

EFFECT OF ENHANCED EFFICIENCY NITROGEN FERTILIZERS AND ANVOL™ ON
WINTER WHEAT YIELD AND PROTEIN CONTENT

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Abstract

Nitrogen (N) plays a critical role in agricultural production, particularly in cereal crops such as winter wheat (*Triticum aestivum* L.), where it influences both yield and grain quality. However, managing nitrogen efficiently remains a challenge in many regions, including Northwestern Ontario, where suboptimal nitrogen use often results in reduced yields and lower plant protein content. This study evaluated the effectiveness of enhanced efficiency nitrogen fertilizers (EEFs), such as Environmentally Smart Nitrogen (ESN), SUPERU™, and urea treated with ANVOL™, in improving winter wheat production under the agroclimatic conditions of Thunder Bay, Ontario. The overarching goal was to determine whether these advanced fertilizers could enhance N use efficiency and address the issue of low plant protein content. The experiment was conducted at the Lakehead University Agricultural Research Station (LUARS; <https://www.lakeheadu.ca/centre/luars>) using N application rate of 120 kg N/ha either from individual N fertilizers or their blends with additional treatments of SUPERU™ at 100 kg ha⁻¹, urea at 160 kg N ha, and a no-N reference treatment. Key parameters such as plant and stem counts, plant heights through critical stages, chlorophyll content, grain yield, and plant protein content were assessed to evaluate treatment performance. Nitrogen source had minor effects on chlorophyll content, with marginal differences in leaf pigmentation among treatments. Similarly, phenotypic traits and grain characteristics showed no substantial variation across N sources or application rates. Grain yields were, however, significantly higher in treated plots compared to reference plots without N. This suggests that EEFs, while aimed at improving N use efficiency, did not translate into higher yields than urea alone under Thunder Bay's specific environmental and soil conditions (pre-seeding nitrate N: 14 ppm and ammoniacal N: 5 ppm). These results underscore the complexity of N management in winter wheat and suggest that factors beyond N application may have a more pronounced impact on yield in this region, in this case a dry summer. Despite the lack of yield improvement, the central question remains whether EEFs can enhance plant protein content—a critical quality determinant for wheat.

Keywords: Nitrogen management, enhanced efficiency fertilizers (EEFs), Environmentally Smart Nitrogen (ESN), SUPERU™, ANVOL, winter wheat, plant protein content, yield, nitrogen use efficiency, chlorophyll content, Thunder Bay, Northwestern Ontario.

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List of Abbreviations

AAC: Agriculture and Agri-Food Canada
 ANOVA: Analysis of Variance
 ANVOL™: urease inhibitor (contains NBPT and Duromide)
 CEC: Cation Exchange Capacity
 CGC: Canadian Grain Commission
 CHLORO1: Chlorophyll content at initial measurement (head-visible stage)
 CHLORO2: Chlorophyll content at second measurement (head-visible stage)
 CHU: Corn Heat Units
 CRFs: Controlled-Release Fertilizers
 CWRW: Canadian Western Red Winter wheat
 CWRS: Canadian Western Red Spring wheat
 DMPP: 3,4-Dimethylpyrazole phosphate (nitrification inhibitor)
 DW: Dry Weight
 EEFs: Enhanced Efficiency Fertilizers
 ESN: Environmentally Smart Nitrogen (polymer-coated Urea)
 FAO: Food and Agriculture Organization
 GDD: Growing Degree Days
 GW: Green Weight
 HENFs: High-Efficiency Nitrogen Fertilizers
 HRWW: Hard Red Winter Wheat
 IFA: International Fertilizer Association
 LUARS: Lakehead University Agricultural Research Station
 LSD: Least Significant Difference
 $Mg\ ha^{-1}$: Megagrams per hectare (equivalent to Metric Tonnes per hectare)
 MT ha^{-1} : Metric Tonnes per hectare
 N: Nitrogen
 NBPT: N-(n-butyl) thiophosphoric triamide (urease inhibitor)
 NI: Nitrification Inhibitors
 NIR: Near-Infrared Reflectance (spectroscopy)
 NO₃-N: Nitrate Nitrogen
 NH₄-N: Ammonium Nitrogen
 NUE: Nitrogen Use Efficiency
 OMAFRA: Ontario Ministry of Agriculture, Food and Rural Affairs
 PH1: Plant Height at Sheath Stage (Zadoks 30–32)
 PH2: Plant Height at Flag Leaf Stage (Zadoks 37–39)
 PH3: Plant Height at Head Visible Stage (Zadoks 50–59)
 PH4: Plant Height at Harvesting Stage (Zadoks 90–92)
 ppm: parts per million
 SPSS: Statistical Package for the Social Sciences
 SUPERUT™: Stabilized Urea fertilizer with urease and nitrification inhibitors
 TKW: Thousand Kernel Weight
 UI: Urease Inhibitors
 UR: Urea
 URAN: Urea treated with ANVOL™

URS: Urea blended with SUPERU™

URSU: Urea + SUPERU™ blends

1.0 Introduction

Efficient nitrogen (N) management is vital to enhance the yield and grain quality of winter wheat (*Triticum aestivum* L.), especially in Northwestern Ontario, where precipitation is uneven. Winter wheat is typically planted from late August to early September and harvested in mid-to-late summer, usually August, of the following year (Top Crop Manager, 2023; Field Crop News, 2019; Fixen et al., 2015n). The methods and timing of N fertilizer application are critically important as they ensure the nutrition of the plants during the requisite periods of growth (Beres et al., 2023; Halvorson & Del Grosso, 2012). Nitrogen is a limiting nutrient in most agricultural soils, and its quantity, usually augmented by crop fertilizers, is directly related to the yield and protein content of grains (Akiyama et al., 2010; Lewu et al., 2020). For many years, scientists directed efforts toward the development of enhanced efficiency fertilizers (EEFs) to overcome problems related to conventional fertilizers, such as leaching, volatilization, and denitrification, which contribute to N lost from fertilizers (Halvorson et al., 2014; Trenkel, 2010). Increased nitrogen loss reduces plant nitrogen uptake, leading to decreased above-ground biomass, grain yield, and plant protein concentration (Brown et al., 2005). Additionally, higher nitrogen losses may elevate the risk of environmental pollution and ecosystem degradation (Snyder et al., 2009).

Among the new EEFs, a polymer-coated urea product known as Environmentally Smart Nitrogen (ESN), made by Nutrien, shows encouraging outcomes under field conditions in enhancing crop N use efficiency (NUE) by synchronizing N release with the plant growth stages that most require it (Halvorson et al., 2014; Beres et al., 2023). The process used by ESN minimizes leaching and N volatilization, reducing total N losses by up to thirty percent

(Halvorson et al., 2014; Fuentes et al., 2024). The proposed ESN technology is suggested to enhance NUE and overall yield especially from the crops grown under difficult agrometeorological environments (Beres et al., 2023; Trenkel, 2010; Blaylock et al., 2004). SuperUTM and ANVOLTM are two other advanced N stabilizers. SuperUTM combines urease and nitrification inhibitors (N-(n-butyl) thiophosphoric triamide [NBPT] and dicyandiamide), effectively reducing N losses from volatilization and leaching (Trenkel, 2010; Beres et al., 2023) and allowing for more extended N availability and uptake (Sahota, 2024; Halvorson & Del Grosso, 2013). Similarly, ANVOLTM contains dual inhibitors (NBPT and Duromide) that slow the conversion of urea into ammonia and nitrate, enhancing N availability during the critical growth periods of winter wheat (Trenkel, 2010; Dell et al., 2014).

Integrated application of ESN, SuperUTM and ANVOLTM has consistently produced higher wheat yields and plant protein content (Fuentes et al., 2024). Findings from studies spanning multiple areas indicate that these modern N fertilizers boost NUE while minimizing environmental pollution, including nitrous oxide emissions (Akiyama et al., 2010; Halvorson et al., 2010). They represent a major step forward as applications in environmentally sensitive areas prone to N leaching and volatilization (Trenkel, 2010; Gao et al., 2015). The integration of controlled-release and stabilized N fertilizers into broader nutrient management strategies is critical for enhancing winter wheat production in areas where climatic variability demands precise timing of fertilizer applications (Lewu et al., 2020; Beres et al., 2023). Furthermore, these fertilizers contribute to environmental sustainability by significantly reducing greenhouse gas emissions, particularly nitrous oxide, from agricultural soils (Akiyama et al., 2010; Dell et al., 2014; Halvorson et al., 2014).

The development of EEFs such as ESN, SuperUTM, and ANVOLTM offers a multi-dimensional approach to improving N management in winter wheat production (IHARF & Agriculture & Agri-Food Canada, 2025). By optimizing NUE, reducing environmental losses, and enhancing grain quality, these fertilizers are poised to play a vital role in sustainable agriculture, particularly in regions facing climatic challenges like Northwestern Ontario (Beres et al., 2023; Sahota, 2024). Ongoing research at the Lakehead University Agriculture Research Station and other similar institutions continues to demonstrate the significant benefits of these technologies for improving both agricultural productivity and environmental sustainability (Fuentes et al., 2024; Halvorson et al., 2014). The objective of this study is to compare the effects of applying traditional urea and ESN, SuperUTM, and ANVOLTM, both individually and in blends at different rates with respect to NUE and crop yield in winter wheat. The study aims to identify N management strategies that optimize N uptake efficiency and maximize agricultural productivity in Northwestern Ontario. One limitation is the study's duration, spanning only a single growing season, which prohibits the observation of long-term effects and renders the outcome vulnerable to seasonal variability in weather patterns.

2.0 Literature Review

2.1 Wheat (*Triticum aestivum* L.)

2.1.1 The Agricultural History of Wheat

Wheat (*Triticum aestivum* L.), with its hexaploid genome ($2n=6x=42$) comprising the AABBDD genome composition, has demonstrated superior adaptability and yield potential, contributing to its dominance among cultivated species (Dubcovsky & Dvorak, 2007; Curtis et al., 2002). It is one of the earliest crops known to be domesticated and is an integral part of the modern complex food systems and the human diet for the past about 10,000 years originating from the Fertile Crescent (Shewry, 2009). The earliest domestication efforts focused on Einkorn (*Triticum monococcum* L.), a diploid wheat species, and Emmer (*Triticum dicoccum* Schrank ex Schübl.), a tetraploid, both of which were selected for their resilience and adaptability in the Fertile Crescent (Heun et al., 1997; Zohary et al., 2012; Feldman & Levy, 2012). These species laid the foundation for the development of modern wheat due to their ability to thrive in early agricultural environments. Due to its adaptability and yield potential, *Triticum aestivum* L. later emerged as a dominant species (Curtis et al. 2022; Rajaram & Macpherson 2002).

The global spread of wheat can be attributed to its genetic versatility, which allows for cultivation under diverse agroclimatic conditions (Pingali, 1999). This adaptability has positioned wheat as a primary cereal crop in terms of area and production, contributing approximately 20% of global caloric and protein intake (FAO, 2021). Wheat is also unique in its high gluten content, which enables the production of a wide variety of food products, including bread, pasta, and pastries (Shewry, 2009). The evolution of wheat has also been

marked by significant genetic changes, driven by natural selection and human intervention (Feldman & Levy, 2012). Key milestones include the emergence of free-threshing wheats and the reduction of seed shattering, traits that facilitate efficient harvesting and processing (Salamini et al., 2002). Modern wheat breeding programs have further enhanced its genetic makeup by focusing on yield, resistance to pests and diseases, and tolerance to abiotic stresses such as drought and salinity (Thomas & Graf, 2014).

The Industrial Revolution and subsequent agricultural advancements in the 19th and 20th centuries transformed wheat production through the introduction of synthetic fertilizers, mechanization, and improved irrigation systems (Pingali, 1999). The Green Revolution in the mid-20th century further boosted wheat yields through the development of high-yielding, semi-dwarf varieties that were more responsive to nitrogen (N) fertilizers (Evenson & Gollin, 2003). These innovations significantly reduced global food insecurity and positioned wheat as a central crop in the fight against hunger.

Today, wheat production faces challenges related to climate change, soil degradation, and increasing global population pressures (Curtis et al., 2002). Research efforts are increasingly focusing on sustainable practices, including the use of enhanced efficiency fertilizers (EEFs) and precision agriculture techniques to address these challenges while maintaining high productivity and quality (Thomas & Graf, 2014). Advances in genetic engineering and molecular biology offer promising avenues for improving wheat's resilience to biotic and abiotic stresses, ensuring its continued role in global food security (Shewry, 2009).

2.1.2 Wheat Production in Canada

Canada ranks seventh among wheat producing countries, with 34.3 million metric tonnes produced per year over the past decade (World Population Review, 2024). Most production occurs in the Prairie provinces, where Saskatchewan alone contributes 50% of Canada's wheat production (Curtis et al., 2002). Canada exports over 70% of its wheat, making it one of the five top wheat exporting nations in the world (FAO, 2021). Although not a significant wheat producing area relative to the Prairies, wheat production has been shown to be possible in Northern Ontario under certain agronomic practices (Shewry, 2009). Because of the region's shorter growing season of 90–120 days, winter wheat or spring wheat varieties that mature early are needed for successful harvests before frost (Thomas & Graf, 2014).

Precipitation in Northwestern Ontario ranges from 450–700 mm annually, which is enough water for wheat growth, but supplemental drainage is frequently needed (Pingali, 1999).

The growth of wheat is dependent on certain environmental conditions: temperature during the growing season between 15 and 24°C; well drained soils; pH 6.0–7.5 (Curtis et al., 2002). Clay rich soils of Northern Ontario create challenges for drainage and nutrient availability, and these conditions are usually overcome by using soil amendments and prudent field management (Shewry, 2009). Wheat is a N demanding crop and the efficient use of N during the wheat tillering and grain-filling stages is critical to maximise grain yield and protein content (Thomas and Graf, 2014).

The average wheat yield in Canada is approximately 2.5–3.5 MT ha^{-1} , although yields in Northwestern Ontario tend to be lower due to soil and climatic constraints (FAO, 2021). Research at the Lakehead University Agricultural Research Station (LUARS) has shown that optimized N management, including the use of EEFs and stabilizers, can improve wheat yields

and protein content in the region (Sahota, 2019). Specifically, applying 100 kg N/ha from a urea and ESN blend (2:1 ratio) can match the dry matter yield and protein content achieved with 150 kg N/ha from urea alone, indicating more efficient N use with the blend. Additionally, ESN has been found to increase plant protein content in winter wheat, with higher N-use efficiency compared to Urea and ammonium sulfate. These findings suggest that incorporating ESN into N management practices can lead to improved crop performance in the region (Sahota, 2019). Enhanced efficiency fertilizers, such as ESN, SUPERUTM, and ANVOLTM, reduce N losses and improve nutrient availability, making them a valuable tool for wheat production in regions like Northwestern Ontario (Curtis et al., 2002).

