Humalite for enhanced wheat and canola production in the Canadian Prairies

by

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Abstract

As the global population approaches 10 billion by 2050, sustainable food production faces unprecedented challenges. Nitrogen (N) fertilizers, crucial for crop yields, contribute significantly to agricultural greenhouse gas emissions and environmental degradation. The 4R Nutrient Stewardship framework (Right source, Right rate, Right time, Right place) aims to optimize fertilizer use efficiency while minimizing environmental impact. In addition, alternatives such as plant biostimulants are advocated to increase plant N uptake and protect them against abiotic stress thereby, making them important for sustainable crop production. One such biostimulant is Humalite, a naturally occurring form of an oxidized coal-like substance containing high levels of humic acid and low amounts of heavy metals. The Prairie Mines and Royalty ULC, Hanna, Alberta holdings (currently WestMet Ag) have large Humalite deposits that are unique due to a higher percentage of humic acid resulting from freshwater deposition. Humalite is known for its ability to improve plant agronomic parameters and increase crop N use. Limited field research exists on the effect of Humalite, application rate, and interaction with urea, especially at reduced rates on grain agronomic parameters. Therefore, a field study was conducted from 2021 to 2023 at three Alberta sites - Battle River Research Group (BRRG), Gateway Research Organization (GRO), and St. Albert Research Station (St Albert), in a split-plot design with four replications, three urea levels (i.e. recommended, half recommend, and zero urea) combined with five Humalite rates (0, 56 (or 112), 224, 448, and 896 kg ha^{-1}). In 2021, the highest wheat yields were observed at half urea rates plus 224 kg ha^{-1} of Humalite at BRRG (35% yield increase), at GRO (8.4% yield increase); and at St Albert (33.5% yield increase). In 2022, canola yields were unaffected by Humalite application rates. In 2023, wheat yields from half-recommended and recommended urea rates plots outperformed zero urea plots across all sites, regardless of Humalite rates. The highest wheat grain protein content values were observed at 224 - 448 kg ha^{-1} of Humalite plus half or recommended urea rate. Depending on the site, the highest net revenue resulted from half urea rates plus Humalite at application rates between 112 – 448 Kg ha^{-1} in wheat i.e. optimal Humalite rate for increased profitability.

Enhanced efficiency N fertilizers (EENFs) are innovative fertilizer products designed to improve N availability to crops while reducing environmental losses. Among these, double inhibitor fertilizers, also known as dual inhibitors, are particularly effective in optimizing N use efficiency by utilizing N inhibitors to improve soil N retention and make it more available to the plant. Building on the field study results, a controlled environment experiment was conducted at the University of Alberta from November 2023 to May 2024 to further investigate the optimal Humalite application rate of 448 kg ha^{-1}. This greenhouse study focused on the interaction between Humalite and EENFs on wheat and canola growth. The experiment compared SuperU®, an EENF, with conventional urea at recommended and reduced (70%) rates, combined with Humalite. A total of seven treatments, including a control, were evaluated over two growth cycles. Results revealed that Humalite was more effective in enhancing wheat growth compared to canola. Under optimal conditions, wheat grown with reduced urea rate plus Humalite demonstrated the highest N fertilizer recovery rate and comparable agronomic N efficiency to the recommended urea rate, however, with significantly lower yields. Interestingly, Humalite application did not show additional benefits when combined with SuperU® under ideal conditions. However, during a heat-stressed cycle, Humalite increased wheat yields when combined with reduced SuperU® compared to recommended SuperU®. This effect was not observed in canola. For wheat, reduced SuperU® was as effective as the recommended rate, producing similar yields as that of the recommended urea rate and resulting in higher protein content than the recommended SuperU®. In contrast, canola responded similarly to the recommended rate of both SuperU® and urea. The results of the two studies suggest that incorporating Humalite and using reduced rates of dual inhibitor EENFs could potentially decrease urea usage, contributing to more sustainable and economically viable wheat production systems.

Preface

This research was funded by Result Driven Agriculture Research (RDAR), WestMet Ag, and Western Grains Research Foundation (WGRF).

This thesis is an original work done by me, Sumedha Vaishnavi Nallanthighal. A version of Chapter 2 of this thesis is published in the Agronomy Journal [Nallanthighal, S. V., Enesi, R. O., Thilakarathna, M. S., & Gorim, L. Y. (2024). Agronomic responses and economic returns from wheat-canola rotation under Humalite and urea applications. *Agronomy Journal.* https://doi.org/10.1002/agj2.21681]. Abstracts of Chapter 2 have also been published as conference abstracts corresponding to poster and oral presentations made during my M.Sc. program. I also referenced this work in field day and industry presentations. The field study was conducted in correspondence with St. Albert Research Station (St. Albert) at the University of Alberta, Gateway Research Organization (GRO), Westlock and Battle River Research Group (BRRG), Forestburg. Experimental site data collection and maintenance were led by the following individuals: Dr. Linda Gorim, Salvador Lopez Benites, Karanjot Gill (St. Albert), Sandeep Nain and Kabal Singh (GRO), and Khalil Ahmed and Alex Olson (BRRG). All locations depended on summer students, lab members, and other research staff.

I co-collected and compiled all the data for chapters 2 and 3. I conducted the literature review, and statistical analysis, and wrote the first draft of chapters 1 and 3. I wrote the literature review and conducted preliminary data analysis for Chapter 2, with major data analysis guidance provided by Dr. Rebecca Enesi. Significant editorial revisions were made to my original drafts by Dr. Linda Gorim and Dr. Rebecca Enesi. I incorporated all the suggestions into the thesis before the final submission.

Dedication

I, Sumedha Vaishnavi Nallanthighal, dedicate this thesis to my loving parents, Renuka and Srinivas Nallanthighal. I am immensely grateful to have you as my parents. You have always supported and encouraged my dreams, my unconventional interests, and taught me the value of hard work.

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To my grandparents, whose prayers have been a source of comfort and strength, thank you for your boundless love and wisdom.

I am also grateful for my efforts in pursuing this roller-coaster of a journey – arguably one of the toughest things that I have set out to accomplish in my life journey so far. I am grateful for who I have become during this time and all the friendships that I have had the fortune to cultivate.

Above all, I offer my deepest gratitude to God and my guru Gurudev Sri Sri Ravishankar for this journey and your constant presence. Your spiritual guidance and support have been my anchor, guiding me through both triumphs and tribulations.

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List of Abbreviations

%	Percent
°C	Degree Celsius
(v/v)	Volume by volume
4R	Right Fertilizer Source, Right Rate, Right Time, Right place
AAC	Agriculture and Agri-Food Canada
AE_N	Agronomic Nitrogen Efficiency
ANLR	Apparent N Loss Rate
ANOVA	Analysis of Variance
AMF	Arbuscular Mycorrhiza Fungi
ANRR	Apparent N Residual Rate
AR_N	Apparent N recovery
BBCH	Biologische Bundesanstalt, Bundessortenamt und Chemical Industry
cm	Centimeter
CDC	Crop Development Centre
CO_2	Carbon Diooxide
CP	Canadian Prairies
CWRS	Canadian Western Red Spring
DAS	Days After Sowing
DCD	Dicyandiamide
EENF	Enhanced Efficiency Nitrogen Fertilizer
Fe	Iron
g	Gram
GC	Growth Cycle
GHG	Greenhouse Gas
GOC	Grain Oil Content
GPC	Grain Protein Content
ha	Hectare
HA	Humic Acid
HI	Harvest Index
HS	Humic Substance
INM	Intrgrated Nutrient Management
K	Potassium
kg	Kilogram
kg ha^{-1}	Kilograms per Hectare
kg N ha^{-1}	Kilograms of Nitrogen per Hectare
m^2	Meters squared
mm	Millimeter
Mg	Magnesium
mg kg^{-1}	milligram per kilogram
Mha	Million Hectares
Mt	Metric tonnes
N	Nitrogen
N_2O	Nitrous Oxide
NBPT	N-(n-butyl) thiophosphoric triamide

NGP	Northern Great Plains of North America
NH3	Ammonia
NH_4^+	Ammonium
NI	Nitrification Inhibitor
NO_3^-	Nitrate
NOB	Number of Branches
NOS	Number of Spikes
NS	Not Significant
NUE	Nitrogen Use Efficiency
NuUE	Nutrient Use Efficiency
P	Phosphorous
PB	Plant Biostimulants
PGPR	Plant Growth Promoting Rhizobacteria
pH	Potential of Hydrogen
ppm	Parts per million
RDwt	Root Dry Weight
RR	Recommended Rate
SA	Soil Amendments
SANR	Soil Apparent Nitrification Rate
SDwt	Shoot Dry Weight
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SPAD	Soil Plant Analysis Development
TDwt	Total Dry Weight
TKW	Thousand Kernel Weight
UI	Urease Inhibitor
USNGP	Northern Great Plains of United States of America
VB	Varietal Blends

Chapter 1.0 Literature Review

1.1 Introduction

The evolution of agricultural practices has profoundly influenced human civilization, starting with the transition from hunter-gatherer societies to settled farming communities around 10,000 BC (Wells & Stock, 2020). Early agricultural practices catalysed population growth, necessitating intensified farming to sustain the growing population (Garbowski et al., 2023; Gignoux et al., 2011; Johnson & Earle, 2000). While these advancements led to significant increases in food production, the intensification of practices such as over-irrigation which led to increased soil salinity, deforestation, shorter crop rotations, conventional tillage, and adopting monocultures over crop diversification proved to be damaging in the long run (Bai et al., 2022; Bennett et al., 2021; Dale & Carter, 1956; King, 1911; Malaj et al., 2020; Malézieux, 2012; Martens et al., 2015; Montgomery, 2012; Ponting, 2007; Tainter, 1988). Intensification practices have resulted in long-term environmental damage, including reduced soil health, loss of biodiversity, and increased greenhouse gas (GHG) emissions, which threaten the very ecosystems that support agriculture (Bellwood, 2023; Diamond, 1998; Goyette et al., 2016; Johnson, 2016; Park et al., 2012; Ponting, 2007; Purwanto & Alam, 2020; Sánchez-Bayo & Wyckhuys, 2019; Stanton et al., 2018). Studies have pointed out that intensive agriculture in the past, combined with early human activities, resulted in the depletion of land and water resources, leading to decreased soil fertility and productivity, and even altered the planet's climate, ultimately reducing the resilience of agricultural ecosystems to changing climatic patterns (Paoletti et al., 2011; Ruddiman, 2005, 2017).

The transition to large-scale industrial agriculture marked a turning point, with the introduction of chemical fertilizers and the Green Revolution heralding unprecedented increases in crop yields (John & Babu, 2021). Fossil fuels, chemical or synthetic fertilizers, and pesticides have significantly contributed to increasing crop yields in the 20th century (Bouwman et al., 2017; Carvalho, 2017; Lu & Tian, 2017). Despite the aim of the Green Revolution to alleviate world hunger, millions of people still face food insecurity today, with around 783 million people (2022) facing hunger and almost 757 million people (2023) facing moderate to severe food insecurity (FAO et al, 2024). Globally, intensive agricultural practices such as monoculture, heavy tillage, and excess synthetic chemical usage have led to a rapid decline in soil health affecting all key indicators of soil quality (Belete & Yadete, 2023; Derpsch et al., 2024; Gupta et al., 2022; Tahat et al., 2020). Furthermore, the excessive use of chemical or synthetic fertilizers and pesticides, aimed at increasing grain yield, has led to reduced soil organic matter, and increased soil salinization, and acidification (Barak et al., 1997; Bouman et al., 1995; Das et al., 2023; Kopittke et al., 2019; Liang et al., 2013; Mandal et al., 2020; Tripathi et al., 2020). These issues have resulted in impaired ecosystem service functions and have stagnated crop productivity (Bai et al., 2022; Buragohain et al., 2018; Lazicki et al., 2020; Ray et al., 2012; Singh, 2018; Wambacq et al., 2022).

On a global scale, reactive nitrogen (N) added to the Earth by anthropogenic activities has increased dramatically, with 90% coming from agricultural production (Fowler et al., 2013; Zhang et al., 2020). In 2021, the global cropland nutrient surplus was 82 million tons (Mt) of N, 8 Mt of phosphorous (P), and 12 Mt of potassium (K) (FAO, 2023) signifying either the over-application of fertilizers and/or the reduced uptake by crops. The overuse of synthetic fertilizers has led to environmental degradation resulting in nutrient imbalances, leaching, and the associated impacts of water pollution - eutrophication, and reduced water quality (Akinnawo, 2023; Dhankhar & Kumar, 2023; Schilling & Spooner, 2006; Turner & Rabalais, 1994; Weyer et al., 2001). In addition to environmental impacts, the widespread application and overuse of these chemical fertilizers have increased food production costs due to the expenses associated with sourcing raw materials, manufacturing, and transportation costs resulting in serious economic and food security challenges for producers and consumers alike (Bonilla-Cedrez et al., 2021; Elser et al., 2014; Hebebrand & Laborde Debucquet, 2023; Khabarov & Obersteiner, 2017).

The global population is projected to reach 9 billion by 2043, significantly increasing food demand. The challenge is to identify sustainable approaches to enhance crop production without repeating past mistakes (Hongguang, 2023). Improving N use efficiency (NUE) has become a primary target for sustainable solutions globally to reduce the over-reliance on synthetic N fertilizers (Rosolem & Husted, 2024). In light of these challenges, there is an urgent need for effective nutrient management systems that can balance fertilizer use while enhancing agricultural productivity and minimizing environmental impacts (Selim, 2020). Conservation practices, including increased crop biodiversity, intercropping, and the application of soil amendments and plant biostimulants have been advocated as viable strategies to address these pressing issues (Bamdad et al., 2022; de Molina et al., 2015; Guo et al., 2018; Li et al., 2020; Martin-Guay et al., 2018; Parr & Hornick, 1992; Tilman, 2020). Amendments such as animal manures, crop residues, green manures, wood ash, biochar, and composted materials play a crucial role in improving soil quality and fertility (Ahmed et al., 2015; Blum, 1992; Debosz et al., 2002; Jones, 2013; Maillard & Angers, 2014; Verma et al., 2020; Wei et al., 2016). Amendments are also essential for providing a sustainable source of nutrients to plants, thereby improving yields and acting as natural fertilizers (Assefa, 2019; Jannoura et al., 2014; Jjagwe et al., 2020; Shaji et al., 2021; Singh et al., 2020; Yadav & Sarkar, 2018). Additionally, plant biostimulants which are substances or microorganisms applied to plants to enhance nutrient efficiency, abiotic stress tolerance, and crop quality traits, offer promising solutions for sustainable agriculture (du Jardin, 2015), especially in the Northern Great Plains (Bartsch et al., 2023; Biederman et al., 2017; Parker et al., 2018; Souza et al., 2019).

1.2 Crop Production in the Northern Great Plains

The Northern Great Plains (NGP) spanning parts of the United States of America and Canada is a critical agricultural region for global food production and food security (Kissinger & Rees, 2009; Kukal & Irmak, 2016; Tanaka et al., 2007). It is an area of highly fertile soils which give rise to high agricultural production (Baulch et al., 2019). The NGP includes arable areas of the three Canadian Prairie (CP) provinces of Alberta, Saskatchewan, and Manitoba, and the agricultural regions of North and South Dakota, Montana, parts of Minnesota and Iowa, and some parts of northeastern Wyoming and Nebraska in the United States (Barker & Whitman, 1988; Baulch et al., 2019; Li et al., 2021; Padbury et al., 2002). This region has a semi-arid to sub-humid climate with extreme summers and winters (Padbury et al., 2002). Agriculture is usually limited by moisture as the region naturally experiences more evapotranspiration than precipitation (Padbury et al., 2002). The CP region has seen a substantial shift from its natural grassland state to agricultural cultivation, a transition that commenced in the 1870s in Manitoba and was nearly completed by the 1920s, reshaping the region's ecological and economic dynamics (Pennock et al., 2011). Agriculture in the CP primarily predominantly takes place on Chernozemic soils, which are rich in soil organic matter (SOM) from native prairie grassland and parkland vegetation (Dumanski et al., 1998). These soils are classified into Black, Brown, and Gray zones based on soil organic carbon (SOC) content and moisture availability, which significantly influence agricultural productivity (Grant & Wu, 2008). Black Chernozemic soils are found in the northern region with higher precipitation and yield the highest crops, while Brown and Dark Brown Chernozems are in the arid southern region, characterized by lower SOC and water availability (Campbell et al., 1990; Pennock, 2021). The Gray soil zones, primarily forested, face limitations in agricultural production due to frosts, lower SOM, and nutrient deficiencies (Landi et al., 2003; Pettapiece et al., 2010). The Black soil zone is the most productive, while the Brown zone experiences the greatest water deficit, affecting crop yields and fertilizer use (Grant & Wu, 2008; Pennock et al., 2011). The NGP significantly contributes to the production and export of staple cereal and oilseed crops (Agriculture and Agri-Food Canada, 2023b; U.S. Department of Agriculture & NASS, 2019); and is renowned for its extensive dryland cereal and oilseed cultivation system, with an increasing trend toward irrigation farming in certain regions (Carr et al., 2021; Cochran et al., 2006; Deines et al., 2020; Evett et al., 2020; Jing et al., 2021; Larney et al., 2004; Wang et al., 2022). The major grain crops grown are wheat, corn, soybeans, and canola, with other significant crops such as oats, barley, sunflower seeds, sugar beets, and edible beans (Johnston et al., 2002; Wienhold et al., 2017). The CP occupies about 51.2 million hectares (Mha), out of the 62.2 Mha of Canada's agricultural land making up around 82% of the country's farmlands (Statistics Canada, 2023). The CP contributes to around 85% of the country's total arable land, making it agriculturally and economically crucial (Statistics Canada, 2023). In 2023, approximately 32 million metric tonnes of wheat were grown on 10.6 Mha across Canada (Statistics Canada, 2024b). The CP accounted for 90% of this production with Alberta contributing 29%, Saskatchewan 45%, and Manitoba 16% (Statistics Canada, 2024b).

1.2.1 Crop production in the Canadian Prairies

The CP is characterized by a short growing season, around 90-120 days, typically from late May to early or mid-September (Baulch et al., 2019; Chipanshi et al., 2021; Mapfumo et al., 2023; Pelster et al., 2023), with cereal and oilseed crops requiring a minimum of 1200 growing degree days to reach physiological maturity (Mapfumo et al., 2023). In 2023, Canada produced approximately 85 million metric tonnes of grains and oilseeds across 27 Mha, and 4 million metric tonnes of pulses on 2 Mha (Agriculture and Agri-Food Canada, 2023a). The CP contributed significantly with 86% of grains and oilseeds and 98% of pulses, accounting for 73% and 97% of the total crop production, respectively (Statistics Canada, 2024b). The CP provinces have historically been the primary regions for wheat cultivation, comprising about

96% of the wheat-growing area from 1908 to 2007, though this figure has slightly decreased to 94% due to diversification into other grains, oilseeds, and pulse crops (McCallum & DePauw, 2008; Statistics Canada, 2024b). Canada is also the largest producer of canola (oilseed rape), cultivating around 18 million metric tonnes on 8 Mha across the country (Statistics Canada, 2024b). The CP accounts for almost 99% of canola production with Alberta, Saskatchewan, and Manitoba contributing 29%, 52%, and 16%, respectively (Statistics Canada, 2024b).

Wheat production in the CP is diverse with nine distinct classes based on the growth habit (spring and winter) and other quality factors such as protein content, kernel hardness, gluten strength, and colour (DePauw et al., 2011; McCallum & DePauw, 2008). The largest CP wheat class grown is the Canadian Western Red Spring (CWRS) which is used for making high-quality bread due to its relatively high protein content and blending capability (Iqbal et al., 2016; Pswarayi et al., 2014). Popular varieties in 2024 include “AAC Brandon”, “AAC Viewfield”, “AAC Starbuck”, “AAC Wheatland”, and “CDC Landmark” (Canadian Grain Commission, 2023; Cereals Canada, 2024). The Hard Red Spring wheat in the U.S. Northern Great Plains (USNGP) is comparable to the Canadian Western Red Spring (CWRS) wheat class. It constitutes approximately 25% of wheat production in the USNGP region, though its bread-making quality is slightly lower than that of CWRS (USDA, 2024). Popular varieties include “LCS Hammer AX”, “LCS Buster”, “LCS Cannon”, “LCS Trigger”, and “LCS Rebel” (Limagrain Cereal Seeds, 2024). Other wheat classes in the CP include Canada Eastern Red Spring (CERS), Canada Eastern Hard Red Winter (CEHRW), Canada Eastern Soft Red Winter (CESRW), Canada Eastern Amber Durum (CEAD), and Canada Eastern White Winter (CEWW) (Canadian Grain Commission, 2023). Optimal wheat growth conditions are achieved between 17-23°C with a precipitation input around 250-1750 mm yr^{-1} (Enghiad et al., 2017; Porter & Gawith, 1999).

Canola/oilseed rape (*Brassica napus* L.) is almost entirely spring sown in the CP and is mostly grown as three main herbicide-resistant or genetically modified systems which are Roundup-Ready®, Liberty-Link®, and Clearfield® canola systems, having bred resistance to glyphosate, glufosinate, and imidazolinone herbicides respectively (Beckie et al., 2004; Health Canada, 2016; Mauro & McLachlan, 2008). Canola typically completes its life cycle in 90-100 days from seeding to maturity, although this duration can vary based on cultivar, soil fertility and moisture, environmental conditions, sunlight intensity, and air temperature (Morrison et al., 2016; N. Harker et al., 2012).

Seeding crops in the CP typically occur from late April to mid-May, although regional variations exist (Dhillon et al., 2022; Johnston et al., 2002; Karamanos et al., 2012). The timing is influenced by soil conditions, snowmelt and runoff, spring precipitation, soil texture and temperature, frost risk, and previous crop residues (Arshad & Azooz, 2003; Grant et al., 2016; Gusta et al., 2004; He et al., 2013). Pre-seed herbicide application is crucial for annual broadleaf and perennial weeds such as kochia [*Bassia scoparia* (L.) A.J. Scott], volunteer canola, redroot pigweed (*Amaranthus retroflexus* L.), common lambs quarters (*Chenopodium album* L.) (Brunharo et al., 2022; Geddes et al., 2022). Pre-seed herbicide applications are usually performed in warm and low-wind conditions to control cool-season annual weeds creating a competition-free environment for the main crops (Chastko et al., 2024; Tidemann et al., 2023). Herbicides such as Axial® (Syngenta) provide broad-spectrum control for grass weeds (Syngenta, 2024). However, prairie producers usually practice a more integrated weed management system due to increased herbicide resistance (Geddes et al., 2022; Tidemann et al., 2023). No-till or minimum tillage practices with standing stubble are preferred during pre-seeding land preparation to preserve soil moisture and nutrients, leading to increased seed yield (Bescansa et al., 2006). Soil temperature plays a crucial role in determining the correct time to seed (Rahman et al., 2020). A soil temperature of 10°C is usually considered the optimum temperature for triggering seed germination (Chen et al., 2005). Choosing the right seeding date is crucial for producers as it significantly impacts crop physiological stages and subsequent yields (MacMillan & Gulden, 2020). Seeding rates are determined by the intended plant density, thousand kernel weight (TKW), and the seed germination percentage (Lafond & Gan, 1999). Producers generally prefer early seeding for both wheat and canola to optimize crop performance by maximizing available soil moisture and the full growing season, with early-seeded wheat being favoured to avoid frost damage and capitalize on early spring moisture and nutrients, resulting in better establishment and growth (Collier et al., 2020; Collier et al., 2021, 2022; He et al., 2012). Ultra-early wheat seeding is currently an advocated practice; characterized by seeding at a soil temperature between 2ºC – 6ºC for the benefits of frost damage and weed infestation avoidance to increase grain yields (Collier et al., 2021; Thilakarathna et al., 2017). However, ultra-early seeding in canola is not feasible, as the optimal seeding temperature required by canola seeds is 10ºC – 20ºC (Chen et al., 2005; Kondra et al., 1983) and may experience seed rotting in cold soils (Chen et al., 2005; Livingston & de Jong, 1990). Additionally, unlike wheat, whose growing point remains protected below the soil surface until BBCH30 (beginning of stem elongation), canola's exposed growing point makes it highly susceptible to frost damage from emergence, significantly limiting the feasibility of ultra-early seeding in the CP (Alt et al., 2020; Fiebelkorn & Rahman, 2016). Seeding rates and target plant densities generally increase from the drier Brown and Dark Brown soil zones to the more moisture-rich Black and Gray soil zones (O'Donovan et al., 2011). Higher seeding rates reduce tiller numbers and lead to uniform maturity and increased yield (Shah et al., 2020). Wheat is typically seeded at a depth of 1.5 to 2.5 inches with an optimal seeding rate of 250-400 seeds/m² (Collier et al., 2021), while canola is placed at a depth of ½ to 1/4th inch with an optimal seeding rate to produce 50-80 plants per square meter (Dhillon et al., 2022). Shallow seeding helps canola seedlings emerge quickly and uniformly, which is important for early growth and development (Hanson et al., 2008). Seeding equipment in the CP varies based on soil conditions and field requirements, with commonly used implements including air seeders, air drills, precision planters, direct or no-till seeders, and disc seeders. Air drills, conventionally used for seeding canola, typically have a 30 cm row spacing (Dhillon et al., 2022). These drills are equipped with double shoots, disc openers, and a seed cup assembly to ensure precise seed placement. Direct-seeding systems commonly employ inorganic fertilizer blends for both cereal and broadleaf crop production (Beckie et al., 1997). Commercial fertilizer blends are labelled with a ratio indicating the proportions of N, P, potassium, and sulphur per unit, each representing essential macronutrients for plant growth (Singh et al., 2023). Common inorganic fertilizer blends include urea N (46-0-0), monoammonium phosphate (11-52-0), triple superphosphate (0-46-0), potassium chloride (0-0-60), and elemental sulphur or ammonium sulphate (21-0-0-24) (Dyck & Puurveen, 2020; Howell et al., 2017). Nutrients are concurrently applied during seeding based on soil test reports for targeted yield goals (Jouany et al., 2021; Khakbazan et al., 2021; Mezbahuddin et al., 2020). Fertilizer granules are typically banded below or beside the seed row during seeding to enhance nutrient availability and uptake (May et al., 2020). One-pass direct seeding, which involves planting directly into the previous crop's residue without prior tillage, has become widely adopted in the CP (Grant et al., 2010; May et al., 2020). Effective crop residue management in the previous growing season is essential, as excessive residue can hinder germination and affect the seeder's ability to accurately place seed and fertilizer, while insufficient residue can lead to excess moisture and cooler soil temperatures, resulting in similar establishment issues (Cutforth et al., 2002; Hu et al., 2015; Liu & Lobb, 2021; Malhi & Lemke, 2007). Wheat seeds are often treated with fungicide seed treatments to protect against common seed- and soil-borne diseases such as common bunt, loose smut, seed rot, and various seedling-related diseases (Aboukhaddour et al., 2020; Kumar et al., 2022; Turkington et al., 2016). In canola crops, flea beetles are a prevalent pest, attacking during early seedling to vegetative stages, potentially causing significant yield losses (Lamb & Turnock, 1982; Lundin, 2020). Canola seeds are commonly treated with neonicotinoid (neonic) insecticides, typically applied as a blue-coloured coating, to protect against flea beetle damage (Tansey et al., 2009). Neonics utilize soil moisture to move from the seed coat to the plant, offering protection, especially in warm, dry conditions during the susceptible stages (Sekulic & Rempel, 2016). The neonicotinoids act as neurotoxins when ingested by flea beetles feeding on the treated plants, effectively reducing crop damage and potential yield losses (Jeschke & Nauen, 2008; Mittapelly et al., 2024).

Post-seeding, producers focus on comprehensive weed, insect, and disease control to ensure a healthy crop (McCallum et al., 2021). Regular field scouting and plant damage assessments are crucial, particularly during susceptible stages of crop development (McCallum et al., 2021). For canola, stems and undersides of leaves are checked for flea beetles till the four-leaf stage, with extra caution when temperatures exceed 14°C (Hoarau et al., 2022). If the threshold is crossed, foliar insecticides are sprayed (Mittapelly et al., 2024). Insecticides such as Malathion and Sevin XLR are applied after mid-July to control flea beetle emergence threatening pod development and yield (Cornelsen et al., 2024; Scagliarini et al., 2023). Additionally, integrated pest management (IPM) strategies, including cultural, physical, biological, and chemical controls, are employed to minimize the impact of pests on canola yields (Hoarau et al., 2022).

The use of inorganic fertilizers in Canada has increased steadily since the 1980s (Yang et al., 2007). In the CP, various inorganic fertilizer blends are commonly applied to cereals and oilseeds, primarily through direct seeding methods (Beckie et al., 1997). However, if not managed properly, inorganic N fertilizers may lead to undesirable consequences, such as increased GHG emissions, particularly nitrous oxide (N_2O) (Glenn et al., 2021; Venterea et al., 2005, 2011). Although N, P, and K fertilizers are the most widely used synthetic macronutrient sources, N fertilizer application has grown exponentially worldwide (Lu & Tian, 2017). Nitrogen is the primary macronutrient associated with increased plant height, crop yield, and quality (Krapp, 2015; May et al., 2012). Plants primarily absorb N in the form of ammonium and nitrate, utilizing it for protein synthesis (Gastal & Lemaire, 2002; Temple et al., 1998). Some plants, particularly legumes, can access N through biological nitrogen fixation – a process where symbiotic soil microorganisms convert atmospheric dinitrogen into ammonia (Compant et al., 2019). However, most crops rely on soil N, and chemical fertilizers have become necessary in many agricultural systems when the N removed through crop harvests exceeds what is naturally available in the soil (Thorburn et al., 2024). Nitrogen fertilizer recommendations for crop production in the NGP, including the CP are highly variable and depend on multiple factors. These factors include soil zone, previous crop, soil texture, application method, crop type, expected yield, soil moisture conditions, and local climate patterns (Grant et al., 2016; Liang et al., 2004; Malhi et al., 2001; McKenzie et al., 2006; Mezbahuddin et al., 2020; St. Luce et al., 2016). Optimal fertilizer rates, placement strategies, timing of application, and nitrogen sources are determined through a combination of soil testing, crop-specific requirements, and regional best management practices (Jégo et al., 2022; Malhi et al., 2001; Zebarth et al., 2009). In the CP, N fertilizer recommendations are commonly based on the measurement of nitrate-nitrogen (NO_3 -N) levels in the top 60 cm of soil during spring sampling, providing information about the available N in the crop root zone (Khakbazan et al., 2018; St. Luce et al., 2015). According to the Alberta Fertilizer Guide (2004), rainfed wheat and canola benefit from N rates between 22 – 99 kg N ha $^{-1}$ and 22 – 112 kg N ha $^{-1}$, respectively, depending on the soil zone and moisture conditions. A recent study in Manitoba found that 30 kg N ha $^{-1}$, combining soil residual and applied fertilizer, was needed for each kg of wheat grain yield to optimize economic returns and protein levels (Mangin et al., 2017). For canola, it typically ranges from 140-196 kg N ha $^{-1}$ for a 2800 kg ha^{-1} yield goal (Wen et al., 2021). Saskatchewan's recommendations range from 17-67 kg N ha^{-1} for wheat and 6-45 kg N ha^{-1} for canola (Saskatchewan Ministry of Agriculture, 2016). In Montana, it is advised to apply 55 g of N per kg of wheat (Jacobsen et al., 2003), while in South Dakota, this rate changes to 40 g of N per kg of wheat (Gelderman & Lee, 2019). Nitrogen fertilizers are placed at different times in the NGP. In the CP, most N fertilizers are applied completely at seeding to reduce machinery and operation costs and limit in-season disturbances (Ma et al., 2006). In the case of oilseeds such as canola, split applications were found to be more effective as N is supplied at appropriate growth stages during crop development (Grant et al., 2012; Ma et al., 2015). In Alberta, split application of anhydrous ammonia at BBCH 14 improved grain yield, and application near BBCH 59-61 increased grain N concentration (Beres et al., 2008).

Phosphorous is applied for rapid root growth, early maturity, and increased yields (Havlin et al., 2014; Ros et al., 2020). The source of P fertilizer is rock phosphate, which is not extensively used in the NGP. The more common commercial P fertilizers available in both granular and liquid form are monoammonium phosphate (11-52-0), diammonium phosphate (18-46-0), ammonium polyphosphate (10-34-0), and triple superphosphate (Froese et al., 2020; Grant & Flaten, 2019). Western Canadian producers predominantly place P fertilizer (mostly monoammonium phosphate) in seed rows or side-banded during seeding (Froese et al., 2020). Potassium is required for plant physiological processes such as photosynthesis, transport of nutrients and water, and stomatal regulation (Bourns & Flaten, 2022). Potassium fertilizers in the CP are sourced from the potash mines located in Saskatchewan (Broughton, 2019).

