EFFECT OF ENHANCED EFFICIENCY NITROGEN FERTILIZERS AND ANVOL^{TM} ON SPRING WHEAT PRODUCTION AND SOIL HEALTH

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by

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Abstract

Nitrogen (N) is an essential macronutrient that plays a critical role in the cultivation of spring wheat, affecting several physiological and developmental processes. The widespread use of N fertilizers can result in environmental contamination, as approximately half of the N applied as fertilizers is lost through various pathways. Urea treated with N stabilizers such as urease inhibitors and nitrification inhibitors could be an effective way to reduce N losses. I hypothesized that application of enhanced efficiency N fertilizers such as polymer-coated urea and urea supplemented with inhibitors of urease and nitrification will improve the growth, yield, and quality of spring wheat, outperforming the traditional application of untreated urea. This study tracked the effects of different N sources at two different application rates (80 kg N ha^{-1} and 120 kg N ha^{-1}) on plant growth attributes, field productivity, soil health metrics, and soil chemical and biological parameters. Nitrogen source had minimal effect on soil health, with only slight changes in microbial composition and nutrient levels. The use of either traditional urea or enhanced efficiency N fertilizers corresponded to the development of beneficial microbial communities. Plant phenotypic traits, grain characteristics, soil nitrate levels, and disease occurrence were not significantly influenced by the choice of N source or application rate, an outcome that can be attributed to limited rainfall during the growing season of the experiment. Grain yields were no higher in any treated plots compared to the no-N reference plots. Plant assimilation of N did occur compared to reference plots, at three times the concentration during booting and two times during tillering stages. Overall, N management strategies that prioritize optimal nutrient absorption, improve soil structure, and promote sustainable agricultural practices are recommended. However, these strategies must be adapted to prevailing environmental conditions.

Keywords: ANVOL™, ESN®, soil health, soil microbial communities, SUPERU™, urea.

Table of Contents

List of Tables

List of Figures

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Introduction

The global agricultural sector places great importance on the crucial objective of increasing food production to meet food requirements of the estimated population of 9.1 billion by 2050 (FAO, 2016). With the restrictions on developing agricultural land, sustainable intensification will become a crucial strategy for all crops (Lambin and Meyfroidt, 2011). Agricultural intensification seeks to enhance productivity by implementing high-yielding cultivars, fertilizers, and mechanization (Bodirsky et al., 2015; Bommarco et al., 2013). Forecasts indicate that there will be an increase in the future need for nitrogen (N) fertilizers simultaneous with the increase in yield potential of new varieties (Wood et al., 2004). Currently, only about half of the total N applied to crops is recovered by crops; the other half either stays in the soils or escapes as ammonia (NH_3), nitrate (NO_3^-), dissolved organic nitrogen (DON), nitric oxides (NO_x), nitrous oxide (N_2O), and dinitrogen (N_2) (Galloway et al., 2004). Strategic N management is crucial to minimizing these losses, mitigating environmental contamination and reducing the energy-intensive production of mineral N fertilizers (Van Bueren & Struik, 2017). Growing concerns about ecological impacts and escalating fertilizer expenses underscore the imperative for heightened N use efficiency.

Canada Western Red Spring Wheat (CWRS) is the most common type of wheat cultivated in Canada, planted on 6.07 million hectares with a production of 21.7 million tonnes of grains annually (Alliance Seeds, 2024). In 2022, 35,000 hectares in Ontario were planted with spring wheat, yielding 0.158 million tonnes of grains. In Thunder Bay, 8,000 tonnes of spring wheat were produced the same year on 332 hectares (Statistics Canada, 2024). CWRS varieties are well known all over the globe for their superior milling and baking properties, with very little loss of protein in the milling process (Canadian Grain Commission, 2022). Nitrogen exerts significant influence on tillering, photosynthesis, and protein synthesis in wheat, necessitating precise adjustments of fertilizer treatments throughout different growth phases (Dobermann & Cassman, 2005). A recent investigation of gaps in cereal yields in Europe highlights the significance of improving N uptake, N use efficiency, and harvest index through various physiological mechanisms and agronomic traits (Schils et al., 2018). Optimizing N use efficiency can be challenging due to the intricate dynamics of N behaviour, its mobile characteristics, and the interactions between genotype and environment.

Spring wheat development ideally requires careful adjustment of fertilizer N supply during various stages of its growth (Kong et al., 2013). The concentration of grain protein is widely recognized as a crucial quality characteristic within the CWRS class (Hucl et al., 2022). Management should take precautions to prevent excessive N availability, which can cause yield losses due to lodging during harvest or the promotion of wheat diseases, as well as its deficiency that can restrict growth and diminish yield. Dealing with N loss is a constant challenge, especially when using conventional N fertilizer such as urea. Using enhanced efficiency fertilizers (EEFs) in on-farm nutrient management could be an effective approach to mitigate N losses. EEFs are created to minimize losses and ensure that N is efficiently taken up by crop plants for optimal growth (Fast et al., 2023). Chemically stabilized, inhibitor-based N fertilizers are a source of N that release~~s~~ N slowly in a controlled manner. Examples of inhibitors used with N fertilizers, especially urea, are NBPT (a urease inhibitor), which helps to decrease the release of ammonia into the air and DCD (a nitrification inhibitor), which decreases leaching of nitrate into groundwater.

EEFs such as ESN (Polymer coated urea) became available for use on farms in the late 1990s and are primarily composed of urea (Wu et al., 2021). They typically contain 44% N, which is released in a steady manner, aligning with the requirements of the crops. The release usually occurs in three stages: 8-15% in 10 days, 40-60% by one month, and 85-90% after 80 days (Golden et al., 2011).

This thesis evaluates conventional N fertilizer (urea), a polymer coated slow-release N fertilizer (ESN®) and their blends, urea supplemented with inhibitors of urease and nitrification (SUPERU™), and urea treated with a dual-action urease inhibitor (ANVOL™). Treatments were applied at two rates of N (80 and 120 kg N ha^{-1}) with a set of reference (untreated) plots, having the goal of monitoring soil health and any increases in growth and yield of CWRS wheat at different stages. The research was conducted at the Lakehead University Agricultural Research Station (https://www.lakeheadu.ca/centre/luars), Thunder Bay, Ontario, Canada. The research aimed to promote modern agricultural practices and aid in strategic policy decisions in the region to optimise N use efficiency.

Physiology of spring wheat

In Canada, there are nine classes of spring wheat (https://grainscanada.gc.ca/en/grain-quality/grain-grading/wheat-classes.html). The CWRS class is the most prominent (McCallum & DePauw 2008). There are eight critical stages of growth in spring wheat: (1) germination/emergence, (2) tillering (May-June), (3) jointing (stem elongation), (4) booting (end of stem elongation in June-July), (5) heading (spike emergence), (6) anthesis, (7) grain filling (July-August), and (8) kernel hardening or maturity (August or early September; Bauer et al., 1992). It is recommended to plant spring wheat as early as possible, as cooler weather during the emergence and early reproductive stages tends to promote tiller formation and the growth of larger heads. Enhanced growth in the initial stages of the season often leads to greater crop productivity. The temperature range required for spring wheat development is between 0 °C and 35 °C (Al-Khatib and Paulsen, 1999). The vegetative development of spring wheat is influenced by specific temperature ranges, which can vary depending on the cultivar (Kobza et al., 1987). The optimal temperature for photosynthesis is 20–22 °C, while higher temperatures of 30–32 °C can hinder the process. The ideal temperature range for anthesis and grain filling is 12–22 °C. If the temperature goes beyond this range, it can have a significant negative impact on grain yield (Tewolde et al., 2006).

Hypothesis

Enhanced efficiency N fertilizers, alone or in combination with urea, or a N stabilizer (Anvol^{TM}) can improve growth characteristics and grain yield, improve nitrate N and ammoniacal N in soil and nitrate N in plant tissue, and improve soil health measures when applied with spring wheat cultivation as compared to conventional N (urea) fertilizer.

The following objectives were set out to test this hypothesis at the Lakehead University Agricultural Research Station in Thunder Bay, Ontario:

i.) To determine the advantages of enhanced efficiency N fertilizers, an N stabilizer, and conventional N fertilizer alone or in combination with other N sources, in terms of their effects on spring wheat growth characteristics and grain yield.

ii.) To determine the advantages of enhanced efficiency N fertilizers, an N stabilizer, and conventional N fertilizer alone or in combination with other N sources, on nitrate N and ammoniacal N in soil and nitrate N in plant tissue of spring wheat.

iii.) To determine the advantages of enhanced efficiency N fertilizers, an N stabilizer, and conventional N fertilizer alone or in combination with other N sources, on soil health measures when applied to spring wheat at seeding.

Literature Review

1. Nitrogen management in agriculture

Production of sufficient crop yields to sustain the current population of 7.8 billion relies heavily on the substantial use of N input. The green revolution achieved significant successes, one of which was the utilization of the Haber Bosch process to produce N-based fertilizers (Matson et al., 2012). The process facilitated not only the enhancement of agriculture, but also the use and advancement of formerly non-agricultural areas, hence enabling the provision of food to a continuously expanding global population. Chemical N fertilizer thus revolutionized agriculture, accompanied by a significant surge in its use over the past 50 years. In 1960, the global N use was 10.8 million Mg, which escalated to 82 million Mg by 2000; it is projected to further increase to 249 million Mg by 2050 (Han et al., 2016; Iannetta et al., 2016). However, reliance on external N input is a fundamental vulnerability in our global food production system (Cassman & Dobermann, 2021).

It is challenging to accurately provide enough N to satisfy the physiological needs of crops, while also managing the movement of reactive N to prevent environmental losses. Consequently, cropping systems that are accountable for most of the world's food production release excessive amounts of nitrate (NO_3^-) N, leading to significant deterioration of water quality, riparian ecosystems, and aquatic environments (Cassman & Dobermann, 2021). The emission of gaseous substances, such as ammonia, nitric oxides (NO_2), and nitrous oxide (N_2O), has a detrimental effect on air quality and is considered a significant factor in the agricultural sector's contribution to climate change. Whereas, insufficient N during crop growth adversely affects plant morphology, growth rate, and the life cycle of crops (Kaplan et al., 2016).

1.1 Nitrogen cycle

Nitrogen (N) exists at around 78% of the Earth's atmosphere mostly as a nonreactive dinitrogen (N_2) molecule, which restricts its accessibility and utilization. Its practical applications are highly restricted as live organisms require it in only minimal amounts. Nevertheless, its compounds form crucial nutrients that when lacking can impede both crop yield and human development (Kuypers et al., 2018). For N_2 to form other compounds, it must undergo dissociation into its two constituent atoms, which can occur by two methods: physical means such as lightning, and biological means through the action of rhizobium bacteria in the root nodules of legumes, known as biological nitrogen fixation (BNF). The N cycle is crucial for maintaining nutritional balance in terrestrial ecosystems, as it involves processes such as N-fixation and mineralization (Hayatsu et al., 2008). The excessive use of N fertilizers in agricultural regions has a significant impact on the N cycle, namely on the processes of nitrification and denitrification (Akiyama et al., 2006). The result is a substantial increase in N_2O emissions and the contamination of groundwater due to the leaching of nitrates from the crop fields.

The N cycle is a sequence of interconnected processes, in which N undergoes conversions from one state to another with the participation of microbes and the non-biological breakdown of intermediate substances (Butterbach-Bahl et al., 2013). Originally, the N cycle was thought to consist of only two main processes: nitrification and denitrification. However, as our knowledge of N transformations has advanced, we now recognize seven interrelated processes within the N cycle (Firestone et al., 1989; Kuypers et al., 2018). In addition to these mechanisms, there are other pathways that facilitate N assimilation and ammonification (Figure 1).

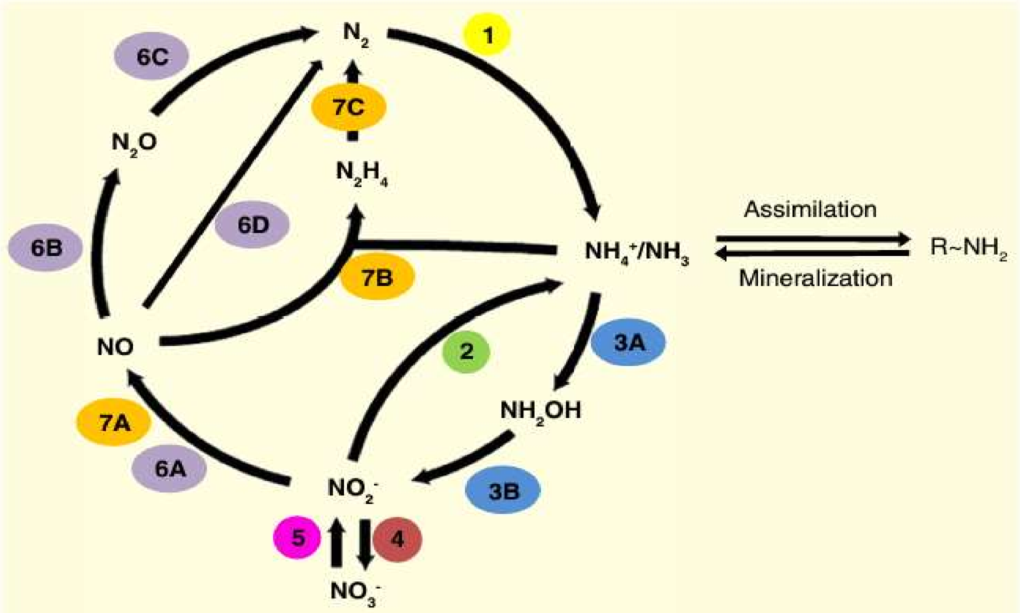

Figure 1. Major processes of the N cycle. Reactions that comprise the seven major processes of the N cycle are represented by the numbered circles. Ammonification may be accomplished either by process 1, reduction of dinitrogen (also referred to as 'nitrogen fixation' or 'Nif'), or by process 2, dissimilatory nitrite reduction to ammonium (DNRA). Nitrification is composed of process 3, oxidation of ammonia to nitrite (also referred to as 'nitritation') – This process is performed by aerobic Ammonium Oxidizing Bacteria (AOB) and the nitrosomonas bacteria, and process 4, oxidation of nitrite to nitrate (also referred to as 'nitratation') typically by means of the enzyme nitrite oxidoreductase and the nitrifying bacteria nitrobacter. Process 5, the reduction of nitrate to nitrite, can be coupled to processes 2, 6 or 7 in a population or a community. Denitrification is shown as process 6, which is also referred to as 'nitrogen-oxide gasification'. Anammox is shown as process 7 and is also referred to as coupled nitrification–denitrification' (Stein & Klotz, 2016).

1.2 Nitrogen management in Canadian agriculture

Globally, there is a tendency of extending N use in the production of all cereal crops (Galloway et al., 2017). This trend is commensurate with the population boom. Approximately 75% of Canada's N fertilizer applications occur in the provinces of Alberta, Saskatchewan, and Manitoba (Statistics Canada, 2016). The reason for the large amounts is the vast area of cropland in the prairie provinces. Amounts of N application are lower in the other provinces. The reason for the large amounts is the vast area of cropland in the prairie provinces. Crop plants on an average take up 30-50% on N applied as fertilizers (Cassman et al., 2002; Janzen et al., 2003). This inefficiency highlights the need for better N control strategies to maximize resource use and limit N losses. Ammonia (NH_3), nitrate (NO_3^-), nitrous oxide (N_2O), and di-nitrogen (N_2) are the various kinds of by products that result from inefficient use of N fertilizers. Qiao et al. (2015) and Janzen et al. (2003) highlighted the negative consequences of such losses to the environment, which may include greenhouse gas emissions and water contamination.

Consequently, farmers in Western Canada make important choices about the composition, rate, location, and timing of applications of N fertilizers. Grant and Pattey (2008) pointed out that operational issues, including product availability, time restrictions, budgetary considerations, and equipment availability also affect the choices made by farmers. Strategies for managing N effectively must consider the various environments where farmers work. To overcome the drawbacks of conventional estimates that rely on linear interpolations, applying a process-based agroecosystem model is recommended (Grant & Pattey, 2003; Grant et al., 2006; Grant et al., 2016; Flesch et al. 2018). These kinds of models provide a thorough simulation of biogeochemical cycles and a means to evaluate the effects of N, taking non-linear feedback into account, and generating continuous estimates of N over time and space.

