

**Effects of urea and urea treated with nitrogen stabilizers on the growth, yield, and quality
of field crops cultivated on Podzols in the boreal climate**

By

Therese Audrey Nzwinda Mbite

A thesis submitted to the School of Graduate Studies
In partial fulfillment of the requirements for the degree of
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Approved:

School of Graduate Studies

Supervisor (Dr. Mumtaz Cheema)

Date

Cosupervisor
Dr. Lakshman Galagedara

Committee member
Dr. Raymond Thomas

Abstract

Nitrogen (N) is one of the prime macro nutrients needed to produce crops. Nutritionally poor soils like Podzols, can be amended with N fertilizers to boost crop productivity. However, intensive use of N fertilizers can cause environmental pollution, because about 50% of N applied as fertilizers gets lost through various pathways. Urea (UR) treated with N stabilizers like urease inhibitors (UI) and nitrification inhibitors (NI) can be used to mitigate N losses. I hypothesized that, UR treated with N stabilizers will increase the growth, yield, and quality of field crops cultivated on podzols in boreal climate compared to the untreated UR application. A two-year field experiment was carried out to determine the effect of UI [N-(n-butyl) thiophosphoric triamide - NBPT] and NIs [nitrapyrin and dicyandiamide] on (1) the growth, yield and quality of silage corn, (2) the growth, yield and quality of faba bean and oats + peas mixture, (3) the growth, yield and quality of cash crops such as canola and wheat. Results revealed that forage quality indices: crude fat, calcium, and magnesium in faba beans were significantly decreased by agrotain and superU (containing agrotain and dicyandiamide) compared to untreated UR. Overall, my thesis findings demonstrate the potential of UR treated with N stabilizers to boost crop production on podzols. This information could be useful to improve sustainable agriculture in boreal climates.

General Summary

Podzol soils are one of the limiting factors to the food and feed self-sufficiency in Newfoundland and Labrador (NL), because these soils are highly acidic and generally poor in fertility thereby reducing agricultural productivity in NL. Nitrogen (N) fertilizer application is of great importance because it can enhance the nutritional quality of podzol soils. However, the intensive use of N fertilizers enhances N loss, and can negatively affect the agricultural sustainability in NL. The use of urea (UR) treated with N stabilizers like urease inhibitors (UI) and nitrification inhibitors (NI) can reduce N losses, by synchronizing N release with N uptake, and can increase crop yields. The field experiments showed that UR treated with N stabilizers did not improve the growth, yield, forage quality indices, N uptake, and nitrogen recovery efficiency of the silage corn, faba bean and oat + pea mixture compared to untreated UR. However, UR treated with N stabilizers can be used to reduce N losses from the Podzols.

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List of abbreviations

FAO – Food and Agricultural Organization

GHG – Greenhouse gas

N₂O – Nitrous oxide

NH₃ – Ammonia

NRE – Nitrogen Recovery Efficiency

NU – Nitrogen uptake

NL – Newfoundland and Labrador

NPK – Nitrogen, phosphorus and potassium

N₂ – Nitrogen gas

N – Nitrogen

BNF – Biological Nitrogen Fixation

NO₃⁻ – Nitrate

NH₄⁺ – Ammonium

AOB – Ammonia oxidizing bacteria

NOB – Nitrite oxidizing bacteria

AMO – Ammonia monooxygenase

NO₂⁻ – Nitrite

NO – Nitric oxide

N₂O – Nitrous oxide

ADF – Acid Detergent Fibre

NDF – Neutral Detergent Fibre

CO₂ – Carbon dioxide

SRF – Slow Release Fertilizers

AAPFCO – Association of American Plant Food Control Officials

DCD – Dicyandiamide

NBPT/ NBTPPT – N-(n-butyl) thiophorictriamide (also known as Agrotain)

HQ – Hydroquinone

UI – Urease inhibitors

NI – Nitrification inhibitors

PPDA – Phenylphosphorodiamidate

CHPT – Cyclohexyl phosphoric triamide

ATS – Ammonium thiosulphate

PPD – Phenylphosphorodiamide

PPDA – Phenylphosphorodiamidate

CTS – Calcium thiosulphate

DMPP – Dimethylpyrazole phosphate

TPTA – Thiophosphoryl triamide

BMP – Best (or Beneficial) Management Practices

RDA- Redundance analyses

DM – Dry matter

UR - Urea

GPC – Grain protein content

Chapter One

General introduction

1.1 Overview

The need to produce more food to feed the estimated 9.1 billion population in 2050 as per FAO projection is of primary importance to the agricultural sector worldwide (Nations, 2015). The reason is that there is a growing demand for food which is mostly characterized by changes in consumption patterns, and dietary and nutritional preferences of the growing population; hence a continuous change in the dynamics of agriculture, which in turn requires a continuous demand for agricultural land for food, fibre, and fuel (Bodirsky et al., 2015; Bommarco et al., 2013). Agricultural intensification can be traced back to the green revolution, where there was a shift from traditional agriculture to modern agriculture characterized by new technologies like the use of high-yielding cereal varieties, heavy mechanization, irrigation water supply and management strategies and chemical fertilizers. The use of nitrogen (N) fertilizers became more popular specifically because of their availability in most markets, coupled with their high-water solubility, rapid uptake by plants, and easy usage on farms. Ever since the introduction of synthetic fertilizers in the agricultural industry, these have become a significant component in the intensive agriculture to satisfy the increased demand for food to feed the growing world population (Erisman et al., 2008; Krol et al., 2020).

The increase in N usage since its introduction during the green revolution has however resulted in the rapid increase of greenhouse gas (GHG) emissions especially nitrous oxide (N_2O) (Migliorati et al., 2015; Modolo et al., 2018; van Beek et al., 2010). N gets lost into the atmosphere, the predominant gaseous losses of reactive N are through N_2O and ammonia (NH_3) volatilization as

well as leaching or runoff. N_2O is a potent GHG that destroys the ozone layer while NH_3 is both an indirect source of N_2O and an environmental pollutant. This can result in eutrophication and acidification of terrestrial and aquatic systems (Krol et al., 2020; Ravishankara et al., 2009). The gaseous losses of N from fertilizers application not only pollutes the environment, but also constitutes a great loss in agricultural productivity due to reduced nitrogen recovery efficiency (NRE) of plants and ultimately yields (Forrestal et al., 2017; Krol et al., 2020). Therefore, N loss mitigation strategies need to be implemented to improve NRE, thereby reducing environmental pollution by N application.

The quest for a sustainable and self-sufficient agricultural sector in Newfoundland and Labrador (NL) has prompted the Government to increase agricultural lands to help intensify agricultural production in the province. According to Canada (2017), there was a drop in the production of major field crops such as silage corn and potatoes in NL in 2016 as compared to 2011. The area under cultivated land decreased from 325 to 206 ha for silage corn and 206 to 149 ha for potatoes, while area under wheat increased from 4 ha in 2011 to 103 ha in 2016. This, therefore, decreased the overall operational farm capacity in the province by about 20.2% from 2011 to 2016. Consequently, supplementary commodities are imported from other Canadian provinces as well as other countries (Canada, 2017). For example in 2009, NL imported about 53% of commodities from other Canadian provinces, 18% of commodities were imported from other countries while about 29% were supplied from within the province (Canada, 2014). The high imports in the province could be justified by the environmental features of NL, mostly dominated by short growing seasons, low temperatures, and podzol soils low in available nutrients; all of which impede the agricultural production of the province (Todd and Spaner, 2003). Therefore, to boost agricultural productivity in the province, synthetic fertilizers like urea (UR) or a combined

formulations comprising of N, phosphorus (P) and potassium (K) (NPK) and organic fertilizers manure or compost, which will help improve the soil nutrient availability, can be used (Ali et al., 2019).

The use of synthetic and organic fertilizers can certainly be beneficial on the podzolic soils used for agriculture in NL, particularly in maximizing yields. However, there are some disadvantages associated with the use of either organic or synthetic fertilizers. Organic fertilizers like animal manure may cause air pollution and pest infections when not properly treated while composting is a long and tedious process that requires various materials for its production. Additionally, organic fertilizers are needed in large quantities to sustain agricultural production (Cuttle et al., 2007). Synthetic fertilizers, on the other hand, are costly and they are the major causes of atmospheric and environmental pollution (GHG, soil acidification, water pollution) especially when nutrients applied are not efficiently used (Bittman et al., 2014; Sutton et al., 2013). Though fertilizers can be used to improve upon nutrient supply from the podzolic soils and to boost the agricultural productivity in NL. Sustainable management practices like reduced or no-tillage, crop rotation, or mixed cropping, and the application of fertilizers following the 4 R stewardship (right source, right rate, right time, and right place) can help maximize nutrient use efficiency and minimize nutrient losses/and environmental pollution.

1.2 Significance of the study

Based on the current increase in the world temperature, mostly caused by climate change, regions of the world characterized by boreal climates like NL should be experiencing greater heating units favorable to crop growth (Altdorff et al., 2017; Todd and Spaner, 2003). However, the predominant soil type in NL are podzols, which are generally known to be less accommodative to agricultural

production because of low fertility and adverse physical properties (Altdorff et al., 2017). This can decrease the potential of a sustainable and self-sufficient agricultural sector in the province. Nevertheless, the sustainable use of N fertilizers by mitigating N loss, can be beneficial to enhance crop production in NL. There are a variety of different N loss mitigation strategies, which could be used to reduce N losses in agriculture. However, my focus of research was on the use of UR and UR stabilized with urease inhibitors (UI) and nitrification inhibitors (NI) to mitigate N losses; thereby increasing the growth, yield, and quality of field crops' produce in a boreal climate. The current study may also be favourable to the quest of the NL government to expand new agricultural lands as well as intensify agricultural activities in the province, making NL more food and feed self-sufficient. Consequently, this study will be making use of new technologies like N fertilizers amended with UI and NI to (i) reduce N losses, (ii) increase NRE and (iii) enhance the growth and development of field crops like silage corn, wheat, and canola, and introduce new crops such as faba bean, oats + peas mixture. Following were the hypotheses of the study:

- i.) Urea treated with N stabilizers will increase the growth, forage dry matter (DM) and forage quality indices of silage of silage corn cultivated on Podzols in boreal climate.
- ii.) Urea treated with N stabilizers will increase the growth, forage DM and forage quality indices of faba beans and oat + peas mixture.
- iii.) Urea treated with N stabilizers will increase the growth, grain yield, 1000 seed/grain weight and seed/grain protein concentration of canola and wheat.
- iv.) Urea treated with N stabilizer will increase N uptake and NRE of field crops cultivated on Podzols in boreal climate.

The following objectives were set out to test these hypotheses:

- i.) To investigate the effect of urea and urea treated with N stabilizers on the growth, forage DM, and forage quality indices of silage corn cultivated on Podzols in boreal climate.
- ii.) To evaluate the effect of urea and urea treated with N stabilizers on the growth, forage DM, and forage quality of faba bean and oat + peas mixture.
- iii.) To elucidate the effect of urea and urea treated with N stabilizers on the growth, seed/grain yield, 1000 seed/grain weight and grain protein concentration of canola and wheat.
- iv.) To correlate the effect of urea and urea treated with N stabilizers on the N uptake and NRE in the field crops cultivated on Podzols in the boreal climate.

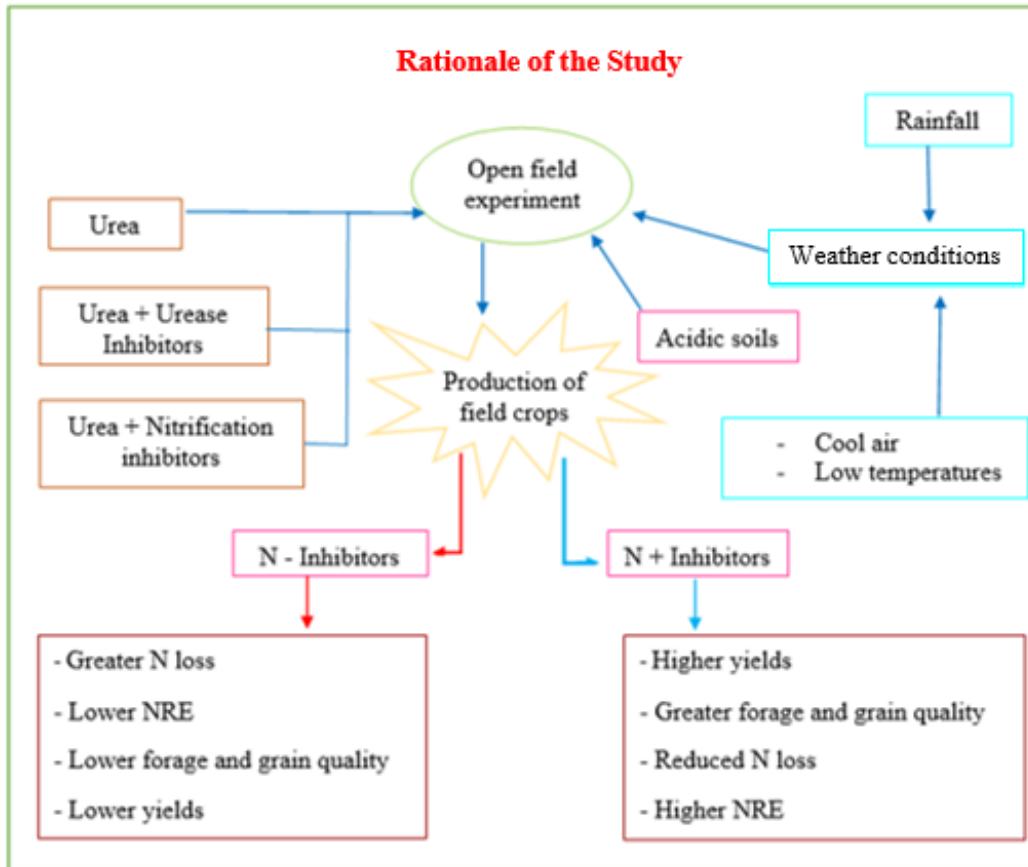


Figure 1.1: A schematic description of the field experiment

1.3 Thesis organization

This thesis is divided into six chapters comprising of the relevant literature reviewed at the beginning of each chapter.

Chapter One: comprises the general introduction and overview of the thesis. It provides background information, rationale, and objective of the thesis.

Chapter Two: provides a detailed review of literature on major concepts and theories relevant to the study.

Chapter Three: gives a detailed knowledge about field experiment on “the effects of urea and urea treated with N stabilizers on growth, yield, forage quality indices, N uptake, and NRE of silage corn grown for two consecutive years”.

Chapter Four: explains the role of urea and urea treated with N stabilizers on the growth, yield, forage quality indices, N uptake, and NRE in legume forage/and forage mixture such as faba bean and oats + peas mixture.

Chapter Five: provides detailed information on another field experiment “the effects of urea and urea treated with N stabilizers on the growth, grain yield, 1000 grain/seed weight, grain/seed protein content, N uptake, and NRE of wheat and canola grown in a boreal climate”.

Chapter Six: makes up of general discussion, conclusions, and recommendations of the study.

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Chapter Two

Literature Review

2.1 Nitrogen uses in agriculture

The dramatic increase in agricultural production mostly in developing countries over the past four decades is a direct effect of the green revolution (Matson et al., 2012). The successes of the green revolution recorded over the years have been immense; one of which was the use of the Haber–Bosch process to manufacture nitrogen (N)-based fertilizers. This has aided not only in the intensification of agriculture but also in the development and exploitation of previously non-agricultural lands; thus, enabling the supply of food to the ever-growing world population (Matson et al., 2012). Since the discovery and production of chemical N fertilizer, N has been the backbone of agriculture as demonstrated by the huge increase in N fertilizer use globally over the last 50 years (from 10.8 million Mg in 1960 to 82 million Mg in 2000 and an anticipated further rise to 249 million Mg in 2050) (Han et al., 2016; Iannetta et al., 2016). Generally, the macronutrients needed by plants are N, phosphorus (P), and potassium (K). However, a greater amount of N is required during crop growth because a deficiency in N leads to a reduction in the plant morphology, growth rate, and life cycle of crops (Kaplan et al., 2016). N is one of the major macronutrients all crops need throughout their growth and development including corn (Kaplan et al., 2016), wheat (Ghafoor et al., 2021), oats + peas mixture (Krga et al., 2021), peas + barley mixture (Hauggaard-Nielsen et al., 2009).

2.2 Nitrogen Cycling

N constitutes about 78% of the atmosphere. Its usable forms are very limited since living organisms need it only in small quantities. However, it is a very important nutrient that can limit both crop production and human growth when in deficit (Kuypers et al., 2018; Smil, 2004). According to Smil (2004), N is present in the atmosphere as a nonreactive dinitrogen (N_2) molecule, which limits its availability and usage. For N_2 to be reactive, it needs to be split into its two constituent atoms. There are two ways through which N_2 can be split before being incorporated either in an organic or inorganic compound; that is physically through lightning and biologically by the rhizobium bacteria in legumes' root nodules through biological nitrogen fixation (BNF) (Smil, 2004).

Relying solely on BNF for N_2 to be incorporated into agriculture before the green revolution was challenging particularly in large-scale farming; consequently, the Haber-Bosch process was created during the latter half of the 19th century to provide a long-lasting solution to future N shortages (Smil, 2004). The Haber-Bosch process refers to the manufacturing of ammonia which is the simplest of all N compounds, from the synthesis of N and hydrogen at high pressure (150-200 atm) and under high temperature (400 to 500 °C) (Murmu et al., 2021). Currently, ammonia is an important raw material in industrial sectors such as medicine, chemical products (Murmu et al., 2021), and most especially the agricultural sector where about 80% of ammonia being produced worldwide goes into fertilizers especially urea (UR) (Kuypers et al., 2018; Smil, 2004). N transformation in agriculture happens chronologically following six distinct processes and for N compounds to be transformed to reactive N, nitric oxide (NO), and N_2 ; various microbes (bacteria, archaea, and fungi) are involved (Kuypers et al., 2018). In summary, the cycle begins with a molecule of N_2 gas which after being fixed to ammonia (NH_3) gets assimilated onto organic N or biomass. Thereafter, organic N gets degraded through the process of ammonification which

involves the release of NH_3 molecule. This NH_3 molecule will then oxidize to nitrate (NO_3^-) via the process of nitrification. To complete the N cycle, denitrifying microbes during denitrification or anaerobic ammonium oxidation will reduce NO_3^- to produce molecular N_2 (Fageria and Baligar, 2005; Kuypers et al., 2018). A pictorial representation of the N cycle is given in Figure 2.1.

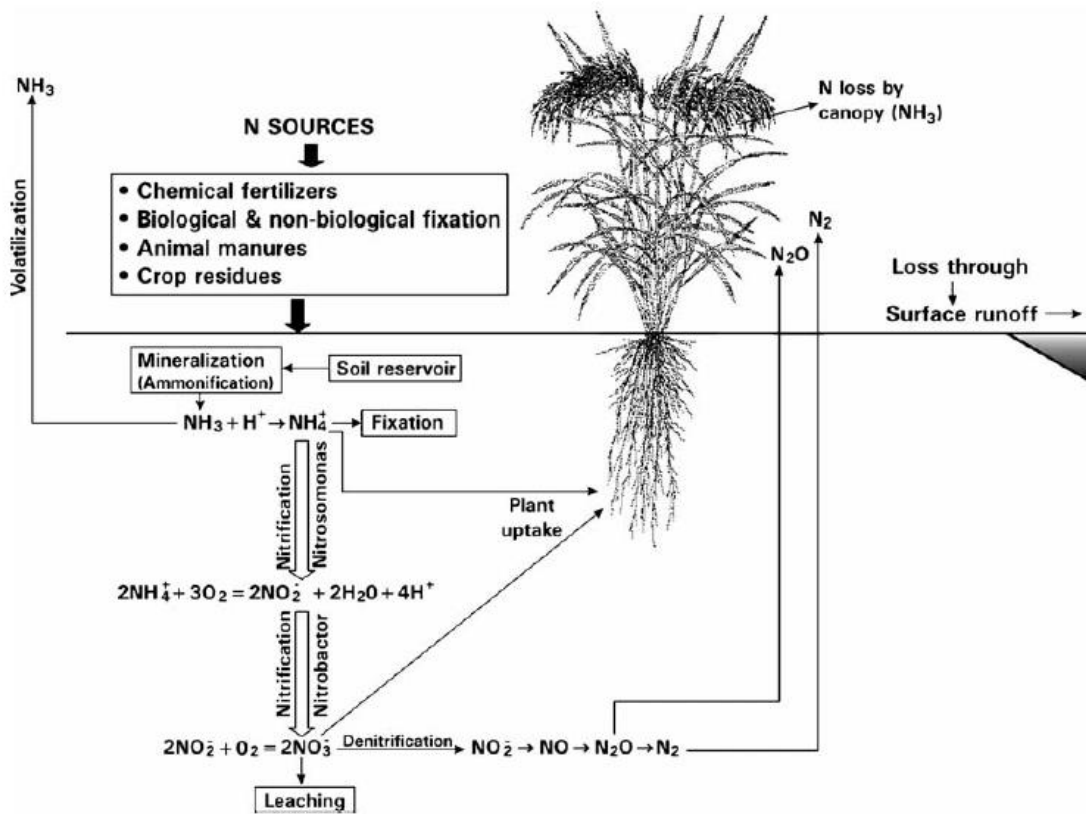
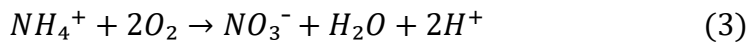
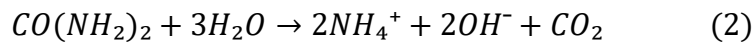
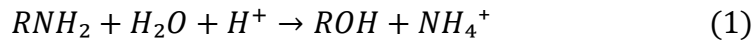


Figure 2.1: A summarized version of N cycling illustrating the processes of fixation, assimilation, ammonification, nitrification, and denitrification along with N loss processes of volatilization, leaching, and runoff in a soil-plant system. Source: Fageria and Baligar (2005).

The major N transformation processes, which take place in agricultural soils are BNF, mineralization, nitrification, and immobilization (Fageria and Baligar, 2005). During mineralization, organic N gets converted to ammonium (NH_4^+) and nitrite (NO_2^-) by microorganisms. Mineralization can also be called ammonification because NH_3 is the first mineral

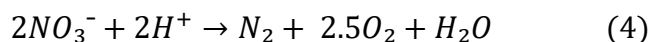
to be formed (Fageria and Baligar, 2005; Kuypers et al., 2018). Ammonification is the hydrolysis of N where the organic and inorganic compounds are catalyzed by enzymes to produce NH_4^+ (Bolan and Hedley, 2003; Fageria and Baligar, 2005). Generally, the first stage of the hydrolysis process yields NH_3 which is a polar molecule that easily gets combined with hydrogen protons (H^+) and produces NH_4^+ (Equations 1 and 2).

During the nitrification process, NH_4^+ gets oxidized to NO_3^- because of the activity of two groups of chemoautotrophic bacteria, the ammonia, and nitrite-oxidizing bacteria (AOB and NOB). The subgroups of AOB β and γ (*Nitrosomonas* spp. and *Nitrosococcus* spp., respectively) mostly the ammonia monooxygenase (AMO) enzyme, begins the nitrification process by oxidizing NH_3 to hydroxylamine (NH_2OH). Thereafter, NH_2OH is oxidized to NO_2^- through hydroxylamine oxidoreductase (Coskun et al., 2017). NOB (particularly *Nitrobacter* spp.) will complete the process by producing NO_3^- through the nitrite oxidoreductase enzyme (Equation 3) (Daims et al., 2016; Hayatsu et al., 2008; Kowalchuk and Stephen, 2001).



Denitrification is the microbial production of NO_2^- , nitric oxide (NO), nitrous oxide (N_2O), and N_2 after the reduction of NO_3^- (Equation 4) (Davidson and Seitzinger, 2006). This reduction process usually takes place in the absence of oxygen by phylogenetically heterogeneous microorganisms which belong to subclasses of bacteria, some archaea, and fungi (Philippot and Hallin, 2005). Alkaline soils are prone to a higher denitrification rate as compared to acidic soils because of high NH_4^+ and NO_3^- concentrations, water content, carbon availability, and

temperature. For example, denitrification is higher in flooded rice culture because of excess water supply (Fageria and Prabhu, 2003; Fageria and Baligar, 2005).



2.3 Nitrogen uptake in plants

The ability of a plant to take up N from the soil and transfer it to the different growing parts for economic purposes is considered nitrogen recovery efficiency (NRE) (Sher et al., 2019). NRE in plants mainly involves N uptake and utilization. However, utilization can be further broken down into assimilation and translocation or remobilization (McAllister et al., 2012). When plants take up NO_3^- with the help of nitrate transporters (NRT1 and NRT2), NO_3^- is converted to NH_4^+ and amino acids before being transported within the plants (McAllister et al., 2012). NH_4^+ assimilation takes place in the plastid or chloroplast through the glutamine synthetase/glutamate synthase (GS/GOGAT) [GS; EC6.3.1.2, NADH-GOGAT; EC1.4.1.13, Ferredoxin (Fd)-GOGAT; EC1.4.7.1] system of reactions (McAllister et al., 2012; Suzuki and Knaff, 2005). After the assimilation of N in the plant, it is transported within the plant through the xylem as glutamine, asparagine, glutamate, and aspartate either for utilization and/or storage (McAllister et al., 2012; Okumoto and Pilot, 2011). When N reaches the sink tissues, assimilation enzymes such as GS1 and glutamate dehydrogenase (GDH)[EC1.4.1.2.] help to remobilize N to the grains during senescence and grain filling (McAllister et al., 2012).

2.3.1 Nitrogen uses in Corn

The two most cost-demanding practices in corn production are irrigation and fertilization (Islam et al., 2010; Kaplan et al., 2016; Khelil et al., 2013). The availability of water and N during corn

growth and development leads to an increase in photosynthesis activity because N constitutes an important component in chlorophyll synthesis (Kaplan et al., 2016). The lack of N in corn induces early flowering, which results in a reduction in tasseling and kernel production. During the grain-filling stage, N in vegetative parts gets transported to the ears and leaflets in the form of sucrose and other sugars. Thus, the high content of N in corn kernels is highly dependent on the N absorbed during the vegetative stage (Kaplan et al., 2016; Spielbauer et al., 2006; Uribelarrea et al., 2004). According to Kumar et al. (2001), an increase in N fertilizer will lead to an increase in corn biomass. Kaplan et al. (2016) stated that N plays a critical role in the metabolic processes of proteins and enzyme synthesis of plants basically because the processes are controlled by the enzymes activity. The authors further explain that the optimum availability of both N and water leads to an increase in plant height, stem diameter, stem, and cob ratios, hence an increase in biomass yield. The forage quality of corn can also be affected by N, since some studies have reported that N fertilizers have led to an increase in silage digestibility and crude protein concentration (Kamalak et al., 2011; Kaplan et al., 2016).

2.3.2 Nitrogen use in legume crops.

The uniqueness of N use in legumes lies in their ability to fix N from the atmosphere through the symbiotic relationship that exists between the legumes and the rhizobium bacteria located in the root nodules (Pandey et al., 2017). This symbiotic relationship is commonly known as BNF whereby atmospheric N is reduced to plant-available forms (Terpolilli et al., 2012).

During BNF, the rhizobia react to plant-obtained flavonoids in the rhizosphere by fusing the Nod factor. Oldroyd et al. (2011) in their review defined the Nod factor as a lipochitooligosaccharide signal molecule that stimulates nodule organogenesis and root hair deformation in the plant. The

rhizobia then contaminate plant roots through the root hair or by crack entry (Sprent, 2009; Terpolilli et al., 2012). The infection thread is later produced after an infolding of the plant's membrane at the infection focal point on the root hair. This thread then grows down the cortical cell layers into the nodule meristem. After forming a micro-colony at the root hair, the bacteria then begin to divide down to become a growing infection thread (Terpolilli et al., 2012). The point of infection becomes a critical juncture for the plant and microbe interaction. As the plant allows access to the rhizobia (which at this point is considered a potential pathogenic threat to the plant) to an intracellular space where a defense mechanism is built to protect the plant yet allowing the infection of the N₂-fixing organism to proceed (Downie, 2010; Terpolilli et al., 2012). For a successful infection, the rhizobia must produce the correct type of Nod factor recognizable by the plant because that is what will induce root curling and initiate nodule organogenesis (Oldroyd and Downie, 2008; Terpolilli et al., 2012).

The infection of the rhizobia happens through cell division and this cell division of the infection thread only occurs at the terminal portion. When the infection thread reaches the developing nodule, the bacteria begin to leave the thread encapsulated by a plant membrane derived from part of the infection thread known as an infection droplet (Terpolilli et al., 2012). These droplets eventually create the symbiosome (the basic nitrogen-fixing unit) of the nodule where the rhizobia will evolve into bacteroids. The exchange of nutrients which happens between the plant and the bacteroids in the symbiosome goes across two membranes; one obtained from the plant (which is reversed outwards to face the bacteria) and the other from the bacteria (Terpolilli et al., 2012). The process of N₂ fixation will be carried out by the rhizobia bacteria which results in the production of NH₃, which is transported and absorbed by the plant (Denton et al., 2000; Garau et al., 2005; Terpolilli et al., 2012; Terpolilli et al., 2008). Though local farmers at the time were not educated

enough to understand the above mechanism, they, however, understood the importance of recycling organic waste alongside the cultivation of legumes (Smil, 2004).