Technological advancements in wheat breeding have significantly enhanced the crop's adaptability and performance in Canada. For instance, the integration of genomic selection techniques has accelerated the development of disease-resistant and high-yielding wheat varieties tailored to the diverse growing conditions of the Canadian prairies. A notable initiative is the \$11.8 million investment over five years aimed at revolutionizing Canadian wheat breeding through advanced genomic technologies (Farmonaut, 2023). Modern varieties are increasingly bred for resistance to diseases such as rust and Fusarium Head Blight, as well as tolerance to drought and frost, traits that are particularly beneficial in Northwestern Ontario's challenging climate (Shewry, 2009). These developments, combined with sustainable farming practices such as crop rotation and reduced tillage, ensure that wheat remains a viable and valuable crop for Canadian farmers (Thomas & Graf, 2014).

2.2 Nitrogen Dynamics and Urea Use in Modern Agricultural Systems

Nitrogen is a critical macronutrient for plant growth and is an essential component of chlorophyll, amino acids, and proteins, which are vital for photosynthesis and plant development (Fathi, 2022). In agricultural systems, N is often the most limiting nutrient, with its availability directly impacting crop yields and quality (Zhao et al., 2020). To address this limitation, Urea is widely used, accounting for over 50% of the global N fertilizer consumption due to its high N content (46%) and cost-effectiveness (Hu & Schmidhalter, 2024).

Urea, known chemically as $\text{CO}(\text{NH}_2)_2$, is very soluble in water, hydrolyzes first to ammonium hydroxide, then to ammonium carbonate and later to nitrate N, the latter as readily available to crop plants (Ahmed et al., 2023). Unfortunately, urea efficiency is also often thrown off by N losses through volatilization, leaching and denitrification, causing significant environmental woes such as greenhouse gas emissions and water pollution (Smith et al., 2019). In favourable conditions, as much as 40 % of the applied urea N may be lost through volatilization (Zhao et al., 2020).

Enhanced efficiency fertilizers, including urease inhibitors like NBPT, nitrification inhibitors such as Dicyandiamide, and polymer-coated Urea have been developed to mitigate N losses from urea application (Fathi, 2022). These inhibitors slow the hydrolysis of urea, reducing ammonia volatilization and improving NUE (Ahmed et al., 2023). Studies have shown that EEFs can increase crop N uptake by 15–20% and reduce N losses by up to 30% compared to conventional Urea (Smith et al., 2019). Urea application rates and timing are critical to optimizing N availability while minimizing environmental impacts (Zhao et al., 2020). Split applications and the incorporation of Urea into the soil have been identified as effective strategies to enhance NUE (Rütting et al., 2018). Integrating Urea with other N

sources such as ESN, SuperUTM, and ANVOLTM can improve yield and reduce the risk of environmental degradation (Ahmed et al., 2023).

The role of N in agricultural systems extends beyond productivity to sustainability. Proper N management is essential for achieving high yields while minimizing environmental footprints, a balance increasingly emphasized in modern agricultural practices (Smith et al., 2019). As agricultural systems face challenges from climate change and resource limitations, innovations in N fertilizers and management strategies remain crucial for global food security (Zhao et al., 2020).

2.2.1 Conventional Nitrogen Rates in Winter Wheat Production in Northwestern Canada

Northwestern Canada has specific climatic and soil conditions, and the critical factor about N management for winter wheat is the optimal timing of N application in this region (Karamanos & Stevenson, 2013). For a standard 2.69 Mg ha⁻¹ winter wheat crop, the N requirement is about 95 kg N ha⁻¹, about 70% of which goes to the grain (67 kg N ha⁻¹) with the remainder in the straw (Karamanos & Stevenson, 2013). While N recovery in cereals is about 25–50 %, the application rates normally needed to meet the crop's requirement are above 50 kg N ha⁻¹ y⁻¹ (Kubota et al., 2017). Recommended N application rates for irrigated Canadian Western Red Spring (CWRWS), and Canadian Western Red Winter (CWRW) wheat in the Alberta Fertilizer Guide (2004) vary from 40–125 kg ha⁻¹ based on soil test results and yield goals. In central Saskatchewan and Alberta, studies have shown that for rainfed systems, N rates of 80–100 kg N ha⁻¹ are usually required to obtain optimum yields (Karamanos & Stevenson 2013). These recommendations are consistent with values reported in Ontario,

where N rates applied to winter wheat are 70–105 kg N ha^{-1} , depending on soil type and topography (OMAFRA, 2017).

In humid regions such as Ontario, pre-plant or early-spring soil nitrate tests often have limited predictive value because nitrate is transient (leaching/denitrification) and spring values can decouple from in-season crop demand (Morris et al., 2018; OMAFRA Field Crop News, 2022). These residual-nitrate tools tend to perform better in drier climates where overwinter losses are smaller (Morris et al., 2018). Ontario relies chiefly on the pre-sidedress nitrate test (PSNT) for corn—an in-season 0–30 cm nitrate-N sample taken around V4–V6—to separate likely responsive from non-responsive fields (OMAFRA Field Crop News, 2021; OSCIA, 2014a). A widely used operational threshold near 21 mg $\text{NO}_3\text{-N kg}^{-1}$ indicates adequate soil N supply where no pre-plant N was applied (OSCIA, 2014b). Where modest pre-plant N has been applied, Ontario research has evaluated a 36 mg $\text{NO}_3\text{-N kg}^{-1}$ “no-top-up” threshold at sidedress (OSCIA, 2015a; OSCIA, 2014a). Because the PSNT quantifies nitrate only—not ammonium or unreleased coated urea—interpretation can be complicated when nitrification inhibitors or controlled-release N are used (Michigan State University Soil & Plant Nutrient Laboratory, n.d.; OMAFRA, 2022). Urease and nitrification inhibitors slow hydrolysis and nitrification, and polymer-coated urea (e.g., ESN) delays N release—mechanisms shown to reduce loss pathways and/or improve N-use efficiency in wetter climates (OMAFRA, 2022; Li et al., 2020; Wang et al., 2020). Split applications, by which a portion of the N is applied at seeding and the other is top dressed in spring, can increase NUE and decrease environmental loss (Akhter et al., 2024). Accordingly, a treatment of applying 30 kg N ha^{-1} in the fall and 90 kg N in the spring was kept in the study reported herein. Split N applications at rates up to 120 kg N ha^{-1} did not reduce grain yield or protein content, but reduced N losses in southern Alberta (Smith

et al., 2019). Bogard et al., (2010) also reported that winter wheat grain yield was not affected by N applications of 60, 75, and 120 kg N ha⁻¹, but protein content increased significantly with the increasing rates of N application.

A series of N response studies in winter wheat point out the diminishing returns to N excessive applications. Walsh et al. (2018) found no significant yield improvements above 135 kg N ha⁻¹ in rainfed systems, with optimal protein concentration with N applications of 90 to 120 kg N ha⁻¹. A prairie wide study indicated that moisture supply related to growing season generally limited yield more than N supply (Ye et al., 2022). Because winter wheat productivity is maximized in Northwestern Canada through integration with water management strategies and N fertilization, it is therefore important to integrate the two practices In Canadian winter wheat systems—particularly Ontario—field trials typically place the most-economic rate of nitrogen (MERN) for soft winter wheat around 80–130 kg N ha⁻¹ when accounting for soil test N, fungicide use, and local yield potential (Ontario Soil and Crop Improvement Association [OSCIA], 2007; OSCIA, 2013). This range should be interpreted as context-specific to Canadian production environments and market classes (e.g., soft vs. hard wheat) rather than universal optima (OSCIA, 2007; OSCIA, 2013). In higher-yield, irrigated or high-rainfall systems of the Pacific Northwest, total crop N requirements scale sharply with yield goals (e.g., ~270–330 lb N ac⁻¹ total supply for 140–180 bu ac⁻¹), so fertilizer N needs can exceed 150 kg N ha⁻¹ after credits for soil, mineralizable, and irrigation water N (Brown, 2001/2001-rev). Likewise, in irrigated durum systems of the U.S. Desert Southwest, typical on-farm rates are ~200–300 kg N ha⁻¹ and research responses have been measured up to ~403 kg N ha⁻¹ under frequent irrigation (Liang et al., 2014). High-yielding environments in China also show yield plateaus near ~250 kg N ha⁻¹, although environmentally balanced rates of

~120–171 kg N ha⁻¹ often optimize the trade-offs among yield, plant protein, and N losses (Ma et al., 2019). Accordingly, claims that rates >150 kg N ha⁻¹ “seldom” increase yield should be qualified as most applicable to many Canadian winter wheat contexts and classes, whereas higher-yielding or irrigated systems can remain responsive to substantially greater N inputs—often with additional late-season N used to meet protein targets for hard wheat (Brown, 2001/2001-rev; Brown et al., 2005; OSCIA, 2013). Efficient N management practices that include split applications and soil testing are important for sustainable and profitable winter wheat production (Dhillon et al., 2020).

2.2.2 Impact of Advanced Nitrogen Fertilization on Yield and Quality in Winter Wheat

Enhanced efficiency fertilizers include urease inhibitors (UIs), nitrification inhibitors (NIIs) and slow- or controlled-release fertilizers (CRFs) developed to address N loss and increase the availability of N to the crops (Karamanos & Stevenson, 2013). They have potential to improve key yield components (e.g., grain yield, Thousand Kernel Weight [TKW], and plant content) in winter wheat systems (Beres et al., 2018). Urease inhibitors such as NBPT delay the hydrolysis of urea, minimizing ammonia volatilization and keeping N within the soils (Smith et al., 2019). By using NBPT treated urea in Alberta, grain yield improvements of 12%, increases in NUE of 15%, and increases in plant protein content of 1.2% were observed in winter wheat as compared to untreated Urea (Beres et al., 2018). NBPT treated N fertilizers resulted in 11% increase in grain yields versus conventional Urea applications, suggesting UIs may be feasible to increase crop performance in areas subject to volatilization (Zaman et al., 2010).

Nitrification inhibitors such as 3,4-dimethylpyrazole phosphate (DMPP) reduce the conversion of ammonium to nitrate and thus reduce Urea losses (Cantarella et al., 2018). Use of DMPP treated Urea resulted in increases of 15% in winter wheat grain yield in field trials in Saskatchewan compared to use of untreated Urea (Cantarella et al., 2018). A study in China's irrigated wheat systems found that a dual inhibitor of NBPT plus nitrapterin allowed total N application rate to be reduced by 20% without affecting grain yield and protein content, demonstrating the efficiency of NIs under high leaching conditions (Tao et al., 2021). ESN is a CRF based on covering Urea with a polymer coating, on the other hand, that releases N slowly and ideally in synchronization with crop demand. In Southern Alberta, applying ESN to a winter wheat crop increased grain yield by 4.3% in a dry year, but decreased grain protein content by 1.3% (Fast et al., 2024). In Montana, ESN increased yields in only 25% of site years, and its performance was highly dependent on environmental conditions such as available soil moisture and temperature (Grant et al., 2012).

SuperUTM, a dual-inhibitor fertilizer, has consistently outperformed other EEFs and conventional Urea in several studies. In Manitoba, SuperUTM increased grain yield by 7% and protein content by 1.5% compared to untreated Urea, emphasizing its efficacy in improving crop performance in N loss-prone environments (Cantarella et al., 2018). However, other trials have reported no significant differences in yield or protein content between EEFs and conventional fertilizers, particularly in environments with low N loss potential. In North Carolina multi-site trials, ESN and other "alternative" N products did not increase wheat or corn grain yield relative to conventional sources (primarily UAN), and wheat straw yields were often lower with ESN, underscoring that responses depend strongly on management and

environment (Cahill, Osmond, Weisz, & Heiniger, 2010; Gatiboni, 2025; Rajkovich, Osmond, Weisz, Crozier, & Israel, 2017).

The North Carolina studies used late, 100% ESN top-dress applications, though not recommended by the manufacturer, and observed that yields were reduced because of insufficient early N supply. This confirms manufacturer recommendations to apply earlier and to blend ESN with other immediately available N sources such as urea or ammonium sulfate to meet near-term crop demand (Smart Nitrogen/ESN, 2025a; Smart Nitrogen/ESN, 2022; Rajkovich et al., 2017). By contrast, in dryland Montana the potential for N loss (and thus the relative upside for EEFs) is often limited by the region's generally cool, dry conditions, so yield/protein benefits from EEFs tend to be smaller unless a specific loss pathway (e.g., volatilization, leaching under wet spells) is clearly at risk (Olson-Rutz, Jones, & Dinkins, 2011). Accordingly, interpretations of EEF performance should explicitly tie outcomes to weather/soil context and management (rate, timing, placement, and blending), rather than generalizing across agroecological zones (Olson-Rutz et al., 2011). The environmental benefits of EEFs include reducing N losses and mitigating greenhouse gas emissions (Abalos et al., 2014). Studies show that EEFs can reduce nitrous oxide (N₂O) emissions by up to 40% compared to conventional urea, with NIs demonstrating the greatest reductions (Zhang et al., 2024). In terms of economics, the price differential between ESN and traditional urea fertilizer fluctuates according to factors such as geographical location, market dynamics, and supplier-specific pricing strategies. Within Northwestern Ontario, specifically in the Thunder Bay area, Thunder Bay Co-operative Farm Supplies has received increased interest in expanding its storage capabilities for ESN, reflecting rising local demand and potentially greater future availability. For precise and updated pricing, direct consultation with regional suppliers is

recommended. In Texan winter wheat systems, ESN reduced N losses but resulted in lower net profits due to its higher cost, making urea the most profitable fertilizer option (Adams et al., 2018). In Western Canada, a 50:50 mix of ESN and conventional urea was found to improve both NUE and cost efficiency, offering a practical compromise for growers (Khakbazan et al., 2013).

The performance of EEFs is highly dependent on three environmental factors: soil texture, precipitation, and temperature (Verburg et al., 2022). Nitropyrene (an NI) increased winter wheat grain yield by 10-15% under waterlogged conditions but had no effect on well drained soils (Kumar et al., 2015). ESN was more effective than conventional Urea in preventing N from becoming depleted in drought prone areas (Fast et al., 2024). In normal moisture conditions, though, the prolonged release of ESN often did not meet N peak demand during critical growth stages, decreasing yield and protein content (Keim & Kronstad, 1981). Barriers to more widespread adoption of EEFs are the higher costs of EEFs and inconsistent performance in low-loss environments. Strategic use of EEFs in N loss-prone regions and additional research to integrate EEFs into site specific agronomic practices may optimize the benefits of EEFs for sustainable wheat production.

2.2.3 Nitrogen Management in the Context of Northwestern Ontario's Wheat Production

Winter wheat cultivation is particularly challenging in Northwestern Ontario, an area characterized by cool summer temperatures and variable rainfall. Soil organic matter is also quite low in much of the area and the risk of N leaching increases during periods of intense seasonal rainfall (Zhang et al., 2024). Nitrogen management is complicated by these environmental constraints, affecting the efficiency of applied fertilizers (OMAFRA, 2022;

Fageria et al., 2011). ANVOL™ acts as a stabilizer that reduces the volatilization of ammonia that can be very helpful in applications under cool, moist conditions such as experienced in Northwestern Ontario (Muir, 2020; Zaman et al., 2009). Research in similar climates to that in Northwestern Ontario has shown that EEFs enhance NUE and are essential in the attainment of desired protein content levels in wheat (Zaman et al., 2013 & Son, 2021; Brown et al., 2005).

