Right Source and Right Time: Reducing Nitrous Oxide Emissions with Enhanced Efficiency Nitrogen Fertilizers

by

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Abstract

Fall applications of synthetic nitrogen (N) fertilizers to cropland induces significant nitrous oxide (N_2O) emissions during spring-thaw and throughout the growing season; impeding N_2O gas reductions for the Canadian agricultural sector. A possible means to reduce N_2O is with enhanced efficiency fertilizers (EEF) which are designed to delay the activity of urea hydrolysis and/or nitrification, or control the availability of plant inorganic N. The objective of this thesis was to quantify and compare cumulative N_2O (ΣN_2O kg N ha^{-1}) and agronomic measurements of Canadian hard red spring wheat (*Triticum aestivum L.*) with EEF and non-EEF sources, and application timing (spring versus fall) in southern Manitoba. Treatments included fall and spring applications of granular urea with and without EEF containing urease inhibitor product- LIMUS, nitrification inhibitor product- eNtrench, double inhibitor product- SuperU, polymer coated urea- ESN, and anhydrous ammonia (AA) with and without a nitrification inhibitor- N-Serve. Using the static-vented chamber technique, treatments were examined in two replicated plot trials in each of three years from 2015 to 2017. Among five out of six sites which had an N source effect, eNtrench and SuperU reduced ΣN_2O by 54 and 43%, respectively. Combining urea sources, the fall-applied ΣN_2O was similar to spring at 4 of 6 sites. Precipitation events induced largest daily emissions from both fall and spring applications. LIMUS protein levels were 0.6 and 0.5% lower compared to eNtrench and ESN. No site observed a significant grain yield response to EEF sources. Grain yields and protein from fall applications were generally similar or lower than spring. eNtrench and SuperU were the most optimal products to reduce N_2O and maintain agronomic variables. Future

studies should investigate whether reduced EEF rates produce similar agronomic and environmental measurements as to this current study.

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1. Introduction

1.1 Agricultural Contribution to N_2O

Major greenhouse gases (GHG), carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), are increasing in atmospheric concentration (IPCC, 2014). The major anthropogenic GHG is CO_2 followed by CH_4 and N_2O accounting for 76, 16 and 6%, respectively, of total anthropogenic GHG emissions (IPCC, 2014). These GHG absorb infrared radiation and emit longwave energy back towards the earth's surface. Thus, as atmospheric concentrations of GHG continue to increase, greater amounts of energy will radiate back to the earth. Increased radiation directed toward the earth's surface is resulting in higher land and ocean surface temperatures (IPCC, 2014). Specifically, the earth's temperature has increased by 0.85°C since 1880 (IPCC, 2014). Presently, higher surface temperatures are increasing atmospheric water vapour amounts which is negatively effecting terrestrial and aquatic ecosystems, increasing the occurrence of extreme weather events and pushing oceanic populations to migrate due to rising sea levels (IPCC, 2014).

Nitrous oxide has the lowest atmospheric concentrations and has not increased to the proportions of CO_2 and CH_4, but is very potent in that it has a global warming potential 265 times that of CO_2 (IPCC, 2014). Canada is a major contributor to global GHG emissions and has committed to decrease emissions by 30% from 2005 levels by 2030 (Environment and Climate Change Canada, 2017). The agriculture sector in Canada is the largest producer of N_2O, accounting for 71% of national N_2O emissions (Environment and Climate Change Canada, 2017). Specifically, the use of inorganic nitrogen (N) fertilizer contributes 22% of agricultural emissions and is increasing with increased fertilizer use (Environment and Climate Change Canada, 2017). However, the use of conventional fertilizers is attributable to 30-50% of crop yields and is expected to increase with greater demands to expand agricultural production and provide for the world's growing population (Stewart et al., 2005; Burton et al., 2008). The Prairies or Northern Great Plains region of Canada is highly dependent on conventional fertilizers where it accounts for 80% of all synthetic N fertilizer use in Canada (Statistics Canada, 2013). Therefore, to efficiently adhere to both commitments, it is imperative to develop, test and compare various N fertilizers that will maintain current yield productions while also mitigating N_2O emissions produced from agricultural soils. Specifically, N_2O indices such as cumulative N_2O emission (ΣN_2O kg N ha^{-1}), emission factor (kg N_2O-N kg^{-1} N) and emission intensity (kg N_2O-N Mg^{-1} yield) are calculated to compare treatment responses.

1.2 Soil N_2O Processes

Nitrous oxide can be produced in soils from multiple pathways of the N cycle (Subbarao et al., 2012). However, N applied as fertilizer is mainly cycled through the nitrification (Subbarao et al., 2012), nitrifier-denitrification (Kool et al., 2011) and denitrification processes (Ryden, 1981; Mosier, 1994). Nitrification is the biological conversion of reduced ammonia (NH_3) to oxidized nitrite (NO_2^-) or nitrate (NO_3^-) by heterotrophic and autotrophic microorganisms, also known as nitrifiers (Norton, 2008). The first step of nitrification is the oxidation of NH_3 to NO_2^- by ammonia-oxidizing bacteria and ammonia-oxidizing archaea (Subbarao et al., 2006; Ward, 2013; Hayatsu et al., 2008; Signor and Cerri, 2013). An intermediate step between the transformation of NH_3 to NO_2^- is the oxidation of NH_3 to hydroxylamine (NH_2OH) by ammonia monooxygenase

(Subbarao et al., 2006). Oxidation of NH_3 to NH_2OH is quickly proceeded by hydroxylamine oxidoreductase to oxidize NH_2OH to NO_2^- (McCarty, 1999; Subbarao et al., 2006). The second step, nitration, is the oxidation of NO_2^- to NO_3^- by nitrite-oxidizing bacteria, catalyzed by nitrite oxidoreductase (Ward et al., 2013; Signor and Cerri, 2013). Nitrification occurs under conditions not restricted by oxygen or moisture as many of the microorganisms in the process require both to transform N. As well, protons and electrons are simultaneously being released and exchanged which increase soil pH and provide the soil a net positive charge (Robertson, 1989).

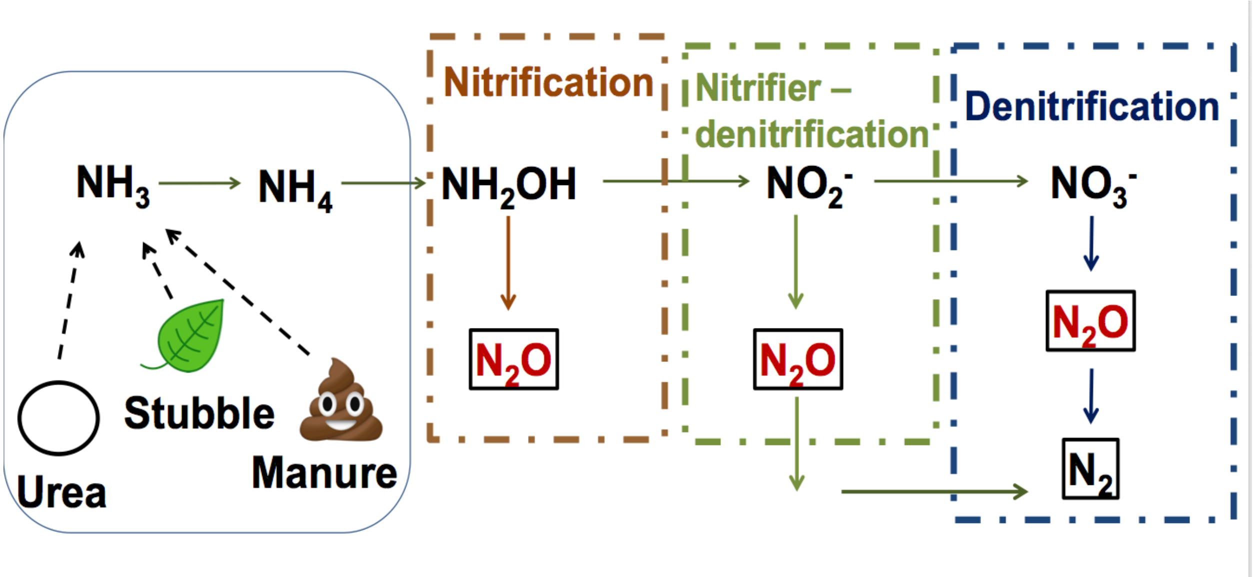

Figure 1.1 Soil nitrogen processes responsible for N_2O production.

During nitrification, when there is a greater availability of electrons for NH_2OH oxidation to NO_2^- compared to NH_3 oxidation, NO_2^- will further be reduced leading to N_2O production (Wunderlin, 2012). Another process known as chemodenitrification follows a very similar pathway but doesn’t require microbial activity as it decomposes intermediates within the oxidation of ammonium (NH_4^+) to NO_2^- (Wrage et al., 2001). Commonly, chemodenitrification occurs in the presence of organic and inorganic iron or copper compounds at a low pH (Wrage et al., 2001). Alternatively, NO_2^- can build up under low oxygen conditions and be used as an electron acceptor for nitrifying bacteria instead of oxygen, known as nitrifier- denitrification (Kool et al., 2011). Ultimately, nitrifying bacteria use the pathway from NO_2^- to dinitrogen (N_2) for anaerobic respiration with N_2O being an intermediate (Ward, 2013; Kool et al., 2011). Nitrifier-denitrification can produce significant N_2O emissions under favourable soil moisture, most commonly at 50 to 70% water-filled pore space (Kool et al., 2011; Kool et al., 2010).

Under low oxygen or complete anaerobic conditions, heterotrophic microorganisms (denitrifiers) use NO_3^- as an electron acceptor in respiration (Wrage et al., 2001). Complete denitrification is the stepwise reduction from NO_3^- to N_2 with NO_2^-, nitric oxide (NO) and N_2O being intermediates. Under increasingly aerobic conditions, incomplete denitrification can occur, inhibiting nitrous oxide reductase responsible for the reduction to N_2 and releasing N_2O as an intermediate (Wrage et al., 2001). Denitrification is the greatest contributor to N_2O production at 75% water filled pore space or greater, or at 20 to 30% soil water content (Mosier et al., 1996; Cai et al., 2016).

1.3 Factors Influencing N_2O Production

The regulation of nitrification and denitrification, and intermediate gaseous release of N_2O is dependent on proximal factors oxygen, carbon (C), and inorganic N in the form of NH_3 or NH_4^+ and NO_3^-. Agro-climatic and soil conditions that influence the availability of proximal factors are soil temperature, moisture and oxygen availability, mineralization/immobilization, plant uptake, cation exchange capacity, microbial respiration rates, soil texture and structure, precipitation events, fertilizing application timing and seasonal changes.

With greater soil concentrations of NH_4^+ and NO_3^- available for microbial consumption, larger N_2O production will subsequently occur. The greatest inputs of NH_4^+/ NH_3 are attributed to N fixation, mineralization of soil organic matter (SOM), synthetic fertilizer (urea and NH_4^+ fertilizers), manure and atmospheric deposition of NH_4^+ (Di and Cameron, 2002; Norton and Stark, 2011). Agriculture crops uptake approximately 30-40% of applied N in the year of application, indicating that a significant amount of N is lost by NH_3 volatilization, NO_3^- leaching and N gas loss by nitrification and denitrification or tied up in soil microorganism processes such as immobilization (Subbarao et al., 2006).

Soil temperature influences nitrifying and denitrifying activity, and resulting production of N_2O. Temperature controls NH_4^+ availability processes, increases or decreases respiration of oxygen diffusion, and alters plant community structure and soil type which controls oxygen availability (Robertson, 1989). The influence of temperature on N_2O is climate specific and in general nitrification rates are highest at 25-35°C (Nortan and Stark, 2011; Subbarao et al., 2006). However, in northern climates as soil temperatures increase from freezing, N conversion by nitrifying and denitrifying microorganism's increase (Signor and Cerri, 2013). As soil temperature rises to over 10°C, N conversion and N_2O production tends to become insensitive to temperature with other factors having a greater influence (Cai et al., 2016).

Similar to soil temperature, soil moisture significantly contributes to microorganism activity and N_2O production. Nitrification rates are increased by the diffusion of oxygen through water films to nitrifiers (Robertson, 1989; Norton and Stark, 2011). At field capacity, nitrification is responsible for the majority of N_2O production. Yet, at increasing soil moisture induced by melting snow, precipitation events or irrigation practices, nitrification conversion to NO_3^- is inhibited but the process of nitrifier-denitrification or denitrification can lead to even greater emissions than nitrification (Kool et al., 2010; Rochette et al., 2008; Phillips et al., 2007).

Soil carbon availability also controls the magnitude of N_2O emissions. Increased soil carbon enhances microbial activity which can lead to immobilization and reduced N_2O since microbial heterotrophs will immobilize N for themselves before poor competitor nitrifiers access remaining NH_4^+ (Robertson, 1989). However, an incubation study by Tenuta et al. (2011) showed higher soluble C and organic matter increased denitrification enzyme activity and N_2O emissions. Therefore, in the presence of high NO_3^- concentrations in combination with high organic matter and oxygen depleted soils, reduction demands increase and significant rates of denitrification and N_2O production occur (Risk et al., 2013).

An important factor controlling the proportion of N_2O emitted from soils is the type and texture of soil (Robertson, 1989; Signor and Cerri, 2013). Soil type and texture can limit aeration, available organic carbon, physical distribution of organic matter and water holding capacity. Soil textures such as sand and clay have different cation exchange capacities, altering the availability of NH_4^+ ions on adsorption sites where nitrifiers grow (Subbarao et al., 2006). Clay soils typically have greater organic matter and carbon contents, limited macropores and greater anaerobic microsites, depending on soil moisture levels, leading to greater N_2O emissions compared to other soil textures (Signor and Cerri, 2013; Wrage et al., 2004).

Many of the above factors are influenced by seasonal changes, management and weather events. In northern climates there are two large episodic N_2O emission periods.

The first episodic period is during spring-thaw (Wagner-Riddle et al., 2008). Tenuta and Sparling (2011) demonstrated significant emissions right after soil thaw where denitrification was the primary process responsible for the production of N_2O. Field studies have showed similar results (Wagner-Riddle et al., 2008; Corre et al., 1996) and indicated that high soil water filled porosity, temperature and NO_3^- accumulation by microbial death and lysis from the freezing action promotes denitrification and large emissions of N_2O (Tenuta et al., 2016; Tenuta and Sparling, 2011, Ryan et al., 2000; Wagner-Riddle et al., 2008). Further increases in N_2O during spring-thaw is usually in response to fall N applications (Tenuta et al., 2016). During fall N fertilizing, if soil temperature is not close to freezing, there is a risk of soil NO_3^- build up during the winter. As the soils start to thaw, NO_3^- will be furthered denitrified to generate N gases (Tenuta and Beauchamp, 2003). This process is enhanced by up to 20% with broadcasted treatments compared to banding (Ridley, 1975).

The second episodic period occurs two to five weeks following spring fertilizer application (Glenn et al., 2012; Asgedom et al., 2014; Tenuta and Beauchamp, 2003). Studies have determined that soils with high soil moisture and temperature, especially after significant rainfall events (Corre et al., 1996; Glenn et al., 2012; Parkin and Hatfield, 2014) will rapidly nitrify and denitrify, and potentially emit N_2O within the first weeks after N fertilizer application (Tenuta et al., 2010). Overall, both fall or spring-N fertilizer applications can significantly contribute to major episodic N_2O emission periods.

1.4 Nitrogen Enhanced Efficiency Fertilizers

Enhanced efficiency fertilizers (EEF) of nitrogen are a potential way to mitigate N_2O emissions from both fall and spring-N fertilizer applications. Twelve years ago the International Panel on Climate Change concluded that multiple EEF may have the potential to cut fertilizer-induced N_2O emissions by 30% (IPCC, 2006). A meta-analysis of 35 field studies found that certain EEF reduce N_2O emissions, on average, by 38% compared to synthetic N fertilizers (e.g. urea and anhydrous ammonia) (Akiyama et al., 2010). Other significant benefits of EEF include increased N use efficiency in the soil, delayed N availability after fertilizer application when crop uptake is generally low, and indirect savings on labour and fuel costs (Akiyama et al., 2010). Inhibitors are best used during conditions where the risk of N loss is high (Subbarao et al., 2006). Specifically, anaerobic soil conditions, high soil moisture levels, rainfall events, and snow melt/soil thaw periods are high risk environments and periods conducive to N losses (Dunmola et al., 2010; Wagner-Riddle and Thurtell, 1998; Tenuta and Sparling, 2011; Mosier, 1994). Enhanced efficiency fertilizers are currently available to producers but are not widely used due to increased costs and lack of yield increases (Olson-Rutz et al., 2009, Grant et al., 2012). However, with growing public concern surrounding climate change and the on-set of national and provincial carbon credits, it now may be very beneficial to regulators and industry to quantify N_2O emissions from EEF applications (Snyder et al., 2009).

The design and function of EEF are different from synthetic fertilizers in that they control biological N transformations responsible for the production of N_2O emissions. Ideally, the availability of inorganic N will synchronize to plant N demand over the growing season (Olson-Rutz et al., 2009; Trenkel, 2010). Enhanced efficiency fertilizers include:

(i) nitrification inhibitors, (ii) urease inhibitors and (iii) controlled-release products. Nitrification inhibitors can be applied with commonly used conventional fertilizers such as urea and anhydrous ammonia (AA). The function of a nitrification inhibitor is to selectively inhibit ammonia-oxidizing bacteria to delay the oxidation of NH_4^+ (Trenkel, 2010; Gao et al., 2015; Subbarao et al., 2006). The most common nitrification inhibitors used in a field setting are nitrapyrin [2-chloro-6-(trichloromethyl) pyridine] and dicyandiamide (DCD) (Subbarao et al., 2006). Nitrapyrin and DCD contain metal chelators and bind to enzyme sites to inhibit ammonia-oxidizing bacteria, specifically *Nitrosomonas europaea,* from oxidizing NH_4^+ to NH_2OH (Ruser and Schulz, 2015; Subbarao et al., 2006). Disadvantages of nitrapyrin is that it is highly volatile and ineffective if broadcasted onto the soil surface (Subbarao et al., 2006). A disadvantage of DCD is that it is highly water soluble and easily leached out of the rooting zone (Akiyama et al., 2010). Common products available to growers that incorporate these ingredients are eNtrench, N-Serve and SuperU.

An indirect approach to inhibiting N_2O emissions is with urease inhibitors. Using a urease inhibitor is an indirect approach because it's mode of action is to inhibit urease enzymes in the soil which hydrolyze urea to NH_3 and NH_4^+, and lowers NH_3 volatilization and indirect N_2O emissions (Trenkel, 2010; Gao et al., 2015; Cai et al., 2016). Depending on the product, the main active ingredients are N-(n-butyl) thiophosphoric triamide (NBPT) and N-(n-propyl) thiophosphoric triamide (NPPT). In the soil, these active ingredients can rapidly convert to an applicable and competing oxygen analog that forms a tridentate ligand with the urease enzyme to delay hydrolysis (Zaman et al., 2009). The use of either NBPT or NPPT can sufficiently block urea hydrolysis into inorganic N forms that lead to N_2O production and directly reduce NH_3 volatilization. Multiple studies have not observed the benefits of NBPT because it inevitably just prolonged the hydrolysis of urea and did not inhibit N_2O production (Awale et al., 2017; Xi et al., 2017). Available fertilizer products incorporated into this current study are LIMUS containing both NBPT and NPPT, and SuperU, along with containing DCD, contains NBPT to act as a double inhibitor.

An alternative EEF which does not have an inhibitory effect are control release fertilizers. Specifically, Environmentally Smart Nitrogen (ESN) uses polymer chemistry and coatings to control the rate at which urea is released into the soil. Polymer coated urea is highly dependent on soil moisture and temperature to release N into the soil (Gao et al., 2015). First, with adequate soil moisture, water hydrates the granule and dissolves N within the granule. The granule does not disperse due to the polymer coating. As soil temperature increases, the N will diffuse out of the granule for crop uptake. The goal of this technology is to release N in parallel with crop N demand which, in theory, will reduce the amount of N that is lost to the environment through nitrification and other N conversion processes (Gao et al., 2015; Halvorson and Del Grosso, 2013). Similar to urease inhibitors, previous studies have observed that polymer coated urea products only delay the release of urea with N accumulating in soils weeks later in the growing season leading to N gas emissions (Awale et al., 2017; Akiyama et al., 2010).

1.5 Previous Experiments Measuring N_2O with EEF

Multiple field experiments have determined that spring-applied EEF technologies can reduce N_2O emissions from agricultural soils (Hyatt et al., 2010; Halvorson et al., 2014; Sistani et al., 2011; Zebarth et al., 2012; Halvorson and Del Grosso, 2013). In the United States, researchers have applied EEF SuperU and ESN to corn and detected greater emission reductions from SuperU than ESN, and with both having less emissions than urea (Halvorson and Del Grosso, 2013; Maharjan and Venterea, 2013). More regionally in the Prairie provinces, Li et al. (2012) sampled N_2O with static-vented chambers from ESN applications at 1 and 1.5 times the recommended rate and observed negligible differences compared to urea. In Manitoba, Asgedom et al. (2014) broadcasted EEF SuperU and ESN, and observed N_2O reductions with ESN but not with SuperU. Using similar methodology to the latter study, Gao et al. (2015) applied SuperU and ESN but instead banded the N resulting in reductions from both SuperU and ESN compared to urea. Therefore, with an abundance of local literature, one can confidently establish that EEF are a viable mitigation technique to reduce N_2O emissions. However, it is important to test fall fertilizer applications and compare with spring applications to give confidence in EEF technologies.

In Manitoba, it is common to apply N fertilizers, mainly AA, in the fall due to lower retail cost, reduced fall workloads, increased seed bed preservation and generally drier soil conditions (Tiessen et al., 2006; Tenuta et al., 2016). In a recent survey asked of agronomists and growers, only 16% of the respondents were not in favour of applying N fertilizers in the fall (Amiro et al., 2017). Due to the potential benefits and popularity of fall N application, researchers have been encouraged to observe N processes and soil losses from fall and spring applications (Bremner et al., 1981; Parkin and Hatfield, 2010; Tenuta et al., 2016; Tiessen et al., 2006). The majority of comparison studies have reported similar or greater N_2O emissions from fall conventional N applications compared to spring.

However, Tenuta et al. (2016) compared late-fall and spring pre-seed AA applications and observed an N_2O reduction of 33% from late-fall application plots compared to spring. Therefore, applying N right before winter soil freezing limits microbial activity and N conversion to inorganic forms conducive to loss.

The application of EEF may compliment the benefit of late-fall applications reducing N loss and maintaining N in urea or NH_4^+ form. Tiessen et al. (2006) recommended applying NBPT and DCD late-fall in areas where denitrification and leaching are high. However, few studies have compared N_2O loss from spring and fall applications of EEF. In the western prairies, the only study that reported N_2O losses from spring and fall-applied ESN observed significantly lower spring emissions compared to fall (Soon et al., 2011). Yet, the demand to reduce N_2O, apply N in the fall and incorporate EEF in farm N management plans is a priority for Manitoba agronomists (Amiro et al., 2017).

1.6 Nitrogen Fertilizer Impact on Agronomic Performance

Wheat grain protein impacts markets and prices available for growers (Westcott et al., 1997). The initial soil available N is used to satisfy grain yield potential, whereas N available in soil at heading is allocated to grain protein establishment (Westcott et al.,1997). However, the initial transformation of N from urea hydrolysis and nitrification is increasing due to higher N application rates and increased dependence on fertilizer applications (Subbarao et al., 2006). In Manitoba and other areas in the Northern Great Plains, it is common to apply N fertilizers before or during seeding. Though, applying conventional fertilizers before or at seeding at high N rates is leading to major N losses and reduced N allocation to protein establishment.

Multiple factors such as seeding date, soil moisture and temperature, cultivar, fertilizer N management and soil type influence grain yields and protein (Thapa et al., 2016; Gao et al., 2012; Terman, 1979; Campbell et al., 1997). However, soil moisture is the primary influencing factor due to the dilution effect where under ideal moisture conditions and N availabilities, especially early in the season, protein concentration will reduce due to dilution from high biomass production (Grant and Flaten, 1998; Gao et al., 2012). Yet, when the opposite occurs under moisture-deficit conditions, protein concentration will increase from low biomass and yield potential (Grant and Flaten, 1998). These latter findings about soil moisture and N application on grain production were based on conventional N fertilizer applications, whereas EEF sources have yet to be thoroughly tested with Manitoba best management practices. Recent work by Thapa et al. (2016) in Minnesota observed no yield or protein differences among urea, nitrification and double inhibitor sources. Using the findings and recommendations from other geographic areas may assist Manitoba growers and agronomists to some extent but without an extensive study in relatable soil zones, recommendations will be expended with a degree of caution.