In as much as legumes can fix and use atmospheric N, according to Kaur et al. (2017), synthetic N will still be needed to supplement N deficiency which may occur during the growth and development of legume crops. Generally, after planting, legumes take about two to three weeks to develop root nodules and until these nodules are mature, BNF is not possible. Therefore synthetic N is needed as a starter. (Jiang et al., 2020; Meakin et al., 2007). From the study carried out on soybean, Kaur et al. (2017) explain that only about 50 to 60% of the N demands of the soybean plants were met through BNF, which was not enough for the plants to thrive all through their life cycle. Krga et al. (2021) and Neugschwandtner et al. (2015) reported that there was a beneficial effect of N fertilizer in an oat and peas mixture; where the percentage of oats planted was higher than that of peas. This is because there will be competition for nutrients amongst the oats crop since they depend on the availability of N in the soil and the BNF of the peas might not produce sufficient N required for both crops. Neugschwandtner and Kaul (2015) also reported that there was a greater N fertilizer economic efficiency in the oats and peas mixture of 100:15% as compared to 100:30% because the lower ratio of peas allowed for greater NRE by the oat plants hence greater yields. Additionally, Ray et al. (2006a) reported a 7.7% and 15.5% increase in soybean yield in irrigated and non-irrigated environments respectively in response to a large amount of N applications in Mississippi (Kaur et al., 2017; Ray et al., 2006b). Similarly, in Mississippi, there was a 4% and 8% increase in soybean seed yield on sandy/silt loam and clay soil, respectively, as a result of N applications (Kaur et al., 2017; McCoy, 2016). The response of legumes to N fertilization is dependent on various factors such as soil properties, temperature, pH, moisture,

irrigation, cultivar, and soil nitrate content before planting (Kaur et al., 2017; Scharf and Wiebold, 2003).

The beneficial effect of N fertilizers on legumes was contradicted by the study of Voisin et al. (2002b) who explained that the availability of mineral N in the soils has a negative impact on the role of BNF in legumes because even the smallest concentration of nitrate in the soil will inhibit the formation of legume nodules and nitrogenase activity. Similar findings were reported by (Hossain et al., 2016; Kiers et al., 2003; Salvagiotti et al., 2009; Schipanski et al., 2010). However, Köpke and Nemecek (2010) support that higher rates of BNF are possible in faba beans even with soil N because faba beans can resist the presence of high amounts of N fertilizers in the soil. Hossain et al. (2016) suggest that the positive and negative correlations between faba bean's percentage of N derived from the atmosphere with soil N uptake and soil mineral N which was observed in their study might be because there was a higher demand for N as compared to the inhibitory effect that N would have on BNF. Mohamed and Babiker (2012) reported that the prior application of N fertilizer at 20kg/ha led to the enhancement of nodule formation in faba bean plants.

The agricultural database of Canada reports that there is a variety of legumes crops grown in the country (Huffman et al., 2006; Yang et al., 2010). The non-edible legume crops grown in the country consist of alfalfa and hay (alfalfa and clover mixed). The edible legume crops include lentils, soybean, dry field peas, common bean, faba beans, and chickpeas (Yang et al., 2010). These different legume crops are produced in different provinces of the country. For example, the production of common beans totals about 60% and it is mostly grown in central Canada (Ontario and Quebec); while lentils and dry field peas are mostly found in the prairie provinces. The land

used for production of hay is 36%, 20%, and 13% in Alberta, Quebec, and Ontario, respectively (Yang et al., 2010).

2.3.3 Nitrogen use in wheat and canola

Nitrogen plays a vital role in the growth and yield of wheat and canola, especially in their protein content (Pampana et al., 2013; Smith et al., 2019). Like other crops, wheat does not require a huge amount of N at the germination stages (Cameron et al., 2013). Pampana et al. (2013) explained that N availability becomes crucial at the tillering and stem elongation stage because it is during this stage that the crops can take up a greater amount of N. If N is deficient at this stage this leads to an increase in shoot mortality, smaller spike size, and hence a limited number of kernels produced per unit area (Arduini et al., 2009; Pampana et al., 2013). To attain maximum grain yield in wheat, N should be applied at various growth stages that is; a split application at seeding and tillering or split application at seeding, tillering, and heading as compared to a full dose at seeding (Ayub et al., 2001; Pampana et al., 2013). This is because the unstable nature of N in the soil will lead to greater N loss when the full dose is applied at seeding, thus reducing the available N in the soil for the crop during its growth cycle. From the findings of Pampana et al. (2013) there will be better NRE, increase in grain yield as well as a reduction in N leaching in wheat when N is split and top dressed prior to stem elongation.

N is a key constituent of proteins, and it is therefore important to ensure that N is applied at the right time. The protein content in wheat grains can be increased by split N application (Fuertes-Mendizábal et al., 2010). Although two doses are more common, Fuertes-Mendizábal et al. (2010) suggested that three applications can supply adequate N during the reproductive stage. The grain yield of wheat is directly linked to the availability of N because N increases tillering density, kernel

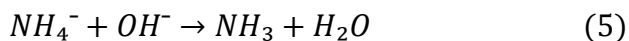
production, kernel weight as well as grain protein content (Szentpétery et al., 2005). Szentpétery et al. (2005) report that the wheat grain protein content was increased by 0.8% after a split application of N as compared to pre-plant application, and by 1.6% after a late N foliar application as compared to no foliar application. The yield, NRE, and grain protein of wheat can also be increased by the split application of controlled-released UR (Beres et al., 2018).

Canola can take up large quantities of N (Avice and Etienne, 2014). However, the NRE of canola (N harvested in seed) is only about 50% (Avice and Etienne, 2014). The low NRE is attributed to N losses through leaching, volatilization, runoff, and denitrification and also to N partitioning in non-harvested portions (Kaefer et al., 2014). N has a positive effect on canola seed germination (Andrade et al. (2020) and the successful establishment of canola is highly dependent on seed germination (Brunes et al., 2015). Sanches et al. (2014) report that an N dose of 90kg ha⁻¹ led to an increase in canola seed weight. Canola oil contents increase when N fertilizer application is split (Narits, 2010) as compared to all N applied at seeding. Although sulphur (S) is needed during canola growth and development (Khoury et al., 2015), N fertilizer aids in the vigorous production of seeds (Andrade et al., 2020). In Brazil, high temperatures led to a drop in the canola yields regardless of N application (Kaefer et al., 2014).

2.4 Nitrogen losses pathways

Nitrogen losses can be associated with NRE in plants because, when the NRE of plants is low, there is an increase in N loss through pathways like leaching, volatilization, denitrification, and runoff (Ullah et al., 2021). After the application of UR, the fertilizer granules will lead to an increase in the soil pH of the surrounding area of the UR granules, and with the help of the urease enzymes, UR hydrolysis immediately takes place (Fageria and Baligar, 2005). Generally, the

hydrolysis of UR will lead to -NH_3 loss through volatilization (Folina et al., 2021). Volatilization happens as a result of the conversion of NH_4^+ to NH_3 gas (Equation 5), which is rapidly subjected to getting lost especially in an alkaline growth medium (Fageria and Baligar, 2005).



Under warm and humid conditions, NH_3 volatilization increases from about 16% to 40% or more (Artola et al., 2011; Cantarella et al., 2018). NH_3 volatilization is influenced by N application time, plant growth stage, soil, and plant N status (Fageria and Baligar, 2005). The N demand by crops varies thus if the N supply is higher than the crop plant N demand, the excess is susceptible to losses of N.

Leaching and runoff are other pathways through which N gets lost in the environment. Since most N fertilizers used on agricultural fields are ammonium-based (urea, anhydrous NH_3 , $(\text{NH}_4)_2\text{SO}_4$, and NH_4NO_3); when the soil particles are not negatively charged to help retain the excess amount of cations like NO_3^- , leaching and runoff occurs (Cameron et al., 2013; Coskun et al., 2017; Halvorson et al., 2014; Lin et al., 2001). Considering that the amount of N loss by leaching is directly proportional to the quantity of N applied, the soil texture or permeability, and the amount of rainfall or irrigation water supplied, sandy soils are prone to high N leaching as compared to fine textured soils (Davis et al., 2003; Fageria and Baligar, 2005).

There are many negative consequences associated with N loss. For example, NO_3^- pollution of freshwater leads to algal bloom, the loss of marine life and diversity due to excessive N deposition, and the contamination of ground water as a result of N leaching (Ghafoor et al., 2021; McAllister et al., 2012). Linquist et al. (2012) explain that the change of state of NO_3^- to N_2O is environmentally unhealthy, because N_2O is both a greenhouse gas (GHG) with a capacity to trap heat 300 times/molecule as compared to carbon dioxide (CO_2) and is the most important destroyer

of the ozone layer in the atmosphere (Ravishankara et al., 2009). Agriculture is currently considered the major source of global anthropogenic GHG emissions with about 60 to 80% of N_2O emissions and 10 to 12% of all other anthropogenic GHG emissions. The loss of N_2O through denitrification of N during the reductase sequence can account for up to 97% of the total N_2O emission from the crop system (Kool et al., 2011; Liu et al., 2016). While the indirect loss of N_2O emission through volatilization, deposition, leaching, and runoff may account for 28 to 37% of global agricultural N_2O production (Coskun et al., 2017; Reay et al., 2012). Consequently, it is of paramount importance that sustainable agricultural practices be carried out through the establishment of alternative N management strategies. Management strategies such as crop rotation, mixed cropping with legumes, no-tillage, the use of legume cover crops, and the use of nitrogen inhibitors are some of the techniques that can be used to mitigate N loss. However, the use of urease and nitrification inhibitors is becoming a more promising strategy for the mitigation of N losses (Migliorati et al., 2015).

2.5 Strategies to mitigate N losses

The most efficient way to mitigate N loss would be by increasing the NRE of plants. This can be achieved by using the 4R principle (Flis, 2017; Ghafoor et al., 2021). However, Stark and Richards (2008) detail four major categories under which N loss mitigation can be targeted that is; land, nutrient, livestock, and manure management.

i.) Land management

- a.) Tillage activities may either increase or reduce N loss. Reduced or zero tillage is the preferred practice as compared to deep tillage because if the mineralization rate of the soil is still high due to high crop residue and organic matter from the previous season, NO_3^-

leaching is at risk of occurring especially in events of heavy rainfall (Spiess et al., 2020). Reduced or zero tillage is also beneficial because it reduces the cost of production while increasing carbon (C) content (Jantalia et al., 2008; Mkhabela et al., 2008).

b.) The efficient use of land and an appropriate farming system like the conversion of arable land to either forestry, extensive grassland farming, or even a complete change for organic farming; may reduce yields but at the same time reduce NO_3^- and N_2O (Aronsson et al., 2007; Saggar et al., 2008).

c.) NO_3^- can be reduced by installing wetlands, farm ponds, and the C amendments of the ground water which will help enhance denitrification. However, this may increase production costs and N_2O loss (Fenton et al., 2008; Hoffmann and Baattrup-Pedersen, 2007).

d.) Methods like crop rotation, cover cropping, irrigation, and the use of high-yielding crop species are also good N loss mitigation mechanisms because they help reduce the number of inputs and stimulate N immobilization thereby reducing NO_3^- and N_2O while increasing yields (Hansen et al., 2007; Hooker et al., 2008).

ii.) Nutrient management

a.) Applying the right quantity of fertilizers according to the N crop demand will maximize NRE since most of the fertilizer N will be taken up by plants. This will reduce cost and excess N application hence a reduction in NO_3^- and N_2O (Shepherd and Chambers, 2007; Van Groenigen et al., 2008).

b.) Since the conventional N fertilizers get hydrolyzed almost immediately after application it is of core importance that these are applied at the right time that is when plants easily and rapidly absorb it. The spring N application as compared to the fall N application led to a

reduction of NO_3^- in the production of corn (Jeong and Bhattarai, 2018) and a corn and soybean rotation in Illinois (Ruffatti et al., 2019). NO_3^- loss can be reduced by N timing by 40%. However, other factors like soil texture and weather conditions other than N application can influence NO_3^- loss (Quemada et al., 2013).

- c.) The type of fertilizer being used also plays a vital role in N loss mitigation. The use of BNF help reduce the need for synthetic fertilizers while slow-release fertilizers help reduce the hydrolysis process of N thereby matching N release to plant N uptake (Lehmann et al., 2003; Sommer et al., 2004; Yanai et al., 2007), this helps reduce NO_3^- , N_2O , and NH_3 while increasing soil properties and biomass yield.
- d.) Chambers et al. (2000) explained that fertilizer application can either be injected, incorporated in the soil, or band applied to help reduce NH_3 loss since the direct exposure of N fertilizers particularly under high temperatures speeds up the hydrolysis process of N fertilizers and hence greater N loss.
- e.) Manure management will involve activities like the storage mechanism or selection of the appropriate manure type. The use of low NH_3 emitting storage facilities should be prioritized to ensure proper storage. Also, solid manure as opposed to slurry will be beneficial in the reduction of NO_3^- , and N_2O because animal slurry is usually in liquid form and may easily leach down the soil profile (Cuttle et al., 2007; Mosquera et al., 2005; Shepherd and Chambers, 2007).

2.5.1 Characteristics of slow-release fertilizers

Slow-release fertilizers (SRF) are also known as controlled availability fertilizers, delayed-release fertilizers, metered-release fertilizers, or slow-acting fertilizers (Fu et al., 2018; Trenkel, 2010).

The discovery of SRF was first reported in the 1920s but they did not gain popularity until the 1960s (Fu et al., 2018). However, the fertilizer industry has currently gained more popularity in the 21st century with about a 6.5% annual growth rate from 2014 to 2019 (Folina et al., 2021; Fu et al., 2018) in the SRF. There are three major characteristics mandated by the European Committee for Stabilization that govern the acceptability of fertilizers to be SRFs (Fu et al., 2018). These characteristics include the ability to release up to 15% and 75% of the fertilizer nutrients within the first 24hrs and 28 d after application, respectively. Finally, a total of at least 75% of the fertilizer should be released within 28days (Fu et al., 2018). The Association of American Plant Food Control Officials (AAPFCO) supports that for fertilizers to be considered slow-release, they need to continuously deplete their nutrients long after their application time (Folina et al., 2021). The main importance of SRF is their ability to have a longer nutrient release time (Yamamoto et al., 2016). This ensures constant nutrient availability for plant growth and a reduction in environmental pollution.

The shortcomings associated with SRFs are mostly high costs as compared to conventional fertilizers (Folina et al., 2021). For example, in 2017 the prices of soluble UR ranged from \$178 to 326 MT⁻¹, controlled-release UR (sulphur coated) ranged from \$327 to 950 MT⁻¹ and controlled-release UR (polymer coated) ranged from \$905 to 2940 MT⁻¹ in the world market (Wesolowska et al., 2021). Also, some SRF are not user-friendly and their efficacy can greatly be affected by the soil pH, soil microbial activity, soil organic matter, temperature, and moisture (Fu et al., 2018; Kakabouki et al., 2020). However, the most commercialized SRF are those containing urease (UI) and nitrification (NI) inhibitors such as N-(n-butyl) thiophosphorictriamide NBPT, hydroquinone HQ, nitrapyrin, and dicyandiamide (DCD) (Folina et al., 2021).

2.5.2 Role of urease and nitrification inhibitors in mitigating N losses and crop production.

One of the main functions of N inhibitors is to amend the fertilizer release pathway by changing the biological activities of N-metabolizing enzymes of soil bacteria. This inhibits the activities of urease and nitrification resulting in N loss during the N cycle (Chien et al., 2009; Timilsena et al., 2015). Generally, SRF which contain urease inhibitors (UI) are manufactured using synthetic compounds which have a similar structure or affinity with urease. There are three groups of synthetic UI compounds namely:

- i. organic or inorganic compounds such as alk(en)ylthiosulfinate, hydroquinone, and p-benzoquinone, which will react with sulfhydryl (mercapto) groups of ureases.
- ii. metal-chelating compounds such as caprylohydroxamic acid and acetohydroxamic acid can form a complex at the active site of the urease with one of the N atoms.
- iii. Competitive inhibitors such as hydroxyurea, phosphoramides, phenyl phosphorodiamidate (PPDA) and N-(n-butyl) thiophosphorictriamide (nBTPT). These are like the UR structure and can bind to the active site of the urease but do not get readily hydrolyzed by the urease (Kafarski and Talma, 2018; Upadhyay, 2012).

The most frequently used SRF are structured with the competitive inhibitor thiophosphorictriamide (NBPT) (Kakabouki et al., 2020; Singh et al., 2008). UIs can also be categorized into solid (e.g., by coating) or liquid fertilizer products and can be applied solely to the soil to help lessen UR hydrolysis by urease. The major role of UI is to slow down the conversion of UR to NH_4 as well as reduce NH_3 volatilization (Saggar et al., 2013; Upadhyay, 2012). According to Panel (2015), NBPT can reduce NH_3 loss from urea by about 66%.

The most widely used UI is N-(n-Butyl) thiophosphoric triamide (NBTPT or NBPT) (Dimkpa et al., 2020). NBPT is commercially known as Agrotain, which was developed by Koch Agronomic Services about two decades ago. Agrotain is commonly applied at approximately 2 to 4 ml kg⁻¹ UR or about 0.2 to 0.4 kg ha⁻¹ (Dimkpa et al., 2020). Compared to other urease inhibitors, cyclohexyl phosphoric triamide (CHPT), ammonium thiosulfate (ATS), phenylphosphorodiamide/phenylphosphorodiamidate (PPD/PPDA), hydroquinone, and calcium thiosulfate (CTS), Agrotain has proven to be able to significantly lessen UR volatilization (Dimkpa et al., 2020). However, factors such as temperature, soil pH, and soil texture can affect the efficacy of NBPT (Abalos et al., 2014; Dimkpa et al., 2020).

Contrary to the UI, nitrification inhibitors (NI) are aimed at retarding the nitrification rate by discontinuing the activity of the nitrifying bacteria (Dimkpa et al., 2020). Generally, the time frame required for the nitrification process to be completed is relatively longer (20 to 28 d) than that of urease hydrolysis inhibition (5 to 7 d). This suggests that the NI will need to prolong their inhibitory activity for as long as the nitrification period lasts, hence this can provide a greater opportunity to synchronize N release with crop N uptake (Singh, 2010), leading to a potential reduction of N₂O emission and NO₃⁻ leaching. The most popular NI used are dicyandiamide (DCD) and 3,4,-dimethylpyrazole phosphate (DMPP) (Harty et al., 2016; Zerulla et al., 2001). The use of SRF with DCD at 1.4 to 1.8 kg ha⁻¹ (Harty et al., 2016) or DMPP at 0.5 to 1.5 kg ha⁻¹ (Zerulla et al., 2001) has proven to significantly reduce NO₃⁻ in the soil as compared to the sole use of UR. Also, Akiyama et al. (2010) reported that other NI such as thiophosphoryl triamide (TPTA) and neem (*Azadirachta indica*) can reduce the N₂O emission on an average by 38%. Though there is some contrasting opinion concerning which of the above is the best NI, these

studies agree that NI can mitigate N loss (Abalos et al., 2014; Akiyama et al., 2010; Dimkpa et al., 2020; Linqvist et al., 2013; Pan et al., 2016).

Aside from N loss mitigation, UI and NI are beneficial for crop production mainly because they make available N for plant uptake hence an increase in yields (Abalos et al., 2014). In the study by Gans et al. (2006), they observed that 231mg of N was taken up by plants per pot as compared to 150 mg N uptake in the control treatments; suggesting that UI increases N uptake in plants. Another SRF commercially known as Limus, which contains UI 25% NPPT + 75% NBPT was used on Fluvoaquic and alluvial soils to grow maize, and the results demonstrated that the soils treated with Limus decreased cumulative NH₃ losses by 84% as compared to untreated soils. The study also reported a 17% increase in N recovery from the treated soils as well as high yields even after 60% reduction in the fertilizer applied (Li et al., 2017; Modolo et al., 2018).

Hydroquinone (HQ) is a urease inhibitor that can reduce the emission of N₂O and NO by around 5% (Modolo et al., 2018). However, when HQ was combined with the NI DCD, there was an increase in the growth of rice and a reduction in N₂O emission as compared to UR treatments without inhibitors (Modolo et al., 2018; Xu et al., 2005). A similar study was carried out to determine the amount of N₂O emission in paddy soils after the amendments of 300 kg N ha⁻¹ with 0.3% HQ + 5.0% of DCD. The results demonstrated that N₂O emissions were reduced in the soil by 24%, 56%, and 17%, respectively before transplanting rice, at the tillering stage, and at the panicle initiation stage as compared to the UR fertilized treatment. The authors also observed that HQ + DCD increased rice grain yields by 10%, 18%, and 6%, respectively, and the straw weight was increased by 16%, 17%, and 8% before transplanting, at tillering and at the panicle initiation stage as compared to the control samples (Li et al., 2009a; Modolo et al., 2018). The above studies suggest that not only is the use of either UIs or NIs as SRF beneficial to crop production, but the

combination of both inhibitors is also good for the mitigation of N loss associated with volatilization, denitrification, leaching, and runoff (Abalos et al., 2014; Cantarella et al., 2018; Forrestal et al., 2017).

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Co-authorship statement for experiment one

Chapter three “The Effect of urea and urea treated with N Stabilizers on the Growth, Yield, and Feed quality of Silage Corn in a Boreal Climate” will be submitted to the journal of Field Crop Research. Therese Audrey Nzwindanda Mbite, will be the primary author of this manuscript. Dr. Mumtaz Cheema will be the corresponding and the last author. The co-authors will be Dr. Lakshman Galagedara, Dr. Raymond Thomas, Dr. Yeukai Katanda, Dr. Muhammad Nadeem, Bilal Javed, Muhammed Farhain, Irfan Mushtaq, and Ayodeji Medaiyese.

Chapter three

The effects of urea and urea treated with nitrogen stabilizers on the growth, yield, feed quality, nitrogen uptake, and nitrogen recovery efficiency of silage corn in a boreal climate

Abstract

Intensive agriculture production demands substantial nitrogen (N) fertilizer application to boost yield but poses higher risks of N losses. Urease inhibitors (UI) and nitrification inhibitors (NI) are known to reduce N losses, synchronize N release to plant N uptake, and consequently enhance growth, and yield. A two-year field experiment was conducted to evaluate the effects of urea (UR) stabilized with UI [N-(n-butyl) thiophosphoric triamide (NBPT)] and NIs [nitrapyrin and dicyandiamide (DCD)] on the growth, forage dry matter (DM), forage quality, N uptake and nitrogen recovery efficiency (NRE) of silage corn. The experimental treatments were: 1) UR; 2) SuperU™ (SU, urea with DCD and NBPT); 3) Agrotain™ (AG, urea with NBPT); 4) eNtrench™ (EN, urea with nitrapyrin) at 115 Kg N ha⁻¹; and 5) No-N as control (CTRL). SU significantly increased forage DM by 53.59% compared to CTRL and NRE by 50% in 2019 compared to UR. These findings suggest that SU could be a potential N stabilizer to enhance forage DM and NRE of silage corn cultivated on podzols in boreal climate.

3.1. Introduction

The dairy and livestock industry of Newfoundland and Labrador (NL) faces challenges of insufficient local forage/silage production due to extreme weather conditions, short growing seasons, infertile and acidic soils, limited available agricultural lands, and little or no forage species diversification (Haverstock, 2013 - 2014; Kwabiah et al., 2003). Currently, the province is about 50% self-sufficient in the production of forages though all other supplements and feed grains are imported from other provinces (Cordeiro et al., 2019). The imports result in heavy economic and environmental burdens due to high shipping prices/transportation, and substantial increases in carbon footprints (Ali et al., 2019). These challenges can be reduced by enhancing and intensifying local forage/fodder/silage/feed production, which can bridge the gap between local production and consumption (Todd and Spaner, 2003).

Silage corn is known as an important forage crop for the dairy industry due to its higher biomass production potential, high energy content, and more uniform quality with one cut forage harvest compared to other forage species (Khan et al., 2015; Kirkland et al., 2005; Rankin, 2015). Additionally, silage-corn is highly palatable, digestible, and easy to ensile due to its highly soluble sugar contents (Karsten et al., 2003). However, the intensive cultivation of silage corn requires optimum N fertilization to enhance growth, development, and forage yield (Kaplan et al., 2016). For example, N in plants helps in the metabolic process of enzyme synthesis which is crucial during photosynthesis activity, the source-to-sink activity in crops is increased by N in plants hence an increase in grain yield and biomass accumulation (Kaplan et al., 2016). Excessive and injudicious use of N fertilizers causes lodging in crops, environmental pollution, and economic losses (ur Rahman et al., 2021). Additionally, N application in the agricultural production system

is lost via different pathways, like volatilization, leaching, runoff, and denitrification (Bittman et al., 2014; Galloway et al., 2008; Sutton et al., 2013).

After application to soil, urea (UR) undergoes hydrolysis via the urease enzyme, causing increases in the soil pH in the surrounding area of the granules and resulting in ammonia (NH_3) losses that average 16% of N applied worldwide and can reach 40% or more in hot and humid conditions (Folina et al., 2021). During the microbial process of nitrification, ammonium (NH_4^+) gets oxidized to nitrate (NO_3^-) and this may lead to NO_3^- leaching and runoff since NO_3^- is mobile and generally unstable in the soil (Coskun et al., 2017). While the microbial process involved in denitrification will reduce NO_3^- and nitrite (NO_2^-) to gaseous forms, such as nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) (Davidson and Seitzinger, 2006). N_2O is a potent greenhouse gas (GHG), with 298 times greater global warming potential than carbon dioxide over a 100-year time scale (IPCC, 2007; Linnquist et al., 2012). To address these challenges, there is a need to introduce some innovative technologies or beneficial management practices (BMPs) to mitigate volatilization, leaching, and gaseous losses of N. For example, reduced tillage (Spiess et al., 2020), crop rotation (Hansen et al., 2007), incorporation of N in the soil (Cantarella et al., 2018), application of slow-release fertilizers (SRFs) (Trenkel, 2010) and UR treated with N stabilizers such as urease inhibitors (UI) and nitrification inhibitors (NI) amongst others have been used to mitigate N losses in different cropping systems (Abalos et al., 2014; Zheng et al., 2021). However, SRFs and UR treated with N stabilizers are getting much attention by researchers, farmers, and policymakers compared to other BMPs (Fu et al., 2018).

Urea treated with N stabilizers such as Agrotain and eNtrench contain UI and NI, respectively whereas, SuperU contains both UI and NI and is therefore considered a promising tool to mitigate reactive N losses from fertilized soils. UI, such as N-(n-butyl) thiophosphoric triamide (NBPT)

block urease activity by binding with the urease enzyme active sites, lowering UR hydrolysis and subsequent NH_3 loss (Amtul et al., 2002; Sha et al., 2020). Recent meta-analyses conducted by Xia et al. (2017), Pan et al. (2016), and (Li et al., 2018) have reported significant reduction in NH_3 (53- 61%) and N_2O (21- 28%) emissions with the application of NBPT. NI inhibit the activity of nitrosomonas by binding with the ammonium monooxygenase enzyme, thereby blocking the first reaction of NH_4 oxidation (Akiyama et al., 2010). Previous studies have reported that NI reduced 38-57% of N_2O emissions (Akiyama et al., 2010; Li et al., 2018; Xia et al., 2017) and 24-81% of NO emissions (Akiyama et al., 2010; Liu et al., 2017) but could increase NH_3 volatilization by 7-27% (Li et al., 2018; Pan et al., 2016; Xia et al., 2017). The ability of UI and NI to slowly release NH_3 in synchrony with plants N need, leads to an increase in nitrogen recovery efficiency (NRE), and an increase in yields (Abalos et al., 2014; Malla et al., 2005; Xi et al., 2017). According to the study of Halvorson and Bartolo (2014), greater yield and NRE of corn were observed with application of N inhibitors. The effectiveness of UI such as NBPT, and NI like nitrapyrin and dicyandiamide (DCD) have been reported to mitigate N loss as well as improve NRE of crops in other ecological regions (Abalos et al., 2014; Cui et al., 2011; Pawlick et al., 2019; Roche et al., 2016; Sanz-Cobena et al., 2008; Zaman et al., 2009). However, the sole application of UI and NI, and their combine application to determine their effects on growth and biomass production is yet to be known in short-term continuous corn crop rotation in a boreal climate.

I hypothesized that urea treated with N stabilizers compared to urea application will increase growth, forage dry matter (DM), forage quality indices, N uptake, and NRE of silage corn cultivated on Podzol in boreal climate.

To test the hypotheses, a field trial was conducted for two years with the following specific objectives:

- To investigate the effects of urea and urea treated with N stabilizers on the growth and yield of silage corn cultivated on Podzols in boreal climate.
- To decipher the role of urea and urea treated with N stabilizers on forage quality indices of silage corn cultivated on Podzols in boreal climate.
- To determine the NRE of silage corn in response to urea and urea treated with N stabilizers cultivated on Podzol in boreal climate.

3.2. Materials and methods

3.2.1. Experimental site and treatments

A two-year field experiment was carried out at (49° 04' 28" N; 57° 33' 23" W), Pasadena, NL, Canada, during the 2019 and 2020 growing seasons. The province is in the boreal region forest zone of the North Atlantic Ocean. The experimental treatments were: 1) urea (UR), 2) Agrotain™ [(AG: urea coated with UI (NBPT)], 3) eNtrench™ [(EN: urea coated with NI (nitrapyrin)], 4) SuperU™ [(SU: urea coated with UI and NI (DCD and NBPT)], and 5) no N fertilizer (CTRL). The experiment was laid out in a randomized complete block design replicated 4 times for a total of 20 experimental units with a plot size of 3 m × 4 m. Before planting in 2019, 10 soil core samples were collected in a zigzag manner from each block at a depth of 0 – 15 cm using an auger and composited a one sample per block to determine the basic soil properties and texture of the experimental site (Table 3.1). The samples were analyzed (Soil & Plant Laboratory, Dept. of Fisheries and Land Resources, St John's, NL) using the hydrometer method (Bouyoucos, 1962), which described the soil texture to be sandy loam for blocks 1, 2, and 3 and loamy sand for block 4. The pH was determined using samples prepared as 50/50 DI water and analyte; and was reported using a Fisher Scientific XL250 Benchtop Meter and pH Electrode. Organic matter (OM) was

obtained after samples were dried overnight at 102°C and then heated in a Muffle furnace at 430°C. Cation exchange capacity (CEC) was calculated using software onsite. Nutrients were extracted from soil samples using Mehlich III (Carter and Gregorich, 2003) and analyzed using a Teledyne Instrument Prodigy High Dispersion ICP as describe in appendix two.