In-crop herbicide application for wheat usually occurs at BBCH 12-22 in late May for weed control, using motorized sprayers (Tidemann et al., 2023). Potential insect pests of wheat include wheat stem sawfly, grasshopper, and wheat midge (McCallum et al., 2021). Wheat midges are uniquely controlled by growing spring wheat varietal blends (VB) such as Shaw VB, Fieldstar VB, Conquer VB, and Utmost VB which contain the single midge-resistant gene

Sm1 in Western Canada (Mbanyele et al., 2024; Vera et al., 2013). Biological or chemical insecticides are applied when pest populations exceed economic thresholds to prevent significant damage and yield loss (Dakhel et al., 2020; Dufton et al., 2022; Sjolie et al., 2024). Wheat diseases include wheat rust, smut, Fusarium head blight, root rots, ergot, and Septoria leaf blotch, with fungicides applied at flag leaf emergence if symptoms are observed (Aboukhaddour et al., 2020). In-crop herbicides for canola have reduced due to the canola varieties being herbicide tolerant. In-crop herbicide for Roundup ready and Truflex canola varieties are usually sprayed from the 2-leaf to 6-leaf stage to reduce yield losses (Harker et al., 2000).

Efficient harvesting practices for wheat and canola in the CP are essential for maximizing yield and quality while minimizing losses. The timing depends on crop maturity, weather, and grain moisture content (Kutcher et al., 2010; Qian et al., 2018). Wheat is ideally harvested at kernel moisture levels of 13-15% to prevent yield and quality losses (Alt et al., 2019). Timely harvest is also important to minimize shattering losses in canola, with pre-harvest desiccants often applied to accelerate and uniformly dry down the crop, ensuring optimal yield and quality (Long et al., 2016). Traditionally, canola is swathed when a 60% seed colour change is observed, then allowed to dry to approximately 10% moisture content before combining (Beres et al., 2023). Swathing or straight-cutting or combining canola depends on various factors, including canola variety, available machinery, crop maturity, climate conditions, and soil type (Brackenreed, 2019; Cavalieri et al., 2014). Recent research has demonstrated that straight-cutting canola can improve seed quality and reduce harvest losses compared to swathing (Brackenreed, 2019; Watson et al., 2007). This shift in harvest methods has been facilitated by the development of shatter-resistant varieties, which can withstand longer field exposure without significant yield loss (Kuai et al., 2016). When chemical desiccation is necessary, producers commonly use products such as diquat (Reglone) or saflufenacil (Heat LQ) to hasten crop dry-down (May et al., 2020). For wheat, proper moisture management is necessary for storage at 8-14.5% moisture content to protect against insects, molds, and mites, while in canola, 8% moisture content is required (Chelladurai et al., 2016; Karunakaran et al., 2001; Sathya et al., 2009).

1.2.1 Need and strategies to build resilience in the Northern Great Plains cropping systems

Agronomic practices such as precision agriculture, crop rotation and diversification, tillage, early seeding, increasing seeding rates, altering row spacing, integrated pest management, and integrated nutrient management are now being incorporated into NGP agriculture to enhance yield and economic benefits (Elliott et al., 2008; Lundin, 2019). Recent agricultural practices have evolved significantly, focussing on conservation agriculture to improve sustainability (Kirkegaard et al., 2020; Lafond et al., 1992). Historically, continuous agricultural practices such as intensive tillage and summer-fallowing led to severe soil degradation i.e. soil erosion, acidification, and salinization (Coote et al., 1981; Voroney et al., 1981). The adoption of conservation agriculture and other regenerative practices over the past four decades has dramatically improved CP soil health and increased crop yields (Awada et al., 2014). Producers in the CP have implemented strategies such as conservation tillage, precision agriculture, diversified cropping regimes, and the use of stress-tolerant crop varieties (Archer et al., 2018; Campbell et al., 2002; Smolik et al., 1995; Tanaka et al., 2010; Zentner et al., 2002, 2011). However, in the current global agricultural context, new challenges are emerging, particularly related to climate change necessitating more sustainable nutrient management (Rashid et al., 2021; Selim, 2020; Wu & Ma, 2015).

To avoid repeating past mistakes and to better prepare for future challenges, crop management strategies must be refined and tailored to current and projected scenarios (Altieri et al., 2015; Lychuk et al., 2019; Martens et al., 2015; Raza et al., 2019; Webb et al., 2017).

These include optimizing crop rotations, further improving reduced or no-tillage practices, enhancing pesticide management, incorporating cover crops, and implementing integrated nutrient management approaches including the use of soil amendments and plant biostimulants (Hanberry et al., 2021; Liu et al., 2022; Lychuk et al., 2017; Mapfumo et al., 2023; Mayer & Silver, 2022; Singh et al., 2020; Van Eerd et al., 2023; Zhang & White, 2021).

Over the past three decades, the CP has witnessed a significant shift from wheat-fallow monoculture systems to diverse crop rotations (Benaragama et al., 2016; Zentner et al., 2002). Before the 1980s, the region primarily employed crop-fallow or crop-crop-fallow rotations, with spring wheat (*Triticum aestivum* L.) as the dominant crop (Campbell et al., 2002). Despite increased productivity, frequent fallow and low-diversity crop rotations led to topsoil loss caused by wind and soil erosion (Janzen, 2001). Crop rotation, the practice of growing a sequence of different crop types in the same field across successive growing seasons has emerged as a solution to these challenges (Iheshiulo et al., 2023; Leteinturier et al., 2006; Munkholm et al., 2013). Crop rotations can significantly influence soil-plant N dynamics due to the residual effects of previously grown crops (Luce et al., 2015; O'Donovan et al., 2014). Moreover, crop rotation enhances crop yields by optimizing nutrient utilization and disrupting pest cycles (Bainard et al., 2017). In response to climate change, market fluctuations, and the need for improved environmental sustainability, traditional crop rotations are being adapted to include a wider variety of pulse crops and more efficient strategies (Martens et al., 2015). Diversifying crop species through rotation not only stabilizes profits (Davis et al., 2012), but also reduces NO_3-N and phosphorus leaching, GHG emissions, and fertilizer requirements in the NGP (Behnke et al., 2018; Kiani et al., 2017; Leteinturier et al., 2006; Lychuk et al., 2021; Malhi et al., 2009; Soon & Clayton, 2002). Despite these benefits, recent trends toward simplified cereal-based rotations have raised concerns about reduced crop diversity, which can jeopardize soil health and crop productivity (Karlen et al., 2006; McDaniel et al., 2014; Ozlu et al., 2019). For instance, continuous or long-term wheat-canola rotations, although beneficial for reducing pest pressures, often result in lower yields (Schillinger & Paulitz, 2018). Historically, crop rotations in the NGP, particularly in the CP, have been dominated by cereals such as spring and durum wheat, with corn prevalent in the NGP of the United States of America (Feng et al., 2021). However, economic pressures such as falling cereal prices and increased input costs, as well as policy changes, have led producers to diversify their cropping rotations from monoculture of cereal crops to include diverse crops such as lentils, canola, chickpea, dry pea, flax, and mustard (Arshad et al., 2002; Chen et al., 2012; Gill, 2018; Harker et al., 2015; Johnston et al., 2002; Lupwayi et al., 1998; Miller et al., 2002; Smith et al., 2017; Tanaka et al., 2007). These pulse and oilseed crops have previously been reported to significantly increase grain yield and quality compared with continuous cereal/wheat systems, attributed to the retention of soil nitrate (Sainju & Pradhan, 2024). A typical CP crop rotation sequence involves cereals (wheat, oats, barley), oilseeds (canola, mustard, sunflower, flax), and legumes (lentils, field peas, beans, chickpeas) spanning across a 3 - 5 year cycle (Dhuyvetter et al., 1996; Grant et al., 2002; He et al., 2021; Miller & Holmes, 2005; Strydhorst & Liu, 2023). Rotation with canola is recommended to be scheduled every two to three years to break insect and disease cycles and promote crop diversification as well as farm profits (Gill, 2018). However, canola being an important economic crop for the CP, mostly continuous canola or 1-in-2-year canola rotations are more common (Town et al., 2023). In the USNGP, rotations also include maize and soybean (O'Brien et al., 2020). Historically, the USNGP area of North and South Dakota and Nebraska includes fodder crops such as grasses and alfalfa for livestock use with 4-yr rotations that include corn, wheat, and soybean which have collectively improved crop yield, soil health, and water use efficiency (Feng et al., 2021; Nebraska Corn Board, 2023; Sainju et al., 2021; Tanaka et al., 2005). Despite setbacks in crop production due to disease outbreaks and low crop value (Aboukhaddour et al., 2020; Byamukama et al., 2021; Nganje et al., 2004), the strategic diversification of crop rotations continues to offer a pathway toward sustainable and resilient agricultural systems.

An advocated strategy being explored is the use of cover crops as a complete or partial replacement for summer fallow in the NGP, with the potential to improve soil health and sustainability (Jones et al., 2020; Khan & McVay, 2019; Nielsen et al., 2016). Single or diverse cover crop species can benefit crop production indirectly by increasing soil stability and water-holding capacity, promoting microbial population and activity, and reducing soil erosion (Dapaah & Vyn, 1998; Jones et al., 2020; Malezieux et al., 2009). Cover crops can directly increase plant available N, improve nutrient use efficiency, and thus, increase crop yields and quality (O'Reilly et al., 2012). Cover crops have also been reported to reduce leaching losses, by around 69% globally compared with fallow as they work as nutrient scavengers taking up residual N left by the previous crop (Li et al., 2021).

Tillage or conventional tillage is an agronomic practice traditionally used for weed elimination, seedbed preparation, decreasing soil compaction, and incorporation of crop residues (Baan et al., 2009; Davies & Payne, 1988; Maillard et al., 2018). Historically, intensive mechanical tillage was commonly employed, particularly during summer fallow years, to control weeds (Curtin et al., 2000). While this approach provided short-term increases in crop yield, it also led to significant long-term drawbacks, such as decreased soil organic carbon (SOC) storage, reduced soil microorganism populations, depletion of plant-available nutrients, and overall decline in soil productivity (Bhattacharyya et al., 2022; McConkey et al., 2012; Yu et al., 2024). Since the 1990s, the NGP shifted to the adoption of no- or reduced-till practices to reduce soil erosion and degradation, optimize water use efficiency, reduce fuel costs required to operate extra machinery and improve nutrient management (Agriculture and Agri-Food Canada, 2009; Horowitz et al., 2010; Lafond et al., 1992, 1993; Nielsen & Vigil, 2010; Unger et al., 2010). By 2016, nearly 80% of CP farmlands were under conservation tillage, including

65% practicing zero-till (Statistics Canada, 2017). Although no or zero-till resulted in several improvements in soil properties and crop yields, some challenges such as excess soil moisture, pest control issues, and cooler soil temperatures are still a cause for hesitancy and feasibility of adoption (Adhikari et al., 2023; Agriculture and Agri-Food Canada, 2009; Sainju, 2020). Despite these challenges, minimum tillage or no-till systems can provide greater resilience to the cropping systems in the context of climate change while being economically beneficial for the producers.

Pest management involves several techniques such as crop rotation, model predictions, monitoring, pheromone traps, biocontrol, and applying pesticides when necessary (McCallum et al., 2021). These pests are tackled by an integrated pest management system which includes crop rotations, seeding rates, row spacing, and other chemicals such as foliar insecticides and neonicotinoid seed treatments (Batallas & Evenden, 2020; Soroka et al., 2018). Pesticides are used to protect crops from weeds, diseases, and pathogens (Chastko et al., 2024; Vankosky et al., 2017). Herbicide-tolerant or genetically modified crops are usually grown in the NGP including corn, soybean, and canola (Brunharo et al., 2022). Glyphosate is the most widely used herbicide for canola, soybean, and corn. More than 93% of the canola-seeded area in CP is glyphosate and glufosinate-ammonia-tolerant (Cornelsen et al., 2024; Kataria & Verma, 1992). Glyphosate is effective on weeds such as wild oats (*Avena fatua* L.), kochia [*Bassia scoparia* (L.) A.J. Scott], and downy brome (*Bromus tectorum*). Other weed species common in the USNGP are giant foxtail (*Setaria faberi* Herrm.), waterhemp [*Amaranthus tuberculatus* (Moq.) J.D. Sauer], and giant ragweed (*Ambrosia trifida* L.). Glyphosate-resistant canola can also act as a volunteer weed in the subsequent crop due to pod shattering in the previous growing season (Jhala et al., 2021). Current plant pathogens mainly affecting canola production include sclerotinia stem rot [*Sclerotinia sclerotiorum* ((Lib.) de Bary)], blackleg [*Leptosphaeria maculans* ((Sowerby) P. Karst)] and clubroot [*Plasmodiophora brassicae*

(Woronin)]; they are managed by fungicides, crop rotation, and resistant cultivars (Del Río et al., 2007; Hwang et al., 2011; Hwang et al., 2016). Canola is currently threatened by flea beetles, cutworms, root maggots, armyworms, diamondback moths, aphids, and leafhoppers (Cornelsen et al., 2024).

Ultra-early seeding of wheat has emerged as an innovative strategy to enhance resilience in the CP agricultural systems (Collier et al., 2024). This approach capitalizes on longer frost-free periods by seeding wheat when soil temperatures reach 2-6°C, regardless of calendar date, to enhance yields by avoiding high temperatures at critical growth stages later in the season (Collier et al., 2020). In the NGP, a short frost-free period can limit grain yields (Collier et al., 2021; Iqbal et al., 2007). The ultra-early wheat seeding system capitalizes on the early season growing degree-day as well as precipitation accumulated to produce higher yield (Collier et al., 2020). Studies in Australia in ultra-early seeding have evaluated and reported increased wheat grain yield due to deeper rooting, better access to soil moisture, reduced temperatures at grain filling, and an overall better establishment of the crop (Hunt et al., 2018; Kirkegaard et al., 2015). An earlier average planting window shift of 0.24 days year^{-1} of "Thatcher" wheat produced a higher yield of 23.5 kg ha^{-1}year^{-1} in six locations in Montana (Lanning et al., 2010), hence, avoiding grain yield reduction due to increased growing season temperatures which affects grain filling (He et al., 2012; Kouadio et al., 2015; Qian et al., 2019). In Western Canada, the highest yields were observed on the earliest seeding dates, with higher seeding rates and a shallow seeding depth at locations south of latitude 51° (Collier et al., 2021). Hence, producers south of latitude 51° are recommended to shift to earlier spring planting in Western Canada to reduce the risk of yield loss due to higher temperatures later in the season (Collier et al., 2020).

The long-term sustainability of agriculture in the NGP hinges on protecting natural resources, building resilience to various biotic and abiotic stresses, as well as maintaining economic viability (Martens et al., 2015). As previously discussed, practices such as crop rotation and diversification, conservation tillage, and ultra-early seeding strategies are being implemented to enhance sustainability, stability, and resilience in the NGP cropping systems (Smith et al., 2017). These approaches have shown promising results, increasing wheat and canola yield and quality while serving as mitigation strategies against variable climate conditions, particularly drought (Smith et al., 2017). However, to maintain and improve current productivity levels, especially in simple crop rotation systems, fertilizer application is often necessary to optimize yield and ensure economic feasibility (Lassaletta et al., 2014). This reliance on synthetic fertilizers as the main anchor for sustaining and increasing crop yields for the growing population has raised concerns about soil health and environmental impacts, highlighting the growing need for Integrated Nutrient Management (INM) (Selim, 2020).

Nitrogen fertilizer production accounts for almost 90% of the entire fertilizer industry (Tyagi et al., 2022). Nitrogen, though vastly available in the atmosphere, cannot be easily taken up by plants (Leigh, 2002). In nature, N is transformed into forms that are readily available to terrestrial ecosystems, which is again converted into other forms of N, eventually circling back into the atmosphere as molecular N (N_2) (Dong et al., 2021). However, natural processes such as biological nitrogen fixation, provide only a limited amount of fixed N which is not enough for increasing crop yields to satisfy the current population demand (Ladha et al., 2022). The solution for increasing N was discovered in the form of the development of synthetic fertilizers based on the effective and efficient Haber-Bosch process (Erisman et al., 2008, 2013) providing great benefits in food production and security since the 1960s (Sutton et al., 2011). Synthetic N fertilizers mostly provide a direct supply of plant-available N in the form of ammonium (NH_4^+) and nitrate (NO_3^-), or urea which breaks down into NH_4^+ and NO_3^- by the activity of urease enzyme and other microorganisms (Drury et al., 2017; Subbarao et al., 2006). This increase in enhanced nutrient supply to plants has exponentially increased crop production and yield over the years, however, N fertilizers are also susceptible to N losses, thus impacting their efficiency (Govindasamy et al., 2023). Nitrogen-based fertilizers in the CP are applied as urea, ammonium nitrate, anhydrous ammonia, and ammonium sulphate (Shen et al., 2019). Applied N fertilizers can be subjected to loss in four major pathways – ammonia volatilization, nitrate leaching, immobilization, and denitrification (Janzen et al., 2003; Qiao et al., 2015). Ammonia in gaseous form can be volatilized into the atmosphere when ammonium-based fertilizers are applied (Pan et al., 2016). Immobilization of soil N occurs when soil microorganisms convert ammonium and nitrate into organic forms of N, thus rendering them unavailable for plant uptake (Yansheng et al., 2020). Denitrification leads to the formation of N_2O from nitrate which has a potential global warming effect 265 times greater than carbon dioxide (Adelekun et al., 2019; Pan et al., 2022). Nitrous oxide is produced mostly due to excess soil N being lost to the environment and soil mediated specifically by soil microbes (Ramzan et al., 2020; Snyder et al., 2009). Though crop production contributes to a relatively smaller percentage of the total GHG emissions, it is an important concern to mitigate N_2O production due to its potency, since it is directly related to sub-optimal methods of soil and fertilizer management in agriculture (Smith et al., 2012; Tenuta et al., 2019). Other land practices such as irrigation and tillage can also contribute to N_2O production (Halvorson et al., 2010; Lee et al., 2006).

1.3 Integrated nutrient management

Integrated nutrient management (INM) is a multifaceted sustainable approach to nutrient use through the judicious and integrated use of organic, inorganic, and biological fertilizer sources (Janssen, 1993; Paramesh et al., 2023). Integrated nutrient management techniques allow for a more balanced nutrient management strategy, combining traditional and modern agricultural fertilizer practices leading to enhanced crop nutrient uptake and soil nutrient retention that mitigates nutrient losses and improves crop productivity and soil quality (Paramesh et al., 2023). Integrated nutrient management practices aim to synchronize the nutrient demand of the crop, with the supply and release of the nutrient sources used, thus, increasing the nutrient use efficiency and reducing losses related to leaching, volatilization, runoff, immobilization, and emission release (Wu & Ma, 2015). Furthermore, INM focuses on the overall improvement of soil physical, chemical, and biological properties for a more sustained agricultural output and productivity (Das et al., 2015). Various studies have shown that INM practices compared to the sole application of chemical fertilizers or organic manures can significantly increase rice, wheat, maize, and cowpea yields and quality, and improve soil health by minimizing nutrient losses and managing nutrient supply (Adediran et al., 2005; Ejigu et al., 2021; Ghosh et al., 2020; Gosal et al., 2018; Hammad et al., 2020;Khan et al., 2007; Ranjan et al., 2023; Urmi et al., 2022; Varatharajan et al., 2022). Integrated nutrient management practices are globally recognized for their effectiveness, yet their adoption varies significantly across regions (Chivenge et al., 2022; Gram et al., 2020; Mohanty et al., 2020). In Asian and African countries, there is a long-standing tradition of implementing INM techniques that combine conventional NPK fertilizers with organic sources such as farmyard manure, compost, vermicompost, and green manure, particularly in grain, pulse, and vegetable crops (Babu et al., 2020; Chakraborty & Kumar, 2017; Chianu & Tsujii, 2005; Thilakarathna & Raizada, 2015; Wabusa et al., 2024). In contrast, Western agriculture primarily relied on manure as the sole nutrient source until the 1930s, when the advent of synthetic N fertilizers via the Haber-Bosch process revolutionized N supply (Cunfer, 2021).

In Canada, agriculture is responsible for approximately 76% of anthropogenic N_2O emissions, with synthetic N fertilizers, particularly urea, being the primary source (Environment and Climate Change Canada, 2022b). Canada's N fertilizer consumption has surged from 0.94 million tonnes in 1981 to 2.5 million tonnes in 2016, with Western Canada experiencing a 200% increase compared to 42% in Eastern Canada (Agriculture and Agri-Food Canada, 2016). This growing reliance on N fertilizers has led to a 43% national increase in N_2O emissions, primarily attributed to N fertilizer application (Agriculture and Agri-Food Canada, 2016). In the CP, N inputs and outputs have varied in the last three decades with Alberta showing the highest yield response (91% N increase, 117% production increase), followed by Saskatchewan (98% N increase, 61% yield increase), while Manitoba demonstrated the lowest efficiency (77% N increase, 26% production increase) (Statistics Canada, 2024b). This corresponded with the change in NUE across the provinces ranging from 67 - 83% for Alberta, 88 - 130% in Saskatchewan, and 61 - 77% in Manitoba (Yang et al., 2024). Despite these increases in N input, net GHG emissions in the CP have remained relatively stable due to enhanced carbon sequestration in soils through best management practices (Agriculture and Agri-Food Canada, 2016). To address the challenges posed by rising N fertilizer use, the Canadian Government dedicated $200 million for the 2021 - 23 season to launch an on-farm climate action program, aiming to reduce GHG emissions by 40% through improved N management (Environment and Climate Change Canada, 2022a). Although nitrate leaching is lower in the CP compared to Eastern Canada due to its semi-arid climatic conditions and lower precipitation rates, nitrate runoff remains a concern (Yang et al., 2023). Studies have indicated that substantial nitrate N losses can occur, especially in late spring, through surface and subsurface runoff during early and late spring snowmelt respectively under the CP climate, which can be exacerbated in wet years following dry years due to potential nutrient flushing (Kokulan et al., 2019, 2022; Tiessen et al., 2010). A recent study calculated that N input in the CP increased from 21.8 - 59.8 kg N ha^{-1} in 1981 to 83.2 – 134.5 kg N ha^{-1} in 2016, an almost three-fold increase, mostly attributed to increased canola production, which requires more N for higher yields (Yang et al., 2023). Additionally, Yang et al. (2024) reported a significant decrease in NUE for the CP provinces, from 91.3% in the early 1980s to 73.3 % in 2016.

Despite this decline, the CP region's NUE remains substantially higher than the global average for major cereals (rice, wheat, and corn), which ranges from 30% to 50% (Bundy & Andraski, 2005; Cassman et al., 2002; Yang et al., 2024). The decrease in NUE for the CP in the 36 years was mainly attributed to the drastic increase in inorganic N fertilizer use and overall increased N losses through N_2O emissions, ammonia volatilization, nitrate losses, and elevated residual soil nitrogen levels (Yang et al., 2024). Hence, there is a pressing need to minimize inorganic N fertilizer dependency as they are directly related to increased fossil fuel usage, as well as optimize the use of inorganic N fertilizer depending on the agro-climatic conditions, crop, soil as well as economic variability in the CP region to improve grain yields and reducing environmental footprint (Mezbahuddin et al., 2020; Snyder, 2017; Yang et al., 2024).

1.3.1 The 4R Nutrient Stewardship

To optimize N fertilizer management, precision agriculture, and variable rate technology have emerged as key strategies in modern agriculture, particularly in the CP, providing site-specific crop management and enabling the practical application of inputs at varying rates across fields (Khakbazan et al., 2021; Mezbahuddin et al., 2020). Building upon these technologies, the 4R Nutrient Stewardship framework offers a comprehensive approach to optimize nutrient use efficiency and sustainability (Bruulsema et al., 2019). The 4R Nutrient Stewardship was developed a decade ago, focusing on the Right *place* and Right *time* of application of the Right *rate* of the Right *nutrient source* to improve crop yields, NUE, soil health, and economic profits for producers and is widely advocated in North America (Bruulsema, 2022). The foundation of the 4R idea was laid down in 1988 by Thorup and Stewart, who emphasized the importance of optimizing nutrient management practices for balancing nutrient cycles for sustainable crop production and reducing extra fertilizer costs (Thorup & Stewart, 1988). Due to the urgency of feeding an increasing population as well as gaining maximum economic yield, the 4R was pushed aside, till it became apparent that improper and N-balanced fertilizer management was leading to serious environmental and economic consequences, as well as nutrient losses and low NUE (Cook et al., 1996; Griffith & Dibb, 1985; Lamb et al., 2008; National Research Council, 1989). The 4R framework is individualized for each farm and locality based on the soil zone/type, different cropping systems, and climate limitations. The performance indicators for efficient 4R management include 9 indicators provided by the International Plant Nutrition Institute (2012) reflecting an area of concern in all cropping systems. The first three indicators are farmland productivity, soil health, and nutrient use efficiency, which are directly measurable at the farm scale. Hence following all 4Rs ensures that fertilizers do not limit crop yields, maintain soil fertility, and optimize nutrient use efficiency.

“Right” timing is crucial for N application, as N availability at the correct growth stage is important for yield and quality parameters (Bogard et al., 2010). The timing of N uptake by crops is not constant during the growing season. In cereals, nitrate supply is crucial at pre-anthesis as well as at the grain-filling stage to obtain higher crop yields and protein content (Martre et al., 2003; Worland et al., 2017). Above-ground N uptake can be as little as 17% during establishment (seeding to tillering) in cereals (McGuire et al., 1998) and oilseeds (Ma & Herath, 2016). During the growth period from tillering to heading, the majority of N uptake takes place, peaking just before anthesis (Malhi et al., 2006). It is in this phase that most of the nitrate and ammonium are absorbed and assimilated in vegetative tissues, which is then mobilized into grains in the later part of the season (Barraclough et al., 2014; Hawkesford, 2014). Malhi et al. (2006) reported marginal N uptake (< 5%) during the grain-filling period in the Black soils in Saskatchewan. In contrast, Mangin et al. (2022) reported almost 21-36% N uptake in wheat post-anthesis, although this uptake was highly dependent on late-season rainfall. The small percentage of post-anthesis N uptake is thought to contribute greatly towards grain N (Kichey et al., 2007; Pask et al., 2012). Standard 2.69 Mg/ha spring wheat, 5.38 Mg/ha barley, and 2.35 Mg/ha canola crops partition 95, 118, and 125 kg N/ha between their seed and straw biomass (Karamanos, 2015). These factors make the timing of N application extremely crucial as both early and late application can lead to N loss. A global meta-analysis reported that late-season applied N increases grain protein but is neutral for crop yields, which is good since there is usually a negative correlation between crop yield and grain N concentration (Giordano et al., 2023). However, in the CP's arid and dry conditions, split application and complete pre-plant application of the recommended rate provided similar yield and protein content (Grant et al., 2012). Hence, it appears that the timing of fertilizer N rate may depend on environmental conditions to provide maximum yield. Late fall application is also sometimes considered as an N management strategy. Because of soil freezing, which diminishes soil N availability, there is a decrease in N_2O emissions in the subsequent season. This indicates that applying anhydrous ammonia late in the fall may prove advantageous compared to spring application in this aspect (Tenuta et al., 2016). Contrastingly, a modeling study found that spring banding was effective in reducing N_2O, N_2, and ammonia emissions compared to fall banding (Mezbahuddin et al., 2020). Split application of urea also reported lower N_2O emissions in potato production in Manitoba (Gao et al., 2017).

Proper N fertilizer placement plays a vital role in ensuring accessibility and efficient N uptake by crops, reducing potential N loss pathways and environmental impact in the NGP's unique soil and climatic conditions (Tenuta et al., 2023). Although most of the NGP applies N fertilizer via side banding, broadcasting is still practiced as an application method in pastures or forages (Grant & Wu, 2008; Mezbahuddin et al., 2020). In the past, broadcast applications were preferred under conventional tillage systems compared to zero-till as tillage enables the fertilizer to be incorporated rather than being exposed on the surface, thus, reducing potential volatilization losses (Malhi et al., 2001). However, since broadcasting can also be environmentally, agronomically, and economically inefficient leading to around 50% more N losses through ammonia volatilization, side banding is currently the preferred way of N application (Gao et al., 2018; Sheppard et al., 2010). Side-banding of N fertilizer has also been demonstrated to either reduce (Nash et al., 2012), increase (Halvorson & Del Grosso, 2013), or even have no effect (Burton et al., 2008) on N_2O emissions.

Conceptually, N application rates should be equal to the crops' requirement for adequate yield that is not satisfied by the initial soil mineral N, mineralizable SOM, and atmospheric N deposition (Cassman et al., 2002; Morris et al., 2018). Nitrogen application rates in the NGP are highly variable as they depend on the crop type, crop requirement, yield goals, soil type and condition, and climatic factors (Cao et al., 2018; Grant et al., 2016). In Manitoba, more than 190 kg N ha^{-1} is recommended for reaching the economically optimal yield and protein content of spring wheat (Mangin et al., 2017). Forage crop production of sorghum in Iowa has a recommendation of 120-140 kg N ha^{-1} for obtaining high yields (Rooney et al., 2007); and 218 kg N ha^{-1} in New Mexico (Marsalis et al., 2010). Therefore, it is crucial to balance N application rates within the 4R network to ensure crop productivity while mitigating environmental impacts in the NGP.

The "Right Source" selection by producers depends on various factors, including but not limited to transportation costs, local availability, soil nutrient deficiency, and soil test reports (Mylavarapu, 2010). Selecting the correct source starts with a soil test assessment which indicates the nutrient requirement moulded for the upcoming season's crops (Bruulsema et al., 2019). In the absence of soil testing, other diagnostic tests such as plant tissue or sap analysis, visual assessment for nutrient deficiencies, crop growth responses, and near-infrared spectroscopy are also used (Fageria & Baligar, 2005; Jones Jr, 2011; Mylavarapu, 2010). The most common N fertilizers used in Canada are urea, anhydrous ammonia, ammonium nitrate, calcium ammonium nitrate, urea ammonium nitrate (UAN), and ammonium sulphate (Statistics

Canada, 2024a). Nitrogen fertilizer source has been found to influence N_2O emissions in crop production systems (Drury et al., 2012; Halvorson et al., 2010; Venterea et al., 2010). Anhydrous ammonia was found to have higher levels of N_2O emissions compared to UAN and urea (Mosier, 1994; Venterea et al., 2005).

Enhanced efficiency nitrogen fertilizers (EENFs) such as polymer-coated "slow-release" urea or stabilized urea which contain nitrification and urease inhibitors, have been designed to adhere to the 4R principles contributing to the "Right Source" aspect (Fast et al., 2024). When urea fertilizer is applied to soil, urease enzymes hydrolyse urea to ammonia, causing N loss due to ammonia volatilization (Drury et al., 2017). The ammonium that results from the hydrolysis of urea can be absorbed by plants, immobilized by soil microbes, attached to the surface of non-exchangeable clay, or proceed to the next transformation to nitrite and nitrate (Sigurdarson et al., 2018). Nitrification is the transformation of ammonium to nitrite followed by nitrate by microbial activity (Subbarao et al., 2006). Nitrate leaching is primarily caused by its greater mobility in soil than ammonium due to its negative charge, which prevents it from being held by the negatively charged soil particles (Meisinger & Delgado, 2002).

Ammonia volatilization is the first avenue of N loss after N fertilizer application. Coating urea with urease inhibitors based on hydroxamic acid and phosphoramides can contribute to reduced ammonia volatilization of urea by half, under various soil and environmental conditions (Lasisi et al., 2019; Silva et al., 2017) by increasing soil ammonium and decreasing soil nitrate levels (Fan et al., 2018). N-(n-butyl) thiophosphoric triamide (NBPT) is the most widely used urease inhibitor due to its ability to bind directly with the active metallocentre site of the urease enzyme (Kafarski & Talma, 2018). Nitrification inhibitors (NI) such as dicyandiamide (DCD) are used to inhibit microorganisms such as Nitrosomonas which aid in converting ammonium to nitrite, thereby reducing nitrate leaching and nitrous oxide emissions (Subbarao et al., 2006; Wissemeier et al., 2001; Zerulla et al., 2001). Application of N inhibitors has been demonstrated to increase wheat and maize yields and NUE because the increased ammonium encourages stronger microbial activity which in turn increases reserve soil N (Fast et al., 2024; Ma et al., 2015). The application of a double inhibitor on urea resulting in a product such as SuperU® (NBPT plus DCD) has the potential to simultaneously reduce nitrate leaching, nitrous oxide emissions, and ammonia volatilization (Drury et al., 2017).

