 2012). The use of slow-release N fertilizers has been demonstrated as one strategy to improve N use efficiency (NUE) and reduce N losses to the environment (Cui et al., 2022).

Ammonia volatilization is a major pathway for N loss from urea-based fertilizers. Ammonia volatilization losses can contribute to air pollution, leading to the formation of secondary pollutants like N_2O, which can have detrimental effects on air quality and human health (Ussiri and Lal, 2012). Ammonia can also react with sulfuric acid or nitric acid in the atmosphere, forming particulate matter ($PM_{2.5}$), which can cause respiratory problems if inhaled, and has been linked to over 2 million premature deaths worldwide (Anderson et al., 2003; Bryce et al., 2005). Ammonia volatilization occurs when N in the NH_4^+ form is transformed to NH_3 gas, which escapes into the surrounding atmosphere under favourable conditions (Bouwman et al., 2002b). High temperatures and high soil pH accelerate urea hydrolysis and can therefore increase NH_3 volatilization (Zhenghu and Honglang, 2000; Sommer et al., 2004; Rochette et al., 2008). The temporary increase in soil pH associated with urea hydrolysis can lead to high NH_4^+ concentrations in soils. This in turn can inhibit the activity of Nitrobacter, the bacteria responsible for the oxidation of NO_2^- to NO_3^-, which can lead to the accumulation of NO_2^- in the soil and negatively impact seed germination and plant growth. When urea-based fertilizers are surface-applied under elevated temperatures and humid conditions, NH_3 losses could reach 40% of applied N (Cantarella et al., 2018).

Nitrate leaching is another significant route for agricultural N loss and occurs when NO_3^- is transported down the soil profile and below the root zone (Addiscott et al., 1996). Water-soluble nitrates are produced through the process of nitrification in which NH_4^+ is converted to NO_3^- during a two-step transformation process facilitated by nitrifying bacteria such as Nitrosomonas and Nitrobacter (Subbarao et al., 2012). Nitrate that enters ground and surface water poses a major risk to human health and the environment via contamination of drinking water sources and eutrophication of surface water bodies under certain conditions such as the absence of N-fixing algae (Di and Cameron, 2002; Paerl et al., 2020).

Nitrous oxide is a highly potent greenhouse gas, with roughly 300 times the global warming potential of carbon dioxide (CO_2) (Myhre et al., 2013). Consequently, N_2O contributes to ozone layer depletion, leading to further adverse environmental impacts and contributing to global warming. Nitrification and denitrification are the primary agricultural routes for N_2O emission (Mosier et al., 1996), with denitrification exceeding nitrification under anaerobic conditions where denitrifying bacteria utilize NO_3^- as an electron acceptor (Mosier and Klemedtsson, 1994). While crops require NO_3^--N for their growth, controlling the nitrification of urea-based fertilizers is crucial for minimizing environmental degradation from excessive N_2O emissions.

High N losses from urea-based fertilizers significantly reduce N uptake by crops, reducing efficiency of fertilizers and increasing the risk of contamination of both groundwater and surface water (Bouwman et al., 2002a; Xu et al., 2013; Cantarella et al., 2018). The low recovery of fertilizer N has led some farmers to resort to excessive application of N fertilizers to optimize crop yields, thereby placing an additional strain on the environment and contributing to low NUE (Giles et al., 2005; Norton and Ouyang, 2019). Therefore, it is crucial to explore beneficial fertilizer additives, such as N stabilizers, to mitigate N losses and promote more sustainable and effective N management practices.

Nitrification inhibitors (NIs), urease inhibitors (UIs), and double-inhibitors (DIs) containing both NIs and UIs have recently emerged as effective technologies to minimize N losses via NH_3 volatilization, N_2O emissions, and NO_3^- leaching, thereby improving NUE in agriculture. The mechanism of action of UIs entails minimizing urease enzyme activity, which slows down urea hydrolysis (Sigurdarson et al., 2018). NBPT is the most widely used active ingredient in UI products, with its effectiveness having been demonstrated in various studies (e.g., Cantarella et al., 2018; Byrne et al., 2020). Producers, however, face challenges in adopting inhibitors due to their high costs, which limit their widespread use. Novel, cost-effective inhibitor products, such as ARMU (18% NBPT), are becoming increasingly popular due to their demonstrated effectiveness at comparatively low application rates relative to alternative commercial products (Lasisi et al., 2020a).

Nitrification inhibitors reduce N losses by temporarily delaying the transformation of NH_4^+ to NO_3^-, thus allowing N to be preserved in a stable, plant-available form that is less prone to loss through leaching and denitrification (Gao et al., 2015). Currently, dicyandiamide (DCD) is the most widely used NI and its efficacy has been widely reported (e.g., Mohanty et al., 2008; Ruser and Schulz, 2015). DMPP is, however, increasingly becoming popular, partly due to environmental concerns associated with DCD usage, as well as its low-dose efficacy, prolonged inhibitory impact, and relative immobility compared to DCD (Zerulla et al., 2001; Danaher and Jordan, 2013). Double-inhibitors (DIs) have been attracting greater attention in recent years. These DIs can simultaneously reduce nitrification and inhibit urease enzymes, leading to reductions in urea hydrolysis and nitrification, thus slowing down the release of NO_3^-

and NH_4^+ to meet plant demands, while reducing losses. This, in turn, enhances crop yields and improves economic returns from fertilizer application (Soares et al., 2012; Zaman et al., 2013).

To our knowledge, the study reported in this thesis is the first holistic investigation of N losses from and the agronomic efficiency of urea-based fertilizers stabilized with novel double-inhibitor formulations of ARMU-Adv. The objective of this research was to determine the efficacy of these novel and potentially relatively low-cost DI formulations in reducing N losses and enhancing wheat yield following broadcast application of granular urea and UAN fertilizers, with or without treatment with ARMU-Adv formulations (NBPT:DMPP ratios of 1:1 and 1:0.5). Minimizing N losses is crucial, not only for ensuring adequate N supply for crops, but also for reducing environmental risks associated with agricultural N losses. We hypothesized that (i) urea-based fertilizers treated with ARMU-Adv formulations would reduce N losses and enhance wheat biomass yield, relative to untreated fertilizers, due to the simultaneous slowing down of hydrolysis and nitrification processes; (ii) ARMU-Adv formulations would increase soil residual NH_4^+-N and reduce NO_3^--N concentration; and (iii) ARMU-Adv formulations would exhibit higher efficacy with urea than with UAN, based on previous findings that DI products are more effective in cases where high N losses are expected.

2.3 Materials and Methods

2.3.1 Soil Sampling

The soil used in the laboratory and growth room experiments was a Gleyed Rego Black Chernozemic soil (Neurhorst series) (0-15 cm layer) from Portage la Prairie, Manitoba, Canada (49º57’9” N, 98º16’0” W). The soil was air-dried, sieved through a 4-mm sieve to remove visible roots and litter, and stored at room temperature (~ 22 ± 2 °C) until treatment application.

Three composite samples (100 g each) were sent to Farmers Edge Laboratory (Winnipeg, MB) for baseline characterization.

2.3.2 Baseline Soil Analysis

Particle size distribution was determined using the hydrometer method (Gee and Or, 2002). Total organic matter content was determined using the loss on ignition method at 375 °C (Hoskins et al., 2002). Soil pH and electrical conductivity (EC) were measured in a 1:2 soil: water suspension (Gardiner et al., 2022). Nitrate-N was extracted with 0.01 M $CaCl_2$ and measured by automated colorimetry following hydrazine reduction and complexing with n(1-naphthyl) ethylenediamine dihydrochloride (Gardiner et al., 2022). Plant-available P was extracted using the Olsen method (0.5 M sodium bicarbonate, pH 8.5; Olsen and Sommers, 1982) and measured by automated colorimetry. Water holding capacity was determined by packing six 100-g soil samples into plastic columns (10 cm diam.) at a bulk density of ~ 1.1 g cm^{-3}. Incremental volumes of water were added to achieve a water-filled pore space (WFPS) range of 50 to 75%. The water was left to drain for at least 6 h and the minimum moisture content required to moisten the entire column, which corresponded of 70% WFPS, was selected as the container capacity.