Producers in Northwestern Ontario realize that protein content in winter wheat is critical not only for internal market quality standards but also for export competitiveness (OMAFRA, 2022). Lower plant protein content can negatively impact grain quality and its market value, (Canadian Grain Commission, 2021; Fast et al., 2023). Optimal enzymatic activity and protein synthesis promote wheat functional properties for milling and baking. However, the regional environmental and soil constraints have posed a challenge to achieving maximum protein levels (Ontario Wheat Board, 2021; Ghimire et al., 2021). Protein development in wheat is dependent on an adequate supply of N at critical stages (Fageria, 2014). Among these stages, most important for protein deposition is the post-anthesis stage, from anthesis (flowering) up to grain filling, where N remobilization from vegetative organs to developing grains occurs (Denys et al., 2006). Maintaining a proper N supply during this stage is essential to achieve high protein content in grain.

Testing of new EEFs and stabilizers has been conducted in similar regions with similar soils and climate to Northwestern Ontario; N retention and availability has been shown to be significantly improved over the use of Urea in low organic matter soils ((Zaman et al., 2009; Cui et al., 2010). Similarly, application of stabilized N fertilizers, such as SuperU™ and ANVOL™, supports maintenance of N availability throughout the growing season and allows for protein accumulation in wheat kernels (Mathlouthi et al., 2022; Abad et al., 2005). In

general, EEFs help in preventing N loss from leaching and volatilization in wet spring conditions (Zaman et al. 2013, Fageria et al., 2011). Research in Western Canada indicated that while application rates of N were associated with increases in protein, though the benefits declined with increasing applications of N to an excessively high rate (Halvorson & Del Grosso, 2013). European studies also found that split N applications during the vegetative and reproductive stages significantly increased yield and plant protein content (Zaman et al., 2013). These results emphasize the critical role of matching N fertilizer application with the patterns of crop uptake to optimize protein synthesis.

2.3 Enhanced Efficiency Fertilizers (EEFs) for Regional Wheat Yield and Protein Optimization

2.3.1 Controlled-Release Fertilizers: ESN

ESN is a polymer-coated Urea that provides for the slow release of N to reduce volatilization and leaching losses. In general, this slow-release mechanism will match crop nitrogen requirements during the growing conditions prevalent in Northwestern Ontario (Smith et al., 2019; Farrer et al., 2006). Multi-year trials at Thunder Bay and New Liskeard (2006–2010) found winter wheat yields and N removal were generally similar for ESN and urea, with post-harvest residual nitrate often comparable and sometimes marginally lower under ESN—consistent with slower N release in cool northern conditions (Sahota & Rowsell, 2011). Ontario strip-trial results likewise showed spring ESN ≈ urea for yield (but higher plant protein), whereas fall-applied ESN reduced yield—highlighting the importance of timing in humid, cool springs (Ontario Soil and Crop Improvement Association [OSCIA], 2008). More broadly in eastern Canada, PCU/ESN performance depends on soil texture, moisture,

temperature, and organic matter, with benefits more evident in wetter seasons or poorly drained sites (Tubeileh et al., 2023). Studies under similar climatic conditions to the region have shown that ESN increases NUE, improves grain yield, and enhances plant protein content (Ma et al., 2024; Wood et al., 2023; Sahota, 2019). In fact, a study conducted in Alberta reported significant improvements in wheat plant protein content with the use of ESN through split application at critical crop growth stages (Zaman et al., 2009). Sahota (2020) analyzed over a decade of data across ten different crops, including winter wheat, and found that ESN not only improved nitrogen-use efficiency but also increased grain and forage protein content by 1-2%, resulting in enhanced yields and providing an economic benefit of \$92.75/ha over Urea. These finding showed similar effectiveness of ESN in fall and spring applications, offering flexibility in management to farmers.

2.3.2 Stabilized Nitrogen Sources: SUPERUTM and ANVOLTM Treated Urea

SUPERUTM contains both urease and nitrification inhibitors, which help maintain N in ammoniacal form and reduce N losses due to volatilization and denitrification, particularly under wet and cool conditions (Farrer et al., 2006; Cui et al., 2010). In regions like Northwestern Ontario, where events of heavy rainfall are quite common, the stabilizing properties of SUPERUTM could offer a potential opportunity to improve N retention (Abad et al., 2005). Investigations carried out in northern climatic regions have indicated that applications of SUPERUTM improve grain yield and protein levels in winter wheat in comparison to untreated Urea, especially under circumstances susceptible to N losses (Muir, 2020; Ghimire et al., 2022). Nonetheless, information pertinent to the distinct climatic and soil

properties of Northwestern Ontario remains limited, underscoring the necessity for localized research to validate these advantages (Smith et al., 2019; Farrer et al., 2006).

ANVOL™ (Koch Agronomic Services) is a urease inhibitor co-formulation of NBPT + Duromide designed to reduce ammonia volatilization from urea-based N sources (Koch Agronomic Services, n.d.-a; Koch Agronomic Services, n.d.-b). Its Duromide component is reported to extend the duration of protection and improve performance across varied soil conditions, including acidic soils where NBPT alone degrades faster (Koch Agronomic Services, 2023a, 2023b). Independent peer-reviewed work likewise shows Duromide + NBPT reduces NH₃ losses more than NBPT alone (up to ~33%) (Cassim et al., 2021). Studies carried out under similar climate conditions have demonstrated that ANVOL™ can improve the NUE and reduce the losses due to volatilization (Zaman et al., 2013). Nevertheless, the exact effect on protein levels in wheat in the short growing periods of Northwestern Ontario remains inadequately addressed, (Ma et al. 2024, Abad et al., 2005).

2.3.3 Blends of Nitrogen Sources (ESN, SUPERU™, and ANVOL™)

A combination of ESN, SUPERU™-, and ANVOL™-stabilized Urea in theory creates a balance between momentary and lasting N availability so that nutrient uptake is optimal throughout the growing season. Blends are generally used in regions with high rainfall, which leaches the N, and low temperatures, which are inhibitory to the absorption of N by plants (Diacono et al., 2013). However, historical weather records indicate that Northwestern Ontario has relatively low yearly precipitation, averaging 720-820 mm, with occasional heavy rainfall (Environment and Climate Change Canada, 2022). Research from other northern regions

would indicate that different fertilizer blends can promote N availability throughout the growing season (Wood et al., 2023; Ghimire et al., 2021). However, there is a lack of specific research into the efficacy of these blends within the very specific environmental conditions found in Northwestern Ontario. The experiment described in the remaining sections was conducted to fill this knowledge gap in determining the effects of ESN, SUPERUTM, and ANVOLTM stabilized Urea blends on yield and plant protein concentration in wheat.

2.4 Other Factors Affecting Wheat Yield in Northwestern Ontario

Weed and pest control are the most important factors in maximizing yield and quality of winter wheat in Northwestern Ontario. The most common problematic weeds in the region are broadleaf weeds. Refine SG, a broad-spectrum herbicide formulated for the control of annual and perennial broadleaf weeds in cereal crops such as wheat, barley, and oats, is typically used by area farmers (FMC Corporation 2023a). This herbicide is effective in targeting significant local weed species, including Canada thistle (*Cirsium arvense* (L.) Scop.), a deep-rooted perennial weed in the family Asteraceae known for its aggressive competition with crops (Minnesota Department of Agriculture, n.d.), cleavers (*Galium aparine* L.), an annual broadleaf weed in the Rubiaceae family that clings to crops and reduces harvest quality, and wild buckwheat (*Fallopia convolvulus* (L.) Á. Löve, formerly *Polygonum convolvulus* L.), a climbing annual vine in the Polygonaceae family that competes for light and space in cereal crops (UC IPM, 2023). These species diminish competition for essential resources such as nutrients, water, and sunlight. The herbicide is flexible with respect to application scheduling

and crop rotation and thus is widely chosen amongst wheat growers in Northwestern Ontario (FMC Corporation, 2023b).

Aphid infestations in the latter part of the Northwestern Ontario growing season represent another major risk to the health and yields of winter wheat. Sevin, containing carbaryl, is a highly active insecticide that affects insect nervous systems, enabling it to provide broad control of aphids and other destructive insects (Pest Control Options, 2023). When uncontrolled, aphids can continue to inflict significant damage on wheat while plants are being nursed through their growth by feeding directly, causing stunted crop growth and the reduction in grain quality and the possibility of virus dissemination (OMAFRA, 2021). The aforesaid integrated pest and weed management strategies are critical for sustainable wheat production in Northwestern Ontario. Using Refine SG for broadleaf weed control and Sevin for aphid control, respectively, farmers can significantly alleviate crop competition and pest-induced stress to achieve higher yields and better grain quality. These techniques are in line with the general objectives of sustainable agriculture with a focus on input minimization and environmental care.

2.5 The Role of Thousand Kernel Weight (TKW) in Assessing Hard Red Winter Wheat Quality and Production in Northwestern Ontario

Seed quality and yield potential in Hard Red Winter Wheat (HRWW) are of agronomic importance, measured as the Thousand Kernel Weight (TKW). TKW represents the mass of 1000 seeds and is a fundamental indicator of kernel size and uniformity. Varietal characteristics, grain development, and production efficiency are evaluated widely using this indicator. TKW correlates with kernel density and milling yield, even though it is not a direct

measure of overall grain quality (Li, 1989). Higher TKW values are generally associated with better milling performance and higher flour extraction rates, as they indicate larger, well-developed kernels (Täufel, 1997). In contrast, smaller kernels often signal poor growing conditions, such as nutrient deficiencies, drought stress, or diseases that limit grain filling (Savdie et al., 1991). HRWW production in Northwestern Ontario prioritizes achieving high TKW values.

Cultivars like AAC Gateway and AAC Redstar, grown under varying management practices and environmental conditions, typically have TKW ranges between 32 and 40 g (Sahota, 2019). These values align with the expected standards for premium-grade HRWW in Canada (Canadian Grain Commission [CGC], 2024). However, TKW is highly sensitive to environmental factors, including temperature, soil fertility, and water availability (Savdie et al., 1991). Late-season moisture stress or early frosts may reduce grain filling, leading to lower TKW values (Lobellet et al., 2011). Annual TKW variability is evident in data from the CGC (2024), which reported slight fluctuations for Canada Eastern HRWW: 37.5 g in 2023 and 38.1 g in 2024. This variability underscores the impact of environmental conditions and highlights the need for adaptive management strategies to maintain consistent quality (Fowler, 2003). A robust TKW reflects effective N management, adequate water availability during grain filling, and crop resilience to environmental stressors (Sahota, 2020). Further research is required to explore the relationship between TKW and enhanced efficiency fertilizers (EEFs) in Northwestern Ontario's unique growing conditions. Overall, TKW serves as a vital metric for evaluating the quality and performance of HRWW, offering insights into kernel size, grain development, and varietal potential (CGC, 2024). Properly managing the factors influencing

TKW allows producers to deliver high-quality wheat that meets industry standards and market demands (Stefanova-Dobreva & Muhova, 2024).

3.0 Materials and Methods

3.1. Study area, climatic and soil conditions, and farm management

The experiment was conducted at the Lakehead University Agricultural Research Station (LUARS), Thunder Bay, Ontario, Canada (48°18'18" N, 89°23'17" W), located in Northwestern Ontario. The region is characterized by a cold climate with a relatively long winter wheat growing season compared to spring-seeded crops. Thunder Bay is in a continental climate zone with extremely cold winters and warm summers. Throughout the growth period of winter wheat, temperatures fluctuate between -10°C and 25°C, and the region receives an average annual precipitation of approximately 720–820 mm, with around 400 mm occurring between May and September, the main wheat growing period (Sahota, 2020). The main soils in the region are loamy clay soils with moderate fertility and good water retention capacity, important attributes for winter wheat.

The relatively cool and wet conditions in the region lead to a prevailing cool soil temperature well into the early growth season that slows down root growth and N uptake (Beres et al., 2018). Soil organic matter (SOM) in Northwestern Ontario is moderate in most soils, promoting the retention of N, but at the same time, the level of SOM increases the risk of N loss through denitrification in saturated conditions (Beres et al., 2023). The dominant climatic and soil conditions in Thunder Bay make N management particularly challenging in terms of time and rate of application.

The environmental conditions during the 2022-2023 winter wheat growing season at the Lakehead University Agricultural Research Station (LUARS), Thunder Bay, Ontario, were characterized by high annual and month-to-month variability in precipitation and temperature, typical of the region's climate (Tables 1, 2). The 2022 growing season experienced greater overall precipitation compared to 2023, particularly during critical growth stages, whereas 2023 was marked by a warmer growing season with higher accumulated Growing Degree Days (GDD) and Corn Heat Units (CHU).

3.2. Experimental design and data collection

The study implemented 15 treatments, including straight nitrogen (N) fertilizers (Urea, ESN, SuperUTM, and ANVOLTM treated Urea/and their blends all at 120 kg N ha⁻¹, SuperUTM at 100 kg N ha⁻¹, and Urea at 160 kg N ha⁻¹, and a no-N reference treatment (Tables 3, 4). Each

Table 1. Monthly weather data showing precipitation, minimum and maximum temperature, growing degree days (GDD), and corn heat units (CHU) recorded at the Lakehead University Agriculture Research Station (LUARS) during the 2022 growing season.

Month	Precipitation (mm)	Max. temp. (°C)	Min. temp. (°C)	GDD	CHU
April	0.0	10	-10	0	0
May	108.7	25	-6	126	239
June	22.0	31	-3	297	496
July	65.3	33	5	338	607
August	53.1	30	4	353	594
Total/Mean	249.1	26	-2	1114	1936

Table 2. Monthly weather as in Table 1 during the 2023 growing season.

Month	Precipitation (mm)	Max. temp. (°C)	Min. temp. (°C)	GDD	CHU
April	69.9	14	-16	0	0
May	46.8	29	-6	162	291
June	56.4	32	2	354	570
July	71.7	31	6	383	633
August	41.7	31	1	327	550
Total/Mean	286.5	27	-3	1226	2044

treatment and the reference were replicated four times in a randomized design for a total of 60 plots (Figure 1). In one treatment, 30 kg N ha⁻¹ was applied at seeding (in the fall) and 90 kg N ha⁻¹ was top dressed in early spring; all the other fertilizer treatments were applied at seeding. Winter wheat variety AAC Gateway was seeded on September 2, 2022, at 450 seeds/m² with an Almaco seed drill at 15-cm row spacings in 5 m x 1.5 m plots with 50-cm space between the plots. Seeds were treated with Vitaflow (<https://www.ipco.ca/vitaflo-sp/>) for protection from plant disease, especially seedling diseases. Refine SG was applied at 30 g ha⁻¹ and 30 mL ha⁻¹ of Sevin insecticide was sprayed in the midseason of the following year. The experimental area was under rainfed conditions.