1.3 Residual soil nitrogen and its management

Residual soil N refers to the quantity of applied N that remains in the soil following the harvest of a crop. It may have a role in determining the quantity of N that will be accessible to the subsequent crops (Chen et al., 2014). Residual soil N can also have enduring impacts on soil structure, nutrient availability, and crop yields over an extended period of time. The following is a summary of the work cited above that is related to the best agricultural practices. Crop plant residues can be managed to make use of residual soil N. When crop residues are reintroduced into the soil, they undergo several processes including biotic immobilization-remineralization, abiotic immobilization, soil organic N mineralization, and plant residue organic N mineralization. The specific mechanism that occurs depends on the type of crop residue and the qualities of the soil. Carbon-to-nitrogen (C/N) ratio in the crop residues is not reliable in predicting their impact. Mineralization consistently enhances the uptake of N by crops and amplifies the likelihood of N loss. Furthermore, while net immobilization is a factor in both immobilization-remineralization and immobilization processes, it does not inevitably result in reduced N uptake by crops (Bird et al., 2001). The results are also influenced by the synchronization between the fluctuating levels of inorganic N in the soil and the uptake of N by the crops. The loss of N during the process of mineralization can be decreased by using a chemical immobilizer. Net N immobilization can be mitigated by altering the time of ploughing and fertilization, or by modifying the positioning of plant residues (Carefoot and Janzen, 1997).

2. Nitrogen uptake in plants

Nutrient use efficiency (NUE) denotes the ratio of nutrient outputs to inputs within an agricultural cropping system. The NUE of crops is determined by the processes of N intake, transport, assimilation, and remobilization (McAllister et al., 2012). The key enzymes involved in the process of N assimilation are nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), and glutamine-2-oxoglutarate aminotransferase, commonly known as glutamate synthase (GOGAT) (Liu et al., 2022). Plants take up NO_3^- with the help of nitrate transporters (NRT1 and NRT2). For nitrate to be assimilated, it ought to first be reduced to nitrite, which is done by the nitrate reductase enzymes in cytoplasm, and then to ammonium in plastid, which is done by nitrite reductase enzymes (Figure 2; McAllister et al., 2012). Roots use NADH as the reducing agent to reduce nitrate in the cytosol. Conversely, leaves use ferredoxin as an electron transporter connected to the light reaction of photosynthesis, resulting in a reduction in both the cytosol and chloroplasts (Liu et al. 2022).

NH_4^+ assimilation occurs in the plastid or chloroplast through a series of events known as the glutamine synthetase/glutamate synthase (GS/GOGAT) system. This system involves the enzymes GS (EC6.3.1.2), NADH-GOGAT (EC1.4.1.13), and Ferredoxin (Fd)-GOGAT (EC1.4.7.1) (McAllister et al., 2012; Suzuki and Knaff, 2005). Following the absorption of N by the plant, it is conveyed throughout the plant via the xylem as glutamine, asparagine, glutamate, and aspartate, which may be used or stored (McAllister et al., 2012; Okumoto & Pilot, 2011). During senescence and grain filling, absorption enzymes such as GS1 and glutamate dehydrogenase (GDH) [EC1.4.1.2.] aid in the remobilization of N to the grains, once it reaches the sink tissues (McAllister et al., 2012).

2.1 The role of nitrogen in wheat production

In wheat production, N application significantly impacts wheat grain yield, grain protein content, and plant growth and development (Triboi et al., 2000). Nitrogen is required for many different metabolic activities, and a healthy supply of N is linked to strong vegetative growth, high photosynthetic activity, and a deep green hue. Several studies have analyzed the individual effects of N fertilizer management strategies on improving wheat production (Zhang et al., 2017; Hu et al., 2023). Similar to other crops, wheat does not require an extensive amount of N during the germination stage, and N availability is of greatest importance during the tillering and stem elongation stages when crops have the ability to absorb a larger quantity of N (Döring & Neuhoff, 2021). Inadequate levels of N at this stage result in elevated shoot mortality, reduced spike size, and a restricted number of kernels per unit area (Döring & Neuhoff, 2021; Mahboob et al., 2023). In order to achieve the highest possible grain yield in wheat, it is recommended to split N application between seeding and tillering, or between seeding, tillering, and heading (Wang et al., 2023).

According to Mizuta et al.’s (2020) research conducted on wheat grown in Japan, splitting and top-dressing of N before stem elongation leads to improved N recovery efficiency, increased grain production, and reduced N leaching. This approach has been found to be more effective than applying the entire dose of N at seeding, which results in a higher rate of N loss (Wang et al., 2023). A consequence is a diminished amount of N accessible to the crop during its growth period (Verma & Sagar, 2020). Despite the fact that rapid uptake does not commence until the stem elongation stage, N fertilizer is typically administered prior to or at planting in the eastern Canadian Prairies because of the short growing season (Pan et al., 2020). This tactic promotes unnecessary early-season vegetative development, which can increase lodging risk due to shadowing effects, and leaves N fertilizer sensitive to losses in

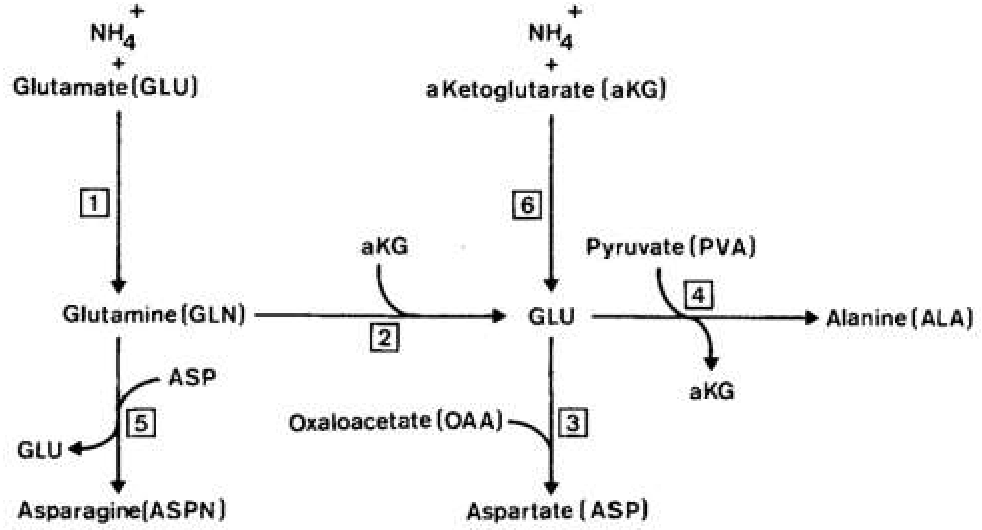

Figure 2. Biochemical pathways for N assimilation. Glutamine is synthesized by glutamine synthetase (1) and is converted to glutamate by glutamate synthetase (2). Aspartate is formed by transanimation from glutamate by aspartate aminotransferase (3); Alanine arises similarly through the action of alanine aminotransferase (4). Aspartate can be transaminated into asparagine by asparagine synthetase (5). Glutamate can also be formed by glutamic dehydrogenase (6), but that reaction seems relatively unimportant in plants (Novoa & Loomis, 1981).

the early growing season (Mangin et al., 2022). High rates of N are needed to get the most out of modern spring wheat cultivars cultivated in western Canada since they produce significantly larger yields than in the past (Mangin et al., 2022; Semagn et al., 2021). Field investigation showed that each increment of N considerably increases plant height, tiller count, 1000-kernel weight, and overall grain yield (Ali et al., 2005). The study additionally emphasizes the importance of applying different nutrients in a balanced manner, along with phosphorus, potassium, sulphur, zinc, and boron (Pandey et al., 2020). In the end, N stands as a major nutrient that greatly affects the productivity of wheat. For sustainable and extended wheat yields, N should be used in aggregate with balanced supply of other nutrients. Researchers and wheat growers trying to increase nutrient loss control strategies for improved wheat yield can learn from the studies indexed here.

2.2 Evaluation of nitrogen sources for spring wheat production

The CWRS class is a high protein wheat that is ideal for producing large quantities of pan breads and specific types of Asian noodles. Additionally, it can be blended with low protein wheat to enhance its quality to meet specific use requirements (Wang et al., 2004). Due to their quality standards, CWRS breeders strive to concurrently select for superior grain productivity and elevated protein levels, even though grain production and protein concentration are inversely correlated (Da Costa & Kronstad, 1994; Simmonds, 1995; Sieling and Kage, 2021). To assist with this trade-off, N losses and shading effects can be decreased, final grain protein levels can be increased, and total N rates can be adjusted by withholding some N fertilizer until the crop is established (Ye, 2023). In summary, to maximize yields using existing cultivars sufficient N fertilizer must be made available through the season, while also taking care to mitigate the danger of lodging (Berry et al., 2004). As an alternative to applying N later in the growing season, controlled-release N products could be used at planting (Ma et al., 2023).

Split N treatment can thus enhance production and protein content in wheat grains. The grain yield of wheat is positively correlated with N availability, which influences tillering density, kernel production, kernel weight, and grain protein content (Oliveira et al., 2021). Applying N to wheat at two separate stages had resulted in an increase of 0.8% in grain protein content compared to applying N before planting. Additionally, using N as a foliar spray at a later stage has led to a 1.6% increase in grain protein content compared to not using any foliar application. While it is more typical to administer two applications, three can ensure N is available during the reproductive phase (Fuertes-Mendizábal et al., 2013). Split application of controlled-released urea has been found to enhance the yield, N recovery efficiency, and grain protein content of wheat (Beres et al., 2018). Likewise, Ali et al. (2018) determined that number of tillers, plant top, spikelets per spike, seeds consistent with spike, biological yield, 1000-kernel weight, grain yield, and harvest index are the traits for which outcomes were much better with staged N applications.

3. Enhanced Efficiency Fertilizers (EEFs)

EEFs are a significant advancement in agriculture. They are specifically developed to minimize nutrient losses and improve N availability compared to conventional fertilizers (Verburg et al., 2022). They also reduce N losses due to leaching, ammonia volatilization, runoff, and microbial tie-up, all of which pose environmental issues (Trenkel, 2010). Today, EEFs are primarily used to reduce the risk of excessive vegetative growth, adjust release patterns, to match N supply with plant nutrient needs, to limit nutrient losses, and to increase nutrient availability (Olson-Rutz et al., 2011). Increased-productivity stabilized fertilizers and controlled or slow-release fertilizers are the two primary categories of EEFs. Controlled or gradual-release fertilizers release nutrients in a more progressive manner than ordinary fertilizers, whereas stabilized fertilizers contain chemical compounds that prevent microbial and enzymatic reactions inside the soil.

The N cycle and the methods by which EEFs can lessen N losses are depicted in Figure 5 (Olson-Rutz et al., 2011; Verburg et al., 2022). At different stages of the N cycle, EEFs have an impact on urea hydrolysis, nitrification, or denitrification. For instance, urease inhibitors such as NBPT reduce ammonia volatilization by postponing the urea-to-ammonium conversion (Olson-Rutz et al., 2011). Temperature, moisture content, and soil characteristics all affect the success of NBPT, making it important to take these factors into account when considering the best possible application periods.

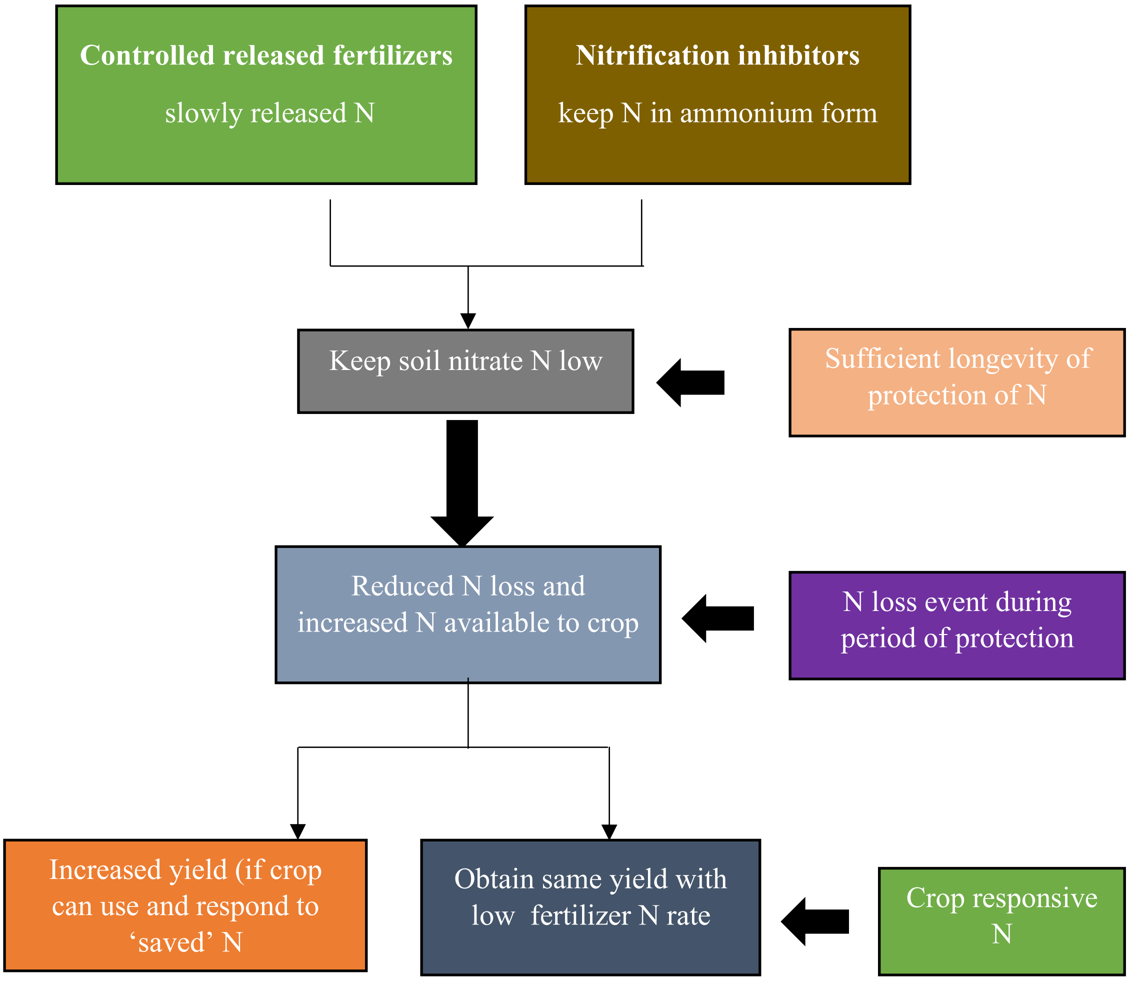

Figure 3. The effectiveness of enhanced-efficiency fertilizers, which rely on controlled release or restriction of nitrification, is contingent on three essential criteria for achieving their environmental and agronomic benefits (Verburg et al., 2022).

Using EEFs may have positive environmental effects in addition to economic ones, such as lowering emissions of nitrous oxide, ammonia volatilization, and groundwater nitrate contamination. Farmers thinking about adopting must weigh these advantages against higher expenses from EEFs (Trenkel, 2010). Farmers ought to make choices based on a complete comprehension of EEF methods, effectiveness requirements, and possible advantages (Di Bella et al., 2014; Dowie et al., 2019). To sum up, enhanced-efficiency fertilizers present a viable way to maximize agricultural yields, reduce environmental effects, and optimize nutrient use efficiency.