2.3.3 Experimental Layout

The experimental layout was a completely randomized design (CRD) with a 2 × 2 factorial-plus-control treatment structure consisting of combinations of fertilizer source (urea and UAN) and NBPT:DMPP ratios (0, 1:1 and 1:0.5) plus a no fertilizer N control (0 N) (Table 2.2). The resulting treatments were urea treated with 1:1 NBPT:DMPP (2.4 L Mt^{-1}) and 1:0.5 NBPT:DMPP (1.8 L Mt^{-1}), UAN treated with 1:1 NBPT:DMPP (1.6 L Mt^{-1}) and 1:0.5 NBPT:DMPP (1.1 L Mt^{-1}), and unfertilized (0 N). The fertilizers were thoroughly mixed with inhibitor ratios and applied to the soil within 24 h. All treatments were replicated four times.

2.3.4 Ammonia Volatilization

Ammonia volatilization was measured using the static chamber method (Grant et al., 1996; Jantalia et al., 2012; Lasisi et al., 2019). The polyvinyl chloride (PVC) chambers (20 cm L × 15 cm diam.) were sealed at the bottom with PVC sheets. Soil was packed into the chambers to a height of 5 cm and a bulk density of 1.1 g cm^{-3}. The chambers were kept in a growth room at a daytime temperature of 22 °C and a nighttime temperature of 16 °C, a relative humidity of 50%, and a light intensity of 270 μmole m^{-2} s^{-1} during the 16-h photoperiod. Urea and UAN were surface-applied by hand to supply an equivalent of 10 g N m^{-2}, corresponding to typical N rates (~100 kg N ha^{-1}) for spring wheat on the Canadian prairies (Manitoba Soil Fertility Guide, 2007). Following treatment application, NH_3 volatilization was monitored by firmly fitting the chambers with two acid-charged foam discs (acid-soaked polyfoam discs 2.5 cm thick × 16 cm diam.). To prepare the acid-charged foam discs, foam discs were washed twice with distilled water and thoroughly squeezed to remove as much water as possible. The discs were then soaked twice in 0.001 M H_2SO_4, wringing out after each soak, and twice by soaking the foam discs in a glycerol-phosphoric acid solution (Grant et al., 1996; Jantalia et al., 2012), wringing out between soakings. Soaking was done on each sampling day before fitting in the columns.

The top foam disc was placed 5 cm above the soil surface to capture the volatilized NH_3. The upper foam disc was placed 5 cm below the upper part of the column to reduce contamination with atmospheric NH_3. On days 1, 2, 4, 7, and 14, the bottom foam disc from each unit was sampled and replaced with a fresh acid-soaked foam disc. Ammonia was extracted from the bottom disc using 2 M KCl. Briefly, after placing the foam disk with trapped ammonia in a Ziploc bag, 125 mL of 2 M KCl was added, followed by careful mixing for 2 min to release NH_4^+ from the foam. The solution collected from the foam disc was transferred to a 250-mL volumetric flask using a funnel. This process was repeated twice to effectively extract NH_3 from the discs. The extracts were frozen until colorimetric analysis with a Technicon auto-analyzer (Grant et al., 1996). The soil from each column was thoroughly mixed, air-dried, ground, and analyzed for residual NO_3^-N using automated colorimetry (hydrazine reduction procedure), while the determination of NH_4-N was carried out colorimetrically with an automated segmented flow autoanalyzer (indophenol blue method) (Maynard et al., 2008).

Ammonia volatilization was calculated as follows:

$$NH_3\text{-N (g m}^{-2}\text{)} = \left(\frac{(Extractant\ (mL) + absorbent\ (mL)) \times NH_3(mg\ N\ L^{-1})}{Area\ of\ chamber\ (m^2) \times 10^6} \right) \qquad [1]$$

Percentage reduction in NH_3 volatilization was calculated by dividing the difference between cumulative NH_3-N volatilized from untreated and treated fertilizer by the cumulative NH_3-N volatilization from the untreated fertilizer.

2.3.5 Nitrous Oxide Emission

Gas sampling for N_2O measurement was performed using a static incubation system (Tenuta and Beauchamp, 2000; Velthof et al., 2003; Chiyoka et al., 2011). Soil was weighed into 1-L mason jars (9 cm in diam.) and packed to a height of 5 cm and a bulk density of 1.1 Mg m^{-3}. The jars were pre-incubated for one week in a walk-in incubator set at 25 °C. To minimize moisture loss and facilitate gaseous exchange during incubation, the jars were covered with perforated parafilm, which was punctured with five pinholes. Humidity was maintained by placing water-filled containers in the incubator to slow down soil moisture loss. After one-week of pre-incubation, treatments were applied to the jars. Urea and UAN were broadcast at 10 g N m^{-2} to approximate the 100 kg N ha^{-1} rate recommended for spring wheat in Manitoba (Manitoba Soil Fertility Guide, 2007). Blank jars were included to account for ambient N_2O concentrations. The study was conducted for 14 d. Water lost due to evaporation was measured (based on weight of individual jars) and replenished with reverse osmosis water every three days. On each sampling day and prior to gas collection, air was purged into each jar headspace to ensure the N_2O concentration in the jar aligned with that in the surrounding environment. Each jar was then securely fitted with a mason jar lid with a rubber septum to prevent gas exchange. Sealed jars were placed in the incubator for two hours to allow gas accumulation.

Gas samples were taken from the headspace of each sealed jar on days 0, 1, 2, 3, 4, 7, 8, 9, 10 and 14 using a 23G1 PrecisionGlide® needle on a 20 mL syringe (BD, Franklin Lakes, NJ). The gas samples were stored in a 12-mL Exetainer vial (Labco, Buckinghamshire, England). N_2O concentration was measured with a gas chromatograph (Varian 3800, Varian Instruments, Walnut Creek, CA). Prior to data analysis, background concentrations of N_2O from the laboratory air were subtracted from the measurements. At the end of the experiment, soil from each jar was thoroughly mixed, air-dried, ground, and analyzed for residual NH_4^+ and NO_3^- concentrations as described above (2.3.3.1). Daily nitrous oxide flux was determined assuming a linear increase in gas concentration with time (Velthof and Oenema, 1995; Velthof et al., 2003). Cumulative N_2O losses were calculated by summing N_2O fluxes over the 14-d incubation duration using linear interpolation to estimate losses on non-sampling days (Tenuta and Beauchamp, 2003).

2.3.6 Potentially-leachable Nitrate

We employed a soil column technique to measure potentially-leachable NO_3^- concentration using a set of customized polyvinyl chloride (PVC) columns based on the method of Qian and Cai (2007). A stopper was placed at the bottom of each column, followed by the careful placement of a fiber mesh screen, GFA filter paper, and cheesecloth to minimize soil displacement when deionized water was added. Soil was packed into the PVC columns (5 cm diam. and 30 cm ht.) to a bulk density of approximately 1.1 g cm^{-3} and a height of 20 cm. All columns were pre-incubated in the dark for one week at 25 °C (Teutscherova et al., 2018). Water lost due to evaporation was determined by weighing each column every 3 d and replenished with deionized water.

At the end of pre-incubation, treatments were carefully broadcast on the surface of each soil column. Urea and UAN rates were the same as described in Sections 2.3.3.1 and 2.3.3.2. One week after treatment application, the columns were leached with 100 mL of deionized water using a graduated cylinder. Leaching was conducted once a week for seven consecutive weeks. After a 30-min equilibration, the leachate (~ 100 mL) was collected from the base of the column under a vacuum (Qian and Cai, 2007). The columns were covered with parafilm containing tiny pinholes to ensure aerobic conditions while maintaining the soil moisture at 70% WFPS. The columns were then returned to the incubator. Upon completion of the experiment, the leachate was filtered and stored in a freezer until analysis for NO_3^- concentration. At the end of the experiment, the soil within each column was thoroughly mixed, air-dried, and sampled for NH_4-N and NO_3-N determination as described above (2.3.3.1).