Table 3.1: Soil analyses report of the experimental site

Year	pH	OM	CEC	P	K	Ca	Mg	S	Cu	Na	Al
		%	cmol kg ⁻¹	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
2019	5.90	3.74	14.75	336.25	85.00	1130.00	192.50	18.50	2.13	18.50	1337.00
2020	6.40	3.97	12.15	378.00	126.75	1285.30	186.25	19.75	2.93	15.50	1435.30

OM: Organic matter, P: Phosphorous, K: Potassium, Ca: Calcium, Mg: Magnesium, S: Sulphur, Cu: Copper, Na: Sodium, Al: Aluminium, CEC: cation exchange capacity

Before seeding in 2019, pre-emergence herbicide (RoundUp Weather Max™) was applied at a rate of 1.67 L ha⁻¹ with a tractor-mounted sprayer (Hardi N-105, Davenport, IA). In the 2020 growing season, the herbicides Credit® Xtreme (active ingredient Glyphosphate) and Eragon® LQ were mixed alongside a blend of surfactant with petroleum hydrocarbons (Merge® Adjuvant) at respective rates of 2.2 L ha⁻¹, 146 mL ha⁻¹, and 1 L ha⁻¹ to control weeds. On June 20, 2019, and June 11, 2020, silage corn (Pride A4415G2RIB) was planted at a seeding rate of 90,900 seeds ha⁻¹ using a Samco system (2 rows 2200 model). UR and UR treated with N stabilizers were applied at 115 kg N ha⁻¹ at seeding whereas, phosphorus and potassium fertilizers were applied respectively at rates (0 kg P₂O₅ ha⁻¹ and 105 kg K₂O ha⁻¹) for 2019 and (0 kg P₂O₅ ha⁻¹ and 60 kg

K₂O ha⁻¹) for 2020 at seeding following soil test reports and regional recommendations. The crop was harvested on October 28 and November 5 in 2019 and 2020, respectively.

3.2.2. Growth parameters

3.2.2.1. Chlorophyll

The chlorophyll content was measured at the tasseling stage. Three plants were randomly selected from the center of the two middle corn rows. Using a SPAD 502 plus chlorophyll meter (Spectrum Technologies, IL, USA), two readings were taken from the fully expanded leaf from the top of the plant (Figure 3.1) (Dwyer et al., 1995; Dwyer et al., 2003). The SPAD readings were taken on opposite sides of the leaf's midvein thus a total of six readings per plot in all four blocks.

3.2.2.2. Photosynthesis

Photosynthesis rate was measured at the tasseling stage on a sunny day. Three plants were randomly selected within the center of the two middle corn rows to measure the photosynthesis rate using a portable photosynthesis system (LI-6400XT) (LI-COR Biosciences, NE, USA). Three photosynthesis measurements were taken from the third most fully expanded leaf from the top (Figure 3.1). Thus, a total of three readings per plot in all four blocks. Before taking measurements, the photosynthesis system was set up at a photon flux density of $1500 \mu\text{molm}^{-2}\text{s}^{-1}$, leaf temperature $25 \text{ }^{\circ}\text{C}$, pump flow rate of $400 \mu\text{mols}^{-1}$, and 400 ppm CO_2 concentration (Nilahyane et al., 2020).



Figure 3.1: Measurement of chlorophyll content (a) and photosynthesis rate (b) of silage corn at the tasseling stage cultivated in a boreal climate with urea and N stabilizer application.

3.2.2.3. *Plant height*

At harvest, ten plants were selected randomly to measure plant height using a measuring rod from each plant's base to the top of the plant (Figure 3.2) (Yin et al., 2010). The mean height of the ten plants was used as the final plant height per plot in all the four blocks.

3.2.2.4. *Forage yield*

Silage corn was manually harvested on October 28, 2019, and November 5, 2020. Five consecutive plants were harvested 5 cm above the ground from the two middle corn rows of each plot, thus a total of ten plants per plot in all four blocks. The fresh weight of the harvested ten plants was measured using a weighing scale (Adam Equipment™ S.N. AE6211313 CBC). Thereafter three plants were subsampled from the ten plants as representative samples. These subsamples were weighed, and chopped with a knife into smaller pieces (Figure 3.2), bagged and oven-dried (Precision Quincy corporation SN 17645, Model No. 40D-350) at 65 °C for 72 h (Ali et al., 2019).



Figure 3.2: Measurement of plant height of silage corn at harvest (a), and manual chopping of the samples (b).

The harvest area was determined by multiplying the average row length by the row width.

After obtaining the dry weights of the samples, the dry matter content (g kg^{-1}) was determined using the original moisture content of each sample (Uzun et al., 2020). However, during the determination of forage DM, the moisture content was corrected to 65% due to varying moisture content. Forage DM (Mg ha^{-1}) was calculated following the method of Clark (2018) Ali et al. (2019); (Clark, 2018) as described in Equation 1 below

$$\text{Forage dry matter} = \left(\left(\frac{10 \text{ plants fresh weight} * 10000}{\text{area}} \right) * \left(\frac{1 - 0.65}{100} \right) / 1000 \right) \dots (1)$$

Dried plant samples were ground with a Wiley mill (A.H. Thomas Co., Philadelphia, PA, USA) and 50 g samples were packed into small Ziplock bags and sent to Activation Laboratories Ltd. (Actlabs, Ancaster ON, Canada), for forage quality analyses. Another subsample was pulverized using a Cryomill (MM 400; Retsch Inc., Newton, PA) and sent to Prince Edward Island Analytical Laboratories (PEIAL, Bioscom Park Charlottetown, Canada) for the determination of N ratio in plant tissues.

3.2.3. Determination of forage quality indices

The forage quality indices were determined using the near-infrared NIR method (Association of Official Analytical Chemists (AOAC), method 989.03) (Gallo et al., 2013). Forage quality parameters analyzed were crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), non-fibrous carbohydrates (NFC), total digestible nutrients (TDN), net energy for lactation (NEL), starch, and minerals (phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg)) concentrations. Finally, the milk production was determined per gram of dry matter to assess milk production per plot (Moreno-Reséndez et al., 2017).

3.2.4. Nitrogen uptake and recovery efficiency

The N uptake was determined based on the N concentration in tissues of silage corn and the NRE was calculated using Equation 2 (Kaur et al., 2018).

$$NRE = \frac{N \text{ uptake (+N)} - N \text{ uptake (CTRL)}}{\text{Amount of N applied}} \times 100 \quad (2)$$

Where:

NRE = nitrogen recovery efficiency

N uptake (+N) = N uptake from fertilized treatments

N uptake (CTRL) = N uptake from control (no N) treatments

3.2.5. Statistical Analyses

A two-way ANOVA technique in XLSTAT (XLSTAT Premium,2018.1.1, NY, USA) was performed to assess the effects of UR and UR treated with N stabilizers on the growth, yield, and

forage quality parameters measured over two years. Treatment means were compared using Fisher's least significant difference (LSD) at $\alpha = 0.05$. Redundant analyses (RDA) was performed to visualize the relationships between treatments and the response variables in XLSTAT. The axis which explained more than 50% of the data were retained (axis 1 and 2) as seen in Figure 3.4.

3.3. Results

3.3.1. Weather data

The weather data was obtained from the weather station in Deer Lake Airport, NL (YDF 71809). The high rainfall experienced in 2019 made the cropping season wetter than that of 2020; although the lowest (9.80 mm) rainfall was recorded in 2019 (Table 3.2). The maximum and minimum temperatures reveal that 2020 was warmer than 2019. The combined interaction of rainfall, maximum and minimum temperatures led to an earlier planting date on June 11, 2020, compared to June 20, 2019.

Table 3.2: Biweekly average air temperature, maximum temperature, minimum temperature, and rainfall of the experimental site during the 2019 and 2020 growing seasons

Growth Period	2019				2020			
	Avg. Temp °C	Max. Temp °C	Min. Temp °C	Rainfall (mm)	Avg. Temp °C	Max. Temp °C	Min. Temp °C	Rainfall (mm)
June 1-15	10.28	16.81	3.74	40.70	10.30	16.10	4.61	32.70
June 16-30	12.55	18.72	6.39	50.40	16.70	23.30	10.1	37.40
July 1-15	12.94	18.02	7.86	53.50	13.80	19.80	7.83	29.60
July 16-31	18.27	25.43	11.12	18.40	16.20	22.40	10	30.50
August 1-15	17.39	24.25	10.53	63.40	17.70	24.90	10.50	19.60
August 16-31	16.30	23.14	9.46	9.80	13.40	19.10	7.70	115.80
Sep. 1-15	11.28	17.22	5.34	55.20	12.30	17.90	6.60	27.80
Sep. 16-30	10.13	14.51	5.74	65.20	10.80	15.70	5.88	26.70
Oct. 1-15	6.05	12.47	-0.37	40	7.33	12.80	1.81	79.10
Oct. 16-31	5.95	10.51	1.39	29.70	3.91	8.66	-0.78	58.60
Nov. 1-15	1.59	5.85	-2.67	130.90	2.39	6.55	-1.75	26.30
Total	122.73	186.93	58.53	557.20	124.83	187.21	62.50	484.10

3.3.2. Growth and forage dry matter of silage corn

The ANOVA results revealed that in 2020, photosynthesis and plant height were significantly increased ($P<0.05$) and forage DM was significantly decreased ($P<0.05$) as compared to 2019 (Table 3.3) while chlorophyll did not show any significant difference between both years. However, N fertilizer treatments had a significant ($P<0.05$) effect on chlorophyll content and forage DM as compared to the CTRL. Only AG and SU significantly ($P<0.05$) increased plant height as compared to the CTRL while no significant differences were recorded within the N treatments in the photosynthesis of silage corn (Table 3.3). SU had an overall increase of 53.59% in forage DM compared to the CTRL. In 2020, photosynthesis rate and plant height were 48%, and 11%, respectively higher while forage DM was 17.4% lower as compared to the 2019 growing season (Table 3.3). The N treatments had significant effects on the growth and forage DM of silage corn (Table 3.3).

Chlorophyll content was significantly ($P<0.05$) increased by SU, AG, EN, and UR applications compared to the CTRL. However, there was no significant increase observed in chlorophyll content in silage corn from UR treated with N stabilizers (SU, AG, and EN) and UR application (Table 3.3). There was a 46%, 45%, 43%, and 42% increase in chlorophyll content, respectively with SU, AG, EN, and UR amended treatments as compared to the CTRL.

The N sources had significant ($P<0.05$) effects on the plant height of silage corn (Table 3.3). Higher plant height (1.99 m and 1.98 m) was observed in AG and SU applied treatments, which increased 27%, and 26% plant height compared to the CTRL. Though, no significant increase in the plant height within UR and UR treated with N stabilizers was recorded.

The N sources had significant ($P<0.05$) effects on the forage DM of silage corn. SU produced a significantly higher forage DM of 36.72 Mg ha⁻¹ compared to the lowest (17.04 Mg ha⁻¹) observed

in the CTRL treatment. There was a 53.59%, 45.33%, 42.04%, and 43.94% increase in forage DM, respectively with SU, AG, UR, and EN applications compared to the CTRL. UR treated with N stabilizers were statistically at par with UR and produced similar forage DM (Table 3.3).

3.3.3. Nitrogen uptake and nitrogen use efficiency

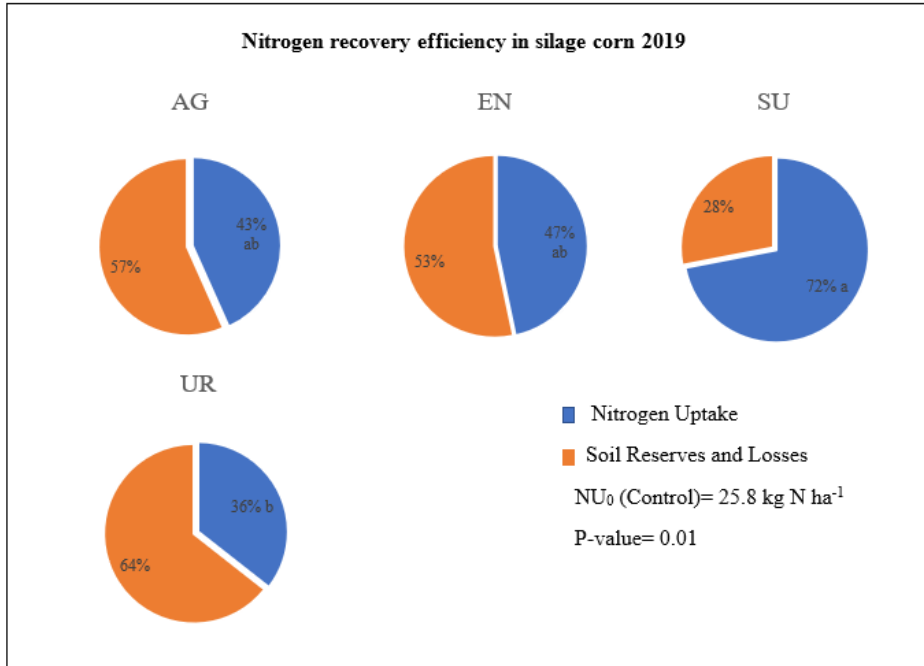
The N treatments and Year had significant ($P < 0.05$) effects on N uptake in silage corn (Table 3.3). A Higher N uptake of 117 kg ha^{-1} was observed in SU treatment and the lowest in CTRL treatment. It was further observed that N uptake was significantly higher in 2020 than in 2019 (Table 3.3). The N stabilizer SU had a significant ($p < 0.05$) effect on the NRE of silage corn in 2019 compared to UR; whereas UR was statistically at par with the UR treated with N stabilizers and produced similar NRE in 2020 (Figure 3.3).

Table 3.3: The effects of urea and urea treated with N stabilizers on the chlorophyll content, photosynthesis rate, plant height, forage dry matter, and N uptake of silage corn.

	Photosynthesis ($\mu\text{mol m}^{-2} \text{sec}^{-1}$)	Chlorophyll SPAD values	Plant height (m)	Forage DM (Mg ha^{-1})	N uptake (kg ha^{-1})
treatments (T)					
CTRL	16.9±1.62	34.3±2.23b	1.57±0.09b	17.04±0.78b	36.3±11.18b
AG	18.1±1.62	49.6±2.23a	1.99±0.09a	31.17±2.82a	93.2±11.18a
EN	19.1±1.62	49.2±2.23a	1.82±0.09ab	30.40±3.36a	84.5±11.18a
SU	22.2±1.62	49.9±2.23a	1.98±0.09a	36.72±1.85a	117.6±11.18a
UR	20.7±1.62	48.7±2.23a	1.90±0.09ab	29.40±1.80a	85.7±11.18a
Year (Y)					
2019	13.4±1.09 b	45.5±1.35	1.74±0.06 b	31.71±1.73 a	71.3±7.56 b
2020	25.5±0.95 a	47.2±1.55	1.96±0.05 a	26.19±1.12 b	95.7±6.55 a
P-value					
Y	0.00	0.45	0.01	0.03	0.02
T	0.20	0.00	0.01	0.00	0.00
Y × T	0.90	0.91	0.79	0.41	0.89

Different letters in the columns indicate significant difference between treatments ($P < 0.05$) and the values are means \pm standard errors. The measured parameters were replicated four times per treatment. CTRL: control, AG: agrotain, EN: eNtrench, SU: superU, UR: urea, forage DM: forage dry matter.

A



B

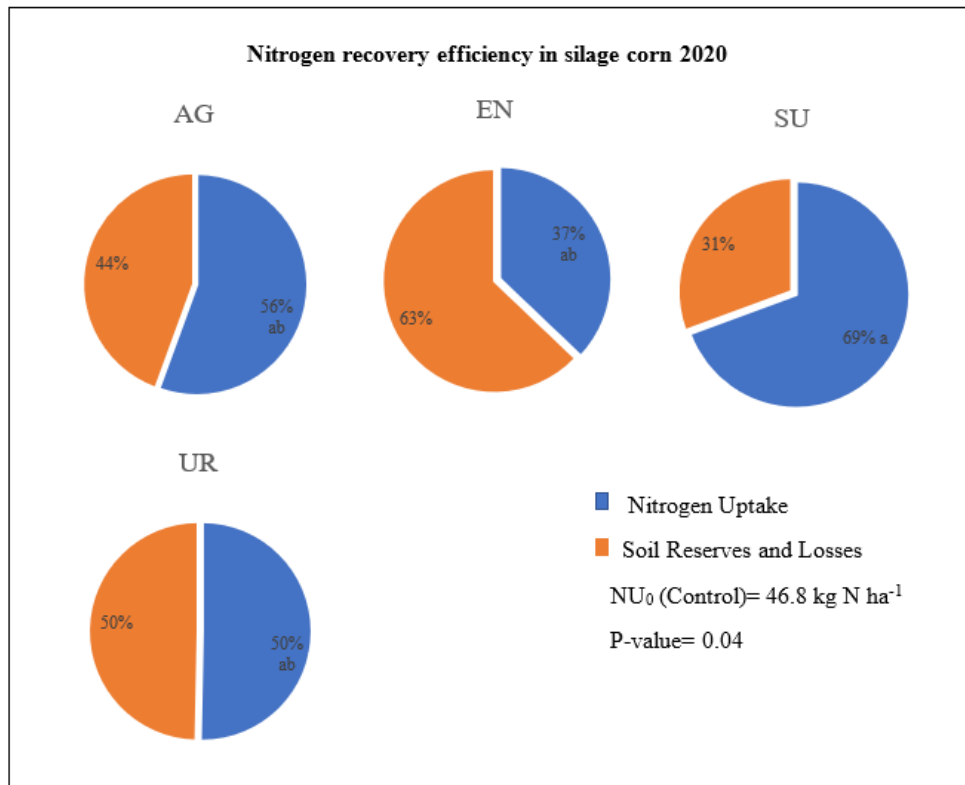


Figure 3.3: The effect of urea and urea treated with N stabilizers on the nitrogen recovery of silage corn in a.) 2019 and b.) 2020

Table 3.4: The effects of urea and urea treated with N stabilizers on the protein and fiber content of silage corn

	CP (g kg ⁻¹)	AP (g kg ⁻¹)	ADF (g kg ⁻¹)	NDF (g kg ⁻¹)	NFC (g kg ⁻¹)	Lignin (g kg ⁻¹)
Treatments (T)						
CTRL	88.7±3.90	79.6±3.69	336.8±7.80	576.2±9.54	270.7±9.82	41.9±1.74
AG	100.7±3.90	92.0±3.69	333.6±7.80	565.9±9.54	272.7±9.82	44.8±1.74
EN	90.6±3.90	82.2±3.69	320.5±7.80	551.9±9.54	297.3±9.82	41.7±1.74
SU	94.4±3.90	86.3±3.69	319.9±7.80	541.9±9.54	302.9±9.82	41.9±1.74
UR	92.8±3.90	84.0±3.69	327.7±7.80	563.8±9.54	287.9±9.82	45.0±1.74
Year (Y)						
2019	114.3±2.64 a	104.8±2.50 a	353.8±5.28 a	588.8±6.45a	221.5±6.64 b	47.5±1.18 a
2020	72.6±2.29 b	64.9±2.17 b	301.6±4.57 b	531.2±5.58 b	351.1±5.74 a	38.7±1.02 b
P values						
Y	0.00	0.00	0.00	0.00	0.00	0.00
T	0.26	0.20	0.46	0.14	0.11	0.47
Y×T	0.89	0.84	0.95	0.85	0.74	0.56

Different letters in the columns indicate significant difference between treatments ($P < 0.05$) and the values are means \pm standard errors. Each analyses was replicated four times per treatment. CTRL: Control, AG: agrotain, EN: eNtrench, SU: superU, UR: urea, CP: crude protein, AP: available protein, ADF: acid detergent fiber, NDF: neutral detergent fiber, NFC: non-fibrous carbohydrates.

Table 3.5: The effect of urea and urea treated with N stabilizers on the digestibility and energy content of silage corn

	TFA (g kg ⁻¹)	TDN (g kg ⁻¹)	SS (g kg ⁻¹)	RFV (%)	NEM (mcal kg ⁻¹)	NEG (mcal kg ⁻¹)	NEL (mcal kg ⁻¹)
Treatments (T)							
CTRL	19.0±0.67	529.0±6.83	109.8±9.40	105.9±3.23	1.0±0.02	0.4±0.02	1.08±0.01ab
AG	18.1±0.67	528.1±6.83	100.7±9.40	106.0±3.23	1.0±0.02	0.4±0.02	1.09±0.01ab
EN	18.8±0.67	518.2±6.83	97.9±9.40	109.8±3.23	1.0±0.02	0.4±0.02	1.11±0.01ab
SU	18.8±0.67	517.5±6.83	98.8±9.40	110.8±3.23	1.0±0.02	0.4±0.02	1.12±0.01a
UR	18.3±0.67	517.5±6.83	104.3±9.40	106.9±3.23	0.9±0.02	0.4±0.02	1.07±0.01b
Year (Y)							
2019	16.7±0.46 b	512.1±4.62 b	91.5±6.36 b	100.7±2.19b	0.92±0.02 b	0.37±0.01 b	1.02±0.01 b
2020	20.6±0.40 a	532.0±4.00 a	113.1±5.51 a	115.1±1.89a	1.01±0.01 a	0.46±0.01 a	1.17±0.01 a
P values							
Y	0.00	0.00	0.02	0.00	0.00	0.00	0.00
T	0.86	0.57	0.89	0.75	0.71	0.71	0.04
Y×T	0.31	0.60	0.87	0.62	0.62	0.60	0.04

Different letters in the columns indicate significant difference between treatments ($P < 0.05$) and the values are means \pm standard errors. Each analyses was replicated four times per treatment. CTRL: control, AG: agrotain, EN: eNtrench, SU: superU, UR: urea, TFA: total fatty acid, TDN: total digestible nutrient, SS: simple sugar, RFV: relative feed value, NEG: net energy gain, NEL: net energy for lactation, NEM: net energy for maintenance.

3.3.4. Forage quality indices

Urea treated with N stabilizers and UR had non-significant effects on CP, AP, ADF, NDF, NFC, and lignin. However, there was a significant increase in CP, AP, ADF, and lignin in 2020 compared to 2019 (Table 3.4). UR and UR treated with N stabilizers had non-significant effects on starch, TFA, TND, simple sugars, RFV, NEM, and NEG. However, N sources, year, and their interaction had significant effects on NEL. SuperU application produced 5% higher NEL compared to UR and 15% higher NEL in 2020 than in 2019. Likewise, TFA, TND, simple sugars, RFV, NEM, and NEG were significantly more in 2020 than in 2019 (Table 3.5).

There was a significant effect of N fertilized treatments (EN, AG, SU, and UR) and year on milk production (Table 3.6); but there was no significant difference between UR treated with N stabilizers (EN, AG, and SU) and UR treatments. SU produced higher milk (34.44 Mg ha^{-1}), whereas the lowest milk production of 17.73 Mg ha^{-1} was observed in the CTRL treatment, though UR treated with N stabilizers and UR had non-significant effects on milk production. Similarly, higher milk production for 2019 and 2002 were statistically at par with each other (Table 3.6).

There was no significant difference observed between UR treated N stabilizers (AG, SU, and EN) and UR application on silage corn minerals (Table 3.6). However, Ca, P, Mg and K significantly decreased in 2020 as compared to 2019 (Table 3.6).

Table 3.6: The effects of urea and urea treated with N stabilizers on milk production and the mineral content of silage corn.

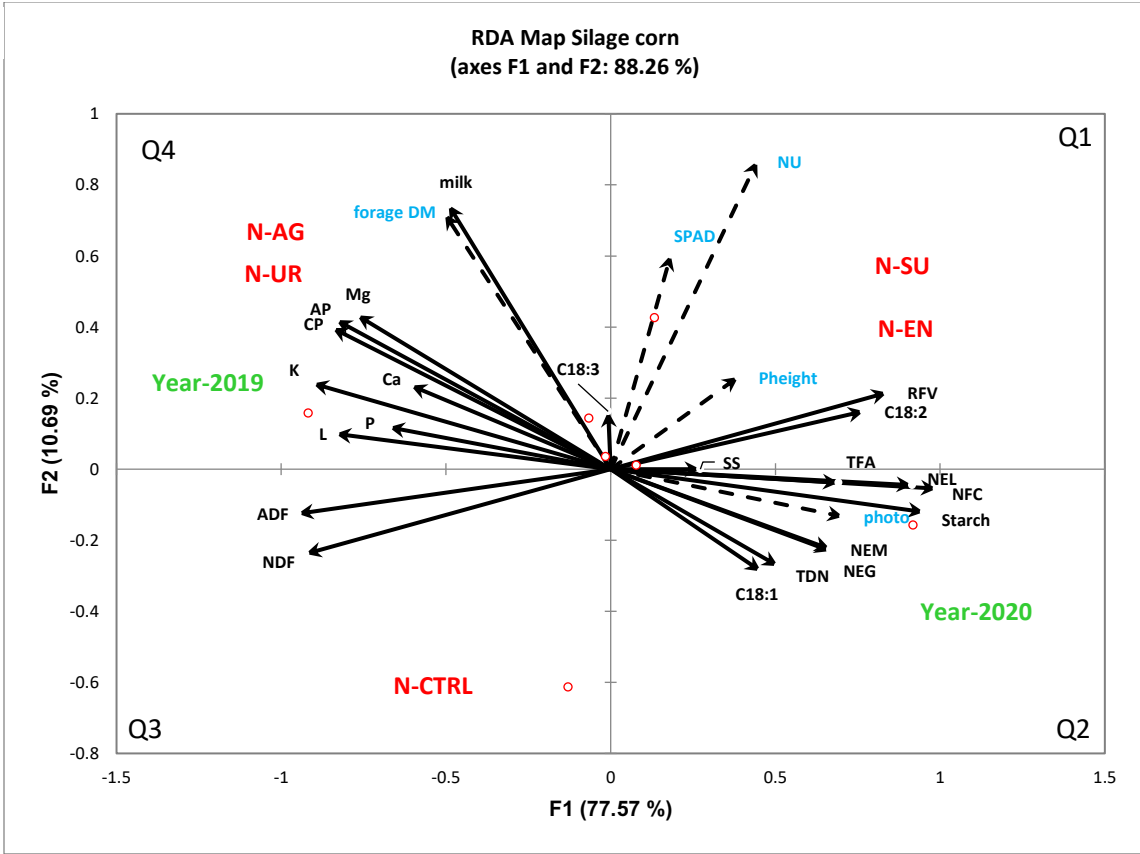
Main factors	Milk (kl ha ⁻¹)	Ca (g kg ⁻¹)	P (g kg ⁻¹)	Mg (g kg ⁻¹)	K (g kg ⁻¹)
Treatments (T)					
CTRL	17.73±1.08b	2.98±0.24	2.5±0.13	1.5±0.12	13.1±0.68
AG	31.83±3.03a	2.56±0.23	2.3±0.13	1.8±0.12	12.9±0.68
EN	29.98±2.78a	2.56±0.23	2.3±0.13	1.5±0.12	12.3±0.68
SU	34.44±1.46a	2.60±0.24	2.1±0.13	1.7±0.12	12.7±0.68
UR	29.87±2.20a	2.74±0.26	2.2±0.13	1.7±0.12	12.1±0.68
Year (Y)					
2019	29.94±1.56	3.51±0.16 a	2.60±0.08 a	2.11±0.08 a	16.9±0.46 a
2020	27.59±1.27	1.86±0.14 b	1.94±0.07 b	1.19±0.07 b	8.36±0.40 b
P values					
Y	0.33	0.00	0.00	0.00	0.00
T	0.00	0.68	0.32	0.43	0.81
Y×T	0.71	0.36	0.36	0.44	0.74

Different letters in the columns indicate significant difference between treatments ($p < 0.05$) and the values are means \pm standard errors. Each analyses was replicated four times per treatment. CTRL: control, AG: agrotain, EN: eNtrench, SU: superU, UR: urea, Ca: calcium, P: phosphorus, Mg: magnesium, K: potassium.

3.3.5. Relationship between response variables and treatments.

The growth parameters, forage DM, N uptake, and forage quality indices of silage corn were used as indicators to assess the use of UR and UR treated with N stabilizers (AG, SU, and EN) on podzols in boreal climate (Figure 3.4a; Tables 3.3-3.6). Few significant associations were observed between the growth parameters, forage DM, N uptake, the forage quality indices, and the treatments (N sources and year). These associations highly influenced the clustering of response variables and the treatments together, however, well separated in different quadrants of the biplot following RDA (Figure 3.4a). Following the RDA, it was observed that axis 1 and axis 2 explained 77.57% and 10.69%, respectively of the total variability in growth parameters, forage DM, N uptake and forage quality indices with different N sources during 2019 and 2020 (Figure 3.4a). For example, in quadrant 1 for the biplot, growth parameters (SPAD and plant height), N uptake, RFV and fatty acid (C 18:2) were clustered together and showed better association with SU treatment (Table 3.3, 3.5). I also observed that forage DM, milk, lignin (L), proteins (crude protein (CP) and available proteins (AP)), and minerals (Mg, Ca, K and P), clustered together in Q4 and showed better association with 2019 growing season (Tables 3.3, 3.4, 3.6). In addition, demsar plots were used following the ANOVA outputs, demsar plots were used to separate the treatments of the statistically significant variables into groups according to their importance. For example, the demsar plot of forage DM separated the treatments into three groups with the least important treatment being CTRL-2020 and the most important being SU-2019 (Figure 3.4b).