1.7 Hypothesis

The first objective was to quantify whether applications of urea and AA combined with enhanced efficiency fertilizer ingredients can mitigate N_2O emissions and maintain Canadian hard red spring wheat (*Triticum aestivum L.*) agronomic measurements compared to urea and AA without EEF. I hypothesized that inhibiting biological nitrogen transformation and slowing down nitrification rates in particular with EEF would reduce cumulative N_2O emissions (ΣN_2O kg N ha^{-1}), emission factors (kg N_2O-N kg^{-1} N) and emission intensities (kg N_2O-N Mg^{-1} yield) compared to ammonia and urea fertilizers without EEF. Agronomic measurements such as yield, protein and nitrogen uptake would be similar between EEF and non-EEF sources since identical N rates among each treatment would provide similar N soil availability to the plant.

The second objective of this current study was to capture spring-thaw N_2O emissions to determine the N_2O reduction potential of fall compared to spring-applied conventional (urea and AA) and enhanced efficiency synthetic N fertilizers. It was hypothesized that preventing nitrification and buildup of soil NO_3^- over winter with fall EEF applications would reduce spring-thaw emissions compared to urea and AA sources without EEF, and fall ΣN_2O emissions would be comparable to spring-applied EEF. Similar to above, agronomic measurements will be similar between fall and spring applications of each treatment.

1.8 Structure of Thesis

This research is presented in two research chapters. Chapter two focuses on the agronomic performance of EEF on hard red spring wheat. Specifically, chapter two presents data and discusses grain yield, protein, nitrogen uptake and residual soil N concentrations. The third chapter investigates the influence of EEF on daily N_2O emissions and cumulative N_2O fluxes, and factors influencing soil N_2O production. Both research chapters have been prepared and formatted to the Agronomy Journal. Following both research chapters is a general discussion outlining the project's accomplishments, unexpected and interesting observations, challenges and improvements, potential future areas of investigation, recommendations to growers and policy makers and highlights of two similar studies conducted in Saskatchewan and Alberta.

1.9 Contributions

I was involved in this research for crop years 2016 and 2017. The project was initiated in the fall of 2014. I was responsible for all gas and soil sampling events, and coordinating with summer students and technicians. Fertilizing, seeding and harvesting operations were conducted by multiple technicians and myself. Data organizing and flux calculations were completed by myself mainly for the 2016 and 2017 crop years. Statistical analysis and organization was conducted by myself with the assistance of professors and associates. Additionally, I took part in multiple speaking events and conferences presenting the results from this research.

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2. Spring Wheat Yield and Protein Responses to Enhanced Efficiency Nitrogen Fertilizers, and Late-Fall and Spring Application Timings in Southern Manitoba

2.1 Abstract

Enhanced efficiency fertilizers (EEF) of nitrogen (N) are one of the leading technologies improving N use efficiency, reducing N losses, and increasing both grain yields and protein levels. These fertilizers are specifically designed to delay the activity of urea hydrolysis and nitrification, or control the availability of plant inorganic N in the soil. The objective of this study was to examine the interactive benefit of EEF N sources in combination with fall and spring applications to Canadian hard red spring wheat (*Triticum aestivum L.*) yield and quality. We tested fall and spring applications of granular urea EEF products containing urease inhibitor product- LIMUS, nitrification inhibitor product- eNtrench, double inhibitor product- SuperU, controlled-release polymer coated urea- ESN, and anhydrous ammonia (AA) with a nitrification inhibitor- N-Serve. Two field trials in each 2015, 2016 and 2017 were conducted within southern Manitoba. Urea and AA treatment plots were separated within the field and arranged in two separate randomized split plot block designs with four replicate plots for each N source. Urea N products were applied mid-row disk subsurface banded and AA treatments knifed in. Nitrogen rates were similar for all treatment combinations for a trial based on soil N test with hard red spring wheat, variety AAC Brandon. Almost all responses to EEF and application timings were significantly greater ($p<0.05$) than the 0 N control treatment; hence 0 N control treatments were excluded in the analysis of variance. Grain yields, protein, biomass and grain nitrogen (NU_{bio} and NU_{grain}) varied with site. Most notably, two sites, LaSalle and Carman in 2016 experienced the highest total growing season precipitation and subsequently had the greatest protein contents. Across all urea products, LIMUS protein levels were 0.6

and 0.5 % lower compared to eNtrench and ESN which can be explained by a higher availability of soil N early and during the growing season with eNtrench and ESN. Protein response to all EEF and non-EEF spring applications were generally greater than fall, and combining sites with urea sources averaged a significant protein increase of 0.2%. Across sites, grain yields ranged from 2.60 to 5.50 Mg ha^{-1}, based on on a standard test moisture weight of 135 g H_2O kg^{-1} of wheat, but no site observed a significant response to EEF. Fall N applied grain yields were either similar or lower than spring, except at the Ridge Road site where greater extractable soil N concentrations and yields were observed throughout the growing season from fall applications. ESN and SuperU sources maintained the highest extractable soil N levels throughout all growing seasons, being 33 and 27% greater than urea, respectively. Overall, the effects of EEF were minimal but the late-fall application of EEF provided some consistent or greater agronomic benefits compared to spring. Therefore, similar grain yields protein and N uptake is possible with fall EEF applications and should be considered in a grower's economic budget.

2.2 Introduction

Canadian hard red spring wheat (*Triticum aestivum L.*) is a major crop grown in the Manitoba. In 2017, approximately 2.1 million acres were sown with Canadian hard red spring wheat in Manitoba (MASC, 2017). Growers who meet desired grain protein levels can be awarded premium pricing for their grain. Yet, under normal or high moisture soil conditions, additional N will be required above recommended rates, in the form of manure or synthetic nitrogen (N) fertilizer to provide for grain filling and protein requirements (Campbell et al., 1996). In addition to price premium for protein, there is increasing economic and societal pressure on growers to increase yields to supply the growing global population.

To ensure high yields, growers often apply additional N above best management recommendations to reduce the risk of yield loss due to insufficient nutrition (Subbarao et al., 2006). Specifically, Canadian growers choose to apply urea and anhydrous ammonia (AA). Urea (46-0-0) is a low-cost granular fertilizer that is converted to NH_4^+ and AA (82-0-0) is ammonia gas that is injected into the soil.

Agricultural N loss occurs through the processes of ammonia (NH_3) volatilization, nitrate (NO_3^-) leaching and N gas production from nitrification and denitrification, respectively. To minimize these problems, industry and government agencies are increasingly promoting and developing technologies to improve N use efficiency, reduce N losses to the environment, and increase both grain yield and protein.

One of the most widely used technologies for achieving higher N use efficiency, reduced N losses and increased agronomic benefits is enhanced efficiency fertilizers (EEF) of N. There have been numerous wheat studies testing EEF in the Northern Great

Plains that have provided inconsistent results but with many observing a similar or greater yield and protein benefits over conventional fertilizers (Walsh and Girma, 2016; Grant, 2014; Karamanos et al., 2014; Tiessen et al., 2008; Malhi et al., 2010; McKenzie et al., 2007; McKenzie et al., 2010; Mohammed et al., 2016; Grant et al., 2012; Thapa et al., 2017; Romero et al., 2017; Grant et al., 2016). The design and function of EEF are to delay the activity of urea hydrolysis and nitrification, or control the availability of plant inorganic N in the soil (Mohammed et al., 2016). Commonly used EEF available to growers either contain a nitrification inhibitor, urease inhibitor, controlled-release urea or mixture of nitrification and urease inhibitor.

The function of a nitrification inhibitor is to selectively inhibit ammonia-oxidizing bacteria to delay to oxidation of NH_4^+ to NO_2^- and NO_3^- (Trenkel, 2010; Gao et al., 2015; Subbarao et al., 2006). Commercially named products, eNtrench and N-Serve, are nitrification inhibitors that contain the active ingredient nitrapyrin [2-chloro-6-(trichloromethyl) pyridine]. Inhibitor product eNtrench is combined with urea, and N-Serve is mixed with AA at application to inhibit nitrifying bacteria such as *Nitrosomonas europaea* in soil (Subbarao et al., 2006).

The mode of action of urease inhibitors is to reduce the rate of urease enzyme activity in soil which hydrolyze urea to NH_3 plus NH_4^+ (Trenkel, 2010; Gao et al., 2015; Cai et al., 2016). A commercially available product, LIMUS, is liquid based applied onto urea before application and contains active ingredients N-(n-butyl) thiophosphoric triamide (NBPT) and N-(n-propyl) thiophosphoric triamide (NPPT) which specifically occupy active sites on the urease enzyme, limiting urea to these sites (Rawluk et al., 2001).

A controlled-release fertilizer used by growers in Manitoba, Environmentally Smart Nitrogen (ESN, 44-0-0), contains a polymer-coating shell around a urea granular, delaying the release of urea until later in the growing season. The goal of ESN is to release urea at a time when plant growth and N uptake is greatest during warm and moist conditions. The polymer coating is less soluble than urea and is influenced by soil temperature and moisture to biodegrade and release N for plant use (Subbarao et al., 2006). Another widely used EEF, SuperU, is a double inhibitor N fertilizer that contains a urease inhibitor, NBPT, and nitrification inhibitor, dicyandiamide (DCD). With DCD, the action of nitrifying bacteria is suppressed resulting in delay in transformation of NH_3/NH_4^+ to NO_3^- (Subbarao et al., 2006).

However, EEF are not the only management factor that affect plant N use, yield and protein development. Environmental conditions, primarily soil moisture and temperature, are major factors. Campbell et al. (1997) reported, under dry and hot conditions, a very low grain yield response to N fertilizer but a protein response with increased rates of N. In the same study, the opposite was reported where grain protein decreased in cool wet conditions, due to the dilution effect, until yield potential was maximized (Campbell et al., 1997). Therefore, when moisture is ideal and N is available, grain yield will dominate over protein until N requirements are met, then protein will increase with any excess N (Grant and Flaten, 1998; Gao et al., 2012). However, when soil is dry, protein may increase but yield restricted (Grant and Flaten, 1998). The variation in grain yield and protein from annual changes in environment and soil conditions has been reported in multiple recent studies in the Northern Great Plains (Mohammed et al., 2016; Thapa et al., 2017; Grant et al., 2012). Therefore, recording local weather events and soil conditions in combination with testing N management techniques is imperative to determine limiting factors affecting agronomic performance.

Due to the major contribution of soil moisture and temperature, the response of grain yield and protein to EEF compared to conventional N fertilizers on Canadian hard red spring wheat has been inconsistent. Growing winter wheat in the Northern Great Plains, many studies have conveyed similar yields, protein and grain N uptake from urea, urea + NBPT, SuperU, N-Serve and ESN (McKenzie et al., 2007; McKenzie et al., 2010; Mohammed et al., 2016). Growing spring wheat in Montana with ESN produced greater yield in only two of eight site-years containing clay loam while coarser soils did not bear any benefits compared to urea (Walsh and Girma, 2016). In that same study in Montana, no trend in grain protein or N uptake with ESN was observed. Malhi et al. (2010) had similar success in a crop rotation containing wheat where applied ESN had 10% greater crop yield and higher N recovery in two of seven site-years that experienced wetter or normal precipitation totals. More locally, in Manitoba, Grant et al. (2011) demonstrated significant yield losses with ESN compared to urea and concluded that delays in N release limited early season N availability. In Saskatchewan, nitrification inhibitors applied with urea and AA (N-Serve) had negligible and inconsistent increases in spring wheat grain yield and protein (Karamanos et al., 2014). Along with urea, NBPT can be applied with urea ammonium nitrate (UAN) which has been more effective compared to urea (Grant et al., 2014). However, urea is the most common form of N fertilizer in Manitoba and there is a demand to increase efficiency with urea products. In Minnesota, even with dramatically different rainfall amounts from year to year, Thapa et al. (2017) did not report any response of a nitrification inhibitor (nitrapyrin) or double inhibitor (NBPT + DCD) applied with urea on grain yield or protein. Past research in the Northern Great Plains has focused on one or two EEF products but there is a gap in the research to test a large breadth of urea and AA based EEF products commercially available to cereal growers in Manitoba.

In an ideal situation, N should be applied to match crop N uptake and inorganic N availabilities in the soil. It has been demonstrated that high N availability earlier in the season will be directed to vegetative production compared to grain protein filling (Fowler, 2003). This poses a problem for Prairie crop producers since the most common and efficient times to apply N fertilizer is in the fall or spring at seeding. A review by Mussell et al. (2015) highlighted that, especially in Prairie soils, spring applications produce greater agronomic benefits compared to fall. The major inefficiency with fall applications is because of N loss prior to freeze-up and during spring-thaw, leaving inadequate N for plant growth and grain production during the growing season (Tenuta et al., 2016; Mussell et al., 2015). The advantages to fall application of N fertilizer, mainly AA in Manitoba, is due to lower fertilizer retail cost, reduced fall workloads, increased seed bed preservation and generally drier soil conditions (Tiessen et al., 2006; Tenuta et al., 2016; Mussell et al., 2015). Growers compensate for the over-winter loss of N by applying excess fertilizer. Another way to maintain N for crops is by delaying fall N applications until soil temperatures reach 5° C (Malhi et al., 2001) which has been shown to reduce losses of N prior to freeze-up and spring-thaw (Tenuta et al., 2016; Tiessen et al., 2008). Overall, losses of N from fall applications is highly dependent on cumulative freezing days, NO_3^- concentrations and number of freeze-thaw events, making it an unpredictable practice for growers due to varying weather patterns from year to year (Tenuta and Sparling, 2011; Wagner-Riddle et al., 2017).

Applying EEF may be an alternative for growers to adaptably apply N in fall or spring. A study by Romero et al. (2017) growing winter wheat observed a protein benefit using NBPT compared to urea from fall applications. Compared to fall applications of AA, N-Serve effectively increased spring wheat grain yield and N uptake after heavy snowfall accumulation (Goos and Johnson, 1999). Yet, compared to spring, studies in the Northern Great Plains have reported inconsistent agronomic benefits with fall-applied EEF and conventional fertilizers with authors ultimately recommending spring applications (Mohammed et al., 2016; Karamanos et al., 2014; Malhi et al., 2010). In Manitoba, few studies have compared fall and spring applications of EEF against conventional N sources. Multiple studies applied ESN in fall and spring but did not witness consistent improvements or similarities in yield, protein or N uptake between timings (Grant et al., 2012; Grant et al., 2016). However, Grant et al. (2016) did find greater protein levels applying fall ESN over fall urea. Other studies in Manitoba growing spring wheat applied NBPT and DCD with urea and observed minimal positive impacts from fall compared to spring-N applications (Tiessen et al., 2008; Grant et al., 2014).

For growers in Manitoba and Northern Great Plains region that rely on fall N applications, using EEF which are designed to match crop N uptake to plant N demand, can be beneficial to grain yield and protein concentrations compared to conventional N sources. However, no study in the Prairies has tested a wide variety of EEF, and compared such EEF to fall and spring applications of Canadian hard red spring wheat. Applying numerous EEF under the same environmental and soil conditions will provide a conclusive comparison on the effectiveness of EEF in southeastern Manitoba. Thus, this research experiment is the most extensive study in Manitoba to evaluate if there is an advantage in fall-applied N EEF in the form of urea and AA. This study adds to the 4R nutrient stewardship data base, specifically Right Source and Right Time, to help deliver growers with best management practices to efficiently use N fertilizer. The objective of this study was to examine the benefit of EEF nitrogen sources containing nitrification and urease inhibitors, and a polymer coating urea in combination with fall and spring applications to spring wheat yield and protein quality.

2.3 Materials and Methods

2.3.1 Experimental Site Descriptions

This field experiment was managed over a period of three growing seasons at six locations from fall 2014 to fall 2017 (two locations per growing season) in southern Manitoba. The locations were no farther than 100 km from Winnipeg, near Glenlea (49°39’3” N, 97°12’34” W), Warren (50°8’32” N, 97°30’21” W), Carman (49°26’16” N, 98°1’19” W), La Salle (49°42’2” N, 97°11’37” W), Stephenfield which will be referred to as Ridge Road (49°26’33” N, 98°20’30” W) and Ritchot which will be referred to as Kelburn (49°41’52” N, 97°7’23” W), Manitoba. Warren and Glenlea sites were used in the 2014-2015 season, Carman and LaSalle in 2015-2016, and Kelburn and Ridge Road in 2016-2017. Field sites changed each year and the selection criteria was based on soil residual NO_3^- concentration being no more than 100 kg N ha^{-1} in the fall, previous crop the season before being soybean and a uniform flat topography.

The soil at Glenlea and LaSalle are mapped in the Red River and Scanterbury series as a clay (MAFRI, 2011; Michalyna et al., 1975) and classified as Gleyed Rego Black Chernozem in the Canadian system and Udic Boroll in the American system (AAFC, 1998). The soil at Kelburn is mapped in the St. Norbert series as a clay (MAFRI, 2011; Michalyna et al., 1975) and classified as Orthic Black Chernozem in the Canadian system and Udic Boroll in the American system (AAFC, 1998). The soil at Warren is mapped in the Warren series as loam to light clay (Ehrlich et al. 1953; MAFRI, 2010; Michalyna et al., 1975) and classified as Rego Black Chernozem in the Canadian System and Udic Boroll in the American system (AAFC, 1998). The soil at Carman is mapped in the Myrtle series as a silty clay to clay (Ellis and Shafer 1943; Michalyna et al., 1975) and classified as Orthic Black Chernozem in the Canadian System and Udic Boroll in the America system. Lastly, the soil at Ridge Road is mapped in the Leary series as a loamy fine sand (Ellis and Shafer 1943; Michalyna et al., 1975) and classified as Orthic Dark Gray Chernozem in the Canadian System and Boralfic Boroll in the American System (AAFC, 1998).

Soil characterizations were taken before fall fertilization or during the growing season at a depth of 0-15 cm and analyzed by Farmers Edge Laboratories. Complete soil descriptions are provided in Table 2.1.

Table 2.1 Average site specific soil descriptions of soil NO_3^-N and PO_4^{-3}-P levels, pH, electrical conductivity (EC), organic matter and soil texture from 0-15 cm soil depth.

Site	NO_3^-N	PO_4^{-3}-P	pH	EC	Organic matter	Particle distribution & soil texture		
	mg kg^{-1}			dS m^{-1}	g kg^{-1}			
						Clay	Sand	Silt
Warren	16	4.0	7.7	0.74	60	310	360	330
Glenlea	8.5	5.6	8.0	1.1	39	660	150	190
Carman	4.9	24	6.1	0.11	61	310	430	260
LaSalle	6.0	7.5	7.6	0.25	71	600	110	290
Kelburn	7.0	12	7.5	0.23	70	†Clay		
Ridge Road	6.0	37	7.2	0.08	15	†Loamy fine sand		

† Lab analysis not provided by Farmers Edge

2.3.2 Experimental and Treatment Design

Treatments of N fertilizer consisted of (i) control with no N application, (ii) urea (46-0-0), (iii) urea + eNtrench, (iv) urea + LIMUS, (v) SuperU (46-0-0), (vi) ESN (44-0-0), (vii) anhydrous ammonia (82-0-0), (viii) anhydrous ammonia + N-Serve. All N sources were applied either in late-fall before freeze-up or at seeding. Late-fall N treatments were applied at soil temperatures around 5°C and spring-N treatments were applied with the seed in early to mid-May. Thus, with two application timings from each N source, there were a total of ten urea treatment combinations, four AA treatment combinations and two 0 N control treatment combinations. However, anhydrous ammonia (vii and viii) and granular urea (ii to vi) treatments had separate experimental designs as they were separated at each site by a 7-meter buffer containing the wheat crop (Figure 2.1; Appendix A). Therefore, urea and AA experiments were analyzed separately throughout the study. The experimental design for each urea and AA experiment was a randomized split plot block design with four replicate plots of 6 by 3.4 m and 15 by 3.4 m, respectively. Specifically, N sources (main-plot) were randomized within each rep but fall and spring application timings (subplot) of the same N source were adjacent to one another and not randomized (Appendix A). Throughout the analysis each fall and spring-N source (subplot) was considered a separate treatment. The 0 N control plots from the urea design were used with the analysis of AA treatments as no 0 N control plots were included within the AA design. Anhydrous ammonia plots were initially seeded to a plot area 15 by 3.4 m but were trimmed down right before combining to 6 by 3.4 m.

Figure 2.1 Experimental layout at all sites. Urea plots to the right and AA plots to the left of weather station buffer area.

2.3.3 Weather Monitoring and Soil Sampling

Long-term annual mean precipitation and air temperature from 1981 to 2010 were obtained from the Environment Canada Winnipeg Richardson International Airport weather station (Environment Canada, 2018). Daily mean air temperature and relative humidity (HMP45C, Vaisala Inc., Woburn, MA) were collected on site if provincial, federal or university weather stations were not within approximately seven km of the field site. Precipitation amounts were measured at the Ridge Road site using a tipping bucket rain gauge (TR-525M Rainfall Sensor, The Texas Electronics Inc., Dallas, TX).

At each growing site in 2015 and 2016, soil samples were collected on six separate occasions: (i) before fall fertilization; (ii) one week after fall fertilization, (iii,iv,v) two, four and six weeks after spring fertilization; and (vi) post-harvest. In 2017, a seventh soil sample was collected in mid-July along with the six other sampling periods mentioned above. For each plot, six samples at a depth of 0-15 cm and six samples at a depth of 15-30 cm, consisting of three on-band and three off-band samples per depth, using a 2.7 cm-diameter Dutch auger, were collected and composited into two samples bags (one per depth). All soil sample periods followed the same procedure as above, except during the post-harvest sample period. During post-harvest in 2015 and 2016, three soil samples at a depth of 0-60 cm were collected and composited into 0-15,15-30 and 30-60 cm samples using a Dutch auger. In 2017, soil conditions were very dry, requiring mechanical sampling of three soil samples at the previously mentioned sample depths with a hydraulic soil auger (#15- TS/model GSRTS, Giddings Machine Company Inc, Windsor, CO). Samples were kept in coolers during sampling days and stored at -20°C until processed. During processing, the samples were thawed for two to four hours and mixed by hand into small aggregates. Samples were either stored again at -20°C or extracted with 2 M KCl and analyzed colorimetrically for NH_4^+ using the Berthelot reaction, and for NO_3^- by reduction using Cu-Cd to NO_2^- and azo dye formation from reaction with sulfanilamide and N-naphthylehylene-diamine dihydochloride (U.S. Environmental Protection Agency, 1983; 1993), using a Technicon Autoanalyzer II system (SEAL Analytical Inc., Mequon, WI).

2.3.4 Agronomic Management of Canadian Hard Red Spring Wheat

Nitrogen rates for Canadian Hard Red Spring Wheat of each source were determined from fall NO_3^- soil sampling (0-60 cm) to achieve a target level of 170 to 190 kg N ha^{-1} in the soil to meet recommendations from Manitoba Fertility Guide and MAFRD N rate calculator (Table 2.2). Fall and spring-N sources were applied at the fertilizer rate. Site-specific application rates, dates, available N applied, residual fall NO_3^-, herbicide and fungicides applications, and hand harvest dates are included in Table 2.2.

Nitrogen EEF products eNtrench and N-Serve were applied on a land area basis at a rate of 2.72 L ha^{-1} and 2.34 L ha^{-1}, respectively. Nitrogen source eNtrench was mixed with urea 24 hours before application and N-Serve was mixed with AA during application with a direct injection system (Sidekick Pro^{TM}, Raven Applied Technology, Sioux Fall, SD) at a rate of 2.34 L ha^{-1} (Figure 2.2). Nitrogen urease inhibitor LIMUS was applied on per tonne of urea basis and mixed with urea 24 hours before application at a rate of 2.6 L $tonne^{-1}$. Control release nitrogen product ESN has a slightly lower N content than urea and required adjustment of product application rates to achieve identical N rate applied in field. In all plots, triple super phosphate (0-46-0) was applied to all treatments at 22 kg P ha^{-1} with the seed.

All treatments were planted to Canadian hard red spring wheat (*Triticum aestivum L.*), variety AAC Brandon, at a rate of 148 kg ha^{-1} and depth of approximately 2.5 cm. Wheat seed was pre-treated with fungicide and insecticide (Cruiser Maxx ® Cereals, Syngenta, Guelph, ON, CA) 24 hours before seeding. All plots were seeded with 20 cm row spacing using an air seeder. Nitrogen fertilizer treatments were midrow-banded of seed row pairs using a Bourgault Mid Row disk-style Bander (Bourgault Industries Ltd., St. Brieux, SK) to a target depth of 2.5 to 5.0 cm. Banding depths varied due to soil type and field conditions. Anhydrous ammonia plots were seeded approximately 2.5 cm deeper than urea since AA was distributed from a steel tube that was 1.5 to 2 cm below where granular urea product was dispensed and applied by pressure (compared to gravity with granular products) which forced product even further below the injection point. Fall and spring 0 N control plots were tilled at time of each respective N application. All sites were managed with commercial herbicides according to label recommendations to mitigate weed competition.

Figure 2.2 Spring AA application at Ridge Ridge on May 4, 2017.