2.3.7 Wheat Biomass Yield and Nitrogen Efficiency Indices

Spring wheat was grown in a walk-in growth room at day/night temperatures of 22 °C/16 °C, a light intensity of 270 µmole m^{-2} s^{-1} during the 16-h photoperiod, and a relative humidity of 50%. Soil (8 kg dry wt.) was weighed into each 12.5-L rectangular plastic container (23.75 cm L × 23.75 cm W × 23.75 cm H) to a bulk density of approximately 1.1 g cm^{-3}. Each pot received inhibitor-treated or untreated urea at the same rates as described above (2.3.3.1). To ensure a balanced supply of nutrients for plant growth, the pots received a N-free, full-strength nutrient solution (Zvomuya et al., 2006). Macronutrient solutions (P, K, and S) were prepared individually, while micronutrient solutions (Zn, Mn, Mo, B, Cu, and Fe) were combined and applied in a single aliquot (Helgason et al., 2007). The pots were sealed with lids and placed in the growth room. Thereafter, 15 spring wheat seeds were planted into each container at the 2.5 cm depth (Manitoba Soil Fertility Guide, 2007). To provide an initial basis for replenishing moisture, each pot was weighed shortly after setup. Water lost due to evaporation was measured (based on the weight of each pot) every 2-3 d and replenished with reverse osmosis water to a target moisture content approximating 70% WFPS . Following germination, the seedlings were thinned to three per pot.

Plants were harvested 55 d after emergence (i.e., at early flowering) by cutting shoots at the soil surface using a pair of scissors. The roots were extracted from each container and thoroughly washed with water. Plant shoots and roots were dried at 60 °C for 48 h after which they were weighed for dry matter yield determination and pulverized to pass through a 0.15 mm sieve prior to laboratory analysis. A 20-g (dry weight) soil sample was taken from each pot, after thoroughly mixing the soil, for NH_4 and NO_3-N determination as described above. Total N concentrations in shoot and root biomass were determined by dry combustion using a NS-2000 Nitrogen Analyzer (Leco Corp., St. Joseph, MI, USA).

2.3.8 Calculations

Nitrogen efficiency indices were calculated as follows (Moll et al., 1982; Dobermann et al., 2007; Chuan et al., 2016; Congreves et al., 2021):

N efficiency ratio (NER): $= \frac{Yield\ (g)}{Tissue\ N\ content\ (g)}$ [2]

Physiological efficiency (PE): $= \frac{Yield\ (g) - Yield\ C\ (g)}{N\ uptake_F\ (g) - N\ uptake_C\ (g)}$ [3]

where N uptake = total N (g kg^{-1}) × aboveground biomass yield (kg), subscript *F* and *C* denote urea-fertilized and unfertilized control treatments, respectively, and yield is the wheat aboveground biomass yield.

Apparent N recovery efficiency (RE) (%) = $\frac{N\ uptake\ F\ (g) - N\ uptake\ C\ (g)}{Quantity\ \mathrm{of}\ N\ applied\ (g)} \times 100$ [4]

Agronomic efficiency (AE) = $\frac{Yield_F\ (g) - Yield_C\ (g)}{Quantity\ of\ N\ applied\ (g)}$ [5]

Partial factor productivity (PFP) = $\frac{Yield\ (g)}{Quantity\ of\ N\ applied\ (g)}$ [6]

Fertilizer contribution rate (FCR) = $\frac{(Yield_F\ (g) - Yield_C\ (g)}{Yield_F\ (g)}$ [7]

2.3.9 Statistical Analysis

Data were analyzed using SAS OnDemand for Academics (SAS Institute Inc., Cary, North Carolina, United States). A repeated measures analysis of variance (ANOVA) was performed using the generalized linear mixed model (PROC GLIMMIX) to determine treatment effects on N_2O emission, NH_3 volatilization and NO_3^- leaching. N_2O flux data were analyzed based on the gamma distribution whereas data for all the other dependent variables were normally distributed. Treatment means were compared using the Tukey multiple comparison procedure at $\alpha = 0.05$.

When treatment interactions with sampling time were significant for cumulative NH_3 volatilization, temporal trends were explored via nonlinear regression using the logistic model (Seber and Wild, 2003):

$$\hat{y} = \frac{\alpha}{1+e^{-(t-\beta)/\gamma}} \quad [8]$$

where $\hat{y}$ is the cumulative NH_3 loss (mg m^2) at time t (d), α is the asymptotic value indicating the peak (maximum) cumulative volatilization, β is the time that it takes to attain 50% of the maximum NH_3 loss (i.e., $\alpha/2$) and corresponds to the curve inflection point representing the time at which the maximum daily NH_3 loss occurs, and γ is used to calculate the maximum daily NH_3 loss (MDL), calculated as:

$$\text{MDL} = \alpha/4\gamma \qquad [9]$$

The logistic model has traditionally been used to model NH_3 volatilization (e.g., Silva et al., 2017; Cantarella et al., 2018; Minato et al., 2023) which typically follows a sigmoidal curve with a gradual increase initially, followed by a rapid increase, and then slows down towards the end, levelling off at a peak value after some time. In this study, the model was fit to the data using PROC NLIN in SAS. Parameters from different treatments were deemed significantly different if their 95% confidence intervals did not overlap.

2.4 Results

2.4.1 Soil Baseline Properties

Initial properties of the soil used in the study included a loam texture, an alkaline pH, and low electrical conductivity (Table 2.1). The soil was high in organic matter, NO_3-N, and Olsen P.

Table 2.1 Selected initial soil properties

Soil Property	Values
Soil pH	8.1
Electrical conductivity (dS m^{-1})	0.35
Soil organic matter (g kg^{-1})	65
Soil organic carbon (g kg^{-1})	37.8
Soil texture	Loam
Sand (%)	43.3
Silt (%)	37.1
Clay (%)	19.6
Nitrate-N (mg kg^{-1})	45
Bicarbonate (Olsen) P (mg kg^{-1})	20.3

2.4.2 Cumulative Ammonia Volatilization

The untreated (no inhibitor) fertilizers showed the highest NH_3 emissions throughout the sampling period. The effects of ARMU-Adv formulation and N source varied with time elapsed since treatment application, as indicated by the significant formulation × day and source × day interactions (Table 2.2).

For all combinations of ARMU-Adv formulations and N sources, cumulative NH_3 loss was adequately described by the logistic model, with an initial low rate of volatilization, followed by a high rate, and then a gradual stabilization to a maximum (Fig. 2.1). The parameter α from the logistic model fit indicates that the asymptotic (maximum) NH_3 volatilization was lowest for the unfertilized (0 N) treatment (81.3 mg m^{-2}) and highest for untreated (no inhibitor) urea (1302 mg m^{-2}) and UAN (824 mg m^{-2}) (Table 2.3). The maximum loss from untreated urea was significantly greater (by 58%) than that from UAN. While, within each N source, both ARMU-Adv formulations (1:1 and 1:0.5 NBPT:DMPP) significantly reduced asymptotic NH_3 volatilization relative to untreated fertilizer, there was no significant difference between the two formulations. When comparing N sources within formulations, asymptotic NH_3 loss was significantly greater for urea than for UAN when treated with the 1:0.5 formulation, but there was no significant difference between the two N sources when treated with the 1:1 formulation.

The time to reach 50% of the maximum cumulative NH_3 loss (β) was 9.53 d for the zero-N treatment (which had a significantly greater β than all the other treatments), and between 2.41 and 5.5 d after fertilizer N application for the rest of the treatments. For both UAN and urea, ARMU-Adv formulations significantly decreased β. However, there was no significant difference in β between the two ARMU-Adv formulations regardless of N source. Similarly, there was no significant difference in β between the two N sources regardless of ARMU-Adv formulation. The maximum daily NH_3 loss (MDL) was significantly lower for the 0 N control (125 mg m^{-2}) than for UAN treated with the 1:0.5 (267 mg m^{-2}) and the 1:1 (301 mg m^{-2}) ARMU-Adv formulations. The MDL ranged from 202 to 301 mg m^{-2} for the N-fertilized treatments but did not differ significantly between any of these treatments.