A



B

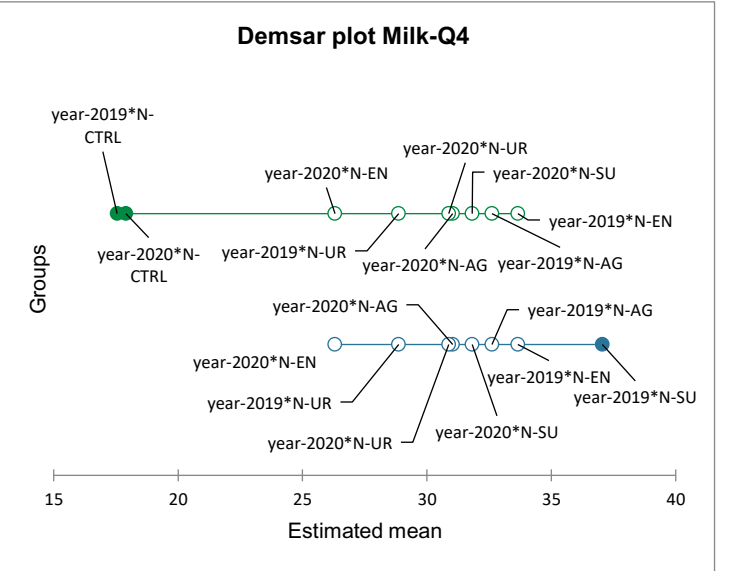
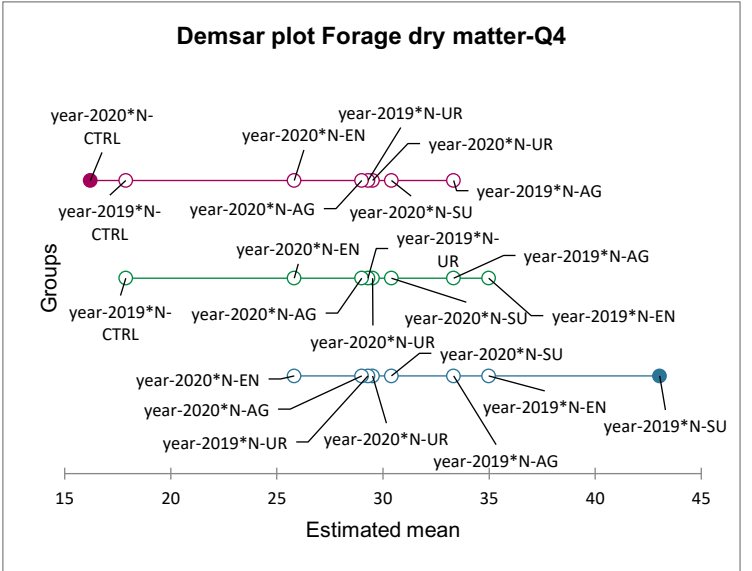
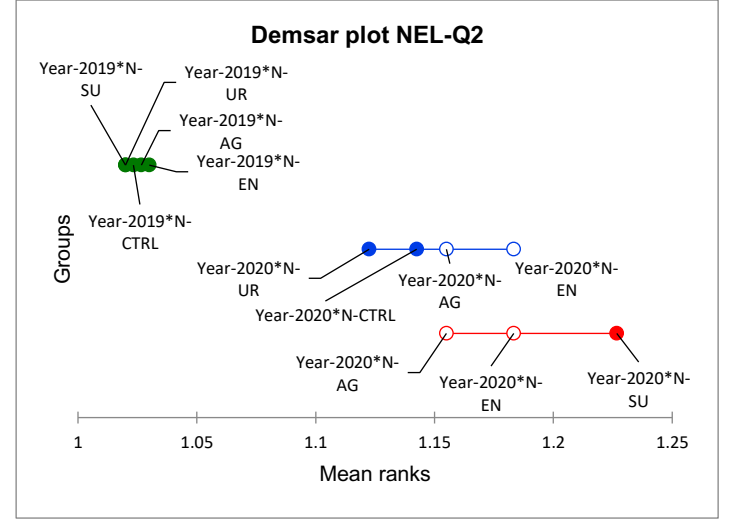
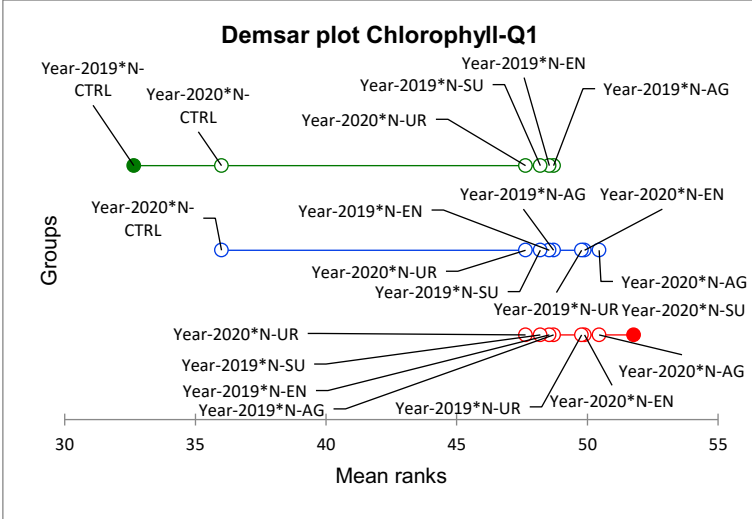
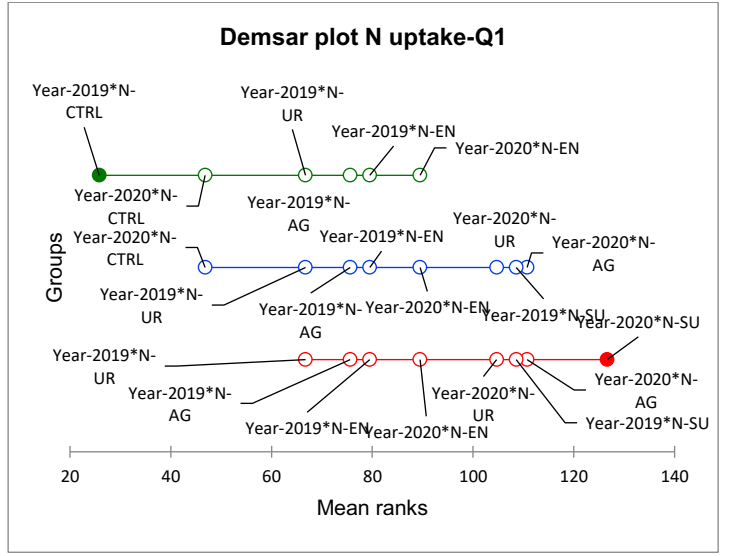
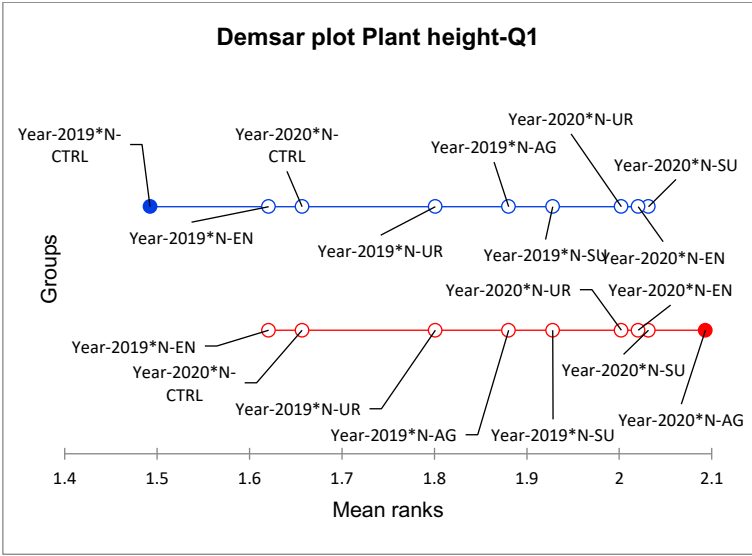


Figure 3.4: RDA biplots showing the association between N sources, growth parameters, forage DM and forage quality indices of silage corn during 2019, and 2020 growing seasons (a) and demsar plots showing the grouping of N treatments according to their importance (b), respectively for plant height, N uptake, chlorophyll, NEL, forage DM, and milk. The active variables are Photo: photosynthesis; SPAD: chlorophyll, PHeight: plant height, forage DM: forage dry matter; NU: nitrogen uptake; CP: crude protein; AP: available protein; ADF: acid detergent fibers; NDF: neutral detergent fibers; NFC: non-fibrous carbohydrates; RFV: relative feed value; TDN: total digestible nutrients; TFA: total fatty acid; C18:2: linoleic acid; C18:3: linolenic acid; Lig: lignin; SS: simple sugars, Mg: magnesium; Ca: calcium; K: potassium; P: phosphorus; NEG: net energy for growth; NEL: net energy for lactation; NEM: net energy for maintenance;

3.4. Discussion

3.4.1. Growth and forage DM of silage corn

N constitutes an important component in chlorophyll synthesis, enhances photosynthesis rate and crop yield/biomass, and therefore is widely used as a fertilizer in all crops. N stimulates leaf growth through the synthesis of proteins involved in cell growth, cell division, cell wall and cytoskeleton synthesis Sun et al. (2017), increasing the photosynthesis rate. In the present study, UR treated with N stabilizers (SU, AG, EN) and UR produced significantly higher chlorophyll content, plant height, and forage DM compared to CTRL treatment, and non-significant effects on photosynthesis rate, though slightly higher in SU treatment (Table 3.3). A previous study conducted by Ghafoor et al. (2021) reported that sulphur and neem-coated UR (N inhibitors) increased chlorophyll contents, photosynthesis rate, and grain yield of wheat at higher N application (130 kg N ha⁻¹) compared to untreated UR, whereas, the lower application rate of neem

coated UR and UR (94, 104, and 117 kg N ha⁻¹) showed non-significant effects on chlorophyll content, and grain yield of wheat in arid conditions. In my study, UR treated with N stabilizers and UR application rate was 115 kg N ha⁻¹ which was very close to the application rate reported by (Ghafoor et al., 2021). UR treated with N stabilizers contain UI and NI compounds which reduce NH₃ volatilization, NO₃⁻ leaching, and N₂O emission. UIs slow down the conversion of UR to NH₄⁺ and, then decline volatilization of NH₃, as a result, a significant percentage of the applied UR is diffused into the soil (Saggar et al., 2013; Upadhyay, 2012) then NIs application lessen the conversion of NH₄⁺ to NO₃⁻ (Subbarao et al., 2006), and N₂O emissions. Additionally, Grant and Bailey (1999) reported a decrease in seed damage due to the addition of NBPT in UR compared with untreated UR, which increased the stand density and promoted a higher yield of barley. In a study conducted on rice, Qi et al. (2012) observed that the urea-coated NBPT reduced the damage to seed germination and increased root growth. NIs usually increase NH₃ volatilization and mixing them with UIs partially offsets the benefits of the latter in reducing NH₃ loss (Cantarella et al., 2018). For example, SU is a blend of both UIs and NIs (NBPT + DCD) also known as a double inhibitor (DI) that demonstrated a significant reduction in all three N losses (NH₃ volatilization, leaching, and N₂O emissions), which significantly enhanced chlorophyll content, and plant height of silage corn (Table 3.3). Lower chlorophyll content in CTRL treatment probably reduced and impaired the chlorophyll synthesis in corn plants resulting in a significant reduction in forage DM (Table 3.3). Generally, N fertilizers enhance the growth of the meristem cells and internodes and hence an increase in plant height (Islam et al., 2010; Kaplan et al., 2016). The significant increase in plant height in 2020 (Table 3.3) can be associated to the high volatilization rate in 2019 due to an increase in inorganic soil N and anaerobic soil conditions caused by the excess rainfall (Table 3.2) (Drury et al., 2017).

SU also known as DI, produced a significantly higher forage DM of 36.72 Mg ha⁻¹ the lowest (17.04 Mg ha⁻¹) in CTRL treatment. UR treated with N stabilizers were statistically at par with UR and produced similar forage DM, although SU produced higher forage DM (Table 3.3) than UR. Higher forage DM with SU application could be attributed to a significant reduction in NH₃ volatilization losses due to UI (NBPT); NO₃⁻ and denitrification losses due to NI (DCD), consequently reduced overall losses and enhanced forage DM, N uptake (Table 3.3), and NRE (Figure 3.3). Li et al. (2017) conducted a meta-analysis on the effects of enhanced efficiency fertilizers (EEF) on yield and NRE in different cropping systems and observed the greatest potential of EEF on enhancing productivity (yield and NRE) while reducing aggregated N losses. They reported that DI increased yield by 11% and NRE by 33% while decreasing aggregated N loss by 47% (84 kg N ha⁻¹). In the present study, SU (DI) enhanced 53.59% forage DM of silage corn compared to CTRL which was higher as reported in the meta-analysis conducted by Li et al. (2017). The results of our study substantiate the findings of Graham et al. (2018) and Zhao et al. (2017) who observed respectively 69.4% and 74.6% higher forage DM in maize with DI (DCD + NBPT) application. The application of DI has led to a significant increase in crop yield compared to a single inhibitor (Kakabouki et al., 2020; Oertli, 1980). However, we didn't observe a significant difference in photosynthesis rate, chlorophyll content, plant height and forage DM among UR treated with N stabilizers and UR application possibly due to reduced inhibitors effectiveness, which can be attributed to low N availability in the soil, less drainage at the experimental site and soil pH (6-8) or short period of effective inhibition or degradation and the limited shelf life (Engel et al., 2015). Additionally, NBPT degrades faster in acidic than in alkaline soils, which affects the longevity of the inhibitor in the soil and undergoes microbial degradation

in the soil. The rate of degradation depends on soil temperature, microbial activity, and soil pH (Engel et al., 2013; Engel et al., 2015).

3.4.2. Nitrogen uptake and nitrogen recovery efficiency

N undergoes various metabolic processes from the moment it is taken up by the plant's roots and gets assimilated before being translocated to various growing parts of the plants, as such maximizing N uptake may increase NRE (McAllister et al., 2012). The present study reveals that the UR treated with N stabilizers and UR treatments significantly increased N uptake in silage corn compared to CTRL (Table 3.2). The use of UI and NI has proven that when urease hydrolysis and nitrification are reduced, there is a reduction in NH₃ volatilization which can be reflected in the N uptake and higher NRE of crops (Abalos et al., 2014). In the present study, UR treated with N stabilizers did not significantly increase N uptake compared to UR; however, N uptake with SU application was 27% higher than with UR application (Table 3.3). Additionally, we observed from the demsar plot that SU in 2020 was an important treatment in the N uptake of silage corn (Figure 3.4c). In 2020, UR treated with N stabilizers were statistically at par with UR and produced similar NRE (Figure 3.3). The lack of significant impact of UR treated with N stabilizers on the N uptake and NRE in the current study may result from the inadequate duration of the inhibitory effect from UR treated with N stabilizers (Burzaco, 2012). However, we observed that SU significantly increased NRE in silage corn in 2019 compared to UR (Figure 3.3). These findings corroborate previous studies, which reported that UR treated with N stabilizers can enhance NRE in corn (Mohd Zuki et al., 2020; Rodrigues Mikhael, 2018).

3.4.3. Forage quality

Forages can differ significantly in protein, fiber, and mineral content. Within the fiber portion, there are several components including hemicellulose, cellulose, and lignin. Research over the last decade has broadened our understanding of these components and their effects on diets. Of the fiber fractions, cellulose is the major fiber fraction digested by the animal. However, lignin can bind the cellulose fraction thus lowering potential forage digestibility. The higher the concentration of lignin, the less digestible the fiber will be. N fertilizers application enhances crop growth and forage DM, however, it is one of the most important nutrients which influences the forage quality of silage and grain crops (Çarpıcı et al., 2017; Uzun et al., 2020). CP represents the amount of N present in the forage (Collins and Fritz, 2003), mainly because N plays a great role in the synthesis of amino acids which are building blocks of protein (Amin, 2011; Zaman et al., 2010). In the current study, UR treated with N stabilizers and UR slightly increased CP compared to CTRL though no significant effects. These findings corroborate that of (Amin, 2011) who observed a significant increase of CP in fodder maize using different N sources (NPK, ammonium sulphate nitrate (ASN), ammonium sulphate (AS), and UR) compared to the CTRL. In the present study, UR treated with N stabilizers AG and SU slightly increased CP compared to UR though no significant effect, suggesting that the inhibitors may have helped to reduce N loss (Table 3.4). Similar results were observed by Huerfano et al. (2018), who reported no significant difference between AS and AS amended with 3,4 dimethylpyrazol succinic acid (DMPSA) or 3,4-dimethylpyrazol phosphate (DMPP) on CP of silage corn. Other studies reported similar trends in the effect of N sources on CP (Fageria and Carvalho, 2014; Safdarian et al., 2014). However, CP significantly increased by 37% in 2019 compared to 2020 (Table 2.4). Higher rainfall during 2019 than in 2020 (Table 3.2) might have enhanced soil moisture and nutrient uptake and consequently

enhanced CP (Wallace et al., 2020). ADF and NDF are the forage quality indices that determine the energy content and digestibility of forages since they represent the roughage portion of the feed that animals tend to least digest (Kaplan et al., 2016; Lamprey et al., 2018; Safdarian et al., 2014). N fertilizers can reduce ADF and NDF concentration in feed (Uzun et al., 2020). The cortex of the stem constitutes lignin, cellulose, and hemicellulose which makes it insoluble while the pith constitutes soluble pectin, waxes, and proteins (Van Soest, 1994). Consequently, as N increases CP through protein synthesis there is an increase in stem diameter hence a concomitant decrease in potential forage fiber constituents like ADF and NDF (Huerfano et al., 2018; Van Soest, 1994). In our study, UR treated with N stabilizers and UR had no significant effects on ADF and NDF, though values of ADF and NDF were slightly decreased by UR treated with N stabilizers and UR compared to CTRL. This corroborates the findings of (Safdarian et al., 2014) who reported no significant difference within N sources. NEL refers to the energy in a diet that is used to produce milk. In my study, SU produced significantly higher NEL compared to UR which produced lower NEL (Table 3.5). This could be attributed due to the combined effects of the UI and NI, reduced N loss, thus an increase in N uptake (Abalos et al., 2014). Significantly higher calculated milk production was observed in SU amended treatment compared to CTRL which produced lowest milk; however, SU was statistically similar with other UR treated N stabilizers and UR (Table 3.6). The higher milk production in SU treatment might be due to higher forage DM, N uptake and NEL that might have enhanced calculated milk production compared to CTRL treatment (Table 3.5, 3.6, 3.7). Additionally, forage DM, milk, P, K, Ca, CP, AP, lignin, Mg were all clustered in the same quadrant suggesting that all these parameters were closely associated with 2019 growing season (Figure 3.4a).

3.5. Conclusions

Nitrogen (N) stabilizers are used to reduce N losses and enhance crop growth, forage dry matter (DM), N uptake, nitrogen recovery efficiency (NRE) and forage quality indices of silage corn. In my study, results revealed that urea (UR) treated with N stabilizers: agrotain (AG), eNtrench (EN), superU (SU) and conventional UR significantly increased the chlorophyll, plant height, forage DM, N uptake, and NRE of silage corn cultivated on podzols in boreal climate. SU was the only N stabilizer which significantly increased net energy for lactation (NEL) by 5% and nitrogen recovery efficiency (NRE) by 50% in 2019 compared to UR. No significant difference between UR treated with N stabilizers and UR in enhancing the growth, forage DM and other forage quality indices of silage corn was recorded. The redundance analyses (RDA) and the demsar plots suggests that the chlorophyll and the N uptake were highly affected by SU in 2020 suggesting that the combination of both urease inhibitors (UI) and nitrification inhibitors (NI) has a positive effect on the production of silage corn cultivated on podzols in a boreal climate. The significant increase in photosynthesis and plant height in 2020 may be because of the higher temperatures experienced during the growth season; since higher temperatures alongside N availability have the potential to favour photosynthetic activity hence an increase in the growth and development of plant cells. The effect of UR treated with N stabilizers on most of the measured parameters did not significantly differ from UR probably because of high temperatures, low soil moisture, low soil pH, and soil type which might have reduced the efficacy of UR treated with N stabilizers. The current findings suggest that the use of UR treated with N stabilizers particularly SU has the potential to increase growth, yield, N uptake, NRE, net energy for lactation (NEL), and milk production of silage corn cultivated on podzols in boreal climates. Further long-term field studies are required to determine

the effect of UR treated with N stabilizers on growth, forage DM, forage quality, N uptake, and NRE in podzolic soils in boreal climate.

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Co-authorship statement for experiment two

Chapter four entitled “the effects of urea and urea treated with nitrogen stabilizers on the growth, yield, nitrogen uptake, nitrogen recovery efficiency and forage quality indices of faba beans and oat + peas mixture in a boreal climate” will be published in the journal of Field Crop Research. Therese Audrey Nzwindi Mbite will be the primary author for this manuscript. Dr. Mumtaz Cheema will be the corresponding and last author. The co-authors will be Dr. Lakshman Galagedara, Dr. Raymond Thomas, Dr. Yeukai Katanda, and Dr. Muhammad Nadeem.

Chapter four

The effects of urea and nitrogen stabilizers on the growth, dry matter yield, N uptake, nitrogen recovery efficiency, and forage quality indices of faba beans and oat + pea mixture cultivated on podzols in boreal climate

Abstract

The high forage quality and diversity needed for the dairy industry in Newfoundland and Labrador can be achieved by growing legumes because of their high protein content. Intensive cultivation generally leads to higher nitrogen (N) usage, hence higher N loss. Urea (UR) treated with N stabilizers like urease inhibitor (UI) [N-(n-butyl) thiophosphoric triamide (NBPT)] and nitrification inhibitors (NI) [nitrapyrin and dicyandiamide (DCD)] can mitigate N loss by synchronizing N release with plant N uptake. A field experiment was carried out to evaluate the effect of UR and N stabilizers UI and NI on growth, forage dry matter (DM), forage quality, N uptake, and nitrogen recovery efficiency of faba bean and oats + peas mixture cultivated on Podzols in boreal climate. Experimental treatments were: 1) UR; 2) SuperU™ (SU, urea with DCD and NBPT); 3) Agrotain™ (AG, urea with NBPT); 4) eNtrench™ (EN, urea with nitrapyrin); at 25 and 40 kg N ha⁻¹ respectively for faba bean and oat + peas mixture and 5) No-N as control (CTRL). SU and AG significantly decreased calcium and magnesium respectively by 16% and 18% compared to UR in faba bean, while no significant treatment effect was recorded on the forage DM in all crops. The findings suggest that UR treated with N stabilizers may not be required to cultivate legumes on Podzols in boreal climate.

4.1. Introduction

The strategy of the Newfoundland and Labrador (NL) Government to clear new land for agricultural purposes will be a greater opportunity to attain self-sufficiency in forage production (Haverstock, 2013 - 2014). Currently, the farming sector in NL is greatly dominated by small-scale farms, which focuses more on greenhouse production, vegetables, fruits, and berries production (Jamieson et al., 2016); a situation that challenges the sustainability of the dairy industry (Haverstock, 2013 - 2014). The dairy industry is one of the most flourishing agricultural sectors in NL, as such its sustainability is imperial; particularly in the production of supplements, feed grains, and forages (Cordeiro et al., 2019). Corn is the crop mostly grown for forage in NL (Canada, 2017). However, for greater forage yield and forage quality, forage mixtures with legumes could be a great alternative (Pandey et al., 2017; Rasmussen et al., 2012).

Legumes have nodules that can fix atmospheric nitrogen (N) in ammonia (NH₃) form for plant use through biological nitrogen fixation (BNF) (Pandey et al., 2017). According to Nyfeler et al. (2011), the combination of both legumes and cereals or grasses is the most efficient way to enhance the BNF of legumes, because more the N is taken up by the cereal crop, the more the legume crop fixes atmospheric N. Studies of Krga et al. (2021) also reported maximum yield as a result of combining legumes with cereals as compared to sole legume cropping. While Neugschwandtner et al. (2015) reported greater N fixation during winter faba beans cultivation, Nyfeler et al. (2011) reported greater N uptake by forage crops in a mixed cropping system. There are studies that suggest that legume crops should be N fertilized, despite their N fixing ability (Jiang et al., 2020; Meakin et al., 2007).

The BNF in legumes may only be possible after the development and maturity of legume root nodules, which usually happens later in the vegetative stage. As such, legume crops will need

starter N before solely depending on BNF (Meakin et al., 2007). Köpke and Nemecek (2010) suggested that legume seed inoculation can be as efficient as N fertilizers since it could promote rapid colonization of the soil by the rhizobia bacteria responsible for BNF. Chen et al. (2017) explained that N fertilization of legumes will help to boost or stimulate plant growth and development, because about 70% of N found in the chloroplast is used to synthesise the photosynthetic apparatus. A study carried out in an organic cropping system in Switzerland with low N fertilization; -and reported that grass-clover contributed about 120 to 140 kg N ha⁻¹ per year through BNF (Oberson et al., 2013; Pandey et al., 2017). However, N loss through volatilization, leaching, and runoff is of major concern when using N fertilizers, thus using urea (UR) fertilizers amended with urease inhibitors (UI) and nitrification inhibitors (NI) can help offset N losses (Migliorati et al., 2015).

The use of UR treated with N stabilizers like UI and NI is one of the best management practices (BMPs) sought nowadays for mitigating reactive nitrogen (Nr) losses from fertilized soils; Since the UI will block urease activity by binding with the urease enzyme active sites, lowering UR hydrolysis and subsequent NH₃ loss (Amtul et al., 2002; Sha et al., 2020). The meta-analyses conducted by Xia et al. (2017), Pan et al. (2016), and (Li et al., 2018) have reported a significant reduction in NH₃ (53- 61%) and nitrous oxide (N₂O) (21- 28%) emissions with the use of UI. NI, on the other hand, will hinder the activity of Nitrosomonas by binding with the ammonium (NH₄⁺) monooxygenase enzyme, thereby blocking the first reaction of NH₄⁺ oxidation (Akiyama et al., 2010). Previous studies have reported that NI reduced N₂O emissions by 38-57% (Akiyama et al., 2010; Li et al., 2018; Xia et al., 2017) and 24-81% of nitric oxide (NO) emissions (Akiyama et al., 2010; Liu et al., 2017), though may increase NH₃ volatilization by 7-27% (Li et al., 2018; Pan et al., 2016; Xia et al., 2017). The ability of UI and NI to slowly release NH₃ in synchrony with

plants' N need, leads to an increase in nitrogen recovery efficiency (NRE), and therefore an increase in yields (Abalos et al., 2014; Malla et al., 2005; Xi et al., 2017).

There is little knowledge of the effect of N inhibitors on legumes, particularly legumes cultivated on podzols in boreal climate. However, several studies disregard N fertilization to legumes because it can be disadvantageous to BNF (Hauggaard-Nielsen et al., 2009; Jiang et al., 2020; Voisin et al., 2002b). For example, Voisin et al. (2002b) opined that even the slightest concentration of nitrate (NO_3^-) in the soil has a negative impact on nodule formation and nitrogenase activity. From the study of Cruchaga et al. (2011), it was observed that the presence of N-(n-butyl) thiophosphoric triamide (NBPT) led to a drastic reduction in the urease activity of the roots of the peas, thereby affecting N metabolism in the plants leading to a deficiency of N; notably in the shoots. Nevertheless, Li et al. (2009b) reported that the best way to offset the negative effect of N fertilizers on legumes is to combine both legumes and cereal crops.

The contrasting ideologies on the benefits of N fertilizers on legumes from various studies give room for more insightful research, particularly on the effects of N inhibitors on legumes. N inhibitors such as NBPT, dicyandiamide (DCD), and Nitrapyrin have been reported to efficiently improve growth, biomass, and NRE of various crops (Abalos et al., 2014). As such, to help improve the forage quality, to diversify forage production, as well as to increase forage self-sufficiency in NL (Haverstock, 2013 - 2014), I hypothesize that the use of urea treated with N stabilizers will significantly increase plant growth, forage dry matter (DM), forage quality indices, N uptake, and NRE of faba beans and oats + peas mixture grown on Podzols (under field conditions) in the boreal climate. The study was conducted with the following objectives:

- To investigate the effects of urea and urea treated with N stabilizers on the growth and forage DM of faba beans and oats + peas mixture cultivated on Podzols in the boreal climate.
- To explore the role of urea and urea treated with N stabilizers on forage quality indices of faba beans and a high nitrogen feed mix of oat + pea cultivated on Podzols in the boreal climate.
- To determine N uptake and NRE of legumes in response to urea and urea treated with N stabilizers.

4.2. Materials and Methods

4.2.1. Experimental site and treatments

A field experiment was carried out at (49° 04' 28" N; 57° 33' 23" W), Pasadena, NL, Canada during the 2019 and 2020 growing seasons. The experimental treatments were 1) urea (UR), 2) Agrotain™ [(AG; urea coated with NBPT)], 3) eNtrench™ [(EN; urea coated with nitrapyrin)], 4) SuperU™ [(SU; urea coated with NBPT and DCD)], 5) CTRL (no N fertilizer). The CTRL treatments in faba bean were 1) CTRL (non-inoculated seeds + no fertilizer) and 2) CTRL+ (inoculated seeds + no fertilizer). Silage corn was seeded in 2019 followed by faba beans and oats + peas mixture grown in 2020 on the same field in rotation. Faba bean seeds were inoculated at 1.5 kg inoculant 900 kg⁻¹ seed 24 hours before seeding except for CTRL (Dubova et al., 2015; Hossain et al., 2016). The experiment was laid out in a Randomized Complete Block Design replicated 4 times for a total of 24 experimental units with a plot size of 3 m × 4 m. Before planting, 10 soil core samples were collected in a zigzag manner from each block at a depth of 0 – 15 cm using an auger and composited to one sample per block to

determine the basic soil properties and texture of the experimental site (Table 4.1). The samples were analyzed (Soil & Plant Laboratory, Dept. of Fisheries and Land Resources, St John's, NL) using the hydrometer method given by Bouyoucos (1962), which described the soil texture to be sandy loam for blocks 1, 2, and 3 and loamy sand for block 4. The pH was determined using samples prepared as 50/50 DI water and analyte; and was reported using a Fisher Scientific XL250 Benchtop Meter and pH Electrode. Organic matter (OM) was obtained after samples were dried overnight at 102°C and then heated in a Muffle furnace at 430°C. Cation exchange capacity (CEC) was calculated using software onsite. Nutrients were extracted from soil samples using Mehlich III (Carter and Gregorich, 2003) and analyzed using a Teledyne Instrument Prodigy High Dispersion ICP as describe in appendix two.

Table 4.1: Basic soil properties of the experimental site

pH	OM	CEC	P	K	Ca	Mg	S	Cu	Na	Al
	%	cmol kg ⁻¹	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
6.40	3.97	12.15	378	126.75	1.28	186.25	19.75	2.93	15.5	1.44

OM: Organic matter, P: Phosphorous, K: Potassium, Ca: Calcium, Mg: Magnesium, S: Sulphur, Cu: Copper, Na: Sodium, Al: Aluminium, CEC: Cation Exchange Capacity.