Enhanced efficiency nitrogen fertilizer products slow the N release by either being encapsulated with protective coatings made of water-insoluble or semi-permeable material to restrict the dissolution rate of the fertilizer or by containing chemicals that inhibit N processes in the soil, thus reducing N loss, in turn providing more N to be available to the plants (Figure 1.1) (Asgedom et al., 2014; Venterea et al., 2011). Studies demonstrate a reduction in N_2O emissions but are inconsistent (An et al., 2021; Graham et al., 2018; Halvorson et al., 2011, 2014; Parkin & Hatfield, 2014). However, no change in N_2O emissions with Environmentally Smart Nitrogen®, a polymer-coated EENF was observed versus conventional urea application (Gao et al., 2017). However, there are some challenges with EENF adoption. Increased costs of EENFs compared to conventional fertilizers restrict consumer adoption (Thapa et al., 2016). Coating damage due to machine handling, from the manufacturing unit to seeding, may also contribute to reduced performance (Beres et al., 2012).

In response to rising N fertilizer prices, CP producers have increasingly turned to soil amendments and plant biostimulants as integral components of their INM strategies to further enhance nutrient use efficiency and reduce N fertilizer dependency (Bartsch et al., 2023). This approach incorporates soil amendments (SAs) and plant biostimulants (PBs) alongside 4R principles to enhance nutrient use efficiency, reduce inorganic N fertilizer dependency, and improve resilience against climate change (Bruulsema et al., 2024; Rubin et al., 2023). Soil amendments such as manure, and composts, enhance soil fertility; and PBs such as microbial and humic substances focus on enhancing plant nutrient uptake, and root development acts as complementary tools for nutrient acquisition potentially reducing synthetic fertilizer requirements (Bhattacharyya et al., 2008; Ghosh et al., 2022; Leoni et al., 2019).

1.4 Amendments: concepts and applications

One effective strategy to address the challenges of increasing crop yields without causing environmental stress, improving soil health, and reducing reliance on synthetic fertilizers, is the use of soil amendments (Garbowski et al., 2023). Soil amendments are aimed at improving soil fertility by increasing water availability to plants, maintaining the biological activity of soil microorganisms, and enhancing nutrient availability and plant uptake (Tejada et al., 2009). Soil amendments also increase soil stability by improving soil aggregate stability, buffer soil pH, enhance bulk density, and improve soil-air-water composition and balance (Figure 1.1) (Tejada et al., 2009; Zhang et al., 2023). Soil amendments include manure, compost, biochar, wood chips, and lime.

Manure is a by-product of livestock production and works as a fertilizer and soil conditioner (Gholami et al., 2016; Kar et al., 2017; Wen et al., 2003). Historically, manure has been utilized to provide nutrients naturally, and for its beneficial soil quality-enhancing properties (Jones, 2012; Liu et al., 2020). Manure can be sourced from cattle, pig/swine, sheep, and chicken; and applied in liquid or solid forms (Asgedom & Kebreab, 2011; Gholami et al., 2016; Hangs & Schoenau, 2023; Weber et al., 2022). Cattle and liquid swine manure have been shown to elevate soil pre-seeding available-N leading to increased wheat, canola, and barley yields, and enhanced N uptake (Mooleki et al., 2001, 2002). Yields either equal to or near the yield produced by synthetic fertilizers have also been observed by manure application (Buckley et al., 2011; Olson et al., 2010; Qian & Schoenau, 2002). Although highly rich in organic matter and a great source of plant nutrients, there are some concerns with sole manure application. Firstly, nutrients present in livestock manure are available sparingly (Biederman et al., 2017; Eghball & Power, 1999; Hangs et al., 2022; Stumborg et al., 2007). Long-term studies have shown that manure application for 15 or more years can proportionately increase mineralizable soil N and P, which contradicts short-term manure application, where N immobilization was observed (Whalen et al., 2001; Zaman et al., 1998). When manure is not applied at least every alternate year, crop yield and N uptake may diminish (Mooleki et al., 2001). Additionally, at least 40% of manure-N is supposed to be plant-available during the entire year, but it can drop to much lower percentages due to lower inorganic N content, cool and dry environment, soil type, and low C:N ratio (Mooleki et al., 2001). Although soils with manure applications have high fertility, repeated and continuous application may lead to excess N build-up, exceeding crop N requirement as well as soil's ability to retain N, eventually causing nitrate and P leaching (Mooleki et al., 2001; Sharifi et al., 2011). Nitrogen and P runoff can lead to algal blooms, eutrophication, killing marine life, and impacting water quality (Liu et al., 2021). Other related problems of manure application are pathogens, salt, greenhouse gas production, and higher costs and feasibility for transport (Whalen & Chang, 2001). Hence, an environmentally safe approach is required along with providing enough nutrients to the crop. An appropriate manure application method would be to reduce its rate and supplement with inorganic N fertilizers to achieve increased nutrient availability and crop yield goals (Iqbal et al., 2019; Sileshi et al., 2019).

Other amendments applied are lime and biochar. Lime application is most commonly used worldwide to alleviate soil acidification i.e. increase soil pH (Fageria & Baligar, 2008; Holland et al., 2018). In the NGP, crop yield loss due to soil acidification is mainly caused by the overuse of N fertilizers (Campbell & Zentner, 1984; Liebig et al., 2006). Previous studies on lime application report increased soil pH resulting in increased alfalfa, wheat, and barley yields in Western Canada (Malhi et al., 1995) with and without N fertilizer application (Hoyt & Hennig, 1982). Lime can be sourced as agricultural lime, sugarbeet lime, and wood ash (Lupwayi et al., 2009). Lime and manure were found to increase CP wheat and canola crop yields and improve plant available P and K and soil microbial quantity in acidic soils (Lupwayi et al., 2009; Malhi et al., 2004; Whalen et al., 2000, 2002). Lime application is also beneficial for crop diseases and insect attacks (Bresnahan et al., 2003; Tinline et al., 1993). However, negative effects due to excess liming have also been reported viz. soil compaction and decreased yields (Cifu et al., 2004; Li et al., 2019). Liming cost is also a barrier to its widespread application in the CP region (Lupwayi et al., 2009). Biochar or black carbon is another amendment used for enhancing soil health via increased nutrient retention and soil carbon sequestering (Joseph et al., 2013). Biochar is a residual product formed during pyrolysis of biomass, a thermochemical decomposition process, subjected to low oxygen under temperatures between 300 and 700ºC, along with other by-products (Bridgwater, 2003; Lehmann & Joseph, 2015). Biochar is reported to increase soil carbon storage, thus, aiding in the mitigation of atmospheric GHG (Fowles, 2007; Kwapinski et al., 2010; Lehmann & Joseph, 2015). Being carbon-rich, biochar has been shown to improve soil fertility (Joseph et al., 2013). The use of biochar as a soil conditioner and as a mitigation strategy to aid soil N and P retention and their increased availability has been reported globally (El-Naggar et al., 2019; Gao et al., 2019). Studies assessing the effect of biochar alone, or in combination with synthetic and/or manure have shown improved soil characteristics (Gao et al., 2022; Šimanský et al., 2018) and crop yields in barley (Agegnehu et al., 2016), maize (Zhu et al., 2015), and wheat (Khan et al., 2022) as well as overall crop yields (Gao et al., 2019; Nguyen et al., 2017). A four-year study assessing the interaction between biochar and manure found enhanced N mineralization and increased water-holding capacity. They also found that biochar was more effective in improving soil properties on Brown soils than Black soils signifying that the soil type has a significant effect on amendment effectiveness (Hangs et al., 2022). In the same study, wheat and canola yields increased under biochar plus inorganic N and P manure compared to biochar plus organic manure. Globally it has been determined that even a small amount of synthetic fertilizer substitution with animal manure or compost can lead to increased yields and improved soil environment (Geng et al., 2019; Zhang et al., 2016).

1.5 Plant Biostimulants and their role in nutrient management

Plant Biostimulants (PBs) differ from soil amendments as they are applied in minute quantities or at rates of or below 100 Liters or kg per hectare (du Jardin, 2015; Rose et al., 2014). The biostimulants industry is currently a fast-growing sector among agricultural industries with a predicted 7.4% annual growth rate and USD 4.6 billion in revenue by 2030 (Critchley et al., 2021). Biostimulants, essentially, offer a novel approach to regulate and/or modify the plants' morphological characteristics such as increased shoot and root growth, and even directly alter the plant's internal efficiency to provide better nutrient uptake capability, and increase yield (du Jardin et al., 2020; Massaya et al., 2022). According to du Jardin et al., (2020) and Michalak et al., (2020), the goal of PB applications is to enhance one or more aspects of the plant rhizosphere, including (i) nutrient use efficiency, (ii) abiotic stress tolerance, (iii) quality traits, and (iv) nutrient availability. Plant biostimulants are targeted at stimulating and modifying natural processes within the plant physiology (Kumari et al., 2022). Biologically derived PBs are defined by their functionality over composition (Bulgari et al., 2015; Calvo et al., 2014). Natural materials-based PBs have garnered attention from both the scientific community as well as industry enterprises in the last 25 years as these substances appear to have great potential in enhancing and improving plant growth and development, providing resilience against abiotic stresses, and improving nutrient use efficiency (Brown & Saa, 2015; Crouch & Van Staden, 1993; du Jardin, 2015; Khan et al., 2009; Maini, 2006; Sharma et al., 2014).

Initially, PBs were regarded as those substances that when applied in minute quantities, would stimulate certain biochemical processes in a living organism to preserve its life, without supplying nutrients (Russo & Berlyn, 1991). A comprehensive list of definitions of biostimulants is assembled by Yakhin et al. (2017), where a chronological approach towards understanding the nature of biostimulants has been provided. Only recently, du Jardin (2015), conducted the first in-depth analysis in understanding the systemization and characterization of PBs, based on the mode of origin and their function. Plant biostimulants are hence becoming increasingly popular for their use in sustainable agriculture systems, and integrated pest and nutrient management programs - to reduce heavy reliance on irrigation water, and synthetic agrochemicals and fertilizers (du Jardin et al., 2020; González-Pérez et al., 2021; Michalak et al., 2020; Wozniak et al., 2020).

Plant biostimulants were first discovered by two independent research teams. The first group observed improvement in shoot and root growth, NUE, and increased drought resistance by some compounds at low doses (Russo & Berlyn, 1991). The product applied was a mixture of humic acids, seaweed extracts, and vitamins. The second group observed similar results with humic and seaweed-based products (Zhang & Schmidt, 2000) on turfgrass. They found that these “hormone-containing products” increased antioxidant levels in plants by influencing plant metabolism through hormonal activity, thus helping the plants to respond better to stress. These studies birthed the idea and proposal of using these products in combination with or by themselves, to reduce fertilizer use, i.e., lowering agricultural inputs and consequently increasing crop yields (Zhang & Schmidt, 2000). Other research studies also pursued understanding PB's mode of action. A review by Kinnersley (1993) identified biostimulants as “phytochelates”– substances that chelate or form a bond with micro and macro-nutrients, thus, promoting plant growth by supplying more nutrients. The chelation property of seaweed extracts improved nutrient optimization and soil structure was one of the major reasons that pushed research (du Jardin et al., 2020). Humic substances (HS) were identified as a type of PB whose chelation bioactivity is provided by carboxyl groups (Vaughan & Malcolm, 1985). The humic acid (HA) and fulvic acids (FA) components of HS enhance metal ion uptake, such as iron (Fe^{+2}) and Magnesium (Mg^{+2}) which are directly required by plants for optimum photosynthesis (Kinnersley, 1993).

In the soil, PBs increase enzymatic along with microbial activity, thus enhancing soil fertility (Hellequin et al., 2020). Although there is growing scientific evidence supporting the efficacy of biostimulants, limitations are preventing their widespread adoption. Research is still evolving in understanding PBs' mechanism of action, optimal application methods, and potential interactions with other agricultural inputs (du Jardin, 2015). Regulatory frameworks, standardization of products, economic viability as well as environmental sustainability, all need to be addressed with interdisciplinary collaboration to enhance the adoption of biostimulants for sustainable agriculture (Brown & Saa, 2015; Calvo et al., 2014; du Jardin, 2015; Yakhin et al., 2017). Plant biostimulants are broadly classified into two categories viz. biological substances which are derivatives of microorganisms or plants, and those not of biological origin (Grammenou et al., 2023). The main categories are seaweed and plant extracts, humic substances, protein hydrolysates, and microbial inoculants (Figure 1.1).

Seaweed extracts are a biostimulant class emerging as a promising tool for agricultural use (Ali et al., 2021). Seaweeds were historically used as agricultural fertilizer in European and Mediterranean countries (Pereira et al., 2019; Temple & Bomke, 1988). Seaweed biostimulants are derived from various species including *Ascophyllum nodosum, Sargassum* spp., and *Laminaria digitata* containing an array of rich bioactive compounds such as polyphenols and phytohormones (Al-Ghamdi & Elansary, 2018; Khan et al., 2009). Chemical and physical methods (mainly alkaline extraction methods) are used for the extraction of biologically active molecules from different seaweeds (Shukla et al., 2019). Seaweed-based biostimulants are reported to promote root development, nutrient uptake, and overall plant growth, while also enhancing abiotic stresses such as drought and salinity tolerance (Ali et al., 2021; Deolu-Ajayi et al., 2022). Various crops including wheat, soybean, chickpea, and rice when treated with seaweed extracts under stress conditions, have recovered with a significant increase in plant growth (Abdel Latef et al., 2017; Sharma et al., 2019; Shukla et al., 2018; Zou et al., 2018). Several studies have indicated an increase in crop yield and quality parameters with a single application of these extracts, or in combination with other fertilizers and PBs (Chen et al., 2021; Hamouda et al., 2022; Nasiroleslami et al., 2021; Nichol et al., 2023; Raj, 2021). However, in some cases, crops treated with these PBs had no significant yields (Di Stasio et al., 2018), while reduced yields were observed in a few stress cases (Trivedi et al., 2018). Despite their widespread use, especially in horticultural sectors, the mechanisms underlying their effects are still an active area of research (Deolu-Ajayi et al., 2022). As agricultural production faces increasing challenges from climate change and soil degradation, seaweed biostimulants offer a viable solution for enhancing crop performance and sustainability (Battacharyya et al., 2015; Hassan et al., 2021; Layek et al., 2018; Michalak et al., 2020; Mukherjee & Patel, 2020). Another type of non-microbial biostimulatory substance is a protein-based product called protein hydrolysates, which are reported to enhance N, P, K, and Mg in crops (Brown & Saa, 2015).

Microbial inoculants such as plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhiza fungi (AMF) have also shown significant promise as PBs (Backer et al., 2018; Sun & Shahrajabian, 2023). Plant growth promoting rhizobacteria directly affect plant growth by releasing phytohormones and volatile compounds that modulate plant signaling pathways, increase nutrient availability through mechanisms such as iron chelation, and inorganic P solubilization, and enhance essential macro- and micro-nutrient uptake (De Freitas et al., 1997; Glick et al., 2007; Podile & Kishore, 2006; Sheng & He, 2006). Additionally, PGPRs have been reported to improve root growth and development, viz. increasing root surface area, volume, and density, supporting further nutrient and water uptake (Mahaffee & Kloepper, 1994; Mantelin & Touraine, 2004; Podile & Kishore, 2006). Similarly, AMF form symbiotic relationships with plant roots, thereby enhancing nutrient and water acquisition, and improving plant stress tolerance against salinity, and drought (Khan et al., 2024; Silva et al., 2023; Sun & Shahrajabian, 2023; Wahab et al., 2023). These fungi act as an extension of plant roots in the form of hyphal networks to enable the plants to increase their nutrient uptake by reaching difficult to access regions in the soil (Sun & Shahrajabian, 2023). Studies in the NGP show an increase in crop yield and soil NPK uptake in maize, wheat, pea, and lentils (Abd El-Azeem & Bucking, 2023; Adesemoye et al., 2008; Biswaray, 2015; Chang, 2008; Floc'h et al., 2022; Germida & Walley, 1996). These findings illustrate the potential of using microbial PBs for sustainable and more resilient agriculture in the NGP, especially under such climatic turbulent conditions.

1.5.1 Humic substances

Humic substances (HSs) are naturally derived macromolecular products formed through the microbial decomposition and transformation of dead plant and animal residues (Tiwari et al., 2023; Zavarzina et al., 2021). Humic substances can be found in various natural environments, including soil, peat, oceans, and freshwater regions, and their properties can vary significantly based on their source and formation processes (Ampong et al., 2022; Bezuglova & Klimenko, 2022; Lanno et al., 2022). Humic substances can be sourced from lignite, leonardite, vermicompost, and other organic materials (Arancon et al., 2006; Canarutto et al., 1996; Huculak-Mączka et al., 2018; Peuravuori et al., 2006; Qian et al., 2015; Tahir et al., 2011; Vlčková et al., 2009). The most common source of HS is leonardite, which is a highly oxidized form of lignite, a coal, but has not reached the state of coal (Conselvan et al., 2017; Petrov et al., 2017; Sun et al., 2020). Humic substances are divided into three components based on their molecular weight and solubility: humic acids (HAs), fulvic acids, and humin (Rathor et al., 2023). Among these, HA is the most active component with a complex structure contributing to its high molecular weight, thus, conferring a recalcitrant property to microbial degradation (Lumactud et al., 2022). Humic acid, a principal component of HSs, acts as a biostimulant that enhances nutrient availability and promotes plant growth (Delfine et al., 2005; Osman & Rady, 2012). It is known for increasing plant growth and grain yield by forming chelated bonds that aid nutrient uptake (Ampong et al., 2022; Calvo et al., 2014; de Melo et al., 2016; Nardi et al., 2017; Olaetxea et al., 2018; Vujinović et al., 2020). Humic substances have the unique ability to form structural complexes with cationic micronutrients through their functional groups - carboxyl, amino, or alcohol groups, chelate micro- and macro-nutrients and enhance their availability to plants (Barton & Abadia, 2007). This chelation process stimulates plant growth by facilitating nutrient accumulation (Chen & Aviad, 1990; Varanini & Pinton, 2000).

Humic acid has been shown to alleviate plant growth under abiotic stresses such as salinity and water stress (Aguiar et al., 2016; Ali et al., 2019; Khaleda et al., 2017; Saidimoradi et al., 2019; Shukry et al., 2023; van Tol de Castro et al., 2022). Humic acids may also enhance heat tolerance in horticultural crops, though their effects on crops are still being explored (Canellas et al., 2024; Cha et al., 2020; Choi et al., 2024; Khan et al., 2020; Poomani et al., 2023). Humic substances are an integral component of soil organic matter and dissolved organic matter, thereby playing a crucial role in soil fertility and plant growth (Canellas & Olivares, 2014; Olaetxea et al., 2020; Stevenson, 1994; Trevisan et al., 2010). Numerous studies have reported the impact of HS on plant and soil functions (Mora et al., 2010; Muscolo et al., 2013; Nardi et al., 2002; Rose et al., 2014; Trevisan et al., 2010). Plants treated with HS had modified root systems and morphology (Rathor et al., 2023, 2024). Humic substances also influence plant physiology and biochemical processes which improve plant photosynthesis and respiration rates thereby increasing crop yields (Olk et al., 2018). In addition to their effects on plant growth, HSs also improve soil physico-chemical properties including soil nutrient content, cation exchange capacity, and microbial population (Figure 1.1) (Gümüş & Şeker, 2015). Good soil structure, influenced by HS, directly affects soil solution and water movement, reduces soil erosion, improves nutrient recycling, and root penetration, and enhances crop yields (Bhatt & Singh, 2022; Khaled & Fawy, 2011; Piccolo et al., 1997; Tahoun et al., 2022). Humic substances such as Humalite combined with recommended N fertilizer significantly increased wheat grain yield by 14-19 %, protein content by 20-30 %, and improved NUE by 14-60 % indoors (Rathor et al., 2024).

1.6 Gaps identified

To achieve the United Nations 2030 Agenda for Sustainable Development Goals, particularly Zero Hunger (Goal 2), Sustainable Consumption and Production (Goal 12), and Climate Action (Goal 13), immediate measures are essential to ensure food security, promote sustainable agricultural practices, and mitigate climate change impacts, thereby building a resilient and sustainable future for coming generations (United Nations, 2015). Agricultural research and development play a pivotal role and responsibility in enhancing global crop production systems to feed the growing world population, reduce dependency on fossil fuels and synthetic fertilizers, and mitigate N_2O emissions, ultimately contributing to a more sustainable and food-secure future. The CP, encompassing Alberta, Saskatchewan, and Manitoba, are of paramount importance to Canada's agricultural sector and economy. This region's diverse soil zones, including Black, Brown, and Gray Chernozems, significantly influence agricultural productivity and necessitate tailored research approaches. Canada has emerged as one of the global leaders in N management, employing advanced technologies such as precision agriculture, the 4R nutrient stewardship program, and EENFs. In Canada's target to reduce

GHG emissions by 40% by 2030, the cropping system management in the CP contributes significantly.

Despite the CP's agricultural progress, the region faces pressing challenges. Over the past 36 years, increased N inputs have led to higher residual soil N levels, raising contamination risks (Yang et al., 2024). This situation underscores the urgent need for improved N management strategies. Compounding these challenges, farm input production costs have risen dramatically, with the N fertilizer farm input price index in Canada more than doubling from 45.3 in 2002 to 105.1 in 2024 (Statistics Canada, 2018). Although EENFs show promise in reducing GHG emissions and improving NUE, location-specific research is required at different rates to test their sustainability as EENFs are costlier than their conventional counterparts, and crop yields are not impacted negatively. Furthermore, with the predictions of a shorter growing season, higher temperatures, and erratic precipitation due to climate change in the CP (Lychuk et al., 2019; Wang et al., 2012), there is a need to conduct research with sustainable options and optimize the use of N fertilizers.

The use of PBs is a natural, innovative, and sustainable technology that is yet to be completely explored in the pursuit of attaining the UN sustainable development goals. The integration of PBs into 4R nutrient stewardship practices faces significant challenges due to limited research and operational examples (Casa & Ronga, 2020), especially in the CP. The diverse range of PBs, with their unique mode of action, complicates the determination of optimal application rates, timing, and placement within the 4R framework making their incorporation a little more complex compared to conventional farm inputs in the CP agricultural systems. The integration of PBs into agricultural practices in the CP presents both opportunities and challenges. Their adoption at the field level remains limited due to producers' concerns about additional costs without guaranteed yield increases. The synergistic action of PBs and N fertilizers to reduce or substitute N fertilizer application while also providing resilience against climate change needs to be explored further from an agronomic perspective. So far, PB with EENFs has been investigated very little, especially in cereal and oilseed crops (Souza et al., 2019). Especially in the CP, with its vast climatic conditions, and different soil zones with different characteristics, there is a dearth of research in the application of PBs in synergistic ways with different N fertilizer sources. There is also a gap between research and its implementation by producers, slowing the pace of adoption of these integration practices. Among PBs, there is a particular lack of research on HSs, especially regarding optimal application rates across different soil zones and environmental conditions in the CP.

Humic substances are a type of PB that have been extensively researched across the world of agriculture, spanning several decades. However, they have several sources that they are derived from making them unique in their action based on the source and the crop and soil that they are being applied on, which makes the standardization of humic products and their characterization methods an ongoing challenge in research thus making it a challenge in optimizing their application. Ampong et al. (2022) identified knowledge gaps in the use of HSs as biostimulants. Although there are global efforts persisting in finding and optimizing tools for N optimization, there is a significant gap in research investigating the use of HSs in combination with conventional NPK fertilizers on N uptake and utilization in agricultural cash crops. Research has shown that HSs need to be tailored in the region where they are being applied (Rathor et al., 2023). To our knowledge, there is a severe lack of research on HS applied with reduced N fertilizer rates and with different N fertilizer sources especially in long-term studies. Such research would provide valuable insight to producers enabling them to make informed decisions regarding optimal sources, rates, frequency, as well as economic viability tailored to their specific local conditions.

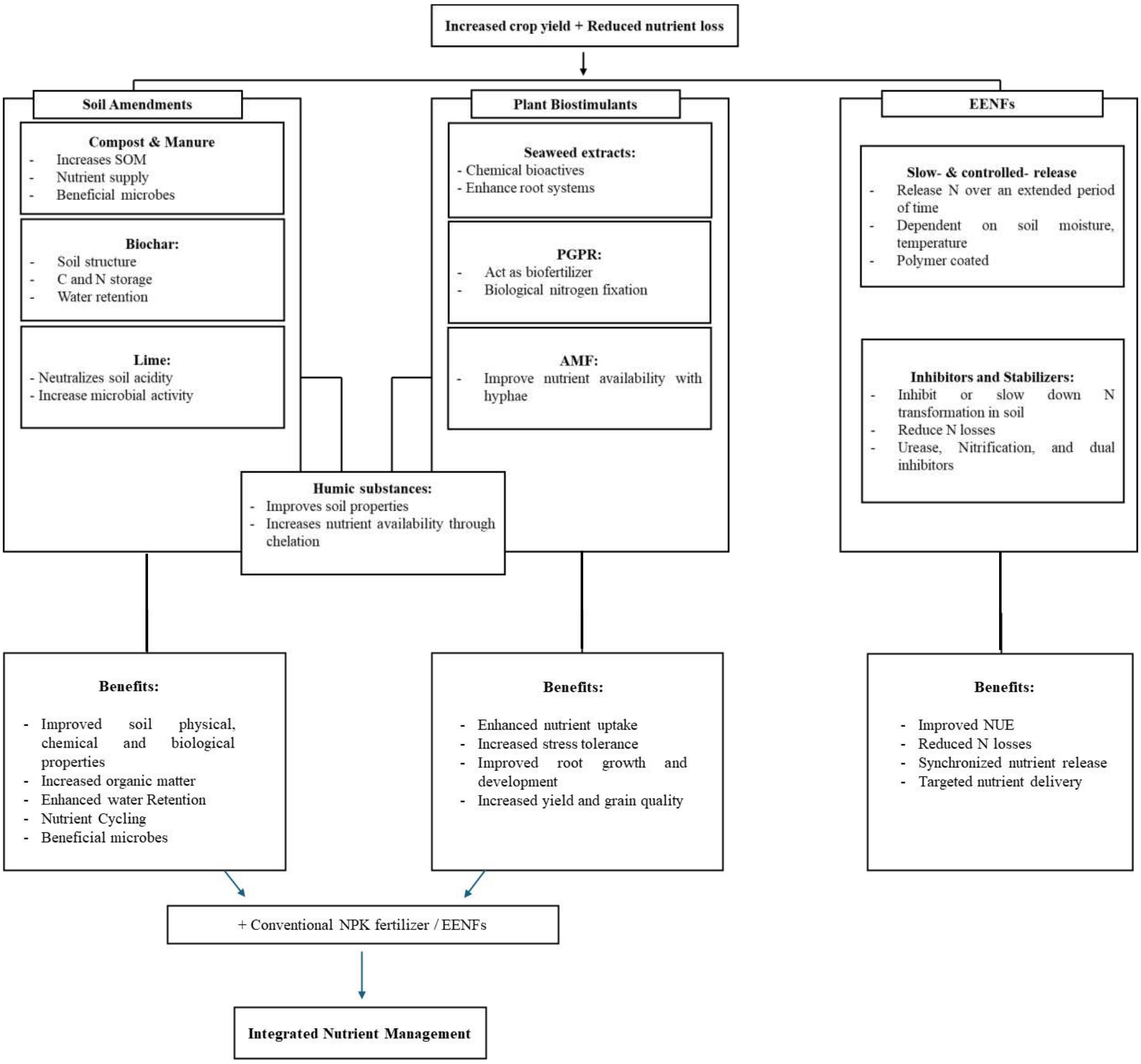

Figure 1.1 Flow chart depicting categories, their action, and benefits of soil amendments, plant biostimulants (PGPR: Plant growth-promoting rhizobacteria; AMF: arbuscular mycorrhiza fungi), and enhanced efficiency nitrogen fertilizers (EENFs) in the context of integrated nutrient management.

Chapter 2.0

Agronomic responses and economic returns from wheat-canola rotation under Humalite and urea applications.

2.1 Introduction

Spring wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.) are the two most predominant grain crops grown in Western Canada. Annual production of wheat and canola in Western Canada is approximately 23.8 and 18.1 metric million tons, respectively (Statistics Canada, 2023). Wheat is a staple grain crop, and it is integral for meeting essential human dietary requirements because of its superior composition of carbohydrates, fats, protein, fiber, zinc, calcium, and vitamins (Irge, 2017). Canola is mainly used as an oilseed crop and is known for its use as an edible oil, biofuel, industrial oil, and a high-protein meal (McVetty & Duncan, 2016). Wheat and canola production is heavily reliant on synthetic fertilizers to provide essential nutrients, such as nitrogen (N), phosphorous (P), and potassium (K), to sustain nutrient supply and support yields (Nyamangara et al., 2020). Nitrogen is required in large quantities to reach optimum crop yields and is widely limited (Elser et al., 2007; LeBauer & Treseder, 2008; Maaz et al., 2021; Marschner, 1995; Yuan & Chen, 2012). Approximately 30-50 percent of applied N is taken up by crops, while the rest is lost through nitrate (NO_3^-) leaching, ammonia (NH_3^-) and nitrous oxide volatilization, and denitrification (Conant et al., 2013; Hood-Nowotny et al., 2010; Malhi et al., 1998; Mosier et al., 1998; Smil, 1999). Excessive N application and N loss adversely cause environmental degradation, resulting in groundwater contamination, greenhouse gas emissions, soil acidification, and impaired microbial activity and function (Cui et al., 2023; Gao et al., 2023; Sun et al., 2023; Hartmann et al., 2015). Crop varieties developed during the Green Revolution of the 1960s improved crop yields. However, these varieties have been found to have relatively poor nitrogen use efficiency (NUE), thus requiring more N fertilizer application to produce higher yields (Gooding et al., 2012; Li et al., 2018). Therefore, there is a need to explore sustainable solutions that enhance the capacity of soils to retain soil N and provide it when needed by crops. To minimize the negative environmental impact of nitrogenous-based fertilizers, it is crucial to investigate alternative strategies that optimize crop yields in wheat and canola systems while minimizing the use of synthetic N sources.

A recent approach involves the use of biostimulants such as Humalite. Humalite is an organic soil amendment with a high concentration of humic acid (HA) and is found in significant quantities in Southern Alberta (Rathor et al., 2024). Humic substances (HSs) (e.g., Humalite) are formed through the microbial decomposition of plant and animal residues from millions of years ago (Lumactud et al., 2022; Olk et al., 2018). These substances are composed of approximately 80% soil organic matter (SOM), and include HA, fulvic acids (FA), and humin (de Melo et al., 2016; Schnitzer, 1978). Humic substances face intense depletion as a consequence of intensive cropping systems (Senesi et al., 2007). As a result, researchers have been attempting to replenish HS in the soil through external applications (Gerke, 2018; Rose et al., 2014). These externally applied HS are obtained from various sources, such as lignite, peat, Humalite, and compost, as well as artificial sources (Akimbekov et al., 2021; Gollenbeek & van der Weide, 2020; Lanno et al., 2022; Rathor et al., 2024; Yang et al., 2021). Humic substances have a positive effect on soil health, improve nutrient uptake and crop yields when applied in crops such as barley, maize, cowpea, canola, mustard, and millet (Arslan et al., 2021; Canellas et al., 2019; Eyheraguibel et al., 2008; García, Santos, et al., 2016; Kahraman, 2016; Laskosky et al., 2020; Malik et al., 2023; Mourad et al., 2021; Olaetxea et al., 2020; Rajpar et al., 2011; Rathor et al., 2024; Rose et al., 2014; J. Shen et al., 2020; Vujinović et al., 2020; Yakhin et al., 2017). Under field conditions, HS improve plant growth and root architecture by enhancing root length, thickness, density, and branching (García, de Souza, et al., 2016; Mora et al., 2010; Tavares et al., 2021). Furthermore, HS enhance NUE by improving nutrient uptake and assimilation (Chen et al., 2004; Nardi et al., 2017; Zhang et al., 2019). This is associated with the chelating ability of HS to form complex but stable natural compounds with metals, thereby improving the bioavailability and solubility of soil nutrients (Chen et al., 2004). However, crop yield responses to HS applications remain inconsistent, resulting in skepticism about the effectiveness of HS products (Billingham, 2015; Bybordi & Ebrahimian, 2013; Hartz & Bottoms, 2010; Mohammed et al., 2019). Some studies have reported no positive effects on crop growth and development after HA application (Albiach et al., 2001; Mukherjee et al., 2014). For example, a combination of biochar and HA did not improve soil fertility and crop productivity (Holatko et al., 2020). Rose et al. (2014), in their meta-analysis, observed that although plant growth responses to HS are generally positive, they are influenced by a variety of environmental and management factors, including the source of the HS; their review also revealed that most successful studies were conducted under controlled conditions.