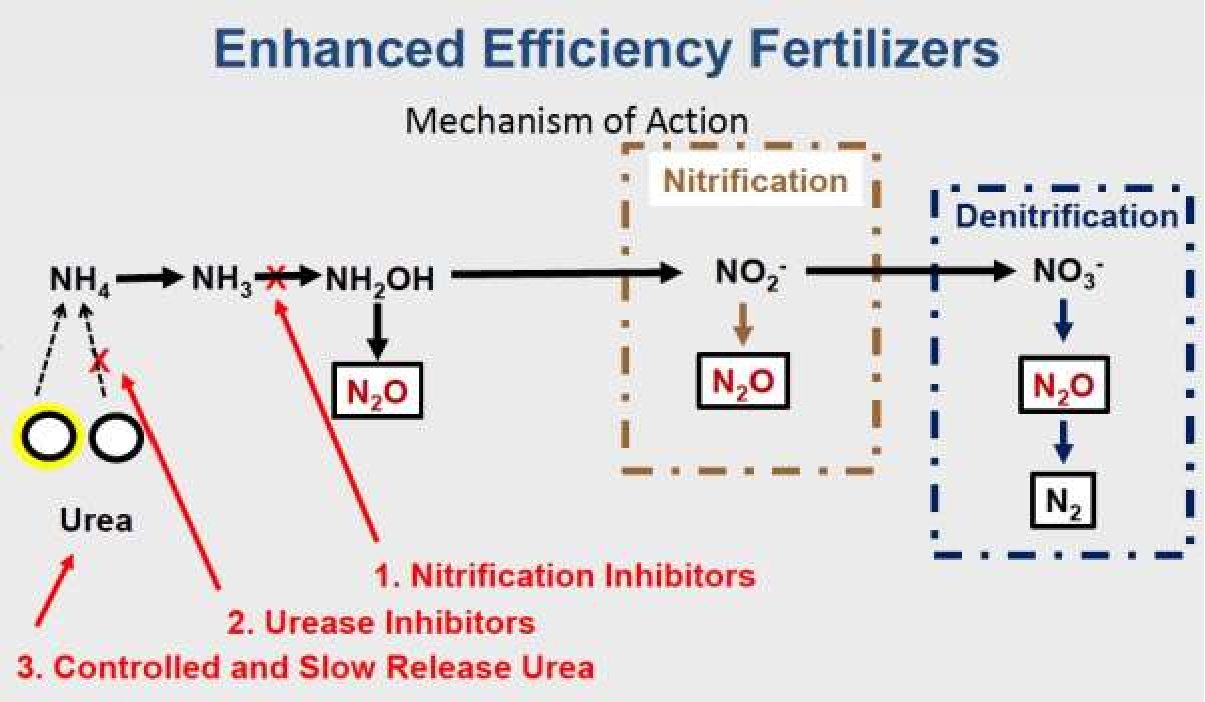

Figure 4. The N cycle and processes by which EEFs may reduce N loss from N-fertilizers (Olson-Rutz et al., 2011).

3.1 Environmentally Smart Nitrogen (ESN®)

The modern approach using Environmentally Smart Nitrogen (ESN®) is intended to improve N use efficiency in contemporary crop production, and ESN® is generically known as a controlled-release fertilizer (Sahota, 2020). Agrium Inc. (now Nutrient) has a patent on ESN®, which is a pale green polymer-coated urea that contains 44 % N. Only when water enters the polymer's microscopic pores does the urea dissolve with a gradual release more in line with the plant needs, a main advantage over applying urea, which dissolves after the first heavy rain. In contrast, ESN® releases around 8-15% of its N in the first 10 days, 40-60% in the first month, and 85-90% within 60 days (Blaylock et al., 2004). Under situations of abundant soil water, urea hydrolyzes rapidly in the soil, and, within 3-10 days, it is transformed into nitrate, which can easily be leached or denitrified. Ammonium hydroxide and ammonium carbonate are the initial byproducts of urea hydrolysis in the soil. Ammonium carbonate is a relatively unstable compound that decomposes into NH_3 (which is lost through volatilization) and H_2CO_3, which further decomposes into H_2O and CO_2 (Sahota, 2006). It is also possible for urea to release N into surface drainage. Thus, the ability to delay N releases by ESN® reduces N losses from soil and thereby environmental protection is another advantage of ESN®.

The primary advantage of slow-release fertilizers is their capacity for an extended duration of nutrient release (Yamamoto et al., 2016). Thus, the European Committee for Stabilization has established criteria that determine whether fertilizers meet the requirements to be termed as "slow release" (Fu et al., 2020). These criteria include the capacity to liberate maximums of approximately 15% and 75% of the fertilizer nutrients within the initial 24 hours and 28 days following application, respectively. The Association of American Plant Food Control Officials (AAPFCO) asserts that for fertilizers to qualify as "slow release," they must consistently deplete their nutrients for an extended period following their application (Folina et al., 2021). Using data from complementary studies by Golden et al. (2011) and extensive research conducted at the Lakehead University Agricultural Research Station (Sahota, 2020), this section examines the multifaceted impact of polymer-coated slow-release

N fertilizer on crop yields, its flexible application techniques, its economic viability, and the environmental implications of its use. However, N release from polymer-coated slow-release fertilizers is moisture and temperature dependent, meaning that it could be too slow in climates with cooler and shorter growing seasons, such as much of North America and Europe. In this case, a mixture of urea and slow-release fertilizer may prove superior in maintaining crop yield to either fertilizer applied alone (Blaylock et al., 2004; Sahota, 2020). In addition, in cases where environmental conditions enhance N losses via denitrification and leaching, the use of polymer–coated urea either alone (in longer duration crops) or in blends with urea in areas with short growing seasons could be promoted for crop production (Sahota, 2020).

Drawbacks associated with slow-release fertilizers are mainly their high costs compared to traditional fertilizers (Folina et al., 2021). For example, in 2017, the prices of urea on the world market ranged from $178 to 326 per MT, while controlled-release (sulphur coated) urea ranged from $327 to 950 per MT, and polymer-coated slow-release fertilizers ranged from $905 to 2940 per MT (Wesolowska et al., 2021). Also, certain slow-release fertilizers are not user friendly, and their performance can be substantially affected by soil pH, soil microbial activity, soil organic matter, temperature, and moisture (Fu et al., 2020; Kakabouki et al., 2020). The most commercialized slow-release fertilizers are those containing urease and nitrification inhibitors, such as N-(n-butyl) thiophosphorictriamide, hydroquinone, nitrapyrin, and dicyandiamide (Folina et al., 2021).

3.2 The function of urease and nitrification inhibitors in reducing nitrogen losses and enhancing crop yield

The primary role of N inhibitors is to modify the channel via which fertilizer is released by altering the functions of N-metabolizing enzymes found in the soil. This process hinders the functions of urea hydrolysis and nitrification, leading to the loss of N (Chien et al., 2009; Timilsena et al., 2015). Typically, slow-release fertilizers that incorporate urease inhibitors are produced using synthetic chemicals that possess a comparable structure or affinity to urease. There are three distinct categories of synthetic urease inhibitors compounds:

- Organic or inorganic substances, such as alk(en)ylthiosulfinate, hydroquinone, and p-benzoquinone, which can undergo a reaction with sulfhydryl (mercapto) groups found in ureases.
- Metal-chelating chemicals, such as caprylohydroxamic acid and acetohydroxamic acid, which can bind to metal ions in the active site of urease using one of the N atoms.
- Competitive inhibitors that include phosphoramides, phenyl phosphorodiamidate, thiophosphoric triamide, hydroxyurea, and N-(n-butyl) thiophosphorictriamide (NBPT), compounds that resemble the urease structure and can attach to the active site of the urease enzyme (Svane et al., 2020).

The drawback of the third category is that the inhibitors are not easily broken down by the urease enzyme (Kafarski, & Talma, 2018; Upadhyay, 2012). Yet, most stabilized fertilizers are formulated with the competitive inhibitor thiophosphoric triamide (Kakabouki et al., 2020). Urease inhibitors can be sold as solid fertilizer products (such as those with a coating), or liquids. The primary function of the urease inhibitor is to inhibit the transformation of urea into NH_4 and minimize the volatilization of NH_3 (Saggar et al., 2013; Upadhyay, 2012).

In contrast to urease inhibitors, nitrification inhibitors are designed to slow down the rate of nitrification by inhibiting the action of nitrifying bacteria (Dimkpa et al., 2020). Typically, the nitrification inhibition process takes longer (20 to 28 days) to complete compared to the inhibition of urease hydrolysis, because they must inhibit for the entire duration of the nitrification period. This, however, implies a better chance to coordinate the release of N with the uptake of nitrogen by crops (Singh, 2008). As a result, there is a potential to decrease the emission of N_2O and the leaching of NO_3^- using nitrification inhibitors. The two most commonly used nitrification inhibitors are dicyandiamide (DCD) and nitrapyrin in the USA and 3,4,-dimethylpyrazole phosphate in Europe (Harty et al., 2016; Zerulla et al., 2001).

3.3 Impact of urea, polymer-coated urea, urease inhibitors and nitrification inhibitors on the soil biological community

Studies conducted by Morales et al. (2015) and Tosi et al. (2020) found that there were only very short-term effects on the microbial community after 12 days with applications of urea + NBPT or Urea + DCD. Fu et al. (2020) found no impact of NBPT on the composition and diversity of the number of bacteria, archaea, or fungi. Castellano-Hinojosa et al. (2019) found that the urea + NBPT treatment resulted in a decrease in the abundance of bacteria and archaea compared to using urea alone, but that there was no difference compared to not using any fertilizer. Several studies have reported a moderate increase or decrease in N_2O emissions when using NBPT in combination with urea, compared to using urea alone (Harty et al., 2016; Roche et al., 2016; Krol et al., 2020). However, Banerjee et al. (1999) found no significant effect of NBPT on soil biomass, N mineralization, arylsulfatase, or phosphatase. The effect of fertilizer type or inhibitor type on microbial function and the abundance of N-cycling communities was minimal, except for a specific effect of DCD on nitrification. Application of DCD, which inhibits activity of urease enzyme, resulted in a decrease in comammox amoA abundance and an increase in both potential mineralisation and N_2O emissions (Duff et al., 2022). In sum, the presence of inhibitors for a prolonged period does not have a major impact on non-target bacterial and fungal communities.

Materials and Methods

1. Study area

The Lakehead University Agricultural Research Station, Thunder Bay (LUARS, formerly Thunder Bay Agricultural Research Station; Figure 5) was established in 1991 by the Ontario Ministry of Agriculture, Food and Rural Affairs, Northwestern Ontario, Canada (48° 22' N, 89° 22' W). In spring, typical temperatures range from -3°C to 26°C. The total precipitation recorded from May to September 2022 was 368.8 mm, with only 140.4 mm occurring during the main crop growing season from June to August, marking it as the lowest ever recorded rainfall at LUARS until 2022 (Table 1), as compared to long-term average weather conditions (Figure 6). There was an intention to repeat the field experiment described in the next section during both 2022 and 2023, but low precipitation in 2023 caused the second experiment to fail as the soil was too dry to support adequate crop growth (Table 2). In 2022, snow persisted throughout the month of May, and there was a significant amount of rainfall (108.7 mm), which resulted in the delay of seeding until the second week of June. With a growing season of approximately 110 days, the spring wheat crop was cultivated in 2022 under rain-fed conditions in the region.

The soil at LUARS is a silty clay loam. Pre-seeding soil tests indicated soil pH of 6.0, organic matter 4.1%, 19.73 mg L^{-1} NO_3^- N, 9.07 mg L^{-1} NH_4^+ N, 41 mg L^{-1} P, 189 mg L^{-1} K, 2438 mg L^{-1} Ca, and 635 mg L^{-1} Mg. The spring wheat production in Thunder Bay and at LUARS has varied considerably from year to year during 2004 to 2022 (Table 3).

Figure 5. Aerial photograph of schematic layout of LUARS indicating experimental plots, barn, and local roadways.

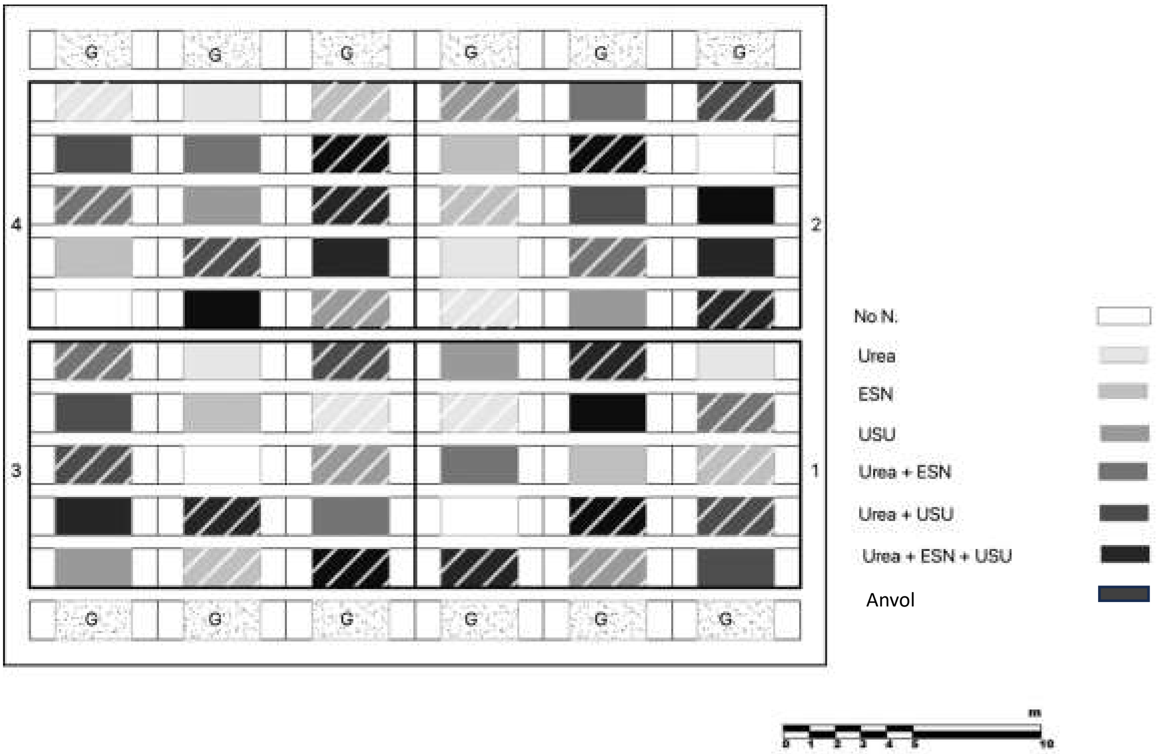

Figure 6. Experimental layout of spring wheat variety “AAC Wheatland” at Lakehead University Agricultural Research Station (LUARS), indicating N application treatments: solid-coloured boxes represent plots with 80 kg N ha^{-1}, and boxes with diagonal lines indicate 120 kg N ha^{-1}. Numerical annotations (1, 2, 3, 4) denote replicate blocks, with 'G' marking guard plots.

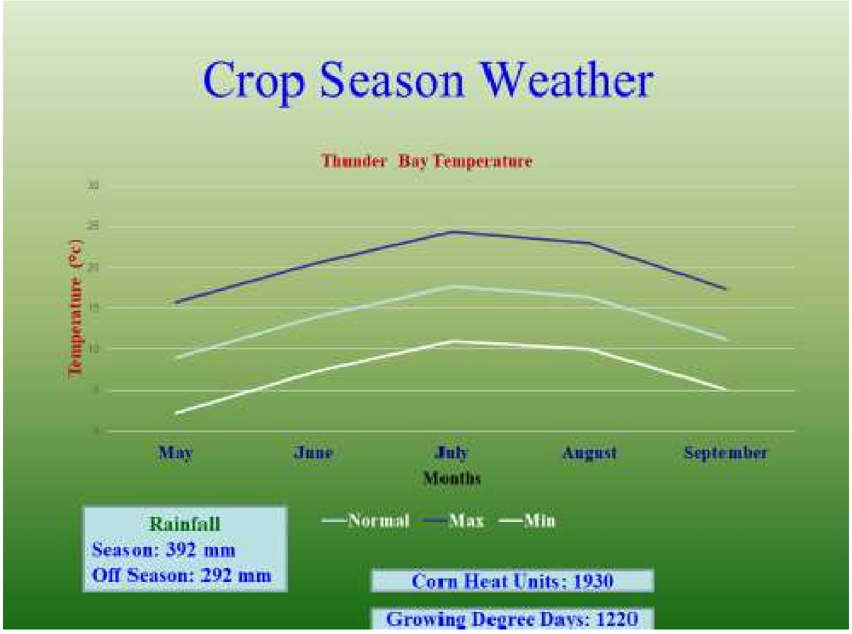

Figure 7. Long-term average weather conditions during 2003-2018 at Thunder Bay, Ontario, Canada (Sahota, 2020).