Before planting, the herbicides Credit[®] Xtreme (active ingredient Glyphosphate) and Eragon[®] LQ were mixed along with a surfactant with petroleum hydrocarbons (Merge[®] Adjuvant) at respective rates of 2.2 L ha⁻¹, 146 mL ha⁻¹, and 1 L ha⁻¹ and applied to control weeds. On June 11th, 2020, faba beans cv. Snowdrop and a 50/50 mixture of oats + peas cv Maxi-Sile were planted at 168 kg seed ha⁻¹ and 55 kg seed ha⁻¹, respectively using a tractor-mounted plot seeder (Wintersteiger XL). UR and UR treated with N stabilizers were broadcasted at 25 kg N ha⁻¹ and

40 kg N ha⁻¹ at seeding for faba beans and oats + peas mixture, respectively. Phosphorus and potassium fertilizers were applied at 0 kg P₂O₅ ha⁻¹ and 60 kg K₂O ha⁻¹, respectively at seeding following soil test reports and regional recommendations. Oats + peas mixture and faba beans were harvested on September 1 and September 17, 2020, respectively.

4.2.2. Growth parameters

4.2.2.1. Chlorophyll content

The chlorophyll content in both faba beans and oats + peas mixture was measured using a SPAD 502 plus chlorophyll meter (Spectrum Technologies, IL, USA). At harvest, five faba bean plants were randomly selected in each plot, of the four blocks and two readings were taken from the fully expanded leaf from the top of the plant (Figure 4.1) (Dordas and Lithourgidis, 2011). Whereas the chlorophyll content for oats + peas mixture was measured at the respective flowering stage of each crop. Eight oat plants were randomly selected from each plot, of the four blocks and one reading was taken at the center of the flag leaf of each plant (Baxevanos et al., 2017; Singh et al., 2019). While six peas plants were randomly selected within the plots, of the four blocks and two readings were taken from the fully expanded leaf from the top of the plant (Abdulmajeed and Qaderi, 2019; Youssef et al., 2018).



Figure 4.1: Chlorophyll measurement of faba beans plants (a), display of different faba beans experimental plots at 6 leaf stage (b), and photosynthesis measurement of faba beans at 6 leaf stage (BBCH 16) (c).

4.2.2.2. *Photosynthesis*

The photosynthesis rate for all crops was measured on a sunny day using a portable photosynthesis system (LI-6400XT) (LI-COR Biosciences, NE, USA). At harvest (BBCH 77-79), Five faba bean plants were randomly selected from each plot of the four blocks, and the photosynthesis measurements were taken from the third most fully expanded leaf from the top of the plants (Figure 4.1) (Li et al., 2009b). At the flowering stage, eight oat plants were randomly selected within each plot of the four blocks and one photosynthesis measurement was taken at the center of the flag leaf of each of the plants. While six peas plants were randomly selected within each plot of the four blocks and one photosynthesis measurement was taken from the third fully expanded leaf from the top of the plants (Abdulmajeed and Qaderi, 2019; Youssef et al., 2018). Before taking measurements, the photosynthesis system was set up at a photon flux density of $1500 \mu\text{molm}^{-2}\text{s}^{-1}$, leaf temperature 25°C , pump flow rate of $400 \mu\text{mols}^{-1}$, and 400 ppm CO_2 concentration (Li et al., 2009b; Parvin et al., 2019).

4.2.2.3. *Plant height*

Ten Faba bean plants were randomly selected at harvest and the plant height was measured using a measuring rod from the plant's base to the top of the plant (Figure 4.2) (Dordas and Lithourgidis, 2011). The mean height of the ten plants was used as the final plant height per plot in all the four blocks.

4.2.2.4. *Forage yield*

The crops were harvested manually; Faba beans on September 17, 2020, at physiological maturity stage 98 (Stoltz et al., 2013) and oats + peas mixture on September 1, 2020, at the soft dough stage

of oat (Omokanye, 2014). Two quadrants of 0.5 m were used to harvest crops 5cm above the soil using grass shears within the sampling unit (Bacchi et al., 2021) (Figure 4.2). The fresh weight of the harvested crops was measured using a weighing scale (Adam Equipment™ S.N. AE6211313 CBC) to determine fresh biomass. Thereafter the crops were bagged and oven-dried (Precision Quincy corporation SN 17645, Model No. 40D-350) at 65°C for 72h (Bacchi et al., 2021).

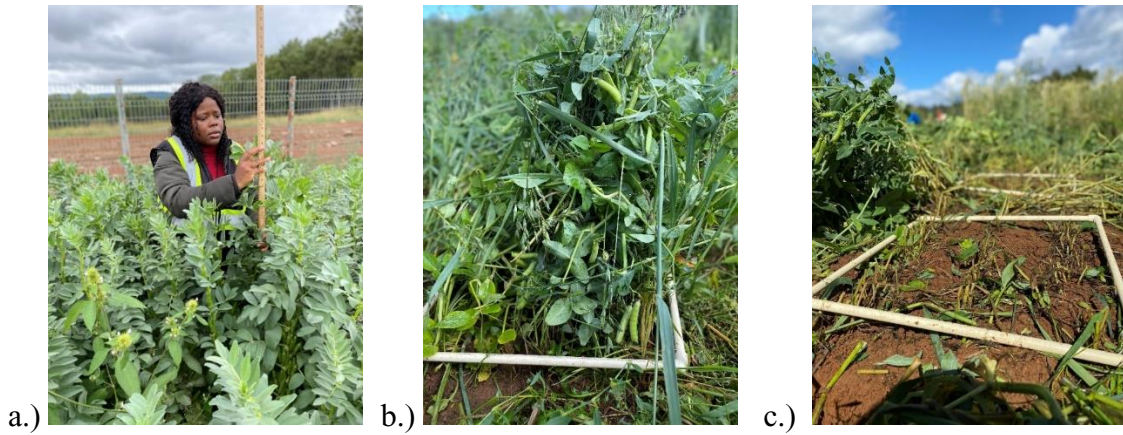


Figure 4.2: Plant height measurement of Faba beans at harvest (a); oat and peas samples prior to harvest (b) and harvested area of oat and peas samples (c).

The harvest area was determined by the averaging the area of two quadrants. After obtaining the dry weights of samples, the forage DM (g kg^{-1}) was determined using the original moisture content of each sample. However, during the determination of forage DM, the moisture content was corrected to 65% following the method of Clark (2018) due to varying moisture content (Equation 1) (Kleinmans et al., 2016; Lithourgidis et al., 2011).

$$\text{Forage dry matter} = \left(\left(\frac{\text{fresh weight} * 10000}{\text{area}} \right) * \left(\frac{1 - 0.65}{100} \right) / 1000 \right) \dots (1)$$

Dried plant samples were ground with a Wiley mill (A.H. Thomas Co., Philadelphia, PA, USA) through a 4 mm screen, and 50 g samples were packed into small Ziplock bags and sent to Activation Laboratories Ltd. (Actlabs, Ancaster ON, Canada), for forage quality analyses. Another subsample was pulverized using a Cryomill (MM 400; Retsch Inc., Newton, PA) and sent to Prince Edward Island Analytical Laboratories (PEIAL, Bioscom Park Charlottetown, Canada) for the determination of N ratio in faba beans and oat and peas tissues.

4.2.3. Determination of forage feed quality indices

The forage quality indices were determined using the near-infrared NIR method (Association of Official Analytical Chemists (AOAC), method 990.03) (Gallo et al., 2013). Forage quality parameters analyzed were crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), non-fibrous carbohydrates (NFC), total digestible nutrients (TDN), net energy for lactation (NEL), starch, and minerals (phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) concentrations.

4.2.4. Nitrogen uptake and recovery efficiency

The N uptake was determined based on the N concentration in tissues of faba beans and oats + peas mixture and the NRE was calculated using the following formula (Kaur et al., 2018).

$$NRE = \frac{N \text{ uptake (+N)} - N \text{ uptake (CTRL)}}{\text{Amount of N applied}} \times 100 \quad (2)$$

Where:

NRE = Nitrogen recovery efficiency

N uptake (+N) = N uptake from fertilized treatments

N uptake (CTRL) = N uptake from control (no N) treatments

4.2.5. Statistical Analyses

A one-way ANOVA technique in XLSTAT (XLSTAT Premium, 2018.1.1, NY, USA) was performed to assess the effects of UR and UR treated with N stabilizers treatments on the growth, yield, and forage quality parameters. Treatment means were compared using Fisher's least significant difference (LSD) at $\alpha = 0.05$. Redundance analyses (RDA) was performed to visualize the relationships between treatments and the response variables in XLSTAT.

4.3. Results

4.3.1. Growth, forage dry matter, nitrogen uptake, and nitrogen recovery efficiency of faba beans and oats + peas mixture

The ANOVA revealed that there was no significant difference ($p > 0.05$) between UR and UR treated with N stabilizers compared to the CTRLs in photosynthesis, chlorophyll, plant height, forage DM, and N uptake of faba beans and oat + peas mixture (Table 4.2, 4.3 and 4.4). UR treated with N stabilizers were statistically at par with UR and produced similar photosynthesis rates, chlorophyll content, plant height, forage DM, N uptake, and NRE of faba beans and oats + peas mixture (Table 4.2, 4.3, 4.4, and Figure 4.3) as with UR.

Table 4.2: The effect of urea and urea treated with N stabilizers on the growth and yield of faba bean

Main factors	Photosynthesis ($\mu\text{mol m}^{-2} \text{sec}^{-1}$)	Chlorophyll (SPAD values)	Forage DM (Mg ha^{-1})	Plant height (m)
Treatment (T)				
CTRL-	19.1 \pm 1.20	37.6 \pm 0.76	36.19 \pm 3.11	0.99 \pm 0.06
CTRL+	17.6 \pm 0.89	37.9 \pm 1.06	33.09 \pm 2.47	0.89 \pm 0.06
AG	18.3 \pm 1.33	38.6 \pm 1.45	33.42 \pm 3.89	0.88 \pm 0.06
EN	19.6 \pm 2.40	37.9 \pm 1.29	35.48 \pm 0.69	0.87 \pm 0.06
SU	18.2 \pm 1.12	38.7 \pm 0.99	34.82 \pm 3.91	0.96 \pm 0.06
UR	17.5 \pm 1.71	39.3 \pm 1.48	31.85 \pm 3.87	0.85 \pm 0.06
P-value				
T	0.95	0.96	0.96	0.43

The values are means \pm standard errors. Each measured parameter was replicated four times per treatment. CTRL-: control without inoculated seeds, CTRL+: control with inoculated seeds, AG: Agrotain with inoculated seeds, EN: eNtrench with inoculated seeds, SU: superU with inoculated seeds, UR: urea with inoculated seeds, forage DM: forage dry matter.

Table 4.3: The effects of urea and urea treated with N stabilizers on the growth and yield of oats + peas mixture

Main factors	Oats	Peas	Oats	Peas	Forage DM (Mg ha ⁻¹)
	Photosynthesis (μmol m ⁻² sec ⁻¹)		Chlorophyll SPAD values		
Treatment (T)					
CTRL	12.3±1.86	19.0±1.83	51.1±1.85	37.0±0.86	32.15±4.08
AG	13.4±1.86	19.0±1.83	50.4±1.85	36.5±0.86	29.85±4.48
EN	16.1±1.86	16.8±1.83	54.5±1.85	38.1±0.86	21.72±2.05
SU	16.2±1.86	15.7±1.83	53.3±1.85	38.7±0.86	25.57±3.00
UR	13.4±1.86	18.7±1.83	52.3±1.85	38.3±0.86	34.79±4.76
P-values					
T	0.37	0.44	0.64	0.47	0.28

The values are means ± standard errors. Each measured parameter was replicated four times per treatment. CTRL: control, AG: Agrotain, EN: eNtrench, SU: superU, UR: urea, forage DM: forage dry matter.

Table 4.4: The effects of urea and urea treated with N stabilizers on the nitrogen uptake of faba bean and oats + peas mixture.

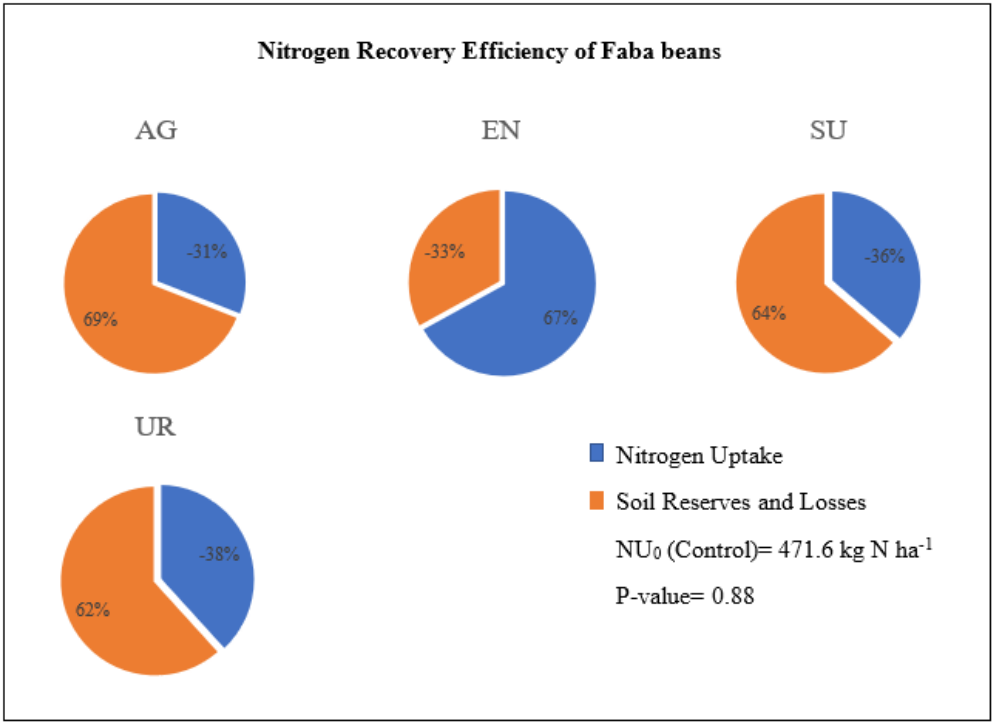
Main factors	Faba beans	Oats + peas
	N uptake (kg N ha ⁻¹)	
Treatments (T)		
CTRL-	450.3±53.65	346.3±43.20
CTRL+	471.6±53.65	-
AG	451.3±53.65	314.2±43.20
EN	520.9±53.65	221.4±43.20
SU	438.7±53.65	255.4±43.20
UR	430.9±53.65	342.9±43.20
P values		
T	0.86	0.22

The values are means ± standard errors. The analyses was replicated four times per treatment. CTRL-: control without inoculated seeds, CTRL+: control with inoculated seeds, AG: Agrotain with inoculated seeds, EN: eNtrench with inoculated seeds, SU: superU with inoculated seeds, UR: urea with inoculated seeds.

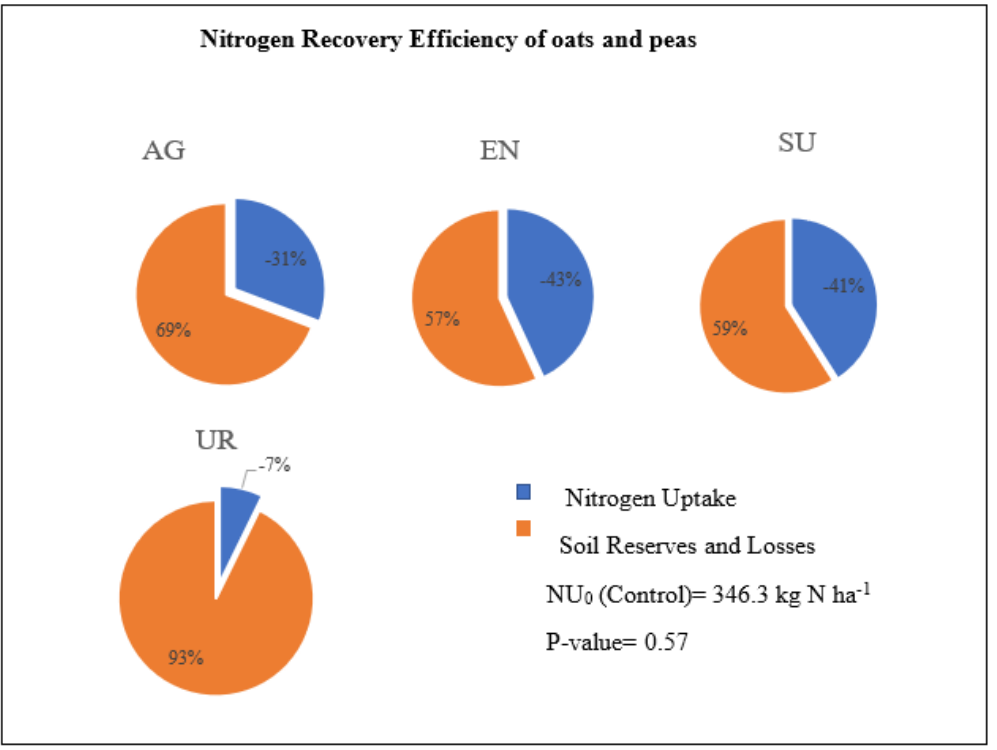
4.3.2. Forage quality indices of faba bean and oats + peas mixture

There was no significant difference ($P>0.05$) between UR and UR treated with N stabilizers in the forage indices CP, acid detergent insoluble protein (ADIP), ADF, NDF, NFC, Lignin (Table 4.5); Starch, CF, TDN, Simple sugar, NEM, NEG, relative feed value (RFV) (Table 4.6); NEL, sodium (Na), Sulphur (S), P, Ca, Fe, Zn, Cu, Mn and K (Table 4.7) of faba beans compared to CTRLs. The EN treatment produced the highest Mg (3.10 g kg^{-1}) and CTRL the lowest Mg (2.58 g kg^{-1}) in faba beans (Table 4.7). UR treated with N stabilizers were statistically at par with UR and produced similar CP, ADIP, ADF, NDF, NFC, and Lignin (Table 4.5); Starch, TDN, Simple sugar, NEM, NEG, RFV (Table 4.6); NEL, S, Na, P and K (Table 4.7) to that by UR in faba beans. However, AG and SU significantly decreased CF by 12% (Table 4.6), whereas AG decreased calcium by 18% and SU decreased Mg by 16% (Table 4.7) as compared to UR.

There was no significant difference ($P>0.05$) between UR and UR treated with N stabilizers in the forage quality indices CP, ADIP, ADF, NDF, NFC, Lignin (Table 4.8); Starch, CF, TDN, Simple sugars, NEM, NEG, RFV (Table 4.9); NEL, S, Na, Ca, P, Mg, Fe, Mn, Cu, Zn and K (Table 4.10) of oats + peas mixture. UR treated with N stabilizers and UR were statistically at par and produced similar CP, ADIP, ADF, NDF, NFC, and Lignin (Table 4.8); Starch, CF, TDN, Simple sugars, NEM, NEG, RFV (Table 4.9); NEL, S, Na, Ca, P, Mg, Fe, Cu, Zn, Mn, and K (Table 4.10) in oats + peas mixture.



A.)



b.)

Figure 4.3: Nitrogen recovery efficiency (NRE) of faba bean (a) and oats + peas mixture (b)

Table 4.5: The effects of urea and urea treated with N stabilizers on the proteins and fibres of faba bean

Main factors	CP (g kg ⁻¹)	ADIP (g kg ⁻¹)	ADF (g kg ⁻¹)	NDF (g kg ⁻¹)	NFC (g kg ⁻¹)	Lignin (g kg ⁻¹)	Starch (g kg ⁻¹)
Treatment (T)							
CTRL-	205.6±5.55	18.8±1.68	306.2±12.49	346.8±11.60	367.5±14.19	47.6±3.57	92.2±9.57
CTRL+	205.0±5.55	16.4±1.68	292.4±12.49	340.7±11.60	378.6±14.19	45.7±3.57	94.9±9.57
AG	213.2±5.55	17.9±1.68	284.9±12.49	320.4±11.60	393.3±14.19	38.2±3.57	113.7±9.57
EN	222.6±5.55	19.1±1.68	290.2±12.49	330.7±11.60	365.0±14.19	44.1±3.57	96.0±9.57
SU	202.7±5.55	16.7±1.68	292.8±12.49	339.3±11.60	382.2±14.19	44.6±3.57	88.1±9.57
UR	218.2±5.55	16.6±1.68	284.9±12.49	324.0±11.60	381.4±14.19	39.2±3.57	103.5±9.57
P values							
T	0.11	0.78	0.85	0.57	0.75	0.39	0.49

The values are means ± standard errors. Each analyses was replicated four times per treatment. CTRL-: control without inoculated seeds, CTRL+: control with inoculated seeds, AG: Agrotain with inoculated seeds, EN: eNtrench with inoculated seeds, SU: superU with inoculated seeds, UR: urea with inoculated seeds, CP: crude protein, ADIP: acid detergent insoluble protein, ADF: acid detergent fibre, NDF: neutral detergent fibre, NFC: non-fibrous carbohydrates.

Table 4.6: The effect of urea and urea treated with N stabilizers on the crude fat, total digestible nutrient, simple sugar, relative feed value, and energy of faba bean

Main factors	CF (g kg ⁻¹)	TDN (g kg ⁻¹)	SS (g kg ⁻¹)	RFV (%)	NEM (g kg ⁻¹)	NEG (g kg ⁻¹)	NEL (mcal kg ⁻¹)
Treatment (T)							
CTRL-	20.7±0.69ab	650.5±9.73	90.9±6.38	176.7±8.92	1.60±0.03	0.87±0.03	1.48±0.02
CTRL+	19.9±0.69ab	661.3±9.73	96.7±6.38	181.4±8.92	1.63±0.03	0.91±0.03	1.50±0.02
AG	19.7±0.69b	667.0±9.73	99.8±6.38	193.8±8.92	1.65±0.03	0.93±0.03	1.52±0.02
EN	22.5±0.69a	662.9±9.73	87.3±6.38	186.7±8.92	1.64±0.03	0.91±0.03	1.51±0.02
SU	19.7±0.69b	660.9±9.73	112.4±6.38	181.9±8.92	1.63±0.03	0.91±0.03	1.51±0.02
UR	22.5±0.69a	667.1±9.73	97.2±6.38	192.8±8.92	1.65±0.03	0.93±0.03	1.52±0.02
P values							
T	0.02	0.85	0.16	0.71	0.84	0.83	0.85

The values are means ± standard errors. Each analyses was replicated four times per treatment. CTRL-: control without inoculated seeds, CTRL+: control with inoculated seeds, AG: Agrotain with inoculated seeds, EN: eNtrench with inoculated seeds, SU: superU with inoculated seeds, UR: urea with inoculated seeds, CF: crude fat, TDN: total digestible nutrients, SS: simple sugars, RFV: relative feed value, NEM: net energy for maintenance, NEG: net energy gain, NEL: net energy for lactation.

Table 4.7: The effects of urea and urea treated with N stabilizers on the macro and micro-nutrients of faba bean.

Main factors	S (g kg ⁻¹)	Na (g kg ⁻¹)	Ca (g kg ⁻¹)	P (g kg ⁻¹)	Mg (g kg ⁻¹)	K (g kg ⁻¹)	Cu(mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn(mg kg ⁻¹)	Zn (mg kg ⁻¹)
Treatment (T)										
CTRL-	0.93±0.07	0.73±0.16	6.80±0.31ab	3.25±0.20	2.58±0.12b	19.0±0.98	12.1±1.13	82.6±6.32	46.9±4.19	71.8±9.82
CTRL+	0.98±0.07	0.90±0.16	7.25±0.31ab	3.30±0.20	2.80±0.12ab	16.3±0.98	12.2±1.13	73.4±6.32	45.5±4.19	68.1±9.82
AG	1.00±0.07	0.78±0.16	6.50±0.31b	3.40±0.20	2.70±0.12ab	15.4±0.98	13.4±1.13	79.9±6.32	41.9±4.19	66.1±9.82
EN	1.03±0.07	0.80±0.16	6.83±0.36ab	3.85±0.20	3.10±0.12a	15.1±1.13	13.4±1.13	81.8±6.32	51.3±4.19	78.8±11.34
SU	0.93±0.07	0.68±0.16	6.68±0.31ab	3.18±0.20	2.60±0.12b	17.3±0.98	12.8±1.13	74.9±6.32	43.4±4.19	61.7±9.82
UR	1.03±0.07	0.90±0.16	7.93±0.31a	3.48±0.20	3.08±0.12a	14.8±0.98	12.0±1.13	78.1±6.32	53.7±4.19	80.9±9.82
P values										
T	0.79	0.89	0.05	0.26	0.02	0.06	0.92	0.88	0.35	0.74

Different letters in the columns indicate significant difference between treatments ($P < 0.05$) and the values are means \pm standard errors. Each analyses was replicated four times per treatment. CTRL-: control without inoculated seeds, CTRL+: control with inoculated seeds, AG: Agrotain with inoculated seeds, EN: eNtrench with inoculated seeds, SU: superU with inoculated seeds, UR: urea with inoculated seeds, S: sulphur, Na: sodium, Ca: calcium, P: phosphorus, Mg: magnesium, K: potassium, Cu: copper, Mn: manganese, Fe: iron, Zn: zinc.

Table 4.8: The effects of urea and N fertilizer stabilisers on the protein and fibres of oats + peas mixture

Main factors	CP (g kg ⁻¹)	ADIP (g kg ⁻¹)	ADF (g kg ⁻¹)	NDF (g kg ⁻¹)	NFC (g kg ⁻¹)	Lignin (g kg ⁻¹)	Starch (g kg ⁻¹)
Treatment (T)							
CTRL	158.9±15.87	1.79±0.22	337.2±11.68	442.3±16.27	287.4±18.25	56.3±2.71	146.9±26.32
AG	145.1±15.87	1.61±0.22	306.7±11.68	413.8±16.27	342.6±18.25	52.3±2.71	140.7±26.32
EN	146.3±15.87	1.29±0.22	297.7±11.68	432.4±16.27	324.7±18.25	50.0±2.71	158.9±26.32
SU	124.0±15.87	1.40±0.22	326.4±11.68	467.1±16.27	306.5±18.25	51.1±2.71	143.9±26.32
UR	144.2±15.87	1.51±0.22	337.3±11.68	452.2±18.79	277.3±18.25	58.2±2.71	87.5±26.32
P values							
T	0.65	0.56	0.09	0.26	0.13	0.21	0.39

The values are means ± standard errors. Each analyses was replicated four times per treatment. CTRL: control, AG: Agrotain, EN: eNtrench, SU: superU, UR: urea, CP: crude protein, ADIP: acid detergent insoluble protein, ADF: acid detergent fibre, NDF: neutral detergent fiber, NFC: non-fibrous carbohydrates.

Table 4.9: The effects of urea and N fertilizer stabilisers on the crude fat, total digestible nutrients, simple sugars, relative feed value, and energy of oats + peas mixture

Main factors	CF (g kg ⁻¹)	TDN (g kg ⁻¹)	SS (g kg ⁻¹)	RFV (%)	NEM (g kg ⁻¹)	NEG (g kg ⁻¹)	NEL (mcal kg ⁻¹)
Treatment (T)							
CTRL	33.0±1.86	626.3±9.09	88.0±7.32	132.3±6.64	1.53±0.03	0.81±0.03	1.42±0.02
AG	34.8±1.86	650.1±9.09	102.3±7.32	147.3±6.64	1.60±0.03	0.88±0.03	1.48±0.02
EN	37.2±1.86	657.1±9.09	104.6±7.32	142.4±6.64	1.62±0.03	0.89±0.03	1.50±0.02
SU	37.1±1.86	634.7±9.09	91.6±7.32	126.9±6.64	1.55±0.03	0.83±0.03	1.44±0.02
UR	31.5±1.86	626.3±9.09	86.5±7.32	124.5±6.64	1.53±0.03	0.81±0.03	1.42±0.02
P values							
T	0.18	0.10	0.32	0.12	0.09	0.09	0.09

The values are means ± standard errors. Each analyses was replicated four times per treatment. CTRL: control, AG: Agrotain, EN: eNtrench, SU: superU, UR: urea, CF: crude fat, TDN: total digestible nutrients, SS: simple sugar, RFV: relative feed value, NEM: net energy for maintenance, NEG: net energy gain, NEL: net energy for lactation.