However, past studies report that HA application in the presence of synthetic fertilizers, such as urea, increased crop yields (Gao et al., 2022; Osman et al., 2013; Zheng, 1991). Humalite-fertilizer interactions have been reported to enhance maize growth, improve fertilizer efficiency, reduce N losses, increase microbial populations and diversity, and improve maize root growth and development (Araújo et al., 2017; Canellas & Olivares, 2014; Puglisi et al., 2013). For example, humic acid urea is an enhanced efficiency organic-inorganic compound fertilizer consisting of a mixture of HA and conventional urea. Humic acid urea has been shown to increase crop yield, biomass, N uptake, and delay urea hydrolysis by inhibiting urea nitrification and ammonification (Rose et al., 2016; Saha et al., 2017; Zhang et al., 2019), and thus, improving NUE (Shen et al., 2020). Several research gaps were identified by Ampong et al. (2022), indicating a paucity of data regarding the application rates of specific HS sources and their interaction with urea fertilizer, especially under field conditions to provide producers with HA source-specific information. Humalite was evaluated because it is a naturally occurring humic organic substance containing high concentrations of HA (61 - 88%), close to zero nutrients, low amounts of micronutrients and heavy metals (Loring Laboratories Ltd. 6835 8St N.E. Calgary Alberta, Canada T2E 7H7 - File No: RC20-0257); the low amounts of micronutrients and heavy metals is due to its unique freshwater depositional environment. Large Humalite deposits are found in Southern Alberta and available to producers who are applying it on their fields. Currently, there are no scientific studies identifying optimum Humalite rates and their interaction with urea under field conditions, particularly in Western Canada. Previous studies have been conducted exclusively under controlled conditions (Rathor et al., 2023, 2024, 2024a; Laskosky et al., 2020). Under controlled environmental conditions, Humalite is reported to enhance wheat growth, grain yield, and protein content by improving soil N availability and nutrient uptake (Rathor et al., 2024). Although Humalite has been applied on-farm by some crop producers, there is a dearth of research on appropriate Humalite application rates and whether Humalite application leads to reduced urea application rates. Therefore, the objectives of this three-year field study were to (1) identify the optimum Humalite application rates at three contrasting sites in Alberta, (2) assess the effect of different Humalite plus urea application rates on wheat and canola yields and protein contents, and (3) evaluate whether producers profit when Humalite is applied in grain systems.

2.2 Materials and Methods

2.2.1 Study sites and growth conditions

Field trials were conducted in small plots over three growing seasons (2021-2023) at three sites in Alberta, Canada. Experimental trials were carried out on no-tilled land at (1) St. Albert Research Station, University of Alberta (53.6929508, -113.6353861), the soil at this site is described as Luvic Chernozem (IUSS Working Group WRB. 2022) and the soil texture is classified as silty clay loam (sand 6%, silt 56%, clay 38%); (2) Gateway Research Organization (GRO) near Westlock (54.0840915, -113.8496014), the soil described as albic solonetz (IUSS Working Group WRB. 2022); and soil texture is classified as loamy soil (sand 38%, silt 40%, clay 22%) and (3) Battle River Research Group (BRRG) near Forestburg (52.522269, -111.962730), the soil described as haplic kastanozem (IUSS Working Group WRB. 2022); and soil texture is classified as loamy (sand 40%, silt 39%, clay 21%). The previous crops at each site were barley at St. Albert, wheat at GRO, and field peas at BRRG. Environmental data was obtained from the Alberta Climate Informational Service website (Government of Canada & Alberta Government, 2020). The monthly average temperatures and total precipitation at each site for the three growing seasons are summarized (Figure 2.1).

A minimum of six random soil cores were taken in the spring, pre-seeding at a depth of 0-15 cm from each block to form a composite sample that was analyzed characterize the soil (Table 2.1) and to determine urea application rates at each site (Table 2.2). Soil pH was measured using a 1:2 soil: water extraction method with a pH meter (McKeague, 1978). Soil Electrical Conductivity (EC) was determined by measuring the electrical resistance in a soil-water mixture between two electrodes, inversing the value, and then multiplying by a conversion rate of 2.06 (McKeague, 1978). Nutrient extraction of water-soluble nitrate, nitrite, available phosphate, and exchangeable potassium from the soil samples was measured using the modified Kelowna extraction solution (Ashworth and Mrazek, 1995). Nitrate, phosphate, and potassium were analyzed using continuous flow colorimetry, and nitrite was analyzed using a SmartChem colorimetric discrete analyzer. Nitrate analysis was performed using a 2.0 M KCl extract by Segmented Flow Analysis (Carter & Gregorich, 2008). Phosphate analysis was performed using the Stannous Chloride method (American Public Health Association et al., 2023). Potassium analysis was measured using an Automated Flame Photometry Method (Dieken & Alberta Research Council, 1996). The obtained data were used to make site-specific fertilizer application rate recommendations for the next crop provided by (A & L Canada Laboratories Inc., 2136 Jetstream Road, London, Ontario, Canada N5V 3P5). Soil organic matter content was measured by oven-drying the sample, followed by burning organic matter, and then calculating the percentage of organic matter from the weight loss on ignition (McKeague, 1978).

Table 2. 1 Three-year (2021-2023) baseline soil chemical properties at three sites before wheat and canola seeding [St. Albert: St. Albert Research Station, University of Alberta, BRRG: Battle River Research Group, GRO: Gateway Research Organization; SOM: Soil organic matter, NO_3-N: Soil available nitrate-nitrogen, CEC: Cation exchange capacity, Mg: Magnesium, Ca: Calcium, Na: Sodium, Al: Aluminum].

Site properties	2021	2022	2023
St Albert			
pH	8	6.8	7.4
SOM (g kg^{-1})	106	77	NA
NO_3-N (mg kg^{-1})	12	8	14
CEC (cmol kg^{-1})	NA	20.5	NA
Mg (mg kg^{-1})	306	302	NA
Ca (mg kg^{-1})	3450	3280	NA
Na (mg kg^{-1})	38	38	NA
Al (mg kg^{-1})	352	378	NA
BRRG			
pH	4.9	4.8	4.9
SOM (g kg^{-1})	52	55	54
NO_3-N (mg kg^{-1})	12	17	15
CEC (cmol kg^{-1})	23.2	20.3	19.9
Mg (mg kg^{-1})	235	215	235
Ca (mg kg^{-1})	970	1170	1080
Na (mg kg^{-1})	41	40	31
Al (mg kg^{-1})	1018	912	905
GRO			
pH	5.5	5.1	5.6
SOM (g kg^{-1})	41	43	46
NO_3-N (mg kg^{-1})	11	14	7
CEC (cmol kg^{-1})	17.1	18	21.6
Mg (mg kg^{-1})	249	232	210
Ca (mg kg^{-1})	1710	1660	1690
Na (mg kg^{-1})	42	49	46
Al (Al mg kg^{-1})	597	803	798

Note: NA(data not available).

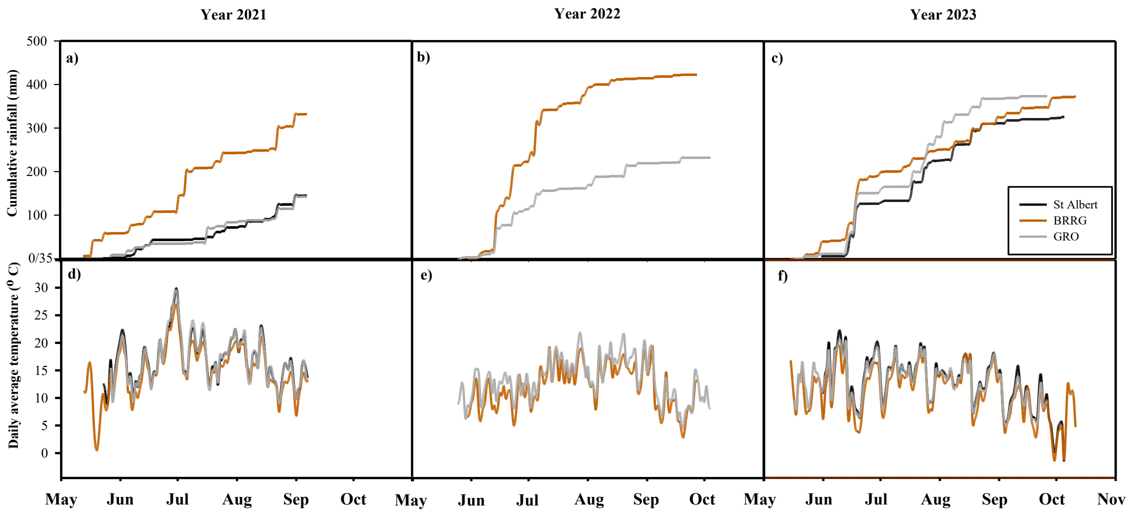

Figure 2.1 Cumulative precipitation (a - c) and daily average temperature (d - f) at St. Albert, Battle River Research Group (BRRG); Gateway Research Organization (GRO) during three crop growing seasons (2021 – 2023).

2.2.2 Experimental design and treatments

The experiment was conducted in a split-plot design with four replications for a total of 60 plots per site. Urea fertilizer levels were the main plot factor, applied at three levels: no urea control, half recommended rate, and recommended urea rate based on soil test at each site viz. St. Albert site: half recommended rate (126, 213 & 112 kg ha^{-1}), and recommended urea rate (251, 409 & 244 kg ha^{-1}) for 2021, 2022, and 2023 respectively; BRRG site: half recommended rate (77, 141 & 129 kg ha^{-1}), and recommended urea rate (154, 281 & 268 kg ha^{-1}) for 2021, 2022, and 2023 respectively; GRO: half recommended rate (106, 123 & 115 kg ha^{-1}), and recommended urea rate (244, 246 & 267 kg ha^{-1}) for 2021, 2022, and 2023 respectively. The subplots were Humalite rates at five levels: i) 0, ii) 112 kg ha^{-1}, iii) 224 kg ha^{-1}, iv) 448 kg ha^{-1}, and v) 896 kg ha^{-1} applied in 2021 and 2022. In 2023, a 56 kg ha^{-1} treatment was introduced, and the 896 kg ha^{-1} treatment was dropped from all trial sites. Humalite, supplied by WestMet Ag (Hanna, Alberta, Canada), was used as a biostimulant in this field study across all sites and years. In 2020, lab analysis indicated that Humalite contains 61 - 88% HAs depending on texture (fine or coarse), close to zero nutrients, low amounts of micronutrients and heavy metals (Loring Laboratories Ltd. 6835 8St N.E. Calgary Alberta, Canada T2E 7H7 - File No: RC20-0257). Chemical constituents and their concentration can be found in the supplementary material of Rathor et al. (2024). Humalite is found exclusively in a 20 km area Southeast of the town of Hanna, Alberta, Canada, specifically within the holdings of the Sheerness Coal Mine (Recently: WestMet Ag), in large deposits. Humalite is extracted from shallow deposits, typically located a few meters below the soil surface. It is believed to have naturally form via the decomposition of organic matter in a freshwater environment when coal was formed during the Ice Age, which distinguishes Humalite from other humic substances that have the likelihood of heavy metals resulting from salt water. Hence, Humalite is unique with high HA content and low heavy metal concentrations.

In all years, P, K, and S at each site were applied at recommended rates based on soil tests (Table 2.2). The source of P was Triple superphosphate (0-45-0), for K was Muriate of Potash (0-0-60), and granulated elemental sulphur for the S. The crop rotational sequence at all sites from 2021 - 2023 was wheat (AAC Brandon) - canola (RR45CM39) - wheat (AAC Brandon). In 2021 and 2022, Humalite was broadcasted and rototilled before seeding; whereas in 2023, both urea and Humalite were side-banded at seeding.

Table 2. 2 Fertilizer application rates (kg ha^{-1}) at three prairie sites for three growing seasons [St. Albert: St Albert Research Station, University of Alberta, BRRG: Battle River Research Group and GRO: Gateway Research Organization; Zero: No urea application, Half: One-half of the recommended urea rate application, Recommended: Recommended urea rate application; TSP Triple superphosphate: MOP: Muriate of Potash].

	2021 (wheat)			2022 (canola)			2023 (wheat)		
Sites	Residual	Half	Recommended	Zero	Half	Recommended	Zero	Half	Recommended
St Albert									
Urea	-	126	251	-	213	409	-	122	244
TSP	120	-	-	75.6	100	88.9	120	111.1	120
MOP	140	-	-	103	130	150	18	10	153
Sulfur	24	0	0	33	40	40	19	0	0
BRRG									
Urea	-	77	154	-	141	281	-	129	268
TSP	24	-	-	49	49	49	38	38	24
MOP	28	-	-	47	47	47	37	37	37
Sulfur	0	0	0	0	7	0	0	0	0
GRO									
Urea	-	106	244	-	123	246	-	115	267
TSP	62	-	-	76	76	76	-	62	62
MOP	28	-	-	37	37	37	-	37	37
Sulfur	20	0	0	13	13	13	-	13	7

2.2.3 Crop establishment and data collection

Crop were seeded using a small plot Seeder, Fabro Drill (Swift Current SK, Canada) mounted with six TechnoTill openers (Wetaskiwin AB, Canada) with nine inches of row spacing at BRRG site, a Fabro zero till (Swift Current SK, Canada) small plot seeder (6 rows) with a nine-inch row spacing at the GRO site, and new Fabro seeder (8 rows) (Swift Current SK, Canada) with 7 inches row spacing at St. Albert site. Seeding and harvesting dates at each site year are reported in Table 2.3. The targeted yield was 4035 kg ha^{-1} and 5380 kg ha^{-1} for canola (2022) and wheat (2021 & 2023), respectively. The targeted plant density was 350 plants/m^2 for wheat and 110 plants/m^2 for canola. Pre-burn and in-crop herbicides applied differed between sites and have been summarized in Table 2.3. Crops were harvested at crop maturity. Data on the grain yield, yield parameters, and grain moisture content were collected during harvest. Crops were harvested with a Zurn 150 plot harvester at GRO and with Wintersteiger Classic harvesters at the other two sites. Quality parameters assessed include thousand kernel weight (TKW) and grain protein. Canola and wheat yields were adjusted to 8% and 14% seed moisture content, respectively, using the following formula:

$$Adjusted\ yield\ (kg/ha) = (Harvest\ yield)\ x\ [(100 - Moisture\ Content\ (\%))/\ (100\text{-}Moisture\ Content\ (\%))]$$

Near-infrared spectroscopy (Mininfra SmarT NIT Analyzer) was used to analyze the protein content of the subsampled wheat grains.

Table 2. 3 Seeding, harvesting, and herbicides applied dates for wheat and canola from 2021 to 2023 growing seasons at three sites in Alberta [St. Albert: St. Albert Research Station, University of Alberta, BRRG: Battle River Research Group near Forestburg, GRO: Gateway Research Organization near Westlock].

Years	Sites	Seeding dates	Harvesting dates	Pre-seed herbicides	In-crop herbicides
2021 (wheat)	St. Albert	23-May	07-Sep	Glyphosate	Stellar™ XL and Axial®
	BRRG	13-May	07-Sep	Glyphosate	Stellar™ XL and Axial®
	GRO	27-May	08-Sep	Glyphosate + Heat®	Viper® + UAN
2022 (canola)	St. Albert	13-May	15-Sep	Glyphosate	Glyphosate + Lontrel™ XC + Poast® Ultra
	BRRG	30-May	27-Sep	Glyphosate	Glyphosate
	GRO	25-May	04-Oct	Not Applied	Glyphosate
2023 (wheat)	St. Albert	31-May	05-Oct	Glyphosate	Stellar™ XL and Axial®
	BRRG	15-May	11-Oct	Glyphosate	Stellar™ XL and Axial®
	GRO	17-May	26-Sep	Glyphosate + MCPA Ester	Curtail M + Axial®

2.2.4. Economic analysis

An economic analysis was conducted to evaluate the profitability of urea and Humalite use in wheat-canola-wheat crop rotation by comparing returns on investment in urea and Humalite, and the control treatment. Urea fertilizer purchase price per tonne was obtained from Sturgeon Valley Fertilizer Ltd, St. Albert, annually at 1548 CAD, 2554 CAD, and 2128 CAD in 2021, 2022, and 2023, respectively. Humalite cost per tonne was obtained from WestMET Group Canada Ltd at 300 CAD dollars. Wheat and canola prices were obtained from the Alberta economic dashboard and the Alberta Canola Producers Commission, respectively (594 CAD per tonne for canola and 438 CAD per tonne for wheat). The net revenue was calculated as gross revenue (crop price multiplied by yield) minus Humalite and urea costs for each treatment per site-year. Other production and field management costs were assumed to cancel out in this economic analysis because they are standard expenditures would have been incurred normally by crop producers. This approach isolates the economic impact of adding Humalite and urea, allowing us to accurately assess their contribution to overall production costs and net revenue.

2.2.5. Data analysis

The data were statistically analysed using R (v4.1.2; R Core Team 2023). Data were subjected to the analysis of variance (ANOVA) using a linear mixed effects model to assess the effects of different treatments across sites. The Akaike Information Criterion (AIC) were employed to evaluate the appropriateness of various models. These criteria serve to measure the quality of each model relative to each other, with a lower value indicating a better fit and model. The three years were analysed separately because of the different Humalite rates in 2023 and the different crops planted in each growing season. In the model, urea and Humalite rates and all possible interactions were fixed factors, while the replicates were the random factors. ANOVA and mean separation were performed using the lmer and emmeans functions. The significance of differences was evaluated using probability levels of $P \leq 0.05$ and $P \leq 0.01$. Boxplots were used to assess the effect of the different treatments on net revenue. Data visualization was plotted using the "ggplot2()" package.

2.3 Results

2.3.1 Environmental conditions during crop growth

Environmental conditions varied between cropping calendars. In 2021, significantly higher temperatures were encountered earlier (June) in the season, with temperatures greater than 30° C in July (Figure 2.1 d-f). Hence, 2021 was considered a dry year as drought conditions persisted, contrary to 2023, where dry conditions were encountered mostly at the beginning of the growing season. St. Albert and BRRG had lower accumulated rainfall compared to BRRG (Figure 2.1a,b) and similar moisture trends throughout the 2023 growing season (Figure 2.1c).

2.3.2 Effects of different Humalite and urea application rates on grain yields, protein, and total kernel weight

There was a significant effect of urea and site on wheat grain yields and there was a significant urea *x* Humalite *x* site interaction in 2021 (Table 2.4). In 2022, the main effects of sites on canola yields were significant, and there was a significant urea *x* site interaction. In 2023, the main effects of urea, Humalite, and site were significant, and there was a significant effect of urea *x* site and urea *x* Humalite *x* site interaction. The average wheat yields were lower than targeted and ranged from 2800 - 4200, 2406 - 4371, and 2142 - 2669 kg ha^{-1} at St. Albert, BRRG and GRO, respectively. At BRRG, significantly higher wheat yields were observed at 224 kg ha^{-1} Humalite rates plus half urea recommended rate. When Humalite was applied at 112 kg ha^{-1} plus half urea rates, wheat yields were higher but not significantly different from the zero-urea treatment (Figure 2.2). When no Humalite was applied, the highest yields were observed when urea was applied at recommended rates. At 448 and 896 kg ha^{-1} Humalite, wheat yields were higher when urea was applied at recommended rates, but these yields were not significantly different to those at half urea rates. At BRRG, wheat protein content ranged from 15.4 (recommended urea rates plus 224 kg ha^{-1} Humalite) to 17.1 % (Table 2.4). Wheat TKW was unaffected by all treatments at all sites (Table 2.5). At GRO, wheat yields were higher at half urea recommended rates irrespective of Humalite application rates, although this increased yield was not significantly different from those at zero and recommended urea rates (Figure 2.2). At GRO, wheat protein content was $\geq$ 17% in all treatments assessed (Table 2.5). The lowest mean wheat yields at St. Albert resulted from zero urea plus 896 kg/ha Humalite rates, zero urea plus 448 and 896 kg ha^{-1} Humalite rates at GRO, and zero urea plus 448 kg ha^{-1}

Humalite at BRRG (Figure 2.2). At St. Albert, the highest wheat yields were observed when urea was applied at the recommended urea rate across all Humalite rates except at 448 kg ha^{-1} Humalite, where half recommended urea rate had significantly higher yields than recommended and zero urea (Figure 2.2). At the St. Albert research site, wheat protein was less than 13.5% in the zero urea plus Humalite application rates and greater than 13.5% at half and recommended urea plus Humalite application rates (Table 2.5).

In 2022, the St. Albert site was hit by hail, so the canola yield from this site was excluded from the analysis as the data was considered unreliable. No significant differences in canola TKW were observed between treatments at St. Albert. Canola yields ranged from 5076 - 6212 and 3577 - 4158 kg ha^{-1} at BRRG and GRO sites, respectively. The lowest canola yields were recorded with recommended urea rates at BRRG and the highest canola yields were observed with half recommended and recommended urea at GRO (Figure 2.3). Humalite and urea rates had no effects on canola TKW at BRRG and GRO.

In 2023, at BRRG, significantly higher wheat yields were observed at recommended urea plus 112, 224, and 448 kg ha^{-1} Humalite rates when compared with 56 kg ha^{-1} and zero Humalite (Figure 2.4). At 56 kg ha^{-1} Humalite rate, similar wheat yields were observed between the half and recommended urea rates, and these yields were significantly different from wheat yields from no urea treatments. Wheat seed protein contents ranged from 12.9 - 14.7 % and increased as urea rates increased (Table 2.5). At the GRO site, wheat yields were significantly higher at half and recommended urea rates compared to zero urea with or without Humalite application and similar between half and recommended urea rates (Figure 2.4). The seed protein content at GRO increased mostly increased urea application (Table 2.5). The highest wheat yield (3830 kg ha^{-1}) was observed at the recommended urea rate plus 224 kg ha^{-1} Humalite rate, while the lowest yield (2641 kg ha^{-1}) was observed at zero urea plus 448 kg ha^{-1} Humalite at St. Albert site (Figure 2.4). When no Humalite was applied, significantly higher wheat yields were observed at recommended urea application, with similar yields for half or no urea rates. At 56 and 448 kg ha^{-1} Humalite rates, higher wheat yields were observed at half urea rates, although these yields were similar to the no and recommended urea rates at 56 kg ha^{-1} Humalite rates and similar to the recommended urea rates at 448 kg ha^{-1} Humalite rates. At 112 kg ha^{-1} Humalite rate, wheat yield at half and recommended urea rates were similar, while at 224 kg ha^{-1} Humalite rate, wheat yield was significantly higher at recommended urea rates compared to no or half urea rates (Figure 2.4). Wheat protein was similar and greater than 13.5% at half and recommended urea irrespective of Humalite rates (Table 2.5).

Table 2. 4 Analysis of Variance (ANOVA) table showing Humalite effects on wheat and canola yields at three sites for three growing seasons (2021 – 2023).

	2021	2022	2023
Source of variation		P - values	
Urea	0.001***	ns	0.001***
Humalite	ns	ns	0.01*
Site	0.001***	0.001**	0.001***
Urea *x* Humalite	ns	ns	ns
Urea *x* Site	ns	0.04*	0.001***
Humalite *x* Site	ns	ns	ns
Urea *x* Humalite *x* Site	0.04*	ns	0.01*

*, **, *** indicate significant differences at $P < 0.05$, $P < 0.01$, $P<0.001$, respectively; ns indicates not significant

Table 2. 5 Effects of Humalite on wheat and canola protein content and total kernel weight at different sites over three years.

			2021 (Wheat)						2022 (canola)		2023 (wheat)		
	HR (Kg/ha)		St Albert		BRRG		GRO		BRRG	GRO	St Albert	BRR	GRO
Urea rates	*2021 &*	*202*	*Protein (%)*	*TKW (g)*	*Protei n (%)*	*TKW (g)*	*Protein (%)*	*TKW (g)*	*TKW (g)*		*Protein (%)*		
Zero	0	0	11.8^{a}	38.1^{a}	16.8^{ab}	32.5^{a}	17.1^{abcd}	38.5^{a}	3.7^{a}	4.3^{a}	12.7^{a}	12.9^{a}	12.3^{ab}
	112	56	12.7^{abc}	39.2^{a}	16.8^{ab}	34.3^{a}	17.0^{abc}	39.6^{a}	3.7^{a}	4.2^{a}	12.5^{a}	13.2^{a}	12.1^{a}
	224	112	13.2^{abc}	39.5^{a}	16.4^{ab}	33.0^{a}	16.9^{ab}	38.3^{a}	3.5^{a}	4.3^{a}	13.0^{ab}	13.7^{a}	13.5^{abc}
	448	224	11.9^{ab}	39.6^{a}	17.1^{b}	32.6^{a}	17.3^{abcde}	38.2^{a}	3.5^{a}	4.4^{a}	12.5^{a}	13.9^{a}	12.7^{abcd}
	896	448	12.3^{ab}	37.2^{a}	17.1^{b}	32.0^{a}	16.8^{a}	38.3^{a}	3.4^{a}	4.2^{a}	13.1^{abc}	13.9^{a}	12.5^{abc}
Half Recommended	0	0	13.7^{abc}	38.8^{a}	16.9^{ab}	34.6^{a}	17.6^{bcde}	38.9^{a}	3.7^{a}	4.1^{a}	13.9^{bc}	14.0^{a}	13.4^{cdef}
	112	56	14.7^{abc}	39.3^{a}	17.0^{b}	32.6^{a}	17.5^{abcde}	39.1^{a}	3.5^{a}	4.2^{a}	14.0^{bc}	14.1^{a}	13.2^{bcde}
	224	112	14.1^{abc}	38.5^{a}	17.0^{b}	33.2^{a}	17.7^{cde}	38.6^{a}	3.5^{a}	4.2^{a}	14.0^{bc}	14.1^{a}	13.3^{bcdef}
	448	224	14.6^{abc}	38.7^{a}	17.1^{b}	33.2^{a}	17.6^{bcde}	39.7^{a}	3.6^{a}	4.2^{a}	13.8^{bc}	14.1^{a}	13.6^{defg}
	896	448	14.5^{abc}	37.8^{a}	16.8^{ab}	33.8^{a}	17.7^{e}	38.3^{a}	3.4^{a}	4.3^{a}	13.9^{bc}	14.2^{a}	13.5^{cdefg}
Recommended	0	0	15.5^{abc}	41.2^{a}	16.3^{ab}	32.9^{a}	17.9^{e}	39.7^{a}	3.3a	4.3^{a}	14.2^{c}	14.3^{a}	14.2^{efg}
	112	56	16.1^{c}	39.7^{a}	16.0^{ab}	31.9^{a}	17.7^{cde}	38.1^{a}	3.4^{a}	4.3^{a}	14.2^{c}	14.5^{a}	14.2^{efg}
	224	112	15.7^{bc}	39.0^{a}	15.5^{a}	33.8^{a}	17.9^{e}	39.2^{a}	3.3^{a}	4.0^{a}	14.2^{c}	14.6^{a}	14.3^{fg}
	448	224	16.3^{c}	39.0^{a}	16.6^{ab}	33.0^{a}	17.8^{de}	38.9^{a}	3.1^{a}	4.1^{a}	14.2^{c}	14.6^{a}	14.4^{fg}
	896	448	15.6^{abc}	39.0^{a}	16.7^{ab}	33.2^{a}	17.9^{e}	37.8^{a}	3.4^{a}	4.1^{a}	14.2^{c}	14.7^{a}	14.2^{efg}

Different letters indicate significant differences at P = 0.05 within each column, HR: Humalite rates. BRRG, Battle River Research Group; GRO, Gateway Research Organization

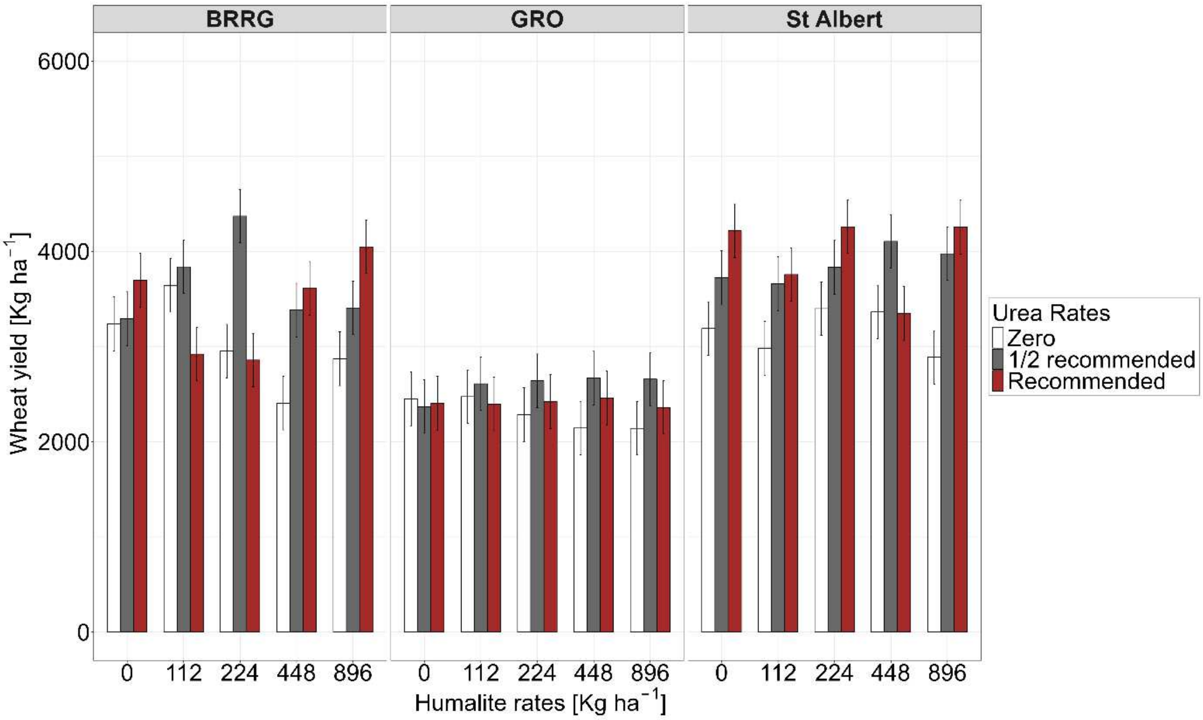

Figure 2.2 Wheat yields under different Humalite and urea rates at different sites in 2021 growing season [Error bars represent standard error of the means; St. Albert: St. Albert Research Station, University of Alberta, BRRG: Battle River Research Group near Forestburg, GRO: Gateway Research Organization near Westlock].