Table 2.2 Nitrogen fertilizer and agronomic management.

Crop Year	Site	N application date		N rate	Fall soil NO_3^--N	Herbicide	Fungicide	Harvest date
		Fall	Spring					
				— kg N ha^{-1} —				
2015	Glenlea	Nov-5	May-11	115	74†	Pyrasulfotole Bromoxynil, Pinoxaden (x2), MCPA ester (x2)	Metconazole	Sep-3
	Warren	Nov-8	May-22	90	100	Pinoxaden	Metconazole	Aug-27
2016	Carman	Oct-22	May-6	123	52	Pinoxaden, Curtail M	-	Sep-2
	LaSalle	Oct-26	May-4	100	68	Pinoxaden, MCPA ester	Pyraclostrobin	Aug-24
2017	Kelburn	Oct-20	May-11	123	50	Thiencarbazone-methyl, Pyrasulfotole Bromoxynil	Pyraclostrobin	Aug-24
	Ridge Road	Nov-4	May-4	123	50	Pinoxaden, MCPA ester	-	Aug-15

† 0-60 cm NO_3^--N concentrations

2.3.5 Yield, Protein and Biomass

Hand harvest was completed prior to combine by sampling two meters of four separate rows to an area of 1.6 m^2 and leaving approximately five cm of stubble in soil. Hand harvest samples were taken at the back of the plot on the second and third rows. Total above ground biomass samples were dried for over 72 hours at 48°C. Dried grain and straw samples were ground to pass through a two mm sieve (Thomas Wiley Laboratory Mill Model 4 Grinder, Thomas Scientific, Swedesboro, NJ) and analyzed for % total N using the combustion method, also known as the modified Dumas method. Grain protein was calculated by multiplying % total N by a conversion factor of 5.7. Sub-samples of grain were dried at 100°C and weighed before and after drying to determine moisture content. Grain (NU_{grain}) and biomass (NU_{bio}) nitrogen uptake were calculated as the product of % total N by yield for grain and straw biomass, separately. Grain yield, % protein, and straw and grain N removal are reported based on the standard moisture content of 135 g H_2O kg^{-1} of wheat grain. Data analysis was completed only with hand harvest yields.

2.3.6 Statistical Analysis

Statistical analyses were completed using Analysis of Variance (ANOVA) using the Statistical Analysis System (version 9.4 software, SAS Institute Inc. 2014) to analyze mean grain yield, NU_{grain}, NU_{bio}, protein and residual extractable soil N (mg N kg^{-1}). The ANOVA was conducted with PROC MIXED procedure and least square means of fixed effects were compared by Tukey's Honestly Significant Difference test (α=0.05). The ANOVA model to test fixed effects were fertilizer source, application timing and site-year. Due to the random split plot block design, the random effects to account for the subplot (application timing) were block and block X fertilizer source. With separate designs, data from the urea and AA plots were analyzed separately. The Proc Univariate function within SAS was used to test for normality of the measured agronomic variables and subjected to the Shaprio-Wilk test in which data were untransformed if the W-statistic was greater than 0.90 or p-value greater than 0.05. Residual extractable soil N was $\log_{10}$ transformed. The 0 N control plots from the urea design was included in the AA design analysis as no 0 N control plots were established in the AA design.

2.4 Results

2.4.1 Weather and Site Conditions

At all sites, average monthly air temperature did not vary considerably from year to year (Table 2.3). Most notable differences were observed for the 2016 sites (LaSalle and Carman) where average air temperature was more than three degrees warmer in May compared to the 30-year normal. Additionally, September average temperature from all sites was greater than the 30-year normal. All other average monthly temperatures were comparable or above the 30-year normal. Cumulative monthly precipitation did vary considerably among site-years. Highest precipitation amounts occurred in August at Warren, Carman and LaSalle, and in July at Glenlea. Precipitation amounts at 2015 sites

Table 2.3 Growing season monthly mean air temperature, total precipitation and historical normal averages for six site-years in southern Manitoba.

	Mean Air Temperature (°C)						Total Precipitation (mm)						
Site	Apr	May	Jun	Jul	Aug	Sep	Apr	May	Jun	Jul	Aug	Sep	Growing season total
Warren	4.4	10.9	17.7	20.0	18.0	14.8	10.4	63.8	52.4	108	135	72.4	443
Glenlea	5.0	11.3	17.6	20.1	18.2	15.4	14.6	82.9	77.8	134	44.9	77.0	432
Carman	3.4	14.7	18.1	20.0	19.1	14.2	41.4	60.4	98.8	86.6	114	71.8	473
LaSalle	3.3	14.9	17.9	20.0	19.2	14.4	35.6	65.7	90.6	59.3	120	71.9	444
Kelburn	5.0	12.1	17.6	19.9	18.5	14.3	24.5	25.5	57.9	56.7	24.2	67.7	257
Ridge Road	5.1	12.1	17.6	20.6	18.4	14.3	15	33.4	85.5	44.4	21.0	95.2	295
Normal (1981-2010)	**4.4**	**11.6**	**17.0**	**19.7**	**18.8**	**12.7**	**30**	**56.7**	**90**	**79.5**	**77**	**45.8**	**379**

*Normal data from Environment Canada for the Winnipeg Richardson International Airport (Environment Canada, 2018).

in April were more than 50% lower and more than 25% greater in July compared to the 30-year normal. High precipitation amounts in July and August of 2015 and 2016 were attributable to heavy downpour events where 25-64% of precipitation fell on one day in the month. Sites in 2017 (Kelburn and Ridge Road) experienced below normal precipitation levels in all months except September and June at Ridge Road, and June at Kelburn. Overall, 2015 and 2016 sites had over 10% and 2017 sites had under 20% total growing season precipitation amounts compared to the 30-year normal.

During fall N applications for the Warren and Glenlea sites, daily air temperatures were between 0-5°C and soil conditions were dry with soil freeze-up occurring in the beginning of November. In fall 2015, daily air temperatures were around 5°C and soil conditions were very moist and sticky due to rain prior to fertilizing and the natural texture of clay soils. Moderate to heavy rain followed fall N applications at Carman and LaSalle until freeze-up in mid to late November. At Kelburn during fall N application, conditions were also sticky and significant rainfall events followed fertilization until freeze-up in early December. At Ridge Road, conditions were very dry during N fertilization and until freeze-up in early December.

Soil conditions during spring-N applications at both 2015 sites were wet and sticky due to rain. Moderate and persistent precipitation events in May pushed back the application date in Glenlea by one week and Warren by almost three weeks then hoped for. Similar to spring 2015, site conditions in spring 2016 were also very wet which was due to a significant spring-thaw and snow melt period. Similar conditions occurred in 2017 at Kelburn with high soil moisture levels between mid-March to late April pushing back spring application by a week. Lastly, Ridge Road had standing water during and after spring-thaw until early April but conditions during spring-N application were very dry due to the sandy soil texture (Figure 2.3).

Figure 2.3 Spring-thaw flooding at Ridge Road.

2.4.2 Grain Yield

Across all sites, grain yield in the 0 N control plots were significantly lower than for any urea or AA source treatment but among N sources there was no yield difference (*P*<0.0001, Appendix A; Figure 2.4). Grain yield was greatest at Kelburn and significantly greater than Ridge Road for both application timings in 2017, resulting in a site-year effect (Table 2.4, 5). Lowest grain yield occurred at Glenlea but there was no significant difference between application timings. Only Ridge Road had a significant application timing difference where fall yields were greater than spring, 3.90 and 3.32 Mg ha^{-1}, respectively, resulting in site-year by time interaction (*P*=0.0005, Table 2.5).

Table 2.4 Three factor ANOVA (p values) of urea granular products (excluding 0 N control) from effects of N source, application time and site-year on grain yield, protein, NU_{grain}, NU_{bio} and residual extractable soil N.

Factors	Yield	Protein	NU_{grain}	NU_{bio}	Residual extractable soil N
Site Yr (S)	<0.001	<0.001	<0.0001	<0.001	<0.001
N Source (N)	0.389	0.001	0.3708	0.192	0.002
Time (T)	0.814	0.047	0.2095	0.003	0.215
S*N	0.608	0.608	0.3138	0.592	0.097
S*T	0.0001	0.198	0.0003	0.001	0.321
N*T	0.815	0.840	0.6122	0.332	0.312
N*T*S	0.999	0.967	0.9538	0.895	0.444

Table 2.5 Site by application timing interaction of mean yield, NU_{grain} and NU_{bio} including all urea N treatments and excluding 0 N control.

Site	Time	Yield	NU_{grain}	NU_{bio}
		Mg ha^{-1}	kg N ha^{-1}	
Warren	Fall	3.14def	85.98de	108.79efg
	Spring	3.40cde	93.49cd	126.39de
Glenlea	Fall	2.60f	68.82f	91.39fg
	Spring	3.01ef	78.74ef	104.07fg
Carman	Fall	3.64bcd	101.77bc	142.87cd
	Spring	3.65bcd	102.60bc	149.77c
LaSalle	Fall	4.01b	105.35bc	160.73bc
	Spring	4.18b	112.32b	177.42ab
Kelburn	Fall	5.50a	131.32a	180.19a
	Spring	5.34a	133.45a	185.16a
Ridge Road	Fall	3.90bc	101.98bc	126.59de
	Spring	3.32de	87.14de	110.11ef

Values followed by different letters in a column are significantly different at $P < 0.05$.

Compared to the Manitoba 10-year average wheat grain yield (3.11 Mg ha^{-1}) from Manitoba Agricultural Services Corporation (MASC), Warren produced similar yields but Glenlea did not for both application timings (MASC, 2016). Specifically, comparing variety

AAC Brandon in the Winnipeg risk area (4.37 Mg ha^{-1}), averages at 2015 sites were well below the variety average. In 2016, grain yields were numerically higher than the 10-year average (3.23 Mg ha^{-1}) at both sites. Additionally, both application timings at LaSalle produced greater yields compared to AAC Brandon variety average in the Winnipeg risk area in 2016 (3.97 Mg ha^{-1}) whereas Carman did not (MASC, 2017). Yields at Kelburn were similar to the average in the Winnipeg risk area (5.31 kg ha^{-1}) and above the 10-year average (3.30 kg ha^{-1}) whereas Ridge Road yields were well below the local area average and similar to the 10-year (MASC, 2018).

Similar to urea sources, both AA and N-Serve had significantly greater yields compared to the 0 N control and thus the 0 N control was not included in the analysis of variance. For AA sources, this study measured a significant difference in grain yield between fall and spring applications (*P*=0.0319, Table 2.6) and among all sites (*P*<0.0001, Table 2.6). Both AA sources from fall applications (3.64 Mg ha^{-1}) were significantly less compared to spring (3.87 Mg ha^{-1}, Table 2.7). Similar to urea N sources, AA sources at Glenlea and Warren 2015 produced the lowest yields (2.71 and 2.92 Mg ha^{-1}, respectively). Kelburn had the greatest grain yields (5.25 Mg ha^{-1}) which were significantly greater than Ridge Road (3.96 Mg ha^{-1}) in 2017. Additionally, the two sites in 2016 had different yields, where conditions at LaSalle (4.13 Mg ha^{-1}) significantly increased grain yields compared to Carman (3.55 Mg ha^{-1}). Between AA sources there was no difference in grain yield. Compared to MASC 10-year and local average, AA sources at all sites had similar trends as urea sources.

Table 2.6 Three factor ANOVA (p values) of AA products (excluding 0 N control) from effects of N source, application time and site on grain yield, protein, NU_{grain}, NU_{bio} and residual extractable soil N.

Factors	Yield HH	Protein	NU_{grain}	NU_{bio}	Residual extractable soil N
Site Yr	<0.001	<0.001	<0.001	<0.001	<0.001
N Source	0.318	0.435	0.442	0.257	0.110
Time	0.032	0.141	0.006	<0.001	0.020
S*N	0.248	0.338	0.584	0.710	0.361
S*T	0.323	0.005	0.072	0.001	0.023
N*T	0.595	0.422	0.498	0.284	0.366
N*T*S	0.981	0.993	0.978	0.967	0.990

Table 2.7 Timing effects on mean grain yield and NU_{grain} including all AA sources.

Effects		Yield	NU_{grain}
		Mg ha^{-1}	kg N ha^{-1}
Timing			
	Fall	3.64b	91.5 b
	Spring	3.87a	99.4 a
Site			
	Warren	2.92d	75.5c
	Glenlea	2.71d	64.5c
	Carman	3.55c	99.3b
	LaSalle	4.13b	108.2b
	Kelburn	5.26a	125.4a
	Ridge Road	3.96bc	99.7b

Values followed by different letters in a column, separated by timing and site, are significantly different at $P < 0.05$.

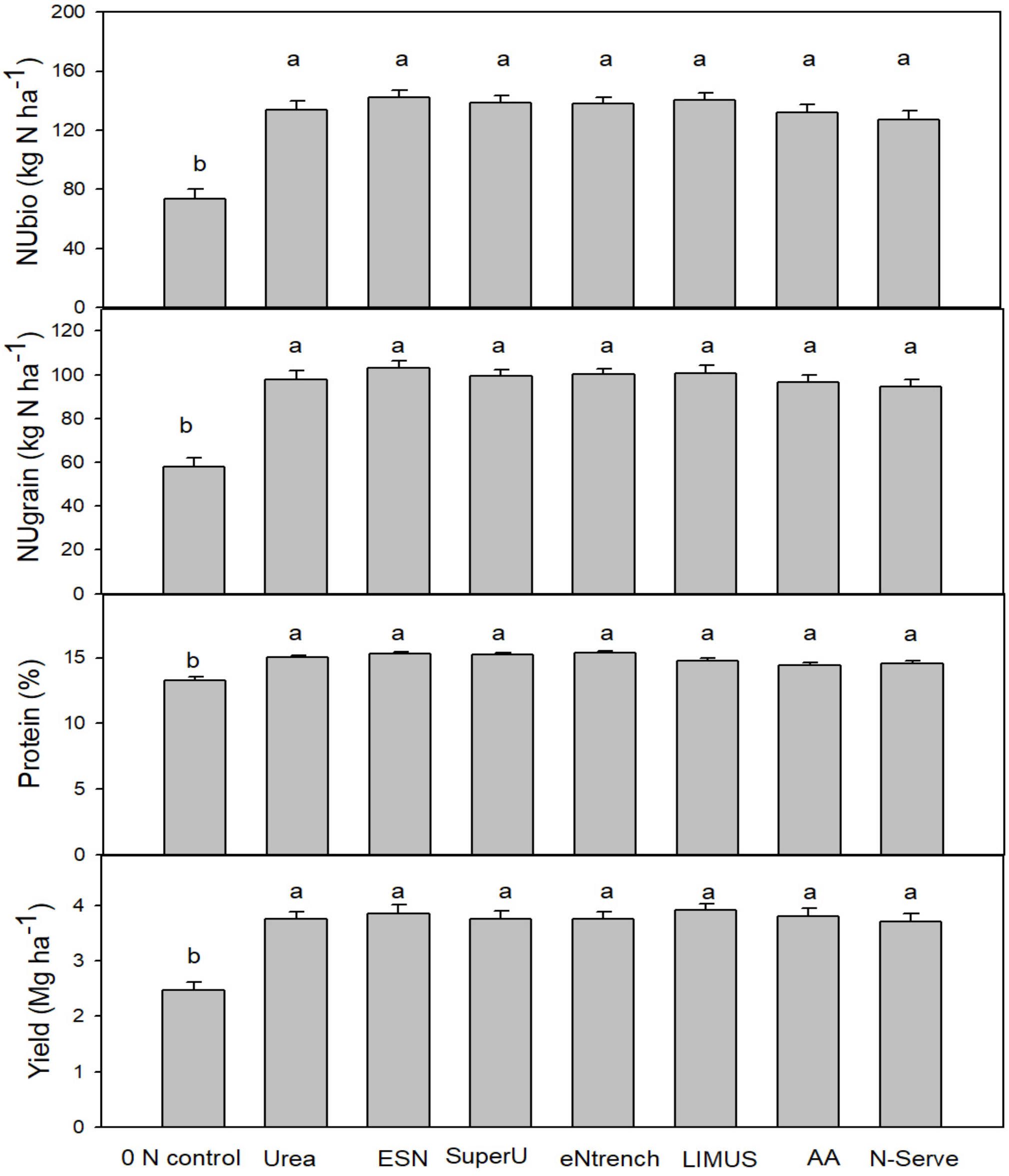

Figure 2.4 Mean grain yield, protein, NU_{grain} and NU_{bio} influenced by N sources over six site-years and spring and fall applications. Urea and AA sources analyzed separately. Bars indicate standard error of the mean.

2.4.3 Protein

Spring wheat grain protein levels had varying responses to urea N sources and timings (Table 2.4). Control treatments (0 N) were 1.2 to 2.0% lower than both urea and AA sources at each application timing, thus the controls were excluded from the statistical analysis (*P*<0.0001, Appendix A; Figure 2.4;). There was an effect of N source on protein (*P*=0.0011, Table 2.4, 8) where ESN and eNtrench had the greatest protein levels across all sites being 15.3 and 15.4 %, respectively, and were significantly greater than the urease inhibitor product, LIMUS (14.8 %). Across all sites, spring applications produced greater protein compared to fall applications, 15.1 and 15.3%, respectively (P=0.0473, Table 2.4). Highest protein values were produced at Carman (15.9%) while lowest proteins were observed at Kelburn (13.6%) and Glenlea (13.5%, Table 2.8). The average Canada Western Spring Wheat protein content in Eastern Manitoba region in 2015, 2016 and 2017 was 14.2, 13.8, 13.3%, respectively (Canadian Grain Commission, 2018). Only one site, Glenlea, was below the Eastern Manitoba protein content average for its study year.

Consistent with urea granular sources, the greatest protein content from AA sources was observed at Carman with spring applications (16.1%), and lowest protein content was at Glenlea with spring applications (12.9%, Table 2.9). At all sites, except Glenlea, spring-N applications had greater protein compared to fall applications, producing a site-year by time interaction (*P*=0.0050, Table 2.6). Compared to the Eastern Manitoba protein content average listed above, only spring-N applications at Glenlea resulted in a decrease in protein, all other treatment timings were either consistent or greater than the Eastern Manitoba average.

Table 2.8 Mean grain protein and residual extractable soil N of urea granular sources and N application timings, all site-years combined.

Effects		Protein	Residual extractable soil N
		%	mg N kg^{-1}
N Source	Urea	15.0ab	23.5b
	ESN	15.3a	31.2a
	SuperU	15.2ab	29.8a
	eNtrench	15.4a	25.1ab
	LIMUS	14.8b	24.6ab
Time	Fall	15.1b	-
	Spring	15.3a	-
Site			
	Warren	14.7b	41.8a
	Glenlea	13.5c	15.9d
	Carman	15.9a	36.1a
	LaSalle	14.9b	21.8bc
	Kelburn	13.6c	18.2cd
	Ridge Road	14.5b	27.5b

Values followed by different letters in a column, separated by N source, timing and site, are significantly different at $P < 0.05$.

Table 2.9 Site by application timing interaction of mean protein, NU_{bio} and residual extractable soil N including all AA treatments, excluding 0 N control.

Site	Time	Protein	NU_{bio}	Residual extractable soil N
		%	kg N ha^{-1}	mg N kg^{-1}
Warren	Fall	14.5bcd	87.1g	40.3ab
	Spring	14.9abcd	101.1fg	36.3ab
Glenlea	Fall	14.1cdef	83.0g	16.8cde
	Spring	12.9f	81.1g	14.1e
Carman	Fall	15.8ab	128.6cdef	33.4abc
	Spring	16.1a	154.6bcd	57.0a
LaSalle	Fall	14.4cde	137.4cde	23.9bcde
	Spring	15.3abc	190.3a	27.4bcde
Kelburn	Fall	13.2ef	160.9abc	13.5e
	Spring	13.9def	185.2ab	16.9cde
Ridge Road	Fall	14.4cde	124.2def	17.4de
	Spring	14.7bcd	121.8ef	34.8abcd

Values followed by different letters in a column are significantly different at $P < 0.05$.

2.4.4 Nitrogen Uptake

Similar to grain yield and protein, 0 N control nitrogen uptake was significantly lower than an N source ($P<0.0001$, Appendix A). There was a consistent pattern among sites where Kelburn had the highest NU_{grain} and NU_{bio} across all urea N treatments (Table 2.5). However, at Ridge Road, there was significantly greater NU_{grain} in the fall compared to the spring which resulted in a site-year by time interaction ($P=0.0003$, Table 2.4). All other sites had similar or higher NU_{grain} with spring compared to fall treatments. Also, an interaction was observed with NU_{bio} ($P=0.0008$, Table 2.4) in which spring was greater or similar to fall treatments at all sites except Ridge Road. No N source had a significant influence on NU_{grain} or NU_{bio} except against the 0 N control treatments, in which all N sources were greater.

Applying AA sources in the spring promoted greater NU_{grain} and NU_{bio} compared to fall applications (Table 2.7, 9). NU_{grain} from spring AA applications was approximately 8% greater than fall across sites. At LaSalle, spring applications had higher quantities of NU_{bio} over fall applications (Table 2.9) which resulted in a site-year by timing interaction ($P<0.0001$, Table 2.6). All other spring applications at each site were either equal or greater than fall applications. No interaction was observed for NU_{grain}, and neither NU_{grain} or NU_{bio} was affected by AA fertilizer sources except against 0 N control. There was large variation in NU_{grain} across sites ($P<0.0001$, Table 2.6). Overall, NU_{grain} averaged across all treatments in Kelburn was 20% greater than Ridge Road in 2017 (Table 2.7). Kelburn had the greatest uptake across all sites with approximately 50% more uptake compared to Glenlea. Sites in 2016 also had significantly greater NU_{grain} compared to sites in 2015.

2.4.5 Inorganic Soil Nitrogen

Inorganic soil NH_4^+ and NO_3^- concentrations were not in the ANOVA as this section serves to highlight the inorganic soil N patterns among treatments. Concentrations of fall extractable soil NH_4^+ peaked at 4 of 6 sites (Warren, Glenlea, Carman and Ridge Road) before soil freeze-up (Figure 2.5). Sources containing urease inhibitors and ESN tended to have lowest measured NH_4^+ concentrations prior to freeze-up. Nitrification followed in 2015 immediately following soil-thaw with highest measured extractable soil NO_3^- concentrations from LIMUS and AA sources. In general, LIMUS had highest measured NO_3^- concentrations among all sites following spring-thaw. Urea rapidly converted to NH_4^+ following application at Warren and preceded with low NO_3^- concentrations in the growing season. eNtrench tended to maintain N in the NH_4^+ form for the longest period following spring-thaw at Glenlea and Carman. Also at Carman, SuperU had the greatest concentrations of NH_4^+ following spring-thaw and lowest measured NO_3^- concentrations after seeding. Conversely, at LaSalle, SuperU maintained the largest concentrations of NO_3^- two and and four weeks after seeding. At Ridge Road, high accumulations of NH_4^+ did not result in high NO_3^- concentrations indicating leaching or available soil N was below the 0-30 cm sampling depth. At Kelburn, very low concentrations of NH_4^+ were detected throughout the year but ESN maintained highest levels of NO_3^- throughout the growing season. Similarly, Glenlea and LaSalle had numerically highest NO_3^- concentrations four and six weeks after seeding from ESN treated plots. In general, soil N concentrations decreased to control levels four to six weeks after seeding,

Spring-N applications followed a similar trend to fall in which all N sources decreased to 0 N control soil N levels four to six weeks after application (Figure 2.6).

Sources eNtrench and ESN in 2015 had the numerically highest NH_4^+ concentrations two and four weeks after application which followed with low NO_3^- amounts during the same time period. Similar to fall, six weeks after seeding, ESN tended to have highest measured NO_3^- concentrations. In general, LIMUS and SuperU had low NH_4^+ and NO_3^- concentrations at all sites throughout the growing season. In 2016, AA plots had numerically higher soil extractable soil N concentrations compared to urea. Conversely at Kelburn, a clear nitrification event occurred two to four weeks after spring applications. At Ridge Road there was no numerical difference among urea EEF sources.

Residual extractable soil N post-harvest mainly consisted of NO_3^-. Levels of residual extractable soil N varied significantly among urea N sources (P=0.0016, Table 2.4) with average residual extractable soil N ranging from 23.5 to 31.2 mg N kg^{-1} (Table 2.8). ESN plots maintained the largest levels of residual extractable soil N followed by SuperU. Interestingly, urea plots did not have significantly higher residual extractable soil N values compared to 0 N control, while all EEF sources had higher residual extractable soil N soil amounts (data not shown). There was no advantage applying in spring compared to fall in order to maintain residual extractable soil N levels. Glenlea had lowest residual extractable soil N amounts along with Kelburn which had highest uptake among all sites.