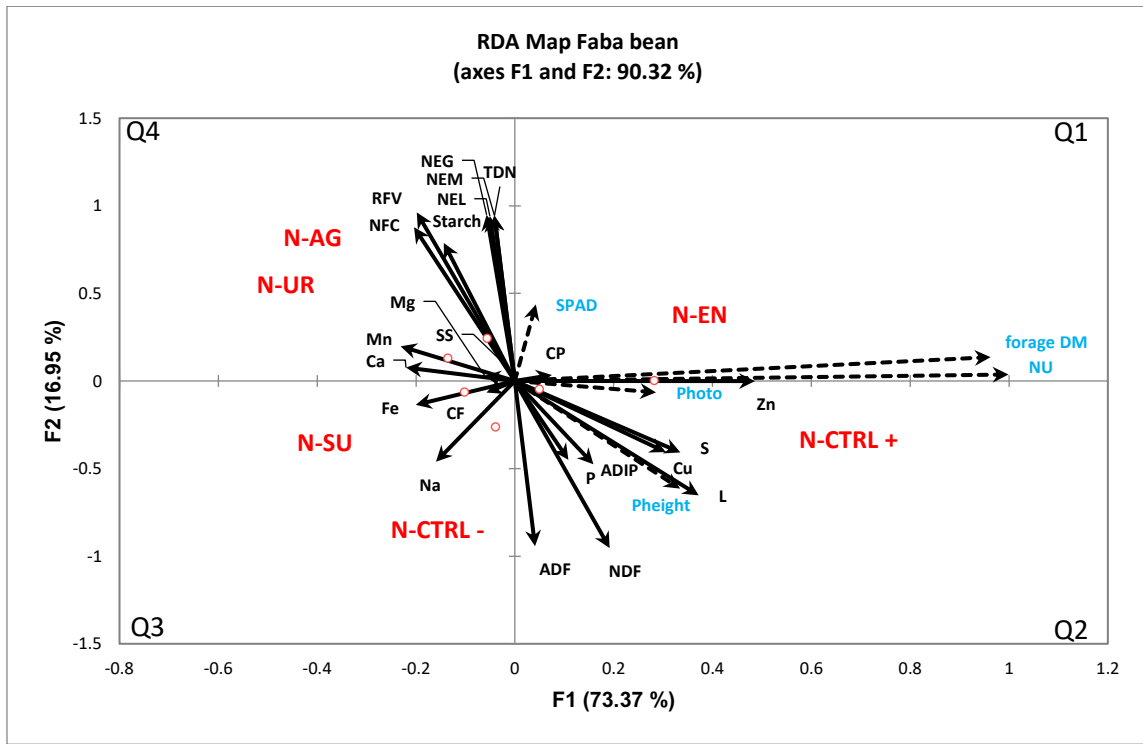
Table 4.10: The effects of urea and N fertilizer stabilisers on the macro and micro-nutrients in oat + peas mixture

Main factors	S (g kg ⁻¹)	Na (g kg ⁻¹)	Ca (g kg ⁻¹)	P (g kg ⁻¹)	Mg (g kg ⁻¹)	K (g kg ⁻¹)	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn(mg kg ⁻¹)	Zn(mg kg ⁻¹)
Treatments (T)										
CTRL	1.25±0.09	0.35±0.04	5.38±0.41	3.28±0.17	2.18±0.09	19.7±0.79	8.29±0.68	411.4±91.96	50.3±5.83	42.1±4.57
AG	1.25±0.09	0.30±0.04	5.33±0.41	3.20±0.17	2.13±0.09	18.1±0.79	7.46±0.68	224.8±91.96	49.5±5.83	40.6±4.57
EN	1.03±0.09	0.30±0.04	4.48±0.41	2.95±0.17	2.05±0.09	18.4±0.79	7.46±0.68	238.2±91.96	41.0±5.83	38.5±4.57
SU	1.08±0.09	0.28±0.04	4.35±0.41	2.93±0.17	1.98±0.09	19.3±0.79	6.79±0.68	261.5±91.96	43.5±5.83	42.0±4.57
UR	1.18±0.09	0.33±0.04	5.10±0.41	3.20±0.17	2.13±0.09	20.6±0.79	7.44±0.68	368.2±91.96	48.4±5.83	40.9±4.57
P values										
T	0.39	0.76	0.29	0.47	0.63	0.21	0.66	0.53	0.75	0.98

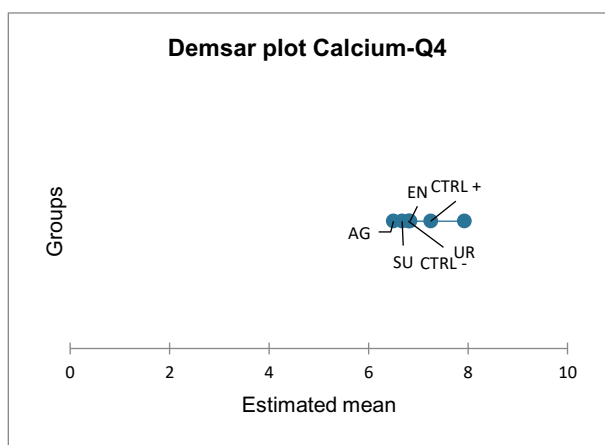
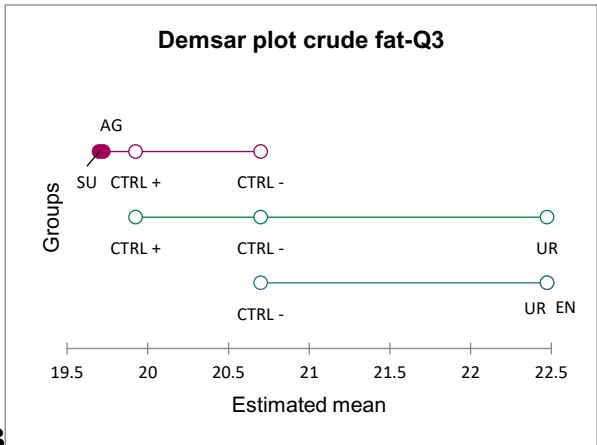
The values are means ± standard errors. Each analyses was replicated four times per treatment. CTRL: control, AG: Agrotain, EN: eNtrench, SU: superU, UR: urea, S: sulphur, Na: sodium, Ca: calcium, P: phosphorus, Mg: magnesium, K: potassium, Cu: copper, Mn: manganese, Fe: iron, Zn: zinc.

4.3.3. Relationship between response variables and treatments.

The growth parameters, forage DM, N uptake, and forage quality indices of faba beans and oat + peas mixture were used as indicators to assess the use of UR and UR treated with N stabilizers (AG, SU, and EN) on podzols in boreal climate (Figure 4.4 and 4.5; Tables 4.2-4.10). I observed some associations between the growth parameters, forage DM, N uptake, the forage quality indices, and the N treatments in faba beans and oat + peas mixture. These associations highly influenced the clustering of response variables and the treatments together, however, well separated in different quadrants of the biplot following RDA (Figure 4.4 and 4.5). From the RDAs it was observed that axis 1 and axis 2 explained 73.37% and 16.95%, respectively of the total variability in growth parameters, forage DM, N uptake and forage quality indices treated with different N sources for faba beans, while axis 1 and axis 2, respectively explained 72.12% and 14.93% of variability in oat + peas (Figure 4.4a and 4.5). For example, in quadrant 2 of the biplot (Figure 4.4a), growth parameters (photosynthesis and plant height), fibres (ADF, NDF, and Lignin), minerals (S, Zn, Cu and P) and ADIP were clustered together and showed a better association with EN treatment (Tables, 4.2, 4.5 and 4.7). While, in quadrant 4 of the biplot (Figure 4.5), photosynthesis in oats, energy (NEM, NEL, NEG), CF, RFV and NFC were clustered together and showed better association with SU and NU treatments (Tables 4.3, 4.8,4.9). In addition, demsar plots were used following the ANOVA outputs, to separate the treatments of statistically significant variables into groups according to their importance. For example, the demsar plot of CF in faba beans separated the treatments into three groups with the least important treatment being SU and AG (Figure 4.4b).

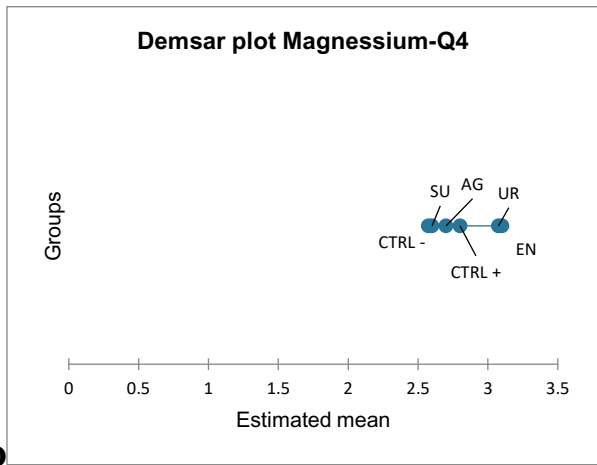


A



B

C



D

Figure 4.4: RDA biplots showing the association between N sources, growth parameters, forage DM and forage quality indices, respectively for faba beans (a), demsar plots showing the grouping of N treatments according to their importance in crude fat (b), calcium (C) and magnesium (D). The active variables are Photo: photosynthesis; SPAD: chlorophyll, PHeight: plant height, forage DM: forage dry matter; NU: nitrogen uptake; CP: crude protein; AP: available protein; ADF: acid detergent fibres; NDF: neutral detergent fibres; NFC: non-fibrous carbohydrates; RFV: relative feed value; TDN: total digestible nutrients; TFA: total fatty acid; C18:2: linoleic acid; C18:3: linolenic acid; Lig: lignin; SS: simple sugars, Mg: magnesium

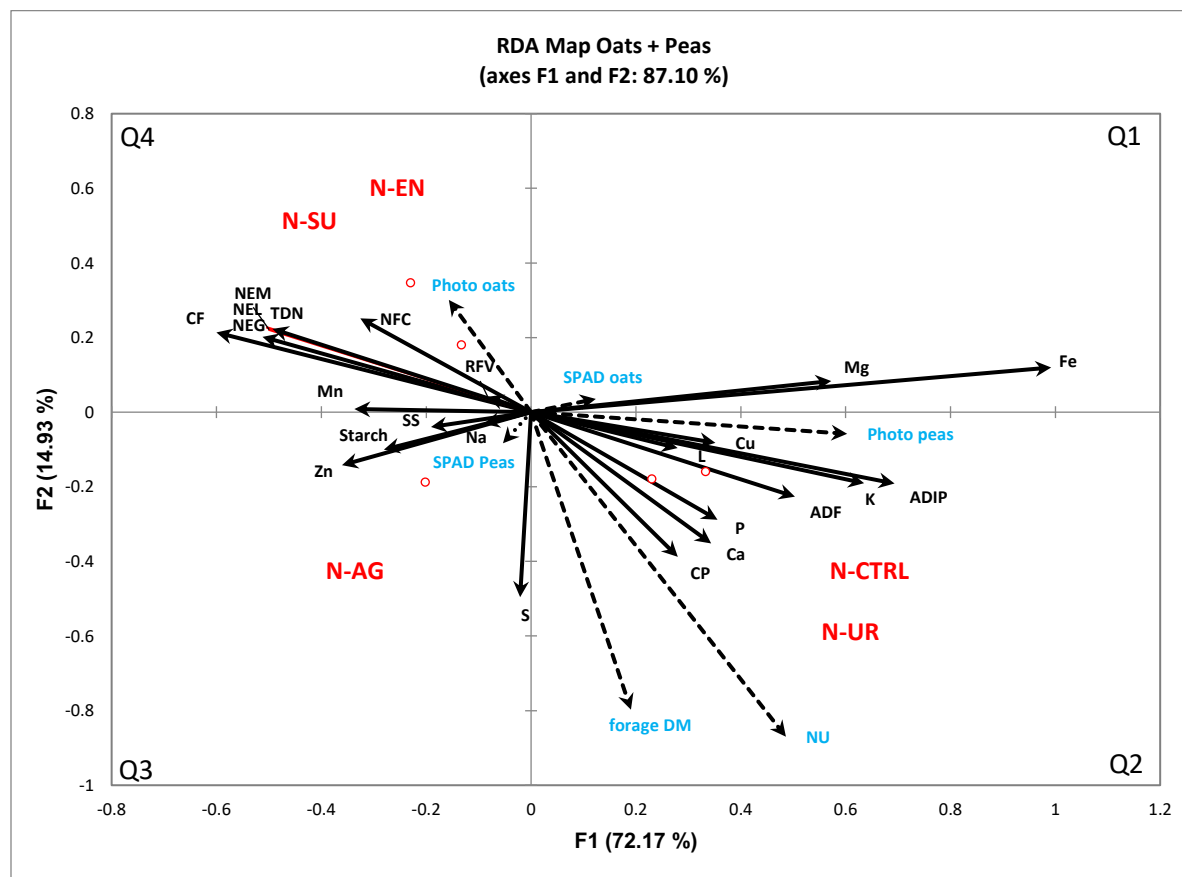


Figure 4.5: RDA biplots showing the association between N sources, growth parameters, forage DM and forage quality indices respectively for oat + peas mixture. The active variables are Photo: photosynthesis; SPAD: chlorophyll, PHeight: plant height, forage DM: forage dry matter; NU: nitrogen uptake; CP: crude protein; AP: available protein; ADF: acid detergent fibres; NDF: neutral detergent fibres; NFC: non-fibrous carbohydrates; RFV: relative feed value; TDN: total digestible nutrients; TFA: total fatty acid; C18:2: linoleic acid; C18:3: linolenic acid; Lig: lignin; SS: simple sugars, Mg: magnesium; Ca: calcium; K: potassium; P: phosphorus; Cu: copper; Zn: zinc; Mn: manganese; Fe: iron; NEG: net energy for growth; NEL: net energy for lactation; NEM: net energy for maintenance.

4.4. Discussion

4.4.1. Growth and forage dry matter of faba bean and oats + peas mixture

N is an important macronutrient for all crops since it promotes the expansion of leaves, which increases sunlight efficiency interception, photosynthesis activity, and overall yield (Cunha et al., 2015; Fageria and Baligar, 2005). Legume crops mostly need a starter dose of N fertilizers to sustain the plants during the early vegetative stage, before BNF, which usually takes place at a later vegetative stage after the development and maturity of the root nodules (Jiang et al., 2020; Meakin et al., 2007). In my study, UR and UR treated with N stabilizers had no significant effects on photosynthesis, chlorophyll, and plant height in the faba beans and oats + peas mixture (Table 4.3). Bernardes et al. (2015) observed that the chlorophyll rate of beans was higher in the CTRL treatment and the lowest in UR + NBPT amended treatments 64 days after seeding. High N mineralization of plant residues from preceding crops can enhance organic N content in untreated plots (Bernardes et al., 2015). This suggests that the lack of significant difference in present study can be due to the left-over plant residues from the corn – faba beans and corn – oats + peas rotation. Some studies have reported that there maybe some level of NH_4^+ toxicity in the legume crops due to an elevated N concentration from the use of UR, or UR amended with N inhibitors such as NBPT; which can impair BNF and the metabolic activities of the legume crops (Cruchaga et al., 2011; Voisin et al., 2002b). However, we did not observe any toxicity symptoms in the present study even though UR treated with N stabilizers were statistically at par with UR in photosynthesis, chlorophyll, and plant height of faba beans and oat + peas mixture (Table 4.3).

Legume crops including faba beans may only require a starter dose of N to avoid the downregulation of BNF (Adak and Kibritci, 2016; Salvagiotti et al., 2009). The efficiency of the starter N dose can be enhanced through the use of Enhanced-efficiency urea-based fertilizers such

as polymer-coated urea (PCU) or NBPT treated UR since these reduce environmental loss of fertilizers through gaseous, leaching, and/or runoff mechanisms and increase fertilizer N uptake when compared to UR (Motavalli et al., 2008). PCU and NBPT have enhanced NRE by reducing gaseous N loss under several cropping systems (Halvorson and Del Grosso, 2012; Jantalia et al., 2012). In the present study, UR alone, UR-coated with UI (AG), NI (EN), a combination of UI and NI (SU), and CTRL produced statistically similar forage yields of faba bean, and oat + peas mixture. The non-significant effects of UR treated with N stabilizers, UR, and CTRL on faba bean and oat + peas mixture biomass could be due to residual N or immobilization because of N application to the previous crops (silage corn – faba bean, and silage corn – oat + peas mixture). Nitrogen management used for silage corn production in the present study, which included a single application of UR treated with N stabilizers as a slow-release fertilizer, may have led to a substantial amount of applied N carried over into the faba bean and oat + peas growing season thereby impacting yield of crops. Our results corroborate the findings of Nash et al. (2012) who observed that 100% PCU and 100% non-coated UR application produced similar yields in double-cropped soybean cultivated in three seasons. Additionally, they also observed that the untreated plots produced high yields like the fertilized ones that parallels with our study in corn – faba beans and corn – oat + peas mixture. In southern Alberta, top dressing of PCU in the spring produced less yield compared to the non-coated urea (NCU) with and without a UI (McKenzie et al., 2010). The lower yields were attributed to excessive delay in N release from the PCU compared to NCU fertilizers. In the present study, also less forage yield of faba bean and oats + peas mixture in UR treated with N stabilizers were recorded as compared to UR, though the differences were non-significance. Additionally, earlier studies reported an increase in winter wheat grain yields with increasing N rate (Halvorson et al., 2004), but yields were unlikely to increase above 120 kg ha⁻¹

of fertilizer N within the soil profile (Olson et al., 1976). Another study conducted by Nelson et al. (2014) reported no significant differences between the PCU, UR + NBPT, ammonium nitrate (AN), and urea ammonium nitrate (UAN) and NCU on the production of wheat inter-seeded with red clover. Salvagiotti et al. (2009) reported that the combined effect of PCU, UR applied as a top dress, and the split application of UR, increased soybean total biomass by 8%, though non significantly as compared to the control. I also didn't observe a significant difference between the inoculated and non-inoculated faba beans treatments though only starter dose of N was applied. The non-significant effects of inoculated treatments on faba beans might be due to the carrying over of residual N in the previous crop applied with 115 kg N ha⁻¹ (Table 4.3).

4.4.2. Nitrogen uptake, nitrogen recovery efficiency, and forage quality

Generally, the BNF in legumes will start about 14 days after planting warranting the need for a starter fertilizer N. However, the response of legume crops to applied N fertilizers and BNF is dependent on factors such as soil temperature, soil moisture, and pH (Osborne and Riedell, 2006). These environmental factors can enhance the hydrolysis of N fertilizers, and consequently N losses. Thus to improve the efficiency of the starter N, polymer coated fertilizers or N inhibitors such as NBPT can be used (Karamanos and Stevenson, 2013). The use of slow release N sources such as PCU, prilled urea, neem-coated urea, and N inhibitors have optimized N uptake and NRE of various crops by reducing the N losses through volatilization, leaching, and runoff (Abalos et al., 2014), leading to greater assimilation and translocation of N by the crops (Kaur et al., 2018; Ren et al., 2017). There are reports that N inhibitors had improved forage quality by enhancing protein content and increasing digestibility via a drop in ADF and NDF concentration in silage corn (Safdarian et al., 2014; Uzun et al., 2020). Bernardes et al. (2015) reported the highest

significant N accumulation in bean plants from PCU. Salvagiotti et al. (2009) reported that PCU and top-dressed UR treatments increased N uptake in soybeans by 10% compared to the CTRL. On the contrary, in the present study, UR and UR treated with N stabilizers had no significant effects on N uptake and NRE in faba beans and oats + peas mixture compared to CTRL (Table 3.4). However, Karamanos and Stevenson (2013) reported that the N uptake and NRE of the 50:50 mixture of smooth brome grass + alfalfa were not significantly affected by PCU, Agrotain treated UR, AN, and UR compared to CTRL. Salvagiotti et al. (2009) also observed that the early and late surface application of AN led to the highest N uptake of soybeans, explaining that since BNF was impaired by the applied fertilizer N, the crops relied on the applied N as a replacement or as trade-off N. Although not significantly, I observed in the present study that the NRE of faba beans and oats + peas were lower, respectively in AG and UR treatments (Figure 4.3), suggesting higher BNF and lower trade-off N or replacement from the applied fertilizer N. Generally, the legume forages do not have a high response to applied N as compared to grass forages (Karamanos and Stevenson, 2013). Voisin et al. (2002a) said that the N nutrition regime of legumes may rely more on the carbon (C) metabolism since the regulatory mechanism is controlled by the C and N fluxes in the phloem, via signaling molecules, either during BNF or N absorption through the roots. Karamanos and Stevenson (2013) reported that the protein concentration of smooth brome grass + alfalfa was not significantly increased by the PCU, Agrotain treated UR, UR, and AN compared to the CTRL. In the present study, the forage quality indices of oats + peas (Table 4.8, 4.9, and 4.10) and that of faba beans (Table 4.6, 4.7, and 4.8) were not significantly increased by UR treated with N stabilizers and UR compared to the CTRL; similar to the findings of (Karamanos and Stevenson, 2013). The unstable availability of N from UR treated with N stabilizers and the possible N loss from the UR treatments may hinder the effectiveness of the N treatments

(Karamanos and Stevenson, 2013). However, I observed that UR treated with N stabilizers, AG and SU, significantly reduced CF compared to UR (Table 4.8), while Ca and Mg were significantly decreased, respectively by the two UR treated N stabilizers AG and SU compared to UR in faba beans (Table 4.7). Some studies observed that an accumulation of NH_4^+ in plant tissues could lead to nutrient imbalances causing essential cations (K^+ , Mg^{2+} and Ca^{2+}) to drop (Britto and Kronzucker, 2002; Chen et al., 2017). The forage quality indices, lignin, CP, Cu, Ca, ADF, P, ADIP, and K, were highly clustered together in the same quadrant suggesting a higher association with the CTRL and UR treatments in oats + peas mixture (Figure 4.5). The inconsistency and nonsignificant difference in the present study are not understood and warrant further investigation to determine the effects of UR treated with N stabilizers on legume crops grown in a boreal climate.

4.5. Conclusion

Nitrogen (N) fertilizer stabilizers have proven to be beneficial in the growth, yield, and N uptake of various crops, since they reduce N losses, thereby making N more available to the crops. In the current study, urea (UR) and UR treated with N stabilizers (Agrotain (AG), eNtrench (EN), superU (SU)) did not significantly increase the growth, forage dry matter (DM), forage quality indices, N uptake and nitrogen recovery efficiency (NRE) of faba beans and oats + peas mixture in boreal climate. A 10% increase in forage DM of faba beans by EN compared to UR was noticed, though not significant. However, a significant decrease of 12% in crude fat (CF) by AG and SU, alongside a significant decrease of 18% and 16% in calcium (Ca) and magnesium (Mg) by AG and SU, respectively compared to UR was observed in faba beans. Additionally, I observed from the demsar plots that SU and AG were the least important treatments in affecting CF, Ca and Mg

of faba beans cultivated on Podzols in the boreal climate. The redundancy analyses (RDA) of oat + peas mixture suggests that the forage DM, N uptake, photosynthesis in peas and forage quality indices (lignin, crude protein (CP), copper (Cu), calcium (Ca), acid detergent fibres (ADF), phosphorus (P), acid detergent insoluble protein (ADIP), and potassium (K)) were highly associated with the CTRL and UR compared to UR treated with N stabilizers. The effect of N stabilizers on most of the measured parameters did not significantly differ from UR, probably because of low soil moisture, high soil mineralization, low pH, and low metabolic activity caused by ammonia toxicity in plant tissues. However, long-term field studies are required to further assess the effect of UR treated with N stabilizers on growth, forage DM, forage quality, and NRE in podzolic soils in boreal regions.

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Co-authorship statement for experiment three

Chapter 5 entitled “the effects of urea and urea treated with nitrogen stabilizers on the growth, grain yield, nitrogen uptake, and nitrogen recovery efficiency of wheat and canola cultivated on podzol in boreal climate” incorporates unpublished material. Therese Audrey Nzwinda Mbite will be primary author for this manuscript, and it will be submitted in the Journal of Field Crop Research. Dr. Mumtaz Cheema will be the corresponding and the last author. The co-authors will be Dr. Lakshman Galagedara, Dr. Raymond Thomas, Dr. Yeukai Katanda, and Dr. Muhammad Nadeem.

Chapter five

The effects of urea and urea treated with nitrogen stabilizers on the growth, grain yield, 1000 grain/seed weight, grain/seed protein concentration, N uptake, and nitrogen recovery efficiency of wheat and canola in a boreal climate

Abstract

Wheat and canola are of great economic importance, but their production is associated with high nitrogen (N) fertilizer use, hence greater N losses. Urea (UR) treated with N stabilizers like urease inhibitors (UI) and nitrification inhibitors (NI) can mitigate N loss by synchronizing N release with crop N uptake. A field experiment was carried to investigate the effect of UR and UI [N-(n-butyl) thiophosphoric triamide (NBPT)] and NIs [nitrapyrin and dicyandiamide (DCD)] on the growth, grain yield, 1000 grain/seed weight, grain/seed protein content (GPC), N uptake and nitrogen recovery efficiency of wheat and canola cultivated on Podzols in boreal climate. Experimental treatments were 1) UR; 2) Split urea (30% starter, 70% side dress); 3) SuperU™ (SU, urea with DCD and NBPT); 4) Agrotain™ (AG, urea with NBPT); 5) eNtrench™ (EN, urea with nitrapyrin); and 6) No-N as control (CTRL). AG significantly increased GPC by 12% compared to UR in canola, while SplitU significantly increased total N uptake in canola by 27% and 30%, respectively compared to SU and EN. No significant difference between UR treated N stabilizers and UR was noted for the grain/seed yield. These findings suggest that AG and SplitU have the potential to enhance GPC and N uptake of canola crops cultivated on Podzols in boreal climate.

5.1. Introduction

Global food security is exerting more pressure on the dynamics of current agricultural practices (Nations, 2015). Not only is more food needed for the growing population, but their consumption patterns and nutritional preferences keep changing. Thus to lessen the impact of food insecurity, more agricultural land is needed to intensify crop production (Bodirsky et al., 2015; Bommarco et al., 2013). Currently, cereal and grain crops such as maize, wheat, and rice are the most cultivated crops worldwide accounting for 12%, 8%, and 8% of the total production (FAO, 2021). In Canada, wheat and canola are widely grown, especially in the Prairie provinces and are considered to be vital for the Canadian economy (Rempel et al., 2014; Xue et al., 2012).

Canola and wheat are both beneficial for human consumption as well as for animal production. Canola is mostly cultivated for its oil content and is considered a good source of mono and polyunsaturated fatty acids; which are healthy for humans (Rempel et al., 2014). The canola meal or press cake can be used as a protein source in animal feed and it is considered to be a good source of vitamins B and E (Rempel et al., 2014). Wheat, on the other hand, is mostly grown for its grains which are a good source of fibres and contains about 13% of protein, beneficial both to humans and animals (Giraldo et al., 2019). Canola and wheat are non-legume crops, thus their production can greatly be limited by the absence of either organic nitrogen (N) or mineral N fertilizers (Grant et al., 2016). N fertilizers are currently one of the most used sources of N for intensive agriculture, mainly because they enhance growth, and boost the yield and grain protein concentrations (GPC) in crops (Grant et al., 2012). Grant et al. (2016) in their study reported that N fertilization led to an increase in the yield and GPC of wheat as well as the seed/oil yield of canola and protein content in the seeds. Brennan (2016) found that N fertilizer will increase canola seed yield and nitrogen recovery efficiency, but the oil content will be reduced. Efreteuei et al. (2016) reported that the

deficiency of N led to a reduction in wheat grain yield. Although N fertilization of wheat and canola is important in an intensive cultivation system, only part of the N fertilizer applied is taken up by the plants while the remainder will get lost through leaching, volatilization, denitrification, and runoff (Cassman et al., 2002; Grant et al., 2012).

N loss mitigation during the production of various crops is essential. Synchronizing fertilizer N release with plant N uptake can increase the photosynthesis activity, chlorophyll content, overall biomass, grain yield, quality, and nitrogen recovery efficiency (NRE) of crops (Abalos et al., 2014; Grant et al., 2012). Nowadays, best management practices (BMPs) such as the use of urease inhibitors UI, N-(n-butyl) thiophosphoric triamide (NBPT), and nitrification inhibitors (NI) such as nitrapyrin, and dicyandiamide (DCD) are being used to mitigate reactive N (Nr) loss in the soil (Amtul et al., 2002). The UI may bind with the active sites of the urease enzyme and block urease activity, thereby reducing urea (UR) hydrolysis and subsequent ammonia (NH₃) loss (Amtul et al., 2002; Sha et al., 2020). A significant reduction in NH₃ (53- 61%) and nitrous oxide (N₂O) (21-28%) emissions with the use of UI was reported by some researchers (Li et al., 2018; Pan et al., 2016; Xia et al., 2017). NI, on the other hand, can bind with the ammonium monooxygenase enzyme to hinder the activity of Nitrosomonas, blocking the first reaction of ammonium (NH₄⁺) oxidation (Akiyama et al., 2010) thus reducing the N leaching losses and enhancing NRE in different cropping systems.

The use of UI and NI has shown beneficial attributes on the growth and yield of wheat and canola in various agro-ecological regions (Andrade et al., 2020; Garrido and López-Bellido, 2001; Gill, 2018; Grant et al., 2011; Zaman et al., 2010). However, little is known about the effect of UR, splitU, UR treated with N stabilizers/inhibitors, and double inhibitors on the growth, yield, N uptake, and NRE of canola and wheat in the boreal climate. Thus, the current study aimed to focus

on the use of UR fertilizer amended with UI (NBPT), NI (nitrapyrin), and the combination of both NBPT and DCD to enhance growth, grain yield, 1000 seed/grain weight, GPC, N uptake and NRE of canola and wheat in a boreal climate. To this effect, I hypothesized that urea treated with N stabilizers will increase the growth parameters, grain yield, 1000 seed/grain weight, GPC, N uptake, and NRE of canola and wheat as compared to conventional urea. To test the hypotheses, a field trial was conducted with the following objectives:

- To investigate the effects of urea and urea treated with N stabilizers on the growth, grain yield, 1000 seed/grain weight and GPC of canola and wheat cultivated on Podzols in the boreal climate.
- To decipher the role of urea and urea treated with N stabilizers on N uptake and NRE of canola and wheat cultivated on Podzols in the boreal climate.

5.2. Materials and Methods

5.2.1. Experimental site and treatments

A field experiment was carried out at (49° 04' 28" N; 57° 33' 23" W), Pasadena, NL, Canada during the 2019 and 2020 growing seasons. The experimental treatments were 1) urea (100% at seeding-UR); 2) split urea (30% at seeding and 70% side dressing); 3) AgrotainTM [(AG; urea coated with NBPT)]; 4) eNtrenchTM [(EN; urea coated with nitrapyrin)]; 5) SuperUTM [(SU; urea coated with (NBPT and DCD)]; and 6) CTRL (no N fertilizer). Silage corn was seeded in 2019 followed by canola and wheat crops grown in 2020 on the same field in rotation. The experiment was laid out in a Randomized Complete Block Design replicated 4 times for a total of 24 experimental units with a plot size of 3 m × 4 m. Before planting in 2019, 10 soil core samples were collected in a zigzag manner from each block at a depth of 0 – 15 cm using an auger and

composited a one sample per block to determine the basic soil properties and texture of the experimental site (Table 5.1). The samples were analyzed (Soil & Plant Laboratory, Dept. of Fisheries and Land Resources, St John's, NL) using the hydrometer method given by Bouyoucos (1962), which described the soil texture to be sandy loam for blocks 1, 2, and 3 and loamy sand for block 4. The pH was determined using samples prepared as 50/50 DI water and analyte; and was reported using a Fisher Scientific XL250 Benchtop Meter and pH Electrode. Organic matter (OM) was obtained after samples were dried overnight at 102°C and then heated in a Muffle furnace at 430°C. Cation exchange capacity (CEC) was calculated using software onsite. Nutrients were extracted from soil samples using Mehlich III (Carter and Gregorich, 2003) and analyzed using a Teledyne Instrument Prodigy High Dispersion ICP as describe in appendix two.