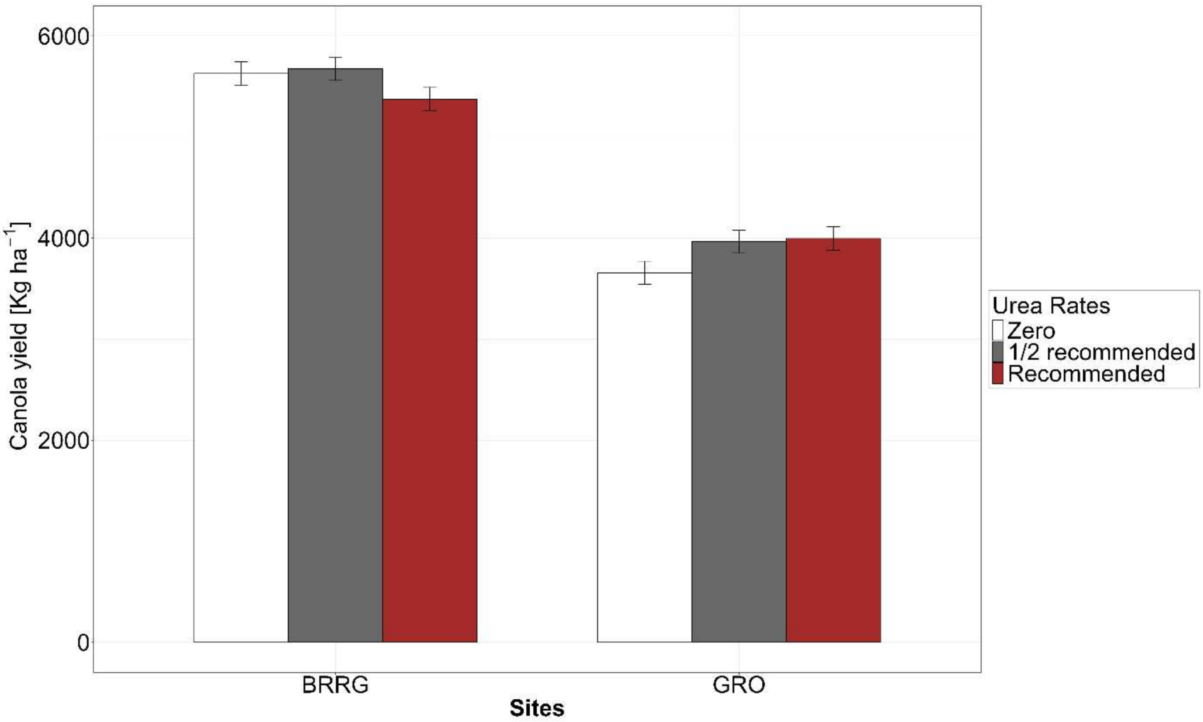

Figure 2.3 Effects of different urea rates on canola yields at two sites. Error bars represent standard error of the means. [Error bars represent standard error of the means; St. Albert: St. Albert Research Station, University of Alberta, BRRG: Battle River Research Group near Forestburg, GRO: Gateway Research Organization near Westlock].

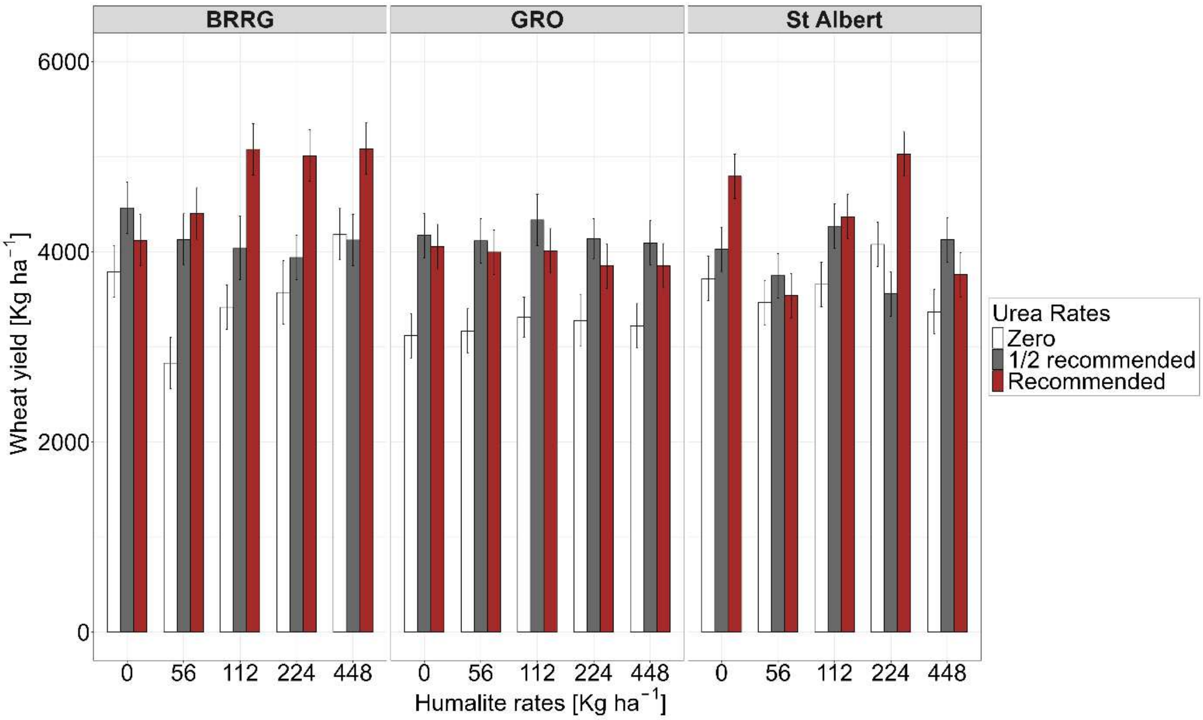

Figure 2. 4 Wheat yields at different Humalite and urea application rates at three sites in the 2023 season [Error bars represent standard error of the means; St. Albert: St. Albert Research Station, University of Alberta, BRRG: Battle River Research Group near Forestburg, GRO: Gateway Research Organization near Westlock].

2.3.3 Economic analysis of Humalite application in the presence of different urea rates

The net revenue generated indicated that profitability depended on site, Humalite, and urea rates. No revenues were lost, i.e., no negative values at all Humalite and urea rates but net revenues across sites and years for each treatment exhibited great variability (Figures 2.5-2.7). More wheat net revenue was generated at St. Albert and BRRG compared to the GRO site in 2021 (Figure 2.5). At the BRRG, the highest net revenue was observed in wheat at 224 kg ha^{-1} Humalite plus half urea rates in 2021 (Figure 2.5). The lowest revenue was generated at 112 and 224 kg ha^{-1} Humalite plus recommended urea application rate. Half and recommended urea plus 448 kg ha^{-1} Humalite rates generated similar net revenues that were higher than the no urea treatment; at 896 kg ha^{-1} Humalite rate, a similar trend was observed although the mean net revenue for the recommended urea treatment was higher (Figure 2.5). At the GRO site, the net wheat revenue was highest when no and 112 kg ha^{-1} Humalite was applied, plus no urea

(Figure 2.5). Net revenues were lowest at all Humalite rates (except at 896 kg ha^{-1} Humalite rate) plus recommended urea. At St. Albert, the highest mean wheat revenue was observed at 112 kg ha^{-1} Humalite plus the recommended urea rate (Figure 2.5). Half urea rates generated a wide range of net revenues with the highest observed at 448 kg ha^{-1} Humalite rate. The lowest net revenues were observed at 896 kg ha^{-1} Humalite at all urea rates.

Based on the net revenue calculations undertaken in 2022, more revenues were generated at the BRRG compared to the GRO site (Figure 2.6). At BRRG, the no urea and recommended urea rates generated the highest and lowest net revenue, respectively. Irrespective of Humalite application rates, half urea rates generated more mean canola net revenues (Figure 2.6). At GRO, the highest net canola revenues were observed at zero and half urea rates irrespective of Humalite application rates; the lowest net canola revenues were generated when urea was applied at recommended rates (Figure 2.6).

In 2023, when no Humalite was applied at the BRRG site, zero and half urea rates had similar net wheat revenues that were significantly higher than the recommended urea rate (Figure 2.7). At 56 kg ha^{-1} Humalite, half and recommended urea rates had significantly higher net wheat revenues compared to the no urea treatment; meanwhile, at 112 and 224 kg ha^{-1} Humalite rates, the recommended urea treatment had the highest wheat net revenue (Figure 2.7). At 448 kg ha^{-1} Humalite, zero urea treatment had the highest net revenue. At GRO, there was a distinct pattern across all Humalite treatments. When half urea recommended rates were applied, net wheat revenue was higher compared to no and recommended urea treatments; the later had the lowest net revenue (Figure 2.7). At St. Albert, the highest wheat net revenue was observed at 224 kg ha^{-1} Humalite rate plus zero urea, while significantly low net revenues were observed at 56 and 448 kg ha^{-1} Humalite rates when urea was applied at recommended rates (Figure 2.7). At 112 kg ha^{-1} Humalite rate, a wide range of wheat net revenues were observed with half urea rates.

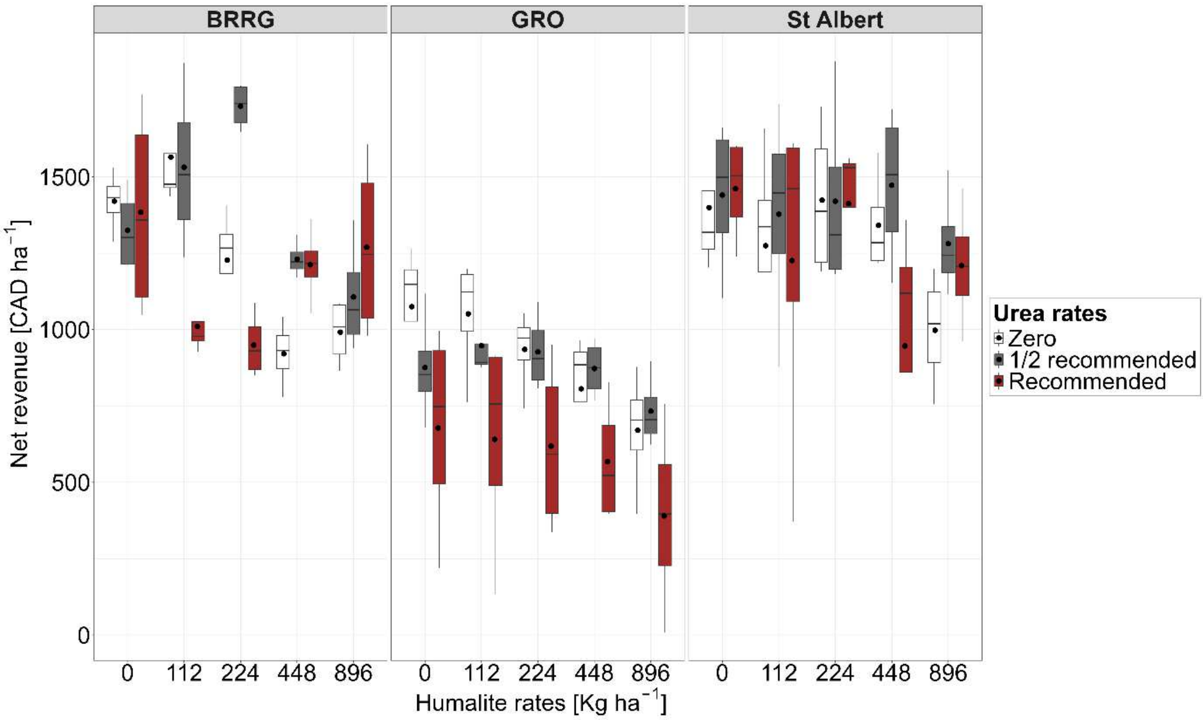

Figure 2.5 Net revenue generated with different Humalite application rates at reduced and recommended urea rates for wheat yields at three sites in 2021. Black dot in each plot indicates the mean. [Error bars represent standard error of the means; St. Albert: St Albert Research Station, University of Alberta, BRRG: Battle River Research Group near Forestburg, Alberta, GRO: Gateway Research Organization near Westlock, Alberta].

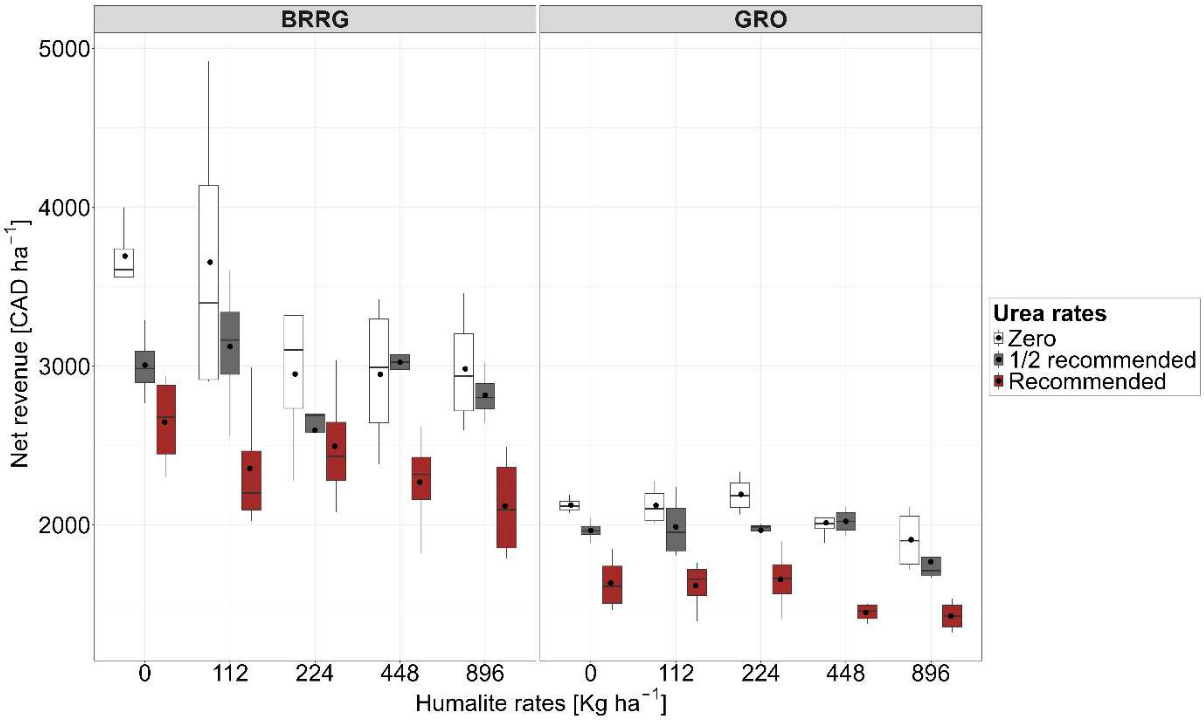

Figure 2.6 Net revenue generated with different Humalite application rates at reduced and recommended urea rates for canola yields at two sites in 2022. Black dot in each plot indicates the mean. [Error bars represent standard error of the means; St. Albert: St Albert Research Station, University of Alberta, BRRG: Battle River Research Group near Forestburg, Alberta, GRO: Gateway Research Organization near Westlock, Alberta].

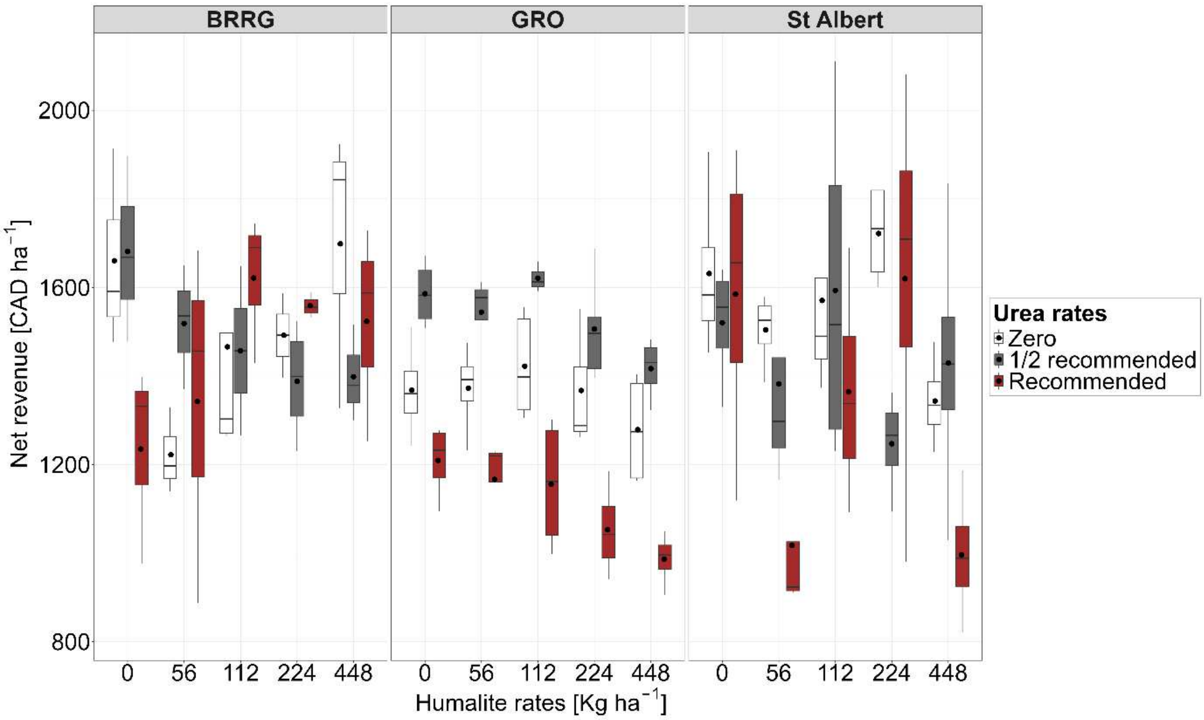

Figure 2.7 Net revenue generated with different Humalite application rates at reduced and recommended urea rates for wheat yields at three sites in 2023. Black dot in each plot indicates the mean. [Error bars represent standard error of the means; St. Albert: St Albert Research Station, University of Alberta, BRRG: Battle River Research Group near Forestburg, Alberta, GRO: Gateway Research Organization near Westlock, Alberta].

2.4 Discussions

Humic substances as biostimulants are reported to either reduce fertilizer inputs and/or promote plant growth and crop yields (Ampong et al., 2022; Brown et al., 2020; Olaetxea et al., 2020; Varanini & Pinton, 2000; Vikram et al., 2022). In a field study conducted in China, the combined HA plus urea application increased maize biomass and grain yields by 11.5 - 21.3 % compared to urea only plots (Zhang et al., 2019). In another 2-year maize-wheat field experiment with various urea and HA-treated urea treatments, both wheat and maize yield increases were also reported. These increases were attributed to reduced N losses and prolonged effects of fertilizer (Kong et al., 2022). In the present study, responses to Humalite and urea application rates varied by site (Figures 2.2, 2.3, 2.4). In 2021, the combination of half urea recommended rates plus 448 kg ha^{-1} Humalite resulted in a yield increase of 8.4% at GRO (Figure 2.2). At BBRG, half urea recommended rates plus 224 kg ha^{-1} Humalite produced a significant yield increase of 34.9%, outperforming control treatments. At St. Albert, the application of full urea recommended rate plus 224 kg ha^{-1} of Humalite recorded the highest yield increase of 33.5% compared to the control (Figure 2.2). A reason for these variable observations could be the amount of soil organic matter (SOM) at these sites (Table 2.1). Humic acids form an integral part of SOM. Therefore, HA (Humalite) application can positively influence soil aggregate stability and nutrient uptake, thus potentially improving crop yields, especially in soils with low clay content and organic matter (Piccolo et al., 1997; Zhou et al., 2019). This may be the reason for better responses to Humalite applications at lower Humalite application rates observed at the BRRG site, which had low SOM levels (5.2%) (Table 2.1). Soil-dissolved organic matter can perform similar functions as applied HS (García et al., 2016). Hence, the high organic matter present in St. Albert (SOM = 10.6%) may be negating the effects of Humalite. This may explain why positive results are mostly observed in sites with low SOM.

Past research shows that the addition of HS with NPK fertilizers results in a significant yield increase, especially in low SOM soils (Duan et al., 2024; Selim et al., 2009; Tahir et al., 2011; Zhou et al., 2019, 2022). Although the lowest wheat yields were observed at GRO, lower Humalite application rates still resulted in increased yields. This suggests that regardless of site and urea rate, the optimal Humalite rate may vary between 112 – 448 kg ha^{-1} depending on SOM content. These findings were similar to results from previous studies indicating that moderate HS application rates are beneficial for plant growth compared to higher rates (Arjumend et al., 2015; Tahir et al., 2011; Tan & Nopamornbodi, 1979).

In 2022, significant effects of Humalite on canola yields were not observed in BRRG and GRO sites. The lowest canola yields were observed at GRO, primarily due to consistently poor rainfall throughout the growing season compared to the BRRG site (Figure 2.3). This absence of rainfall, particularly at the onset of the growing season may have resulted in moisture stress, thus hindering the plants' ability to absorb water and nutrients at a critical phase of the plant development. Furthermore, urea application significantly influenced canola yields and trends were inconsistent in BRRG and GRO sites. At BRRG, the highest canola yields were recorded in control and half urea recommended rates, while for GRO, yields consistently increased with higher urea rates (Figure 2.3). Yield responses to increasing N fertilizer rates vary depending on factors such as rainfall, soil moisture content, soil type, and residual fertility (Cheema et al., 2001).

The observed consistent benefit of applying Humalite at lower rates on crop yields led to the adjustment of Humalite application strategy in 2023, involving the eradication of 896 kg ha^{-1} and addition of 56 kg ha^{-1} (Figure 2.4). Similar results were reported by Tahir et al. (2011) in Pakistan on calcareous and non-calcareous soils where applying HA at high rates did not improve growth, yields and nutrient uptake in wheat plants. In our study, the optimal Humalite rate ranged from 112 - 224 kg ha^{-1} when combined with recommended urea rates at all sites.

For half urea recommended rate, an optimal Humalite range of 56 – 112 kg ha^{-1} was observed. Humic substances improve soil physical, chemical and biological properties, thereby improving plant performance and yields (Gümüş & Şeker, 2015; Li et al., 2019; Mosa et al., 2020; Trevisan et al., 2010). The effects of Humalite and urea on wheat yields were particularly prominent in 2023, especially at the GRO site, compared to previous years. This increased response could be linked to the application of organic materials (Humalite), which can enhance soil physicochemical properties over time. Such improvements often require a longer duration to become obvious. Additionally, the residual effects of organic materials on soil properties and crop production can persist for several years, contributing to the observed yield increases (Diacono & Montemurro, 2010). This research aimed to identify conditions under which crop yields could be maximized with reduced urea application. Yield trends varied by location; at GRO, applying half urea recommended rates was most effective across all years irrespective of Humalite application rate. In a study by Lindsey et al. (2021), the application of HS with reduced N fertilizer provided equivalent turfgrass quality and cover relative to full N rates. In another study with rice, a lower dose of HA applied with reduced N fertilizer resulted in significant yields and fertilizer efficiency comparable to full N application (Suhardjadinata et al., 2015). However, at BRRG and St. Albert, urea applied at recommended rates mostly outperformed other urea application rates, with a few exceptions, particularly at reduced rates of Humalite. The high yields observed at low Humalite rates, particularly in wheat, suggest that the effects of HSs on plant growth and yields is non-linear (Rose et al., 2014). This indicates that increasing concentrations of HSs do not uniformly enhance plant performance (Rose et al., 2014). Instead, the response is influenced by several factors, including the application rate, method of application, source of HSs, crop type, and climatic conditions (Akimbekov et al., 2021). These interactions highlight the complexity of predicting and ensuring consistent responses to HSs (Rose et al., 2014 and Akimbekov et al., 2021).

Therefore, further research is needed to improve our understanding and predictability of how HSs affect plant growth and yield under varying conditions.

Thousand kernel weight (TKW) at all sites were similar, irrespective of treatments. However, protein contents in grains were affected by urea application across all sites. Across all urea levels, the highest protein content in wheat was predominantly observed with Humalite rates between 224 and 448 kg ha^{-1}. These results are supported by a pot experiment conducted by Rathor et al. (2024), which found that wheat seed protein content was higher when Humalite was combined with inorganic fertilizers. In another field experiment conducted by Li et al., (2019) in China, it was observed that protein contents in peanut was higher in the first year of experiments when HA was combined with inorganic fertilizer. In a review conducted by Ampong et al. (2022), it was reported that HA increased crop N uptake, regardless of the amount or form of N fertilizer applied. Additionally, the review suggested that HA affects protein content differently, depending on the rates of HA applied, the method of application, and the crop type. Therefore, there is still an inconsistent trend in N assimilation and crop protein content under varying HA types. Therefore, the higher protein content observed at higher urea rates across all sites could be associated with higher N availability and uptake (Rathor et al., 2024). Humalite is expected to chelate nutrients, making them available to plants (Boguta et al., 2019; Van Dijk, 1971). Our results indicate that moisture deficit may be a significant factor in the St Albert resulting in wheat protein contents below 13.5% when no urea was applied; meanwhile, in other sites (BRRG and GRO), wheat protein content was significantly higher as expected.

Along with yield increase, the influence of return on investment should also be considered when contemplating the application of biostimulants. Crop producers are currently looking for strategies to reduce input costs and increase profitability, considering the ongoing surge in fertilizer prices in the global market. In 2021, because wheat yields were higher at half urea recommended rates plus 112 - 224 kg ha^{-1} Humalite, wheat net revenue values remained higher than other treatments and rates at BRRG. While at GRO, the highest wheat net revenues were generated with no urea application because there was no significant yield advantage to urea and Humalite application. This can be linked to the low canola yields. The poor yield performance is likely a result of insufficient rainfall at the onset of the growing season, which hindered plant establishment and growth, leading to early plant stress during this critical period. Hence affecting the overall productivity and profitability of the crop. At St. Albert, the highest net revenue gains were recorded with 112 and 448 kg ha^{-1} of Humalite under recommended and half urea rates, respectively (Figure 2.5). In the subsequent year for canola, at GRO, net revenue margins continued to drop with higher urea and Humalite rates and profitability was maximum at half urea recommended rate (Figure 2.6). Increasing Humalite rates plus urea at recommended rates reduced net revenue margins (Figure 2.6). In 2023, wheat net revenue margins mostly declined with higher urea rates across Humalite treatments. Crop producers may find minimal incentive to utilize high Humalite and urea rates, as yield variations across treatments did not lead to substantial gains in increasing net revenue margins (Figure 2.5). This study suggests that the optimal Humalite rate for crop producers at GRO is half the urea recommended rate plus 112 kg ha^{-1} Humalite. The highest net revenue resulted from 448 kg ha^{-1} Humalite plus zero urea application rates at the BRRG meanwhile at St. Albert, 224 kg ha^{-1} Humalite plus zero urea application rates resulted in the highest net revenue. Generally, higher net revenue gains occurred with lower input combinations. It should be noted that recommendation is dependent on soil type, climatic conditions in the growing season, and farming systems and can change depending on other factors. Currently, a better profit margin can be attained with lower inputs for crop producers. The gains from applying Humalite may arise more from soil health benefits in the long term.

2.5 Conclusions

The effect of Humalite on crop yield in the presence of different urea rates was weather, crop, and site-specific. Wheat seems to respond to Humalite application more than canola. This response may be linked to factors such as differences in their root architecture (Rathor et al., 2024; Wu et al., 2017) and possibly the role that canola and wheat root exudates play in nutrient uptake in the presence of Humalite (Canarini et al., 2019). The optimal application rates of Humalite varied depending on soil type, climate, and crop species, with lower Humalite plus reduced urea resulting in higher net revenue for crop producers. At GRO and BRRG, half-recommended urea application rates plus 112 - 448 kg ha^{-1} Humalite resulted in yields similar to recommended urea application rates. In sites such as St. Albert with high SOM, yield increases were observed mostly at recommended urea rates. Reduced N rates plus Humalite can allow for reduced use of N fertilizers that will be profitable for producers and protect the environment. Considering the rising fertilizer prices and environmental concerns, the use of biostimulants such as Humalite offers a promising avenue for improving crop yields while reducing input costs. Nonetheless, further research is needed to identify Humalite application strategies, such as the method and timing of application, and evaluate the interaction of Humalite with different N sources and other nutrients in major crops. Additionally, there is the need to assess whether Humalite is beneficial in dry years long term, especially in a changing climate.

Chapter 3.0

The impact of Humalite application in the presence of different nitrogen sources on wheat and canola agronomic soil and nitrogen related parameters - A Greenhouse Study

3.1 Introduction

Nitrogen (N) availability is one of the most limiting factors for crop productivity. Nitrogen is the most used plant nutrient for optimal plant growth and productivity, making N-fertilizer a critical component of agriculture (Lassaletta et al, 2014; Mezbahuddin et al., 2020). Currently, N fertilizer accounts for more than 50% of the global food production (Zhang et al., 2015). However, globally around 30-50% of N is taken up by crops (Bindraban et al., 2020; Linquist et al., 2012). This reduced nitrogen use efficiency (NUE) results in the inability of the crops to access soil N and maximize their yield potential (Cassman et al., 2002; Mezbahuddin et al., 2020). There are other consequences including economic loss due to higher investments in fertilizer inputs, excess fertilizer application-related costs, and food insecurity (Sharma & Bali, 2018). Moreover, N-losses create a negative environmental footprint contributing to greenhouse gas (GHG) emissions, underground water and air pollution, and eutrophication (Linquist et al., 2012).

Currently, global NUE has decreased since the 1960s though N fertilizer input has increased nine-fold since then (Gastal et al., 2015; Lassaletta et al., 2014; Tilman et al., 2002). A meta-analysis by Lassaletta et al. (2014) reported and predicted a disproportionately low yield increase to increased N fertilization. However, Yang et al. (2024) reported that though NUE for the Canadian Prairies is higher than the global average, NUE in the prairies has significantly reduced from 1981 to 2016, even though N fertilizer inputs have increased drastically. With the increased N fertilizer inputs, increasingly higher residual soil N has been observed in the prairie soils along with an increase of nitrous oxide emissions by 43% (Yang et al., 2023, 2024). Additionally, Statistics Canada (2018) reported a substantial increase in the farm input price index, with the N fertilizer index increasing from 45.3 in 2002 to 105.1 in 2024. Thus, there is an economic, environmental, and agronomic motivation to improve fertilizer NUE. The 4R Nutrient Stewardship, a technology to improve N management, was developed a decade ago to improve crop yields, NUE, and soil health, as well as provide economic profits to the producers (Snyder, 2017). 4R stands for the Right Place, Right Time, and Right Rate for the Right Source of N fertilizer to be applied (Bruulsema, 2022). Therefore, there has been a persistent push to enhance N fertilizer management that aims to retain soil N and improve NUE, reflecting a commitment to sustainable agricultural practices (Khakbazan et al., 2021; Snyder, 2017; Trenkel, 2010). One of the methods of achieving reduced GHG emissions as well as improving NUE through incorporating the 4R principles is to incorporate the use of enhanced efficiency N fertilizers (EENFs) (Grant & Wu, 2008; Snyder, 2017).

Enhanced efficiency N fertilizers have been designed to (i) delay the release of plant-available N into the soil, thus increasing nutrient availability and uptake by plants, leading to higher NUE, and (ii) reduce N loss and environmental impact caused by the loss (Snyder, 2017; Trenkel, 2010). Enhanced efficiency N fertilizers have been proposed to provide better benefits over conventional urea (Shaviv, 2001; Trenkel, 2010). A type of urea-based EENFs are coated with urease inhibitors (UI), nitrification inhibitors (NI), or both (Byrne et al., 2020). Urease or nitrification inhibitors delay N release which permits plants to capture ammonium ions for an extended period and reduces ammonia volatilization and nitrate leaching loss (Dawar et al., 2011). Impregnation with NI suppresses soil nitrifying microorganisms delaying the conversion of ammonium to nitrite and nitrate (Lam et al., 2022). This facilitates greater ammonium adsorption into soil exchangeable sites instead of lost due to leaching, nitrous oxide emissions, and denitrification, thereby improving NUE of crops (Degenhardt et al., 2017). Urease and nitrification inhibitors combined called dual inhibitors (DI) are added together to fertilizers to further enhance the individual application of either UI or NI. The most common UI and NI used in agriculture are N-(n-butyl) thiophosphoric triamide (NBPT) and Dicyandiamide (DCD ($C_2H_4N_4$)) respectively (Dai et al., 2014; Modolo et al., 2018). SuperU® (KochTM Fertilizer), is a popular DI used in North America that conglomerates both NBPT and DCD, with urea granules (Koch Fertilizer, 2021).