Table 2.2 Ammonia volatilization (NH_3) as a function of nitrogen source, ARMU-Adv formulation (NBPT:DMPP), and sampling time.

Effect	Cumulative NH_3 loss (mg N m^{-2})
Source	
Unfertilized	32.4
UAN	287
Urea	331
Formulation	
0	634
1:0.5	221
1:1	209
Sampling day (Day)	
1	53.8
2	147
4	308
7	470
14	590
21	619
28	634
p-value	
Source	**0.05**
Formulation	**< 0.0001**
Day	**< 0.0001**
Source × Formulation	**0.03**
Source × Day	**< 0.0001**
Ratio × Day	**< 0.0001**
Source × Formulation × Day	0.20

† Means in the same column followed by the same letter are not significantly different according to the Tukey multiple comparison procedure (α = 0.05).

Table 2.3 Logistic model parameters for ammonia volatilization as affected by nitrogen fertilizer and ARMU-Adv formulation.

N Source	Formulation	α†	SE	LCL	UCL	β	SE	LCL	UCL	γ	SE	LCL	UCL	MDL
Control (0 N)	None	81.37	7.81	64.1	98.5	9.53	1.96	5.21	13.85	6.18	1.43	3.02	9.33	125b
UAN	0	824	16.8	789	858	2.55	0.14	2.25	2.84	0.98	0.12	0.73	1.24	202ab
	1:0.5	429	16.2	396	462	5.13	0.49	4.12	6.14	2.49	0.46	1.55	3.42	267a
	1:1	475	12.1	450	500	5.55	0.35	4.84	6.26	2.54	0.31	1.89	3.18	301a
Urea	0	1302	49.9	1200	1405	2.41	0.25	1.90	2.92	0.75	0.21	0.32	1.17	250ab
	1:0.5	528	23.7	480	577	4.90	0.49	3.90	5.90	1.87	0.40	1.04	2.70	249ab
	1:1	499	22.4	453	545	5.52	0.52	4.45	6.58	1.99	0.43	1.10	2.88	249ab

† α is the asymptotic value indicating the peak (maximum) cumulative volatilization; β is the time that it takes to attain 50% of the maximum NH_3 loss; and γ is used to calculate the maximum daily NH_3 loss (MDL) as $MDL = \alpha/4\gamma$; SE, standard error of the model parameter; LCL, lower confidence limit of the parameter, UCL, upper confidence limit of the parameter.

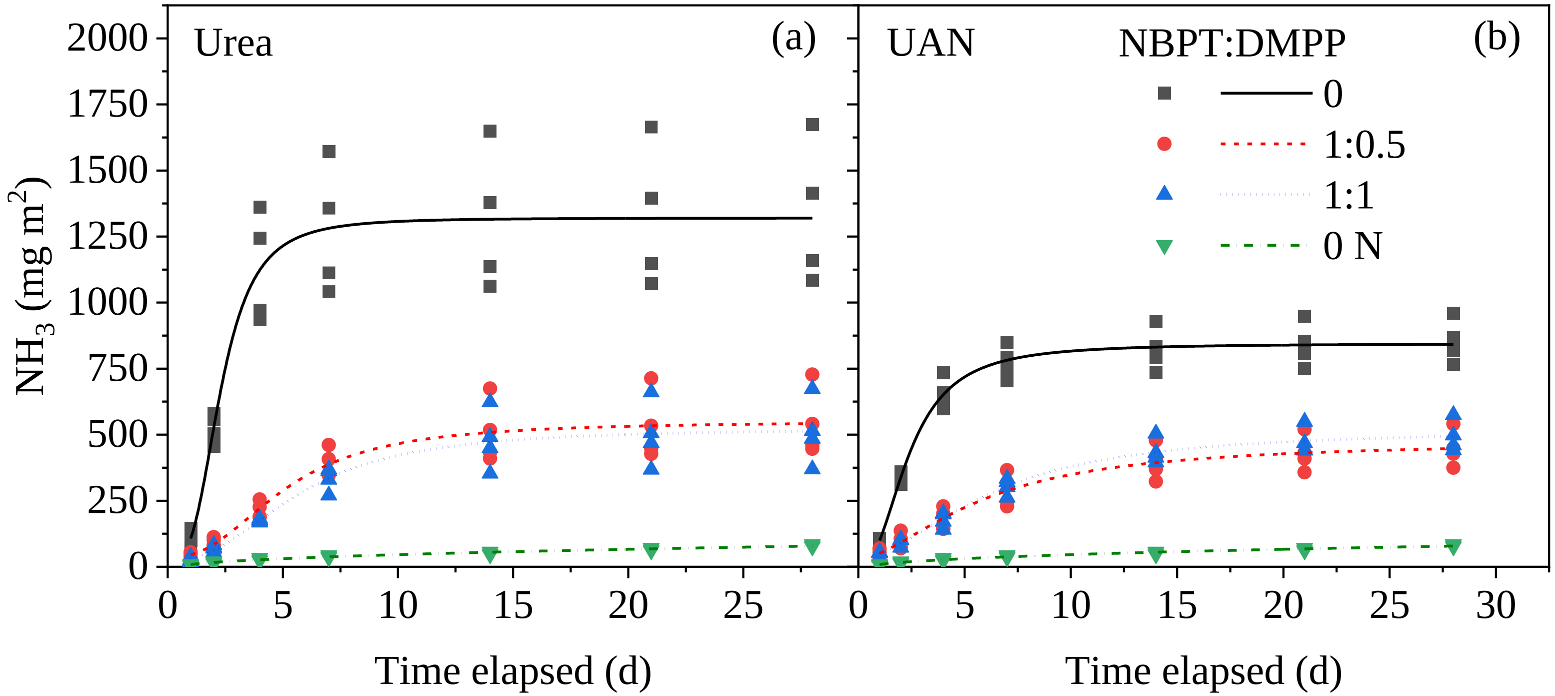

Figure 2.1 Logistic model fits for ammonia volatilization as affected by nitrogen fertilizer and ARMU-Adv formulation (NBPT:DMPP ratio).

2.4.3 Nitrous Oxide Emission

There was a significant N fertilizer source by ARMU-Adv formulation by sampling day interaction for N_2O flux (Table 2.4; Fig. 2.2). For both ARMU-Adv formulations, N_2O fluxes were detected during the first 10 d of the experiment, after which there were no detectable fluxes measured. While the flux was generally greater for urea than UAN, the difference between the two fertilizers was more pronounced on Days 2 and 4 (Fig. 2.4). Specifically, all formulations exhibited peaks on Days 2 and 4 (which had the higher peaks), during which the flux was highest for the untreated fertilizers, except on Day 4, when untreated urea displayed slightly lower peak emissions relative to inhibitor treatments. Untreated urea led to increased N_2O emissions during the initial sampling days, culminating in a notable peak on day 2. Subsequently, N_2O flux for untreated urea began to decrease in comparison to the inhibitor treatments.

Overall, despite some variations over time, the differences in N_2O loss mitigation between the two ARMU-Adv formulations were less apparent when compared to the disparities between the two fertilizers. Both inhibitor formulations significantly reduced N_2O flux from UAN relative to untreated UAN, and the reduction was more pronounced for the 1:1 ratio. Similarly with urea, both inhibitor formulations performed comparably from day 3 till the last sampling day, whilst the 1:1 formulation displayed a lower peak emission on Day 2 compared to the 1:0.5 formulation.

There was a significant N source by inhibitor formulation interaction for cumulative N_2O emission over the 14 d (Table 2.4). While N_2O flux from untreated fertilizers did not differ between urea and UAN, it was significantly higher for urea than UAN with both inhibitor formulations (Fig. 2.3). Both inhibitor formulations reduced N_2O flux from UAN relative to untreated UAN, and the reduction was significant for the 1:1 ratio. In contrast, inhibitor application had no significant effect on the emissions relative to untreated urea, regardless of the formulation. As expected, N_2O flux was significantly lower for the zero-N treatment compared with all the other treatments.

Table 2.4 Nitrous oxide emission as a function of nitrogen source, ARMU-Adv formulation (NBPT:DMPP), and sampling time.