Between AA sources there was a site-year by time interaction from which fall 2016 and 2017 sites had lower and 2015 sites had higher residual extractable soil N (P=0.0227, Table 2.6) compared to spring applications. Warren and Carman maintained the highest levels of residual extractable soil N over the other sites among AA sources. Fertilizing in the spring with AA and N-Serve had higher levels of residual extractable soil N compared to fall applications (99.4 and 91.5 kg N ha^{-1}, respectively).

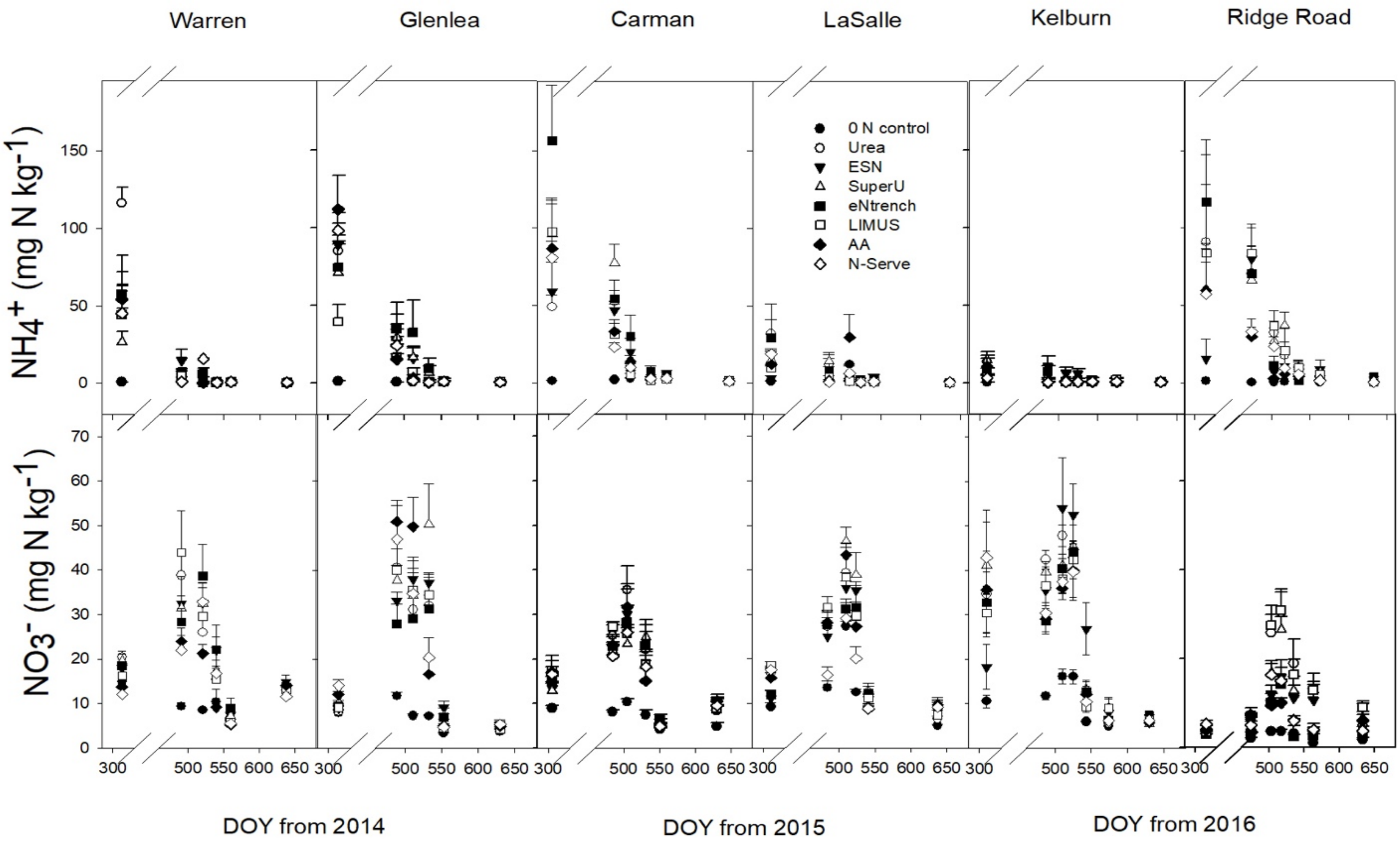

Figure 2.5 Extractable soil NH_4^+ and NO_3^- affected by fall fertilizer N sources for six site-years. Bars indicated standard error of the mean.

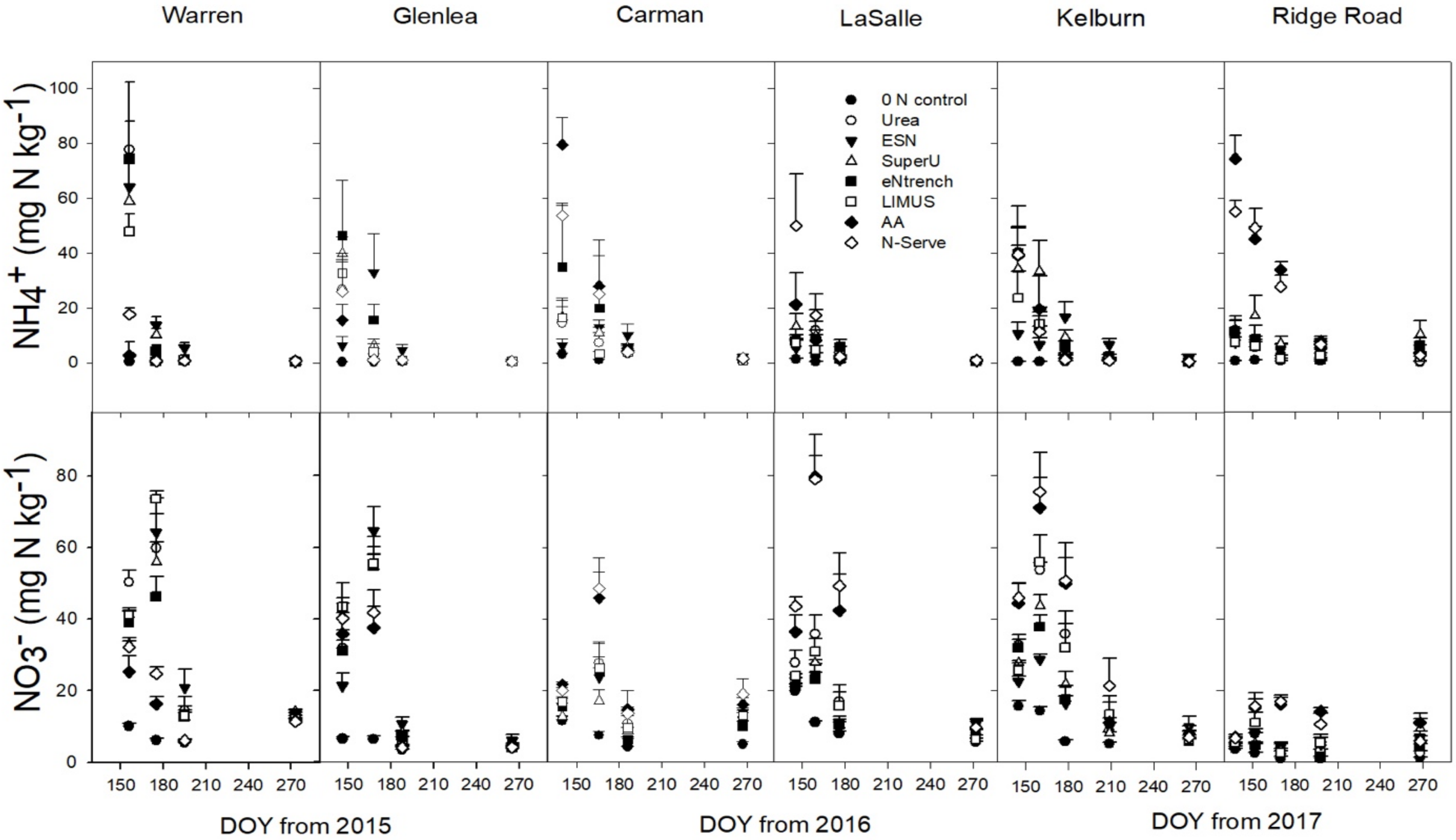

Figure 2.6 Extractable soil NH_4^+ and NO_3^- affected by spring fertilizer N sources at six site-years. Bars indicated standard error of the mean.

2.5 Discussion

2.5.1 Grain Yield

Grain yields varied among site-years, ranging from 2.60 to 5.50 Mg ha^{-1} for both urea and AA sources. Thapa et al. (2015) proposed that yield benefits under dry conditions using EEF are minimal since they are not conducive to high N loss compared to high moisture prone soils. Yet, in poorly drained fields that are conducive to high moisture conditions, the use of EEF can reduce N losses and promote greater yields over conventional fertilizers (Tiessen et al., 2008; Thapa et al., 2016; Grant et al., 2016). This study was subject to a mix of weather conditions from 2014-2017 but no site was observed to have an N source effect on grain yield.

Among urea and urea based EEF, there was a site-year by time interaction where fall-applied yields at 2015 sites were below spring-applied, both 2016 sites and 2017 Kelburn were similar between application timings, and 2017 Ridge Road fall-applied yields were greater than that of spring. Therefore, one of the most important factors influencing grain yields were environmental and soil conditions. The 2014-2015 season experienced very wet conditions in the fall and spring, which may have led to significant losses of inorganic N during spring-thaw or before soil freeze-up. At 2016 sites, fall applications were applied close enough to freeze-up to discourage N losses and maintain N in the urea or NH_4^+ form. The similar yields observed across application timings at LaSalle and Carman is paralleled with similar fall and spring NH_4^+ and NO_3^- concentrations throughout the growing season. Similar to this current study, Tiessen et al. (2008) measured N soil concentrations and yields with nitrification and urease inhibitor products and observed similar yields between late-fall and spring-N applications. Therefore, this current study established that it is difficult to predict a safe fall application date to prevent significant N losses, but if N is applied late enough in fall, consistent yield benefits can be produced compared to spring applications.

Surprisingly, in the sand based soils at Ridge Road, grain yields tended to be greater with fall compared to spring applications. Grant et al. (2012) attributed similar yields and N accumulation from fall and spring applications in sandy loam soils due to N being more limited compared to clay soils. If this were true, then both spring and fall should have had similar yields. Yield measurements from AA sources did not confirm that N was limiting throughout the whole field since fall applications were consistently less compared to spring. Therefore, a significant event must have occurred to the urea granules to promote more available N to the plant in the fall compared to spring. Speculation on this event is discussed further below. Overall, similar inconsistencies across years and sites have been observed by previous studies in the Northern Great Plains and have concluded no increase in yield from fall-applied EEF compared to spring-applied urea or EEF (Grant et al., 2016; Karamanos et al., 2014). Averaging all timings and sites together, there was no significant grain yield benefit to applying N with EEF over conventional sources of urea or AA.

2.5.2 Protein

Averaged across sites, protein levels from all N sources (including urea and AA sources) were significantly greater than the 0 N control. However, at LaSalle and Carman, 0 N control protein measurements were not significantly different than any other N source from each respective application timing. Yet, Carman and LaSalle had the highest protein values of any other site which indicates that the grain yield potential was satisfied for both 2016 sites resulting in high protein across all treatments. The source of excess N cannot be explained from seasonal soil testing as N levels in 2016 were similar to levels in 2015 and 2017, except at Ridge Road. A study by Alkier et al. (1972) reported a greater relationship between soil N and protein when sampled to a depth of 122 cm compared to 61 cm. Thus, the excess available N for protein filling is attributable to N further down in the soil profile. Additionally, in Grant and Flaten (1998), the authors stated legume residues nitrify rapidly resulting in higher protein concentrations for the following cropping year compared to those which nitrify slowly. With average 0 N control proteins above 13%, protein levels in this current study agree with Grant and Flaten (1998) in which soybean residues provided a significant portion of inorganic N for grain filling to the following crop. Therefore, the combination of potential available N below 60 cm and mineralized N from soybean residue was sufficient to supply high grain protein. However, past years cropping history beyond soybean was not known at any field site but should be acknowledged that previous crops before soybeans may of influenced N availability and organic carbon pools.

In regards to environmental influence on grain protein, LaSalle and Carman recorded above normal precipitation amounts, which is consistent with Gao et al. (2012) who also reported highest protein levels in the year of most cumulative growing season precipitation. This precipitation influence on protein is inconsistent with Campbell et al. (1996) in which N was diluted by yield, but this was under conditions where limited N was available to meet the requirements of protein synthesis. Campbell et al. (1996) observed that with increasing rates of N to satisfy grain yield potential any excess N was used for protein filling, confirming that when sufficient N is available in the soil to meet yield then the remaining N will be directed to protein. The opposite occurred at Glenlea which had lowest protein from both urea and AA sources. This can be due to very large precipitation events during the grain filling period in July reducing available N and being lost to leaching or denitrification. Specifically, the saturated soil conditions from the clay soil texture at Glenlea retained a lot of moisture further increasing its susceptibility to N loss.

The soil at Kelburn has a reputation for having very high mineralizable N throughout the growing season. Unpublished soil tests 20 meters from the Kelburn field site from April 2017 indicated soil residual N levels greater than 100 kg N ha^{-1} to a depth of 120 cm (data not shown; Don Flaten, personal communication). Thus, a large amount of N was likely available early in the growing season for crop yield. In addition to high available soil N, there was limited moisture available later in the growing season which discouraged N uptake post-anthesis (Grant and Flaten, 1998). Therefore, due to the high yield potential from early season available N, and dry conditions limiting N movement, the protein accumulation was restricted. In Manitoba, Gao et al. (2012) had adequate field moisture and low yields resulting in N accumulation during grain filling. This present study confirms previous research in which consistent N supply and larger yields will result in decreased protein due to N dilution (Campbell et al., 1996; Campbell et al., 1977). To confirm the latter, residual extractable soil N levels were lowest from urea and AA sources at Kelburn compared to the other sites. This lack of residual extractable soil N means there was insufficient N for grain filling processes later in the growing season.

In the current study, there was a significant difference in protein between EEF urea sources. Specifically, LIMUS (urease inhibitor) protein levels were 0.6 and 0.5% lower compared to eNtrench and ESN, respectively. LIMUS is a urease inhibitor which retains N in the urea form. It is possible that compared to eNtrench and ESN, LIMUS inhibited early season biomass production by maintaining N in the urea form. Grant and Flaten (1998) stated that absorption of N from soil to grain is minor compared to the translocation of biomass N. Thus, limited N uptake early in the season retarded the ability for available

N to translocate from the biomass to the grain for protein maintenance. The most apparent decrease in protein by LIMUS was observed at Kelburn (data not shown) where dry conditions may have inhibited the translocation of assimilates from the straw to the grain (Campbell et al., 1977). The enhanced benefit of using eNtrench and ESN was most likely due to the availability of N early and throughout the growing season. Early season available soil NH_4^+ was high enough to promote early season uptake but not nitrification to NO_3^- where it is prone to loss. Yet, compared to urea, there was no significant benefit or disadvantage to using EEF. This is consistent with multiple studies in southern Alberta and Montana under winter wheat which observed no response to an NBPT inhibitor compared to urea (McKenzie et al., 2010; Mohammed et al., 2016). N-Serve had no benefit to increasing grain protein over AA alone which is consistent with Karamanos et al. (2014) who observed no increase in protein using a nitrification inhibitor over urea and AA. Additionally, ESN has been tested extensively in the Northern Great Plains region under varying soil conditions and, similar to this current study, was inconsistently effective at promoting increased protein compared to urea (Malhi et al., 2010; Grant et al., 2012; Walsh and Girma, 2016). In general, protein levels were fairly high throughout the six site-years with small increases by ESN and eNtrench compared to LIMUS, but further investigation should be completed to understand the effects of EEF under dry conditions.

Lastly, urea and AA sources had a time effect and site-year by time interaction, respectively. Urea sources including all EEF had higher protein from spring applications compared to fall. However, this cannot be explained by available soil N throughout the growing season because by early June most of the available N between fall and spring applications were comparable at each site. Therefore, similar amounts of N must have been lost from nitrification or denitrification at the early stages of the growing season.

Grant et al. (2016) tested ESN and SuperU in southwestern Manitoba on spring wheat and found higher protein with fall banded ESN compared to fall and spring urea on a silty clay and clay loam soils, but this was inconsistent across years. As well in that same study, spring-applied ESN was recommended over SuperU. Mohammed et al. (2016) grew winter wheat in the Northern Great Plains and reported greater protein content from spring compared to fall-applied SuperU. Across other studies in the Northern Great Plains measuring fall and spring applications of EEF, results are inconsistent, but they all provide the same conclusion in that EEF are best used in conditions that are conducive to N loss (Malhi et al., 2010; Grant et al., 2016; Karamanos et al., 2014). Yet, in this current study, the protein content only differed by 0.2% between fall and spring which shows that if it is time saving and economical to apply fertilizer late-fall compared to spring, minimal protein losses would occur and revenue lost by the farmer would be negligible.

2.5.3 Nitrogen Uptake

The highest levels of NU_{grain} from urea and AA sources, and NU_{bio} from urea sources was identified at Kelburn which confirms findings from above that this site had a high yield and uptake potential due to management history. The patterns between yield and N uptake were generally consistent, causing a site-year by time interaction, in that spring 2015 sites had larger uptake than fall, and fall Ridge Road had much greater fall NU_{grain} and NU_{bio} compared spring. Ridge Road fall N applied NO_3^- concentrations were much higher throughout the growing season compared to spring. It appears that spring-N sources converted to NH_4^+ but volatilized as NH_3 due to dry and warm soil conditions right after application. Greater concentrations of NH_3 near the surface will promote greater N losses from volatilization (Grant et al., 2014; Francis et al., 2008). Nitrogen losses will be even more accelerated in coarse textured soils due to the low cation exchange capacity to hold NH_4^+ ions. Fall sources were not conducive to significant N losses compared to spring because the N granules moved further down the soil profile during spring-thaw, reducing risk for NH_3 volatilization but still maintaining a form not conducive to leaching. With minimal losses over spring-thaw and limited rainfall during the growing season, N was available for plant uptake throughout the growing season. To our best knowledge, this is the first experiment conducted on a loamy fine sand based soil testing fall and spring applications of EEF in Manitoba.

Additionally, there was a trend at LaSalle in which spring had considerably more NU_{grain} and NU_{bio} than fall. There is no explanation for this as Carman had similar weather conditions but LaSalle had a much larger clay content which may have fixed more NH_4^+ during spring-thaw. Therefore, it may be beneficial to retain the N in the urea form for as long as possible but this was not confirmed from the current experiment since ESN and LIMUS had similar N uptake compared to nitrification inhibitor sources. The urea results from LaSalle were consistent with AA sources where spring-N applications had greater NU_{bio} over fall applications. Compounding the AA measurements confirmed that large amounts of NH_4^+ fixed in the heavy clays during spring-thaw. Tiessen et al. (2008) observed soil N concentrations with urease and nitrification inhibitors, and witnessed a greater amount of N remaining in the soil at freeze-up compared to urea. Thus, the longer retention time in the form of NH_4^+ may increase ammonia volatilization during spring-thaw (Thapa et al., 2015). Likewise, to other agronomic parameters measured in this study and previous studies in the Northern Great Plains, N sources and application timings provide inconsistent N uptake measurements with spring being the recommended application timing over fall to maximize N uptake (Malhi et al., 2010; Mohammed et al., 2016; Grant et al., 2012).

Lastly, excess N may have been supplied by EEF during this experiment. This hypothesis may be conclusively answered by residual extractable soil N levels measured at the end of the growing season. Two EEF sources, ESN and SuperU, had significantly higher residual soil N amounts compared to urea. Specifically, these sources had 33 and 27% more residual soil N remaining at the end of the growing season compared to urea. Therefore, if rates were reduced by approximately 25%, would similar yield and protein levels be observed over 100% urea? Thapa et al. (2017) also concluded that the non-response from nitrification and double inhibitors was because adequate N was provided to meet wheat grain requirements. Locally, in Manitoba, the only study that tested EEF at different rates with spring wheat (SuperU and ESN at 0.5x, 1.0x and 1.5x recommended rates against urea) recommended urea over any EEF (Grant et al., 2016). Therefore, from the lack of response by EEF in this present study, it may be beneficial to test a range of EEF N rates and provide a conclusive agreement on which EEF rate is most effective in the Manitoba region.

2.6 CONCLUSION

Protein levels were highest at sites with greatest total growing season rainfall. As well, averaging among sites, LIMUS had significantly lower protein compared to ESN and eNtrench which may suggest that the LIMUS fertilizer did not supply adequate inorganic soil N due to its design to maintain N in urea form instead of slowly releasing inorganic N or retaining in NH_4^+ form. Yet, fall applications of urea sources had lower proteins levels to spring when all sites were combined. Grain yields were inconsistent from year to year but among urea and AA sources, grain yields did not differ between application timings or N sources.

The agronomic consistency and advantage of EEF to grain yield and protein may also be environment dependent but this was outside of the scope of this study. However, varying soil textures and conditions such as soil moisture and temperature, and precipitation amounts were evident in this study and likely affected N availabilities for grain and protein. This study does provide merit for fall applications since similar observations between fall and spring treatments were observed when averaging N sources (urea and AA sources separately) together. In particular, the 2016 results highlighted the benefit of applying N fertilizer as close as possible to freeze-up to maximize yields. Additionally, in sand textured soils, it may be beneficial to apply EEF in the fall to limit NH_3 volatilization and move urea granules further down into the soil profile without being leached. Yet, in heavy clay soils, the abundance of negatively charged ions may retain NH_4^+ during spring-thaw and limit use by the crop. There was no difference between AA and N-Serve, but SuperU and ESN had greater residual extractable soil N levels compared to urea which can imply that reduced rates of EEF may produce similar yields and protein.

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3. Right Time and Right Source Nitrogen Management to Reduce N_2O emissions in Southern Manitoba

3.1 Abstract

The annual application of synthetic nitrogen (N) fertilizers to cropland is a major factor impeding nitrous oxide (N_2O) gas reductions for the Canadian agricultural sector. Fall N applications are common in Manitoba but induce significant N_2O emissions during spring-thaw. One solution to mitigate N_2O emissions may be with enhanced efficiency fertilizers (EEF) of N but not much is known about EEF on thaw N_2O. The objective of this study was to quantify and compare N_2O emissions of EEF and non-EEF, and application timings in southern Manitoba from Canadian hard red spring wheat (*Triticum aestivum L.*). Using the static-vented chamber technique, both late-fall and spring applications of urea and anhydrous ammonia (AA) using urease inhibited, nitrification inhibited, and controlled-release EEF sources were examined in two replicated plot trials over three years. Fall and spring-N sources were applied at soil temperatures around 5°C and with the seed in May, respectively. Urea N products were applied mid-row banded and AA knifed in. Fluxes were determined on approximately 20 days per site and linear interpolated between sampling dates to determine area-scaled cumulative N_2O emissions. Among urea sources, five out of six sites had a N source effect with N sources eNtrench and SuperU containing nitrification inhibitors and polymer-coated ESN having lower cumulative N_2O emissions compared to urea in at least one of five sites. Among the five sites, eNtrench and SuperU reduced ΣN_2O by 54 and 43%, respectively, whereas ESN, the urease inhibitor LIMUS and the nitrification inhibitor N-Serve, did not over spring-applied urea and AA. Peak N_2O emission periods coincided with precipitation events > 10mm. Combining urea sources, fall-applied cumulative N_2O emissions were similar to that for spring at 4 of 6 sites. No

timing effect was recorded between AA sources. Nitrogen applied-scaled emission factor (EF) and yield-based emission intensity (EI) followed similar trends as ΣN_2O where eNtrench = SuperU < ESN = LIMUS = urea among five sites with an N source effect. From this study, fall and spring-applied urea plus eNtrench and SuperU can be recommended as a best management practice as they reduced ΣN_2O emission by over 40% at multiple sites compared to fall and spring-applied urea.

3.2 Introduction

In Canada, the agriculture sector is the leading source of nitrous oxide (N_2O) emissions, accounting for approximately 70% of total emissions (Environment Canada, 2016). Lesser amounts of N_2O are produced from organic cropping systems (Westphal et al., 2018) whereas anthropogenic nitrogen (N) inputs in the form of fertilizer and manure accelerate and amplify the rate of N_2O emissions (Gao et al., 2015; Asgedom et al., 2014; Glenn et al., 2012). With N_2O being a major radiative forcing agent and having a global warming potential 265 times that of carbon dioxide (CO_2), the current rate of N_2O emissions cannot be sustained to mitigate rising surface temperatures and climate change (IPCC, 2014).