Several studies have been conducted to observe the agronomic efficacy of different EENFs, especially with inhibitor-added fertilizers. Apart from reducing N loss, EENFs are implemented to prolong and/or increase plant N availability and crop yields (Yang et al., 2016). Inhibitor-based EENFs have been shown to increase grain yield and other agronomic parameters compared to conventional urea applications (Beres et al., 2018; Fast et al., 2024; Owens et al., 2023; Thapa et al., 2016; Wang et al., 2023). Theoretically, this would lead to an increase in NUE and crop yields, however, consistent results have not been supported by research. The use of NIs has been shown to indirectly improve agricultural and horticultural crop yields by 2 - 4.5% (Pasda et al., 2001). A meta-analysis conducted by Thapa et al. (2016) reported a 7% increase in cereal (rice, wheat, maize) yields with the use of NIs. A similar yield increase was observed in a previous meta-evaluation by Wolt (2004) with a soil N retention increase of 28%. Some studies reported addition with UIs increased crop yield compared to NIs (Chuan et al., 2010). Findings from another recent meta-analysis which included the newer inhibitors reported an overall significant increase in crop yields (3-5%), with UI being more effective than NI and DI (Fan et al., 2022). The addition of urea with DIs has also not been supported with consistent results. The application of dual inhibitors has led to significant yield and NUE increases compared to a single inhibitor and conventional urea (Adams et al., 2018; Cui et al., 2022; Kakabouki et al., 2020; Qi et al., 2022; Zaman et al., 2013). A significant increase of 2-5% in crop yields with the use of NBPT+DCD was reported by a meta-analysis (Fan et al., 2022). Field and laboratory experiments show the effectiveness of SuperU® (DI) in increasing yields and agronomic N- efficiency under favourable conditions of adequate rainfall, but not under dry conditions (Afshar et al., 2018). In some cases, however, the application of DI did not affect yields significantly compared to conventional urea (Tenuta et al., 2023). Some studies indicate that DIs may not always enhance soil mineral N, N uptake, or yields, as one inhibitor might negate the effects of the other (Frame, 2017; Rozas et al., 1999; Soares et al., 2012). A study found that the addition of NBPT to urea improved cotton yields and other parameters, however with the addition of DCD to NBPT + urea fertilizer, limited NBPT performance which resulted in reduced N uptake, NUE, and crop yields (Kawakami et al., 2012). Some studies have not observed any significant increase in yield with DIs compared to conventional urea but did find a reduction of N losses without compromising on crop yield and quality (Thapa et al., 2015; Torralbo et al., 2022).

On the other hand, plant biostimulants (PBs) such as Humalite have been postulated to increase N uptake, crop yields, and yield quality parameters, as well as NUE (Ampong et al., 2022; Rathor et al., 2023, 2024; Rose et al., 2014). Humalite like other humic substances (HS) is naturally occurring, a highly stable organic substance derived from the transformation of dead and decayed biota (Canellas et al., 2015; Lumactud et al., 2022; Nardi et al., 2017; Rathor et al., 2023, 2024). A major constituent of humic substances is humic acid (HA), which acts as an N fertilizer synergist and is directly involved in promoting crop growth and yield, prolonging N use efficiency, and reducing N losses (Araújo et al., 2017). The complex structure contains carboxyl and other oxygen-containing functional groups that form complex bonds with N in the urea or ammonium ions (Jin et al., 2023; Liu et al., 2024) allowing it to chelate cations in the soil contributing to increased nutrient uptake, availability, and transport (Goel et al., 2021). Thus, HSs can bind and stabilize ammonium ions in the soil, thereby prolonging their availability to the plants (Dong et al., 2009; Laskosky et al., 2020; Rose et al., 2014; Shen et al., 2020; Zhang et al., 2013). Hence, research supports the role of HAs in enhancing plant growth parameters, including biomass, chlorophyll content, plant height, grain yield, and related quality parameters (Canellas et al., 2019; Canellas & Olivares, 2014; Jannin et al., 2012; Muscolo et al., 2013; Scaglia et al., 2016; Zandonadi et al., 2013).

The synergistic effect of humic substances with urea has been well studied. Humic acids have been shown to inhibit urea hydrolysis in some cases (Shen et al., 2020). Other studies show contrasting results where HAs increased urea hydrolysis and reduced ammonium nitrification to nitrate (Laskosky et al., 2020). Humic acid urea, a fertilizer made with humic acid and urea, is reported to significantly improve crop yields and N fertilizer recovery (Liu et al., 2019; Zhang et al., 2019). Canadian producers employing 4R nutrient management strategies are concurrently applying both SuperU and Humalite in their farms. Currently, there are no prairies-specific assessments evaluating the impact of humic products such as Humalite and SuperU applications on grain crop agronomic parameters and nutrient use. Secondly, considering that humic products are considered biostimulants, the jury is out there on whether reduced N should be applied in the presence of humic products and SuperU.

Therefore, the objectives of this project are:

(1) To assess the interaction of different N sources and Humalite on crop agronomic parameters.

The null hypotheses were:

i. Humalite interacts with N sources resulting in increased wheat and canola agronomic parameters.
ii. Humalite plus reduced N rates had similar effects on grain crop agronomic parameters.
iii. Wheat and canola responded differently to Humalite plus SuperU applications.

(2) To assess the interaction of different N sources and Humalite on nitrogen use and selected soil parameters.

The null hypotheses were:

i. Humalite interacted with different N sources resulting in increased N use and increases in soil chemical parameters.
ii. Humalite plus reduced N rates had similar effects on N use and other soil parameters.

3.2 Materials and Methods

3.2.1 Experimental design and data collection

An experiment was conducted from November 2023 – April 2024 at the Plant Growth Facility, University of Alberta. The greenhouse was maintained at 23 ± 3˚C with a 16-h light and 8-h dark cycle. The soil used for the experiment was obtained from a silage corn stubble research farm (Ornithic Black Chernozem soil), South Campus, University of Alberta. The soil was first sieved through an 11mm mesh followed by uniform mixing with sand (Target Products Ltd.) at a ratio of 1:2 (v/v) soil: sand ratio. Six kilograms (bulk density 1.5 g/cm^3) of soil-sand mixture (soil) was filled into each 6.52L plastic pot. The soil was sent to Elements Laboratory, Edmonton for soil analysis and wheat/canola fertilizer recommendation. The experiment was a completely randomized design with seven treatments replicated 8 times and repeated twice with each phase referred to as a cycle. A modern high-yielding CWRS cultivar 'AAC Brandon' and a roundup-ready canola variety, RR45CM39, were assessed. Treatments comprised of two N sources (urea and urea coated with dual inhibitors i.e. SuperU®) at two N rates: recommended rate - RR and reduced by 30% RR, and no N applied as the control. Phosphorous, K and S fertilizer application were applied as per the fertilizer recommendation provided by Elements Laboratory. The treatments were as follows: (i) No N Control (T1) (ii) Urea at RR (T2) (iii)

Urea (RR) + Humalite (T3) (iv) Urea at 70% RR + Humalite (T4) (v) SuperU at RR (T5) (vi) SuperU at 70% RR (T6), and (vii) SuperU at 70% RR + Humalite (T7). Additionally, phosphorous (Triple superphosphate 0-45-0) and sulphur (elemental sulphur 35%) were applied at 100 ppm and 50 ppm respectively to canola from March 5th – 10th, 2024 in the second cycle due to deficiency symptoms observed. The 70% RR was referred to as the reduced N treatment. Humalite (WestMet Ag, AB, Canada) was applied at 448.3 kg/ha based on field trials (Nallanthighal et al., 2024). The fertilizer and Humalite amounts were calculated based on soil bulk density for each pot. Available NPK analysis by Elements Laboratory, Edmonton was performed by extracting the nutrients on Modified Kelowna solution and analyzing further by continuous flow calorimetry. A 5 cm hole was made in the middle of the topsoil where the treatment mixtures were added. Six wheat and canola seeds were sown at 1" depth around the fertilizer hole and thinned down to one plant per pot one week post-emergence. Plants were fully watered throughout the experiment and run in two cycles. Each cycle had two sets of plants, a set for destructive sampling (DS) at BBCH 65 and another sampling (whole sampling-WS) at seed maturity (Table 3.1). Dates when sampling activities were conducted and a list of agronomic parameters collected are found in Table 3.1 and Table 3.2, respectively. For Powdery Mildew prevention in canola, several fungicides (Regalia Maxx Biofungicide (Marrone Bio Innovations) @ 2.5 ml/1L, and Ivory Dish Soap and Potassium Bicarbonate mixture @ 10ml/1L ±10g/1L) applications were performed. Ference (Syngenta Canada Inc.) at 5ml/10L was applied to prevent Aphids and Thrips in wheat.

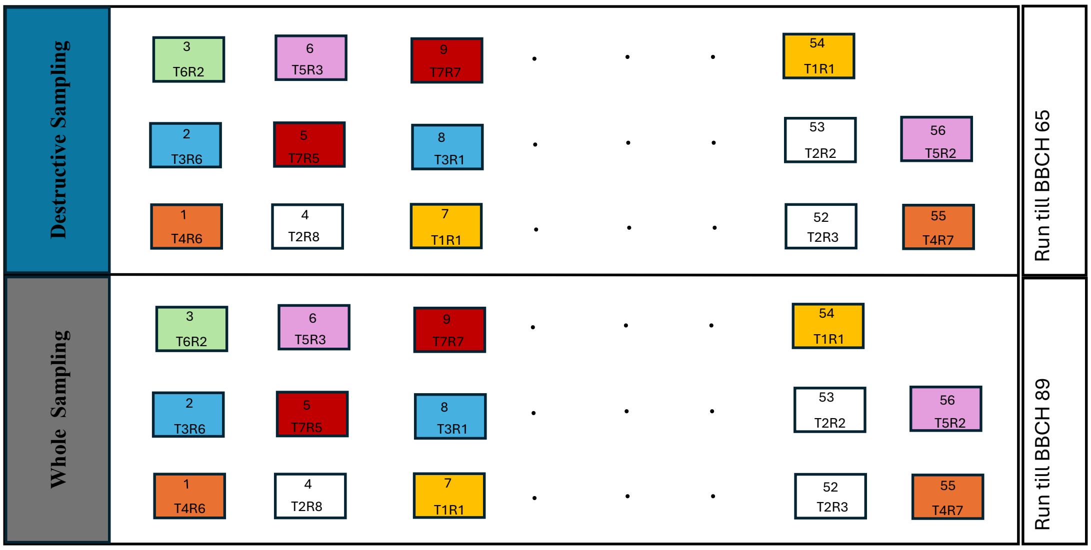

Figure 3.1 Experimental design (completely randomized design) conducted at the plant growth facility, University of Alberta indicating one crop cycle.

Table 3.1 Dates during which agronomic activities were conducted at each cycle in wheat and canola [(W.S) Whole sampling; (D.S.) Destructive Sampling].

	Canola				**Wheat**			
	Cycle 1		Cycle 2		Cycle 1		Cycle 2	
Activities	W.S.	D.S	W.S.	D.S	W.S.	D.S	W.S.	D.S
Seeding	Nov 3/23	Nov 3/23	Jan 4/24	Feb 13/24	Nov 8/23	Nov 8/23	Jan 8/24	Jan 8/24
Harvesting	Feb 15/24	Dec 23-24/23	Apr 9/24	Apr 2/24	Mar 23/24	Jan 24/24	May 1/24	Mar 27/24
SPAD(BBCH 65)	Dec 19-28/23	-	Mar 5-10/24	-	Jan 15-25/24	-	Mar 18-21/24	-
Plant Height	Dec 19-28/23	-	Mar 5-10/24	-	Mar 21/24	-	Mar 18-21/24	-
Biofungicide (Powdery mildew)	Nov 23/23	Nov 23/24	Jan 19/24	Feb 15/24	-	-	-	-
Insect biocontrol	Dec 19/23	Dec 19/24	Feb 17/22	Feb 17/23	Dec 19/23	Dec 19/24	Feb 17/24	Feb17/24

Table 3.2 List of wheat and canola agronomic and soil parameters collected during the experimental cycles I & II

	Canola				Wheat			
Parameters	Cycle I		Cycle II		Cycle I		Cycle II	
	Whole	Destructive	Whole	Destructive	Whole	Destructive	Whole	Destructive
Yield	✓	—	✓	—	✓	—	✓	—
Thousand kernel weight	✓	—	✓	—	✓	—	✓	—
Protein/Oil content	✓	—	✓	—	✓	—	✓	—
SPAD	✓	—	✓	—	✓	—	✓	—
Plant height	✓	—	✓	—	✓	—	✓	—
Days to flowering	✓	—	✓	—	✓	—	✓	—
Shoot dry weight	—	✓	—	✓	—	✓	—	✓
Root dry weight	—	✓	—	✓	—	✓	—	✓
Shoot total nitrogen content	—	✓	—	✓	—	✓	—	✓
Soil ammonium-N	—	✓	—	✓	—	✓	—	✓
Soil nitrate-N	—	✓	—	✓	—	✓	—	✓

✓ Done — Not required

3.2.2 Evaluation of shoot, root biomass, and soil parameters

Plants destined for DS were harvested at crop flowering (BBCH 65) stage. The shoot was cut at the soil surface and separated from the root. Roots were collected, thoroughly washed, and placed in labelled paper bags, dried at 60°C for 48-72 hours, and weighed on a digital scale balance (Denver Instrument S234.3 Summit Series Analytical Balance, 230g x 0.1 Mg). Dry weights were recorded as root dry weight (RDwt) and shoot dry weight (SDwt). Root shoot (Root/Shoot) ratio was calculated by dividing RDwt by SDwt, and total dry weight (TDwt) was calculated by adding both RDwt and SDwt. For total N content (TNC), plant materials were finely ground using a Thomas Model 4 Wiley mill and sent to the Natural Resources Analytical Laboratory (NRAL), the University of Alberta for analysis. The soil mix was also air dried for 1 week, ground, and sent to NRAL for soil ammonium (NH_4-N), nitrate-N (NO_3-N), pH, and TNC analysis. Total organic carbon (TOC) and TNC were analyzed by combustion elemental analysis (Thermo FLASH 2000 Organic Elemental Analyzer, Thermo Fisher Scientific Inc., Bremen, Germany 2016).

3.2.3 Other agronomic data collected

In canola and wheat, SPAD readings were taken using a SPAD-502 Plus chlorophyll meter (Minolta) at BBCH 65. In wheat, SPAD values were obtained from the tip of the flag leaf and the second fully developed leaf; and in canola, were collected from the tips of any three fully developed leaves. In each pot, plant height from ground level to the tip of the leaves was recorded for canola at BBCH 65, and before harvest for wheat. The total number of branches (NOB) in canola was counted at BBCH 65. The total number of spikes (NOS) was counted before wheat harvest. Wheat grains were threshed using a Hege 16 laboratory thresher (Wintersteiger, Ried im Innkreis, Austria). Canola plants were covered with plastic covers and tied at the bottom to contain the pods inside and minimize shattering losses. When pods started to change their colour, the canola plants were cut and set to dry in the greenhouse for a uniform dry down, after which pods were crushed and seeds were collected. The harvested seeds per pot were cleaned, dried to a moisture content of 4%, and weighed on a digital scale balance (Denver Instrument S234.3 Summit Series Analytical Balance, 230g x 0.1 Mg) for grain yield per pot. The wheat grain protein content (GPC) and canola oil content (GOC) were analyzed using TANGO FT-NIR spectrometer (Bruker Optics, Ettlingen, Germany). Thousand kernel weight (TKW) was calculated by multiplying the weight of 200 grains by five for both crops. Harvest Index (HI) was calculated by dividing grain yield by SDwt collected at DS (Dai et al., 2016).

Crop NUEs were calculated based on equations from Congreves et al. (2021) thus:

***Apparent N recovery (AR_N) %** = ((Total N uptake of plant from the treatment – Total N uptake from the control) / Total applied N of the fertilizer in the treatment) x 100*

***Agronomic Nitrogen Efficiency (AE_N(kg kgN^{-1}))** = (Grain yield from the treatment – Grain yield from the control) / Total applied N of the fertilizer in the treatment*

Agronomic N efficiency reflects crop yield increase per unit N applied, whereas AR_N measures the proportion of applied N taken up by the crop (Li et al., 2023)

Other N-related parameters were calculated as follows (Li et al., 2023; Qi et al., 2021):

***Soil apparent nitrification rate (SANR) %** = NO_3^- -N/(NH_4^+-N + NO_3^- -N) × 100*

***Apparent N residual rate (ANRR) %** = (Residual N in fertilized treatment − Residual N in unfertilized treatment)/N rate × 100*

***Apparent N loss rate (ANLR) %** = 100 − AR_N − ANRR*

3.2.4 Data analysis

All data analyses were conducted using R version (v4.1.2; R Core Team 2023). A linear mixed-effects model was employed to identify significant sources of variation. This model accounts for the fixed effects of the seven treatments and two growth cycles, their interaction, and the random effect of replication. The agronomic and soil parameters were considered as response variables. The model was implemented using the lme function from the nlme package in R, with the following structure:

*model <- lme(Yield~ Treatment*Cycle, random=~1 | Replication, data=dataset)*

Significance levels between different treatments, cycles, and their interactions were assessed at $p < 0.001$, $p < 0.01$, and $p < 0.05$. Post-hoc analysis was performed using the emmeans package with Fisher's Least Significant Difference (LSD) test, and letter groupings were obtained to identify significant variables. Graphs were created using SigmaPlot® Version 11.

3.3 Results

3.3.1 Environmental conditions during crop growth

The soil combined with sand and used in this experiment was composed of nitrate-N (10 ppm), phosphorous (>80 ppm), potassium (501 ppm), sulphate-S (13 ppm), total nitrogen (0.12 mg kg^{-1}), total organic carbon (1.62 mg kg^{-1}), organic matter (3.5%), a soil pH of 6.0, and an electric conductivity (0.30 dS/m). Temperature varied significantly for growth cycles (GC) when wheat and canola were grown. Growth Cycle I (GCI) experienced relatively lower temperatures compared to Growth Cycle II (GCII) (Figure 3.1). A significant temperature fluctuation was observed as a pronounced dip in temperatures from 52 - 82 days after sowing (DAS) in GCI and from 0 - 20 DAS in GCII (Figure 3.1). Notably, GCII was characterized by a marked increase in temperature, especially from 70 DAS onwards (Figure 3.1). Temperature readings ranged from 21ºC to almost 34ºC towards the end of GCII, resulting in hot and dry conditions within the greenhouse at the pod/grain filling stages (Figure 3.1). High temperatures in GCII potentially induced heat stress in the crops. During GCI, canola plants reached the full flowering stage (BBCH 65) more quickly than wheat, taking approximately 47 - 55 days compared to wheat's 70 - 86 days. In GCII, both crops showed a slight shift in their flowering timelines. Canola flowered between 57 - 63 days, while wheat reached full flowering slightly earlier, between 69 - 72 days; probably due to the heat. The time to maturity also varied between the two growth cycles for both crops. In GCI, canola matured in 104 days, while wheat required

135 days to reach full maturity. In GCII, there was a reduction in maturation time for both crops, with canola maturing in 96 days and wheat in 114 days.

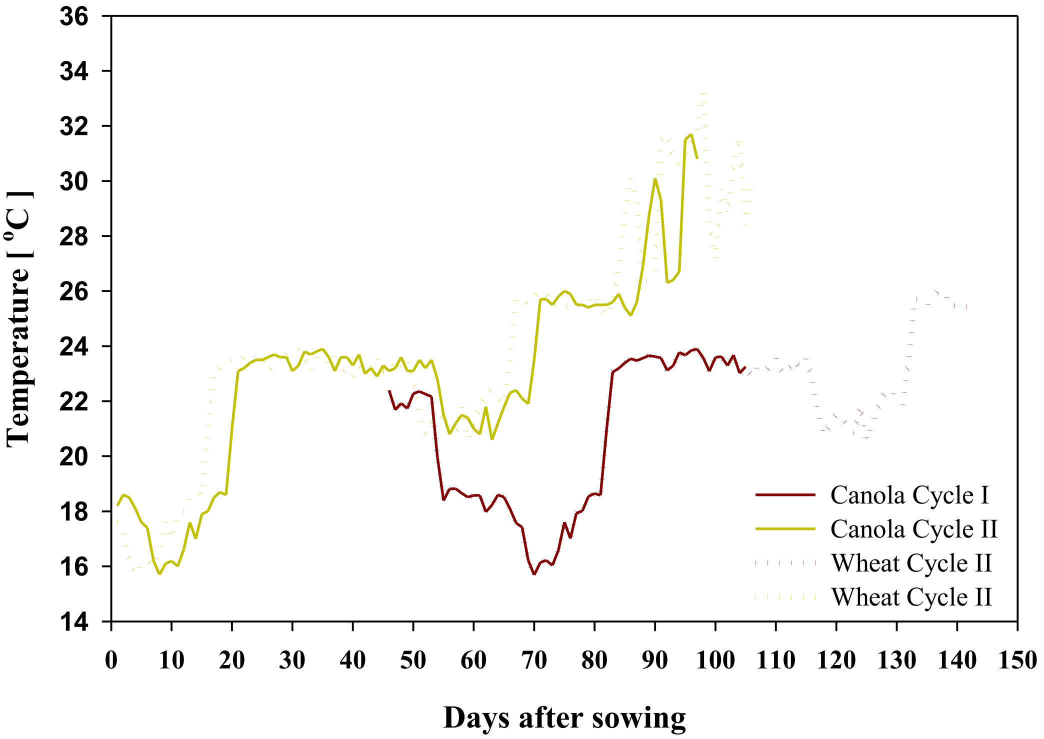

Figure 3.2 Average daily temperatures during the two growth cycles of wheat and canola growth

3.3.2 Humalite *x* different N sources interaction effects on crop agronomic parameters

The effects of different treatments, growth cycles (GCs), and their interactions on wheat and canola agronomic parameters are summarized in the Analysis of Variance (ANOVA) table (Table 3.3). For wheat, the ANOVA indicate a significant two-way interaction between treatment *x* cycle for several parameters, viz. SPAD ($P < 0.01$), SDwt ($P<0.001$), RDwt ($P<0.01$), TDwt ($P<0.001$), NOS ($P < 0.05$), GPC ($P<0.001$), yield ($P<0.001$), and HI ($P<0.001$) (Table 3.3a). The treatment effect was significant only for wheat height (Ht)

(P<0.05), while cycle significantly affected wheat height (P<0.001), R/S ratio (P<0.001), and TKW (P<0.001) (Table 3.3a).

Table 3. 3 Analysis of Variance (ANOVA) table showing the effects of N treatments, cycles, and their interaction on wheat and canola agronomic parameters [Plant height: Ht (cm), SPAD, shoot dry weight: SDwt (g), root dry weight: RDW (g), total dry weight: TDW (g), Root/shoot ratio: R/S, number of spikes in wheat: NOS or number of canola branches: NOB, protein content: PC (g $plant^{-1}$) and oil content: OC (g $plant^{-1}$), thousand kernel weight: TKW (g), Yield: grain yield (g), harvest index: HI].

Source of variation	Ht	SPAD	NOS/NOB	SDwt	RDwt	R/S	TDwt	Yield	TKW	GPC/OC	HI
a) Wheat											
Treatment	*	**	***	***	**	ns	***	***	ns	***	ns
Cycle	***	***	ns	***	***	***	***	***	***	***	ns
Treatment *x* Cycle	ns	**	*	***	**	ns	***	***	ns	***	***
b) Canola											
Treatment	***	**	***	***	***	*	***	***	ns	***	ns
Cycle	***	**	***	ns	ns	ns	ns	***	***	***	***
Treatment *x* Cycle	ns	ns	ns	ns	ns	ns	ns	***	ns	***	**

*, **, *** indicate significant differences at P < 0.05, P < 0.01, P<0.001, respectively; ns indicates not significant

Wheat height was significantly lower in GCII (57.9 ± 0.78 cm) compared to GCI (73.2 ± 0.79 cm) (Figure 3.4A). The urea treatments (T2-T4), even with Humalite addition or reduced urea had similar heights (Figure 3.3). In contrast, the application of SuperU at a reduced rate or in combination with Humalite (T7) resulted in significantly taller plants compared to SuperU at RR which had the shortest (62.4 ±1.23 cm) plants among all treatments (Figure 3.3). SPAD values were significantly higher in GCII, with no significant differences among treatments (Figure 3.4A). In GCI, urea at RR and reduced rates plus Humalite had similar SPAD values (Figure 3.4A). Similarly, SuperU at RR and SuperU at reduced rate plus Humalite had similar SPAD values, although the latter had higher SPAD values (Figure 3.4A). Notably, SuperU at reduced rate plus Humalite had SPAD values that were comparable to all urea treatments (T2-T4). Overall, SDwt was higher in GCI across all treatments (Figure 3.4C). In GCI, urea at reduced rates plus Humalite had significantly higher wheat SDwt compared to urea at RR but this was similar to urea at reduced rate without Humalite (Figure 3.4C). The application of SuperU at a reduced rate plus Humalite significantly decreased wheat SDwt compared to SuperU at RR; SuperU at reduced rate had similar SDwt with urea and SuperU at RR. Similar SDwt was observed among GCII treatments (Figure 3.4C). Wheat RDwt was consistently higher in GCII compared to GCI across all treatments (Figure 3.4D). In GCI, no significant differences in RDwt were observed among urea (T2-T4) or SuperU treatments (T5-T7). However, both urea treatments with Humalite had significantly higher RDwt than all SuperU treatments (Figure 3.4D). In GCII, only SuperU at reduced rate plus Humalite showed a significant increase in RDwt compared to the control but was similar to SuperU at a reduced rate, urea at RR, and urea at reduced rate plus Humalite. Significantly low TDwt were observed for urea at RR plus Humalite, urea at reduced rate plus Humalite, and SuperU at RR (Figure 3.4E). In GCI, the Humalite effect varied depending on the N source applied. Urea at reduced rate plus Humalite had significantly higher (22%) wheat TDwt compared to urea at RR, whereas SuperU at reduced rate plus Humalite significantly decreased wheat TDwt compared to the other SuperU treatments (Figure 3.4E). Wheat Root/Shoot ratio was significantly higher in GCII compared to GCI (Figure 3.5B). The total NOS was similar between cycles for most treatments except for urea and SuperU at RRs (Figure 3.4B). In GCI, urea and SuperU at RRs had similar but significantly higher spike numbers compared to all other treatments irrespective of cycles (Figure 3.4B).

Wheat yield varied significantly between growth cycles, with higher yields observed in GCI for all N treatments (T2-T7) (Figure 3.4F). In GCI, among urea treatments (T2-T5). Applying urea at RR resulted in the highest and significant wheat yields. Interestingly, these yields were similar to those resulting from SuperU at reduced rates plus Humalite application (Figure 3.4F). All SuperU treatments had similar wheat yields during both cycles except for T7 with significantly lower yield during GCII. Thousand kernel weight was significantly higher in GCI compared to GCII wheat plants (Figure 3.5C). Wheat grain protein content (GPC) was higher in GCI compared to GCII in all N treatments, though it was only significant for urea at RR, SuperU at RR, and SuperU at a reduced rate (Figure 3.4G). In GCI, urea at RR resulted in the highest and significant GPC compared to all other treatments irrespective of cycles (Figure 3.4G). Humalite did not have a pronounced effect on wheat protein content as was expected for both N sources. Wheat HI was variable between GC depending on treatments (Figure 3.4H). In GCI, urea at a reduced rate plus Humalite application resulted in a significantly lower wheat HI compared to urea at RR (Figure 3.4H). Conversely, SuperU at a reduced rate plus Humalite resulted in a significantly higher wheat HI compared to SuperU at RR. In GCII, urea at a reduced rate plus Humalite application resulted in significantly higher HI than urea at RR; similar wheat HI between SuperU treatments were observed.

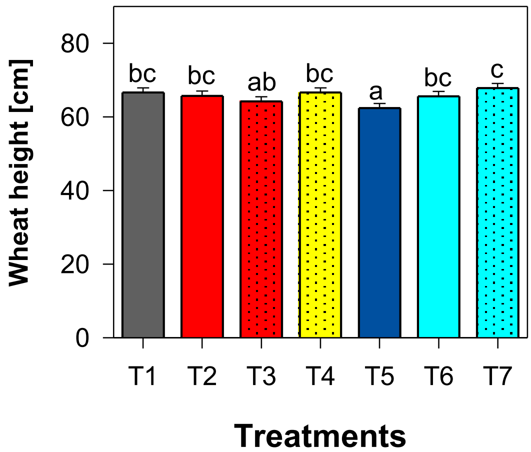

Figure 3.3 Wheat height under different treatments [T1: Control; T2: Urea at recommended rate (RR); T3: Urea at RR plus Humalite; T4: Urea at 70% RR plus Humalite; T5: SuperU at RR; T6: SuperU at 70% RR; T7: SuperU at 70% RR plus Humalite; Error bars represent standard error; different letters represent significant differences at $p \leq 0.05$].

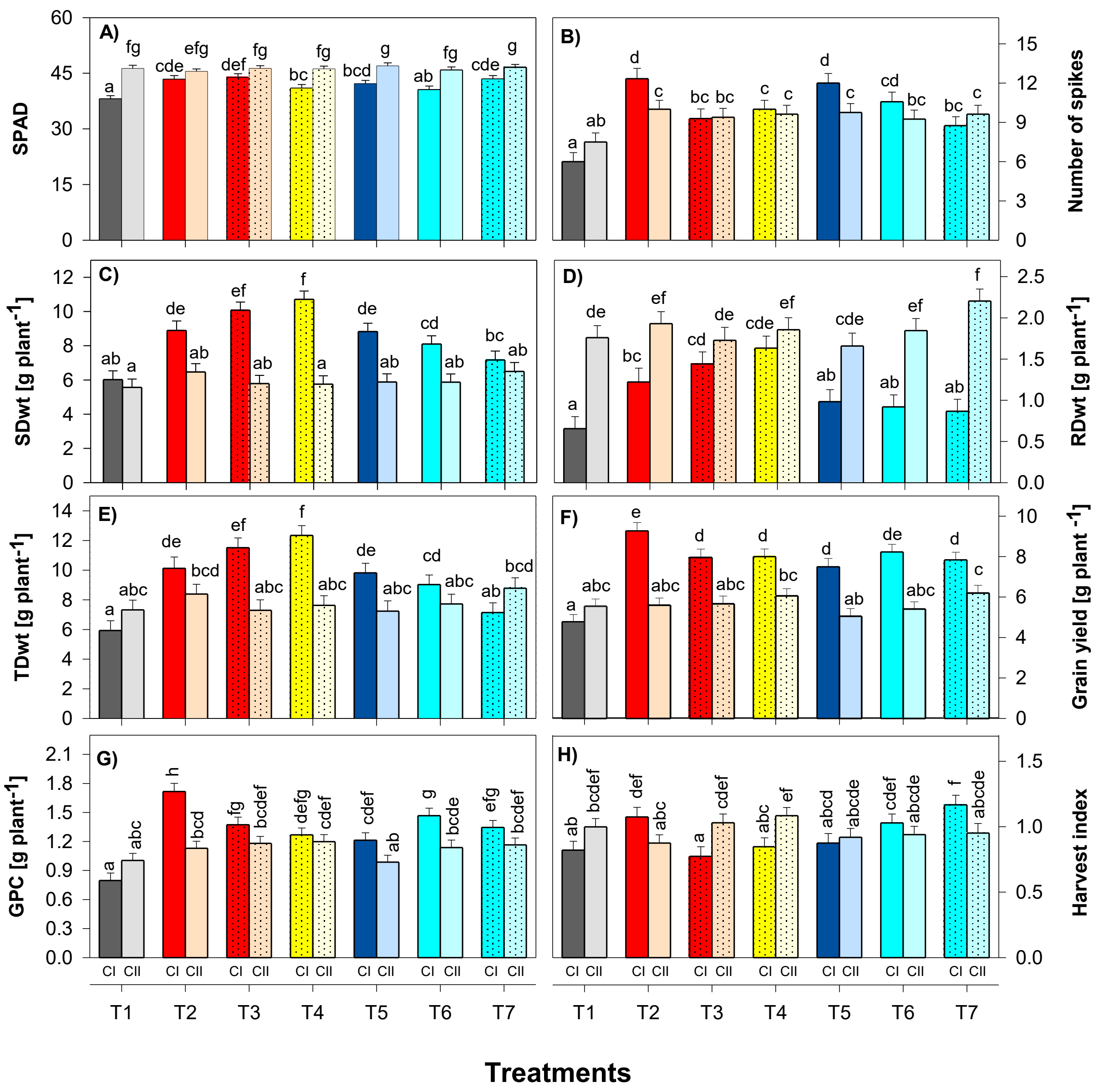

Figure 3.4 Effects of different treatments x cycle interaction on wheat agronomic parameters [T1: Control, T2: Urea at recommended rate (RR), T3: Urea at RR plus Humalite, T4: Urea at 70% RR plus Humalite, T5: SuperU at RR, T6: SuperU at 70% RR, T7: SuperU at 70% plus Humalite; shoot dry weight: SDwt, root dry weight: RDwt, total dry weight: TDwt, grain protein content: GPC; Error bars represent standard error of means, different letters represent significant differences at $p \leq 0.05$].