Effect	N_2O Flux (μg kg^{-1} d^{-1})	Cumulative N_2O loss (μg kg^{-1})
N Source		
Unfertilized	0.93‡	21.2
UAN	9.22	260
Urea	12.7	517
Formulation		
0	12.1	433
1:0.5	10.1	372
1:1	10.3	361
Sampling day†		
0	2.67	
1	26.0	
2	47.4	
3	32.0	
4	74.5	
7	23.0	
10	1.75	
14	0.60	
	p-value	
Source	**0.01**	**< 0.0001**
Formulation	0.18	0.18
Source × Formulation	**0.01**	**0.01**
Day	**< 0.0001**	
Source × Day	**< 0.0001**	
Formulation × Day	**< 0.0001**	
Source × Formulation × Day	0.03	

† Sampling day for inhibitor treatments only

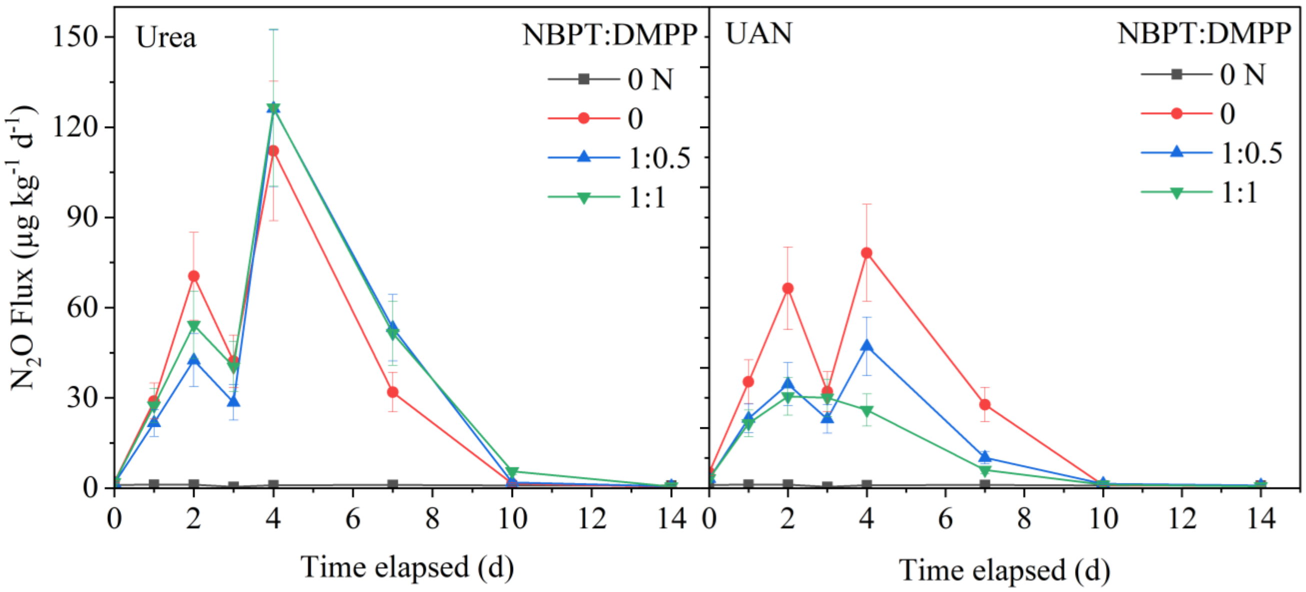

Figure 2.2 Daily N_2O emissions following surface application of urea and UAN treated with different ARMU-Adv formulations (NBPT:DMPP). Error bars represent standard errors of the mean (n = 4).

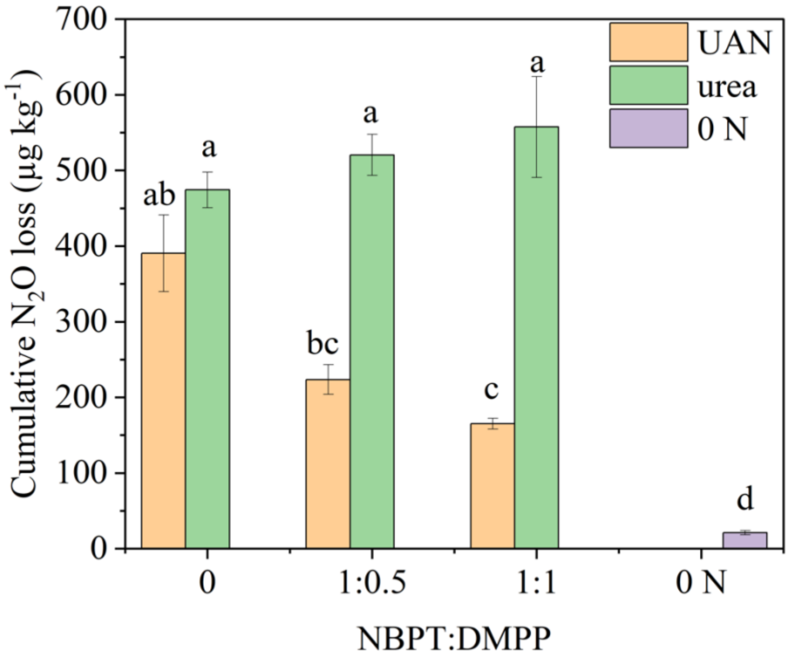

Figure 2.3 Cumulative N_2O emission following surface application of urea and UAN treated with different ARMU-Adv formulations (NBPT:DMPP). Error bars represent standard errors of the mean (n = 4). Bars with the same letter are not significantly different according to the Tukey multiple comparison procedure (α = 0.05).

2.4.4 Nitrate Leaching

Weekly NO_3^- leaching losses, averaged across N sources and ARMU-Adv formulations, varied significantly over the 7-wk leaching period but were not significantly affected by N source or inhibitor ratio (Table 2.5). With no fertilizer application (Control), NO_3^- leaching peaked in Week 1. Overall, NO_3^- leaching was greatest in the first week following treatment application and declined gradually thereafter. Treatment effects on total NO_3^- leaching over the 7-wk period were not significant (Table 2.5).

Table 2.5 Nitrate leaching as a function of nitrogen source, ARMU-Adv formulation (NBPT:DMPP), and sampling time.

Effect	Weekly NO_3^-N leached	Total NO_3^-N leached
	mg kg^{-1}	
N Source		
Unfertilized	22.2	155
UAN	24.0	168
Urea	25.7	180
Formulation		
0	23.9	168
1:0.5	25.4	178
1:1	25.1	175
Sampling time (week) †		
1	75.2a‡	-
2	35.0b	-
3	19.0c	-
4	13.7cd	-
5	10.8d	-
6	10.1d	-
7	9.80d	-
	p-value	
Source	0.4	0.4
Formulation	0.82	0.8
Source × formulation	0.05	0.1
Week	**< 0.0001**	
Source × week	0.59	
Formulation × week	0.9	
Source × formulation × week	0.4	

† Excludes the unfertilized control.

‡ Means in a column followed by the same letter are not significantly different according to the Tukey multiple comparison procedure (α = 0.05).

2.4.5 Wheat Biomass Yield and Nitrogen Efficiency Indices

There were no significant main or interaction effects on wheat shoot and root biomass yields (Table 2.6). The 1:0.5 inhibitor formulation significantly increased N accumulation in the aboveground biomass (shoots) relative to untreated (no inhibitor) fertilizers. In contrast, treatment effects on N accumulation in the root biomass were not significant. Similarly, treatment effects on N efficiency indices were not significant, with the exception of ANR, which was significantly greater for the 1:0.5 inhibitor formulation than the no-inhibitor control, but did not differ significantly between the two inhibitor formulations (Table 2.7).

Table 2.6 Wheat biomass yield as a function of nitrogen source, ARMU-Adv formulation (NBPT:DMPP), and sampling time.