Table 1. Weather data for the 2022 growing season at the Lakehead University Agriculture Research Station (LUARS). Start date for Corn Heat Units (CHU) was May 1, 2022, and end date was September 27, 2022.

Month	Precipitation (mm)	Max temp. (°C)	Min temp. (°C)	Growing Degree Days	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
September	119.8	30	-5	191	340
October	34.9	21	-6	67	137
Total/Mean	403.7	26	-3	1372	2413

Table 2. Weather data for the 2023 growing season at LUARS. Start date for Corn Heat Units (CHU) was May 1, 2023, and end date was October 7, 2023.

Month	Precipitation (mm)	Max temp. (°C)	Min temp. (°C)	Growing Degree Days	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
September	23.8	34	0	285	470
October	22.5	27	-10	77	119
Total/Mean	332.8	28	-3	1588	2632

2. Spring wheat variety

Canada Western Red Spring (CWRS) wheat variety “AAC Wheatland,” which has a moderate growth habit, was used for the field experiment. Average flag leaf length, plant height at maturity, and spike length of this cultivar are 18.6, 91.5, and 9.7 cm, respectively (Canadian Food Inspection Agency, 2020). It has shorter awns than spikes. This cultivar is also resistant to the orange wheat blossom midge (*Sitodiplosis mosellana*), a fly in the Cecidomylidae family and a significant pest of wheat (Chavalle et al. 2017).

Table 3. Spring wheat grain yield and production in the Thunder Bay area, 2004–2022 and grain yield (CV Sable) at LUARS (Statistics Canada, 2024).

Year	Area seeded (ha)	Area harvested (ha)	Yield (kg/ha)	Area in production ('000 T)	Grain yield at LUARS (kg ha^{-1})
2004	400	400	3,335	0.5	7,976
2005	700	700	2,078	0.6	5,010
2006	549	500	2,421	0.5	3,258
2007	1400	1400	2,959	1.7	4,216
2008	-	-	-	-	5,237
2009	-	-	-	-	6,309
2010	216	155	3,671	0.2	4,880
2011	744	158	4,041	0.3	4,594
2012	2,633	2,516	3,840	3.9	3,175
2013	1,121	1,121	1,264	0.6	3,476
2014	-	-	-	-	5,846
2015	2,100	2,100	4,304	3.6	4,370
2016	-	-	-	-	5,899
2017	800	800	2,750	0.9	5,200
2018	1,400	1,352	3,705	2.0	5,776
2019	-	-	-	-	
2020	400	393	3,026	-	
2021	583	583	2,535	0.6	
2022	332	332	5,958	0.8	

Note: CV Sable was not grown at the research station after 2018.

3. Field experiment

The seeding of wheat was done on June 9, 2022, with a 10-row, tractor-operated seed drill at a seeding rate of 400 seeds per m^2. The field was divided into 72 plots, of 5-m length and 1.5-m breadth (plot areas of 7.5 m^2), of which 60 plots were designated to the main experiment featuring four replicates of 14 different treatments and a set of four reference (no-N) plots (Figure 5). Additionally, there were 12 guard plots. A randomized complete block design constituted the experiment (Table 4). Nitrogen fertilizers were applied broadcast at seeding, at two rates, 80 kg N ha^{-1} and 120 kg N ha^{-1}. Nitrogen sources were applied alone or in combination, including urea, polymer-coated urea (ESN®), and urea supplemented with inhibitors of urease and nitrification (SUPERU™) and urease double inhibitor (Anvol™) treated urea, making a total of 7 N sources. Combinations of urea with polymer-coated urea (ESN®), and urea supplemented with inhibitors of urease and nitrification (SUPERU™) were applied at a ratio of 2:1 on N basis, and the combination of urea with both polymer-coated urea and urea supplemented with inhibitors of urease and nitrification was applied at a ratio of 1:1:1 on N basis, at both the N rates; 80 kg N ha^{-1} and 120 kg N ha^{-1}. In addition to N, phosphorous @ 20 kg P_2O_5 ha^{-1} and potassium @ 20 kg K_2O ha^{-1} were applied as 0-45-0 and 0-0-60, respectively, at seeding. Application of 80 kg N ha^{-1} is the recommended rate, and 120 kg N ha^{-1} is an enhanced rate, reflecting the popular understanding that higher N fertilizer application results in higher grain yields.

Table 4. List of treatments applied in the experiment. Figure symbol refers to figures presented in the Results section.

Figure symbol	**Treatment**
1	No N
2	Urea 80 kg N ha^{-1}
3	ESN® 80 kg N ha^{-1}
4	SUPERU™ 80 kg N ha^{-1}
5	Urea 53 kg N ha^{-1} + ESN® 27 kg N ha^{-1}
6	Urea 53 kg N ha^{-1} + SUPERU™ 27 kg N ha^{-1}
7	Urea 27 kg N ha^{-1} + ESN® 27 kg N ha^{-1} + SUPERU™ 27 kg N ha^{-1}
8	Urea 120 kg N ha^{-1}
9	ESN® 120 kg N ha^{-1}
10	SUPERU™ 120 kg N ha^{-1}
11	Urea 80 kg N ha^{-1} + ESN® 40 kg N ha^{-1}
12	Urea 80 kg N ha^{-1} + SUPERU™ 40 kg N ha^{-1}
13	Urea 40 kg N ha^{-1} + ESN® 40 kg N ha^{-1} + SUPERU™ 40 kg N ha^{-1}
14	Urea treated with ANVOL™ 80 kg N ha^{-1}
15	Urea treated with ANVOL™ 120 kg N ha^{-1}

No N: Reference (control), Urea: Conventional N fertilizer, ESN®: Polymer coated slow-release N fertilizer, SUPERU™: Urea supplemented with urease and nitrification inhibitors, ANVOL™: dual action urease inhibitor (NBPT and Duramide), SE: Standard error, NR: Nitrogen rate, NS: Nitrogen source.

4. Soil data collection

Soil samples were collected from the 15 treatments from all plots (Table 4) from 0-15 cm for analysis of soil health, chemical and biological analyses, and nitrate concentration. Soil samples were collected on four occasions: seven days pre-treatment on June 1, 2022, seven days post-treatment on June 17, 2022, mid-season on August 13, 2022, and seven days pre-harvest on September 22, 2022. Samples from four replicates were mixed to form one composite sample for each treatment. Additional soil samples were collected from each replicate from 0-30 cm depth seven days prior to seeding and seven days prior to harvesting for nitrate N and ammoniacal N tests. Analysis of soil samples was done by A & L Canada Laboratories Inc., London, Ontario.

5. Plant data collection

Plant tissue samples were collected for evaluation of NO_3^-N % concentration in wheat plants. These samples were collected at four growth stages: tillering on July 4, 2022, booting on July 28 ,2024, hard dough on September 11, 2022, and maturity on September 25, 2022. Five to six plants were randomly selected and excised from the ground level, from the two rows of each replicate. These specimens were dried in an oven and then sent to A & L laboratories for analysis. Plant height was measured from five randomly selected plants from the centre of each of the plots, from ground level to the tip of the tallest spike at maturity. Plant count (per m^2) was determined by counting the number of plants in fifty cm west to east from 2nd or 3rd inner row of each plot:

$$\text{Plant count (per m}^2\text{)} = \frac{\text{Number of plants 50 cm row}}{0.075},$$

where 0.075 m^2 is the area covered by a 50-cm row. The number of tillers (per m^2) was calculated by counting both the main stem and tillers in 50 cm west to east from 2nd or 3rd inner row of each plot:

$$\text{Number of tillers (per m}^2\text{)} = \frac{\text{Number of tillers per 50 cm row}}{0.075},$$

where 0.075 m^2 is the area covered by a 50-cm row. Spike weight was determined by harvesting and drying the spikes from five randomly chosen plants in each plot, and then weighing them. In addition to spike weight, the spike length of spring wheat was measured using a ruler on five randomly selected spikes from each plot.

The middle two rows of wheat from each plot were harvested at ground level to minimize edge effects, covering a total area of 0.9 m^2. Gross yield was measured by weighing the harvested wheat crop on a balance. Biomass in spring wheat represents the total mass of plants including all the parts of the wheat plants such as stems, leaves, and grains. Biomass per hectare was calculated as:

$$\text{Biomass (MT ha}^{-1}\text{)} = \frac{\text{Gross Yield}}{0.9} \times 10,$$

where 0.9 m^2 is the area covered by two harvested middle rows, and 10 is the conversion factor of MT ha^{-1} from kg m^{-2}. Grain yield was determined by weighing the grain obtained from threshing the harvested wheat from each plot. The harvest index is a metric used to quantify the difference between the potential and actual yield. The terms are expressed as the proportion of grain yield in relation to plant yield and serves as an indicator of efficiency for each treatment in converting absorbed nutrients into grains. This index was calculated as:

$$\text{Harvest index (\%)} = \frac{\text{Grain Yield}}{\text{Biological Yield}} \times 100.$$

Grain yield in MT per hectare was determined as:

$$\text{Grain yield (MT ha}^{-1}\text{)} = \frac{\text{Adjusted grain yield to standard 14\% grain moisture content}}{0.9} \times 10,$$

where 0.9 m^2 is the area covered by two harvested middle rows, and 10 is the conversion factor of MT ha^{-1} from kg m^{-2}. Thousand kernel weight was measured by counting 1000 grains with the seed counter, and weighing them on an electronic balance. Test weight was determined by filling a standardized hectolitre container known as a Chondrometer with grains. The grains were carefully levelled to the top of the container without any compaction and weighed on an electronic balance. Test weight was expressed in grams. Straw yield was calculated by drying the straw obtained after threshing the harvested wheat from each plot at 65°C for 72 hours in an oven. The dried straw was then weighed using an electronic balance. The data were recorded in kilograms and subsequently converted into MT per hectare.

In the context of spring wheat cultivation, the term "grain per kg nutrient" refers to the ratio of the grain yield to the total nutrients applied to the crop and was calculated by dividing grain yield ha^{-1} with the total amount of nutrients applied ha^{-1} per plot:

$$\text{Grain per kg nutrients} = \frac{\text{Grain yield kg ha}-1}{\text{Total amount of nutrients applied kg ha}-1} .$$

6. Disease data

To calculate disease incidence of Net Blotch, Take All, Barley Yellow Dwarf Virus (BYDV) and Fusarium Head Blight (FHB) as a proportion of infected plants, each disease was evaluated with a standardized severity index on the scale of 0-9, where 0 = free from infection and 9 = 89 % infection.

7. Data analysis

Principal Component Analysis (PCA) was performed on chemical, biological, and soil health data utilizing R Studio (version 4.3, R Core Team 2020). The PCA was carried out to understand relationships among the seven different N sources (NS) and the two N application rates (NR), soil chemical and biological indicators, and soil health index parameters. The latter included *Pseudomonas* population, total gram negative bacteria, gram positive bacteria, ratio of gram positive to gram negative bacteria, ratio of fungi to bacteria, total gram negatives associated with pH, ratio of fungi to bacteria associated with pH, ratio of fungi to bacteria associated with organic matter, *Pseudomonas* population associated with PERP, total gram negative bacteria associated with CEC, total microbial activity associated with CEC, total fungi associated with CEC, total gram negatives associated with K to Mg ratio, *Rhizobium* related bacteria associated with an overall biological index, an overall microbial sustainability index, percent organic matter, GFI, PMN, dissolved CO_2 -carbon, and an overall soil health index. Separate PCAs illustrate the four sampling periods: pre-treatment, post-treatment, mid-season and pre-harvest.

Two-way analysis of variance (ANOVA) assessed the effects of the different enhanced efficiency fertilizers and ANVOL^{TM} treated urea (N sources), N application rates (NR), and their interactions on NO_3^- N, NH_4^+ N, on spring wheat production measures (plant count, number of tillers, plant height, spike weight, spike length, harvest index, grain per kg nutrient, 1000 kernel weight and test weight, and grain, straw, and biomass yields. Means were compared using the Bonferroni correction factor to adjust for 26 parameters being compared. The interaction term tested the possibility that differences in parameters according to N sources were not the same for the two application rates. The Bonferroni correction set significance levels at $*P < 0.0017$ and $**P < 0.00035$, which are equivalent to alpha levels of 0.05 and 0.01. All analyses were run on the ratio of the measures for each N treatment relative to the reference plots. The field and plant data were analysed with SPSS (Version 26).

Results

1. Chemical and biological soil characteristics

Before the treatments were applied, background variation in soil chemical and biological parameters across the plots was mostly in estimated nitrogen release, total bacteria, gram-negative bacteria, and *Actinomycetes*, collectively contributing to 29.5% of explained variation (along principal component 1, PC1), along with the ratio of K to Mg, sodium, other cations, mineralizable N, nitrate nitrogen, and pH, collectively contributing to 21.4% of explained variation (along principal component 1, PC2; Figure 7a). Pre-harvest, treatments with the final set of soil samples scoring highest in chemical and biological parameters along PC1 were polymer-coated urea, urea with polymer-coated urea, and urea with urea supplemented with inhibitors of urease and nitrification, all applied at the normal rate of 80 kg N ha^{-1} (Figure 7d), corresponding to high values of phosphorus- Bray-P1 (PBIC), active carbon (CREC), estimated nitrogen release (ENR), phosphorus- bicarbonate (PBI), cation exchange capacity (CEC), calcium (CA), sodium (NA), iron (FE), sulphur (S), and aluminium (AL), all in a positive direction. Urea and urea supplemented with inhibitors of urease and nitrification alone at the normal application rates scored lowest along PC1. On the other hand, urea with urea supplemented with inhibitors of urease and nitrification, urea with both polymer-coated urea, and supplemented with inhibitors of urease and nitrification (at the normal application rates), and urea with urease double inhibitor (at both application rates) scored high along both PC1 and PC2, corresponding to high values of iron (FE), sulphur (S), aluminium (AL), sodium (NA), and mineralizable N (NMIN).

All treatments with elevated N application rates (120 kg N ha^{-1}), except for urea with urease double inhibitor at the higher application rate (120 kg N ha^{-1}), clustered at lower values along PC2, corresponding to high values of N fixers (NFIX), fungus (FUNG), general bacteria (BACT), the anaerobic *Trichoderma* (TRIC) and *Pseudomonas* populations (PSEU), total bacteria (TBAC), gram-negative bacteria (Grne), and *Rhizobium* related bacteria (RHIZ) (Figure 7d). None of these patterns occurred in post-treatment or mid-season soil samples (Figures 7b-c). Conversely, treatments of urea with polymer-coated urea and urea with urea supplemented with inhibitors of urease and nitrification at normal application rates scored high along PC1, corresponding to high values of phosphorus - Bray-P1 (PBIC), active carbon (CREC), estimated nitrogen release (ENR), phosphorus - bicarbonates (PBI), potassium (K), sodium (NA), N-fixing bacteria (NFIX), and mineralizable N (NMIN) in mid-season and pre-harvest, but not in the early post-treatment samples.