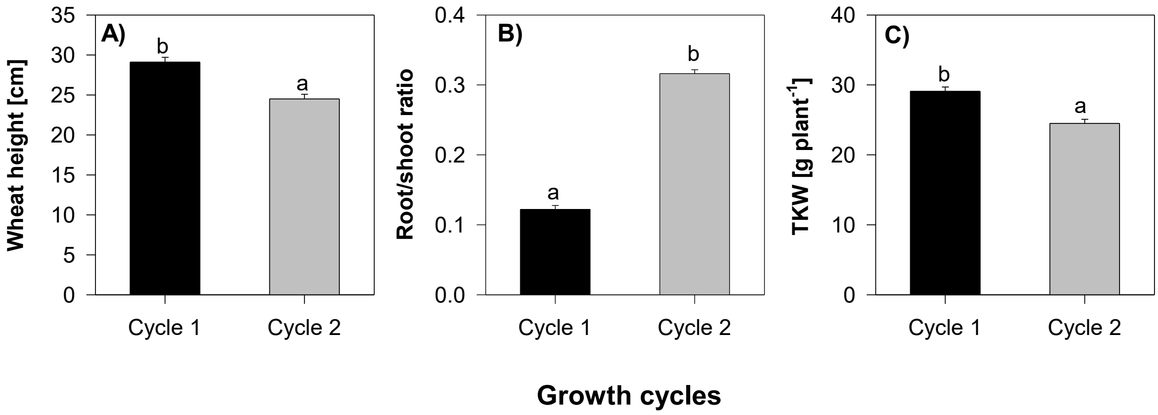

Figure 3.5 Effect of experimental growth cycles on wheat height (A), Root/Shoot ratio (B), and thousand kernel weight (TKW) (C) [Error bars represent standard error of means, different letters represent significant differences at $p \leq 0.05$].

For canola, the ANOVA revealed a significant two-way interaction between treatment *x* cycle for GOC ($P<0.001$), yield ($P<0.001$), and HI ($P<0.01$) (Table 3.3b). Treatment effects were significant for canola height ($P<0.001$), SPAD ($P<0.01$), SDwt ($P<0.001$), RDwt ($P<0.001$), R/S ratio ($P<0.05$), TDwt ($P<0.001$), and NOB ($P<0.001$) (Table 3.3b). The growth cycle significantly affected canola height ($P<0.001$), SPAD ($P<0.01$), NOB ($P<0.001$), and TKW ($P<0.001$) (Table 3.3b). Canola height was significantly higher in GCII (90.4 ±0.88 cm) compared to GCI (84.4 ± 0.79 cm) (Figure 3.6A). Application of urea at RR resulted in significantly taller plants compared to urea at RR plus Humalite (Figure 3.7A). Canola plants had similar heights when SuperU treatments were applied; all treatments resulted in significantly taller plants compared to the control as expected (Figure 3.7A). SPAD values were significantly higher in GCI compared to GCII (Figure 3.6B). SPAD values were similar between all N treatments and this was significantly higher than the control (Figure 3.7B). The highest canola SDwt was observed in urea at RR which was similar to urea at RR plus Humalite but significantly different from all other treatments (Figure 3.7D). A significant decrease in canola SDwt was observed in urea at a reduced rate plus Humalite; similar canola SDwt were observed in all SuperU treatments. Similar canola RDwt was observed between all N treatments, and this was significantly higher than the control (Figure 3.7E). The highest canola Root/Shoot ratio was observed when urea at a reduced rate plus Humalite was applied; this was similar to SuperU treatments but significantly higher than urea treatments and the control (Figure 3.7F). Similar canola TDwt was observed between SuperU at a reduced rate and SuperU at a reduced rate plus Humalite, but canola TDwt when subjected to SuperU at a reduced rate plus Humalite is significantly lower than that in SuperU at RR. All N treatments applied at RR had similar and higher, albeit significantly higher canola TDwt in 2 out of 3 treatments (Figure 3.7G). Canola TDwt was lower when urea at a reduced rate plus Humalite was applied compared to the other urea treatments; all treatments resulted in significantly higher canola TDwt compared to the control as expected.

Canola yields were treatments and GC dependent (Figure 3.8A). Yields were generally higher in GCI in all N treatments. In GCI, the highest canola yields were observed in urea at RR (T2 and T3) irrespective of whether Humalite was applied; this yield was similar to that resulting from SuperU at RR application; similar canola yields were observed between SuperU treatments irrespective of cycles (T5-T7) (Figure 3.8A). In GCII, Humalite application significantly reduced the canola yields under urea treatment. Thousand kernel weight was significantly higher in canola plants from GCII compared to GCI (Figure 3.6C). Grain oil content also varied significantly between growth cycles (Figure 3.8B); higher canola GOC was observed in GCI in all N treatments. The addition of Humalite did not significantly increase canola GOC (Figure 3.8B). Similar canola GOC were observed between SuperU treatments (T5-T7). In GCII, urea at RR plus Humalite had a significantly lower canola GOC; this was similar to SuperU at RR and the control. Among SuperU treatments, both reduced rate treatments (T6 and T7) resulted in significantly higher canola GOC than SuperU at RR. Canola HI was lower in GCII for all N treatments (Figure 3.8C).

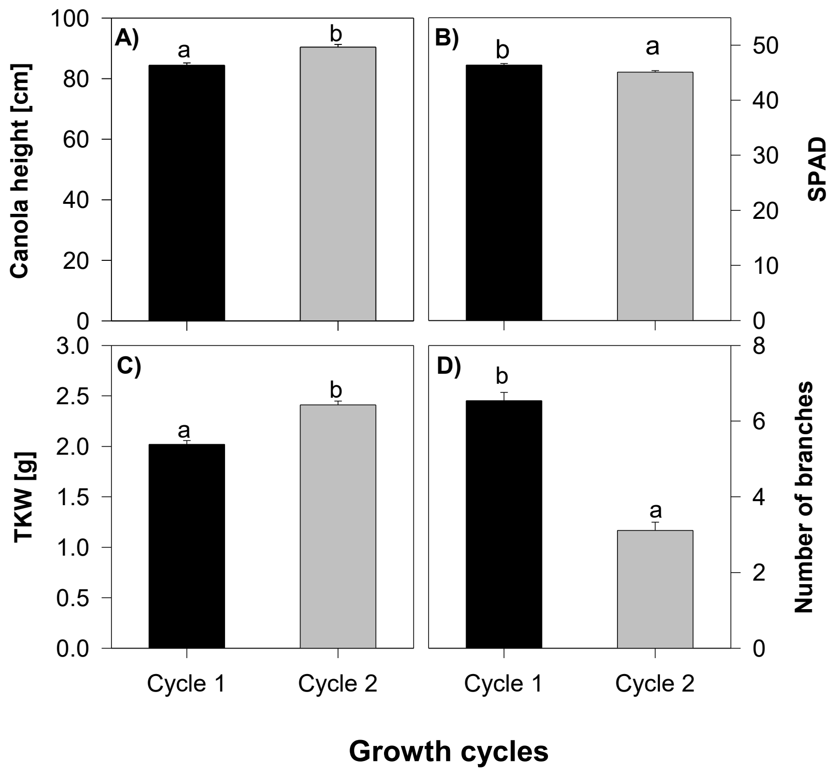

Figure 3.6 Effect of experimental growth cycles on canola height (A), SPAD (B), thousand kernel weight (TKW) (C), and number of branches (D) [Error bars represent standard error of means, different letters represent significant differences at $p \leq 0.05$].

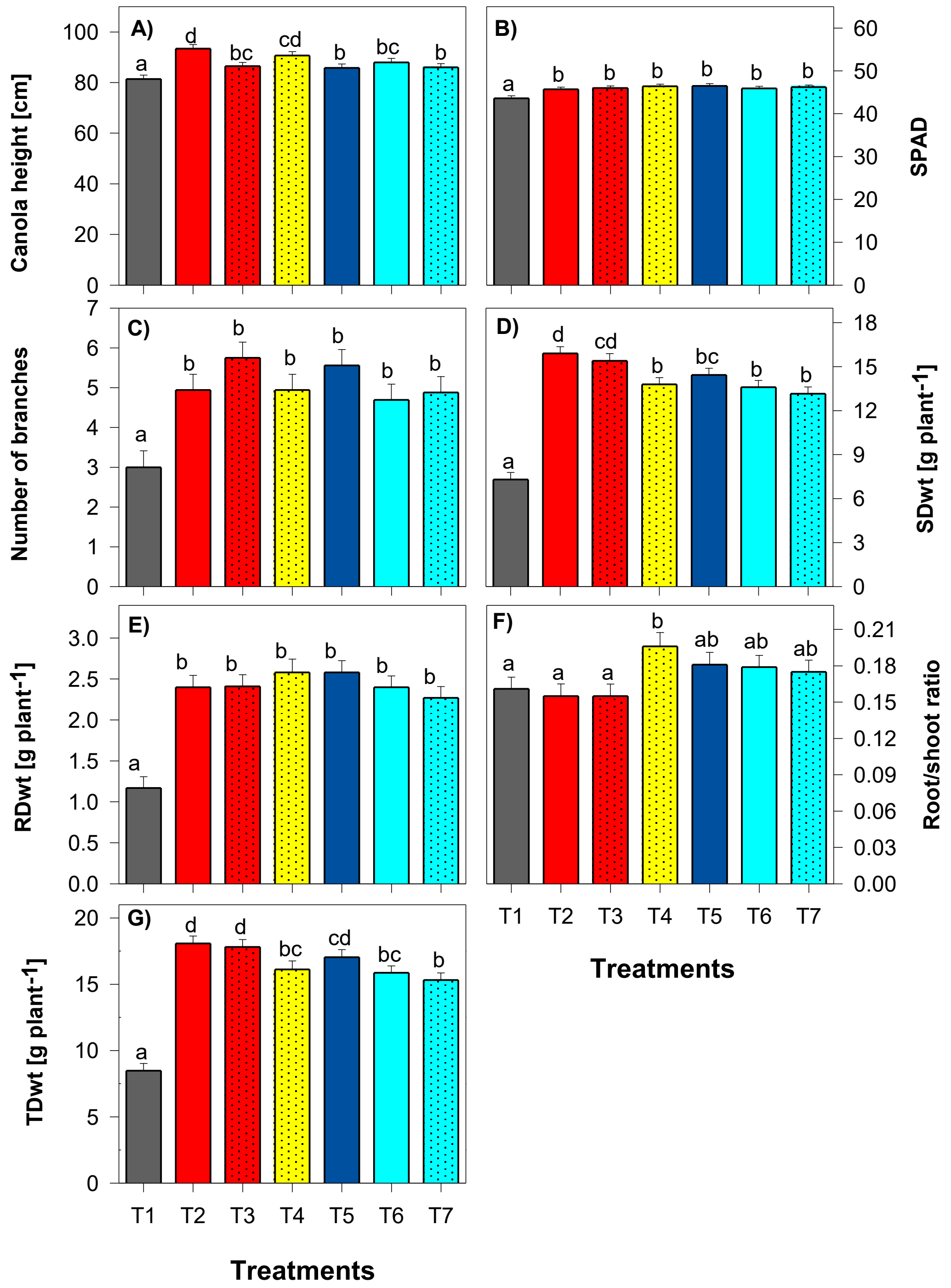

Figure 3.7 Effects of different treatments on canola agronomic parameters [T1: Control; T2: Urea at recommended rate (RR); T3: Urea at RR plus Humalite; T4: Urea at 70% RR plus Humalite; T5: SuperU at RR; T6: SuperU at 70% RR; T7: SuperU at 70% RR plus Humalite; shoot dry weight: SDwt, root dry weight: RDwt, total dry weight: TDwt; Error bars represent standard error of means; different letters represent significant differences at $p \leq 0.05$].

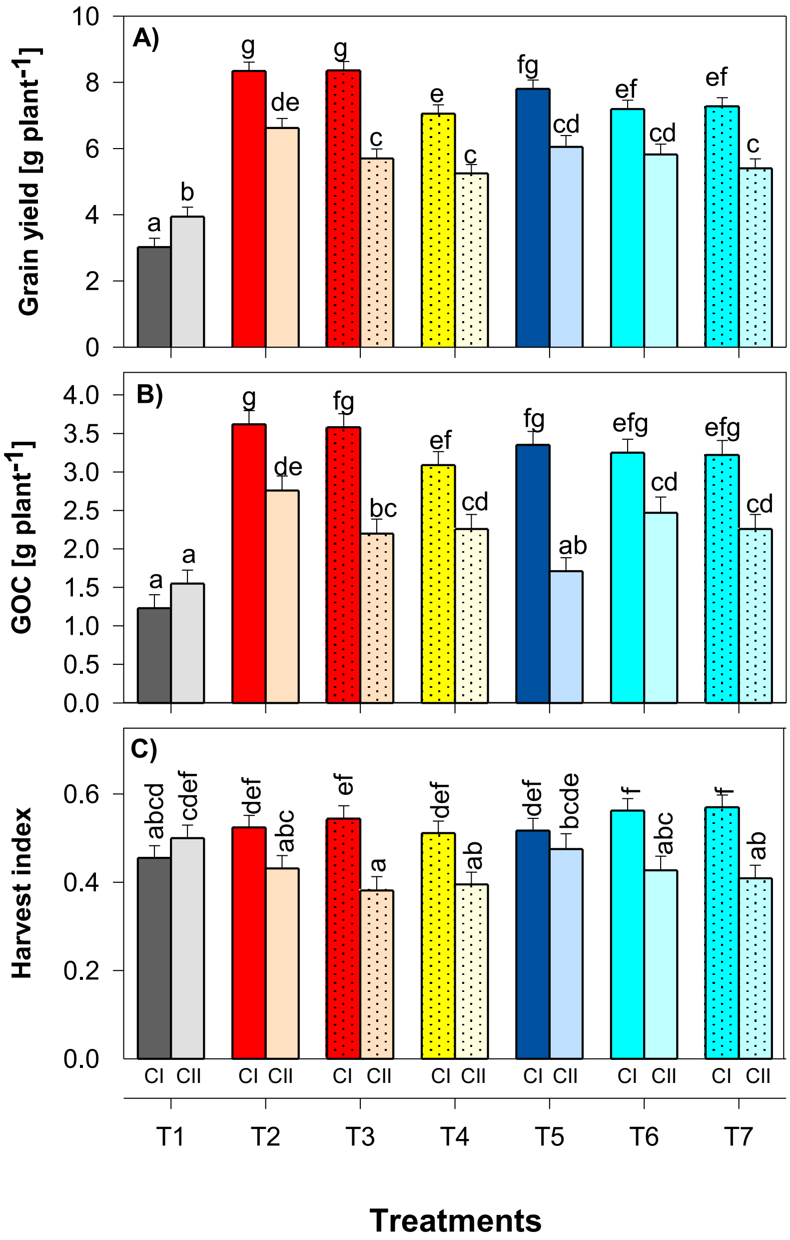

Figure 3.8 Effects of different treatments x cycle interaction on canola agronomic parameters [T1: Control; T2: Urea at the recommended rate (RR); T3: Urea at RR plus Humalite; T4: Urea at 70% RR plus Humalite; T5: SuperU at RR; T6: SuperU at 70% RR; T7: SuperU at 70% RR plus Humalite; grain oil content: GOC; Error bars represent standard error of means; different letters represent significant differences at $p \leq 0.05$].

3.3.3 Humalite *x* different N sources interaction effects on soil nitrogen use and nitrogen related parameters

The effects of different treatments, GCs, and their interaction on wheat and canola N use and N-related parameters are summarized in Table 3.4. In wheat, ANOVA indicates that a significant treatment *x* cycle interaction was observed for TNC (P<0.001), AR_N (P<0.01), NH_4-N (P<0.05), NO_3-N (P<0.001), and ANRR (P<0.05) (Table 3.4a). Treatments were significant for AE_N (P<0.01), soil pH (0.01), and SANR (P<0.001); GCs have a significant impact on AE_N (P<0.001), soil pH (P<0.001), SANR (P<0.001), and ANLR (P<0.01) (Table 3.4a).

Table 3. 4 Analysis of Variance table showing the effects of N treatments, growth cycles, and their interaction on wheat and canola N use and N-related parameters [Total nitrogen content: TNC (mg kg plant^{-1}), agronomic N efficiency: AE_N (kg N^{-1}), apparent N recovery efficiency: AR_N (%), Soil pH: pH, soil ammonium-N: NH_4-N (mg kg plant^{-1}), soil nitrate-N: NO_3 -N (mg kg plant^{-1}), soil apparent nitrification rate: SANR (%), apparent N residual rate: ANRR (%), apparent N loss rate: ANLR (%)].

Source of variation	TNC	AE_N	AR_N	pH	NH_4-N	NO_3-N	TIN	SANR	ANRR	ANLR
a) Wheat										
Treatments	***	**	ns	**	ns	***	***	***	ns	ns
Cycles	***	***	***	**	***	***	***	***	***	**
Treatment *x* Cycle	***	ns	**	ns	*	***	***	ns	*	ns
b) Canola										
Treatments	***	ns	ns	ns	ns	***	***	***	ns	ns
Cycles	***	***	**	***	***	***	***	***	***	***
Treatment *x* Cycle	*	ns	*	***	ns	***	***	***	ns	ns

*, **, *** indicate significant differences at P < 0.05, P < 0.01, P<0.001, respectively; ns indicates not significant

Wheat shoot TNC varied significantly across treatments and GC (Figure 3.9A). In GCI, all N treatments resulted in significantly higher wheat shoot TNC compared to the control. The application of urea at RR plus Humalite resulted in significantly higher shoot TNC meanwhile similar shoot TNC were observed when urea at recommended rate plus Humalite and urea at reduced rate plus Humalite were applied (Figure 3.9A). In contrast, reducing the rate of SuperU, with or without Humalite significantly decreased shoot TNC compared to SuperU at RR (T5). Additionally, both reduced rate SuperU treatments resulted in similar shoot TNC.

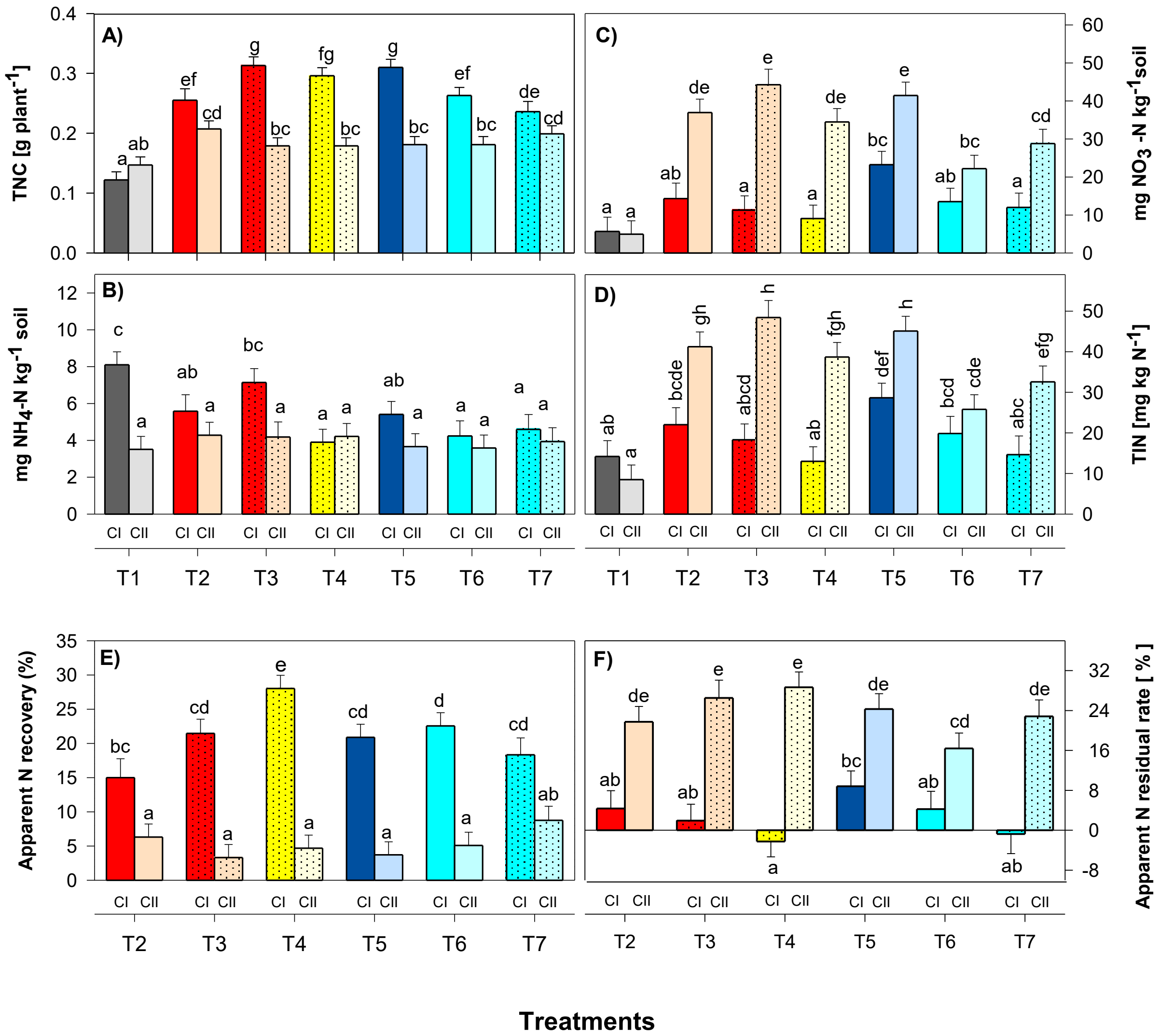

Figure 3.9 Effects of different treatments x cycle interaction on wheat soil and N-based parameters [T1: Control; T2: Urea at recommended rate (RR); T3: Urea at RR plus Humalite; T4: Urea at 70% RR plus Humalite; T5: SuperU at RR; T6: SuperU at 70% RR; T7: SuperU at 70% RR plus Humalite; shoot total nitrogen content: TNC, ammonium-N: NH_4-N, nitrate-N: NO_3-N, and total inorganic nitrogen: TIN; Error bars represent standard error of means; different letters represent significant differences at $p \leq 0.05$].

Agronomic N efficiency was significantly higher in wheat plants grown in GCI compared to GCII (Figure 3.10A). Among urea treatments, Humalite application plus urea at RR did not significantly improve AE_N compared to urea at RR (Figure 3.11A). However, Humalite plus urea at a reduced rate resulted in significantly higher AE_N. Application of SuperU treatments with reduced rates with and without Humalite resulted in significantly higher AE_N compared to SuperU at RR (Figure 3.11A). SuperU treatments at a reduced rate and urea at RR also had similar AE_N (Figure 3.11A). Apparent N recovery (AR_N) varied significantly across treatments and cycles (Figure 3.9F). Apparent N recovery values were generally lower across all GCII treatments than GCI (Figure 3.9F). In GCI, the AR_N ranged from 15% to 28%, with the highest AR_N observed in wheat plants treated with urea at a reduced rate plus Humalite compared to all other treatments (Figure 3.9F). The application of urea at RR plus Humalite increased AR_N compared to urea at RR, though not statistically significant. Among SuperU treatments, both SuperU at reduced rate treatments showed similar AR_N to SuperU at RR (Figure 3.9F).

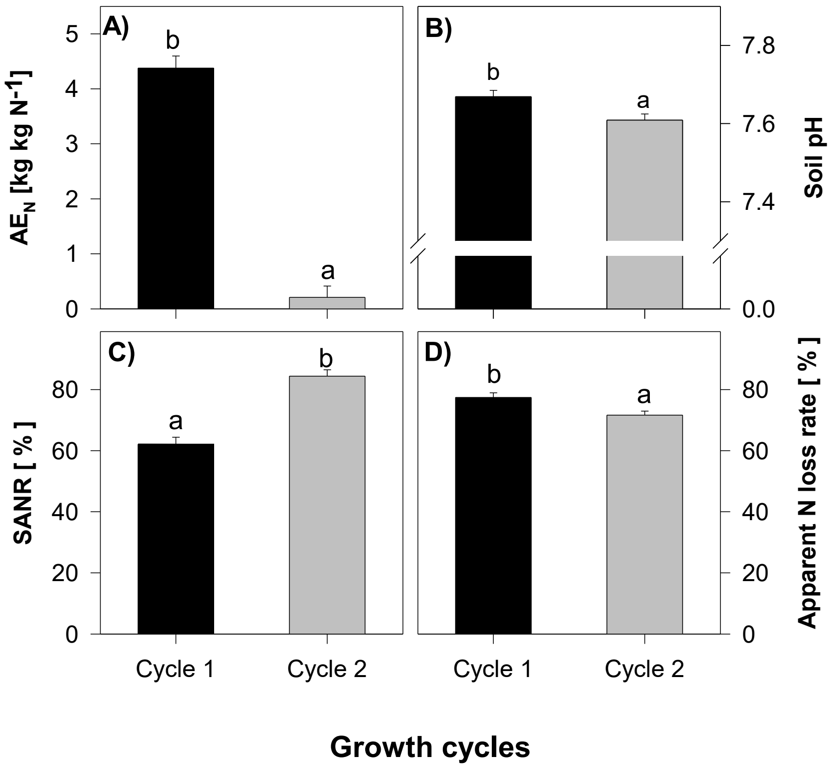

Figure 3.10 Effect of experimental growth cycles on wheat agronomic nitrogen efficiency (AE_N) (A), soil pH (B), soil apparent nitrification rate (SANR) (C), and apparent N loss rate (D) [Error bars represent standard error of means; different letters represent significant differences at $p \leq 0.05$].

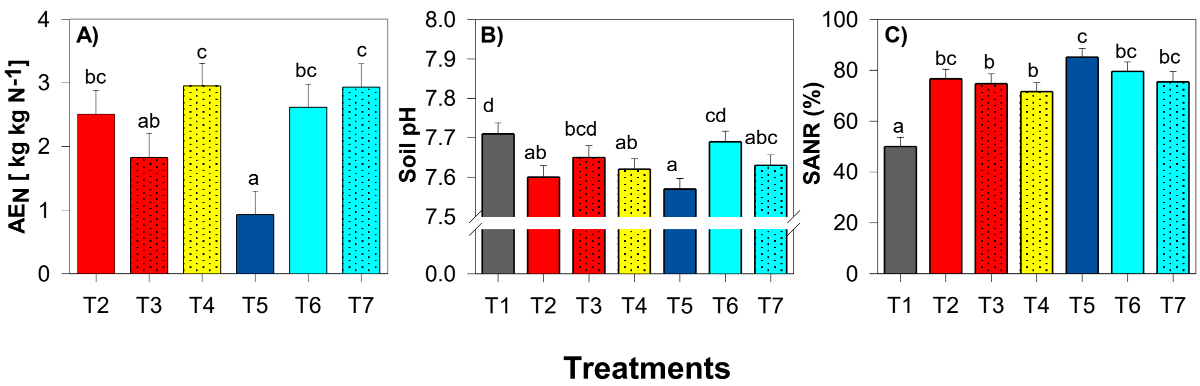

Figure 3.11 Effects of treatments on wheat soil and nitrogen related parameters [T1: Control; T2: Urea at the recommended rate (RR); T3: Urea at RR plus Humalite; T4: Urea at 70% RR plus Humalite; T5: SuperU at RR; T6: SuperU at 70% RR; T7: SuperU at 70% RR plus Humalite; agronomic nitrogen efficiency: AE_N, soil apparent nitrogen rate: SANR; Error bars represent standard error of means; different letters represent significant differences at $p \leq 0.05$].

Soil pH varied significantly across treatments (Figure 3.11B). Soil pH was significantly higher in GCI compared to GCII (Figure 3.10B). The control treatment had the highest pH that was significantly higher than most of the N treatments except for urea at RR plus Humalite and SuperU applied at a reduced rate (Figure 3.11B). Humalite plus urea at RR slightly increased soil pH compared to urea at RR but this increase was insignificant. Application of SuperU at reduced rate plus Humalite also resulted in higher but insignificant soil pH compared to SuperU at RR (Figure 3.11B). Soil ammonium-N levels varied significantly across treatments and growth cycles (Figure 3.9B). In GCII, NH_4-N levels were generally lower (ranging from 3.5 - 4.3 mg kg^{-1}) but similar between treatments. In GCI, the control treatment had the highest NH_4-N level, similar to urea at reduced rate plus Humalite application, but significantly higher than all other N treatments. Soil NH_4-N levels were lower but insignificant for reduced N treatments (T4, T6, and T7). Urea at RR plus Humalite slightly increased NH_4-N but this increase was insignificant. Similar soil NH_4-N was observed between urea and SuperU at RRs (Figure 3.9B). Soil nitrate-N levels were generally higher in GCII compared to GCI in all N treatments (Figure 3.9C). In GCI, only SuperU at RR resulted in significantly higher NO_3-N levels, all other N treatments were similar to the control. For SuperU treatments, SuperU at a reduced rate plus Humalite, and SuperU applied at reduced rates resulted in similar NO_3-N levels, which were significantly lower than SuperU at RR (Figure 3.9C). In GCII, the control had the lowest NO_3-N level, which was similar to all N treatments except for SuperU at RR Urea treatments had similar NO_3-N (Figure 3.9C). However, only SuperU at RR resulted in significantly higher NO_3-N levels compared to SuperU at reduced rate treatments.

Soil apparent nitrification rate in wheat was significantly higher in GCII compared to GCI (Figure 3.10C). All N treatments resulted in significantly higher SANR compared to the control (Figure 3.11C). Among the urea treatments (T2-T4), no significant differences were observed (Figure 3.11C). Similarly, there were no significant differences among the SuperU treatments (T5-T7), although SuperU at RR had slightly higher SANR than at a reduced rate (Figure 3.11C). The ANRR was significantly higher in GCII compared to GCI (Figure 3.9F). In GCI, although not statistically significant, application of urea plus Humalite (T3 and T4) tended to reduce ANRR, with urea at a reduced rate plus Humalite showing a negative value (Figure 3.9F). Among SuperU treatments, no significant differences were observed as well, regardless of whether N was reduced or Humalite applied. However, SuperU at a reduced rate plus Humalite also resulted in a negative ANRR value (Figure 3.9F). In GCII, urea treatments had similar ANRR, although treatments with Humalite (T3 and T4) tended to have increased ANRR (Figure 3.9F). Similar ANRR was observed among SuperU treatments even when reduced N and Humalite were applied (Figure 3.9F). Apparent nitrogen loss rate differed significantly between the two growth cycles with GCI showing a significantly higher N loss rate compared to GCII (Figure 3.10D).

In canola, the ANOVA table indicates that significant treatment *x* cycle interaction for TNC ($P<0.05$), AR_N ($P<0.05$), soil pH ($P<0.001$), NO_3-N ($P<0.001$), and SANR ($P<0.001$) (Table 3.4b). Treatment alone did not have a significant impact on any parameter but GC had a significant impact on all parameters thus: AE_N ($P<0.001$), NH_4-N ($P<0.001$), ANRR ($P<0.001$), and ANLR ($P<0.001$).

Canola shoot TNC varied significantly across treatments and GC (Figure 3.12A). In GCII, all treatments had significantly higher canola shoot TNC compared to GCI, though it was not significant for urea at RR plus Humalite and SuperU at RR (Figure 3.12A). In GCI, the application of urea at reduced rate plus Humalite resulted in significantly decreased shoot TNC (Figure 3.12A). In contrast, reducing the rate of SuperU, with or without Humalite significantly decreased shoot TNC compared to SuperU at RR (T5) in GCI; this shoot TNC was similar to SuperU at RR in GCII. Both SuperU at reduced rate treatments resulted in similar shoot TNC (Figure 3.12A). Meanwhile, urea at RR resulted in significantly higher shoot TNC compared to SuperU at RR in GCII. Agronomic N efficiency was significantly higher in canola plants grown in GCI compared to GCII (Figure 3.13A). Apparent N recovery varied significantly across treatments and cycles (Figure 3.12F). In GCII, the AR_N was generally higher for most treatments compared to GCI (Figure 3.12F). Similar AR_N was observed for all urea treatments in both cycles. In contrast, in GCII, SuperU treatments at reduced rates, with or without Humalite (T6 & T7) had significantly higher AR_N values compared to SuperU at RR (Figure 3.12F).