Effect	Shoot biomass	Root biomass	Shoot N uptake	Root N uptake
	g pot^{-1}		mg pot^{-1}	
N Source				
Unfertilized	70.7	39.5	522	270
UAN	90.0	51.3	922	375
Urea	89.4	50.7	890	361
Formulation				
0	88.4	46.7	854b†	345
1:0.5	90.5	49.7	939a	369
1:1	90.2	56.5	925ab	391
		p-value		
Source	0.66	0.89	0.17	0.64
Formulation	0.45	0.39	**0.02**	0.48
Source × Formulation	0.07	0.19	0.22	0.06

† Means followed by the same letter are not significantly different according to the Tukey multiple comparison procedure ($\alpha = 0.05$).

Table 2.7 Nitrogen efficiency indices as a function of nitrogen source, ARMU-Adv formulation (NBPT:DMPP), and sampling time.

Effect	NER†	AE	PE	PFP	FCR	ANR/RE
	mg kg^{-1}				%	
N Source						
UAN	98.1	29.7	3.87	138	21.3	61.5
urea	101	28.8	3.94	137	20.8	56.5
Formulation						
0	104	27.2	3.97	136	20.0	51.0b‡
1:0.5	96.5	30.4	3.85	139	21.8	64.1a
1:1	98.1	30.0	3.89	139	21.6	61.8ab
p-value						
Source	0.39	0.66	0.54	0.66	65.8	0.17
Formulation	0.19	0.45	0.63	0.45	41.6	**0.02**
Source × formulation	0.89	0.07	0.64	0.07	6.80	0.22

† NER, nutrient efficiency ratio; AE, agronomic efficiency; ANR, apparent N recovery efficiency; PFP, partial factor productivity; FCR, fertilizer contribution rate; PE, physiological efficiency.

‡ Means in the same column followed by the same letter are not significantly different according to the Tukey multiple comparison procedure ($\alpha = 0.05$).

2.4.6 Residual Soil N Concentration

Residual N (NH_4^+ and NO_3^-) concentrations in the soil at the end of the NH_3 volatilization, N_2O emission, and NO_3^- leaching experiments, and the spring wheat bioassay were mostly not significantly affected by N source and inhibitor formulation or their interaction (Table 2.8). Exceptions were residual NH_4^+-N concentration in the N_2O emission experiment, which was greater for inhibitor-treated UAN than inhibitor-treated urea and untreated fertilizers (albeit insignificant differences), and residual NO_3-N concentration in the NO_3^- leaching experiment, which was significantly lower for the 1:0.5 inhibitor formulation than untreated fertilizers (no inhibitor) but did not differ significantly between the two inhibitor formulations (Table 2.8).

Table 2.8 Treatment effects on residual soil NH_4-N and NO_3-N at the end of the ammonia volatilization, nitrous oxide emission, nitrate leaching, and winter wheat experiments .

	NH_3 volatilization		N_2O emission		Nitrate leaching		Wheat yield	
	mg kg^{-1}							
Effect	NH_4^+-N	NO_3-N	NH_4^+-N	NO_3-N	NH_4^+-N	NO_3-N	NH_4^+-N	NO_3-N
N Source								
Unfertilized	0.78	199	14.7	210	7.09	28.0	6.65	1.39
UAN	0.87	233	16.2	348	7.92	36.1	5.17	1.43
Urea	1.03	253	13.4	341	8.02	35.2	6.79	1.46
Formulation								
0	1.02	259	15.3	340	7.91	42.6a†	5.80	1.45
1:0.5	0.96	223	14.3	350	7.78	28.1b	6.24	1.47
1:1	0.87	248	14.8	344	8.22	36.2ab	5.90	1.43
				p-value				
Source	0.54	0.24	**0.04**	0.19	0.86	0.85	0.07	0.68
Formulation	0.88	0.20	0.81	0.33	0.84	**0.02**	0.91	0.90
Source × Formulation	0.15	0.16	0.10	0.38	0.33	0.12	0.97	0.63

† Means in the same column followed by the same letter are not significantly different according to the Tukey multiple comparison procedure ($\alpha = 0.05$).

2.4.7 Percentage Reduction in Gaseous N Losses

The percent reduction in NH_3 volatilization relative to untreated fertilizer, averaged across inhibitor ratios, was significantly greater for urea (60.5%) than UAN (44.3%) (Table 2.9). In addition, fertilizer treatment with ARMU-Adv formulations decreased NH_3 losses by 53% (mean of the two formulations), for both N sources. The two ARMU-Adv formulations reduced N_2O emission to a significantly greater extent for UAN (50%) compared with untreated UAN, with the 1:1 ratio showing a significantly greater percent reduction (58%) than the 1:0.5 ratio (43%). In contrast, inhibitor treatment of urea did not significantly reduce N_2O losses relative to untreated urea.

Table 2.9 Percentage reduction in ammonia and nitrous oxide losses as a function of nitrogen source, ARMU-Adv formulation (NBPT:DMPP) cumulative loss.

Effect	NH_3 Volatilization	N_2O Emissions‡
	—————%	—————
N Source		
Urea	60.5a†	-
UAN	44.3b	50
Formulation		
1:1	52	58a
1:0.5	53	43b
	p-value	
Source	**0.01**	**-**
Formulation	0.85	**0.03**
Source × Formulation	0.41	-

† Means in the same column followed by the same letter are not significantly different according to the Tukey multiple comparison procedure ($\alpha = 0.05$).

‡ Excluding percent reduction for urea (negative inconclusive value).

2.5 Discussion

2.5.1 Ammonia Volatilization

Parameters from the logistic model indicated that both ARMU-Adv formulations significantly reduced peak cumulative NH_3 volatilization relative to untreated fertilizer. However, peak cumulative NH_3 volatilization (α) and time to attain 50% of the maximum NH_3 loss (β) did not differ significantly between the two ARMU-Adv formulations. Furthermore, our study showed that increasing the proportion of DMPP in the formulation (i.e., changing the NBPT:DMPP ratio from 1:0.5 to 1:1) had no significant effect on the efficacy of ARMU-Adv. Noteworthy is that the observed peak cumulative NH_3 loss was higher for urea compared to UAN at the 1:0.5 ratio, whereas volatilization loss did not differ significantly between the two N sources when treated with the 1:1 ratio.

Consistent with our results, Forrestal et al. (2016) reported NH_3 losses commencing on the first day following application of urea-based fertilizers, despite the fact that granular urea requires time to break down and dissolve before volatilization can begin (Grant et al., 1996). This contrasts with the findings of Lasisi et al. (2019) and Lasisi et al. (2020b), which showed that volatilization commenced two or more days after fertilizer application. This may be attributed to the fact that, unlike those previous field studies, our controlled-environment study was carried out under optimum conditions that were more conducive to rapid hydrolysis, resulting in elevated NH_3 concentrations and increased volatilization rates (Fageria and Baligar, 2005; Frame, et al., 2017; Jiang et al., 2023). Further research is needed to fully understand the complex relationship between DIs and their efficacy under varying field conditions (Gioacchini et al., 2002; Ding et al., 2011).

Following the initial period of low NH_3 losses observed from Day 1 in our study, there was a gradual increase in emissions following the application of N fertilizers, culminating to a subsequent plateau. The gradual increase was likely due to the limited formation of NH_3 as optimal conditions for volatilization began to emerge (Bouwman et al., 2002). The subsequent increase in NH_3 volatilization before peaking to a maximum on Day 7 is consistent with results from previous studies, which showed that the hydrolysis process typically occurs within 3-7 days following fertilizer application under conducive conditions (Peng et al., 2015; Lasisi et al., 2019; Jiang et al., 2023). Understanding these emission patterns is essential for enhancing N management techniques and reducing environmental risks associated with fertilizer application.

Previous studies have shown a one-day delay in the peaking of NH_3 volatilization when DCD was added to urea in the presence of NBPT (Zaman et al., 2008; Soares et al., 2012). By comparison, our study, incorporating DMPP and NBPT, showed a 2.4- to 5.6-day delay in the peak volatilization when urea-based fertilizers (urea and UAN) were treated with ARMU-Adv containing different ratios of NBPT and DMPP. The relatively long delay in peak emissions indicates that the ARMU-Adv formulations were effective in delaying NH_3 volatilization. Nevertheless, the subsequent peak highlights the diminishing inhibitory effect of DIs with time (Lasisi et al., 2019). There was no significant difference between the formulations with respect to MDL, regardless of N source. However, it is worth noting that, the maximum daily NH_3 loss (MDL) following the incorporation of UAN with both inhibitor ratios displayed a significant distinction, in comparison to the control (0 N) while none were observed for urea. This disparity indicates that urea has a higher susceptibility to increased NH_3 loss than UAN, with its characteristics being the primary drivers of NH_3 emissions, irrespective of inhibitor ratio. This may be attributed to the higher urea content available for hydrolysis to NH_3 in urea than UAN (Peng et al., 2015; Lasisi et al., 2019).