Composite soil samples were collected on September 15, 2022, randomly from four designated sites within the experimental area before seeding winter wheat. The samples were analyzed at A&L Canada Laboratories, Inc. The goal of the analysis was to establish baseline soil fertility parameters, including soil organic matter, concentrations of phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), sulfur (S) and zinc (Zn), as well as pH, cation

exchange capacity, and base saturation. At the start of the trial, just after germination, plants were counted along a 50 cm length of the 3rd row in each plot (fall counts; September 19, 2022). Surviving plants were counted along the same 50 cm length after snow melt (spring counts; May 10, 2023). A third stem count occurred at the flag-leaf stage (June 19, 2023), again in the same 50 cm of the third row, in this case recording all tillers per plant in that section, giving an indication of tillering intensity. Plant height (cm) was measured at four critical growth stages, as defined by the Zadoks scale. These were the sheath stage (Zadoks 30-32), where the leaf sheath elongates and stem elongation begins, the flag leaf stage (Zadoks 37-39), when the final leaf appeared and unfolded, critical for photosynthesis and yield potential, the head visible stage (Zadoks 50-59), during which the wheat head emerged from the sheath, and the harvesting stage (Zadoks 90-92), representing full maturity and readiness for harvest.

Plant chlorophyll content was predicted at two key phenological positions, first when the plant head was not visible, and later when the plant head was visible. The predictions were

Table 3. Fertilizer products applied to the winter wheat experiment at the Lakehead University Agriculture Research Station (LUARS).

Urea Type	Acronym*	Brand Name	Manufacturer	Active Ingredient
Urea	U	Generic urea	Various	
Environmentally Smart Nitrogen	ESN	ESN	Nutrien	Polymer coated
Super Urea	SU	SuperU™	Koch Agronomic Services	NBPT (N-(n-butyl) thiophosphoric triamide) + DCD (Dicyandiamide)
Stabilized urea with inhibitors	ANVOL	ANVOL	Koch Agronomic Services	NBPT + Duromide

*Acronyms used in this thesis.

Table 4. Summary of treatments applied to the winter wheat experiment at LUARS starting in September 2022 with harvest in August 2023. Code refers to enumeration in text and analysis refers to the way data are presented in figures in this thesis. Plot numbers refer to Figure 1.

Code	Treatment	Analysis	Plot numbers
T1	No N (reference plot)	1	411, 208, 112, 304
T2	ESN @ 120 kg N/ha	1	211, 408, 115, 306
T3	Urea @ 120 kg N/ha	1, 2	412, 314, 209, 108
T4	Urea @ 90 kg N/ha + ESN @ 30 kg N/ha	1, 3	212, 414, 101, 308
T5	Urea + ESN + SUPERU TM each @ 40 kg N/ha	1	207, 403, 111, 301
T6	Urea @ 60 kg N/ha + SUPERU TM @ 60 kg N/ha	1, 3	215, 413, 104, 307
T7	Urea @ 120 kg N/ha treated with Anvol TM	1	201, 404, 107, 309
T8	Urea @ 30 kg N/ha fall and 90 kg N/ha in spring	1	410, 105, 210, 312
T9	SUPERU TM @ 120 kg N/ha	1, 2	409, 202, 113, 310
T10	Urea @ 160 kg N/ha	2	416, 215, 303, 109
T11	SUPERU TM @ 100 kg N/ha	2	214, 414, 101, 308
T12	Urea @ 30 kg N/ha + ESN @ 90 kg N/ha	3	206, 405, 313, 103
T13	Urea @ 30 kg N/ha + SUPERU TM @ 90 kg N/ha	3	213, 407, 102, 305
T14	Urea @ 60 kg N/ha + ESN @ 60 kg N/ha	3	415, 204, 114, 311
T15	Urea @ 90 kg N/ha + SUPERU TM @ 30 kg N/ha	3	203, 401, 315, 106

based on measurements using the Apogee Chlorophyll Concentration Meter. Plant weights measured at the dough stage (July 11, 2023) by cutting entire plants at ground level at the same 50-cm section of the 3rd row where the plant counts were taken. After green weights were measured, samples were dried in a forced-air oven at 60 °C and weighed again dry. Gross yield (or biological yield) was measured after a total harvest in August 2023 as the above-ground biomass (grain + straw + chaff) per unit area (ha). Grain yield was then estimated in kg ha⁻¹ by weighing the cleaned grains from each plot after threshing. Thousand kernel weight was determined by randomly selecting grain kernels from each plot and using an automatic seed counter to count exactly 1000 seeds. A high-accuracy digital balance was used to weigh the collected seeds. A Cox funnel apparatus placed over a standard 0.5-litre container was used for weight measurement. To allow the grain to flow evenly in the container, the slide at the seating

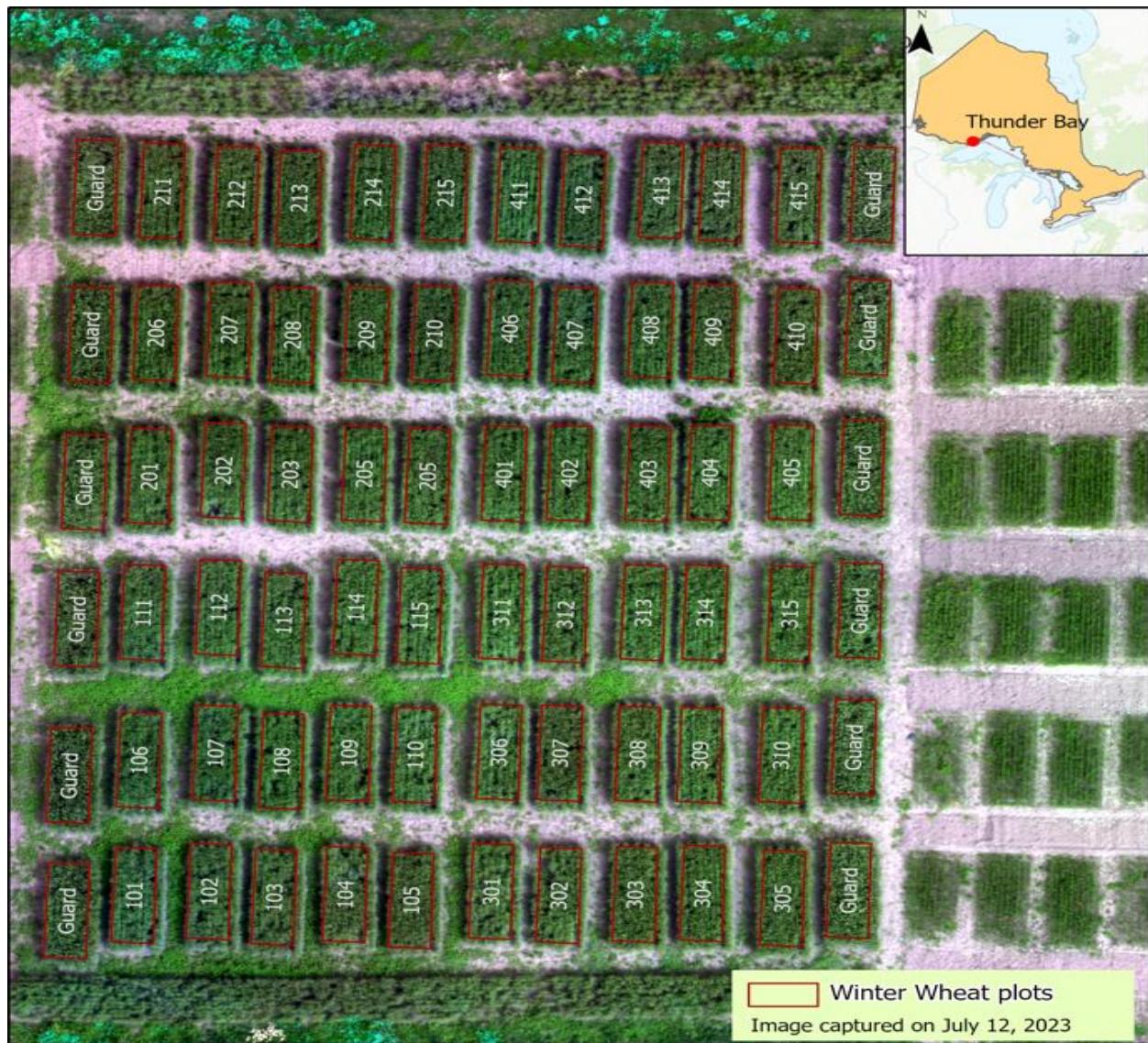


Figure 1. Experimental plots constituting four replicates of winter wheat trials (see Table 4) at the Lakehead University Agriculture Research Station (LUARS).

of the funnel base was quickly removed. Once filled, the level was flattened off by a flat striker and the filled container was weighed to determine test weight as weight per unit volume.

To assess plant protein content, A&L Laboratories (London, Ontario) was contracted. To reduce costs, only one block of plots was subsampled for the assessment. Near-infrared reflectance (NIR) spectroscopy was used. This method, known for its accuracy and speed,

works by passing infrared light through the samples and measuring the reflectance at various wavelengths. The reflected light is correlated with the chemical composition of the sample, allowing for quick and efficient estimation of protein levels in both "as fed" and "dry matter" forms, providing a comprehensive assessment of the wheat's protein quality. A calibrated moisture meter was used to determine grain moisture content, whereby a representative grain sample was introduced into the chamber of the meter, the lid secured, and the unit powered. Electrical conductivity analysis was then used to display the moisture content on electrical conductivity analysis to ensure precise and reliable readings. All procedures were standardized for accuracy, reproducibility and the minimal error of operator.

3.3 Data analysis

Data analysis was carried out with SPSS software (version 27), UNIANOVA procedure for univariate Analysis of Variance (ANOVA) to assess the contribution of N source, rate and blends to plant counts (fall and spring), stem counts, plant height at four growth stages, chlorophyll content at two stages, and plant weight, gross yield, grain yield, and Thousand Kernel Weight (TKW) at harvest. Three separate analyses were conducted to assess treatment effects, using Bonferroni corrections to account for the multiple tests (thirteen measures tested). For each test, the data entering the ANOVA is a relative score above or below 1, dividing the measure for each treated plot to the measure in the reference plot for the same block. The first analysis explored the role of various N sources and their blends, applied as a total of 120 kg ha⁻¹, to determine the influence of different fertilizer sources on plant and stem counts, plant growth, yield, and protein content, grain yield and TKW in a one-way ANOVA

(Treatments T2 to T9; Table 4). The second analysis dealt with the effects and interaction between N source and N rate, comparing urea and SUPERUTM applied at higher (Treatments T10, T6) and lower rates (Treatments T3, T11) in a two-way ANOVA on each of the same thirteen variables. The third analysis investigated the effects of different N blends and timing of application in a one-way repeated-measures ANOVA, comparing urea + ESN (Treatments T10, T6, T9 and T11), and urea + SUPERUTM (Treatments T12-14 and T6, T4 and T15) in combinations of 90 kg ha⁻¹ applied in winter and 30 kg ha⁻¹ the following spring, 60 kg ha⁻¹ in both seasons, and 30 kg ha⁻¹ applied in winter and 90 kg ha⁻¹ the following spring, again for the same thirteen variables. Normalized means were compared using a Fisher's Least Significant Difference (LSD) test whenever ANOVA treatment effects were significant at $P \leq 0.05$. Bar charts were used to show variation across treatments by displaying means and standard error for each measured variable in each treatment.

4.0 Results

4.1 Soil analysis

Noticeable differences occurred in macronutrient levels across the four sampling areas (Appendix 1). Soil organic matter content ranged from 3.4-6.0 %, i.e., soils were rich content in organic matter, allowing good nitrogen (N) mineralization. Nitrate N (NO₃-N) ranged from 46-73 ppm, while ammoniacal N (NH₄-N) occurred at relatively low levels, 3-5 ppm. Phosphorus ranged from low (12 ppm) to slightly higher (21 ppm), while potassium was consistently low across all samples. Calcium levels were high in all samples, ranging from 644-754 ppm, suggestive of good soil structure and stability. Magnesium levels varied from

low (69 ppm) to moderate (161 ppm). Sulphur was low (<3 ppm) and suggested to be limiting in all soil samples. Micronutrient levels also varied. Manganese levels were low to moderate, while soil iron content was consistently high. Zinc, like sulphur, was identified as a limiting nutrient at low to medium levels. Sodium levels ranged from low (2850 ppm) to medium (3160 ppm). Soil pH values were near neutral (6.3-7.4), and Cation Exchange Capacity (CEC) ranged between 21.4 and 29.5 meq/100g, suggesting satisfactory nutrient-binding ability.

4.2 Plant and stem counts

Although plant counts were lowest in the urea @ 90 kg N/ha + ESN @ 30 kg N/ha fertilizer treatment, where they were lower than plant counts in the reference plots (Figure 2), the counts did not differ significantly by treatment in either fall ($F_{7,24} = 0.9$; $P = 0.52$) or spring ($F_{7,24} = 1.2$; $P = 0.38$) for applications at a total rate of 120 kg N ha⁻¹. Comparing stem counts at the flag-leaf stage about six weeks later in the growing season, all plots except those treated with urea @ 60 kg N/ha + SUPERUTM @ 60 kg N/ha showed a higher tillering rate than the reference plots (Figure 3). There were no differences among the eight treatments ($F_{7,24} = 0.3$; $P = 0.95$). In comparisons of urea and SUPERUTM applied at higher (Treatments T10, T6) and lower rates (Treatments T3, T11), plant counts were higher than in reference plots in spring for both fertilizers at the higher application rates and for both application rates for SUPERUTM (Figure 4).

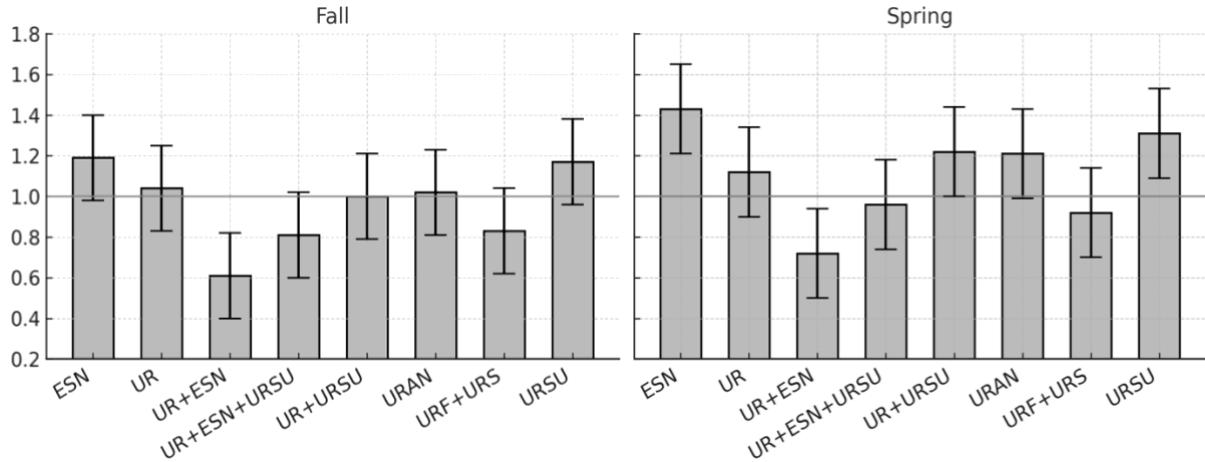


Figure 2. Relative winter wheat plant counts at the Thunder Bay Agriculture Research Station, in fall after germination (September 19, 2022) and spring after snowmelt (May 10, 2023), comparing eight nitrogen (N) treatments applied at a total rate of 120 kg N ha^{-1} , including ESN, urea (UR), UR+ESN, UR+ESN+SUPEROTM (URSU), UR+URSU, UR+ANVOLTM (URAN), UR in fall and spring (URF+URS), and URSU (Treatments 2-9; Table 4). Counts are relative to the reference (no-N) plot in each block; bars show means with standard error.