Soil pH varied significantly across treatments and cycles (Figure 3.12B). Soil pH was generally lower for all N treatments in GCII than GCI, except for the control (Figure 3.12B), which showed a slight increase, which was not significant. In GCI, SuperU at RR had significantly higher soil pH, comparable to urea at RR plus Humalite (Figure 3.12B). SuperU at a reduced rate resulted in significantly reduced soil pH regardless of Humalite application, but increased soil pH in GCI, compared to SuperU at RR. Application of urea at RR plus Humalite resulted in a slight, but insignificant increase in soil pH compared to urea at RR only; the control treatment maintained a relatively stable pH across both GCs.

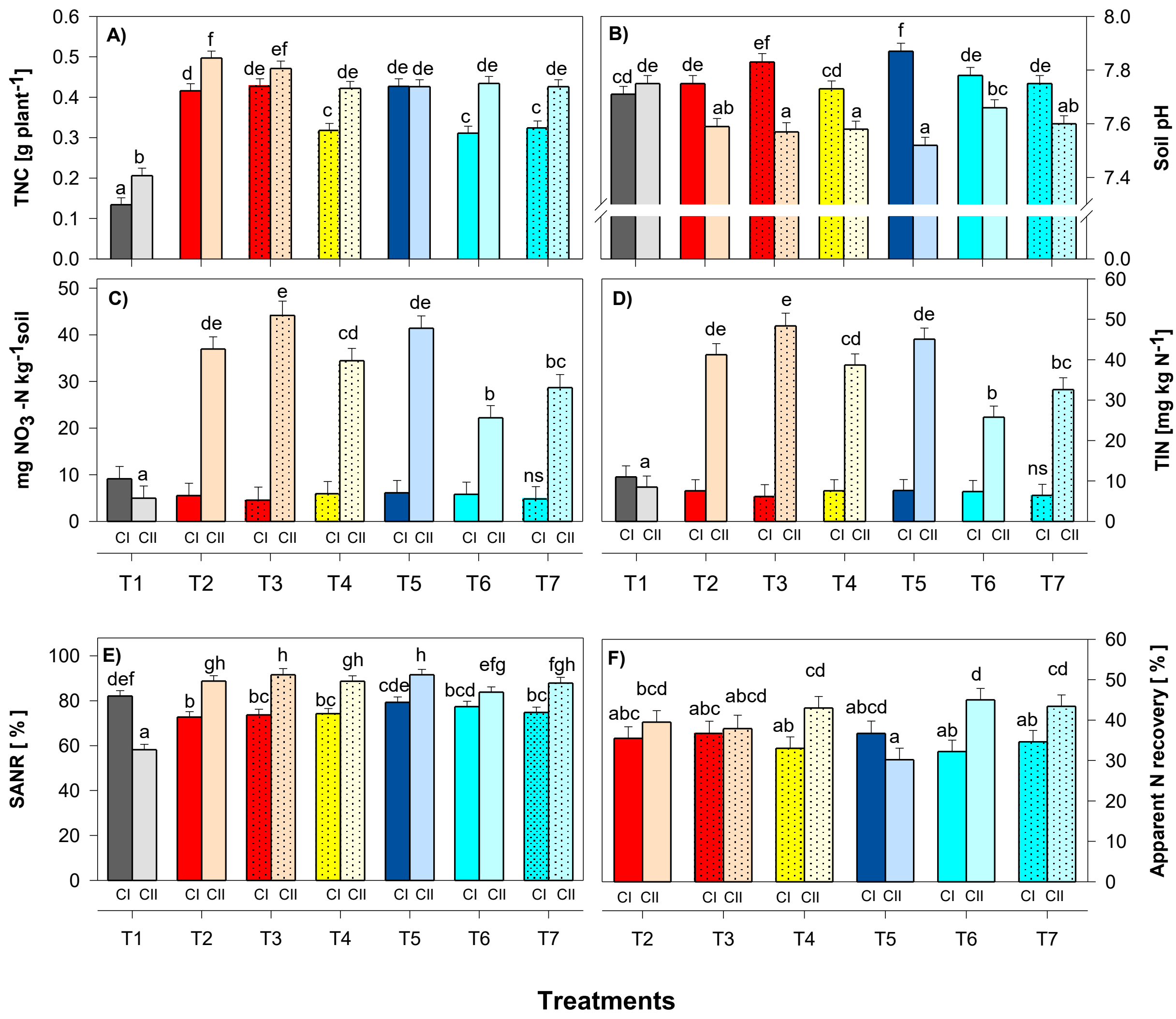

Figure 3.12 Effects of different treatments x cycle interaction on canola soil and N-based parameters [T1: Control; T2: Urea at recommended rate (RR); T3: Urea at RR plus Humalite; T4: Urea at 70% RR plus Humalite; T5: SuperU at RR; T6: SuperU at 70% RR; T7: SuperU at 70% RR plus Humalite; shoot total nitrogen content: TNC, nitrate-N: NO_3-N, total inorganic nitrogen: TIN, soil apparent nitrification rate (SANR); Error bars represent standard error of means; different letters represent significant differences at $p \leq 0.05$].

In canola, soil NH_4-N was significantly higher in GCII compared to GCI (Figure 3.13B). Soil NO_3-N levels varied significantly across treatments and cycles (Figure 3.12C). Soil NO_3-N levels were generally higher in GCII compared to GCI for all N treatments, with no significant differences observed among treatments in GCI (Figure 3.12C). Application of urea at RR plus Humalite resulted in a slight but insignificant increase in soil NO_3-N levels;

when applied with urea at a reduced rate had similar NO_3-N levels as urea at RR (Figure 3.12C). SuperU at RR had similar NO_3-N levels to all urea treatments; these NO_3-N levels were significantly higher than SuperU at reduced rate treatments (T6 & T7). Application of SuperU at a reduced rate plus Humalite resulted in a slight, though statistically insignificant increase in soil NO_3-N levels (Figure 3.12C).

Soil apparent nitrification rate varied significantly across treatments and cycles (Figure 3.12E). Soil apparent nitrification rates were significantly higher in GCII compared to GCI for all N treatments; in contrast with the control treatment decreasing significantly in GCII (Figure 3.12E). In GCI, SANR in control was significantly higher than all urea and Humalite treatments but similar to SuperU at recommended and reduced rates. In GCI, SANR ranged from 73% to 79% for N treatments, while in GCII, it increased to 84 - 92%. No significant differences were observed among urea treatments in both cycles and SuperU treatments in GCI. In contrast, in GCII, canola plants showed significantly higher SANR for all N treatments compared to the control (Figure 3.12E). However, in GCII, SuperU at RR had significantly higher SANR compared to SuperU at a reduced rate; this SANR was similar to that of SuperU at a reduced rate plus Humalite. The ANRR and ANLR showed significant variation across cycles (Figure 3.13C, D). The ANLR was significantly low in GCI, compared to GCII, exhibiting negative values in GCI (Figure 3.13C). In contrast, ANLR was significantly higher in GCI compared to GCII.

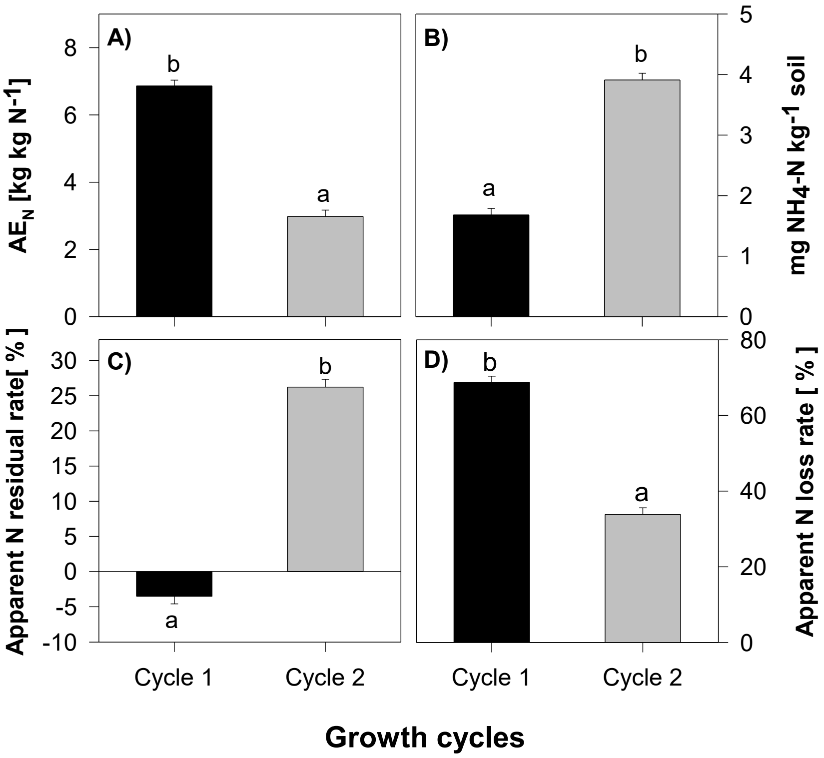

Figure 3.13 Effect of experimental growth cycles on canola agronomic nitrogen efficiency (AEN) (A), soil available ammonium-N (NH4-N) (B), apparent N residual rate (ANRR) (C), and apparent N loss rate (D) [Error bars represent standard error of means; different letters represent significant differences at $p \leq 0.05$].

3.4 Discussions

3.4.1 Effect of environmental condition in the presence of Humalite and different nitrogen sources on grain agronomic, and nitrogen-related parameters

This study explored the effects of different N sources, application rates, Humalite, and their interaction on the agronomic, and N-related parameters in wheat and canola under controlled environment. Growth conditions in the growth facility played a significant role, evident in the variations observed in the two GCs. Wheat and canola are cool-season crops that are highly sensitive to increasing temperatures, especially during reproductive and grain/pod-filling stages (Angadi et al., 2000; Liu et al., 2014; Singh et al., 2008; Ullah et al., 2022; Vignjevic et al., 2015; Weymann et al., 2015). Wheat agronomic parameters collected demonstrated that

GC significantly affected most variables. In wheat, parameters such as plant height, SDwt, TDwt, grain yield, GPC, TKW, NOS, and HI were lower in GCII compared to GCI. Conversely, RDwt and SPAD values were higher in GCII. For canola, SPAD values, NOB, yield, GOC, and HI were adversely affected in GCII. The temperature variations between GCs likely contributed to these differences. The hot and dry conditions in GCII, particularly after 70 days after sowing (DAS) when temperatures exceeded 27°C, may have influenced crop physiological processes and reduced fertilizer efficiency, thereby affecting yield and protein content. This aligns with existing literature indicating that high temperatures negatively impact wheat yield due to heat stress during the grain-filling period, known as terminal heat stress, which is particularly severe (Farooq et al., 2011; Kumar et al., 2019; Rehman et al., 2021; Satorre & Slafer, 1999). Optimal temperatures for wheat anthesis and grain filling range from 12-22°C, and exposure beyond this range can significantly reduce grain yield (Tewolde et al., 2006). Heat stress disrupts physiological processes such as photosynthesis (Al-Khatib & Paulsen, 1999), consequently leading to protein degradation (Sairam et al., 2000), and hastening leaf senescence (Zhao et al., 2007). This results in decreased grain weight and size, reduced yields, and lower protein content quality (Dias & Lidon, 2009; Hurkman et al., 2009; Nuttall et al., 2018), as observed in GCII wheat in the current study.

For canola, SPAD values, NOB, grain yield, GOC, and HI were significantly lower in GCII compared to GCI. Studies have shown that high temperatures affect canola yields and oil content (Faraji et al., 2009; Lohani et al., 2022; Pokharel et al., 2021; Si et al., 2003). Heat stress during the flowering stage can lead to canola flower blast, characterized by aborted flowers, poorly filled pods, and blank sections on the stem, resulting in significant yield reductions as high temperatures are known to induce male and female sterility (Morrison & Stewart, 2002; Polowick & Sawhney, 1988). Canola exposed to temperatures above 27°C can suffer significant yield losses (Morrison & Stewart, 2002). High temperatures during seed filling can shorten the seed-filling duration, reducing yield and related parameters (Hocking et al., 1997; Morrison & Stewart, 2002). However, TKW in canola has been shown to increase under heat stress conditions due to the crop's phenotypic plasticity to mitigate stress damage (Pokharel et al., 2021; Rivelli et al., 2024), which was observed in this study as TKW was higher in GCII.

Regarding N fertilization, research has not reached a consensus on its impact on crop yield improvement under high-temperature stress. Previous research has indicated that grain yield, protein content, and TKW in wheat do not increase with higher N supply under high-temperature conditions (Zahedi et al., 2004). Moreover, some studies suggest that higher N application can further increase sensitivity to heat stress, thereby reducing grain yields (Ordóñez et al., 2015; Slafer & Savin, 2018). Consistent with these findings, all N-fertilized treatments for both wheat and canola in GCII produced lower yields than in GCI. In contrast, a study by Hassan et al. (2015) reported that increasing N fertilizer concentration can alleviate heat stress effects. Meanwhile, Zhou et al., (2024) suggested that reducing N application by 20% can help tolerate heat stress. In this study, during GCII, both RR and reduced rate N applications resulted in wheat yields that were similar to control treatments. However, for canola, N treatments outperformed the control, with urea at RR producing significantly higher yields than at a reduced rate, while SuperU at a reduced rate produced similar yields as that of SuperU at RR. This could suggest that different types of crops react differently with N under heat stress conditions. Plant biostimulants are designed to improve physiological processes by providing increased tolerance to abiotic stresses, including heat stress to improve production quality (Carmody et al., 2020; Cocetta et al., 2022; Repke et al., 2022). Humic substances are a type of PB that have been shown to increase heat tolerance in plants by activating Heat-Shock Protein coding genes (Cha et al., 2020). In liquid forms, HSs have been shown to improve abiotic tolerance in diverse crops such as wheat, maize, pepper, and soybean (Kaya et al., 2018;

Kıran et al., 2019; Merwad, 2019). However, solid HSs have a lower impact on stress tolerance compared to their liquid counterparts but are reported to remain longer in soil and can further improve NUE, and N availability (Qin et al., 2023; Qin & Leskovar, 2020). In the current study, the observed increase in wheat HI for Humalite combined with urea under high-temperature conditions can be justified by the potential of Humalite to enhance nutrient uptake and mitigate stress effects, which aligns with previous findings (Maignan et al., 2020; Qin et al., 2023; Qin & Leskovar, 2020). However, when urea was applied at the RR, the HI decreased in the heat-affected cycle, likely due to ammonia-N volatilization and reduced efficiency under stress (Cameron et al., 2013). The lack of comparable results with SuperU treatments suggests that Humalite's effect might be counteracted by the use of dual inhibitors. Xiao et al. (2024) found that HA interacted differently with NBPT in various soil and pH conditions, delaying urea hydrolysis by binding to NH_4-N ions in black soils. Given that HA can also act as natural urease inhibitors in agriculture (Liu et al., 2019; Zhang et al., 2019), the combination of Humalite and dual inhibitors might result in the binding of available N, negating their individual effects. However, Humalite application did not significantly improve canola agronomic performance, aligning with our field study findings (Nallanthighal et al., 2024). Plant biostimulants including HSs have been shown to increase abiotic stress tolerance in canola (El-Shazly, 2020; Passandideh et al., 2022). However, there have been inconsistencies in their results with rapeseed or canola (Osvalde et al., 2024). Moreover, studies have been conducted using liquid HS or humic acid, whereas in the present study, we applied HS in a solid form.

In the current study, wheat also showed significantly lower AE_N, soil pH, TNC, NH_4-N, and AR_N under high temperatures. Similarly, canola exhibited lower AE_N, and soil pH in GCII. Previous studies have demonstrated that heat stress also affects enzymes responsible for NH_4-N and NO_3-N metabolism, impacting their assimilation regardless of N applied (Giri et al., 2017; Klimenko et al., 2006; Mishra et al., 2023). In GCII, a significant increase in SANR and significantly lower soil pH were observed for all N treatments in both crops. This aligns with literature indicating that increased temperature enhances nitrification rates leading to reduced soil pH (Dal Molin et al., 2020; Li et al., 2020; Schroder et al., 2011).

In this study, wheat treated with urea at reduced rate plus Humalite resulted in an increase in RDwt in GCII, though not significantly. However, the increase was significant with SuperU at a reduced rate plus Humalite when compared to its RR counterpart. This aligns with previous research indicating that HA can enhance lateral root growth by activating the auxin signal transduction pathway (Canellas et al., 2002; Elmongy et al., 2020; Malik & Azam, 1985; Olaetxea et al., 2018; Rathor et al., 2023, 2024; Zandonadi et al., 2007).

3.4.2 Effect of nitrogen sources and Humalite on grain agronomic, soil, and nitrogen-related parameters

Inhibitor-based EENFs and HSs have demonstrated their effectiveness in slowing urea release in soil and retaining soil-N, thereby enhancing uptake when crops require it most (Beres et al., 2018; Fast et al., 2024; Gao et al., 2022; Saha et al., 2019). In the current study, SuperU at RR significantly reduced plant height in wheat and canola compared to urea at RR (Figure 3.3A and 3.6A), an important aspect considering the impact of lodging on wheat production; a similar finding was observed in cotton, suggesting reduced lodging and potential yield and quality loss prevention (Kawakami et al., 2012). This contrasts with other studies where increased plant height was observed with single or dual inhibitors (Ge et al., 2023; Hussain et al., 2021). Similar plant height was observed with the application of urea at reduced rate plus Humalite and urea applied at RR (Figure 3.3A and 3.6A), indicating that Humalite application negated the effect of reduced N application on plant height.

No significant differences were observed between both N sources applied at recommended rates on most agronomic parameters except wheat yield and protein in GCI

(Figure 3.4F, G). This is consistent with Recio et al. (2020) and Sistani et al. (2014), where no differences in above-ground biomass were found between conventional urea and EENFs in corn. Furthermore, the addition of Humalite increased SDwt (Figure 3.4C) and N uptake with urea (Figure 3.9A), however there was no significant change observed when applied with SuperU. This aligns with research showing that increased shoot biomass correlates with N uptake, and that humic acid-urea fertilizer enhanced both parameters compared to urea (Gao et al., 2022). However, the combined effect of SuperU with Humalite may have negated the effect of Humalite on N uptake and consequently SDwt.

In wheat GCI, urea at RR and SuperU at a reduced rate produced the highest yields (Figure 3.4F). Notably, SuperU at reduced rate showed similar growth parameters (Figure 3.4A-H), except for significantly higher protein content compared to SuperU at RR (Figure 3.4G), consistent with findings in cereal crops where inhibitors at lower urea rates were more effective (Khan et al., 2013; Yan-hong et al., 2018). No effect of N sources on TKW was observed, similar to the findings by (Mangin et al. (2022). A protein penalty was noted with SuperU as well as Humalite application compared to urea at RR (Figure 3.4G). Our results are supported by previous research demonstrating the lack of EENF efficacy in increasing wheat grain protein (Beres et al., 2010; Mangin et al., 2022; Thapa et al., 2016). Inconsistent results with HA on grain protein have been reported, with some studies showing increased protein content (Nasiroleslami et al., 2021; Rathor et al., 2024), and others reporting no difference (Li et al., 2019; Radwan et al., 2014). Our study revealed a protein penalty associated with Humalite and urea application, while SuperU application showed no effect. This finding underscores that Humalite is dependent on the N source used.

Agronomic N efficiency, is a fertilizer-based NUE perspective that provides a measure of the yield gain per unit of applied N fertilizer, offering insight into the economic and environmental of N fertilizers (Congreves et al., 2021). SuperU at RR had the lowest wheat

AE_N and reducing SuperU regardless of Humalite application comparatively increased AE_N to levels comparable with urea at RR (Figure 3.11A). As mentioned previously, lower rates of urea plus inhibitors are more effective at improving NUE expressed as AE_N (Khan et al., 2013; Yan-hong et al., 2018). A meta-analysis by Rose et al. (2018) further reported that EENFs may not increase yields and NUE compared to conventional N application, with sub-optimal rates being more effective, aligning with our results. Urea at a reduced rate plus Humalite resulted in similar AE_N (Figure 3.11A), and SANR (Figure 3.11C) as urea at RR. SuperU at a reduced rate plus Humalite resulted in similar AE_N and SANR values compared to SuperU at a reduced rate. This may indicate that reducing urea and applying Humalite would provide similar NUE as well as nitrification as that of conventional rates, providing economic and environmental benefits, but may not provide such benefits when applied with SuperU. Humalite application also increased AR_N when applied with urea, however had similar values as that of SuperU at a reduced rate (Figure 3.9E). This suggests that Humalite combined with conventional N fertilizer enhances NUE, expressed as AE_N and AR_N. Despite higher AR_N with urea at a reduced rate plus Humalite, this did not translate to increased grain yield or protein content, corroborating findings that inhibitors and HA can increase NUE without significantly boosting yield (He et al., 2018). These results suggest that while Humalite enhanced N uptake, utilization, and recovery efficiency, it may have altered the plant's N allocation patterns. The additional N might have primarily been directed towards vegetative growth, such as leaves and stems, rather than reproductive structures like grain. Alternatively, Humalite could have affected the plant's ability to remobilize N from vegetative tissues to the grain after flowering. Research shows that under controlled conditions, N uptake in wheat can continue till close to maturity, contributing to yield and protein (Glass, 2009; Masclaux-Daubresse et al., 2010). Apparent N residual rates for Humalite treatments are slightly lower than the other treatments, potentially reducing N availability (Figure 3.9F). However, the interpretation of the current N-related results must be done with caution because AE_N was calculated at harvest, while AR_N and ANRR were assessed at wheat flowering. These results suggest that reduced N can be applied as a split application with Humalite to prolong NUE to obtain significantly higher yields. Furthermore, no significant impact on soil pH was observed with Humalite in wheat (Figure 3.11B) although studies show HA's pH buffering ability can increase soil pH (Korsakov et al., 2023).

In canola, results for N sources and Humalite application differed. In GCI, results suggest that both N sources applied at RR produced the highest yields (Figure 3.8A). SuperU, when applied at a reduced rate produced yield and GOC (Figure 3.8B) similar to SuperU at RR, regardless of Humalite application. However, these yields were significantly lower than those achieved with urea at RR. Previous research suggests that while single inhibitor N fertilizers can increase yield and oil content in rapeseed, double inhibitor N-based fertilizers may not have the same effect (Bečka et al., 2024; Mikusova & Ryant, 2021). Our study findings further agree with Mourad et al. (2021), that humic substances with reduced N fertilizer linearly decreased plant height (Figure 3.7A), grain yield (Figure 3.8A), and oil content (Figure 3.8B). Applying urea at a reduced rate significantly decreased SDwt and TDwt, but did not affect RDwt. In contrast, SuperU applied at a reduced rate resulted in agronomic parameter values similar to those observed when SuperU was applied at the RR. These results therefore suggest that SuperU at reduced rates can be applied instead of recommended rates but may result in slightly lower yields than urea at RR. Humalite application resulted in similar SDwt and RDwt in canola signifying that Humalite application did not increase biomass (Figure 3.7D,E). Similarly, no effect of Humalite on SDwt and RDwt was observed when applied with SuperU. Urea at a reduced rate plus Humalite resulted in a significantly higher Root/Shoot ratio (Figure 3.7F) which corroborates with research that HSs stimulate root growth and improve root architecture (Ampong et al., 2022; Delfine et al., 2005).

For both N sources, recommended rates outperformed reduced rates for canola shoot TNC regardless of Humalite application (Figure 3.12A), suggesting that irrespective of N sources, Humalite did not have a significant impact on N uptake in canola. Nitrification rates were similar across all urea treatments as seen in wheat (Figure 3.12E). Soil pH decreased under heat stress conditions in GCII, due to increased nitrate and nitrification rate, which releases hydrogen ions, acidifying the soil (Han et al., 2017; Weber & Gainey, 1962). However, in GCI, urea at RR plus Humalite resulted in a slight, though not statistically significant increase in soil pH which aligns with the pH buffering capacity of HSs in literature (Pertusatti & Prado, 2007). No effect on NUE parameters from N sources or Humalite application was observed (Figure 3.12G), consistent with studies showing no effect of HA on canola yield and components (Eyni et al., 2023). In contrast, other studies have foliar application in canola enhanced agronomic traits (Alizadeh et al., 2022; Amiri et al., 2020; Barekati et al., 2019; Gürsoy & Kolsarıcı, 2017; Hemati et al., 2022).

In our study, it was observed that wheat and canola responded differently to dual inhibitors and Humalite. Where some studies found enhanced parameters with the effect of EENFs and Humalite as mentioned before, some studies found no impact of dual inhibitor application on yield or NUE parameters in both wheat and canola (Lasisi et al., 2022). While dual inhibitors or Humalite may not always increase yields, further investigation into their potential to reduce N losses through ammonia volatilization, nitrate leaching, and nitrous oxide emissions would be valuable.

3.7 Conclusions and Recommendations

This study revealed significant differences in the agronomic, soil, and N-based parameters of wheat and canola across different growth cycles, N sources, and Humalite applications. Both wheat and canola, as cool-season crops, were highly sensitive to increased temperatures in the second GC, especially during reproductive and grain/pod filling stages, resulting in reduced yields and protein content. The application of SuperU resulted in mixed results for both wheat and canola, not consistently improving yields or NUE, possibly due to the interaction of inhibitors negating the chelating effects of Humalite. In wheat, a reduced rate of SuperU was more effective in increasing wheat yields and grain protein content when compared to the recommended rate. Humalite when applied with reduced urea significantly increased N uptake and NUE but did not translate to increased yield or protein content. There was no significant influence of SuperU or Humalite in canola. The study highlights the importance of tailoring N management strategies according to the crop and the crop's needs and suggests further research on field scale level with different rates on reduced N fertilizer sources with Humalite under stress conditions.

Chapter 4.0 General discussion, conclusions, and recommendations

Sustainable solutions for optimizing N fertilizer application to improve NUE and enhance or maintain grain yields while reducing N losses are a necessity for future crop production systems in the Canadian Prairies. Nitrogen, being a critical nutrient for plant growth and development, requires careful management to balance yield optimization with environmental stewardship, particularly in the face of climate change. Nitrogen is usually supplied as granular urea fertilizer in the Canadian Prairies for most crops, however, its susceptibility to losses in the current changing environmental conditions necessitates innovative approaches. Plant biostimulants, such as Humalite, a humic substance, and EENFs which have been coated with chemicals such as urease and nitrification inhibitors are important in tackling these challenges. These products also actively target N losses and make N more available to plants. It is important to choose biostimulants and EENFs based on the environmental conditions during the field season, crop grown, and soil type.

Findings from our field study (chapter 2) underscore the variable impacts of Humalite and urea application on crop yields and economic returns across different soil zones and environmental conditions. Three urea rates (zero, full recommended rate, and half recommended rate) and five Humalite rates (0, 112 kg ha^{-1}, 224 kg ha^{-1}, 448 kg ha^{-1}, and 896 kg ha^{-1}) were applied in a split-plot design at three research sites St Albert, BRRG, and GRO which had black, brown, and gray soil zones respectively, for three years 2021-2023 in a wheat-canola-wheat rotation. In the year 2023, due to a change in the method of Humalite application from broadcasting to side-banding, a new rate of 56 kg ha^{-1} was added, and the highest rate 896 kg ha^{-1} was dropped. At the BRRG site, characterized by low SOM, applying half the recommended rate of urea combined with 224 kg ha^{-1} of Humalite led to a significant yield increase of 35%. In contrast, at St Albert, which has higher SOM, the highest yield increase of 34% was observed with the full recommended rate of urea plus 224 kg ha^{-1} of Humalite. At the

GRO site, the combination of half urea recommended rates plus 448 kg ha^{-1} Humalite resulted in wheat yield increase of 8.4% in 2021. These results indicate that the effectiveness of Humalite is influenced by the SOM levels, with more pronounced benefits in soils with low organic content.

Furthermore, the study also highlights the significant impact of moisture conditions on crop response to Humalite and urea treatments. In 2022, canola yields at GRO were consistently low due to poor rainfall throughout the growing season. This moisture stress hindered the plants' ability to absorb water and nutrients, especially during critical development phases. Conversely, in years with adequate rainfall, the effects of Humalite were more pronounced, particularly in wheat. The residual effects of Humalite application became more evident over time, with increased responses observed in 2023 compared to previous years, especially at GRO. This suggests that the long-term benefits of Humalite on plant growth, nutrient availability, and soil physicochemical properties may take several years to fully manifest. Furthermore, wheat was more responsive towards Humalite compared to canola, which may suggest that Humalite may be more effective with cereals crops than oilseed crops in the Canadian Prairies. However, further research with different crops is advocated.

Economically, the study revealed that lower input combinations often resulted in higher net revenue gains. At BRRG, the highest net revenue was achieved with 448 kg ha^{-1} of Humalite and no urea application, while at St Albert, the optimal economic return was observed with 224 kg ha^{-1} Humalite and no urea. At GRO, the highest net revenues for wheat were generated with no urea application in dry year (2021), while for canola, profitability was maximized at half the recommended urea rate. Though GRO had overall lower yields compared to BRRG and St Albert, Humalite had the most pronounced effect in the gray soil zone. These findings suggest that Humalite can be a cost-effective alternative to higher urea rates, especially in environments where SOM is low and adequate soil moisture availability.

Therefore, based on the study results, specific recommendations for producers in different soil zones are as follows: At the BRRG site, characterized by low soil SOM, producers should consider applying half the recommended urea rate combined with 224 kg ha^{-1} of Humalite to achieve significant yield increases. At St. Albert, with higher SOM, the optimal strategy is to apply the full recommended urea rate plus 224 kg ha^{-1} of Humalite, which resulted in the highest yield increase. For the GRO site, where the lowest wheat yields were observed, applying half the recommended urea rate plus 448 kg ha^{-1} of Humalite led to a yield increase of 8.4%. These site-specific recommendations highlight the importance of tailoring Humalite and urea applications to local soil zones and SOM levels.

For future research, it is crucial to conduct long-term studies to better understand the residual effects of Humalite on soil properties and crop production over time. Expanding research to include a wider range of soil types, climatic conditions, and cropping systems will help develop more comprehensive application guidelines. Investigations can focus on the specific mechanisms by which Humalite interacts with different soil types and fertilizers to enhance nutrient availability and uptake. Additionally, exploring various application methods and timings can maximize the efficiency of Humalite use. Detailed economic analyses can also be determined for a long-term cost-benefit ratio of Humalite application under various production scenarios. These research efforts will improve the predictability and consistency of Humalite effects on plant growth and yield, ultimately aiding producers in optimizing their fertilization strategies.

The controlled environment study (chapter 3) further investigated the interaction between different N sources, application rates, and Humalite on wheat and canola conducted twice or in two growth cycles. The N sources included urea and SuperU, which is an inhibitor-based EENF, applied at the recommended rate and 70% of the recommended rate. A Humalite rate of 448 kg ha^{-1} was selected based on the field study results. The experiment revealed significant interactions between Humalite and N sources, with varying effects on crop yields and agronomic parameters. The effect of Humalite varied depending on the crop, N source used, and the environmental conditions. Notably, the second cycle unexpectedly was subjected to heat stress, adversely affecting crop performance. Wheat and canola, both cool-season crops, showed reduced yields and protein/oil content under these high-temperature conditions, highlighting the sensitivity of these crops to heat stress. In wheat, Humalite combined with urea at a reduced rate showed promise in improving NUE parameters such as (AE_N) and (AR_N). This combination resulted in similar NUE values as urea applied at the recommended rate, suggesting potential economic and environmental benefits. However, these improvements in NUE did not translate to increased grain yield or protein content, indicating that the additional N uptake may have been directed towards vegetative growth rather than reproductive structures. For canola, the effects of Humalite were less pronounced. Humalite application did not significantly impact root and shoot dry weights or N uptake, regardless of the N source used. However, when applied with urea at a reduced rate, it did result in a higher root/shoot ratio, suggesting potential benefits for root growth and architecture. The interaction between Humalite and N sources varied between crops and cycles. In wheat, Humalite combined with urea showed more positive effects on N-related parameters compared to its combination with SuperU. In canola, the effects were less clear, with SuperU at reduced rates performing similarly to recommended rates for most parameters, regardless of Humalite application.

Further research may be conducted with field trials to validate the results of this study. Research can also explore the potential of split N applications in combination with Humalite to optimize NUE and yield throughout the growing season. Additionally, studies could examine the mechanisms behind the different responses of wheat and canola towards Humalite which would provide a better understanding of which crops respond to Humalite more effectively. Lastly, given the observed impact of high temperatures, investigating the potential of Humalite to mitigate heat stress effects on crop performance could be crucial, especially in the context of climate change.

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