Table 5.1: Basic soil properties of the experimental site

pH	OM	CEC	P	K	Ca	Mg	S	Cu	Na	Al
	%	cmol kg⁻¹	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
6.40	3.97	12.15	378.00	126.75	1.28	186.25	19.75	2.93	15.50	1.44

OM: Organic matter, P: Phosphorous, K: Potassium, Ca: Calcium, Mg: Magnesium, S: Sulphur, Cu: Copper, Na: Sodium, Al: Aluminium, CEC: Cation Exchange Capacity.

Before planting, the herbicides Credit[®] Xtreme (active ingredient Glyphosate) and Eragon[®] LQ were mixed alongside a blend of surfactant with petroleum hydrocarbons (Merge[®] Adjuvant) and were applied at 2.2 L ha⁻¹, 146 ml ha⁻¹, and 1L ha⁻¹, respectively to control weeds. On June 11th, 2020, canola cv. Invigor L234PC and wheat cv. AC Scotia were planted at a seed rate of 5.6 kg ha⁻¹ and 190.5 kg ha⁻¹, respectively using a tractor-mounted plot seeder (Wintersteiger XL). UR was applied either at seeding or in split application, whereas UR treated with N stabilizers were manually broadcasted at seeding at 100 kg N ha⁻¹. The phosphorus and potassium fertilizers were

applied at 0 kg P₂O₅ ha⁻¹ and 60 kg K₂O ha⁻¹, respectively at seeding following the soil test report and regional recommendations. Canola was harvested when 50% of pods turned brown and the wheat was harvested at physiological maturity on September 22 and October 1, 2020, respectively.

5.2.2. Growth Parameters

5.2.2.1. Chlorophyll

The chlorophyll content was measured at the respective flowering stage of each crop using a SPAD 502 plus chlorophyll meter (Spectrum Technologies, IL, USA). Ten wheat plants were randomly selected from each plot in all four blocks and one reading was taken at the center of the flag leaf of each plant (Tosti and Guiducci, 2010). While five canola plants were randomly selected at 50% flowering, from each plot in all four blocks, and one reading was taken from the most fully expanded leaf from the top of the plant (Men et al., 2020; Naderi et al., 2012; Seepaul et al., 2016).

5.2.2.2. Photosynthesis

The photosynthesis rate was measured at the respective flowering stage for each crop on a sunny day using a portable photosynthesis system (LI-6400XT) (LI-COR Biosciences, NE, USA). Five wheat plants were randomly selected within each plot in all four blocks and one photosynthesis measurement was taken at the center of the flag leaf of each of the ten plants (Gaju et al., 2016). While five canola plants were randomly selected at 50% flowering, from each plot in all four blocks, and one photosynthesis measurement was taken from the most fully expanded leaf from the top of each plant (Men et al., 2020; Naderi et al., 2012; Seepaul et al., 2016). Before taking measurements, the photosynthesis system was set up at a photon flux density of 1500 $\mu\text{molm}^{-2}\text{s}^{-1}$, leaf temperature 25°C, pump flow rate of 400 $\mu\text{mol s}^{-1}$, and 400 ppm CO₂ concentration.

5.2.2.3. *Plant height*

Ten wheat and canola plants were randomly selected before harvest and the plant height was measured using a measuring rod from the base of the plant to the top (Figure 5.1) (Sani, 2014; Todd and Spaner, 2003). The mean of the ten plants was used as the final plant height per plot in all four blocks.



Figure 5.1: Plant height measurements in wheat (a) and canola (b) before harvest.

5.2.2.4. *1000 seeds/grain weight and grain yield measurements*

Canola plants were swathed using grass shears from two quadrants (0.5m) within each plot in all four blocks on September 22, 2020, and allowed to dry down in the field (Vera et al., 2007). The plants were swathed when the seeds in the pods started changing colour from green to yellow or light brown (Smith, 2010). Whole plant samples were allowed to dry in the field after swathing and collected after three weeks (October 13th, 2020), when the seed colour had completely changed to dark brown or black. The wheat plants were manually harvested five centimeters above the

ground from two quadrants at physiological maturity (October 1, 2020). Canola and wheat plant samples were oven dried at 65°C for 72 h; thereafter the samples were manually threshed to obtain the grains. Grain yield (kg) per plot was scaled to Mg ha⁻¹ following (Equation 1), while 1000 seeds were counted and weighed using weigh balance to determine the 1000 seed weight (g). The plant tissues of both canola and wheat crops were ground with a Wiley mill (A.H. Thomas Co., Philadelphia, PA, USA) and sieved using a 4 mm mesh screen. About five grams of these ground samples were further ground alongside a five-gram sub-sample of canola and wheat grains using a Cryomill (MM 400; Retsch Inc., Newton, PA) and sent to Prince Edward Island Analytical Laboratories (PEIAL, Bioscom Park Charlottetown, Canada) to determine N uptake in plant tissues and grains.

$$\text{Grain yield} = (\text{Grain yield}(kg))/1000) \times 10000/\text{Harvest area} \quad (1)$$

5.2.3. Nitrogen uptake and recovery efficiency

The N uptake was determined based on the N concentration in the grains and the plant tissues of canola and wheat. N uptake in the whole plant was calculated by adding the N in seeds/grains and N in plant tissues of both crops (Lasisi et al., 2022). The NRE was calculated using (Equation 2) (Kaur et al., 2018); while the GPC was determined using (Equation 3).

$$NRE = \frac{N \text{ uptake (+N)} - N \text{ uptake (CTRL)}}{\text{Amount of N applied}} \times 100 \quad (2)$$

$$GPC = \%N \text{ in grain} \times 6.25 \text{ for canola and } 5.7 \text{ for wheat} \quad (3)$$

Where:

NRE = Nitrogen recovery efficiency

N uptake (+N) = N uptake from fertilized treatments

N uptake (CTRL) = N uptake from control (no N) treatments

5.2.4. Statistical Analyses

A one-way ANOVA technique in XLSTAT (XLSTAT Premium, 2018.1.1, NY, USA) was performed to assess the effects of UR and UR treated with N stabilizers on the growth, yield, and NRE of canola and wheat. Treatment means were compared using Fisher's least significant difference (LSD) at $\alpha = 0.05$. Redundance analyses (RDA) was performed to visualize the relationships between treatments and the response variables in XLSTAT.

5.3. Results

5.3.1. Growth, grain yield, and 1000 seed weight of wheat and canola

The ANOVA results revealed that UR and UR treated with N stabilizers had no significant ($P > 0.05$) effects on the photosynthesis rate of canola and wheat (Table 5.2). UR and UR treated with N stabilizers had significant ($P < 0.05$) effects on the chlorophyll content, plant height, and grain yield of canola and wheat. However, there were no significant ($P > 0.05$) effects of UR and UR treated with N stabilizers on 1000 seed/grain weights of canola and wheat (Table 5.3).

The AG treatment showed higher chlorophyll content (45.6) compared and the lowest (38.96) in the CTRL treatment in canola. Whereas UR, split urea and all UR treated N stabilizers were statistically at par with each other and produced similar chlorophyll content in canola (Table 5.2). In wheat, significantly higher chlorophyll content (42) was observed with EN application, though statistically at par with other UR treated N stabilizers, UR and splitU. The lowest chlorophyll content (31.9) was recorded in CTRL. Similarly, AG (N stabilizer) produced significantly higher plant height (1.11m) than (0.90 m) in CTRL treatment, which was the lowest, in wheat. UR, splitU,

and SU were statistically at par with AG and produced similar plant heights in wheat (Table 5.2). The splitU application produced a significantly higher seed yield (2.69 Mg ha^{-1}) of canola, though statistically at par with UR and UR treated with N stabilizers. Whereas the lowest (1.63 Mg ha^{-1}) grain yield was observed in CTRL. In wheat, a higher grain yield (3.93 Mg ha^{-1}) was recorded with UR application and the lowest (2.10 Mg ha^{-1}) in CTRL. The AG, EN, SU, and splitU applications produced 3.68, 3.30, 3.68, and 3.59 Mg ha^{-1} of wheat grain yield, which was statistically similar to the grain yield from UR application (Table 5.3).

Table 5.2: The effects of urea and urea treated with N stabilizers on photosynthesis, chlorophyll, and plant height of wheat and canola

Main factors	Photosynthesis ($\mu\text{mol m}^{-2} \text{sec}^{-1}$)		Chlorophyll (SPAD values)		Plant height (m)	
	Canola	Wheat	Canola	Wheat	Canola	Wheat
Treatment (T)						
CTRL	22.9±2.31	16.2±2.23	38.9±1.10b	31.9±1.27b	1.06±0.04	0.90±0.03b
AG	26.7±2.31	21.8±2.23	45.6±1.10a	41.8±1.27a	1.21±0.04	1.11±0.03a
EN	29.8±2.31	16.6±2.23	45.2±1.10a	42.2±1.27a	1.21±0.04	-
SU	26.5±2.31	17.4±2.23	44.8±1.10a	41.7±1.27a	1.17±0.04	1.10±0.03a
SplitU	26.3±2.31	20.8±2.23	43.6±1.10ab	40.1±1.27a	1.19±0.04	1.02±0.03ab
UR	27.3±2.31	18.6±2.23	42.2±1.10ab	40.3±1.27a	1.13±0.04	1.06±0.03a
P-value						
T	0.50	0.42	0.01	0.00	0.12	0.00

Different letters in the columns indicate significant difference between treatments ($P < 0.05$) and the values are means \pm standard errors. Each measured parameter was replicated four times per treatment. CTRL: control, SplitU: split urea application, AG: Agrotain, EN: eNtrench, SU: superU, UR: urea.

Table 5.3: The effects of urea and urea treated with N stabilizers on the grain yield and 1000 seed weight of wheat and canola.

Main factors	Seed/Grain yield (Mg ha ⁻¹)		1000 Seed/Grain weight (g)	
	Canola	Wheat	Canola	Wheat
Treatment (T)				
CTRL	1.63±0.14b	2.10±0.27b	2.65±0.10	36.9±2.26
AG	2.41±0.14a	3.68±0.27a	2.93±0.11	41.3±2.26
EN	2.28±0.14ab	3.30±0.27ab	2.90±0.10	44.7±2.26
SU	2.41±0.14a	3.68±0.27a	2.85±0.10	44.7±2.26
SplitU	2.69±0.14a	3.59±0.27a	2.93± 0.10	39.9±2.26
UR	2.34±0.14a	3.93±0.27a	2.63±0.10	41.7±2.26
P-value				
T	0.00	0.00	0.17	0.18

Different letters in the columns indicate significant difference between treatments ($P < 0.05$) and the values are means \pm standard errors. Each measured parameter was replicated four times per treatment. CTRL: control, SplitU: split urea application, AG: Agrotain, EN: eNtrench, SU: superU, UR: urea.

Table 5.4: The effects of urea and urea treated with N stabilizers on the N uptake and grain protein concentration of Canola and wheat

Main Factor	N uptake in canola (kg N ha ⁻¹)		N uptake in wheat (kg N ha ⁻¹)		Seed/Grain protein content	
	Seed	Total uptake	Grain	Total uptake	Canola	Wheat
Treatment (T)						
CTRL	44.2±4.75c	77.2±9.88c	40.7±5.78b	61.1±8.27b	17.0±0.59c	10.7±0.39b
AG	76.6±4.75ab	139.4±9.88ab	78.9±5.78a	119.1±8.27a	19.8±0.59ab	12.2±0.39ab
EN	66.0±4.75abc	118.2±9.88bc	75.6±5.78a	115.5±8.27a	18.2±0.59abc	13.1±0.39a
SU	72.8±4.75ab	123.9±9.88b	83.1±5.78a	126.6±8.27a	18.9±0.52abc	12.9±0.39a
SplitU	86.6±4.75a	169.6±9.88a	84.5±5.78a	114.8±8.27a	20.1±0.52a	13.4±0.39a
UR	65.3±4.75bc	116.8±9.88bc	80.3±5.78a	111.2±8.27a	17.4±0.59bc	11.7±0.39ab
P-value						
T	0.00	0.00	0.00	0.00	0.01	0.00

Different letters in the columns indicate significant difference between treatments ($P < 0.05$) and the values are means \pm standard errors. Each measured parameter was replicated four times per treatment. CTRL: control, SplitU: split urea application, AG: Agrotain, EN: eNtrench, SU: superU, UR: urea.

A)

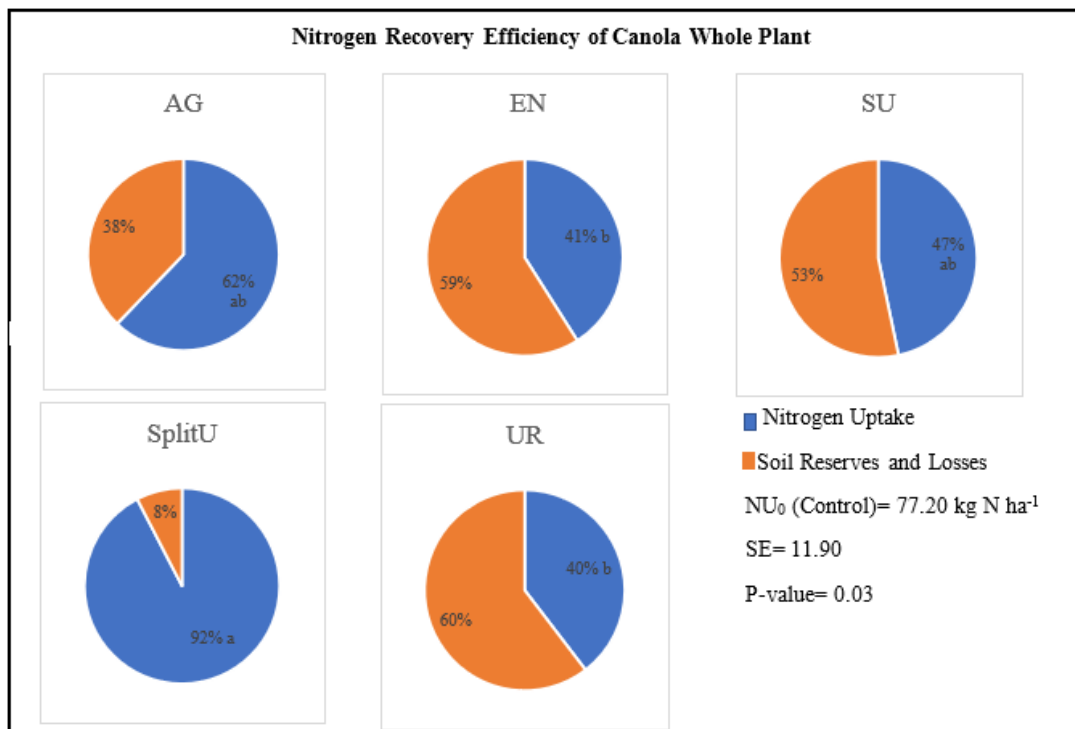
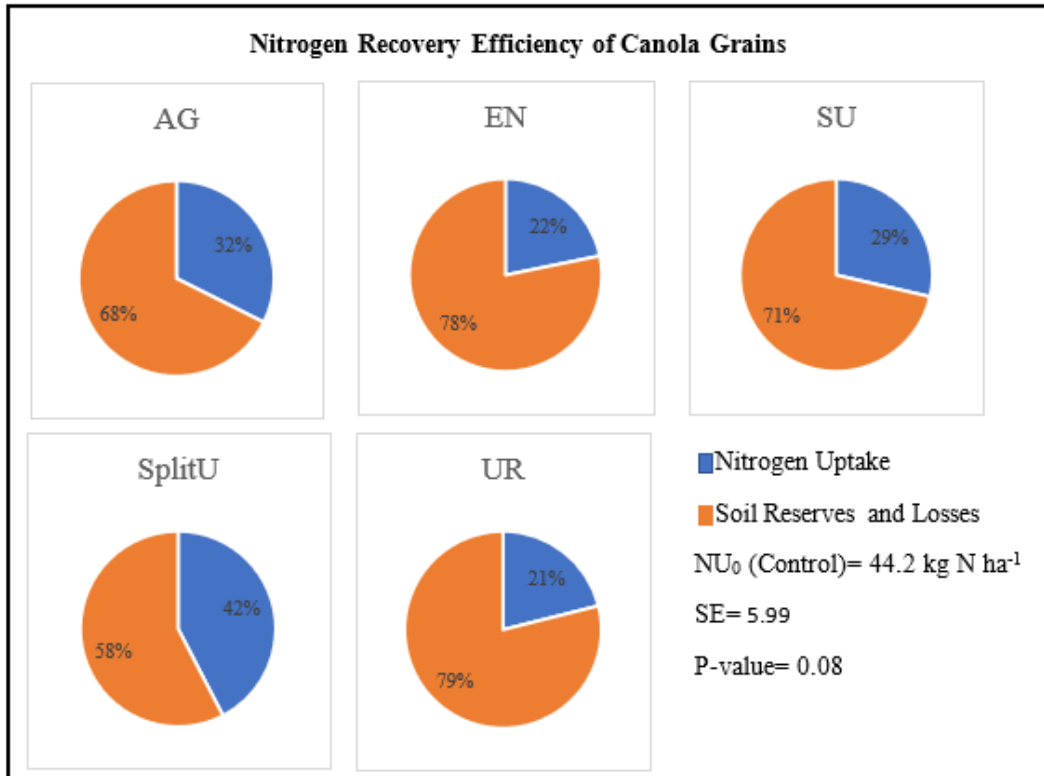


Figure 5.2: Effects of Urea and urea treated with Nitrogen stabilizers on Nitrogen Recovery Efficiency (NRE) of canola A) seeds and B) whole plants.

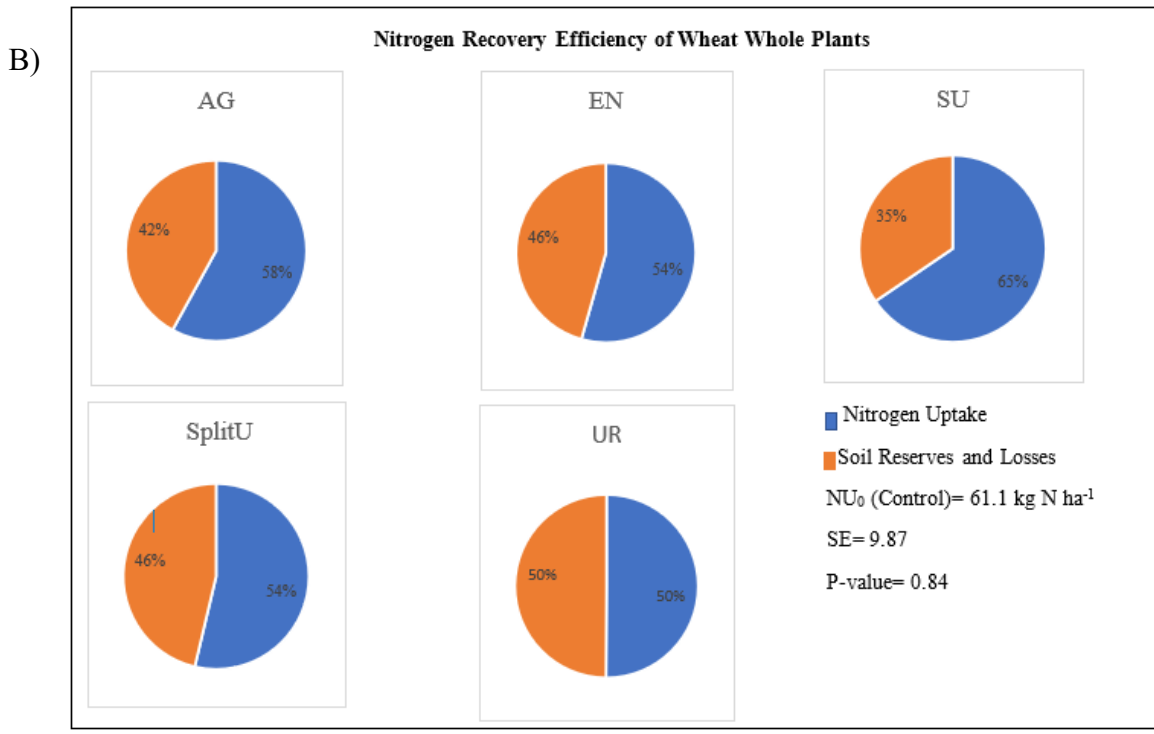
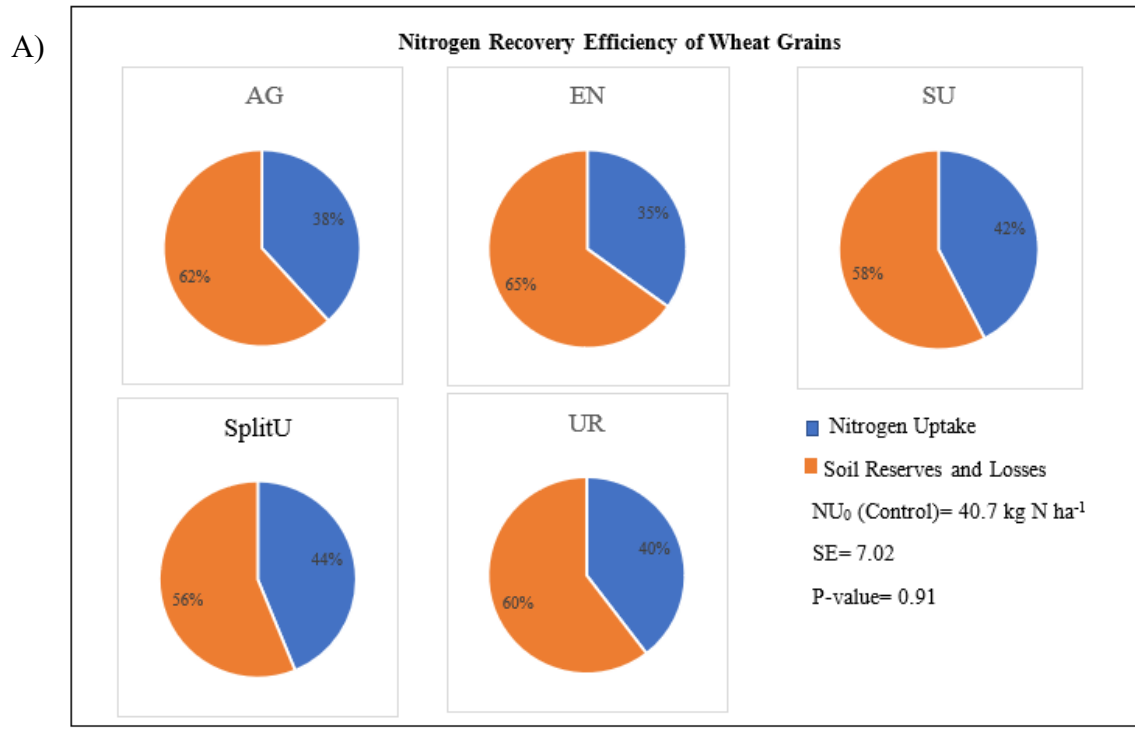


Figure 5.3: Effects of Urea and urea treated with nitrogen stabilizers on Nitrogen Recovery Efficiency (NRE) of wheat a) grains and b) whole plants.

There was a significant difference ($P < 0.05$) between UR and UR treated with N stabilizers on the N uptake in seeds/grains, whole plants, and the NRE of canola and wheat plants compared to CTRL (Table 5.4 and figure 5.2). The splitU treatment showed significantly higher N uptake (86.6 kg ha^{-1}) and (169 kg ha^{-1}) in seeds and whole plant of canola, respectively, though statistically at par with AG and SU. N uptake by seeds (44.2 kg ha^{-1}) and whole plants (77.2 kg ha^{-1}) was the lowest in CTRL (Table 5.4). In wheat, higher N uptake in grain was observed in SplitU application, whereas higher N uptake in the whole plant was recorded in SU though statistically at par with other UR treated with N stabilizers and UR application. The lowest N uptake in grains and the whole wheat plant was recorded in CTRL (Table 5.4). There was greater NRE in splitU (92%) compared to UR (36%) and EN (37%) (Figure 5.2 and 5.3), whereas UR treated with N stabilizers were statistically at par with UR and produced similar NRE in canola whole plant (Figure 5.2 and 5.3). The SplitU and UR treated with N stabilizers treatments significantly ($P < 0.05$) increased seed/GPC in canola and wheat compared to CTRL (Table 5.4). The SplitU produced the highest GPC of 20.1% and 13.4% compared to the lowest (17.0%, 10.7%) in CTRL in canola and wheat, respectively. The EN, SU, and UR produced statistically similar seed protein content (GPC) in canola, whereas AG and SplitU produced significantly greater seed protein content (GPC) than UR (Table 5.4). The SplitU and UR treated with N stabilizers were statistically at par with UR and produced similar GPC in wheat (Table 5.4).

5.3.2. Relationship between response variables and treatments.

The growth parameters, grain yield, N uptake, and seed/GPC in canola and wheat were used as indicators to assess the use of UR and UR treated with N stabilizers (AG, SU, and EN) on Podzols in the boreal climate (Figure 5.4a and 5.5a; Tables 5.2-5.4). Several significant

associations were observed between the growth parameters, grain yield, N uptake, GPC, and the N treatments. These associations highly influenced the clustering of response variables and the treatments together, however, well separated in different quadrants of the biplot following RDA (Figure 5.4a and 5.5b). Following the RDA, it was observed that Axis 1 and Axis 2 explained 94.53% and 2.10%, respectively of the total variability in growth parameters, grain yield, N uptake and GPC in wheat applied with different N sources (Figure 5.5a). Axis 1 and Axis 2 explained 96.54% and 1.78% of total variability in canola (Figure 5.4a). For example, in quadrant 1 for the biplot, seed yield and total N uptake in canola were clustered together and showed better association with SplitU treatment (Table 5.3, 5.4). I also observed 1000 grain weight, total N uptake, GPC, and chlorophyll, clustered together in Q1 and showed better association with SU and EN in the biplot of wheat (Figure 5.5a) (Tables 5.2, 5.3, 5.4). In addition, demsar plots were used following the ANOVA outputs, to separate the treatments of statistically significant variables into groups according to their importance. For example, the demsar plot of seed PC in canola separated the treatments into three groups with the least important treatment being CTRL and the most important being SplitU (Figure 5.4b). While the demsar plot of the grain yield in wheat separated the treatments into two groups with the least important being CTRL and the most important being UR (Figure 5.5b).