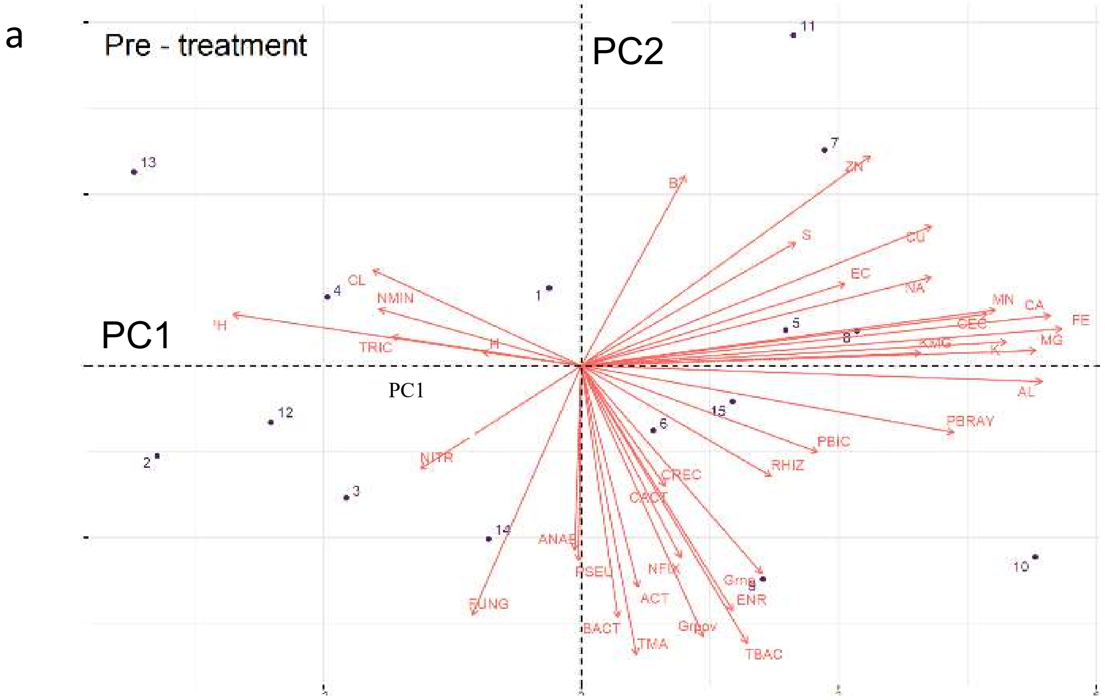

Figure 8: Principal components analysis (PCA) of soil chemical and biological characteristics at four sampling periods (a-d). For symbol definitions, see Table 4 (continued on following pages).

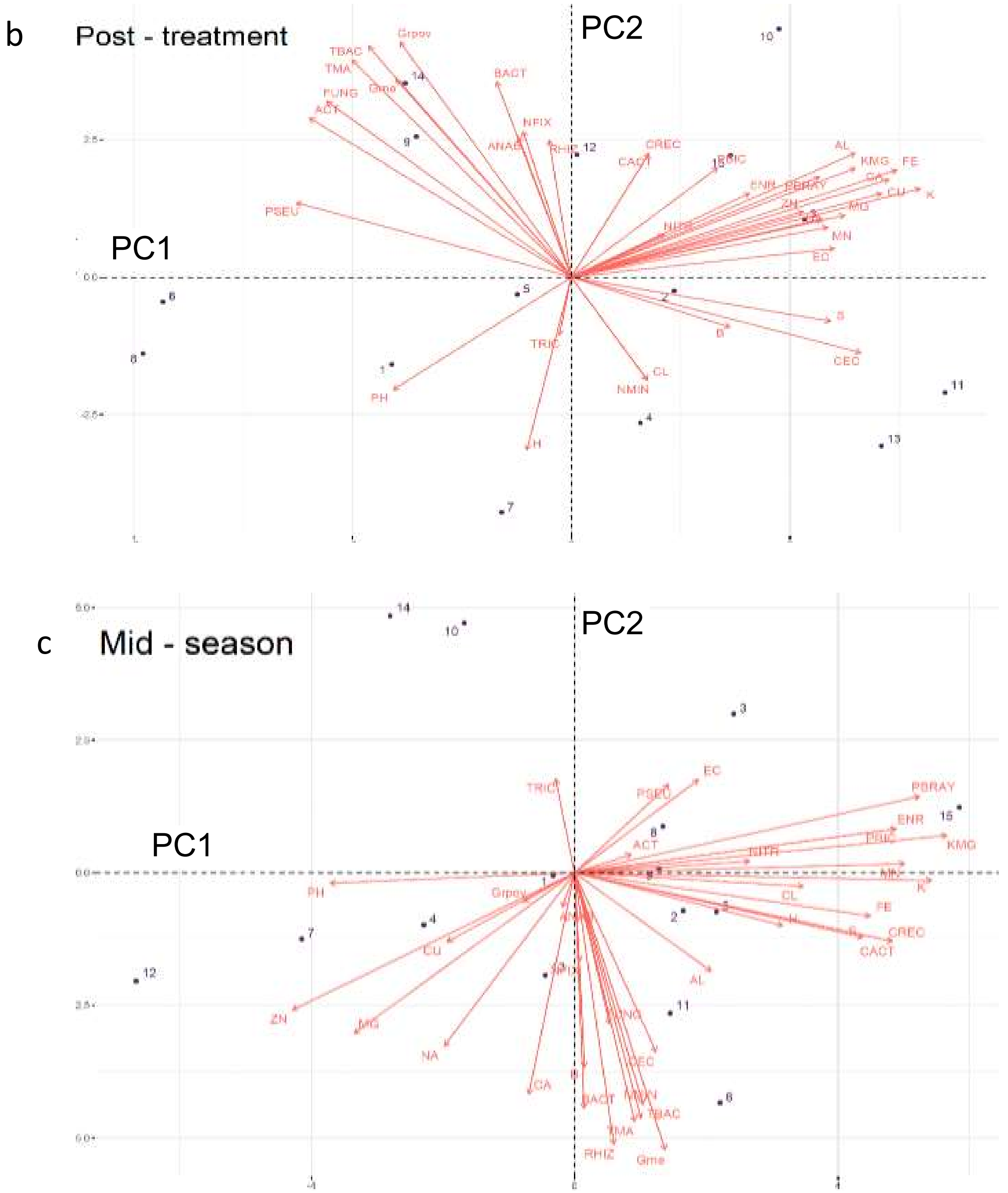

Figure 7 (continued). Principal components analysis (PCA) of soil chemical and biological characteristics at four sampling periods (a-d). For symbol definitions, see Table 4.

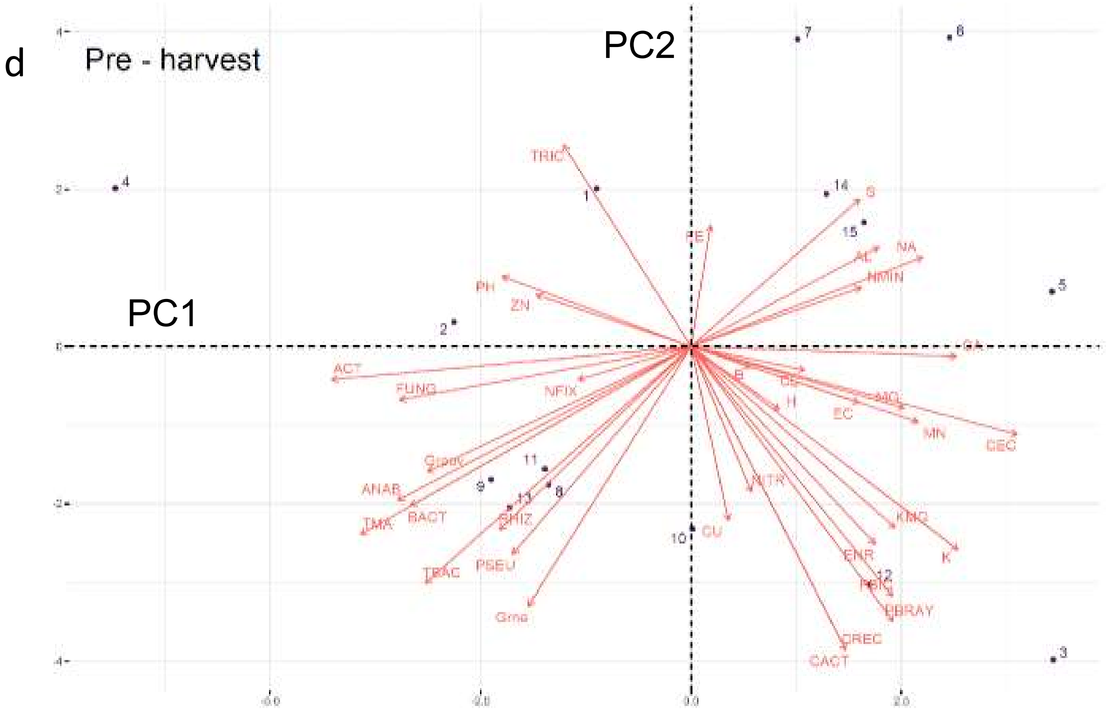

Figure 7 (continued). Principal components analysis (PCA) of soil chemical and biological characteristics at four sampling periods (a-d). For symbol definitions, see Table 4.

2. Soil heath

Background variation in soil health metrics among the soil samples taken before treatment application was due to estimated N release, total gram negatives associated organic matter, total gram negatives associated pH, overall microbial sustainability, and fungus-associated CEC, collectively contributing to 35.6% of explained variation (along PC1), and rhizobium related associated boron, PMN and dissolved CO_2^-- carbon, total gram negatives-associated CEC, total gram negative bacteria associated with the ratio of K to Mg, and percent organic matter, collectively contributing to 26.4% of explained variation (along PC2; Figure 8a). Pre-harvest, treatments scoring highest along PC1 were urea with polymer-coated urea, and urea with urea supplemented with inhibitors of urease and nitrification at the elevated application rate – 120 kg N ha^{-1}, and urea with urease double inhibitor at the normal application rate – 80 kg N ha^{-1} (Figure 8d), corresponding to high values of percent organic matter (SOM), the *Pseudomonas* population associated with percent phosphorous (PERPSH1), total gram negative bacteria associated with pH (PHSH2), ratio of fungi: bacteria associated with organic matter (OMSH5), total gram negative bacteria associated with ratio of K to Mg (KMGSH2), and gram positive bacteria associated with soil health index (SH3). In contrast, polymer coated urea, urea with urea supplemented with inhibitors of urease and nitrification, and the triple combination of urea with polymer-coated urea, urea supplemented with inhibitors of urease, and nitrification at the normal application rate scored lowest along PC1. Treatments with urea at the normal application rate, polymer-coated urea at the elevated application rate, and urea supplemented with inhibitors of urease and nitrification at both the application rates all scored high along both PC1 and PC2, corresponding to high values of total gram negatives-associated with CEC (CECSH2), general fertility index (GFI), and the ratio of fungi to bacteria associated with pH (PHSH5). Treatments with urea alone, polymer coated urea and urea with polymer coated urea at the normal N application rates clustered at lower values along PC2, corresponding to high values of, total microbial activity associated with CEC (CECTMA), total gram negatives associated with soil health index (SH2), the ratio of gram positive to gram negative bacteria associated with soil health index (SH4), the total gram negatives associated with CEC (CECSH2). None of these patterns occurred in post-treatment or mid-season soil samples (Figures 8b-c). For normal application rates, variation in soil health did not show any specific patterns, similar to what was observed with the chemical and biological data.

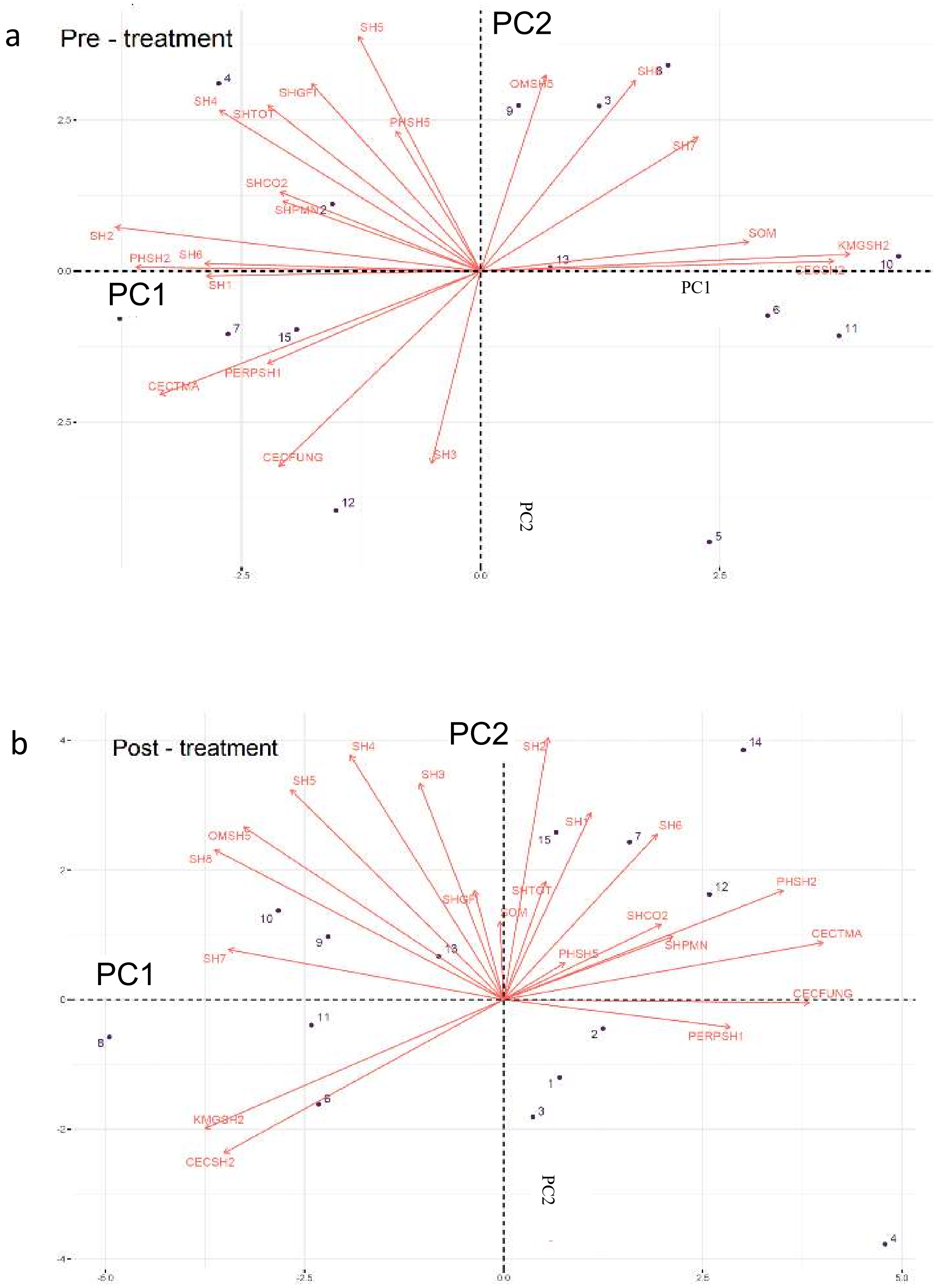

Figure 9. PCA of soil health metrics at four sampling periods (a-d). For symbol definitions, see Table 4.