Specifically, the application of synthetic N fertilizer is the greatest source of N_2O emissions (Environment Canada, 2015). The Northern Great Plains of Canada accounts for 80% of all national synthetic N fertilizer use which makes it the largest producer of N_2O from soils (Statistics Canada, 2013). Additionally, N fertilizer use in Canada is increasing substantially (58% increase from 1990-2008; Environment Canada, 2011). The use of commercial fertilizers, mainly N, phosphorus, and potassium is attributable to 30-50% of crop yields (Stewart et al., 2005). However, the success of increasing N fertilizer use is clouded by the overwhelming demand by the public to reduce Canada's greenhouse gas (GHG) emissions to 70% of 2005 emission levels (Environment and Climate Change Canada, 2016). Conversely, with a growing global population, Canada is aspiring to expand agricultural production and be a key global food provider (Burton et al., 2008).

Urea and anhydrous ammonia (AA) are the most common N fertilizers in the Northern Great Plains (Tenuta et al., 2016; Gao et al., 2015). Generally, N applied as fertilizer is mainly cycled through nitrification and denitrification processes, but more recently nitrifier-denitrification has been observed to be a significant N_2O source (Subbarao et al., 2006; Ryden, 1981; Mosier, 1994; Kool et al., 2010). Nitrification is the biological conversion of reduced ammonia (NH_3) to oxidized nitrite (NO_2^-) or nitrate (NO_3^-) by heterotrophic and autotrophic microorganisms, also known as nitrifiers, in aerobic environments (Norton, 2008). The intermediate hydroxylamine (NH_2OH) is the molecule responsible for N_2O production when there is a greater availability of electrons for NH_2OH oxidation compared to NH_3 oxidation (Signor and Cerri, 2013; Wunderlin, 2012). Denitrification is the reduction of NO_3^- to dinitrogen gas (N_2) where denitrifying bacteria use NO_3^- as a terminal electron acceptor when aerobic respiration is limited in low oxygen environments (Wrage et al., 2001). Under increasingly aerobic conditions, incomplete denitrification can occur, inhibiting N_2O reductase and releasing N_2O as an intermediate (Wrage et al., 2001). Nitrifier-denitrification is an equally important pathway where N_2O is released under intermediate or low oxygen conditions as NO_2^- acts as an electron acceptor and nitrifiers use the pathway from NO_2^- to N_2 for anaerobic respiration (Wrage et al., 2001; Shaw et al., 2006; Ward, 2013; Kool et al., 2011).

The production of N_2O is seasonally variable with two large episodic emission periods in northern climates (Maas et al., 2013). The first event that occurs in a calendar year is during spring-thaw (Wagner-Riddle et al., 2008). In Canada, spring-thaw can account for over half of total N_2O annual emissions in agriculture soils (Wagner-Riddle et al., 2007, 2017). It is well known that denitrification is the main process contributing to spring-thaw N_2O production but it has been debated as to at which soil depth are microbial organisms active and contributing to N_2O production (Wagner-Riddle et al., 2008). Recent studies in Canada have confirmed that the majority of emissions occur from the top soil layer in which death and lysis of soil microorganisms provide N dissipation (Ryan et al., 2000; Tenuta and Sparling, 2011; Wagner-Riddle et al., 2008). Similar findings have indicated that high soil moisture, rapid increases in soil temperature, length of soil freezing, NO_3^- concentration and soil depth control microbial activity and the magnitude of spring-thaw N_2O emissions (Wagner-Riddle et al., 2008; Tenuta et al., 2016; Tenuta and Sparling, 2010). The second episodic event is weeks after spring-N application which is attributable to high available N increasing microbial activity, N mineralization, and temperature and oxygen respiration following rainfall (Glenn et al., 2012; Asgedom et al., 2014; Tenuta and Beauchamp, 2003; Gao et al., 2015). The production of N_2O is influenced by environmental and soil conditions that control soil N processes such as availability of NH_3/ammonium (NH_4^+), aeration, soil temperature and moisture, pH, rainfall events, NO_3^- concentrations, soil texture and organic matter (Eagle et al., 2017; Liu et al., 2017; Westphal et al., 2018; Tenuta and Beauchamp, 2000; Cai et al., 2016).

Enhanced efficiency fertilizers (EEF) of N are a potential solution to mitigate N_2O loss from synthetic N applications. The design and function of EEF is to control biological N transformations and sync the availability of inorganic N to plant N demand over the growing season (Olson-Rutz et al., 2009; Trenkel, 2010). Most commonly, EEF target urea hydrolysis or nitrification, or delay N release from urea granules. Nitrification inhibitor products such as eNtrench and N-Serve consist of main active ingredient nitrapyrin which selectively inhibits ammonia-oxidizing bacteria and reduces the oxidation of NH_4^+ to NO_3^-. In contrast, urease inhibitor product LIMUS contain main active ingredients N-(n-butyl) thiophosphoric triamide (NBPT) and N-(n-propyl) thiophosphoric triamide (NPPT) which restrict urease enzymes from hydrolyzing urea to NH_3 plus NH_4^+ (Trenkel, 2010; Gao et al., 2015). SuperU is a common product in the Northern Great Plains containing both a nitrification, dicyandiamide (DCD), and urease inhibitor, NBPT. Lastly, EEF product, Environmentally Smart Nitrogen (ESN) does not inhibit biological activity but delays the release of urea with a polymer-coating shell. With ESN, soil conditions such as soil moisture and temperature control the rate of urea release into the soil solution (Gao et al., 2015).

Multiple reviews have been conducted comparing the effectiveness of urease and nitrification inhibitors, and polymer-coated urea products. Akiyama et al. (2010) reported reduced N_2O emissions from nitrification inhibitor and polymer-coated N fertilizers by 38 and 35%, respectively, compared to conventional fertilizers, whereas urease inhibitors had no significant reduction. Additional reviews by Qiao et al. (2015) and Gilsanz et al. (2016) observed similar findings with an average reduction of 44 and 42%, respectively, from nitrification inhibitors over conventional fertilizers. In the most recent review, SuperU was recommended over ESN and a urease inhibitor fertilizer containing NBPT for reducing N_2O emissions (Eagle et al., 2017). Lastly, researchers from the Northern Great Plains region comparing EEF across multiple soil types and management conditions have reported reductions of 38, 30 and 19% with nitrification inhibitors, double inhibitors and controlled-release N fertilizers (Thapa et al., 2016).

It appears the evidence is overwhelming in that EEF can significantly reduce N_2O emissions over conventional N fertilizers. However, in Manitoba, it is common to apply N fertilizers in the fall due to lower retail costs, reduced fall workloads, increased seed bed preservation and generally drier soil conditions (Tiessen et al., 2006; Tenuta et al., 2016). The few studies that have reported on fall and spring N_2O emissions reported greater or similar emissions from fall compared to spring applications (Burton et al., 2008; Parkin and Hatfield, 2010; Soon et al., 2011). Contrary to the previously mentioned studies, applying N fertilizer late-fall when soil temperatures are around 5°C slows nitrification and reduces the presence of NO_3^- in the soil before winter (Tiessen et al., 2006). A recent study by Tenuta et al. (2016) confirmed the benefit of late-fall application with lower cumulative N_2O emissions from fall AA applications compared to spring. Additionally, the increased effectiveness of banded EEF over broadcasted has been confirmed in Manitoba soils (Gao et al., 2015). Thus, there is a need for a field study to test an array of polymer-coated, nitrification and urease inhibitor products, and apply recently tested management practices to understand the efficacy of these EEF in mitigating N_2O soil emissions.

This research experiment was the first of its kind to be tested in Manitoba in that it evaluated fall-applied N enhanced efficiency fertilizers in the form of urea and AA, which is currently not considered a fertilizer best management practice. This study captured N_2O emissions from thaw to freeze-up, and explored any significant differences in N_2O emissions with each fertilizer product. The objectives of this study were to (i) investigate if urea and AA based EEF reduce area-scaled cumulative N_2O, and N_2O emissions during spring-thaw and post-spring fertilization compared to conventional urea and AA fertilizers, and (ii) applying EEF in late-fall results in similar cumulative N_2O emissions compared to spring EEF and non-EEF applications.

3.3 Materials and Methods

3.3.1 Experimental Site and Soil Descriptions

This field experiment was managed over a period of three growing seasons at six locations from fall 2014 to fall 2017 (two locations per growing season) in southern Manitoba. The locations were no farther than 100 km from Winnipeg, near Glenlea (49°39’3” N, 97°12’34” W), Warren (50°8’32” N, 97°30’21” W), Carman (49°26’16” N, 98°1’19” W), La Salle (49°42’2” N, 97°11’37” W), Ridge Road (49°26’33” N, 98°20’30” W) and Kelburn farms (49°41’52” N, 97°7’23” W), Manitoba. Warren and Glenlea sites were managed in the 2014-2015 season, Carman and LaSalle in 2015-2016, and Kelburn and Ridge Road in 2016-2017. Field sites changed each year and the selection criteria was based on soil residual NO_3^- concentration being no more than 100 kg N ha^{-1} in the fall, previous crop the season before being soybean and a uniform flat topography.

Complete soil descriptions and complete soil analysis from each site are provided in chapter two. In general, the soil at Glenlea and Warren in 2015 consisted of a clay and clay loam, respectively. At Carman and LaSalle in 2016 the soil was a silty clay loam and clay, respectively. Lastly, in 2017, Kelburn and Ridge Road soils were composed of a clay and loamy fine sand, respectively.

3.3.2 Experimental Design

The complete treatment description and experimental design is provided in chapter two. In brief, N fertilizers were applied in late-fall or at seeding and consisted of (i) control with no N application, (ii) urea (46-0-0), (iii) urea + eNtrench, (iv) urea + LIMUS, (v) SuperU (46-0-0), (vi) ESN (44-0-0), (vii) anhydrous ammonia (82-0-0), (viii) anhydrous ammonia + N-Serve. The experimental design was a randomized split plot block design with four replicate plots of 6 by 3.4 m. Urea and AA treatments were analyzed individually being separated by a 7-meter buffer at each site.

3.3.3 Weather and Soil Monitoring

Climate averages and on-site weather monitoring for each site is provided in chapter two. During gas sampling days, soil temperature at a depth of 2.5 centimeters and air temperature 60-120 cm above ground (in the shade) was taken with a Traceable Longstem Thermometer (Fisher Scientific^{TM}, Ottawa, ON). Soil volumetric moisture content (VMC), at a depth of 5 centimeters in soil, was measured with a HH2 Moisture Meter (Delta-T Devices Ltd, Cambridge, UK) within 15-30 cm of a chamber. Each site in 2016 and 2017 measured mean daily soil temperature at a depth of 5, 10 and 20 centimeters and VMC at a depth of 5 to 10 centimeters. A copper-constantan thermocouple (Type-E, OMEGA Engineering Inc., Laval, QC) measured mean daily soil temperature and a capacitance probe (5TM, Decagon Devices Inc., Pullman, WA) measured mean daily soil VMC. On-site weather stations at 2016 and 2017 sites were positioned in between urea and AA plots, and used when manual measurements during daily gas sampling periods were not retrieved.

3.3.4 Nitrous Oxide Gas Sampling and Analysis

Sampling for N_2O emissions occurred during the months of October to December (after fall N applications) and in the following year from March to October at each site. The sampling interval after fall N application was one to two times per week until soil freeze-up. The sampling interval during spring-thaw and after spring-N fertilizer application was one to two times per week and then once every one to two weeks later in the growing season from mid-June to October. Major gaps between gas sampling days occurred in August and September periodically at each site due to harvest and soil sampling activities. Only fall-applied and 0 N control treatments were sampled after fall N application, during spring-thaw and days prior to seeding. After seeding and spring-N treatment applications, both fall and spring-N treatments were sampled for N_2O emissions. Total number of days with flux measurements at each site for fall and spring treatments, respectively, were: Glenlea 16,11; Warren 21,14; Carman 16, 12; LaSalle 23,14; Kelburn 31,16; Ridge Road 24,16.

The static-chamber sampling method was used to capture N_2O emissions (Tenuta et al., 2010). A plastic acrylic chamber consisted of a rectangular collar base with dimensions approximately 0.40 m x 0.20 m; 0.10 m height and a rectangular lid 0.40 m x 0.20 m with an internal vent tube to equilibrate pressure and temperature. Collars were inserted approximately five cm deep into the soil immediately after soil N fertilizer application. Four collars were placed in each plot between separate plant rows over two fertilized bands and two non-fertilized bands. Each pair of chambers, one on fertilized and one on non-fertilized bands, were placed on opposite sides of the plot to establish adequate spatial representation (Figure 3.1). Each collar remained open during the growing season, except during periods of gas collection. During sampling, lids were placed on top of the collar and sealed by wrapping rubber bands over the chamber. The gas samples from each chamber were collected by extracting air from within the headspace of the chamber, by a rubber septum on the lid, with a 20 mL disposable syringe (Becton-Dickinson, Franklin Lakes, NJ) at consistent intervals of 0, 20, 40, and 60 minutes (Figure 3.2). Extracted gas samples were immediately injected into 12 mL, twice helium-flushed and evacuated to 0.05 MPa, glass vials. Before each sample, except for the zerominute gas extraction, air was drawn from the chamber and released back into the chamber to remove stagnant air in the sampling port. Gas sampling took place between 9 am and 2 pm. All vials were transported back to the University of Manitoba Soil Ecology lab and stored in darkness until analysis.

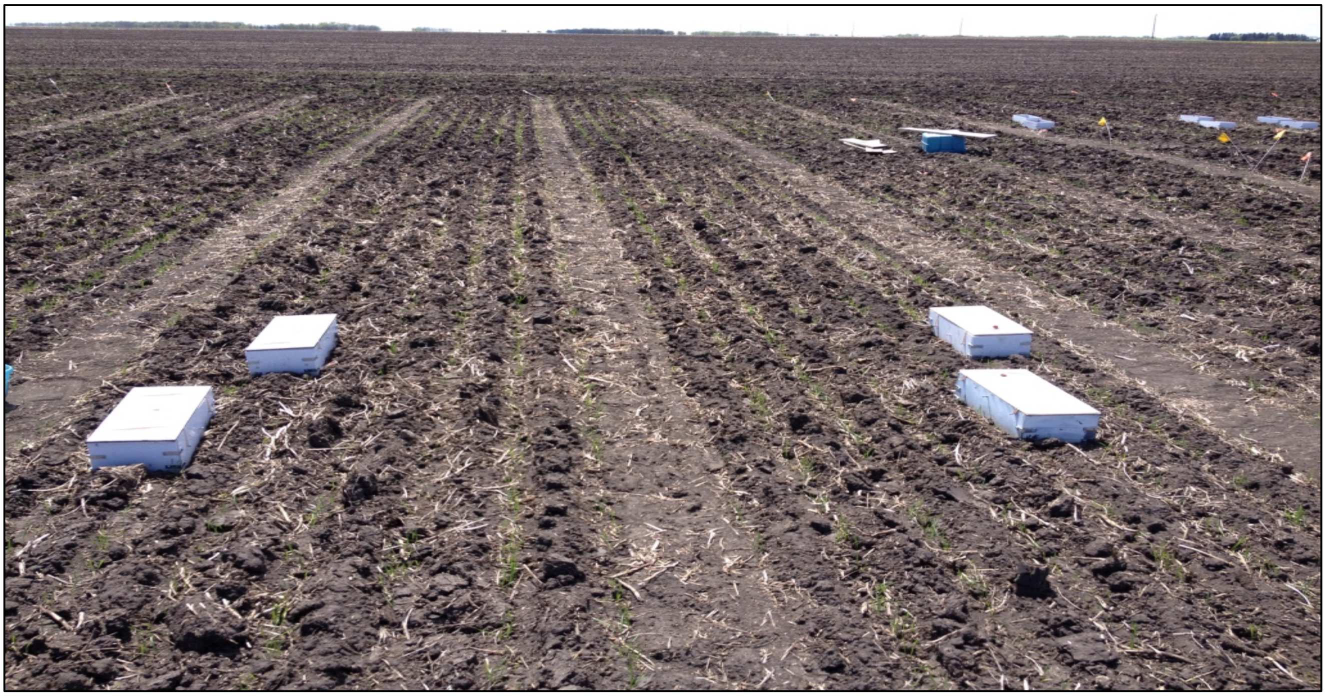

Figure 3.1 Chamber placement in a plot with a chamber place on band and off band at each side of the plot.

Concentrations of N_2O in each vial were determined using a gas chromatograph equipped with an electron capture detector (Varian CP-3800, Bruker Daltonics LTD., Milton, ON) and a CombiPAL robotic sample introduction system (CombiPAL, Zwingen, Switzerland). The chromatograph was calibrated using a dilution of pure N_2O gas (Welders Supplies, Winnipeg, MB). Certified reference standards were positioned at every 10th vial position during gas chromatography in which if the concentration measured


Figure 3.2 Sampling for N_2O with a static-vented chamber.

differed by more than 5% from expected concentration then the sample analysis or calibration was repeated. The concentrations analyzed from each chamber were used to calculate N_2O emission rates (ng N m^{-2} min^{-1}) using the HMR package (Pedersen, 2015) implemented with the R language (R Core Team, 2017). The HMR package advised to apply one of three regression approaches to estimate N_2O gas emissions from sampling. A nonlinear model was recommended if the rate of N_2O accumulation decreased with time. A linear model was recommended if the rate of N_2O accumulation was consistent with time or in the absence of a clear trend in gas concentration with time (Gao et al., 2015).

All four chambers were averaged to acquire a plot flux. Occasionally, a chamber would be discovered to be inadequate or a chamber septum would break from the lid rendering the sample not usable. The chamber malfunction would not be re-sampled on that day and plot flux would decrease to three, and on rarest occasions to two chambers per plot. Specifically, to include a plot where only two chambers were usable, one chamber on adjacent sides of the plot must have been sampled. If only one chamber remained, then the plot was omitted. At the Kelburn site during spring-thaw, the third replicate was under water for multiple sampling days. Thus, the third replicate was omitted from all sampling days to maintain consistent statistical power throughout the season. All the other sites included four replicates in the calculations and analysis. Daily estimated summations of N_2O plot fluxes were linearly interpolated between each gas sampling date to determine an area-scaled cumulative emission (ΣN_2O kg N ha^{-1}) for each plot. It was assumed that the N_2O emission rate measured on sampling days was representative of the average daily emission rate of that day. To account for background N_2O production in a field, fall 0 N control ΣN_2O from fall N application to seeding were added to all spring treatment ΣN_2O values. Cumulative N_2O emissions were then expressed as an index of spring urea or AA emissions. This index indicates that spring-applied urea or AA N_2O emissions were set to 100%. All urea and AA sources were then calculated against spring urea and AA to determine a percentage reduction or increase over spring-applied urea or AA. Thus, each urea and AA treatment within a replicate was expressed as a percentage of spring urea or AA ΣN_2O emissions at each site.

Yearly fertilizer N applied-scaled emissions factors (EF) were expressed as a percentage of kg N_2O-N kg^{-1} N applied, which was calculated by the following equation:

$$\mathrm{EF} = \frac{(\mathrm{N_2O_{fert}} - \mathrm{N_2O_{control}})}{\text{Applied N}} \times 100,$$

where N_2O_{fert} is the ΣN_2O (kg N ha^{-1}) for a N fertilizer treatment, N_2O_{ctrl} is the ΣN_2O (kg N ha^{-1}) of the 0 N control, and applied N (kg N ha^{-1}) is the application rate of N fertilizer.

Grain yield-based emission intensities (EI) were calculated as a ratio of growing season ΣN_2O to grain yield for each treatment, overall expressed as kg N_2O-N Mg^{-1} yield.

The reporting years for ΣN_2O emissions begun on January 1st of the crop year starting with fall N fertilizer applications. Specifically, fall-applied ΣN_2O emissions, EF and EI were expressed during the periods after fall fertilization (from the date of fall N application to the first date of daily average soil temperature at 5 cm $< 0°C$), spring- thaw (from the first date of average air temperature $> 0°C$ to the date of spring planting and N fertilizer application), growing season and post-harvesting. During soil freezing over the winter period it was assumed zero N_2O was emitted. Spring-applied N_2O emission factors and intensities were expressed only during the growing season and post-harvesting periods.

3.3.5 Agronomic and Nitrogen Management

Complete description of N and agronomic management conducted in this study can be found in chapter two. Nitrogen rates were applied to achieve a target level soil N level of 170 to 190 kg N ha^{-1} considering fall NO_3^- soil test (0-60 cm). Fall and spring-N sources were applied with the same fertilizer rate (Table 3.1). Fall N sources were applied as close to predicted freeze-up as possible. All treatments were planted to Canadian hard red spring wheat (*Triticum aestivum L.*) and urea N fertilizer treatments were midrow-banded to a depth of 2.5 to 5.0 cm and AA plots were placed approximately 2.5 cm deeper than urea sources at each site.

Table 3.1 Nitrogen application date, rate and pre-fall application NO_3^--N levels.

Crop Year	Site	N application date (DOY)		N application rate	Fall NO_3^--N
		Fall	Spring (with seeding)		
				kg N ha^{-1}	
2015	Glenlea	Nov-5-14 (309)	May-11-15 (496)	115	74†
	Warren	Nov-8-14 (312)	May-22-15 (507)	90	100
2016	Carman	Oct-22-15 (295)	May-6-16 (492)	123	52
	LaSalle	Oct-26-15 (299)	May-4-16 (490)	100	68
2017	Kelburn	Oct-20-16 (295)	May-11-17 (496)	123	50
	Ridge Road	Nov-4-16 (309)	May-4-17 (489)	123	50

† 0-60 cm NO_3^--N concentrations

3.3.6 Soil Sampling and Analysis

Complete soil sampling description and analysis is provided in chapter two. In brief, soil samples were collected throughout the year on six or seven separate occasions. Post-harvest samples were collected to a depth of 60 cm whereas all other samples were collected to a depth of 30 cm. Samples were then analyzed colorimetrically for NH_4^+ using the Berthelot reaction, and NO_3^- by reduction using Cu-Cd to NO_2^- and azo dye formation from reaction with sulfanilamide and N-naphthylehylene-diamine dihydochloride (U.S.

Environmental Protection Agency, 1983; 1993), using a Technicon Autoanalyzer II system (SEAL Analytical Inc., Mequon, WI).

3.3.7 Statistical Analysis

Statistical analyses were completed using Analysis of Variance (ANOVA) using the Statistical Analysis System (version 9.4 software, SAS Institute Inc. 2014) to analyze mean ΣN_2O emissions, spring urea and AA indexed ΣN_2O emissions, EF and EI. The ANOVA was conducted with PROC MIXED procedure and least square means of fixed effects were compared by Tukey's Honestly Significant Difference test ($\alpha=0.05$). Fixed and random effects, and urea and AA experimental designs were identical to chapter two. Urea indexed ΣN_2O emissions were square root transformed for ANOVA, but when all sites were analyzed separately, no transformation was required to attain a normal distribution. Urea and AA emission intensities were also square root transformed to be normally distributed. Cumulative N_2O for AA sources were $\log_{10}$ transformed when sites were analyzed together. When sites were analyzed independently, sites required some transformation. Untransformed mean values are presented.

3.4 Results

3.4.1 Weather, Site Conditions and Soil Inorganic Nitrogen

Detailed seasonal weather and site conditions during fall and spring-N applications have been provided in chapter two. In general, growing season (May-September) air temperature from all sites did not vary considerably from the 1981-2010 long-term average (16.0°C; Environment Canada, 2018). Total growing precipitation in 2015 and 2016 sites were at least 17% greater than the long-term average (349 mm) with the highest precipitations at Warren and Carman of each year, respectively. Precipitation at Kelburn and Ridge Road were well below long-term average being 232 and 280 mm, respectively. The number of days with >10 mm in the growing season totaled; 12 Warren, 14 Glenlea, 18 Carman, 13 LaSalle, 9 Kelburn and 9 Ridge Road (Appendix B). Note that heavy precipitation events in early May, the spring-N application at Warren was delayed by three weeks (May 22, DOY 507).

After fall N fertilization, most sites did not experience significant precipitation events (>10 mm) except at Carman (1 event) and Kelburn (2 events). Onset of soil freeze-up (air temperature< 0°C) occurred from approximately DOY 312-435 at 2014-2015 sites, DOY 322-436 at 2015-2016 sites and DOY 335-447 at 2016-2017 sites. Precipitation totals throughout the non-growing season (November-April) were lower at Warren (75 mm) and Glenlea (50 mm), similar at Kelburn (107 mm) and Ridge Road (113 mm) and greater at Carman (130 mm) and LaSalle (138.1 mm) than the long-term average (108 mm).

Soil temperature on the first sampling date after fall N applications was generally around 5°C and sampling occurred until surface soil temperatures were close to or below 0°C (Figure 3.3). During spring-N applications and seeding, soil temperatures in 2015 were approximately 7 and 18°C at Glenlea and Warren, and 15°C at both sites in 2016 and 2017. Soil temperatures increased throughout the growing season to approximately 25 to 30°C, with highest maximum temperatures at Ridge Road and Carman. Soil moisture was inversely related to soil temperature where lowest moisture coincided with highest soil temperature, especially in June. During spring-thaw in 2015 and 2016, soil moisture increased to over 0.50 m^3 m^{-3}. Soil texture of each site contrasted in 2017 which resulted in strong retention of soil moisture at Kelburn and heavy leaching at Ridge Road with soil moistures peaking at approximately 0.60 and 0.20 m^3 m^{-3}, respectively. However, it should be noted that Ridge Road was completely submerged in water until the beginning of April.