Differences in fall plant counts above the reference plots did not occur, and there were no significant differences among the four treatments, neither for the effect of which fertilizer was applied nor for the effect of application rate, and neither for the fall plant counts ($F_{3,11} = 0.5$; $P = 0.70$) nor for the spring plant counts ($F_{3,11} = 0.6$; $P = 0.64$). Stem counts at the flag-leaf stage were higher than the reference plots for SUPEROTM at the higher application rate and for urea at the lower application rate (Figure 5). However, as for the plant counts, neither effect was significant comparing the four treatments ($F_{3,11} = 0.7$; $P = 0.59$). The final comparison shows varying time of application with lower than reference plant counts in both seasons for urea with ESN applied at 60 kg N ha^{-1} in fall and 60 kg N ha^{-1} in spring, and in fall for urea with

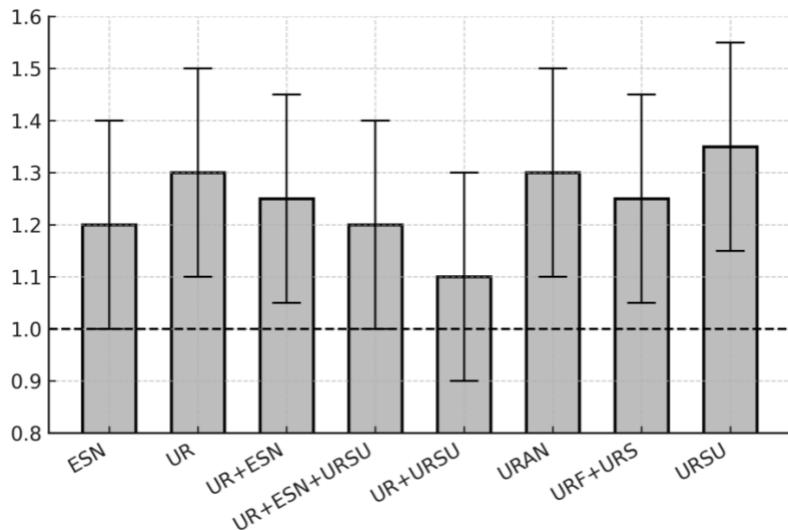


Figure 3. Relative winter wheat stem counts at the flag-leaf stage (June 19, 2023), comparing the same eight N treatments as in Figure 2.

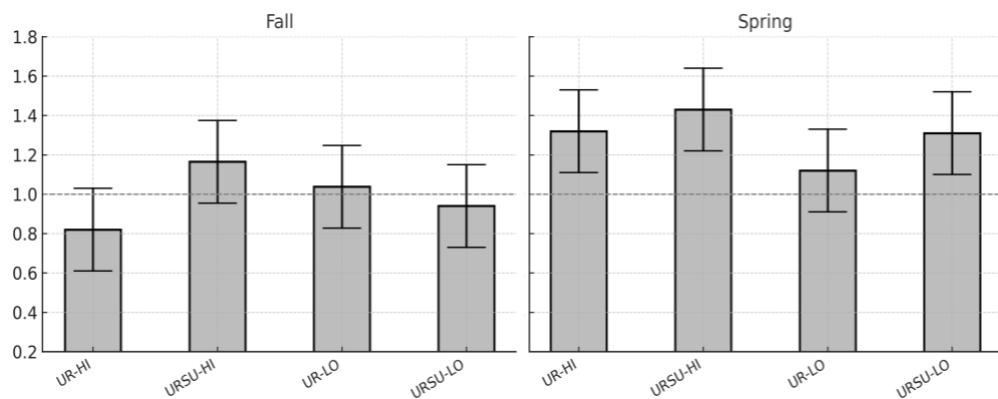


Figure 4. Relative winter wheat plant counts as in Figure 2, comparing two nitrogen (N) treatments, urea (UR) and SUPERU™ (URSU), each applied at two rates, UR-HI (160 kg N ha⁻¹) and UR-LO (120 kg N ha⁻¹), URSU-HI (120 kg N ha⁻¹) and URSU-LO (100 kg N ha⁻¹).

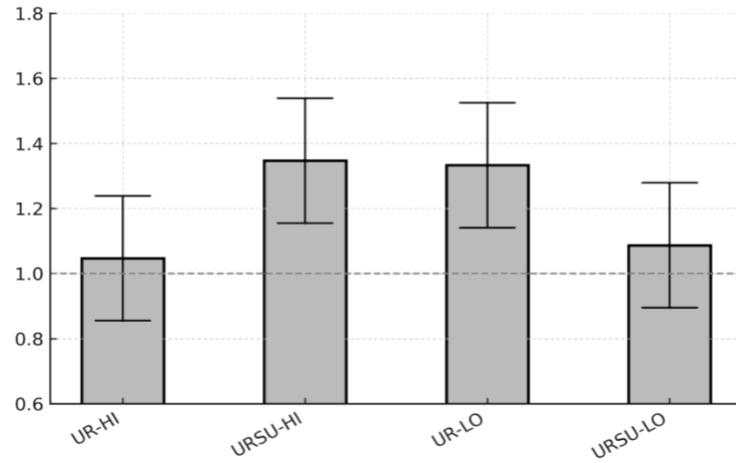


Figure 5. Relative winter wheat stem counts at the flag-leaf stage, comparing treatments as in Figure 4.

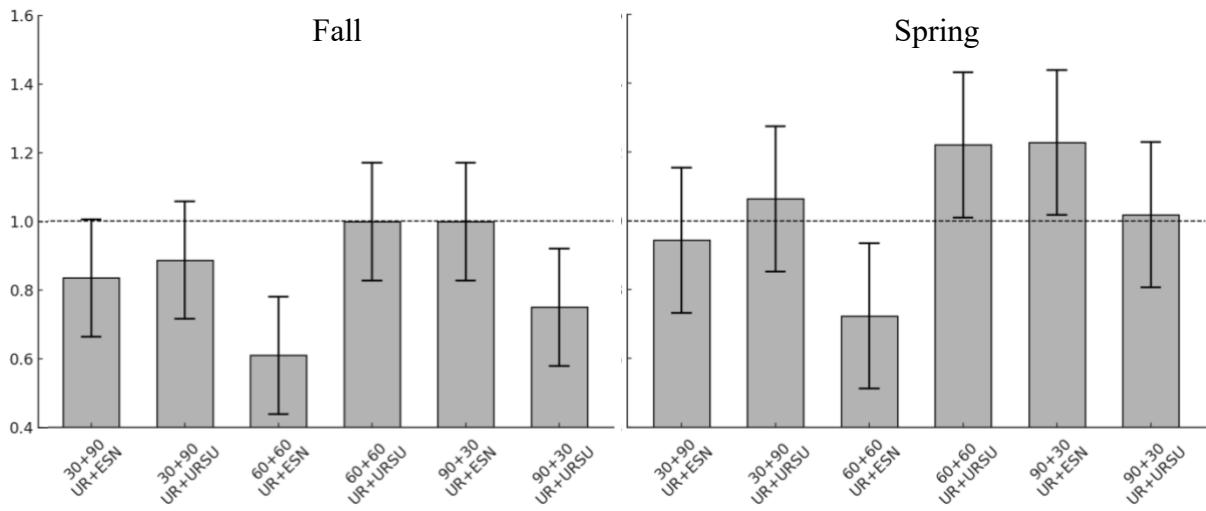


Figure 6. Relative winter wheat plant counts as in Figure 2, comparing two nitrogen (N) treatments, urea with ESN (UR+ESN) and urea with SUPERUTTM (URSU), each applied at a total rate of 120 kg N ha⁻¹ distributed in three ways, 30 kg N ha⁻¹ in fall and 120 kg N ha⁻¹ in spring (30+90), 60 kg N ha⁻¹ in fall and 60 kg N ha⁻¹ in spring (60+60), and 90 kg N ha⁻¹ in fall and 30 kg N ha⁻¹ in spring (90+30).

SUPERUTTM applied at 90 kg N ha⁻¹ in fall and 30 kg N ha⁻¹ in spring (Figure 6). Again, neither the effect of which fertilizer was applied nor the effect of time of application were significant in comparing the plant counts across the six treatments, neither in the fall ($F_{5,23} = 0.8$; $P = 0.58$) nor in the spring ($F_{5,23} = 0.8$; $P = 0.57$). Stem counts at the flag-leaf stage were higher

than the reference plots for all treatments except for urea with ESN applied at 60 kg N ha⁻¹ in fall and 60 kg N ha⁻¹ in spring (Figure 7). There were no significant differences created by varying time of application or for the two fertilizer types on flag-leaf stage stem counts ($F_{5,23} = 0.7$; $P = 0.62$).

4.3 Plant height

Differences in plant height among the treatment applications totalling 120 kg N ha⁻¹ occurred only at the sheath stage owing to significantly lower performance of the urea with

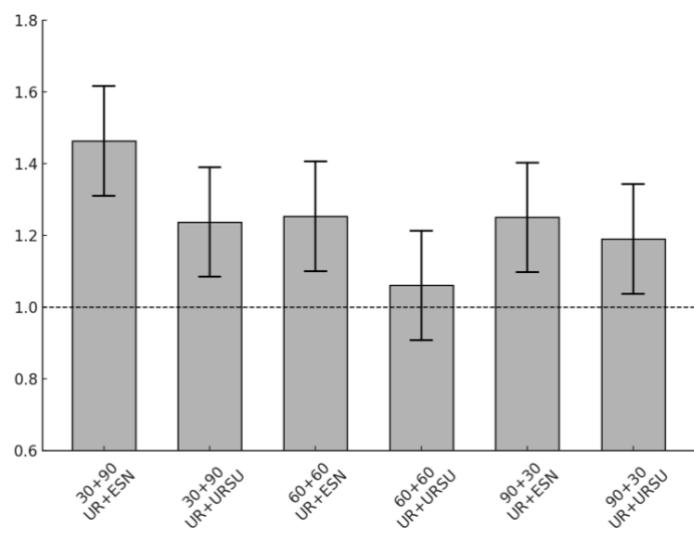


Figure 7. Relative winter wheat stem counts at the flag-leaf stage, comparing treatments as in Figure 6.

SUPERUTTM treatment ($F_{7,31} = 2.2$, $P = 0.05$; Figure 8); there were no differences across treatments at the second measure at the flag-leaf stage ($F_{7,31} = 0.3$, $P = 0.96$), at the third measure at the head completely visible stage ($F_{7,31} = 1.4$, $P = 0.22$), and at the fourth measure

at the harvesting stage ($F_{7,31} = 2.1, P = 0.08$). In most of the treatments at all four times of measurement, the fertilized plants were taller than in the reference plots. Comparing the treatments that included two application rates for urea and for urea with SUPERUTM, most fertilized plots had taller plants than the reference plots at all four stages, and differences by fertilizer type or by application rate did not occur across the four treatments at any of the four stages (Figure 9; sheath stage, $F_{3,15} = 1.4, P = 0.29$; flag-leaf stage, $F_{3,15} = 2.0, P = 0.17$; head completely visible stage, $F_{3,15} = 1.0, P = 0.40$; harvesting stage, $F_{3,15} = 0.7, P = 0.56$). All fertilizer blends with any combination of spring and fall applications had taller plants in the flag-leaf and head completely visible stages, but there was no longer any height advantage through to the harvesting growth stage except for the balanced and fall-loaded applications of urea with

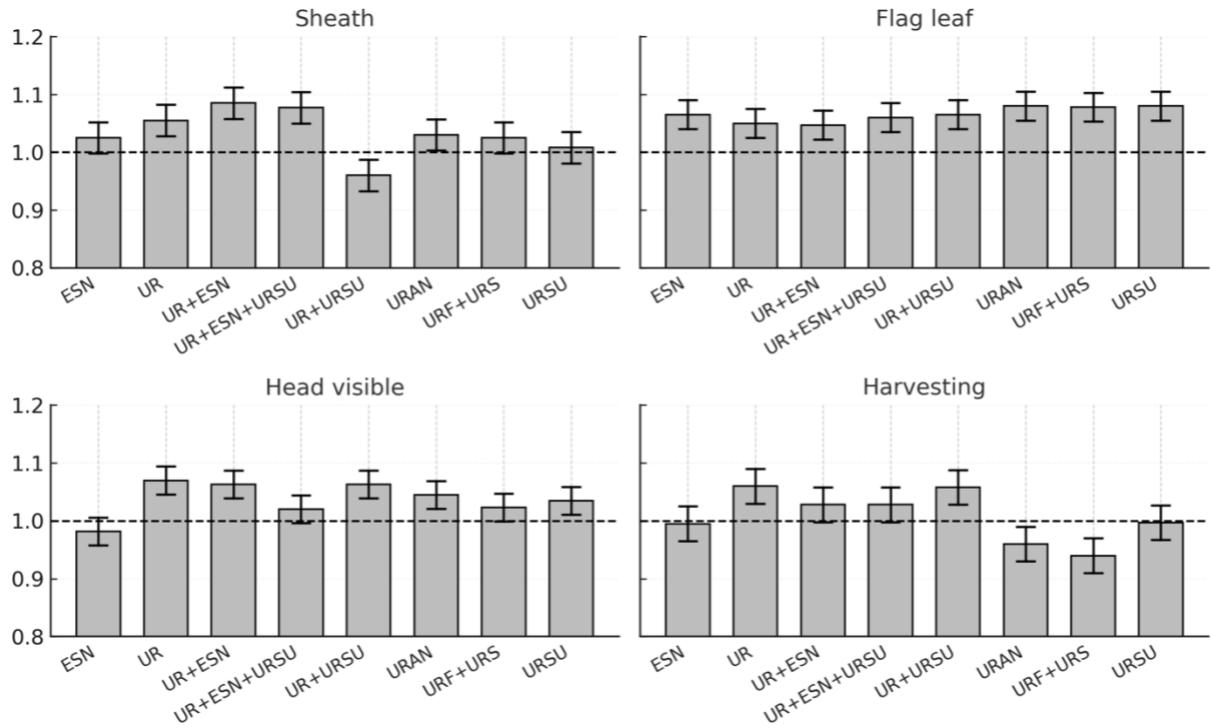


Figure 8. Relative differences in plant height at four stages (sheath, flag-leaf, head completely visible, and harvesting) for the eight treatments as in Figure 2. Plant heights are relative to the reference (no-N) plot in each block; bars show means with standard error.