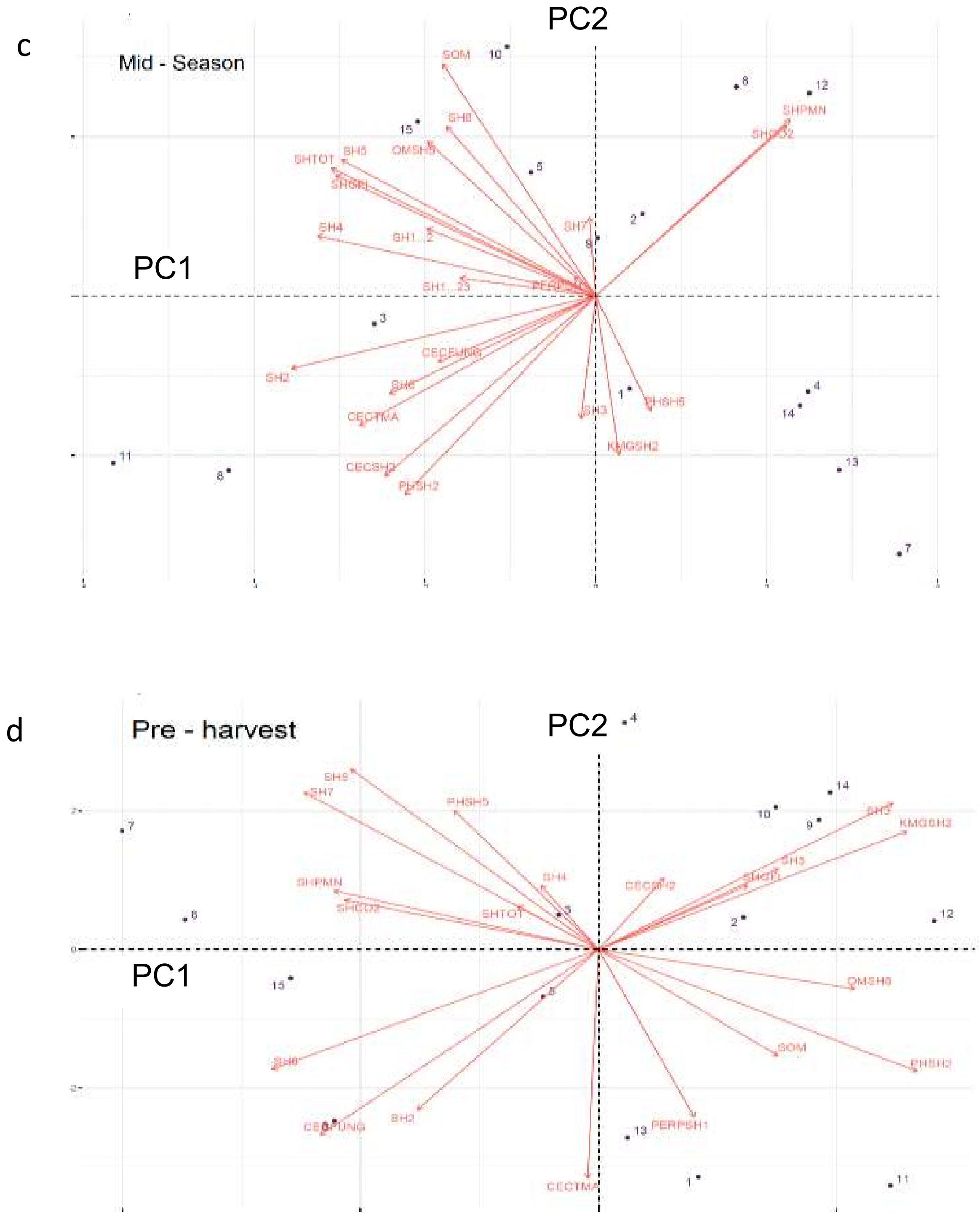

Figure 8 (continued). PCA of soil health metrics at four sampling periods (a-d). For symbol definitions, see Table 4.

Soil NO_3^- N and NH_4^+ N concentration was not different by N sources and did not differ from no N at post-treatment and pre-harvest sampling stages (Figure 9; Table 5).

3. Plant analyses

Different N application rates and sources demonstrated no distinct impact on plant count and tiller numbers. Similarly, variation in N application rates and sources did not affect plant height. The weight and length of the spike also remained unaffected by differing N rates and sources. Furthermore, N rates or sources did not induce alterations in the harvest index; however, a decline in the harvest index was observed across all N sources relative to the reference plot (Table 6).

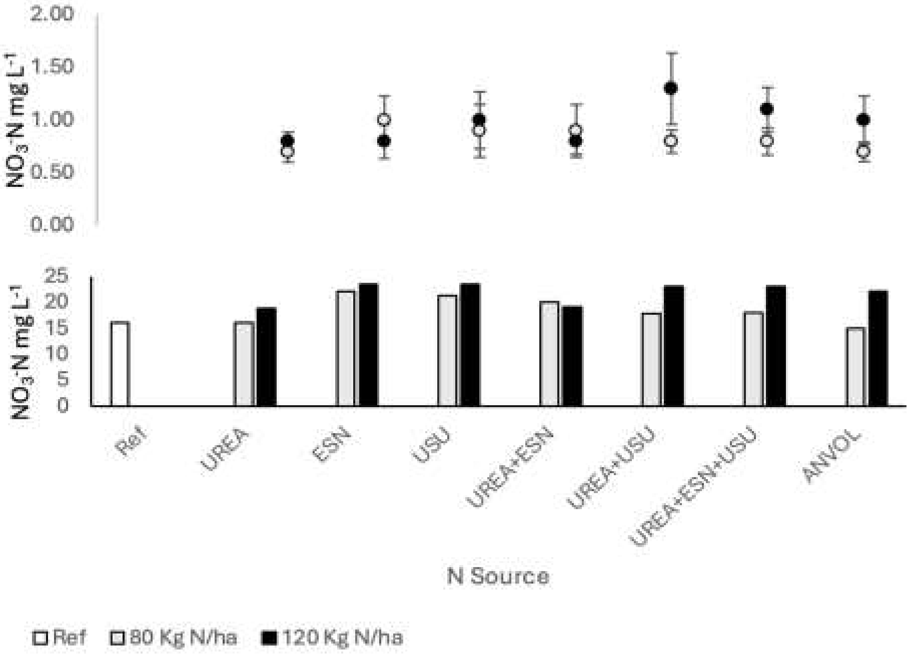

Figure 10. Nitrate N concentration in soil at pre-harvest sampling stage comparing treatments to reference plots. Upper symbols are mean ratio of treated plots to no N, with bars representing standard error. Lower bars are mean absolute values of yield for the four replicate blocks; lighter shading represents the lower application rate (80 kg N ha^{-1}) and darker shading the higher (120 kg N ha^{-1}).

Table 5. Effect of different rates and sources of N on NO_3^- N and NH_4^+N at pre-treatment and pre-harvest stages (Average values over four replicate blocks).

Treatments	Pre-treatment		Pre-harvest	
	NO_3^- N	NH_4^+ N	NO_3^- N	NH_4^+ N
No N	18.75	7.75	16.00	8.75
80 kg N ha^{-1}	20.61	8.85	18.64	10.89
120 kg N ha^{-1}	19.00	9.46	23.04	12.71
Mean	19.73	9.06	20.52	11.60
SE	1.05	0.27	1.11	0.65
N source (NS)				
Urea	18.13	9.00	17.38	9.50
ESN®	18.63	8.38	22.75	13.63
SUPERU™	18.25	9.00	22.38	12.00
Urea + ESN®	18.75	9.13	19.50	10.75
Urea + SUPERU™	27.50	10.00	23.38	12.75
Urea + ESN® + SUPERU™	18.75	9.63	21.50	12.25
Urea + ANVOL™	18.63	9.00	19.00	11.75
Mean	19.73	9.07	20.52	11.60
SE	1.05	0.27	1.11	0.65
Fixed effects (*P*)				
NR	0.42	0.32	0.10	0.21
NS	0.23	0.82	0.83	0.80
NR*NS	0.16	0.29	0.82	0.68

NO_3^- N: Nitrate Nitrogen, NH_4^+ N: Ammoniacal nitrogen, No N: Reference (control), Urea: Conventional N fertilizer, ESN®: Polymer coated slow-release N fertilizer, SUPERU™: Urea supplemented with urease and nitrification inhibitors, ANVOL™: dual action urease inhibitor (NBPT and Duramide) treated urea, SE: Standard error, NR: Nitrogen rate, NS: Nitrogen source.

Grain per kg nutrients (partial factor productivity of nitrogen) displayed a significant decline with increasing nitrogen rates relative to the reference plot. The highest grain per kg nutrients decline was observed with 120 kg N ha^{-1} that is 22 kg kg^{-1}, whereas the reference plot recorded the highest grain per kg nutrients, that is 98.0 kg kg^{-1}. However, there were no significant effects observed with different N sources or their interaction with N rates on grain per kg nutrients. Grain yield, thousand kernel weight and test weight remained unchanged

Table 6. Effect of different rates and sources of N on plant count, number of tillers, plant height, spike weight, spike length, and harvest index. Average value represents four replicate blocks.

Treatment	Plant count (m^2)	Number of tillers (m^2)	Height (cm)	Spike weight (g)	Spike length (g)	Harvest index (%)
N application rate (NR)						
No N	329	620	79	1.29	14.8	49.2
80 kg N ha^{-1}	333	584	76	1.35	14.9	50.5
120 kg N ha^{-1}	294	580	76	1.36	15.0	44.8
Mean	319	595	77	1.35	14.9	48.2
SE	9	13	1	0.02	0.0	1.2
N source (NS)						
Urea	301	560	77	1.42	15.0	48.5
ESN®	338	595	75	1.33	15.0	46.7
SUPERU™	323	601	77	1.36	14.8	48.7
Urea + ESN®	301	567	74	1.40	15.0	49.1
Urea + SUPERU™	311	580	77	1.42	15.0	48.2
Urea + ESN® + SUPERU™	326	603	76	1.28	14.8	45.3
Urea + ANVOL™	297	568	77	1.30	14.8	47.2
Mean	314	582	76	1.36	14.9	47.7
SE	9.43	12.7	1	0.02	0.0	1.2
Fixed effects (*P*)						
NR	0.08	0.87	0.59	0.89	0.06	0.07
NS	0.94	0.94	0.89	0.34	0.20	0.98
NR*NS	0.98	0.05	0.19	0.58	0.10	0.50

No N: Reference (control), Urea: Conventional N fertilizer, ESN®: Polymer coated slow-release N fertilizer, SUPERU™: Urea supplemented with urease and nitrification inhibitors, ANVOL™: dual action urease inhibitor (NBPT and Duramide) treated urea, SE: Standard error, NR: Nitrogen rate, NS: Nitrogen source.

with different N sources in comparison to the reference plot (Table 7). Grain yields were no higher in any N-treated plots compared to the no-N reference plots (Figure 10). There were no notable alterations observed in straw and biomass yields (Table 7) with the treatments. Grain per kg nutrients, thousand kernel weight, test weight, grain, straw, and biomass yields were not influenced by N rates or N sources (Table 7). Plant assimilation of NO_3^- N was enhanced by the N fertilizer @ 80 kg N ha^{-1} as compared to the reference plots, at three times the concentration during booting and two times during tillering stages (Table 8, Figures 11-

12). However, there were no differences at the higher application rate (120 kg N ha^{-1}) consistently observed across the N sources.

Table 7. Effect of different N rates and sources on grain per kg nutrients, grain, straw, and biomass yields and grain weight (Average values over four replicate blocks).

		Yield (Mg ha^{-1})				
Treatment	Grain per kg nutrients (kg kg^{-1})	Grain	Straw	Biomass	1000 K weight (g)	Test weight (kg hl^{-1})
N application rate (NR)						
No N	98.0	3.42	4.65	6.93	40.0	82.0
80 kg N ha^{-1}	31.0	3.62	4.80	7.22	39.0	75.0
120 kg N ha^{-1}	22.0	3.43	5.46	7.75	39.0	77.0
Mean	50.3	3.49	4.97	7.30	39.0	78.0
SE	2.51	0.09	0.14	0.15	0.18	0.29
N source (NS)						
Urea	28.9	3.80	5.34	7.88	41.0	79.0
ESN$^{®}$	27.5	3.53	5.36	7.72	39.0	75.0
SUPERU^{TM}	27.0	3.55	4.97	7.35	41.0	76.0
Urea + ESN$^{®}$	27.3	3.66	4.97	7.42	40	74.0
Urea + SUPERU^{TM}	27.9	3.69	5.37	7.83	38	75.0
Urea + ESN$^{®}$ + SUPERU^{TM}	25.4	3.36	5.47	7.72	39	78.0
Urea + ANVOL^{TM}	23.5	3.07	4.97	6.46	40	77.0
Mean	26.5	3.52	5.21	7.48	40	76.0
SE	2.51	0.09	0.14	0.14	0.18	0.29
Fixed effects (*P*)						
NR	<0.001	0.357	0.04	0.09		
NS	0.34	0.383	0.49	0.22		
NS*NR	0.14	0.189	0.06	0.50		

No N: Reference (control), Urea: Conventional N fertilizer, ESN$^{®}$: Polymer coated slow-release N fertilizer, SUPERU^{TM}: Urea supplemented with urease and nitrification inhibitors, ANVOL^{TM}: dual action urease inhibitor (NBPT and Duramide) treated urea, SE: Standard error, NR: Nitrogen rate, NS: Nitrogen source.

Table 8. Effect of different N sources and application rates on N content in plant tissue at different wheat growth stages. Average values over four replicate blocks.

Treatment	Wheat growth stage			
	Tillering	Booting	Hard dough	Maturity
N application rate (NR)				
No N	0.05	0.05	0.01	0.00
80 kg N ha^{-1}	0.11	0.11	0.02	0.00
120 kg N ha^{-1}	0.12	0.11	0.03	0.00
Mean	0.11	0.11	0.03	0.00
SE	0.00	0.02	0.00	0.00
Source of N (NS)				
Urea	0.12	0.11	0.41	0.00
ESN®	0.10	0.22	0.02	0.00
SUPERU™	0.09	0.07	0.03	0.00
Urea + ESN®	0.10	0.09	0.03	0.00
Urea + SUPERU™	0.13	0.10	0.02	0.00
Urea + ESN® + SUPERU™	0.13	0.09	0.02	0.00
Urea + ANVOL™	0.12	0.09	0.03	0.00
Mean	0.11	0.11	0.03	0.00
SE	0.00	0.02	0.00	0.00
Fixed effects				
NS	0.10	0.22	0.39	0.62
NR	0.48	0.90	0.04	0.29
NS*NR	0.65	0.36	0.98	0.51

No N: Reference (control), Urea: Conventional N fertilizer, ESN®: Polymer coated slow-release N fertilizer, SUPERU™: Urea supplemented with urease and nitrification inhibitors, ANVOL™: dual action urease inhibitor (NBPT and Duramide) treated urea, SE: Standard error, NR: Nitrogen rate, NS: Nitrogen source.

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Disease incidence of Net Blotch, Take All, BYDV and FHB were also not affected with N sources or the N rates (Table 9).

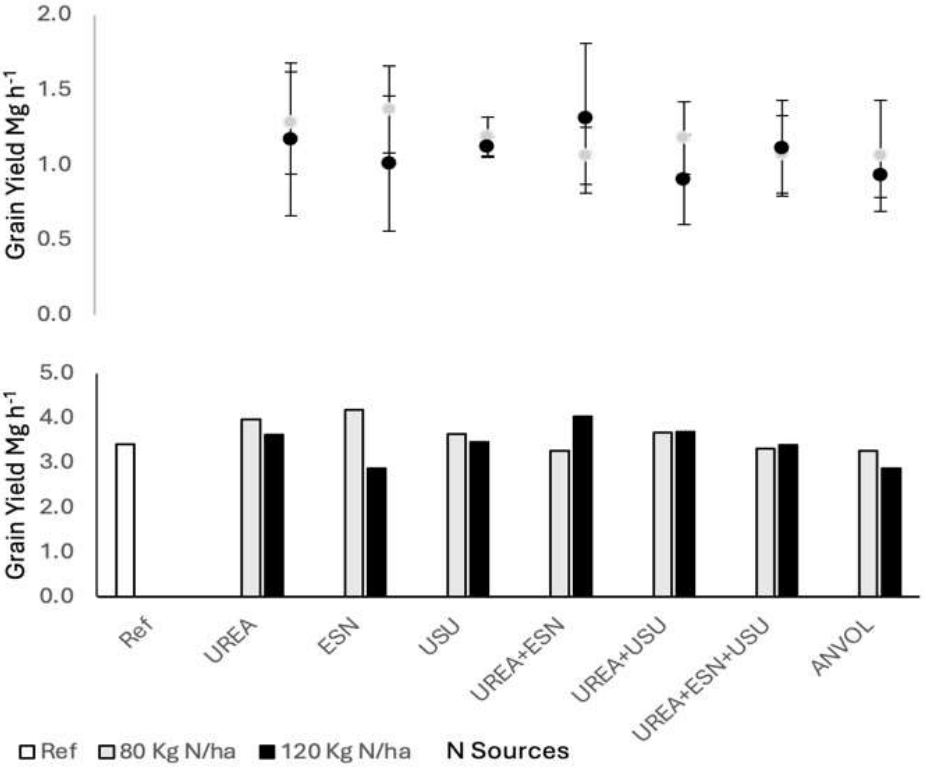

Figure 11. Grain yield comparing treatments to reference plots. Upper symbols are mean ratio of treated plots to no N, with bars representing standard error. Lower bars are mean absolute values of yield for the four replicate blocks; lighter shading represents the lower application rate (80 kg N ha^{-1}) and darker shading the higher (120 kg N ha^{-1}).