Soil inorganic N concentrations are described in full in chapter two. Urease inhibitor LIMUS tended to have greatest measured NO_3^- concentrations following spring-thaw. At multiple sites, nitrification inhibitors eNtrench and SuperU preserved N in the NH_4^+ form for the longest period following spring-thaw. Controlled-release product ESN prolonged the highest measured concentrations of NO_3^--N later in the growing season. All N sources had similar soil N concentrations to the control four to six weeks after seeding.

3.4.2 Daily N_2O Emissions

Control plots typically followed a similar emission pattern to all treatments but the magnitude of control emissions from observed N_2O episodic events was up to ten times lesser than N treatments (Figure 3.4). Therefore, emphasis of the results and discussion is in regards to N addition treatments.

The 2015 and 2016 growing seasons had normal to above normal precipitation totals. Specifically, significant precipitation events (>10 mm) triggered N_2O emissions. In 2015 and 2016, all sites experienced highest N_2O emissions two to four weeks following

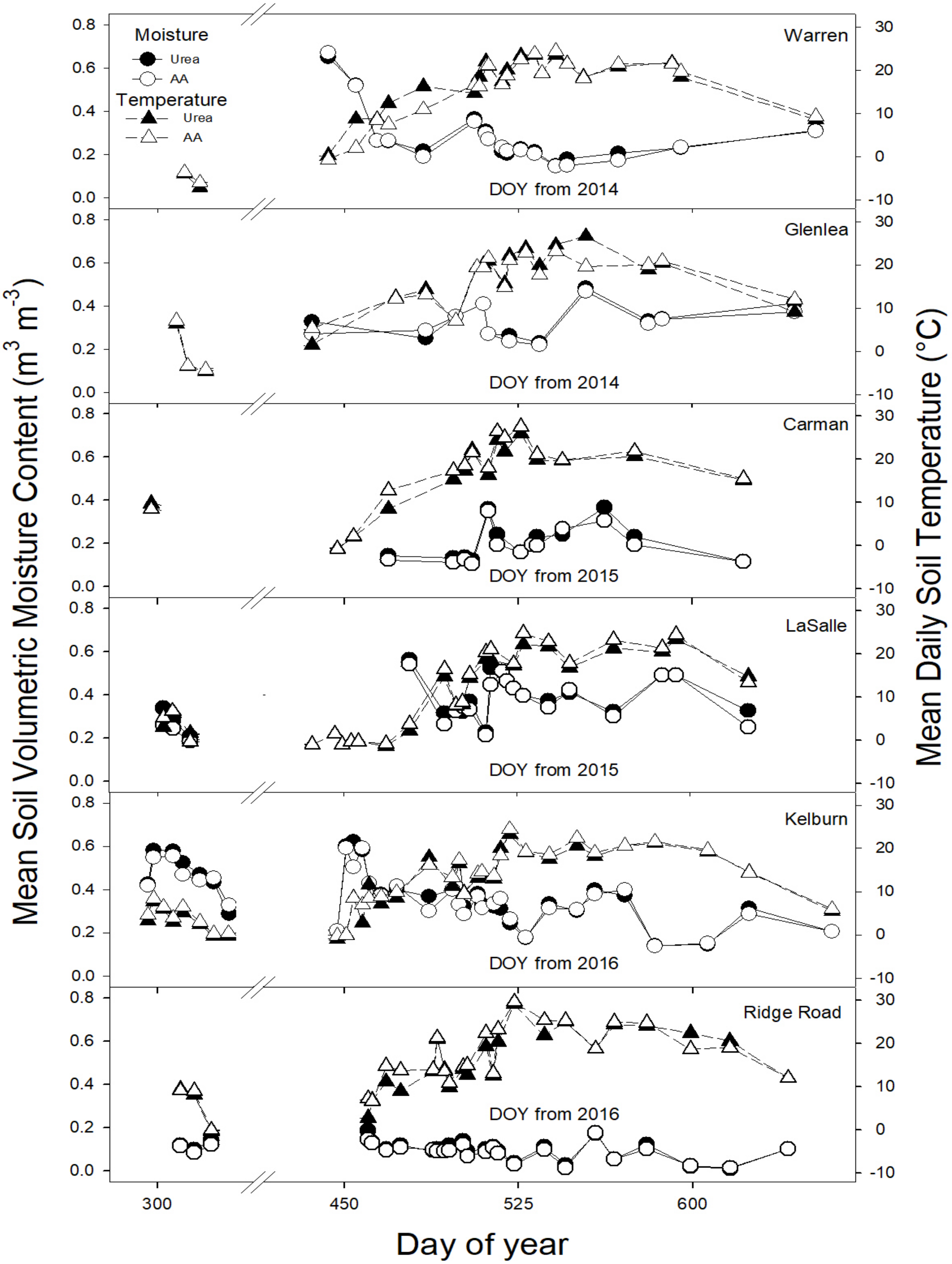

Figure 3.3. Mean soil volumetric moisture content and soil temperature measurements on each gas sampling date at all sites. Mean values from all N source treatments and applications times are shown.

seeding coinciding with rainfall events (Figure 3.4). Emissions following the aforementioned precipitation events were generally greater from spring-applied sources compared to fall except at Glenlea. Daily N_2O returned to control levels approximately six to ten weeks following seeding at all sites in 2015 and 2016.

Sources containing nitrification inhibitors had the lowest emissions following rainfall events during the growing season. Controlled release product, ESN, generally had similar N_2O emissions to SuperU and eNtrench. However, ESN applied at Glenlea and in the fall at Carman ended up having a delayed N_2O release during the growing season resulting in substantially greater emissions than nitrification inhibited sources. Urease inhibitor, LIMUS, produced emissions equal or greater to urea at all sites in 2015 and 2016. Applications of AA with N-Serve varied by site and year but generally applications of AA with and without N-Serve had similar N_2O emissions. As mentioned previously, sites in 2017 received much less rain than the 2015 and 2016 growing seasons resulting in emissions from all N sources being similar in magnitude to the control.

During spring-thaw, the largest magnitude of daily emissions occurred at the Kelburn site with emissions as high as 270 g N d^{-1} ha^{-1} (Figure 3.4) over ten days (DOY 451 to 461). During this major spring-thaw N_2O event, emissions were highest from eNtrench followed by ESN, urea, LIMUS and SuperU. Plots applied with AA with and without N-Serve were also subject to this spring-thaw emission event but to a lesser magnitude compared to urea sources. Lastly, control plots had a maximum daily emission of 23.0 g N d^{-1} ha^{-1}. Moisture conditions at Kelburn over the days were near saturation and soil temperature increased by 11.6°C. Spring-thaw daily emissions from all other sites were no greater than 20 g N d^{-1} ha^{-1}. However, due to heavily saturated conditions and

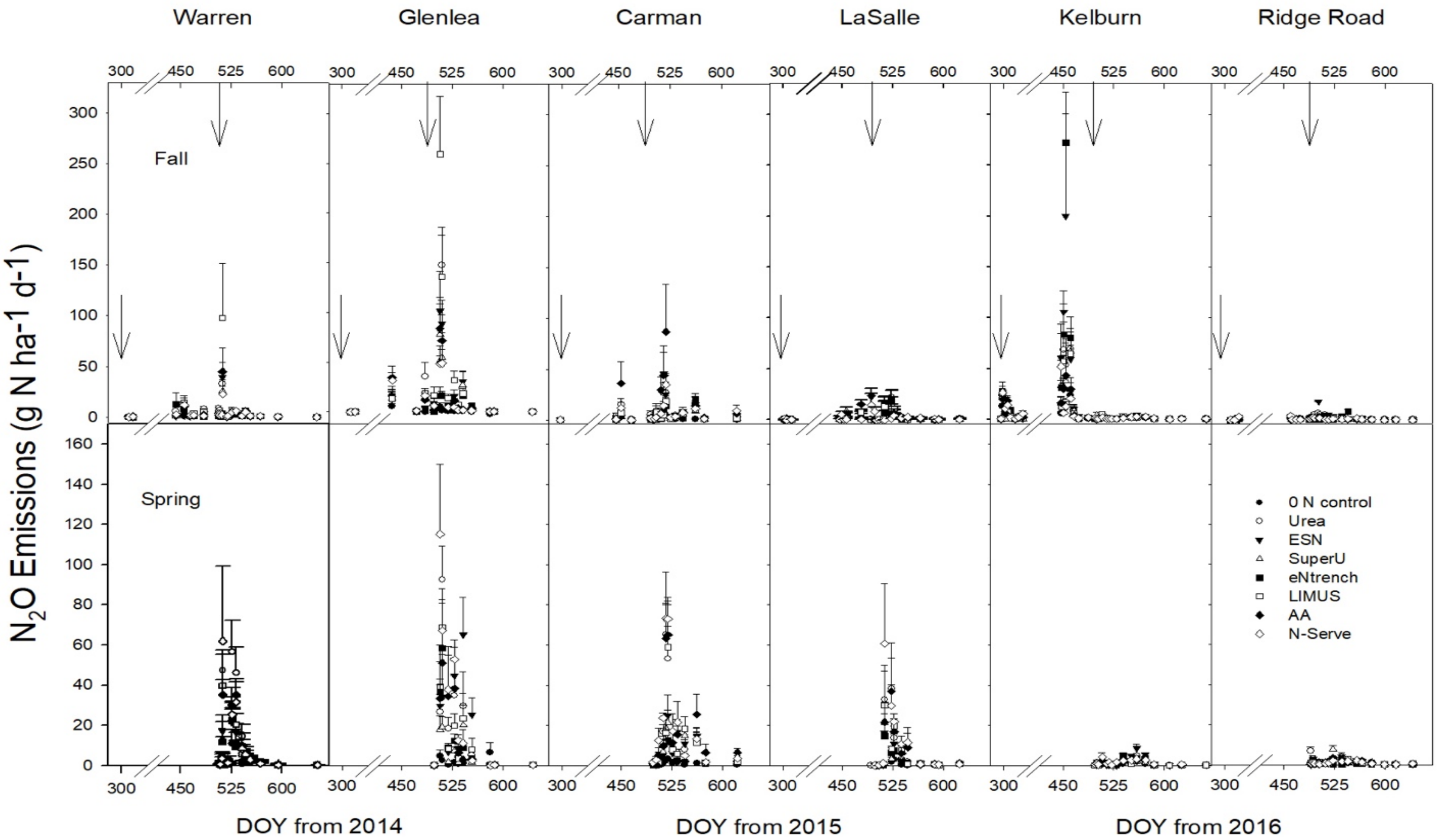

Figure 3.4 Total daily spring and fall N application mean daily N_2O emissions affected by N fertilizer sources. Bars indicate standard error of the mean. Arrows indicate fall and spring-N fertilizer application dates.

ponding at Glenlea and Ridge Road, a significant portion of the spring-thaw period was not sampled.

During the fall, most sites were fertilized close enough to freeze-up to limit microbial activity and N_2O emissions above background control levels. (Figure 3.4). Yet, the site at Kelburn produced emissions double to that of the control 3 d following fall N application. Specifically, emissions at Kelburn ranged from 21 to 26 g N d^{-1} ha^{-1} among all N sources.

3.4.3 Nitrogen Sources

All N_2O indices, including ΣN_2O, indexed N_2O, EF and EI, had similar patterns of N sources among all sites. At all sites, the 0 N control had significantly less ΣN_2O, indexed N_2O, EF and EI than any N source ($P<0.001$; Appendix C). At five of six sites (Glenlea, Warren, Carman, LaSalle and Ridge Road) an N source effect was significant among all N_2O indices (Table 3.2; Figure 3.5). Individual or both fall and spring applications of eNtrench had reduced ΣN_2O at the Warren, Carman, Ridge Road and LaSalle sites than urea. Individual or both fall and spring applications of SuperU resulted in reduced emissions than urea at the Glenlea, Warren, Carman and LaSalle sites. ESN only reduced emissions over urea at the Warren and LaSalle site. In contrast, the urease inhibitor LIMUS increased emissions compared to urea at Glenlea.

Nitrogen sources and application timings were further analyzed as a ratio (index) of spring urea to quantify the reduction potential of EEF against conventional N sources. Among the five sites with an N source effect, eNtrench and SuperU had reduced index ΣN_2O emissions by 54% and 43% of spring-applied urea (Figure 3.5). Likewise, to N_2O, eNtrench and SuperU (0.14 and 0.18 kg N Mg^{-1}, respectively) had significantly less EI compared to urea (0.31 kg N Mg^{-1}) among sites with an N source effect ($P=0.003$).

Emission factor measurements were the exact same as EI where eNtrench and SuperU increased the efficiency of N with values of 0.27 and 0.34%, respectively, of applied N compared to 0.75% for urea. Therefore, all N_2O results confirm that SuperU and eNtrench sources were most effective at reducing N_2O emissions over urea. At each site, there was no N source effect detected from AA plots with and without N-Serve for all N_2O measurements (Table 3.3; Figure 3.5; Appendix D).

Table 3.2 Urea based N fertilizer mean ΣN_2O.

		ΣN_2O (kg N ha^{-1})					
N sources	Time	Glenlea	Warren	Carman	LaSalle	Kelburn†	Ridge Road
Urea	Fall	2.47ab	0.62abc	0.97ab	0.54ab	1.31ab	0.33a
	Spring	1.67bc	1.06a	1.15a	0.70a	0.53b	0.24ab
ESN	Fall	2.14abc	0.44bc	0.97ab	0.45ab	2.36ab	0.25ab
	Spring	2.13abc	0.51bc	0.93abc	0.31b	0.67ab	0.18ab
SuperU	Fall	1.61bc	0.35c	0.59bc	0.48ab	1.15ab	0.20ab
	Spring	0.55c	0.37c	0.75abc	0.32b	0.51b	0.14ab
eNtrench	Fall	0.90bc	0.29c	0.64bc	0.36ab	2.64a	0.12ab
	Spring	1.04bc	0.40bc	0.47c	0.31b	0.56ab	0.08b
LIMUS	Fall	3.63a	0.85ab	0.68abc	0.51ab	1.08ab	0.28ab
	Spring	1.32bc	0.66abc	1.11a	0.54ab	0.48b	0.17ab
		ANOVA (p value)					
	N Source (N)	0.0031	0.0004	0.0013	0.0354	0.4888	0.0199
	Time (T)	0.0041	0.1718	0.1179	0.5830	0.0013	0.2630
	N*T	0.0370	0.0918	0.0910	0.4087	0.7570	0.9481

† Kelburn values $\log_{10}$ transformed.

Values followed by different letters in a column are significantly different at $P < 0.05$.

3.4.4 Timing and Site-Year

At two of six sites, N_2O indices were affected by timing which resulted from lower emissions with spring-applied urea granular sources compared to fall at the Glenlea and Kelburn sites (Table 3.2; Figure 3.6). However, at the other four sites fall-applied ΣN_2O

was comparable to spring. An N source by time interaction (*P*=0.037) at Glenlea was a result of reduced ΣN_2O emissions of spring LIMUS compared to fall. Besides from the latter, when sites were analyzed separately, all other N sources had similar ΣN_2O between both application timings. At Warren and Ridge Road, ΣN_2O emissions of fall-applied AA sources were significantly less than spring (Table 3.3). Specifically, at Warren, combining AA sources resulted in a significant N_2O reduction from fall-applied (0.51 kg N ha^{-1}) over spring (0.79 kg N ha^{-1}). In general, emission rates were greatest at Glenlea and lowest at Ridge Road with average emission rates at each site being 1.79 (Glenlea), 1.54 (Carman), 0.80 (LaSalle), 0.67 (Kelburn), 0.65 (Warren) and 0.44 kg N ha^{-1} (Ridge Road). Calculating the indexed ΣN_2O, excluding the Kelburn site, fall-applied was 13% greater than spring (*P*=0.018; Appendix D).

Urea EF and yield-based EI was affected by time effects as well as site-year interactions by N source and time, and a three-way interaction of N source by time by site-year (Appendix D). Site-year interactions occurred for both EF and EI measurements because of greater values at Glenlea compared to the other sites. When excluding the Kelburn site, applying in the spring with urea granular products resulted in a reduction of 0.09 kg N Mg^{-1} and 0.16% compared to fall for EI and EF, respectively (*P*=0.002; 0.002). Differences only occurred among site-years with AA based EF and EI measurements, with no differences between application timings (Figure 3.6; Appendix D).

3.4.5 Spring-Thaw N_2O

The fraction of spring-thaw ΣN_2O emissions varied from site to site but ESN had the lowest, averaging 8% across five sites with an N source effect. Compared to urea, averaging 23%, applying SuperU and LIMUS reduced spring-thaw emissions with ratios of 20 and 18%, respectively, of whole-year emissions. Surprisingly, eNtrench averaged 26% of whole-year emissions which mainly was due to high spring-thaw emissions at Warren being 50% of whole-year emissions. Applying AA with N-Serve in the fall resulted in 29% of emissions occurring during spring-thaw which was greater than AA alone, averaging 22%. At Kelburn and LaSalle, spring-thaw N_2O emissions accounted for over 90% and between 20 to 40%, respectively, of whole-year emissions among all urea and AA based sources.

Table 3.3 AA based N fertilizer mean ΣN_2O emissions.

		ΣN_2O (kg N ha^{-1})					
N Sources	Time	Glenlea	Warren	Carman†	LaSalle	Kelburn †	Ridge Road†
AA	Fall	2.00	0.53	1.83	0.83	0.88	0.23b
	Spring	1.36	0.78	1.67	0.78	0.52	0.80a
N-Serve	Fall	1.60	0.49	1.07	0.51	0.88	0.30b
	Spring	2.19	0.80	1.60	1.09	0.52	0.41ab
		ANOVA (p value)					
	N Source (N)	0.6151	0.9228	0.5353	0.9569	0.7568	0.4807
	Time (T)	0.9492	0.0369	0.4894	0.1188	0.3890	0.0110
	N*T	0.1724	0.7632	0.3392	0.0735	0.6954	0.2162

† Carman, Kelburn and Ridge Road $\log_{10}$ transformed.
Values followed by different letters in a column are significantly different at $P < 0.05$.

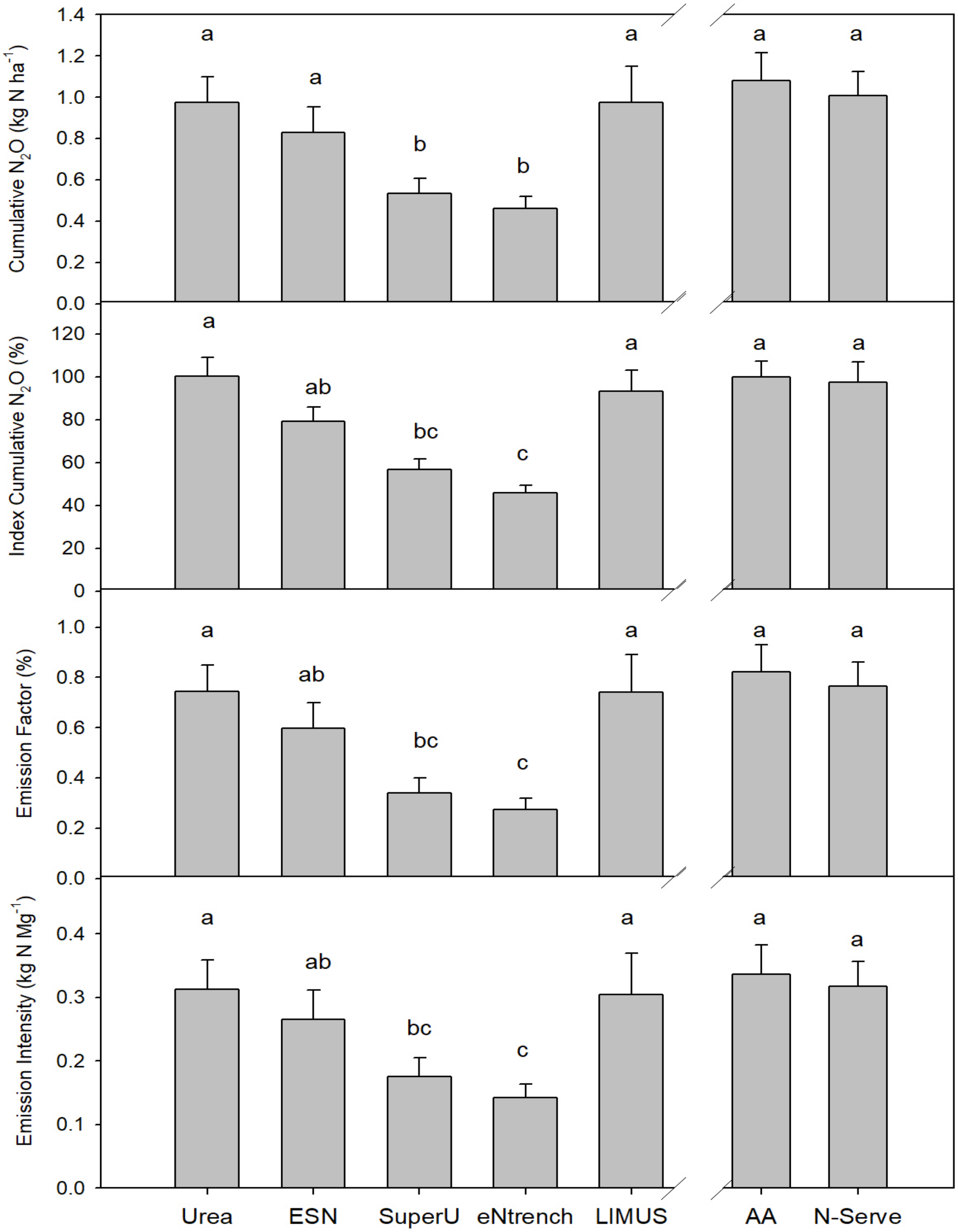

Figure 3.5 Mean ΣN_2O emissions, spring urea index ΣN_2O emissions, EI and EF across sites with an N source effect (Warren, Glenlea, Carman, LaSalle and Ridge Road) as influenced by N sources.

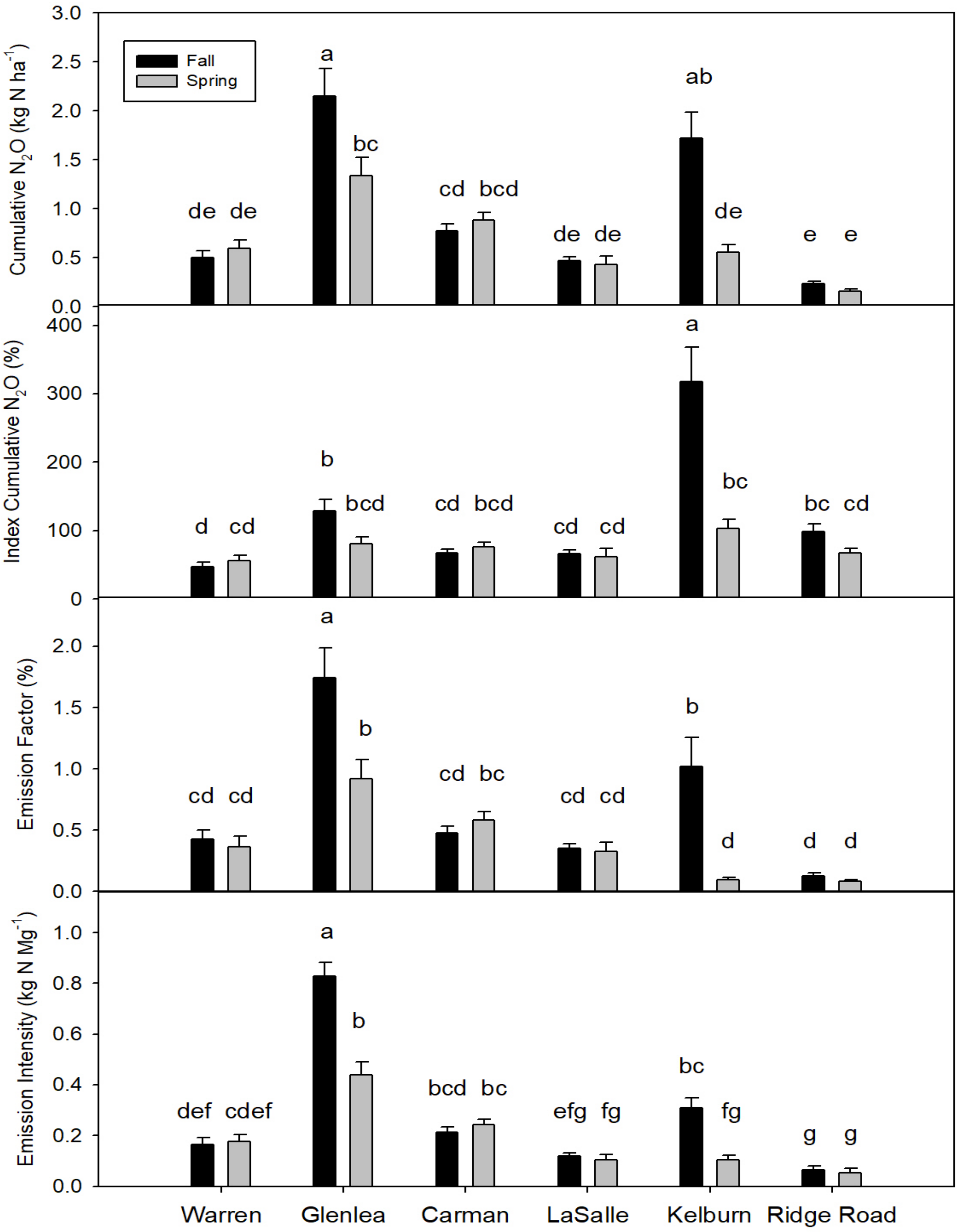

Figure 3.6 Mean ΣN_2O emissions, spring urea index ΣN_2O emissions, EI and EF across all granular urea sources with fall and spring application timings.