ESN (Figure 10). Moreover, the outcome in plant height neither by treatment nor across the application rates varied significantly (sheath stage, $F_{5,23} = 2.2$, $P = 0.10$; flag-leaf stage, $F_{5,23} = 0.0$, $P = 1.00$; head completely visible stage, $F_{5,23} = 0.4$, $P = 0.84$; harvesting stage, $F_{5,23} = 0.9$, $P = 0.50$).

4.4 Chlorophyll content

There were no differences in chlorophyll content between plants in treated and reference plots and no differences among the eight treatments with applications totalling 120

kg N ha^{-1} (Figure 11; head completely visible stage, $F_{7,31} = 0.4, P = 0.88$; flowering stage, $F_{7,31} = 0.1, P = 0.99$). Similarly, application type and rate did not affect plant chlorophyll content,

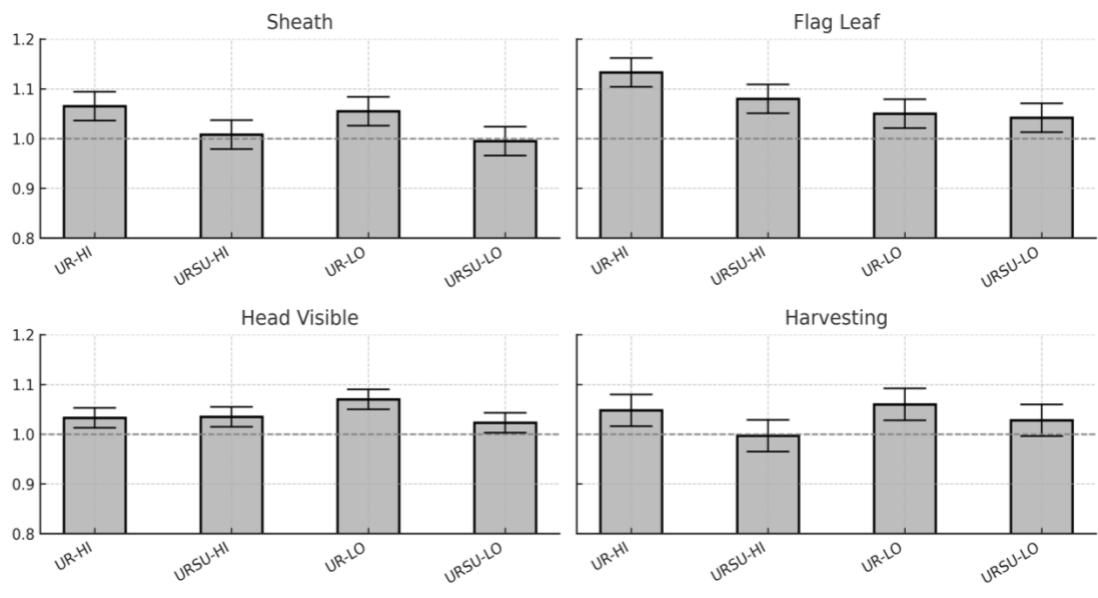


Figure 9. Relative differences in plant height at four stages as in Figure 8, comparing treatments as in Figure 4.

with only one case, the higher application rate of for urea with SUPERUTM, having plants with a different chlorophyll content than the plants in the reference plots (Figure 12; head completely visible stage, $F_{3,15} = 1.2, P = 0.34$; flowering stage, $F_{3,15} = 0.1, P = 0.95$). Finally, in the third set of comparisons of plant chlorophyll content, there were likewise no differences to report among treatments or with between the treatments and the reference plots (Figure 12; head completely visible stage, $F_{5,23} = 0.2, P = 0.97$; flowering stage, $F_{5,23} = 0.4, P = 0.87$).

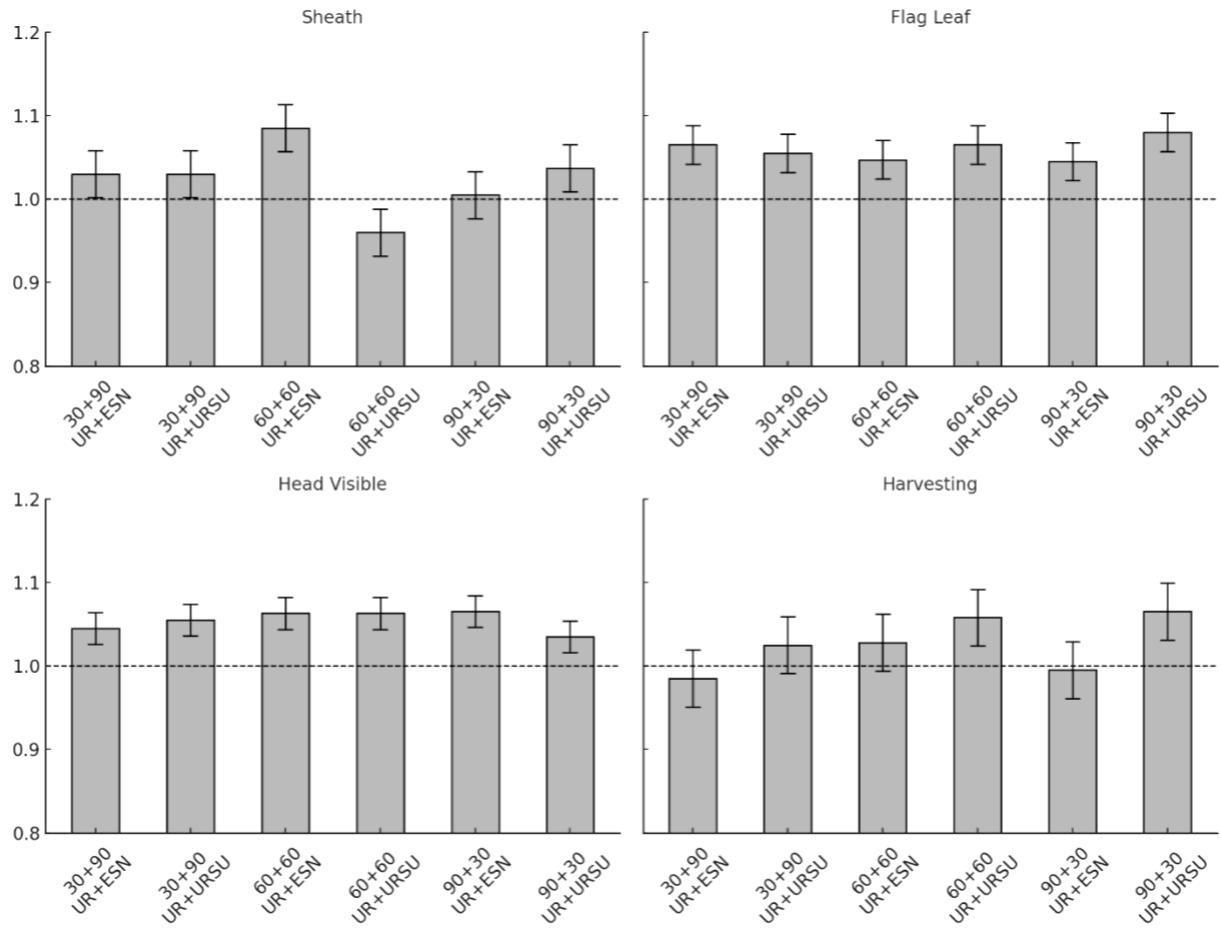


Figure 10. Relative differences in plant height at four stages as in Figure 8, comparing treatments as in Figure 6.

4.5 Plant green and dry weights

Plant weights did not differ among the eight treatments with applications totalling 120 kg N ha⁻¹ (green weights, $F_{7,31} = 1.7$, $P = 0.16$; dry weights, $F_{7,31} = 1.6$, $P = 0.17$). However, the plants in the plots treated with urea and ESN had weights lower than in the reference plots (Figure 14). Comparing the urea and the urea with SUPERUTM treatments at two application rates, application type and rate did not affect plant weights (green weights, $F_{3,15} = 2.1$, $P = 0.11$; dry weights, $F_{3,15} = 0.5$, $P = 0.67$), but plants treated with the lower application rates

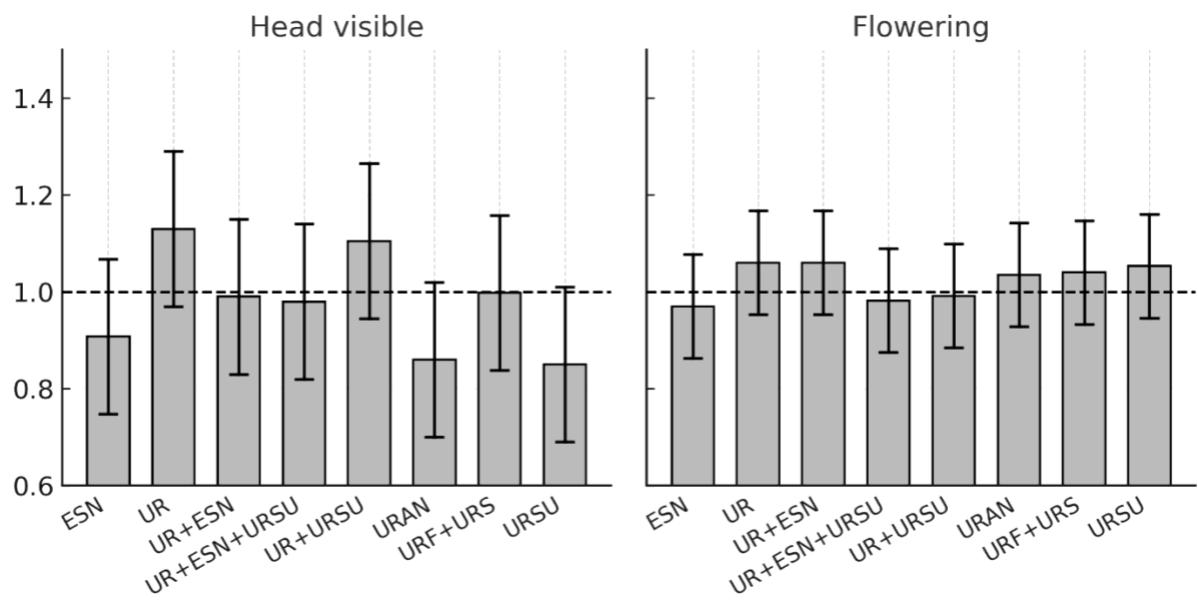


Figure 11. Relative differences in chlorophyll content of winter wheat at the head completely visible and flowering stages, comparing treatments as in Figure 2.

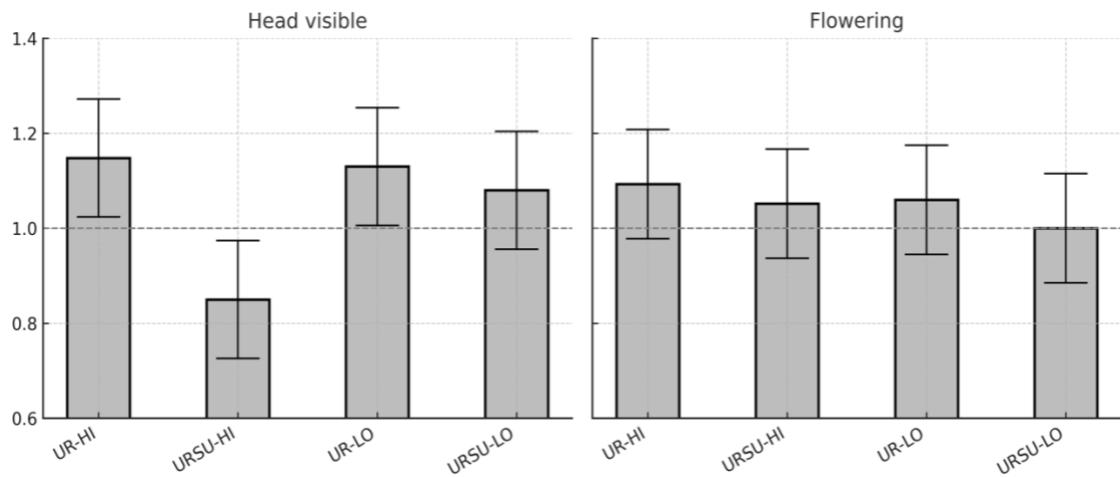


Figure 12. Relative differences in chlorophyll content as in Figure 11, comparing the treatments as in Figure 4.

were heavier than plants in the reference plots (Figure 15). Comparing the urea with SUPERUTTM and urea with ESN treatments with the variable fall and spring application rates, application type and rate did not affect plant weights (green weights, $F_{5,23} = 0.5$, $P = 0.67$; dry weights, $F_{5,23} = 2.1$, $P = 0.12$), but plants treated with the lower application rates were heavier than plants in the reference plots (Figure 15).

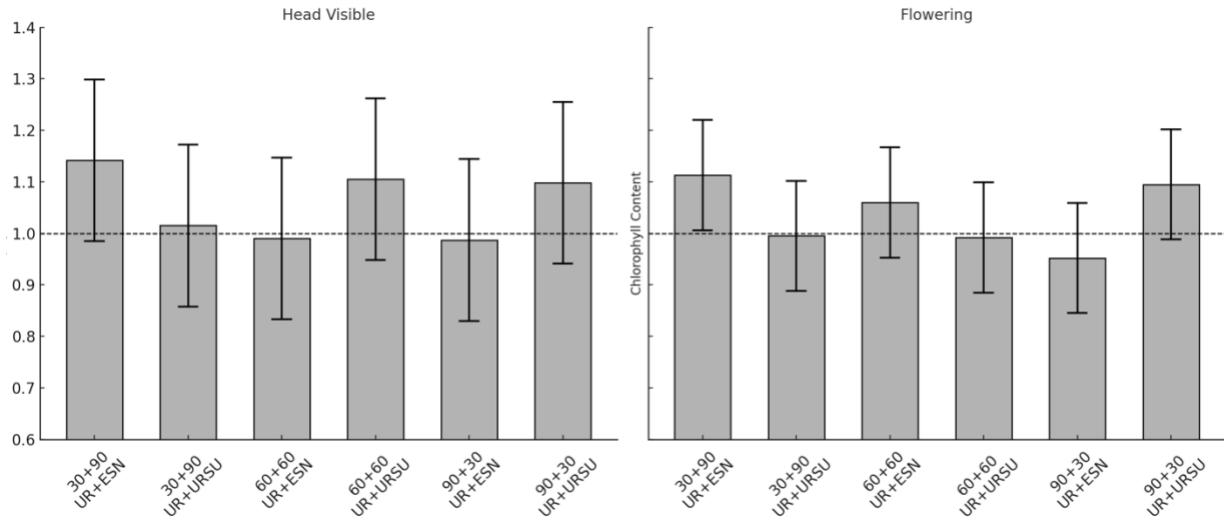


Figure 13. Relative differences in chlorophyll content as in Figure 11, comparing the treatments as in Figure 6.