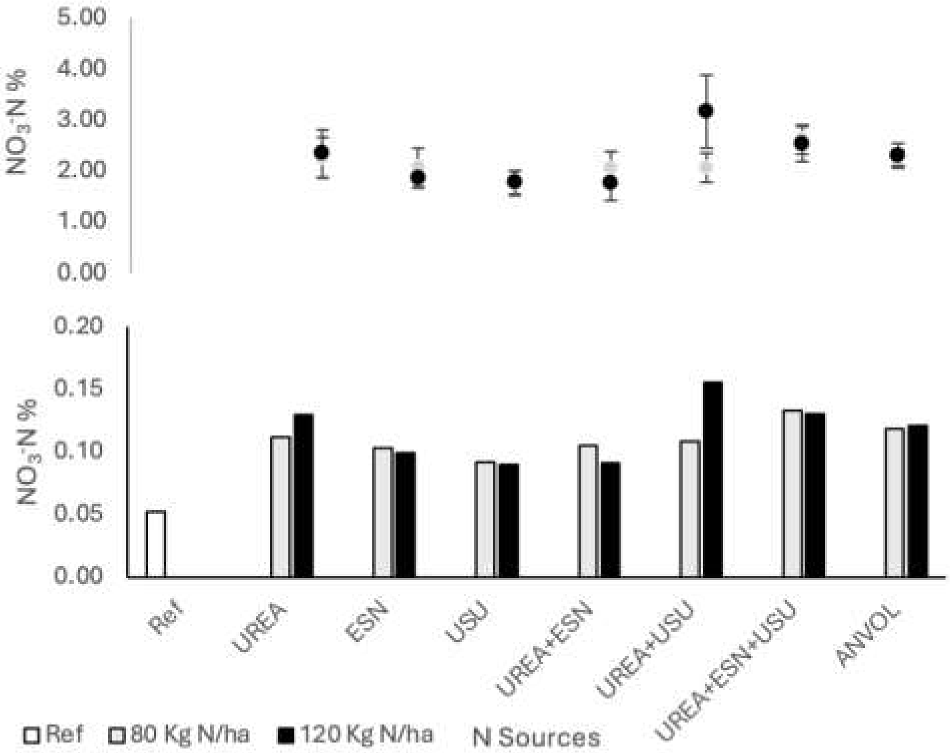

Figure 12. Nitrate N % in whole plant at tillering stage comparing treatments to reference plots. Upper symbols are mean ratio of treated plots to no N, with bars representing standard error. Lower bars are mean absolute values of yield for the four replicate blocks; lighter shading represents the lower application rate (80 kg N ha^{-1}) and darker shading the higher (120 kg N ha^{-1}).

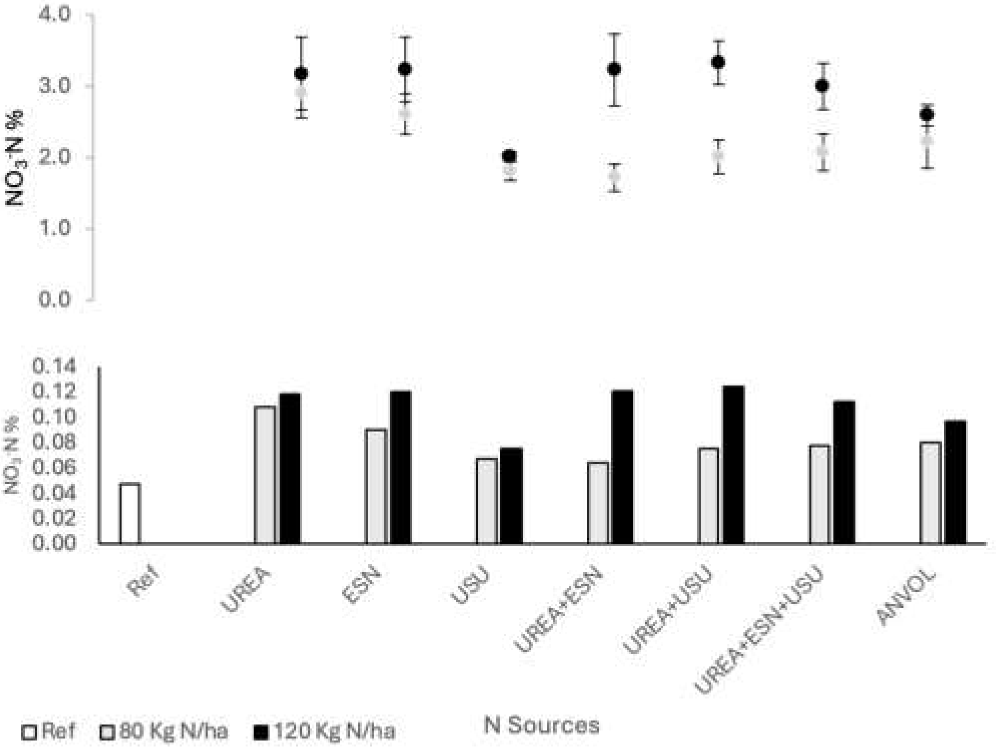

Figure 13. Nitrate N % in whole plant at booting stage comparing treatments to reference plots. Upper symbols are mean ratio of treated plots to no N, with bars representing standard error. Lower bars are mean absolute values of yield for the four replicate blocks; lighter shading represents the lower application rate (80 kg N ha^{-1}) and darker shading the higher (120 kg N ha^{-1}).

Table 9. Effect of N treatment on Net Blotch, Take All, Barley Yellow Dwarf Virus (BYDV), and Fusarium Head Blight (FHB) incidence in spring wheat. Average value represents four replicate blocks.

Treatment	Net Blotch	Take all	BYDV	FHB
No N	1.75	0.00	1.25	0.00
Urea 80 kg N ha^{-1}	1.00	0.00	1.25	0.25
ESN® 80 kg N ha^{-1}	1.00	0.50	1.00	0.25
SUPERU™ 80 kg N ha^{-1}	1.25	0.25	1.50	0.25
Urea 53 kg N ha^{-1} + ESN® 27 kg N ha^{-1}	1.50	0.50	1.50	0.50
Urea 53 kg N ha^{-1} + SUPERU™ 27 kg N ha^{-1}	1.25	0.25	1.25	0.00
Urea 27 kg N ha^{-1} + ESN® 27 kg N ha^{-1} + SUPERU™ 27 kg N ha^{-1}	1.25	0.25	1.00	0.25
Urea 120 kg N ha^{-1}	1.00	0.50	1.00	0.25
ESN® 120 kg N ha^{-1}	1.25	0.75	1.25	0.00
SUPERU™ 120 kg N ha^{-1}	1.00	0.00	1.00	0.25
Urea 80 kg N ha^{-1} + ESN® 40 kg N ha^{-1}	0.75	0.50	1.00	0.50
Urea 80 kg N ha^{-1} + SUPERU™ 40 kg N ha^{-1}	0.75	0.25	0.75	0.50
Urea 40 kg N ha^{-1} + ESN® 40 kg N ha^{-1} + SUPERU™ 40 kg N ha^{-1}	1.00	0.50	1.25	0.75
Urea treated with ANVOL™ 80 kg N ha^{-1}	1.25	0.00	1.25	0.25
Urea treated with ANVOL™ 120 kg N ha^{-1}	1.50	0.25	1.25	0.00
Mean	1.17	0.30	1.17	0.27
$P > F$	0.19	0.73	0.32	0.32
SE	0.06	0.08	0.05	0.06

No N: Reference (control), Urea: Conventional N fertilizer, ESN®: Polymer coated slow-release N fertilizer, SUPERU™: Urea supplemented with urease and nitrification inhibitors, ANVOL™: dual action urease inhibitor (NBPT and Duramide) treated urea, SE: Standard error, NR: Nitrogen rate, NS: Nitrogen source.

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Discussion

Adding nitrogen (N) enhances tillering and promotes photosynthesis and leaf growth by stimulating the production of proteins essential for cell growth, cell division, and the synthesis of cell wall and cytoskeleton components in wheat grain (Haderlein et al., 2001; Sun et al., 2017). The role of N is crucial due to its impact on several physiological and developmental processes of the plant. According to Schils et al. (2018), N is a limiting factor in the yield and production of spring wheat and is extensively employed as a fertilizer. This study investigated the effect of various of N sources at two application rates (80 and 120 kg N ha^{-1}) on plant growth, field productivity, chemical, biological and soil health metrics in the cultivation of spring wheat (CV AAC Wheatland) at the Lakehead University Agricultural Research Station (LUARS), Thunder Bay. At LUARS, changes in phenotypic and agronomic traits of wheat were not observed with different N sources or application rates as compared to the reference plots with no N.

The findings at LUARS could be attributed to high soil N mineralization and presence of residual N that may obscure the expected effects, as well as limited rainfall during the growing season that may not have released N from the polymer-coated slow-release application. With low rainfall, conventional N fertilizers will not suffer the same leaching losses, resulting in no advantage to enhanced efficiency fertilizers, for which effectiveness will be similarly hindered by low moisture available for adequate nutrient absorption. This discussion highlights the complex relationship between environmental factors and the efficacy of fertilizers in general. A recent study conducted in the Canadian Prairies found no advantage in using a urease inhibitor, either alone or in combination with a nitrification inhibitor, on wheat grain production and N use efficiency when there was below-average rainfall throughout the growing season (Lasisi et al., 2022). However, Karamanos et al. (2004) observed that the use of a seed-placed urease inhibitor resulted in higher plant density compared to the use of urea in various crops systems. At the same time, the use of the urease inhibitor, a dual inhibitor, and a polymer-coated urea resulted in an increase in plant count, but a decrease in the number of heads per plant compared to the use of urea alone.

In the LUARS study reported here, there were no discernible variations in plant count, spike weights or spike lengths with different N sources and application rates compared to the reference (no N) plots. Nitrogen availability is particularly important during the tillering and stem elongation stages, when crops have a higher capacity to absorb a larger quantity of nutrients (Arduini et al., 2009; Pampana et al., 2013). Inadequate levels of N at these stages can result in elevated shoot mortality, reduced spike size, and consequently a restricted number of kernels produced per unit area (Mahboob et al., 2023). In the LUARS study, N sources alone or in combination also showed no differences in effects on straw and biomass yields of spring wheat. The lack of variation in these measures may be due to the crop's optimal N requirement and rapid growth cycle, N use efficiency, the soil characteristics at the experimental station (a reasonably good pre seeding levels of 19.73 mg L^{-1} NO_3^- N and 9.07 mg L^{-1} NH_4^+ N), and other environmental conditions such as low rainfall, all of which can restrict the benefits of additional N in promoting biomass production (Ranjan et al., 2023). Plants have physiological constraints in effectively processing N for photosynthesis, protein synthesis, and cell development (Ye et al., 2022). Elsewhere, N inhibitors such as NBPT, dicyandiamide, and Nitrapyrin have been found to effectively enhance the growth, biomass, and N use efficiency (Ábalos et al., 2014). Analysis of the spring wheat data collected at LUARS indicated that adjusted grain, grain yield, thousand kernel weight, and grain per kg nutrients (partial factor productivity) did not differ with the N treatments.

The outcome at LUARS matches the established fact that the impact of N treatments on yield and protein concentration of spring and winter wheat is highly influenced by moisture and temperature (Campbell et al., 1988; Gan et al., 2000). A recent study conducted in Manitoba revealed that the use of inhibitors or stabilizers did not have a significant effect on grain yield in small grains like wheat (Lasisi et al., 2020; McKenzie et al., 2010; Mohammed et al., 2016; Tao et al., 2021; Thilakarathna et al., 2020). In this case, the residual N in the soil, combined with natural N mineralization processes, likely provided enough N to optimize grain yield with no N addition. As a result, any potential agronomic benefits from using inhibitors or double inhibitors were not apparent. However, this interpretation contradicts more recent outcomes in studies conducted by Beres et al. (2018), Fast et al. (2023) and Wang et al. (2023, 2023b), where an increase in grain production in winter wheat occurred when exposed to the same dual inhibitor used in this study (SUPERU^{TM}). Winter wheat has much longer duration than the spring wheat; hence the two varieties may respond differently to N sources and application rates. A worldwide study conducted by Thapa et al. (2016) revealed that the application of a nitrification inhibitor and dual inhibitor increased wheat grain yield, while no improvements occurred with the application of polymer-coated urea. However, also in contrast to the LUARS study, Ghafoor et al. (2021) reported that sulphur-coated slow-release N fertilizer increased chlorophyll content, photosynthesis rate, and grain yield of wheat at a higher N application (130 kg N ha^{-1}).

The balance and availability of nitrate and ammonium in the soil are essential for the overall health and vigour of plants during various stages of growth. However, the behaviour of fertilizers differs greatly under a typical weather condition. In the current study, NO_3^- N in plant tissue doubled during the tillering and hard dough stages, and nearly tripled during the booting stage with higher rate of N, regardless of the source. However, the increase was statistically non-significant due to non-uniformity along the sources. The non uniformity along the N sources on N uptake may be attributed to various factors, including soil type, pH, organic matter, and the biodegradation of nitrification and urease inhibitors, which could have diminished the effectiveness of urea treated with N stabilizers/inhibitors (Fisk et al., 2015;

Shi et al., 2016; Yan et al., 2012). For example, because DCD (the nitrification inhibitor in SuperU^{TM}) has high hydrophilicity and mobility, it may be able to separate itself more spatially from $NH4^+$ point sources (Li et al., 2017) and nitrifying microorganisms (Ruser & Schulz, 2015). This particular outcome of the LUARS study emphasized the importance of adjusting fertilizer strategies to match the existing environmental conditions and achieve the goal of improving nutrient management and promoting agricultural sustainability. Similarly, Wood et al. (2024) observed low soil NO_3^- for both fall and spring applications immediately following application, which gradually increased in May due to increase in soil water content, temperatures, and N_2O fluxes.

At the LUARS, N accumulation in soils was higher with the higher N application rate, as reported earlier by Zheng et al. (2020) and Pin et al. (2021b). The proportion of NH_4^+ N to N in soil is influenced by the presence of favourable conditions for nitrification, which is further affected by pH levels and anaerobic conditions (Barber, 1984). In contrast, higher NH_4^+ N soil content was observed in spring wheat with UAN treatments (0-30 cm depth; Torabian et al., 2023). Similarly, UAN combined with urease or nitrification inhibitor in summer wheat significantly improved the NO_3^--N and NH_4^--N (0-20 cm depth) with reduced leaching losses of NO_3^- N (Ren et al., 2023). Fu et al. (2020) observed increase in ammonia-oxidizing bacteria in response to urea and urea supplemented with urease inhibitor, whereas the activity was low when nitrification inhibitor was present. Similarly, Wood et al. (2024) reported that nitrification inhibitors along with urease inhibitor (SuperU^{TM}) effectively curtailed N_2O emissions by 37-57%, while maintaining wheat productivity. Ammoniacal N (NH_4^+N) can be lost to volatilization, while nitrate N can be lost through leaching, both of which pose challenges in field conditions (Wang et al., 2015). The LUARS study, like past studies, highlights the significance of aligning fertilizer programs with existing environmental circumstances to maximize nutrient management and ensure environmental sustainability.