3.5 Discussion

3.5.1 Introduction

This study is unique as it is the first to incorporate a wide array of nitrification, urease and polymer coated N sources, and compare their effectiveness in reducing N_2O of fall and spring applications. However, this is not the first study conducted in the Northern Great Plains region or even southern Manitoba that has looked at the effectiveness of EEF products compared to conventional products and the difference between fall and spring applications of EEF measuring N_2O emissions (Asgedom et al., 2014; Bremner et al., 1981; Soon et al., 2011; Burton et al., 2008). Yet, there is still research required to determine the efficacy of EEF to reduce N_2O versus conventional sources using best management practices (Sistani et al., 2011; Zebarth et al., 2012). Specifically, this current study differed from previous studies that compared between fall and spring-applied EEF in that it incorporated best management practices of applying N fertilizers late-fall when soil temperatures were approximately 5°C (Tenuta et al., 2016). As well, this current study banded EEF instead of broadcasting which has been confirmed to be more effective to reduce N_2O emissions (Gao et al., 2015).

3.5.2 Enhanced Efficiency Fertilizer Cumulative N_2O

Multiple previous studies that had broadcasted EEF followed by soil incorporation did not measure consistent reductions in N_2O compared to applying conventional urea fertilizer. Parkin and Hatfield (2014) compared broadcasted urea to SuperU and ESN in a corn field at 168 kg N ha^{-1} and observed no significant difference between treatments with ΣN_2O emissions of about 2.9 kg N_2O-N ha^{-1}. Another corn study in Ontario which applied urea containing NBPT and SuperU in 2013 and 2014 at a rate of 130 kg N ha^{-1} observed higher ΣN_2O in 2014 from NBPT and similar in both years from SuperU compared to urea with ΣN_2O values as high as 3.18 kg N ha^{-1} (Drury et al., 2017). More locally in southern Manitoba, Asgedom et al. (2014) measured a significant reduction in N_2O with ESN but not SuperU with ΣN_2O values higher than this current study. The previously mentioned study by Gao et al. (2015) reported significantly lower ΣN_2O in three of four site-years from ESN and SuperU compared to urea with ΣN_2O values ranging from 0.16 to 2.0 kg N ha^{-1}. The present study used current best management practices and hypothesized that inhibiting biological N transformation and slowing down nitrification rates with EEF will reduce ΣN_2O emissions. The EEF eNtrench and SuperU were confirmed in this present study to be effective N sources to mitigate N_2O emissions.

The most effective EEF to lower N_2O emissions in the current study that has not been tested as consistently as SuperU and ESN is urea formulated with nitrapyrin, commercially known as eNtrench or Instinct in the United States. Within North America, eNtrench has only been tested in a laboratory setting with sandy loam and silty clay soils from the Northern Great Plains, reducing N_2O emissions by 23.5 and 34.1%, respectively compared to urea (Awale et al., 2017). The current study reported a reduction in emissions at four of six sites with eNtrench, averaging 54% lower ΣN_2O over urea among the five sites with an N source effect. The three sites with the greatest reduction in N_2O using eNtrench were Warren and Carman followed by Ridge Road. However, denitrification was likely not the major source of N_2O production at these sites during the growing season due to coarse texture soils, VMC levels being around 0.20 m^3 m^{-3} and relatively sustained NO_3^- levels. Thus, the conditions at these three sites were favourable for moderate aeration and another N process being responsible for N_2O production. Nitrifier-denitrification is the reduction of NO_2^- to N_2O or N_2 under both aerobic and anaerobic conditions (Wrage et al., 2001; Kool et al., 2011; Shaw et al., 2006). Therefore, from the soils inability to create anoxic conditions, highest N_2O reductions were likely under nitrifier-denitrification with 0.15-0.30 m^3 m^{-3} VMC levels.

The site at LaSalle had higher clay contents and greater VMC levels over 0.30 m^3 m^{-3} than Warren, Ridge Road and Carman. With prolonged high soil moisture during the growing season in combination with second lowest ΣN_2O emissions of any site, first being Ridge Road, it is likely that complete denitrification occurred producing dinitrogen gas. Therefore, in heavy clay soils during wet seasons, the reduction potential of eNtrench may not be as great compared to conditions conducive to nitrifier-denitrification.

The product SuperU has been found to be advantageous in reducing N_2O emissions due to its ability to limit oxidation of NH_3 with nitrification inhibitor DCD (Thapa et al., 2015, Gao et al., 2015). Similar to eNtrench, SuperU effectively reduced N_2O in four of six sites, but combining sites with an N source effect resulted in a reduction of 43% over urea. This study contrasted multiple other studies which broadcasted and incorporated SuperU, and observed no reductions in N_2O over conventional N sources (Drury et al., 2017; Asgedom et al., 2014). However, it is plausible that SuperU was not as effective as eNtrench due to high organic matter concentrations restricting the effectiveness and increasing decomposition rates of NBPT and DCD (Jacinthe and Pichtel 1992; Reddy, 1964; Asgedom et al., 2014). The effect of organic matter restricting SuperU can be furthered confirmed at the Glenlea site which had the second lowest organic matter but greatest reductions in N_2O from SuperU of any site. Overall, the difference between urea nitrification products was not significant highlighting the imperativeness to band nitrification inhibitors to limit decomposition and sorption with negatively charged organic molecules.

Surprisingly, ESN was not as an effective source as the urea nitrification products to reduce N_2O over urea, especially during wet seasons, releasing only 20% less emissions among sites with an N source effect. In this current study, ESN delayed the release of N which it is designed to do, but peak emission magnitudes were higher than eNtrench and SuperU. Golden et al. (2011) tested the N retention in ESN and observed N release was not affected so much by soil moisture but by increasing soil temperatures. During the 2016 season in the current study, soil temperatures increased rapidly after seeding which may have resulted in rapid release of N and increased nitrification, and potentially denitrification under high soil moisture (VMC > 0.30 m^3 m^{-3}) from high NO_3^- concentrations later in the growing season. Thus, warmer soil temperatures accelerated release of N from ESN granules producing N_2O presumably by nitrification and denitrification. Once inorganic N was available, precipitation events later in the growing season catalyzed peak emission events explaining the delay in N_2O release from the ESN product. Yet, similar to past studies, largest reductions from urea were observed when ESN was applied in coarse soils (Gao et al., 2015; Li et al., 2012). Therefore, consistently high moisture levels during the growing season in clay based soils will only delay the release of N_2O but not lower.

Using a urease inhibitor alone to reduce N_2O emissions was shown to be ineffective, not only in the current study, but as well as multiple prior (Drury et al., 2017; Awale et al., 2017; Akiyama et al., 2010). The main action of urease inhibitors is to delay urea hydrolysis and mitigate NH_3 volatilization but not to inhibit nitrification. Without sufficient N uptake, NH_4^+ will accumulate once the inhibitor expires resulting in significant nitrification and denitrification, and increased N_2O emissions (Akiyama et al., 2010; Awale et al., 2017). Although not monitored in the current study, urease inhibitors may have a significant role in reducing NO_2^- accumulation from lower NH_4^+ formation and soil pH by urea hydrolysis in sand based soils where denitrification is a lesser risk for N loss (Cai et al., 2016).

The application of AA is a common practice in Manitoba (Tenuta et al., 2016) and N-Serve, containing the nitrification inhibitor nitrapyrin, has been examined in various studies in other regions (Cai et al., 2016; Chen et al., 2010; Parkin and Hatfield, 2010). Nitrapyrin is beneficial in reducing N_2O by retaining soil NH_4^+ levels, limiting nitrification and resultant denitrification (Wolt, 2004; Qiao et al., 2015). Unlike previous studies, the current study demonstrated that mixing N-Serve with AA did not result in lower N_2O emissions. In the current study there were perhaps mechanical difficulties that may have provided incomplete delivery of N-Serve. Specifically, in 2015 and 2016, the flow of N-Serve from the side kick pump system occurred as pulses. To provide a consistent and controlled stream of N-Serve, a smaller diameter tube was fitted in the spring of 2017. However, conditions in 2017 were not conducive for N_2O production. With caution in mind, future studies with AA or N-Serve are required to be conclusive regarding the effectiveness of N-Serve to reduce N_2O emissions.

3.5.3 Fall and Spring N_2O

The significance of not accounting for spring-thaw emissions in northern climates can lead to an underestimation of N_2O emissions by 17 to 28% (Wagner-Riddle et al., 2017). Unless N is applied close to freeze-up, applying in the fall is not advisable due to greater potential for N loss before freeze-up and during spring-thaw (Soon et al., 2011; Tenuta et al., 2016). In the current study, it was hypothesized that fall and spring EEF applications would result in similar area-scaled ΣN_2O emissions. Except for LIMUS at the

Glenlea site, all EEF had similar ΣN_2O between fall and spring applications. Combining all urea N sources resulted in two sites with greater fall-applied emissions compared to spring. Overall, the most effective EEF sources, eNtrench, SuperU and ESN did not differ between application timings at each site and confirms that applying in late-fall produces emissions similar to spring.

The two of six sites which had higher fall N_2O than spring were Glenlea and Kelburn These sites had vertisol soils which swell and shrink depending on the soil moisture levels. Thus, during and immediately following spring-thaw at these two sites, moisture was greatest coinciding with highest N_2O from fall applications. Most notably at Glenlea, peak emissions from all N fall applications not only aligned with spring but the magnitude was more than double for some sources which is not uncommon for urea in Manitoba (Burton et al., 2008). Therefore, applying fall fertilizers to a vertisol soil may lead to greater inefficiencies and increased soil N losses before and following spring seeding. Yet, all other sites in this current study observed fall EEF applied soil NO_3^- concentrations peaking two to four weeks following seeding whereas urea peaked two weeks after seeding. The delayed appearance of NO_3^- shows that EEF sources effectively maintained soil N in NH_4^+ form at reduced soil temperatures during spring-thaw, thus mitigating N_2O loss.

At Kelburn, over 90% of N_2O was emitted during spring-thaw with no other significant N_2O periods due to very dry conditions during the growing season. On top of being a vertisol soil, this high release of N_2O during thaw may be due to the fact that soil freeze-up did not occur until over a month after application, allowing adequate time for urea hydrolysis, nitrification and build-up of soil NO_3^- concentrations in the top 0-6-inch layer. The magnitude of emissions during spring-thaw at Kelburn is similar to previous organic, fertilizer or manure studies (Asgedom et al., 2014; Wagner-Riddle et al., 2007;

Westphal et al., 2018) but is unique in that thaw N_2O was the greatest fraction contributing to total N_2O. This study is in agreement with past studies in that significant bursts of N_2O during spring-thaw occur in top layer of unfrozen, water-logged soils promoting denitrification at VMC levels above 0.30 m^3 m^{-3} (Tenuta and Sparling, 2011; Matzner et al., 2008; Wagner-Riddle et al., 2008; Ryan et al., 2000). Therefore, in combination with extremely dry conditions during the growing season and highest NO_3^- concentrations before soil freeze-up among any site, conditions were very favourable for high spring-thaw and low growing season N_2O emissions.

Anhydrous ammonia has been a more common N source to test at varying application times due to its popularity as a fall fertilizer (Amiro et al., 2017; Parkin and Hatfield, 2010). Four of six sites in the current study had similar fall and spring emissions from AA with and without N-Serve. One of the first N_2O studies measuring fall and spring AA applications found significantly higher fall emissions but reductions of 63 and 87% with nitrapyrin in the fall and spring, respectively, compared to AA alone (Bremner et al., 1981). Conversely, Parkin and Hatfield (2010) did not measure any reductions from fall and spring applications of nitrapyrin compared to AA in an Iowa corn study. Recently, Tenuta et al. (2016) applied fall and spring AA and observed very different moisture patterns in that the first year spring-thaw was very wet corresponding with largest emissions during spring-thaw whereas the second year observed drier conditions corresponding to both a spring-thaw and early summer emission peak. The second year of Tenuta et al. (2016) was very similar to the current study in that during a normal to dry spring, the emissions from fall applications were greatest during both spring-thaw and early summer when rain events resulted in transformation of AA to nitrate and N_2O. Overall, Tenuta et al. (2016) observed greater ΣN_2O emissions from spring than fall applications whereas another local study had similar or greater fall N_2O than spring (Burton et al., 2008) The results of the current study agree more with Burton et al. (2008) where fall-applied AA emissions are more likely to equal spring for AA sources.

The majority of spring-thaw emissions in this study, except at Kelburn, accounted for 10-30% of total N_2O loss. Previous studies in southern Manitoba reported ΣN_2O thaw emissions from 23 to 35% of total annual N_2O using an array of N sources and management practices (Glenn et al., 2012; Dunmola et al., 2010). Preliminary statistical analysis of spring-thaw emissions indicated no significant difference between conventional and EEF sources (data not shown). In contrast, Tiessen et al. (2006) observed reduced nitrification from EEF containing NBPT and DCD compared to conventional sources. Substantial nitrification can occur during the fall if conditions are not anaerobic and temperatures are above freezing for a significant period of time (Nyborg and Malhi, 1986; Malhi and Nyborg, 1979; Signor and Cerri, 2013). Specifically, it can take as little as seven to ten days for a third of urea to hydrolyze and up to 60% can be nitrified before freeze-up with early fall N applications (Malhi and Nyborg, 1979). In Alberta and Saskatchewan, Soon et al. (2011) reported 1.5-fold lower N_2O from spring than fall polymer-coated urea, attributing time of application to be largest effect than N source. This current study adopted recommendations from Tenuta et al. (2016) to apply fall N applications around 5 °C. Using these recommendations resulted in significant hydrolysis and mineralization from the presence of high NH_4^+ concentrations, but nitrification was limited as indicated by low fall NO_3^- concentrations. This study is novel as no previous work has monitored N_2O with late-fall applications and observed the majority of sites having similar N_2O indices to spring applications; thus solidifying the importance of late-fall applications in reducing spring-thaw emissions and incorporating EEF in the fall can result in losses parallel to spring applications.

3.5.4 Site Differences

The varying N_2O emission magnitudes during the growing season from the current study is consistent with other studies testing EEF products. Most interestingly in the current study was the absence of N source differences at the Kelburn site. The lack of precipitation during the growing season and high organic matter were ideal conditions to suppress any differences between N sources. Instead of microbes nitrifying available N from fertilizers, high organic matter and resulting mineralization may have increased nitrification and denitrification producing N_2O and continuous build-up of NO_3^-(Awale et al., 2017: Parkin and Hatfield, 2014).

The soil at the Glenlea had the highest ΣN_2O of any site ranging from 0.55 to 3.63 kg N ha^{-1}. All other sites in this current study ranged from 0.08 to 2.64 kg N ha^{-1}. Previous local studies that broadcasted conventional and EEF sources reported emissions ranging from 0.9 to 5.5 kg N ha^{-1} (Burton et al., 2008; Glenn et al., 2012; Asgedom et al., 2014). Most recently, Tenuta et al. (2016) reported ΣN_2O ranging from 3.2 to 9.2 and 5.1 to 14.0 kg N ha^{-1} from fall and pre-plant applied AA, respectively. However, Gao et al. (2015) was the first and only local study to band EEF and reported growing season ΣN_2O emissions similar to this current study ranging from 0.16 to 2.04 kg N ha^{-1} among urea sources. Therefore, banding EEF in fall or spring has the potential to produce yearly ΣN_2O emissions lower than one kg N ha^{-1}. The greater emissions at Glenlea is attributable to urea, LIMUS and ESN sources experiencing high soil moisture from precipitation and the moisture retentive nature of gleyed soils. Therefore, these sources were not able to retain

N in the urea or NH_4^+ form under heavy soil moisture. Alternatively, Ridge Road experienced the lowest emissions of any site due to hot and dry conditions, low organic matter and coarse soil texture limiting urease hydrolysis and nitrification processes that produce N_2O.

3.5.5 Nitrous Oxide Emission Factor and Emission Intensity

Emission factors (EF) ranged from 0.08 to 1.74% applied N among all N sources and application timings with significantly lower EF and emission intensity (EI) values from eNtrench and SuperU sources. Similarly, Gao et al. (2015) reported similar EF values ranging from 0.23 to 0.80% with ESN and SuperU. Burton et al. (2008) banded ESN and observed a 0.60% reduction compared to urea. However, when broadcast incorporated, SuperU was not as effective as ESN in reducing EF, highlighting the inefficiency of broadcasting vs banding nitrification inhibitors (Asgedom et al., 2014). To our knowledge, Awale et al. (2017) is the first study to report on urea + nitrapyrin (eNtrench) in the Northern Great Plains region reporting on average 5.7 and 4.5 % of applied N in sandy loam and silty clay soil, respectively, and not statistically different than urea. As well, the latter study reported slightly higher EF with urea + NBPT being 6.5 and 5.2 % of applied N in the two different soil textures. The inclusion of the current study provides baseline EF for eNtrench and LIMUS in southern Manitoba. Similar to Burton et al. (2008), fall-applied EF were significantly greater than spring, averaging 0.62 and and 0.46 %, respectively, of applied N among all urea N sources (excluding Kelburn). Yet, the combined fall and spring EF among all EEF sources ranged from 0.27 to 0.76 % applied N which is similar to the black soil zone average being 0.33 % of applied N (Rochette et al., 2018), providing new estimates to account for fall and spring applications of EEF.

Yield-scaled emission intensity (EI) is a way to relate the agronomic performance of the crop to ΣN_2O emissions. The same trends were observed from EI as EF, with lowest values from eNtrench and SuperU. Among all sources, Glenlea had highest values and Ridge Road lowest. Identical N rates among each treatment combined with no statistical grain yield differences, highlighted in chapter two, are responsible for the similar trends between EF and EI. It is evident that the applied N was not used by the crop as efficiently in Glenlea due to consistently high soil moisture causing lower yields which resulted in greater EF and EI values. The EI values in this current were lower than previous studies in Manitoba growing wheat (Asgedom et al., 2014). More recently, banding of SuperU and ESN produced similar intensities to those of the current study (Gao et al., 2015). Additionally, the values in this study were similar to a long-term organic crop rotation study outside of Winnipeg, Manitoba (Westphal et al., 2018). Therefore, the advantages of both eNtrench and SuperU are evident from the current study and are effective tools to not only decrease N_2O but increase N efficiency for the crop.

3.6 Conclusion

The recommendation from IPCC (2014) to include nitrification inhibitors as a mitigation technique to reduce N_2O emissions was confirmed in this current study. Specifically, the EEF products eNtrench and SuperU reduced peak emissions following significant precipitation events resulting in lower ΣN_2O by over 40% compared to spring-applied urea. Both urea nitrification products delayed N transformations to NO_3^-, and likely reduced N_2O most efficiently during conditions favourable for nitrifier-denitrification. Nitrogen source ESN also delayed NO_3^- accumulation but eventually similar emissions occurred as with urea later in the growing season following precipitation events. At Kelburn, increased mineralization from high organic matter levels and very dry conditions likely masked any source effect to reduce N_2O emissions. Ultimately, eNtrench and SuperU were the most consistent and beneficial products to reduce N_2O emissions in this current study. Specifically, under normal to above normal precipitation conditions, applications of eNtrench, SuperU and ESN emitted lower N_2O compared to spring urea. However, at two sites which contained moisture retentive vertisol soils, fall emissions were much greater than spring due to high precipitation events and saturated spring-thaws. Additionally, late-fall application timing had a huge effect on reducing spring-thaw emissions with no measurable N source differences until the growing season. Therefore, it is ultimately recommended to apply nitrification inhibitors every season as a protective investment for growers to limit significant N losses. Additionally, nitrification inhibitor and polymer coated urea products can be applied in late-fall at soil temperatures around 5°C, adding to current best management practices that in the past have only recommended spring applications. However, lower net costs are required to make EEF cost effective for growers in Canada. Future work is required to include different N rates with sub-surface placement as EEF increased N use efficiency and lower rates may provide similar efficiencies to higher rates of urea. Additionally, crops requiring long growing seasons such as corn are increasing in popularity in Manitoba, enabling future studies to establish application times and sources most beneficial to increasing yields and decreasing N losses.

3.7 References

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4. Synthesis

4.1 Project Accomplishments

Manitoba is highly dependent on conventional fertilizers and is a significant contributor to national and global crop production with Canadian hard red spring wheat (*Triticum aestivum L.*), variety AAC Brandon, comprising over 1 million acres of agricultural land in the province (MASC, 2018). Conventional fertilizers have a niche in agriculture as they are cheap and easy to apply at high rates. However, applying at high rates results in significant N losses. Generally, losses in the form of N_2O are low with an emission factor of 0.33% of applied N in the black soil zone (Rochette et al., 2018). Yet, with a global warming potential 265 times that of CO_2, N_2O is a significant contributor to the greenhouse effect. Enhanced efficiency fertilizers of N have been successfully tested to reduce N_2O emissions in southern Manitoba (Gao et al., 2015; Asgedom et al., 2014). Additionally, applying N fertilizers in the spring has been the long standing recommended practice in Manitoba, albeit producers still choose to adopt fall applications. In the current study, the static-vented chamber design was implemented at six sites over three years to test a large array of EEF available or soon to be commercially available to growers. This study was successful in capturing N_2O soil emissions, from fall and spring applications of EEF and conventional N fertilizers. More importantly, major episodic periods such as after fertilization and during spring-thaw were monitored.

Previous studies within Manitoba and North America have tested single or multiple EEF. No study in Manitoba has tested five EEF on a field scale. This study incorporated major inhibitor active ingredients and a delayed release product in combination with two application timings, fall and spring. Using the static-vented chamber method, four chambers per plot were sampled to capture a spatial and temporal representation of N_2O emissions being produced from the soil. Additionally, with three years of sampling in changing soil environments, this experiment was able to provide a more comprehensive investigation on emissions across southern Manitoba. As well, this study captured changing climatic conditions from year to year with 2015 and 2016 growing seasons having above normal precipitation and 2017 extremely low precipitation.

The two objectives of this thesis were to calculate cumulative N_2O emissions and observe agronomic parameters to first compare EEF and conventional N sources, and second to compare fall and spring applications of N sources. This current study indexed cumulative N_2O as a ratio of spring urea, enabling easy comparisons among N sources. At sites with an N source effect, product eNtrench reduced N_2O emissions by 54% over spring-applied urea, especially under conditions that were conducive to N loss via nitrifier-denitrification. Additionally, product SuperU had significant reductions in cumulative N_2O emissions over spring urea by 43%. All other products did not work as effectively as eNtrench and SuperU. Applying late-fall at soil temperatures close to 5°C promoted comparable cumulative N_2O emissions against each respective N source spring application. Major differences between sources occurred following precipitation events whereas negligible differences were observed during spring-thaw. This similarity between application timings was apparent observing grain yield within each site. Therefore, along with the type of N fertilizer, applying as late as possible in the fall has a beneficial impact on N efficiencies, N_2O loss (especially during spring-thaw) and crop production.

4.2 Surprising and Interesting Observations

Grain yield did not vary among N sources which was due to identical N rates being applied. However, protein levels were highest with eNtrench and ESN, and significantly greater than LIMUS because more soil N was available early and throughout the growing season. Surprisingly, LIMUS had lowest protein levels and comparable or higher N_2O emissions to urea. As well, to a lesser extent as LIMUS, ESN did not reduce N_2O emissions which is in disagreement with previous work in Manitoba (Gao et al., 2015; Asgedom et al., 2014). Therefore, in above-normal precipitation years with consistent rainfalls throughout the growing season, controlled release products only delay the release and loss of inorganic N in the form of N_2O instead of increasing the efficiency of N use by the crop.