2.5.2 Nitrous Oxide Emission

Our study showed a significant reduction in cumulative N_2O losses for ARMU-Adv formulations relative to the no inhibitor treatment but only for UAN. This demonstrates the effective inhibitory effect of DMPP in limiting the availability, from UAN, of NO_3^-, a key precursor for N_2O production (Wang et al., 2020). Although the two inhibitor ratios showed similar cumulative N_2O emissions when added to UAN, only the 1:1 ratio showed a significant reduction in N_2O emission relative to untreated UAN. This is likely due to the higher DMPP concentration in the 1:1 ratio, resulting in a greater inhibitory effect on nitrification, compared to the 1:0.5 ratio (Adhikari et al., 2021). This notion is corroborated by a study by Di and Cameron (2012), which showed a 66% decrease in N_2O emissions at a DMPP rate of 5 kg ha^{-1}, but no significant reduction in emissions at a lower rate of 1 kg ha^{-1} (Marsden et al., 2017). This indicates a negative correlation between DMPP rate and N_2O losses, suggesting that there might be an optimal rate of ARMU-Adv for minimizing N_2O loss from UAN. On the contrary, other studies have shown no correlation between the NI application rate and N_2O emissions, suggesting the NI efficacy is not solely dependent on the application rate (Adhikari et al., 2021, Cai and Akiyama, 2017).

Inhibitor treated UAN showed significantly lower N_2O losses relative to treated or untreated urea in our study. Several studies have demonstrated higher N_2O emissions from granular urea compared to liquid UAN (Grant et al., 2001; Ren et al., 2021; Jiang et al., 2023). This is consistent with the observation that the N_2O global warming potential (GWP_{N2O}; 39.3%) and N_2O greenhouse gas intensity ($GHGI_{N2O}$; 52.4%) of urea were greater than those for UAN (Ren et al., 2021). Lower N_2O emissions from UAN-fertigated maize than urea-fertilized crops have also previously been reported (Halvorson and Del Grosso, 2012). Moreover, the combination of high urea N content (in urea) and rapid hydrolysis can result in elevated

NH_4^+/NH_3 concentrations in the soil, which can be subsequently converted to NO_3^- and, ultimately, N_2O (Halvorson and Del Grosso, 2013; Ren et al., 2021).

2.5.3 Potentially-leachable Nitrate

Most of the NO_3^- leaching losses during the 7-wk leaching period occurred during the first week following fertilizer application. This may be due to processes, such as nitrification, from fertilizer N application, resulting in the formation of NO_3^- (Jiang et al., 2023). Nitrate leaching is most prevalent under conditions of higher soil moisture and elevated soil NO_3^- concentrations (Zvomuya et al., 2003). For instance, inhibitors like DMPP have been shown to demonstrate greater efficacy under conditions conducive to greater NO_3^- leaching (Abalos et al., 2014; Acharya et al., 2022). Previous studies have shown that UAN results in higher nitrate leaching when compared to urea (Byrne et al., 2020; Jiang et al., 2023). This is because half of the N in UAN is in the highly soluble NO_3^- form, while urea forms require time to convert to NH_4^+ and NO_3^- respectively, a slow process that facilitates greater plant uptake (Legg and Meisinger 1982, Grant et al., 2001).

The crop-specific fertilizer N rate and controlled moisture conditions in our experiment may have provided high enough NO_3^- concentrations and sufficient moisture for enhanced NO_3^- leaching to occur (Acharya et al., 2022). However, Arregui and Quemada (2006) reported that very wet soil conditions may minimize the efficacy of DMPP, leading to an increased amount of NO_3^- available for leaching. Furthermore, the high SOM content of our soil might have had a significant effect on the leaching process by inducing a priming effect whereby the addition of the NI enhanced SOM mineralization, resulting in greater release of soil organic N, thereby diminishing the efficacy of the NI (Gioacchini et al., 2002).Overall, our results suggest that the efficacy of the ARMU-Adv formulations tested is limited with respect to NO_3^- leaching and may not be reliable for prolonged NO_3^- leaching management especially under conditions that enhance the degradation of the inhibitor, such as high SOM and SOC (Gao et al., 2015; Friedl et al., 2020).

2.5.4 Wheat Biomass Yield and Nitrogen Efficiency Indices

Our study showed a significant increase in shoot N uptake and ANR for the 1:0.5 ARMU-Adv formulation relative to untreated fertilizer. Increased shoot N uptake presents several advantages, such as enhanced crop yield, and lower N losses to the environment (Chen et al., 2008; Mohammed et al., 2016). The enhanced ANR from inhibitor application is particularly beneficial as it reflects the effective supply of crop-available N, resulting in enhanced uptake of N by the crop (Gagnon et al., 2012; Chiyoka et al., 2014).

With the mitigation of N losses, it is expected that ARMU-Adv treated fertilizers would enhance N availability for crop uptake. However, other studies have demonstrated limited to no benefits on crop yield from the use of DIs due to the complex nature of soil conditions, such as moisture levels and environmental factors (Mohammed et al., 2016; Muller et al., 2023). For instance, Gao et al. (2015) demonstrated that the low N_2O emissions from the use of SuperU (a DI-treated urea) relative to untreated urea did not translate to an improvement in N uptake and grain yield.

The lack of significant treatment effects on wheat yield and some N efficiency indices in our study may be due to the high N concentration and high soil organic matter content of the soil used, which may have diminished the effectiveness of the DI by creating favorable conditions for wheat yield, thereby reducing the response to DI-treated N fertilizer (Gao et al., 2012). Moreover, the relatively high SOC content of the soil used in our study may have enhanced the breakdown of ARMU-Adv formulations (Gao et al., 2015).

Other studies have shown that the lower N_2O and NH_3 losses associated with inhibitor-treated urea-based fertilizers could potentially improve the availability of N in the soil at later stages of plant growth, thereby enhancing agricultural productivity and promoting environmental sustainability (Cui et al., 2022). Overall, the 1:0.5 NBPT:DMPP ratio demonstrates greater efficacy in comparison to the 1:1 ratio, ultimately offering a trade-off between efficacy and cost in comparison to the 1:1 ratio. Despite the lack of wheat yield benefit from inhibitor treatment, the use of ARMU-Adv led to a significant reduction in gaseous N losses and enhanced N uptake. Therefore, the treatment of urea-based fertilizers with either ARMU-Adv formulation is a potentially effective approach to minimize N losses from agricultural systems without compromising spring wheat yield.

It is noteworthy that, averaged across ARMU-Adv formulations, crop N uptake rates for urea and UAN were similar, despite various studies reporting greater N uptake from UAN (Wang et al., 2018; Ren et al., 2021). This disparity has been attributed to the fact that the N in UAN is partitioned equally between ammonium nitrate and urea forms and can therefore result in the enhancement of photosynthetic efficiency, thereby increasing crop yield and N uptake (Fageria and Baligar, 2005; Rochette et al., 2009b).

Ren et al. (2021) reported a 9.1% increase in maize yield under fertigation with UAN relative to urea. The authors noted that UAN offered the benefits of different N forms (i.e., urea, NH_4^+, and NO_3^-), which likely enhanced crop biomass yield and N recovery efficiency. They attributed the lower yield for urea to the greater susceptibility of urea to N loss and the poor distribution of the granular form relative to liquid UAN (Ren et al., 2021). The absence of significant differences in wheat biomass yield and uptake between urea and UAN in our study suggests that the difference in the N loss mechanisms between urea and UAN did not translate to differences in biomass yield and plant uptake.