In the LUARS study with spring wheat, Net Blotch, Take All, Barley Yellow Dwarf Virus (BYDV) and Fusarium Head Blight (FHB) occurrence was not affected by various N sources and application rates. Net Blotch, a notable wheat disease, is caused by the pathogen *Bipolaris sorokiniana* and can be affected by N management (Roy et al., 2023). BYDV, a viral pathogen affecting cereal crops such as wheat, can be indirectly affected by N levels. Elevated N levels can stimulate the development of wheat, potentially increasing the appeal of the plants to aphids, the main carriers of BYDV. However, the nutritional condition of the plant can influence the intensity of disease symptoms and the overall well-being of the crop. Sufficient fertilization may result in enhanced tolerance to virus infections. Research has indicated inconsistent findings: certain studies observed that higher N levels can worsen the severity of FHB due to the promotion of fungal development caused by abundant vegetative growth (Matic et al., 2022). Two investigations indicated that N supply can result in increases in both FHB occurrence and mycotoxin concentrations (Lemmens et al., 2004; Heier et al., 2005). This effect is due to increased N supplies that can cause luxuriant plant growth, a microenvironment that is favourable to pathogen development, and changes in plant metabolism that lessen disease resistance, all of which can lead to an increase in FHB incidence and mycotoxin concentrations (Chami et al., 2022). The impact of N on FHB can also be influenced by additional factors, including the susceptibility of the cultivar, climatic circumstances, and the timing of N application (Matic et al., 2022). Another study indicated that FHB was more severe when there was a partial N deficit (reducing the N application rate to 50% of the full requirement; Chai et al., 2021). FHB infection can be more severe under N-deficient conditions due to weakened plant defences and altered physiological states that favour pathogen infection (Xu et al., 2022).

In the LUARS investigation, just at the pre-harvest stage, treatments with polymer-coated urea, urea with polymer-coated urea, and urea with urea supplemented with inhibitors of urease and nitrification corresponded to increased levels of phosphorus, active carbon, extractable N, and various cations, indicating higher soil fertility and microbial activity with these fertilizers (see Appendix, Tables 10 and 11). The polymer-coated urea along with inhibitors activity might have provided a slow and controlled release of N, fostering a more active and diverse microbial community resulting in greater microbial biomass enhancing organic matter decomposition leading to nutrient cycling (Zaman et al. 2009). Active carbon, indicative of organic matter decomposition and nutrient cycling, plays a crucial role in maintaining soil structure, moisture retention, and microbial habitat (Mann, 1986). Nitrogen application enhances the availability of phosphorus and potassium by improving root growth and the plant's ability to take up nutrients (Grunes, 1959). In contrast, at LUARS, treatment with urea alone resulted in apparently poorer soil conditions. Such conditions are possibly attributed to imbalanced soil microbial communities, which hinder nutrient cycling and organic matter decomposition (Lehmann and Kleber, 2015).

Treatments of urea in combination with urea supplemented with inhibitors of urease and nitrification, polymer-coated urea, and urea supplemented with inhibitors of urease and nitrification, urea with both polymer-coated urea at normal application rate and urease double inhibitor at both application rates were associated with favourable soil conditions with high quantities of important nutrients such as iron, sulphur, aluminium, and sodium, as well as significant mineralizable N. Thus, N treatment with a normal application rate may enhance the availability of other nutrients associated with crop productivity and suggests a unique microbial community composition with elevated levels of N-fixing bacteria, fungi, general bacteria, anaerobic *Trichoderma* and *Pseudomonas* populations (see Appendix). These patterns were not present in soil samples taken just after application of treatments or during the middle of the season, indicating a delayed impact of N injection on microbial communities.

Treatments with polymer-coated urea and urea in combination with urea supplemented with inhibitors of urease and nitrification with normal application rates (80 kg N ha^{-1}), corresponded to substantial amounts of phosphorus, active carbon, extractable N, potassium, sodium, N-fixing bacteria, and mineralizable N in mid-season and pre-harvest samples (see Appendix). In a previous study, N fertilizer application led to an increase in net N mineralization, net nitrification, and microbial NUE, while decreasing microbial respiration (Jiang et al., 2023). In the soil heath measures at LUARS, Pre-harvest, soils scoring highest along PC1 were from plots treated with urea with polymer-coated urea, urea with urea supplemented with inhibitors of urease and nitrification at the elevated application rate, and urea with urease double inhibitor at the normal application rate. These treatments may have corresponded to increased levels of organic matter, ratio of fungi to bacteria, associated organic matter, total gram negative bacteria associated with potassium and magnesium, gram-positive bacteria, and cation exchange capacity, which were observed in some of the N treatments in the LUARS study (see Appendix). This interpretation matches that of another study in which optimal soil conditions for microbial activity and nutrient availability come with N fertilizers (Bardgett and van der Putten, 2014). Bacteria and fungi play a crucial role as the main consumers in the process of decomposition, determining whether the decomposition pathways are driven by bacteria or fungi for energy production (Wang et al., 2019). However, the ratios of fungus to bacteria, determined by measuring microbial biomass, respiration, or growth, only provide a brief overview of the energy flow within a certain period, rather than giving a complete understanding of the total contributions across time.

The results at LUARS emphasize the intricate relationship between soil characteristics and microbial communities, underscoring the significance of comprehensive soil management strategies in preserving soil health and production. Hao et al. (2019) observed a similar correlation between introduction of N and the structure and diversity of the soil microbial community, which in turn can alter the inherent physical and chemical characteristics of the soil. At LUARS, treatments with urea alone, polymer-coated urea and urea with polymer-coated urea at the normal N application rates corresponded to high values of, total microbial activity, total gram-negative bacteria, the ratio of gram positive to gram negative bacteria, the General Fertility Index, and total gram-negative bacteria associated with CEC. These patterns were not observed from soil samples taken just post treatment or during the middle of the growing season, suggesting again a delayed impact of increased N application on soil microbial activity (Hirsch et al., 2017). Normal N application rates may not cause substantial changes to soil microbial activity or community composition when compared to treatments with higher N application rates in the short term (Marschner et al., 2011). Heightened microbial activity associated with increased N application rates have demonstrated the stimulatory effect of N fertilization on soil microbial communities, particularly in terms of increased enzymatic activities and N cycling processes (Fierer et al., 2012). At a minimum, the findings of the LUARS study indicate that N fertilizers influence the soil microbial community. Hence, long-term studies are required to determine the effectiveness of nitrogen sources and their application rates on plant growth, productivity, chemical, biological and soil health metrics in spring wheat.

Conclusion

Nitrogen (N) is an important macronutrient essential for increasing wheat production. Therefore, enhancing nitrogen recovery efficiency (NRE) and minimizing N losses through the use of urease inhibitors (UI) and nitrification inhibitors (NI) is vital across numerous agroecosystems. The present investigation observed no effect of N sources and application rates on the phenotypic and agronomic traits of wheat. However, NO_3^- N levels in plant tissue doubled during the tillering and hard dough stages and nearly tripled during the booting stage with N application, regardless of the N source. The LUARS study also found no effect of different N sources and N rates on wheat growth, grain yield, and 1000 kernel weight. The impact of the inhibitors was likely obscured by excessive soil mineralization and leftover N fertilizer from the previous cropping season. Additionally, restricted rainfall during the growing season highlighted the complex relationship between environmental factors and fertilizer impact. Under suboptimal precipitation conditions, both conventional N fertilizer and enhanced efficiency N fertilizers performed similarly, likely due to limited leaching from conventional urea, reducing the expected benefits of enhanced formulations. Soil health parameters showed minimal variation across different N treatments, with delayed soil microbial activity responses to elevated N application rates. Polymer-coated urea at elevated rates, and urea supplemented with urease and nitrification inhibitors (SUPERU^{TM}) at both rates improved soil conditions, enhancing essential nutrient levels and microbial populations. These findings underscore the complex interplay between N fertilization, environmental factors, soil health, and crop production, highlighting the necessity for N management strategies that adapt to current environmental conditions, maximize nutrient absorption, improve soil quality, and support sustainable farming practices.

Long term field investigations are required to evaluate the effects of enhanced efficiency N fertilizers on the growth, grain yield, and 1000 kernel weight of a variety of crops growing on silty clay loam soil. The observed inconsistency in N uptake among urea treatments with N stabilizers/inhibitors and conventional fertilizer applications warrants further investigation into the roles of soil pH, soil type, soil temperature, soil organic matter, and the mechanisms of conventional N fertilizer treated with N stabilizers/inhibitors in reducing N losses during crop production in silty clay loam soil.

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Appendix

Table 10. Correlation scores for the chemical and biological characteristics of soil in pre-treatment, post-treatment, mid-season, and post-harvest periods with principal components from four analyses one per period.

	Pre-treatment		**Post-treatment**		**Mid-season**		**Post-harvest**	
Characteristics	**PC1**	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**
Pseudomonas population	-0.01	0.59	-0.71	0.26	0.24	-0.28	-0.44	0.54
Nitrogen-fixing bacteria	0.20	0.58	-0.13	0.50	0.01	0.28	-0.28	0.09
Rhizobium related fungi	0.38	0.33	-0.06	0.47	0.10	0.86	-0.47	0.48
Gram positive bacteria	0.25	0.82	-0.44	0.81	-0.13	0.09	-0.65	0.33
Actinomycetes	0.12	0.67	-0.68	0.55	0.14	-0.06	-0.88	0.09
General bacteria	0.07	0.76	-0.19	0.67	0.02	0.74	-0.69	0.42
General fungi	-0.22	0.76	-0.63	0.60	0.09	0.48	-0.71	0.14
Trichoderma	-0.38	-0.09	-0.03	-0.20	-0.05	-0.30	-0.31	-0.53
Anaerobic bacteria	-0.01	0.56	-0.14	0.48	-0.03	0.11	-0.72	0.40
Total gram-negative bacteria	0.36	0.63	-0.45	0.68	0.23	0.87	-0.40	0.68
Total bacteria	0.34	0.84	-0.53	0.79	0.17	0.77	-0.65	0.62
Total microbial activity	0.11	0.88	-0.57	0.75	0.15	0.78	-0.81	0.49
Phosphorous – bicarbonate (mg L^{-1})	0.48	0.26	0.38	0.38	0.82	-0.14	0.49	0.65
Phosphorous – Bray – P1 (mg L^{-1})	0.75	0.20	0.64	0.35	0.88	-0.24	0.50	0.72
Potassium, K (mg L^{-1})	0.86	-0.07	0.90	0.30	0.91	0.02	0.65	0.53
Magnesium, Mg (mg L^{-1})	0.92	-0.05	0.71	0.21	-0.56	0.51	0.52	0.72
Calcium, Ca (mg L^{-1})	0.95	-0.15	0.82	0.34	-0.12	0.70	0.65	0.53
Sodium, Na (mg L^{-1})	0.71	-0.27	0.65	0.20	-0.33	0.55	0.57	0.16
Sulfur, S (mg L^{-1})	0.43	-0.37	0.67	-0.15	0.73	0.20	0.41	0.03
Zinc, Zn (mg L^{-1})	0.58	-0.64	0.60	0.23	-0.72	0.43	-0.38	-0.23
Manganese, Mn (mg L^{-1})	0.84	-0.17	0.66	0.17	0.84	-0.03	0.56	-0.39
Iron, Fe (mg L^{-1})	0.97	-0.11	0.84	0.37	0.75	0.14	0.05	-0.14
Copper, Cu (mg L^{-1})	0.71	-0.42	0.80	0.29	-0.32	0.22	0.09	0.20
Boron, B (mg L^{-1})	0.21	-0.58	0.41	-0.17	0.03	0.61	0.15	-0.32
Aluminum, Al (mg L^{-1})	0.95	0.05	0.74	0.43	0.35	0.31	0.46	0.46
Cation exchange capacity (meq 100 g^{-1})	0.82	-0.16	0.75	-0.26	0.21	0.57	0.80	0.05
K/Mg ratio	0.69	-0.04	0.74	0.38	0.95	-0.12	0.50	-0.26
%H	-0.20	-0.04	-0.12	-0.59	0.53	0.17	0.22	0.23
pH	-0.70	-0.16	-0.46	-0.39	-0.62	0.03	-0.46	0.48
EC (ms cm^{-1})	0.53	-0.25	0.68	0.10	0.31	-0.29	0.41	0.16
Nitrate – N (mg L^{-1})	-0.32	0.31	0.24	0.15	0.45	-0.04	0.15	-0.18
Chloride, CL (mg L^{-1})	-0.42	-0.29	0.20	-0.35	0.58	0.04	0.28	0.15
Reactive C (mg L^{-1})	0.17	0.36	0.20	0.43	0.81	0.22	0.38	0.38
Estimated nitrogen release	0.31	0.74	0.46	0.29	0.82	-0.14	0.45	0.06
Active carbon (mg L^{-1})	0.17	0.36	0.20	0.43	0.81	0.22	0.38	0.79
Mineralizable nitrogen (mg L^{-1})	-0.41	-0.17	0.20	-0.36	0.17	0.73	0.42	-0.16

Estimated N release: Estimated N release refers to the anticipated amount of N becoming available for plant uptake within a specified timeframe based on soil properties, microbial activity, and environmental factors.

Table 11. Correlation scores for the soil health characteristics of soil in pre-treatment, post-treatment, mid-season, and post-harvest periods with principal components from four analyses one per period.

	Pre-treatment		**Post-treatment**		**Mid-season**		**Pre-harvest**	
Characteristics	**PC1**	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**
Pseudomonas population	0.67	-0.02	-0.24	0.63	0.49	0.22	0.86	-0.09
Total gram-negative bacteria (TGN)	0.89	0.17	-0.12	0.88	0.94	-0.24	0.77	-0.28
Gram positive bacteria	0.12	-0.75	0.23	0.73	0.04	-0.41	-0.77	0.06
Ratio gram positive to negative bacteria	0.64	0.62	0.42	0.82	0.88	0.21	0.12	0.24
Fungi to bacteria ratio (fungi:bacteria)	0.30	0.91	0.58	0.71	0.79	0.46	-0.30	-0.02
pH associated with (TGN)	0.84	0.02	-0.77	0.37	0.59	-0.66	-0.37	-0.77
pH associated with fungi:bacteria	0.21	0.54	-0.17	0.12	-0.16	-0.38	-0.15	0.63
Organic material associated with fungi:bacteria	-0.16	0.76	0.71	0.58	0.51	-0.66	-0.18	-0.48
PERP associated with *Pseudomonas* population	0.52	-0.36	-0.62	-0.09	0.04	-0.38	0.05	-0.61
CEC associated with TGN	-0.86	0.04	0.77	-0.52	0.65	0.51	-0.51	0.18
CEC associated with total microbial activity	0.79	-0.48	-0.88	0.19	0.73	0.05	0.50	-0.69
CEC associated with total fungi	0.49	-0.76	-0.84	-0.01	0.51	-0.21	0.81	-0.20
K/Mg ratio associated with TGN	-0.91	0.07	0.82	-0.43	-0.04	-0.52	-0.91	-0.02
B associated with rhizobium-related bacteria	0.68	0.03	-0.42	0.56	0.66	-0.32	0.82	0.06
Overall Biological Index	-0.53	0.52	0.75	0.17	0.03	0.27	0.13	0.87
Overall Microbial Sustainability Index	-0.38	0.74	0.79	0.51	0.48	0.58	0.03	0.87
Organic Matter %	-0.66	0.11	0.01	0.26	0.47	0.78	-0.14	-0.55
General Fertility Index	0.41	0.73	0.08	0.37	0.82	0.41	-0.31	-0.02
Potential mineralizable nitrogen (mgL^{-1})	0.49	0.27	-0.46	0.21	-0.61	0.59	0.35	0.51
Solvita CO_2^- C (mgL^{-1})	0.49	0.31	-0.43	0.25	-0.60	0.57	0.35	0.47
Soil Health Index	0.52	0.64	-0.12	0.40	0.83	0.44	0.12	0.22

General Fertility Index: The General Fertility Index is a composite measure that combines various soil fertility parameters, such as organic matter content, nutrient levels, pH, and texture, to provide an overall assessment of the soil's ability to support plant growth and productivity, Potential Mineralizable N: Potential Mineralizable N refers to the amount of N that soil microbes can convert from organic to inorganic forms, indicating its availability for plant uptake.