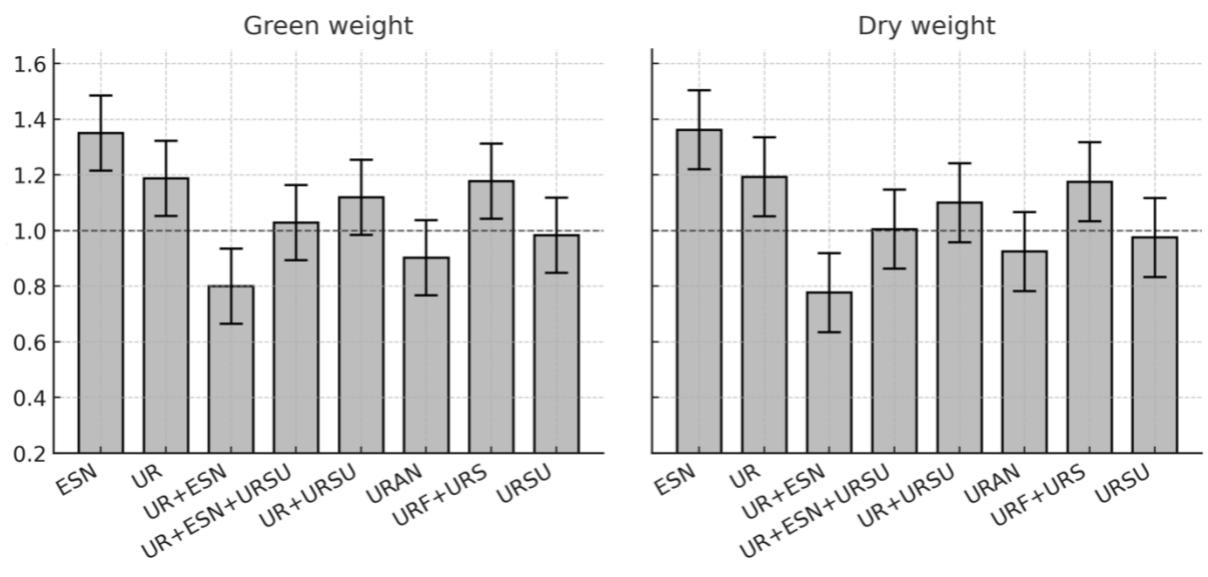


Figure 14. Relative differences in plant weights, comparing treatments as in Figure 2.

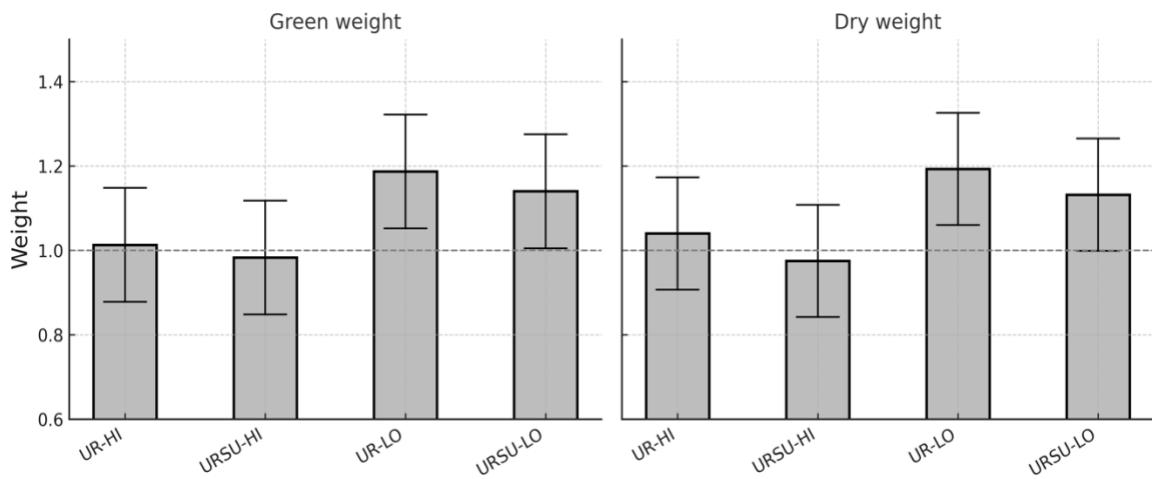


Figure 15. Relative differences in plant weights, comparing treatments as in Figure 4.

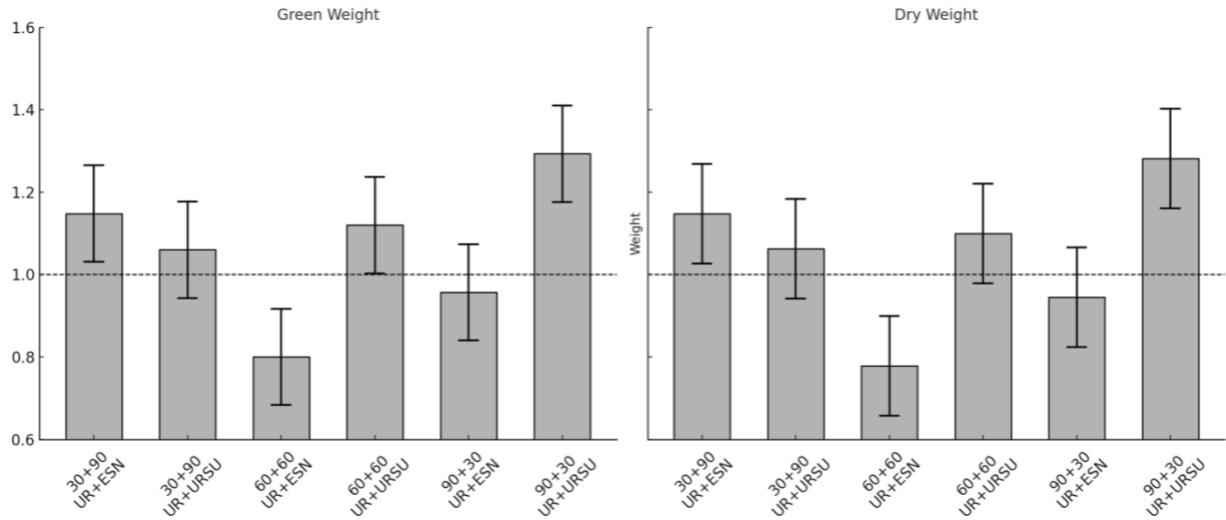


Figure 16. Relative differences in plant weights, comparing treatments as in Figure 6.

4.6 Grain yield, Thousand Kernel Weight, and plant protein content

Grain yields were higher for any treated plot with applications totalling 120 kg N ha^{-1} compared to the yields in the reference plots (Figure 17), but Thousand Kernel Weight (TKW) was higher than in the reference plots only for the urea with ANVOLTM treatment and for the treatment with urea applied in fall and spring (Figure 18). Across the treatments, there were no significant differences in grain yield ($F_{7,31} = 1.7, P = 0.99$) or TKW ($F_{7,31} = 1.7, P = 0.16$). Similarly, grain yield was greater for both urea and urea with SUPERUTM treatments at both lower and higher application rates, than yields in the reference plots, but only for urea at the higher application rate was TKW higher than for the reference plots (Figure 19). No significant differences occurred between the treatments by type of fertilizer or application rate for either grain yield ($F_{3,15} = 0.04, P = 0.99$) or TKW ($F_{3,15} = 0.1, P = 0.94$). Somewhat in contrast, comparing urea with ESN and urea with SUPERUTM treatments at the variable fall and spring application rates, only grain yields from plots treated with urea and SUPERUTM at rates of 60

kg N ha^{-1} in fall and spring or 90 kg N ha^{-1} in fall and 30 kg N ha^{-1} in spring outperformed the yields from the reference plots, and only the latter treatment produced higher TKW than the reference plots (Figure 20). No significant differences occurred among any of the treatments in grain yield ($F_{5,23} = 0.3, P = 0.88$) or TKW ($F_{5,23} = 0.4, P = 0.84$). Plant protein content was at the same level as in the reference plots for one treatment only, urea applied in fall and spring; three treatments (urea and SUPERUTM at rates of 60 kg N ha^{-1} in fall and spring, at rates of 30 kg N ha^{-1} in fall and 90 kg N ha^{-1} in spring, and at a rate of kg N ha^{-1} in spring) achieved less than 75% of the protein content of the reference plots (Figure 21).

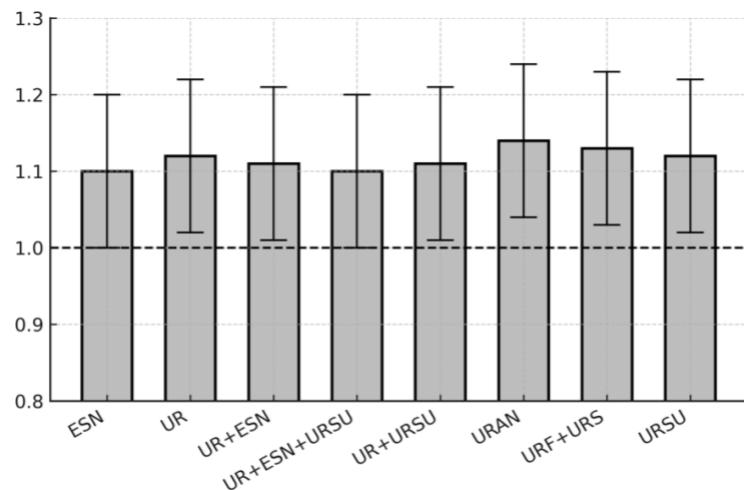


Figure 17. Relative differences in grain yield, comparing treatments as in Figure 2.

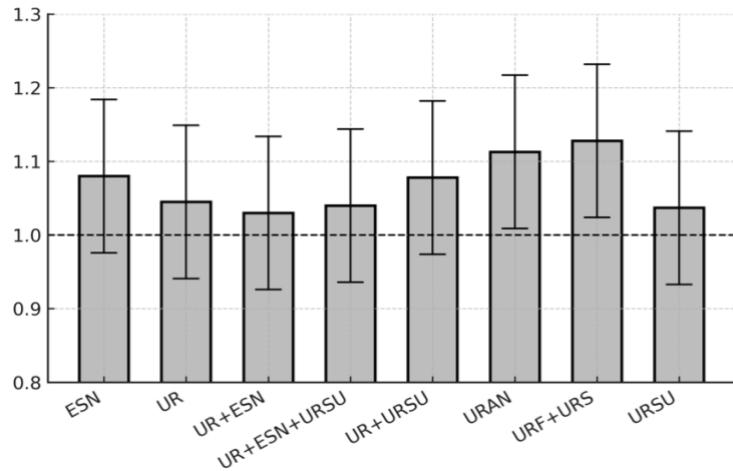


Figure 18. Relative differences in Thousand Kernel Weight, comparing treatments as in Figure 2.

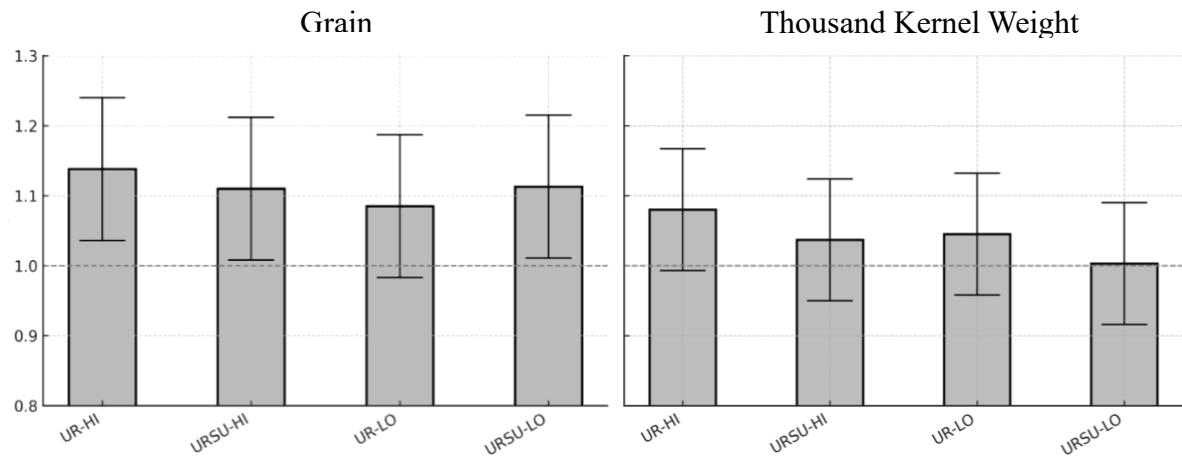


Figure 19. Relative differences in grain yield and Thousand Kernel Weight, comparing treatments as in Figure 4.

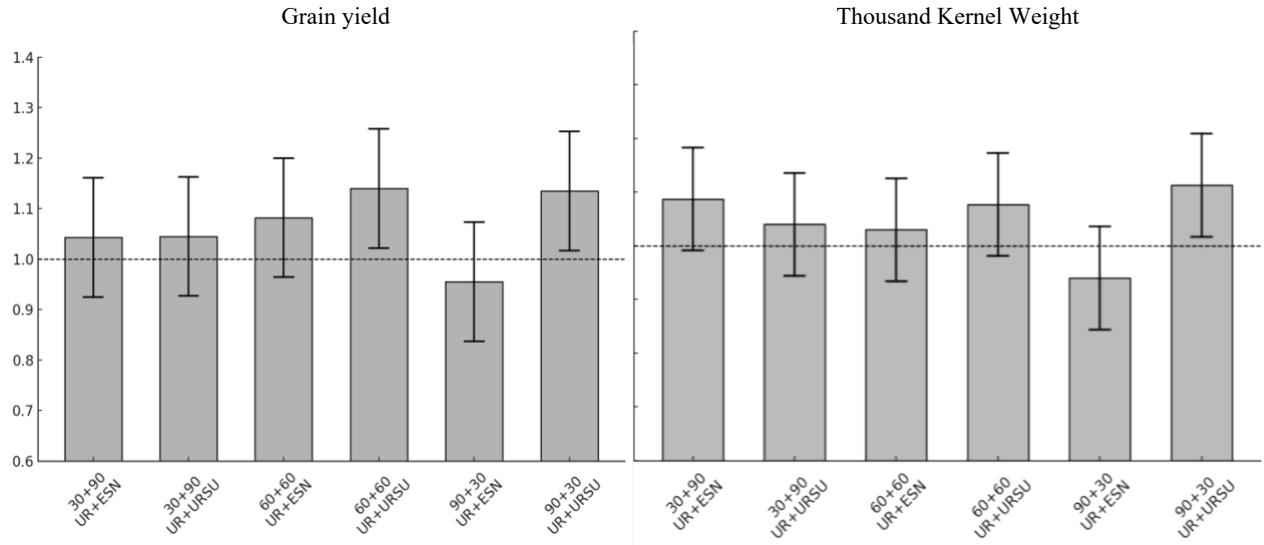


Figure 20. Relative differences in grain yield and Thousand Kernel Weight, comparing treatments as in Figure 6.