2.5.5 Residual Soil Nitrogen

The ability of DIs to simultaneously inhibit nitrification and urea hydrolysis may lead to the accumulation and retention of plant-available NH_4^+ and a temporary reduction in the production and accumulation of NO_3^- due to the inhibitory effect of DMPP (Guo et al., 2014; Wang et al., 2020; Zaman and Nguyen, 2012).

The low accumulation of soil NO_3^- associated with the 1:0.5 formulation relative to untreated fertilizer following the NO_3^- leaching experiment demonstrates that this ARMU-Adv formulation was effective at slowing down the production of NO_3^-, which would otherwise translate to N losses via N_2O emission and NO_3^- leaching, particularly in the absence of crop N uptake (Park et al., 2012, Subbarao et al., 2006).

It noteworthy that, despite the lack of a significant difference in total NO_3^- leaching between DI-treated and untreated fertilizers, the significantly lower residual soil NO_3-N observed with the 1:0.5 formulation relative to untreated fertilizer highlights possible ARMU-Adv efficacy. The lack of a significant effect of the added inhibitors on residual soil NH_4-N and residual NO_3-N may be attributed to the immobilization of applied N by soil microorganisms (Zaman and Nguyen, 2012; Ma et al., 2015; Wang et al., 2020).

Additionally, residual soil NH_4-N may have been fixed between clay mineral interlayers (Juma and Paul, 1983). Nevertheless, similar to our results, Lasisi et al. (2021) observed no significant difference in the residual NO_3-N levels between DI-treated and untreated urea or UAN following canola and wheat crops.

2.5.6 Percentage Reduction in Nitrogen Losses

Our study showed that the percentage reduction in NH_3 volatilization from ARMU-Adv treatment was significantly greater for urea (60%) than for UAN (44%). This reflects the larger urea-N content of urea (100%) compared with UAN (50%) and underlies the greater vulnerability to NH_3 volatilization of urea relative to UAN (Chambers et al., 2009, Lasisi et al., 2020). On the other hand, the relatively low N_2O emissions and high percentage reduction in N_2O emission from UAN (50%) highlight the potential of UAN as a preferred N source for the reduction of N_2O emissions. The greater percent reduction in N_2O emission from UAN treated with the 1:1 NBPT: DMPP formulation (58%) than the 1:0.5 formulation (43%) reflects the greater concentration of the nitrification inhibitor, DMPP, in the 1:1 formulation and underscores the importance of determining the optimal inhibitor ratios for achieving the desired outcomes without compromising efficacy. Nonetheless, it is important to note that the effectiveness of a DI formulation can be influenced by various factors, including N source, moisture content and temperature.

By comparison, inhibitor ratio had no significant effect on NH_3 volatilization, suggesting that there is no benefit, in this regard, to increasing (doubling in this case) the DMPP concentration of ARMU-Adv beyond the lower optimal level. Overall, our results indicate that the use of ARMU-Adv holds significant potential for minimizing N losses associated with urea-based fertilizers and mitigating their adverse environmental impacts.

2.6 Conclusion

Surface application of urea and UAN treated with formulations of the double-inhibitor ARMU-Adv significantly reduced cumulative ammonia volatilization relative to untreated fertilizers. Despite the difference in their DMPP concentrations, the two ARMU-Adv formulations (1:1 and 1:0.5 NBPT:DMPP) were equally efficacious in reducing NH_3 volatilization from urea and UAN. Percent reduction in NH_3 volatilization was significantly greater for inhibitor-treated urea (60.5%) than the UAN equivalent (45%). Relative to untreated UAN, ARMU-Adv formulations produced a 50% reduction in N_2O emission. Relative to untreated UAN, the 1:1 formulation produced a significantly greater percent reduction in N_2O emission (58%) than the 1:0.5 formulation (43%). The 1:0.5 inhibitor-treated fertilizers produced a notable increase in shoot N uptake and ANR relative to untreated fertilizers. ARMU-Adv demonstrated greater efficacy with urea than with UAN in reducing NH_3 losses as well as in improving wheat shoot N uptake and ANR. On the other hand, ARMU-Adv exhibited superior efficacy in mitigating N_2O losses when applied to UAN in comparison with urea. Overall, these findings suggest that the ARMU-Adv formulations hold a significant potential for minimizing N losses from urea-based fertilizers, thereby potentially mitigating adverse environmental impacts without compromising crop productivity. The low NBPT and DMPP dosages relative to most commercially-available DI products may make ARMU-Adv more cost-effective than other double inhibitors currently in use. Future studies should test ARMU-Adv formulations under field conditions and examine the economics of their use relative to other N stabilizers on the market.

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3. OVERALL SYTHESIS

3.1 Research Findings

This research investigated the effectiveness of a novel double inhibitor (DI) product, ARMU Advanced (ARMU-Adv), in reducing NH_3 volatilization, N_2O emissions, and NO_3^- leaching from urea-based fertilizers, as well as improving wheat biomass yield and residual soil NH_4^+ and NO_3^- under controlled environment conditions. The DI formulations consisted of urease inhibitor (N-(n-Butyl) thiophosphoric triamide (NBPT)) to nitrification inhibitor (3,4-Dimethylpyrazole phosphate (DMPP)) ratios of 1:1 and 1:0.5 and were applied to urea ammonium nitrate (UAN) and urea.

The results showed a significant reduction in NH_3 losses when the two fertilizers were treated with the ARMU-Adv formulations. The 1:1 NBPT:DMPP formulation significantly reduced N_2O losses from UAN but had no significant effect on N_2O emissions from urea. The DI had no effect on NO_3^- leaching regardless of the fertilizer source. However, the 1:0.5 ARMU-Adv formulation decreased residual soil NO_3^- concentration following the NO_3^- leaching experiment.

The 1:0.5 formulation significantly enhanced shoot N uptake and apparent N recovery (ANR) by wheat relative to untreated fertilizer. Overall, our results indicated the efficacy of ARMU-Adv in reducing NH_3 and N_2O losses and enhancing wheat shoot uptake and ANR relative to untreated fertilizers, showcasing its promise as a valuable additive in crop production. Additionally, ARMU-Adv demonstrated superior efficacy with urea than with UAN as the N source, except in the N_2O study. Although DIs presents an additional cost to producers, the long-term benefits in terms of reduced fertilizer losses and environmental sustainability may offset the extra expense.

3.2 Implication of the Research

Our results indicate that ARMU-Adv formulations have the potential to effectively reduce agricultural N losses and improve fertilizer N efficiency. The low NBPT and DMPP rates provide a potential cost-effective option for farmers relative to other DI products on the market. The significant improvement in wheat shoot uptake and ANR in our study with the incorporation of ARMU-Adv formulations relative to untreated fertilizers can potentially benefit the Canadian economy by influencing wheat production, as wheat is a significant economic crop in the prairies.

Nitrogen losses are a significant contributor to environmental degradation via eutrophication of surface water bodies, contribution to–global climate change, and human health problems. Our findings contribute towards understanding and establishing effective mitigation techniques that will aid the formulation of agricultural strategies aimed at mitigating negative impacts of urea-based fertilizers. Incorporating DIs into sustainable agricultural systems can ultimately help accomplish desirable environmental and agronomic outcomes, thereby establishing a more effective and sustainable food production system through mitigation of N_2O losses, hence climate change.

3.3 Future Studies

The promising and favourable outcomes from our laboratory study need to be tested at field-scale to thoroughly evaluate the efficacy of ARMU-Adv under practical agricultural settings. Field studies will enable the examination of ARMU-Adv impacts and interactions with various environmental variables, thus offering useful perspectives for enhancing its implementation in practical agricultural systems. Future studies should also examine novel DI formulations that can enhance the shelf life of treated fertilizers (Cantarella et al., 2018). More research is needed to explore the impact of varying fertilizer application depths on the efficacy of ARMU-Adv formulations in reducing N losses from urea (Zaman et al., 2008; Haitao Wang et al., 2020). An assessment of the economic feasibility of the novel inhibitor formulations will inform farmers on the profitability of cropping systems incorporating the products (Cui et al., 2011).

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