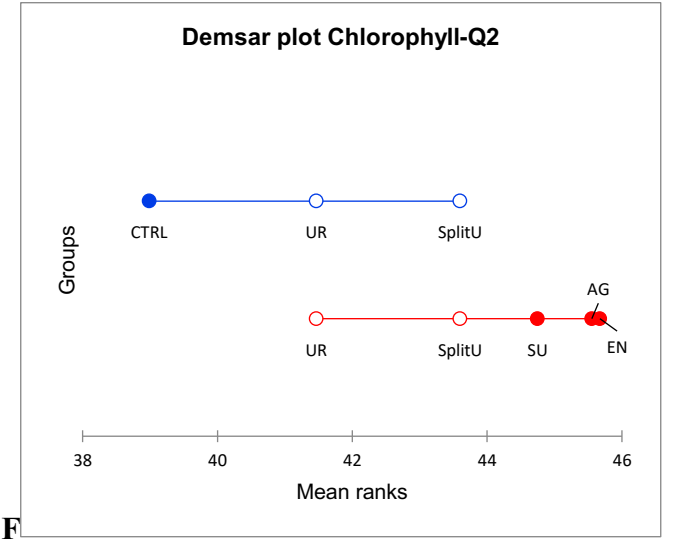
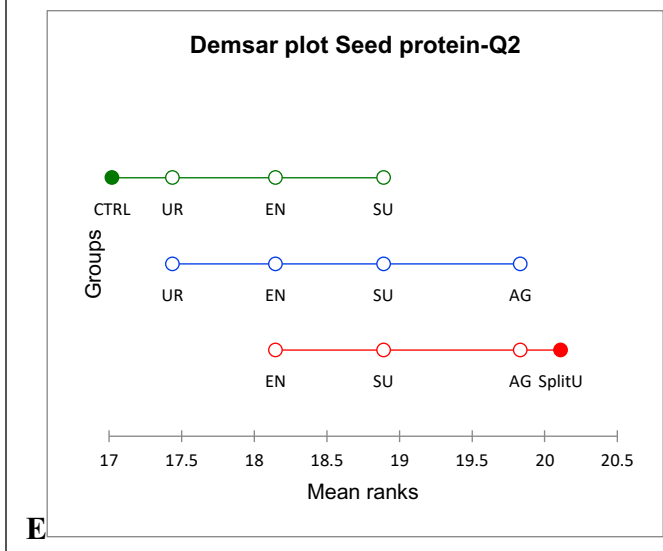
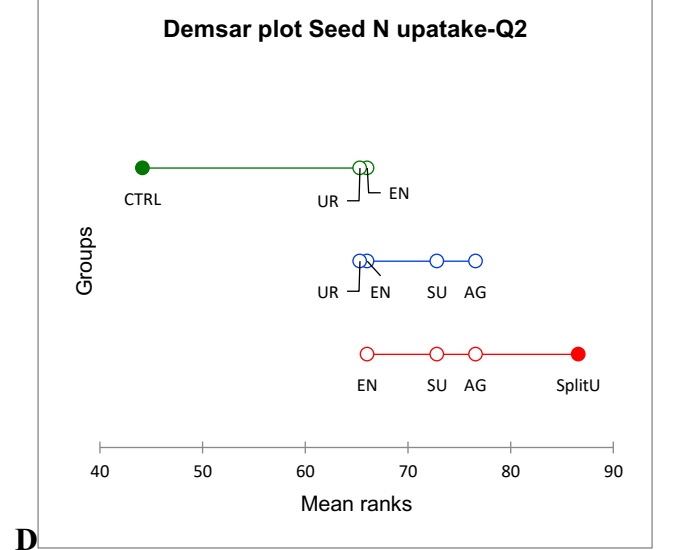
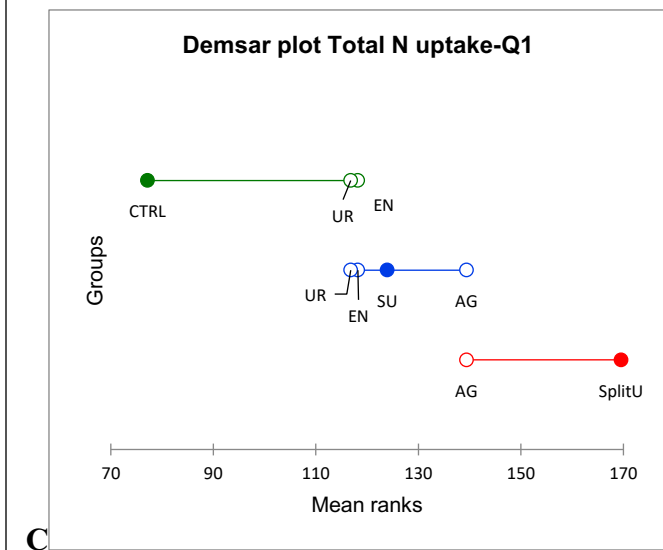
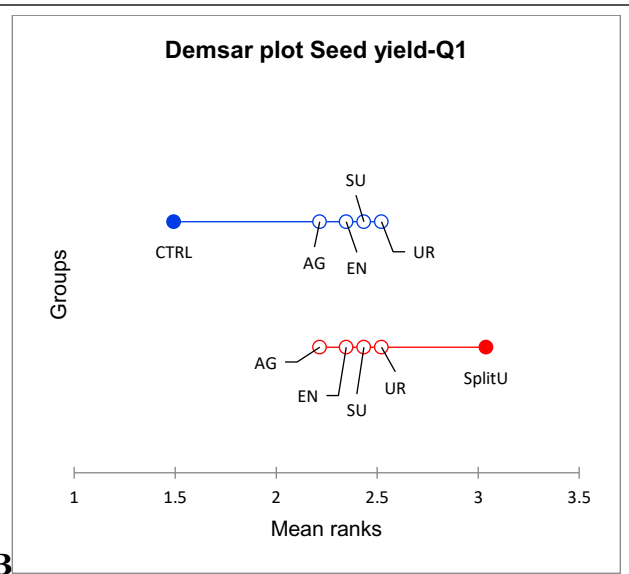
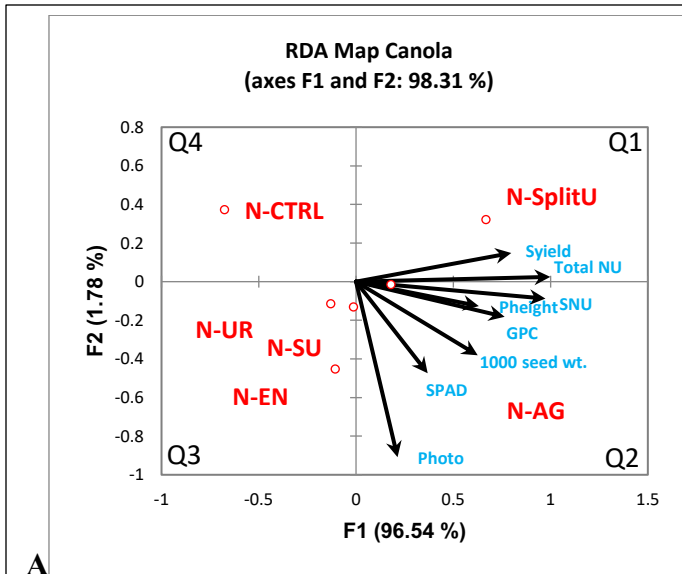
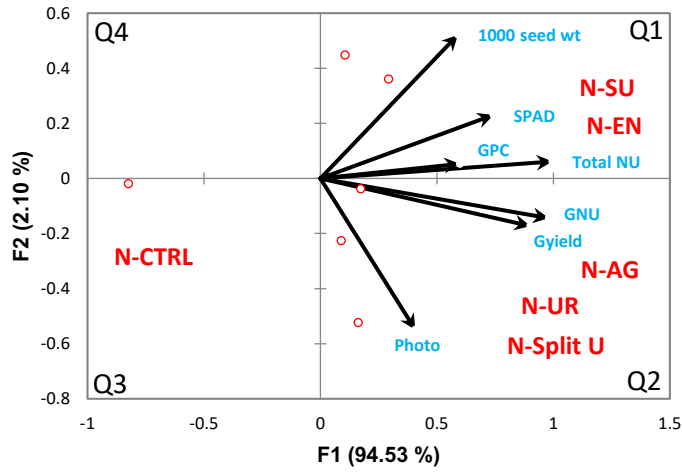
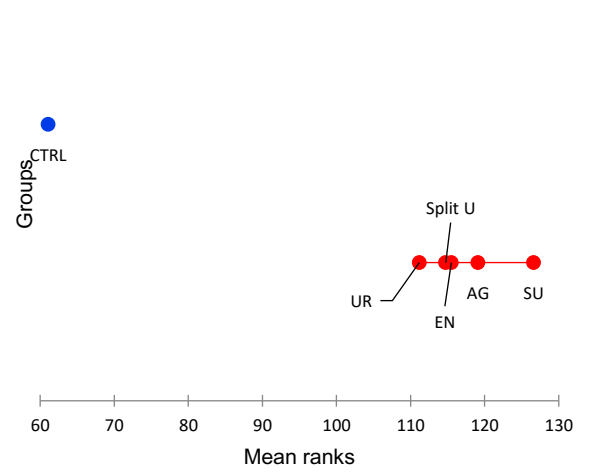


Figure 5.4: RDA biplots showing the association between N sources, growth parameters, seed yield, 1000 seed weight, protein content and N uptake of canola (a), demsar plots showing the grouping of N treatments according to their importance in seed yield (b), total N uptake (c), seed N uptake (d), seed protein (e), and chlorophyll (f). The active variables are Photo: photosynthesis; SPAD: chlorophyll, PHeight: plant height, Syield: seed yield; SNU: seed nitrogen uptake, Total NU: total nitrogen uptake, 1000 seed wt: 1000 seed weight.

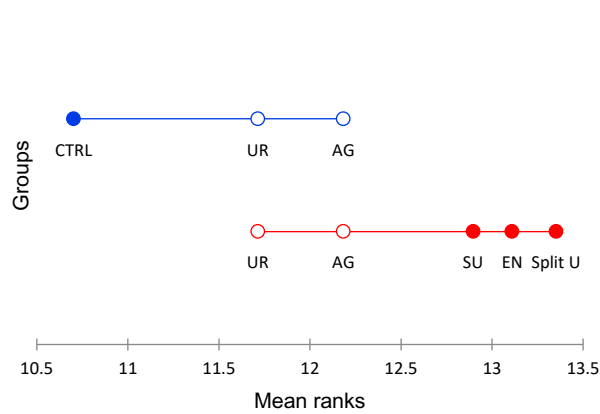
RDA Map Wheat
(axes F1 and F2: 96.64 %)



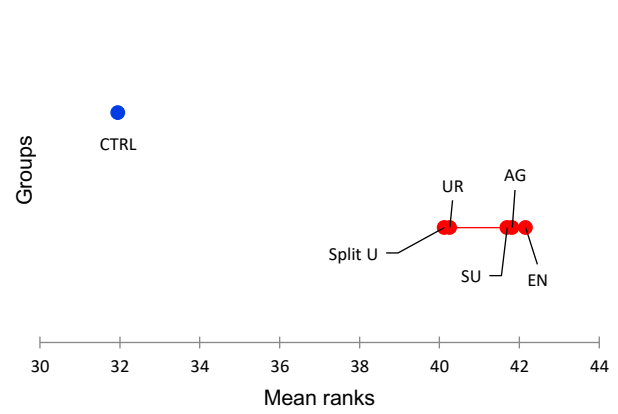
Demsar plot Total N uptake-Q1



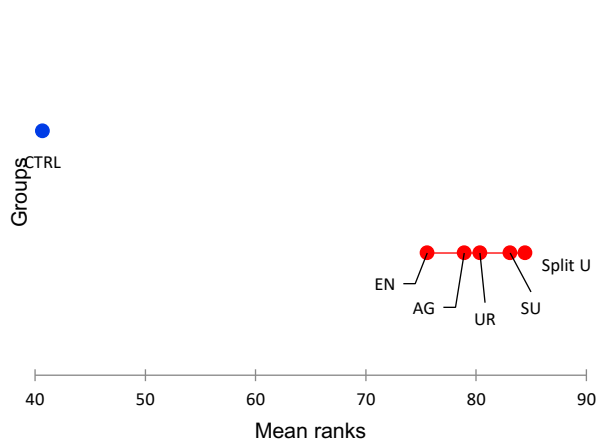
Demsar plot Grain protein-Q1



Demsar plot Chlorophyll-Q1



Demsar plot Grain N uptake-Q2



Demsar plot Grain Yield-Q2

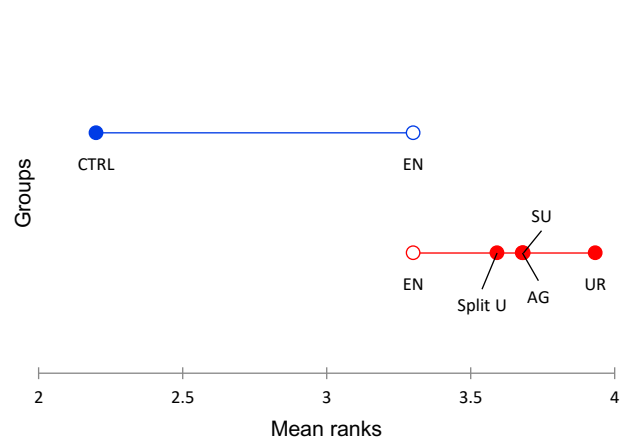


Figure 5.5: RDA biplots showing the association between N sources, growth parameters, grain yield, 1000 seed weight, protein content and N uptake of wheat (a), demsar plots showing the grouping of N treatments according to their importance in total N uptake (b), grain protein content (c), chlorophyll (d), grain N uptake (e), and grain yield (f). The active variables are Photo: photosynthesis; SPAD: chlorophyll, PHeight: plant height, Gyeild: grain yield; GNU: grain nitrogen uptake, Total NU: total nitrogen uptake, 1000 seed wt: 1000 seed weight.

5.4. Discussion

5.4.1. Growth of Canola and Wheat

Chlorophyll content in plants is very sensitive to changes in N fertilizer application as N is a major constituent of chlorophyll in plants; thus the chlorophyll pigments will increase as N fertilizer increases (Tranavičienė et al., 2007). Grant et al. (2011) observed higher chlorophyll content in urea ammonium nitrate (UAN) and UAN coated with NBPT (UI) in canola plants compared to the CTRL. Giannoulis et al. (2020) also observed higher chlorophyll content in cotton crops fertilized with Agrotain (UR coated with NBPT), whereas lower chlorophyll was noted in the control. In the current study, AG (UR coated with NBPT) produced significantly higher chlorophyll content in canola than the CTRL, though statistically at par with other UR treated with N stabilizers. Differences in the chlorophyll content with UR, SplitU, and CTRL were non-significant. Higher chlorophyll content in AG treatment can be attributed to the presence of NBPT which strongly blocks three active sites of the urease enzyme. Thus, forming a bond of tridentate nature, with two nickel centers and one oxygen from the carbamate bridge linking both metals. This will reduce the probability of UR to reach the nickel atom and increase its efficiency in delaying UR hydrolysis (Manunza et al., 1999). In wheat, UR treated with N stabilizers, UR, and

SplitU produced higher chlorophyll content compared to the CTRL (Table 5.2). In my study, all UR treated with N stabilizers and UR applications produced significantly higher plant height in wheat compared to the CTRL, whereas UR treated with N stabilizers and UR had no significant effects on the plant height of canola (Table 4.2). UR treated with N stabilizers such as AG, EN, and SU did not significantly increase plant height in wheat as compared to UR (Table 4.2). No doubt, UR treated with N stabilizers/inhibitors decreased N loss, it does not guarantee the increased uptake of N by the plants since the N may get immobilized in the soil and eventually get lost through leaching or volatilization (Ashraf et al., 2022).

5.4.2. Grain yield, N uptake, NRE (nitrogen recovery efficiency), and protein content of canola and wheat

N is an important macro nutrient and N fertilizers application plays a significant role in the growth and yield of canola and wheat crops (Brennan, 2016; Efreteui et al., 2016; Grant et al., 2012; Wallace et al., 2020). However, N losses associated with N fertilizers application reduce growth, yield, N uptake, and NRE of different crops, enhancing the cost of production and environmental pollution. Different studies have reported the benefits of N inhibitors in enhancing crop yield, N uptake, and NRE in various agro-ecosystems (Abalos et al., 2014). In the present study, there was no significant effect of UR treated with N stabilizers (AG, EN, SU), UR, and SplitU on grain yield, N uptake, and NRE of canola and wheat (Table 4.3). Though UI (NBPT) plus NI (nitrapyrin and DMPP) (SU - double inhibitor) has the potential of simultaneously reducing NH_3 volatilization, N_2O emission, dinitrogen gas (N_2) emission, and nitrate (NO_3^-) leaching. Despite the reduction of N losses from UR by NBPT and NIs, the conserved N didn't

consistently increase crop yield and/or the NRE, particularly in small grains with relatively low N requirements (Lasisi et al., 2020; McKenzie et al., 2010; Mohammed et al., 2016; Tao et al., 2021; Thilakarathna et al., 2020). Other studies reported that crops that require large amounts of N such as corn exhibited higher yield responses to NBPT and NIs application (Drury et al., 2017; Liu et al., 2019; Martins et al., 2017). A recent study conducted by Lasisi et al. (2020) on the Canadian prairie, in Manitoba, reported that the lack of significant effect of inhibitors/stabilizers compared with UR and SplitU on grain yield and N uptake in wheat and canola may be because the residual N plus soil mineralization supplied sufficient N to optimize grain yield, thereby masking any agronomic benefit from the use of inhibitors or double inhibitors usage. The results observed in the current study also demonstrated no significant effects of UR treated with N stabilizers/inhibitors, UR, and SplitU application on the yield of canola and wheat probably due to residual N plus soil mineralization supplied enough N to optimize grain/seed yield of wheat and canola. Specifically, I observed inconsistency of UR treated with N stabilizers/inhibitors on wheat grain yield vis-à-vis UR application and that needs further investigation. The current results are also in agreement with those reported by Lasisi et al. (2020) who observed the use of UR treated with N stabilizers/inhibitors provided yield benefit for fall-applied UR but not for spring-applied UR. While inhibitors may not always provide yield benefits to farmers, these are environmentally friendly, and allow flexibility in farm operations, as UR treated with N stabilizers/inhibitors are used at seeding, hence reducing the cost of split application or top dressing.

Additionally, soil factors; particularly soil type, can have a major influence on the persistence and effectiveness of NIs. For example, certain NIs may persist in the soil for a long time but are not bioactive because they are adsorbed on the soil colloids. The adsorption of other NIs, such as DCD, 3,4-Dimethylpyrazole phosphate (DMPP), and nitrapyrin, to soil organic matter, has been

recognized as an important factor influencing inhibitory efficacy (Fisk et al., 2015; Shi et al., 2016; Yan et al., 2012). In the current study, SplitU application significantly increased N uptake in canola and wheat though statistically at par with SU and AG and the lowest N uptake was recorded in CTRL (Table 5.4). The lack of significant effects of UR treated with N stabilizers/inhibitors compared with UR and SplitU on N uptake and NRE could be due to multiple factors, such as soil type, pH, organic matter, and biodegradation of NBPT and NIs that might have reduced the efficacy of UR treated with N stabilizers/inhibitors. For example, nitrapyrin has high bioactivity for a short period but the inhibitor is rapidly degraded into 6-chloropicolinic acid, making it less effective as a NI. Due to the higher hydrophilicity and mobility of DCD (NI in SuperU stabilizer) that might have enhanced spatial separation of DCD from the NH_4^+ point sources (Li et al., 2017) and nitrifying microorganisms (Ruser and Schulz, 2015) and hence DCD may be leached away from N-application zones in the crop rhizosphere. It has been reported that the SplitU application can better optimize canola seed yield and NRE compared to its single application at seeding, probably due to minimum losses (Riar et al., 2020). Bouchet et al. (2016) also explained that NRE in canola can be affected if there is a lack of synchrony in the translocation of N from source to sink. In the present study, N uptake in canola seed and whole plant was significantly higher in SplitU, though statistically at par with AG and SU; the lowest N uptake was observed in CTRL (Table 5.4).

N content in grains is likely to increase with an increase in N because it helps in the synthesis of amino acids, which are building blocks in protein -(Amin, 2011; Grant et al., 2011; Zaman et al., 2010). In the current study, SplitU and AG significantly increased seed/GPC in canola, whereas SplitU and UR treated with N stabilizers significantly increased GPC in wheat compared to CTRL (Table 4.4) contrary to the results reported by Grant et al. (2011) who observed higher protein

content in canola seeds in UAN compared to UR and UAN + NBPT treatments. Espindula et al. (2014) also observed higher grain N concentration in UR compared to UR + NBPT all applied at full dose in canola. In my study, UR treated with N stabilizers were statistically at par with UR in wheat, whereas EN and SU were statistically at par with UR in canola and produced similar seed/GPC. However, AG produced significantly higher seed/GPC in canola compared to UR (Table 5.4). Additionally, grain yield, grain N uptake, and photosynthesis in wheat were all clustered in the same quadrant suggesting that all these parameters were closely associated with SplitU, UR and AG (Figure 5.5a). The inconsistency of N uptake, NRE, and GPC among UR treated with N stabilizers/inhibitors and UR applications in the current study warrants further investigation to decipher the role of soil pH, soil type, soil temperature, soil organic matter and mechanism of UR treated with N stabilizers/inhibitors in reducing N losses in Podzolic soils in the boreal climate.

5.5. Conclusions

Nitrogen (N) is one of the most important macronutrients required to produce wheat and canola at a higher level of production, thus maximizing nitrogen recovery efficiency (NRE) and reducing N losses with the use of urease inhibitors (UI) and nitrification inhibitors (NI) is very critical in different agroecosystems. In the current study, urea (UR) treated with N stabilizers (Agrotain (AG), eNtrench (EN), superU (SU)) and UR significantly increased the chlorophyll, seed/grain yield, N uptake, and seed/grain protein concentration (GPC) of canola and wheat cultivated on Podzols in the boreal climate compared to CTRL. However, there was no significant difference between the UR treated with N stabilizers and UR on the growth, grain yield, 1000 seed/grain weight, and N uptake of canola and wheat. The effect of UR treated with N stabilizers

on most of the measured parameters was not statistically different than that from UR possibly because of high soil mineralization and inherent N fertilizer in the soil from the previous cropping season which may have masked the effectiveness of the inhibitors. Nevertheless, it was observed that the SplitU significantly increased total N uptake in canola as compared to SU and EN by 27% and 30%, respectively. AG significantly increased GPC in canola by 12% compared to UR. I also observed from the demsar plots that SplitU was the important treatment in increasing seed/GPC and seed/grain N uptake of canola and wheat cultivated on Podzols in the boreal climate. The current findings do not reject the null hypotheses possibly due to the short duration of the experiment; thus long-term field studies are required to further determine the effectiveness of UR treated with N stabilizers on growth, grain yield, and 1000 grain weight of various grain crops cultivated on Podzols in the boreal climate.

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Chapter Six

General discussions and conclusions

6.1. General discussions and conclusions: The objectives of the present study were:

- i.) To investigate the effect of urea and urea treated with nitrogen (N) stabilizers on the growth, forage dry matter (DM), and forage quality indices of silage corn cultivated on Podzols in the boreal climate.
- ii.) To evaluate the effect of urea and urea treated with N stabilizers on the growth, forage DM, and forage quality of faba bean and oat + peas mixture.
- iii.) To elucidate the effect of urea and urea treated with N stabilizers on the growth, grain yield, 1000 seed/grain weight and grain protein concentration (GPC) of canola and wheat.
- iv.) To correlate the effect of urea and urea treated with stabilizers on the N uptake and nitrogen recovery efficiency (NRE) of field crops cultivate on Podzols in the boreal climate.

The above objectives were not archived in any of the studies. All three studies did not provide evidence that urea (UR) treated with N stabilizers can increase the growth, yield, and quality of field crops as compared to conventional UR. However, all three experiments provided strong evidence that UR treated with N stabilizers can be used as substitute fertilizers to grow various crops under field conditions in a boreal climate.

In the study one, different UR treated with N stabilizers (urease inhibitors (UI) and nitrification inhibitors (NI)) Agrotain (UI), eNtrench (NI), and SuperU (UI + NI) were broadcasted in the field at seeding time to grow silage corn for two consecutive years. The measured parameters such as photosynthesis, chlorophyll, plant height, forage DM, forage quality indices, N uptake, and NRE

were used to test the efficacy of N stabilizers treated UR as compared to the conventional UR. The results of this study provided strong evidence that Agrotain (AG), eNtrench (EN), and SuperU (SU) did not have a significant effect on the growth, forage DM, forage quality indices, N uptake, and NRE of silage corn as compared to UR. Specifically, UR treated with N stabilizers did not significantly increase or decrease the growth of silage corn as compared to UR. The forage quality indices of silage corn were not significantly affected by UR treated with N stabilizers as compared to UR except for net energy for lactation (NEL) which was increased significantly (7%) by SU as compared to UR. The N uptake of silage corn was not significantly affected by UR treated with N stabilizers as compared to UR; though AG and SU had higher N uptake (9% and 37%, respectively) while EN had lower N uptake (1.4%) compared to UR in silage corn. The NRE of silage corn in 2019 had a significant increase of 50% in SU compared to UR, whereas UR treated with N stabilizers were statistically at par with UR in 2020. However, the growth, forage DM, forage quality indices, N uptake, and NRE of silage corn was affected by the different cropping seasons. Additionally, the demsar plots suggests that SU is an important treatment to increase forage DM, N uptake, NEL, chlorophyll and milk production of silage corn cultivated on Podzols in a boreal climate.

In the study two, UR treated with N stabilizers (UI and NI) Agrotain (UI), eNtrench (NI), and SuperU (UI + NI) were applied at seeding to determine the growth, forage DM, forage quality indices, N uptake, and NRE of legume crops/mixture such as faba bean and oat + peas mixture. A 50/50 combination of oats + peas mixture and faba beans alone were planted in two different experiments under field conditions in rotation with silage corn to test the effect of UR treated with N stabilizers on the plant height, photosynthesis, chlorophyll, forage DM, forage quality indices, N uptake, and NRE of these crops as compared to UR. It was observed that UR treated with N

stabilizers did not significantly affect the growth, forage DM, forage quality indices, N uptake, and NRE of the faba beans and oats + peas mixture as compared to UR. Specifically, UR treated with N stabilizers (AG, EN, SU) did not significantly increase the growth and forage DM of both faba beans and oats + peas mixture compared to UR. Rather, a decrease of 14%, 38%, and 27%, respectively by AG, EN, and SU on the forage DM of oats + peas mixture compared to UR was observed. In faba beans, EN, AG, and SU slightly increased forage DM by 10%, 4.9% and 9.3%, respectively compared to UR. The forage quality indices of faba beans and oats + peas mixture was not significantly increased or decreased by UR treated with N stabilizers as compared to UR except for crude fat (CF) in faba beans, which was significantly decreased by 12% by AG and SU as compared to UR. AG also significantly decreased calcium (Ca) content in faba beans by 18%, while SU significantly decreased magnesium (Mg) by 16% as compared to UR. The N uptake and NRE of both faba beans and oats + peas mixture was not significantly affected by UR treated with N stabilizers. Additionally, the demsar plots suggests that SU and AG are the least important treatments to increase CF, Mg and Ca of faba beans cultivated on Podzols in the boreal climate.

In the study three, UR treated with N stabilizers (UI and NI) Agrotain (UI), eNtrench (NI), and SuperU (UI + NI) were applied at seeding to grow canola and wheat. To evaluate the efficacy of these UR treated with N stabilizers parameters such as photosynthesis, chlorophyll, plant height, grain yield, 1000 seed/grain weight, seed/GPC, N uptake, and NRE of both canola and wheat were measured. The results of the study demonstrated that UR treated with N stabilizers did not significantly affect the growth, grain yield, 1000 seed/grain weight, seed/GPC, N uptake, and NRE of canola and wheat. Specifically, photosynthesis, chlorophyll, plant height, and grain yield of canola and wheat were not significantly increased by UR treated with N stabilizers (AG, EN, SU) as compared to UR. AG, EN, and SU slightly decreased grain yield in wheat by 6%, 16%, and 6%,

respectively while seed yield in canola was slightly increased by 3% by AG and SU as compared to UR. EN slightly lowered the canola seed yield by 3%. UR treated with N stabilizers did not significantly affect the 1000 seed/grain weights of canola and wheat. AG, EN, and SU increased 1000 seed weight of canola by 11%, 10%, and 8%, respectively, while there was a 7% increase by EN and SU and only 1% drop by AG in 1000 grain weight of wheat as compared to UR. The seed/GPC, N uptake and NRE of canola and wheat were not significantly increased or decreased by UR treated with N stabilizers fertilizers. However, EN and SU significantly decreased N uptake in canola by 30% and 27%, respectively compared to the split application of urea (SplitU). Additionally, the demsar plots suggest that SplitU is the most important treatment to increase seed/grain N uptake, and seed/GPC of canola and wheat cultivated on Podzols in a boreal climate. The above studies demonstrates that UR treated with N stabilizers do not have the potential to significantly increase the growth, yield, and quality of field crops cultivated on Podzols in a boreal climate as compared to the conventional UR.

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Appendix One

Biweekly average air temperature, maximum temperature, minimum temperature, and rainfall of the experimental from the weather station in Deer Lake Airport, NL (YDF 71809) site during 2020 growing season.

Growth Period	Avg. Temp °C	Max. Temp °C	Min. Temp °C	Rainfall mm
June 1-15	10.30	16.10	4.61	32.70
June 16-30	16.70	23.30	10.10	37.40
July 1-15	13.80	19.80	7.83	29.60
July 16-31	16.20	22.40	10	30.50
August 1-15	17.70	24.90	10.50	19.60
August 16-31	13.40	19.10	7.70	115.80
Sep. 1-15	12.30	17.90	6.60	27.80
Sep. 16-30	10.80	15.70	5.88	26.70
Oct. 1-15	7.33	12.80	1.81	79.10
Oct. 16-31	3.91	8.66	-0.78	58.60
Nov. 1-15	2.39	6.55	-1.75	26.30
Total	124.83	187.21	62.50	484.10

Appendix two

A.) Mehlich III extraction method

i.) Materials and reagents

- Reciprocating shaker
- Erlenmeyer flasks 125 mL
- Filter funnels
- Filter paper (Whatman #42)
- Disposable plastic vials
- Instrumentation common in soil chemistry laboratories such as: spectrophotometer for conventional colorimetry or automated colorimetry (e.g., Technicon AutoAnalyzer; Lachat Flow Injection System); flame photometer; or ICP-OES or ICP-MS
- M3 extracting solution:
 - a.) Stock solution M3: (1:5 M NH_4F þ 0:1 M EDTA). Dissolve 55.56 g of ammonium fluoride (NH_4F) in 600 mL of deionized water in a 1 L volumetric flask. Add 29.23 g of EDTA to this mixture, dissolve, bring to 1 L volume using deionized water, mix thoroughly, and store in plastic bottle.
 - b.) In a 10 L plastic carboy containing 8 L of deionized water, dissolve 200.1 g of ammonium nitrate (NH_4NO_3) and add 100 mL of stock solution M3, 115 mL concentrated acetic acid (CH_3COOH), 82 mL of 10% v=v nitric acid (10 mL concentrated HNO_3 in 100 mL of deionized water), bring to 10 L with deionized water and mix thoroughly.
 - c.) The pH of the extracting solution should be 2.3 ± 0.2 .

- Solutions for the manual determination of phosphorus:
 - a.) Solution A: dissolve 12 g of ammonium molybdate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 250 mL of deionized water. In a 100 mL flask, dissolve 0.2908 g of potassium antimony tartrate in 80 mL of deionized water. Transfer these two solutions into a 2 L volumetric flask containing 1000 mL of 2:5 M H_2SO_4 (141 mL concentrated H_2SO_4 diluted to 1 L with deionized water), bring to 2 L with deionized water, mix thoroughly, and store in the dark at 48C.
 - b.) Solution B: dissolve 1.056 g of ascorbic acid in 200 mL of solution A. Solution B should be fresh and prepared daily.
 - c.) Standard solution of P: use certified P standard or prepare a solution of 100 mg mL⁻¹ P by dissolving 0.4393 g of KH_2PO_4 in 1 L of deionized water. Prepare standard solutions of 0, 0.5, 1, 2, 5, and 10 mg mL⁻¹ P in diluted M3 extractant.
- Solutions for K, Ca, Mg, and Na determination by atomic absorption:
 - a.) Lanthanum chloride (LaCl_3) solution: 10% (w=v).
 - b.) Concentrated solution of cesium chloride (CsCl) and LaCl_3 : dissolve 3.16 g of CsCl in 100 mL of the 10% LaCl_3 solution.
 - c.) Combined K and Na standard solutions: use certified atomic absorption standard and prepare solutions of 0.5, 1.0, 1.5, 2.0 and 0.3, 0.6, 0.9, 1:2 mg mL⁻¹ of K and Na, respectively.
 - d.) Combined Ca and Mg standard solutions. Prepare 2, 4, 6, 8, 10 and 0.2, 0.4, 0.6, 0.8, 1:0 mg mL⁻¹ of Ca and Mg, respectively.
- Standard solution for Cu, Zn, and Mn determination by atomic absorption:

a.) Combined Cu and Zn standard solution: 0, 0.2, 0.4, 0.8, 1.2 to 2.0 mg mL⁻¹ of Cu and of Zn in M3 extractant.

b.) Mn standard solutions: prepare 0, 0.4, 0.8, 1.2 to 4 mg mL⁻¹ of Mn in diluted M3 extractant.

ii.) Extraction procedure:

- Weigh 3 g of dry soil passed through a 2 mm sieve into a 125 mL Erlenmeyer flask.
- Add 30 mL of the M3 extracting solution (soil:solution ratio 1:10).
- Shake immediately on reciprocating shaker for 5 min (120 oscillations min⁻¹).
- Filter through M3-rinsed Whatman #42 filter paper into plastic vials and store at 4°C until analysis.
- Analyze elements in the filtrate as soon as possible using either an automated or manual method as described below.