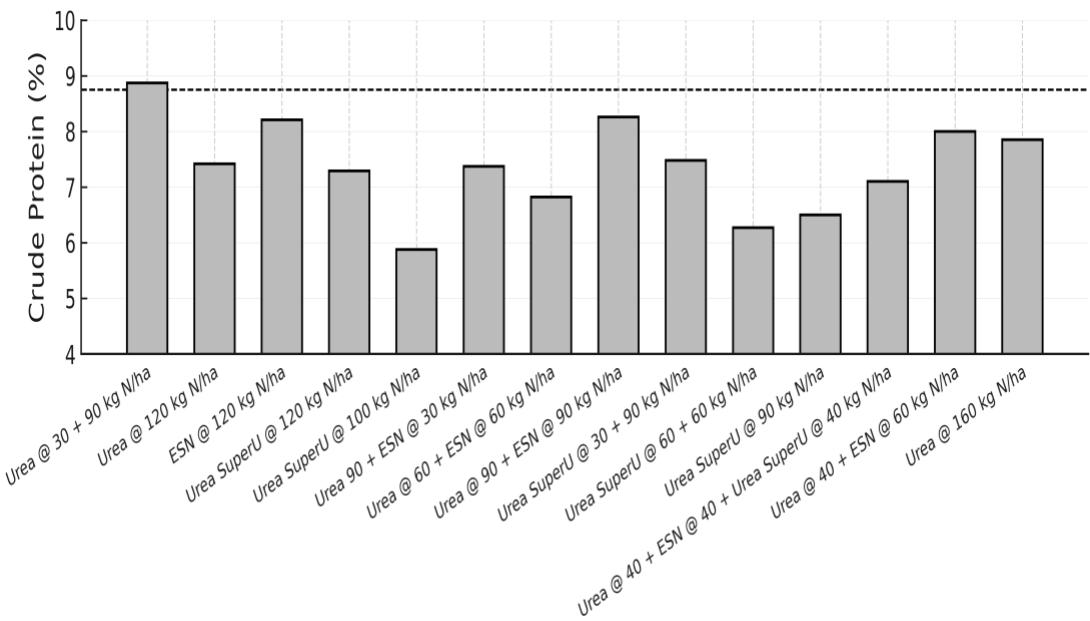


Figure 21. Plant protein content by Near-Infrared Reflectance (NIR) spectroscopy (A&L Laboratories). All treatments for one block only (without replication) are shown with a bar indicating crude protein (%) in the grain sample from that treatment plot; the horizontal dashed line is the crude protein in the plants from the reference plot without nitrogen fertilizer.

5.0 Discussion

The weather pattern in the 2022-23 season explains the lack of differences among EEFs or between EEFs and untreated urea. During the active growth window (May–Aug 2023), site rainfall totalled ~216.6 mm (46.8, 56.4, 71.7, and 41.7 mm, respectively), while maximum daily temperatures during June–August reached ≥ 30 °C (32 °C in June; 31 °C in July and August). These conditions represent both a drier-than-typical moisture supply and episodic heat stress that can compress grain fill and limit yield response to N source. By contrast, long-term climate data suggests the normal is ~400 mm precipitation from May–September in Thunder Bay (Sahota, 2020). With less water moving through the profile, conditions conducive to N loss via leaching or denitrification were limited; correspondingly, any expected advantages of stabilized or controlled-release N (i.e., loss mitigation and better N synchronization) would be muted.

Mechanistically, two concurrent factors likely reduced any practical separation among sources. First, the low in-season rainfall meant less opportunity for leaching/denitrification—the pathways where EEFs often create value—so conventional urea could perform similarly once crop demand was capped by moisture. Second, repeated ≥ 30 °C events in June–August likely shortened grain filling and constrained N uptake and remobilization, further limiting the payoff from prolonged N release or hydrolysis/nitrification control. This interpretation is consistent with literature summarized in the review (Section 2): EEF performance is strongly contingent on soil texture, precipitation, and temperature; benefits tend to appear in loss-prone (wet) or waterlogged contexts, while trials in low-loss environments frequently report no yield/protein advantage over urea. Looking ahead, this framing helps growers: in seasons or fields where heavy spring rains are likely (surface-applied N, delayed incorporation, or fine-

textured soils prone to saturation), inhibitors or controlled-release sources may be worth the premium; in dry, warm windows like 2023 at LUARS, the marginal value diminishes and differences among sources can be statistically and agronomically negligible.

Enhanced efficiency fertilizers (EEFs) have become a major innovation to increase the nitrogen use efficiency (NUE) and reduce nitrogen (N) losses in agriculture. Through regulation of N transformations and/or by the timing of N release, EEFs limit losses from volatilization, leaching, and denitrification, thus maximizing the supply of N to crops over the crop growth periods (Asadu et al., 2024). These aspects of the effectiveness of the EEFs were not tracked in this study, except as outcome measures in differences in plant height, chlorophyll content, and plant weights at harvest, comparing various EEFs applied in a randomized block design that included treatments with urea alone, EEFs, blends of urea with EEFs and a reference plot without N fertilizer. Some of the measured crop-response variables were significantly different between added N treatments and a 0-N reference treatment but were not significantly different among EEF product treatments. Where positive differences at the various growth stages did occur between treated and reference plots, they were most frequently and often highest for the treatments with urea alone. These larger positive differences for urea applied alone occurred in plant height and chlorophyll content, especially for high rates of spring application (160 kg N ha^{-1}) at early growth stages, and for plant weights at harvest for the lower rate of application (120 kg N ha^{-1}). Relatively dry spring and summer conditions in 2023 likely either were not conducive to N loss and therefore negated potential EEF benefits or rendered the EEF treatments less effective at supplying optimum N forms at optimum times.

What is left to consider are any positive effects of the EEFs on emergence rates (plant counts in the fall), survival of plants (counts in spring), and tillering rates (stem counts in June 2023), along with any positive outcome measures in the grain yield, thousand kernel weight (TKW), and plant protein content. Treatments with ESN and urea with ESN in combination had somewhat higher plant counts in fall and spring than urea alone or AnvolTM treated urea. The continuous N supply provided by ESN in this case likely facilitated root growth and carbohydrate reserves, both of which play important roles in overwintering survival (Xue et al., 2014). The present finding is consistent with the research of (Wood et al., 2023), who emphasized the necessity of N immobilization to promote and sustain higher plant density during winter wheat systems.

Variation in number and in harvest weights of plants across some treatments, especially for N blends such as urea, ESN and SUPERUTM in combination, highlights the importance of differential N release as a factor influencing early growth and overwintering success. The results are consistent with previous studies suggesting that EEFs in combination can buffer against N losses during critical growth periods, particularly in regions with high precipitation during the fall (Lewu et al., 2020). Stem counts, a measure of the tillering potential and early biomass yield, exhibited best outcomes also for ESN and urea with ESN. This observation aligns with results from Abalos et al. (2016), who reported that the application of fixed N fertilizers stimulates tillering and vegetative growth in cereal plants. Still, a detailed investigation is required to determine the long-term consequences of these findings in a wide range of environmental conditions.

The most encouraging results in this study are positive outcome measures in grain yield, the final product of crop growth. Here, the EEF-treated plots were not outperformed by

those with urea alone, and nearly every treated plot (the exceptions being most treatments with fall and spring application rates) had higher grain yields compared to the no-N reference treatment. These results are consistent with work by Grzebisz & Biber (2024), in which the authors attribute grain yield improvements to stabilization of N sources and subsequent prevention of N losses and provision of adequate N supply during the critical growth stages. In this experiment, most N-fertilized treatments out-yielded the 0-N reference, and at the matched seasonal rate of 120 kg N ha⁻¹ the EEF sources performed similarly to urea. (Sahota & Rowsell, 2011; OSCIA, 2013).

Because lower N levels were not matched across sources (e.g., SUPERUTTM at 100 kg N ha⁻¹ without an analogous urea-100 treatment), these data do not support the claim that EEFs enabled a lower N rate to achieve comparable yield; a balanced rate series by source is required to test that hypothesis (Morris et al., 2018). This interpretation is consistent with regional trials showing ESN \approx urea at equal total N in Northern Ontario and with studies in North Carolina reporting no consistent yield advantage to loss-prevention amendments over conventional sources under typical management (Sahota & Rowsell, 2011; Rajkovich et al., 2017). More broadly, extension syntheses indicate EEF gains are context-dependent and are most evident when loss risks (volatilization, leaching, denitrification) are appreciable—helping explain parity with urea in seasons where loss pressure is modest (Olson-Rutz et al., 2011). To directly test whether EEFs permit lower total N for equivalent yield, implement a balanced rate series within each source (e.g., 0/100/120/150 kg N ha⁻¹ for both urea and SUPERUTTM—and parallel rates for any ESN blend) with identical timing/placement, and evaluate source \times rate via within-source response curves (slope/plateau comparisons) in a multi-site, multi-year design.

Less convincing in terms of fertilizer effectiveness were (1) much less evidence for increase in TKW, even for the EEFs in combination and SUPERU™ at a higher application rate, over the no-N reference treatment, and (2) plant protein concentration ranged from 5.9 to 8.9 %, with the counter-intuitive pattern that most N-treated plots showed lower plant protein than the 0-N reference, except the urea split 30 kg N ha^{-1} (fall) + 90 kg N ha^{-1} (spring), which matched or exceeded the 0-N level. This pattern is consistent with the documented nitrogen-dilution effect in winter wheat: when biomass accumulates faster than plant N uptake, tissue N (and thus protein) concentration declines unless N supply is sustained (Zhao et al., 2020).

Maintaining N availability into grain fill—for example via split or late N—tends to raise protein with little effect on yield, highlighting the role of timing in the effect of N (Brown et al., 2005). Mechanistically, nitrification inhibitors (e.g., DMPP) can prolong NH_4^+ availability and reduce NO_3^- losses, while polymer-coated urea staggers N release—approaches that may help protect plant/tissue protein when late-season N is limiting, though responses are site-year dependent (Wang et al., 2020; Qi et al., 2021). Finally, the broader yield pattern in this study—fertilized treatments exceeding 0-N—aligns with Ontario winter-wheat rate-series work, underscoring that as N programs increase biomass and yield, protein dilution can occur unless post-anthesis N needs are met (Ontario Soil and Crop Improvement Association [OSCIA], 2013).

A yield–protein trade-off was evident for two urea+SUPERU™ split programs: (i) the blend applied at the same split as urea alone— 30 kg N ha^{-1} in fall + 90 kg N ha^{-1} in spring—which produced higher TKW than the 0-N reference but among the lowest plant-protein concentrations; and (ii) the $60 + 60 \text{ kg N ha}^{-1}$ (fall:spring) blend, which delivered one of the highest grain yields while plant-protein concentration remained comparatively low. These two

split sources likely supplied enough N early to drive biomass and kernel set, which lifted yield and TKW, but did not sustain N availability into grain filling, so plant-protein concentration fell via N-dilution—the well-documented tendency for tissue protein % to decline when growth outpaces N supply unless late-season N is maintained (Jones, 2020; Zhao et al., 2020; Abiola et al., 2024).

Although SUPERUTTM (urea stabilized with the urease inhibitor NBPT and nitrification inhibitor DCD) reduces losses from volatilization and nitrification, stabilization does not substitute for late-season N; protein is most sensitive to N around flag leaf to shortly after anthesis, so programs that front-load N can increase yield while leaving protein comparatively low if post-anthesis N is inadequate (Koch Agronomic Services, n.d.; NutrientStar, n.d.; Brown et al., 2001; Orloff, 2012; Linquist et al., 1992). Consistent with this mechanism, extension and meta-analyses show that late/split N tends to raise wheat protein (often with little yield change) and that the magnitude is context-dependent—largest when loss risk or in-season N shortfalls are likely, smaller when conditions limit N response (Jones, 2020; Orloff, 2012).

Ontario guidance likewise notes that achieving protein targets can require more/appropriately timed N than that needed to maximize yield alone, underscoring the importance of timing (OMAFRA, 2022, Pub. 811, Ch. 4). In general, EEF treatments resulted in lower protein concentrations than conventional untreated urea. Normally, the increased protein content of treatments with EEFs can be explained by their capacity to control N status during grain-filling, an important window for protein synthesis (Woodard and Bly, 1998). The results of this study certainly do not align with others who reported higher values of wheat protein with stabilized nitrogenous fertilizer than with traditional fertilizer.

The findings here provide useful insights for N management in Northwestern Ontario, even though plant protein levels in the region—such as those reported at LUARS in Thunder Bay—have generally met minimum market standards. Generally, strategic use of N fertilizers, particularly through the integration of EEFs or blended N sources, can help optimize protein content to meet premium quality targets (Brown et al., 2005). Beyond productivity, EEFs offer environmental benefits, including reduced nitrogen losses and lower greenhouse gas emissions, supporting more sustainable agronomic practices in the region (An et al., 2024). From an economic perspective, although EEFs involve higher upfront costs, their potential to improve NUE and boost protein yields may result in higher returns through access to premium markets for high-protein wheat (Woodard & Bly, 1998). In our site-year, conditions were drier than normal during the period from green-up through early grain fill (site weather records), reducing the risk of NO_3^- leaching and denitrification; under such conditions, nitrification-focused EEFs (e.g., DCD/DMPP products and PCU/ESN) are not expected to deliver large yield gains over urea (Olson-Rutz, Jones, & Dinkins, 2011; Lawrencia et al., 2021).

Likewise, urease inhibitors mainly protect against NH_3 volatilization when surface-applied urea/UAN faces warm, moist conditions without rapid incorporation; in cool/dry conditions or when rainfall/tillage incorporates N promptly, agronomic benefits and return on investment decline (Cantarella et al., 2018; OMAFRA Field Crop News, 2023). This weather-indexed expectation aligns with Northern Ontario experience: multi-year comparisons at Thunder Bay and New Liskeard frequently found similar grain yields for ESN and urea at equal N, with advantages appearing mainly in seasons/fields with higher loss potential. (Sahota & Rowsell, 2011). Taken together, our data and prior work support a risk-indexed approach to EEFs in rainfed systems: prioritize urease inhibitors when surface applications are exposed to

conditions that favor volatilization (warm, moist, no quick incorporation), and prioritize nitrification-focused options or PCU/ESN on poorly drained soils or in wet springs where leaching/denitrification is likely; recognize that in dry years, well-timed urea may perform comparably at lower product cost. (Cantarella et al., 2018; OMAFRA Field Crop News, 2023; Olson-Rutz et al., 2011; Lawrenca et al., 2021). This framing also explains why our results differ from some wetter site-years and studies: EEF performance and return of investment are contingent on weather, soil, loss pathway, application method, and price, so effects are highly variable across environments—an important caveat often under-discussed in EEF reports. (Abalos et al., 2014; Sahota & Rowsell, 2011). Long-term trials are required to compare the long-term cumulative effect of EEFs on soil health and the N cycle.

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