Ridge Road was a rather interesting site where soil texture and growing season weather conditions were much different compared to the other five sites in this current study. Most shockingly, fall-applied yields were greater than spring-applied which can be attributed to a combination of reasons such as soil profile characteristics, spring-thaw conditions and a rapid increase in soil temperatures. Bedrock was present less than 60 cm below the soil surface. Therefore, ponding during spring-thaw likely relocated fall-applied N further down the soil profile instead of leaching to groundwater, allowing the crop to attain N during the growing season. However, spring-applied N that was not influenced by spring-thaw was most likely volatilized as ammonia. Thus, the ponding event actually benefited the crop during the 2017 growing season.

4.3 Challenges and Improvements

The static-chamber technique is advantageous as it can detect small differences of N_2O emissions, is low cost and does not require in-field power consumption or on-line measurements (Denmead, 2008). However, the technique applied in this current study required labour, a minimum four persons for five hours per sampling period. Additionally, in the lab, a maximum of 360 gas samples could be analyzed for N_2O in a day because only two gas chromatograph analyzers were available. Due to the high personnel demand, capturing soil N_2O, especially during high emissions periods, could not be completed daily. Failure to sample everyday left uncertainty as to whether the release and magnitude of soil N_2O was accurately portrayed in this study. Incorporating automated chambers within the experimental design would have provided reassurance as to whether a significant emission episode was captured appropriately. Automated chambers provide the benefits of static-vented chambers plus less bias of seasonal and annual emissions due to consistent and greater temporal resolution (Wang et al., 2013). The use of automated chambers would have been especially valuable during spring-thaw when the field was not attainable by personnel due to saturated soil conditions. Therefore, future projects should consider deploying automated systems to confirm static-chamber measurements.

The mixing of AA with nitrification inhibitor N-Serve requires rate and equipment testing before small plot fertilization. Testing is required for small plot experiments because N-Serve has to be applied at a very low rate to replicate large farm applications. In this current study, lack of testing may have resulted in error but this was inconclusive. In 2017, sufficient testing and equipment maintenance was completed but climatic and soil conditions were not conducive to highlight EEF potential. Therefore, requesting support from N-Serve manufacturing technicians would be beneficial before implementing a future project.

4.4 Future Work

Products ESN and SuperU had over 25% more residual extractable soil N levels in the soil compared to urea averaged among all sites. A recent study measuring spring wheat in Minnesota suggested the recommended rate of fertilized applied with EEF is more than sufficient to meet the N requirements of the crop (Thapa et al., 2017). Therefore, it is plausible that reduced EEF rates would be sufficient to meet the N requirements of spring wheat compared to current conventional N source rates. Reduced rates would cut down costs of more expense EEF, ultimately encouraging more growers to use EEF. It is recommended for future work to investigate whether decreased EEF rates by up to 25% would translate into similar agronomic measurements than from applying 100% N rate. This recommendation can be furthered by measuring N_2O and observing if emissions also decrease significantly with reduced rates. Also, it is important to reduce the costs of EEF to incentivize growers to frequently use EEF as they are currently more expensive than conventional fertilizers. Overall, if similar yields and proteins could be maintained with lower rates along with reduced prices of EEF, then the economics regarding consistent EEF usage may be justified within the agriculture community.

Understanding that N_2O is not produced homogenously across a landscape is vital to establishing an accurate emission budget within an area. Small, even microsite, areas can produce large amounts of soil N_2O and are referred to as hotspots. These hotspots can occur by N mineralization of organic matter, especially in high organic matter soils, or by anthropogenic inputs. Besides from N inputs, the landscape type, soil porosity, water content, temperature, respiration rates and climatic events can influence the occurrence of N_2O hotspots (Nicolini et al., 2013). A recent study by Mason et al. (2017) attributed close to 40% of total N_2O emissions to hotspots. Therefore, using static-vented chambers risks the opportunity to capture these significant hotspots. Due to the randomness of these hotspot areas and significant contribution to total N_2O production, techniques must be designed to sufficiently capture hotspots. Such techniques include micrometeorological methods such as the eddy covariance or flux-gradient which provide large spatial representation and high temporal resolution. These micrometeorological methods should be used in conjunction with chambers to ensure significant N_2O loss periods are not missed. Additionally, future work implementing static or automated chambers should determine the source area strength to the total flux (Molodovskaya et al., 2011). Implementing this technique, perhaps a season before chamber deployment, will provide greater knowledge on chamber placement within a plot area.

4.5 Recommendations for Growers and Policy Makers

The use of EEF in this current study did not translate into greater grain yield or protein. Therefore, the use of EEF by growers will not translate into greater profits as the cost of EEF is currently greater than conventional urea or AA. However, the reduction potential of EEF, especially nitrification inhibitors, to reduce N losses is significant. Thus, it is imperative for policy makers to provide subsidies to growers to lower the cost of EEF.

Another option is to provide growers with carbon credits with the adoption of EEF to their farm management plan. These subsidiary actions should be especially important for federal policy makers as Canada has committed to the Paris Climate Agreement to reduce greenhouse gas emissions from all major industry sectors. Yet, the adoption of late-fall EEF applications can be both cost and time effective to growers since fertilizers tend to cost less in the fall and fall workloads are less than spring. This current study also found similar agronomic benefits to spring with late-fall EEF applications but only one site year had greater fall applied yields than spring. Therefore, growers must still use a degree of caution if applying EEF in fall but late-fall applications can be recommended to provide similar grain yield and protein amounts compared to spring.

4.6 Testing EEF Across the Prairies

Two other prairie provinces, Saskatchewan and Alberta, participated in the same project to evaluate EEF applications in late-fall and spring. The project in Saskatchewan included four site-years with two being non-irrigated and two being irrigated in 2016 and 2017. The project in Alberta included two site-years in 2016 and 2017. In total, across the prairies, twelve site years were completed. Results in Saskatchewan and Alberta were consistent to Manitoba in that SuperU and eNtrench reduced N_2O emissions, area-, applied N and yield-scales more effectively than any other EEF. Specifically, eNtrench reduced N_2O at all four Saskatchewan sites and SuperU reduced N_2O at three Saskatchewan sites. Similar to this study, EEF LIMUS, ESN and N-Serve did not effectively reduce N_2O over conventional urea and AA. At two Saskatchewan and one Alberta site-year, fall-applied cumulative N_2O was greater than spring-applied. However, there was one site-year in each Saskatchewan and Alberta that measured greater spring-applied N_2O versus fall-applied. In respect to agronomy, fall applications reduced grain yield at one site in Saskatchewan. All EEF sources did not negatively affect yield at any site in Saskatchewan and Alberta. The results from the other prairie provinces reaffirms the conclusions from the current study in Manitoba in that urea nitrification products can reduce N_2O emissions and maintain crop productivity.

4.7 References

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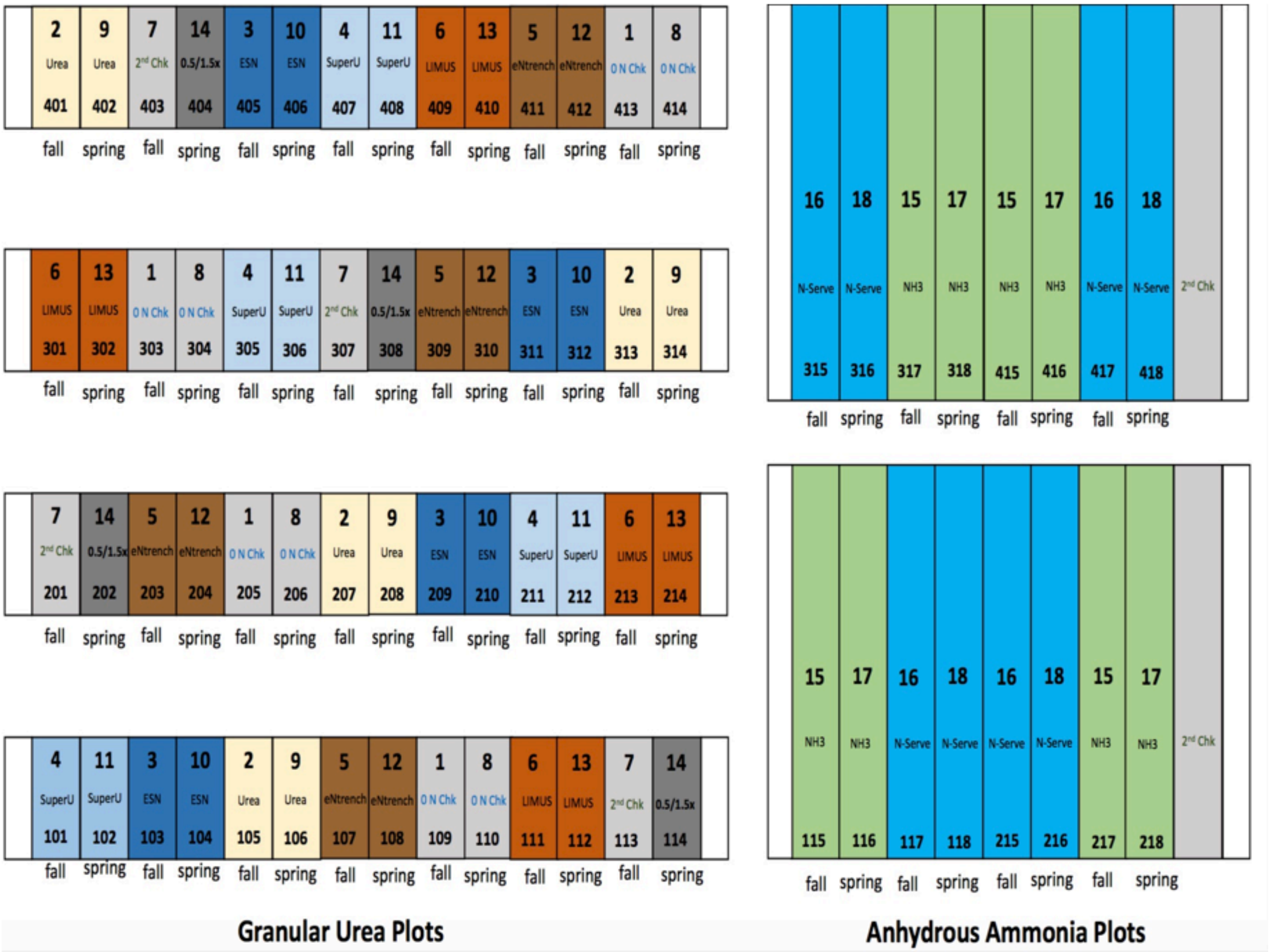
Appendix

A. Urea and AA Site Design



2	9	7	14	3	10	4	11	6	13	5	12	1	8
Urea	Urea	2nd Chk	0.5/1.5x	ESN	ESN	SuperU	SuperU	LIMUS	LIMUS	eNtrench	eNtrench	0 N Chk	0 N Chk
401	402	403	404	405	406	407	408	409	410	411	412	413	414
fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring

6	13	1	8	4	11	7	14	5	12	3	10	2	9
LIMUS	LIMUS	0 N Chk	0 N Chk	SuperU	SuperU	2nd Chk	0.5/1.5x	eNtrench	eNtrench	ESN	ESN	Urea	Urea
301	302	303	304	305	306	307	308	309	310	311	312	313	314
fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring

7	14	5	12	1	8	2	9	3	10	4	11	6	13
2nd Chk	0.5/1.5x	eNtrench	eNtrench	0 N Chk	0 N Chk	Urea	Urea	ESN	ESN	SuperU	SuperU	LIMUS	LIMUS
201	202	203	204	205	206	207	208	209	210	211	212	213	214
fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring

4	11	3	10	2	9	5	12	1	8	6	13	7	14
SuperU	SuperU	ESN	ESN	Urea	Urea	eNtrench	eNtrench	0 N Chk	0 N Chk	LIMUS	LIMUS	2nd Chk	0.5/1.5x
101	102	103	104	105	106	107	108	109	110	111	112	113	114
fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring	fall	spring

Granular Urea Plots

16	18	15	17	15	17	16	18	
N-Serve	N-Serve	NH3	NH3	NH3	NH3	N-Serve	N-Serve	2nd Chk
315	316	317	318	415	416	417	418	
fall	spring	fall	spring	fall	spring	fall	spring	

15	17	16	18	16	18	15	17	
NH3	NH3	N-Serve	N-Serve	N-Serve	N-Serve	NH3	NH3	2nd Chk
115	116	117	118	215	216	217	218	
fall	spring	fall	spring	fall	spring	fall	spring	

Anhydrous Ammonia Plots

Figure I. Treatment map for each site illustrating N source and application timing plots.

B. Urea and AA agronomic comparison including 0 N control

Table I. Analysis of variance and comparison of urea N sources from effects and interaction of N source and site-year on yield, protein, NU_{grain} and NU_{bio}.

	Yield (Mg ha^{-1})	Protein (%)	NU_{grain} (kg N ha^{-1})	NU_{bio} (kg N ha^{-1})
N sources				
0 N Control	2.47 ± 0.15b	13.30 ± 0.20c	57.98 ± 3.54b	73.84 ± 4.57b
Urea	3.75 ± 0.13a	15.01 ± 0.16ab	97.78 ± 3.01a	133.67 ± 5.00a
ESN	3.86 ± 0.16a	15.33 ± 0.13a	103.01 ± 3.81a	141.98 ± 5.79a
SuperU	3.76 ± 0.15a	15.23 ± 0.13ab	99.47 ± 3.38a	138.97 ± 5.37a
eNtrench	3.76 ± 0.13a	15.38 ± 0.16a	100.13 ± 2.89a	137.77 ± 4.41a
LIMUS	3.91 ± 0.13a	14.80 ± 0.15b	100.84 ± 2.85a	140.72 ± 4.71a
Site				
Warren	3.07 ± 0.08d	15.27 ± 0.20b	82.72 ± 2.53c	107.65 ± 3.84d
Glenlea	2.57 ± 0.09e	14.49 ± 0.19c	66.38 ± 2.67d	87.61 ± 3.64e
Carman	3.48 ± 0.08c	15.83 ± 0.10a	96.90 ± 2.41b	138.11 ± 3.64c
LaSalle	3.87 ± 0.08b	14.98 ± 0.10b	102.22 ± 2.49b	155.50 ± 5.10b
Kelburn	5.22 ± 0.09a	13.62 ± 0.16d	125.56 ± 3.15a	171.25 ± 4.76a
Ridge Road	3.29 ± 0.17cd	14.87 ± 0.19bc	85.43 ± 4.19c	106.84 ± 5.00d
	ANOVA (p value)			
Source (N)	<0.0001	<0.0001	<0.0001	<0.0001
Site Yr (S)	<0.0001	<0.0001	<0.0001	<0.0001
N*S	0.2744	0.0016	0.1570	0.2261

Values followed by different letters in a column, separated by N source and site, are significantly different at $P < 0.05$.

Means ± standard error.

Table II. Analysis of variance and comparison of AA N sources from effects and interaction of N source and site-year on yield, protein, NU_{grain} and NU_{bio}.

	Yield (Mg ha^{-1})	Protein (%)	NU_{grain} (kg N ha^{-1})	NU_{bio} (kg N ha^{-1})
N sources				
0 N Control	2.47 ± 0.15b	13.30 ± 0.20b	57.98 ± 3.54b	73.84 ± 4.57b
AA	3.81 ± 0.14a	14.45 ± 0.16a	96.52 ± 3.54a	131.85 ± 5.81a
N-Serve	3.71 ± 0.15a	14.57 ± 0.17a	94.35 ± 3.51a	127.36 ± 5.81a
Site				
Warren	2.63 ± 0.09d	14.19 ± 0.22b	66.21 ± 3.14d	82.03 ± 4.07d
Glenlea	2.28 ± 0.15d	12.95 ± 0.22c	52.77 ± 4.00e	67.04 ± 5.21d
Carman	3.25 ± 0.11bc	15.65 ± 0.17a	89.67 ± 3.60bc	126.74 ± 5.62b
LaSalle	3.68 ± 0.16b	14.61 ± 0.16b	95.20 ± 4.81b	138.43 ± 9.33ab
Kelburn	4.91 ± 0.14a	13.11 ± 0.22c	114.08 ± 4.57a	153.43 ± 7.51a
Ridge Road	3.22 ± 0.28c	14.15 ± 0.25b	79.76 ± 6.80c	98.43 ± 8.35c
	ANOVA (p value)			
Source (N)	<0.0001	<0.0001	<0.0001	<0.0001
Site Yr (S)	<0.0001	0.0071	<0.0001	<0.0001
N*S	0.0011	0.4724	0.0201	0.0144

Values followed by different letters in a column, separated by N source and site, are significantly different at $P < 0.05$.

Means ± standard error.

C. Individual site air temperature and total precipitation

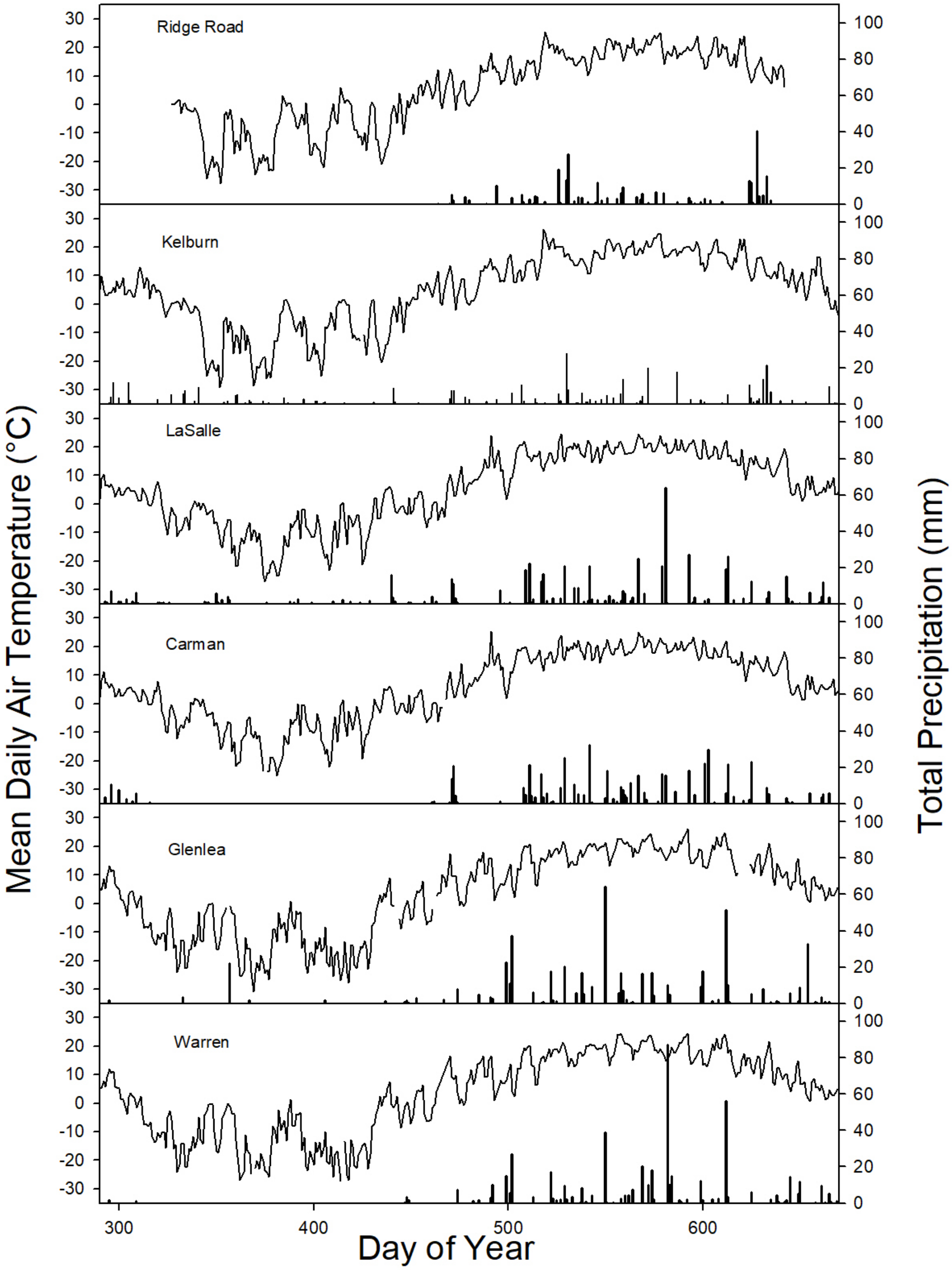


Figure II. Daily total precipitation and mean air temperature at each site from fall fertilizer application to last gas sampling date.

D. Urea and AA N_2O comparison including 0 N control

Table III. Analysis of variance and comparison of urea N sources from effects and interaction of N source and site-year on ΣN_2O, EF and EI.

	ΣN_2O (kg N ha^{-1})	EF (%)	EI (kg N Mg^{-1})
N sources			
0 N Control	0.19 ± 0.03c	----	0.08 ± 0.01c
Urea	0.97 ± 0.11a	0.70 ± 0.10a	0.29 ± 0.04a
ESN	0.92 ± 0.14a	0.63 ± 0.11a	0.28 ± 0.04a
SuperU	0.58 ± 0.07b	0.34 ± 0.06b	0.17 ± 0.03b
eNtrench	0.61 ± 0.10b	0.36 ± 0.07b	0.16 ± 0.02b
LIMUS	0.95 ± 0.16a	0.68 ± 0.14a	0.28 ± 0.06a
Site			
Warren	0.49 ± 0.05cd	0.39 ± 0.06b	0.16 ± 0.02b
Glenlea	1.49 ± 0.17a	1.33 ± 0.16a	0.55 ± 0.06a
Carman	0.72 ± 0.06c	0.53 ± 0.05b	0.20 ± 0.01b
LaSalle	0.40 ± 0.04de	0.34 ± 0.05bc	0.10 ± 0.01c
Kelburn	1.02 ± 0.15b	0.56 ± 0.14b	0.19 ± 0.03b
Ridge Road	0.18 ± 0.02e	0.11 ± 0.01c	0.06 ± 0.01d
	ANOVA (p value)		
N Source (N)	<0.0001	<0.0001	<0.0001
Site Yr (S)	<0.0001	<0.0001	<0.0001
N*S	<0.0001	<0.0001	<0.0001

Values followed by different letters in a column, separated by N source and site, are significantly different at $P < 0.05$.

Means ± standard error.

Table IV. Analysis of variance and comparison of AA N sources from effects and interaction of N source and site-year on ΣN_2O, EF and EI.

	ΣN_2O (kg N ha^{-1})	EF (%)	EI (kg N Mg^{-1})
N sources			
0 N Control	0.19 ± 0.03b	----	0.08 ± 0.01b
AA	1.03 ± 0.12a	0.74 ± 0.10a	0.31 ± 0.04a
N-Serve	0.96 ± 0.11a	0.68 ± 0.09a	0.24 ± 0.04a
Site			
Warren	0.50 ± 0.06c	0.50 ± 0.09c	0.18 ± 0.02bc
Glenlea	1.26 ± 0.20a	1.37 ± 0.16a	0.49 ± 0.07a
Carman	1.08 ± 0.20ab	1.11 ± 0.19ab	0.31 ± 0.05b
LaSalle	0.57 ± 0.09c	0.69 ± 0.09bc	0.14 ± 0.02cd
Kelburn	0.61 ± 0.09bc	0.19 ± 0.10c	0.12 ± 0.02cd
Ridge Road	0.31 ± 0.08d	0.30 ± 0.08c	0.10 ± 0.02d
		ANOVA (p value)	
N Source (N)	<0.0001	0.5184	<0.0001
Site-Year (S)	<0.0001	<0.0001	<0.0001
N*S	0.0044	0.8296	0.0016

Values followed by different letters in a column, separated by N source and site, are significantly different at $P < 0.05$.

Means ± standard error.

E. Three-Way analysis of variance

Table V. Analysis of variance of urea and AA N sources (excluding 0 N control) from effects and interactions of site-year, N source and time on spring urea and AA indexed ΣN_2O emissions, fertilizer-based emission factor (EF), and yield-based N_2O emission intensity (EI) among all sites. Square root transformation performed on urea indexed ΣN_2O, and urea and AA EI values. Urea spring index ΣN_2O analyzed across 5 of 6 sites with an N source effect (Glenlea, Warren, Carman, LaSalle and Ridge Road).

	Urea			AA		
Factors	Index ΣN_2O (5 of 6 sites)	EF	EI	Index ΣN_2O	EF	EI
Site Yr (S)	<0.001	<0.001	<0.001	0.937	<0.001	<0.001
N Source (N)	<0.001	<0.001	0.002	0.864	0.518	0.626
Time (T)	0.018	<0.001	<0.001	0.307	0.431	0.330
S*N	0.588	<0.001	0.001	0.941	0.830	0.986
S*T	0.003	<0.001	<0.001	0.351	0.702	0.401
N*T	0.380	0.564	0.606	0.307	0.120	0.124
N*T*S	0.293	0.003	0.019	0.351	0.477	0.512