Soil Nitrogen Cycling and Nitrous Oxide Emissions: Interactive Effects of N Fertilization Options, Soil Management, Cropping Systems and Climatic Factors in Central Alberta

by

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Abstract

Increases in nitrous oxide (N_2O) emissions derived from inorganic nitrogen fertilization has become a major environmental concern due to the significant contribution of N_2O to global warming, and the deterioration of the stratospheric ozone layer. Effective N fertilization management strategies can reduce the N losses to the environment and enhance the N use efficiency by crops which is required to meet the global demand for food production.

The effect of N fertilization options (conventional vs. enhanced efficiency N fertilizers (EENFs)) and application timing (fall N vs. spring N) on N_2O emissions and plant productivity were studied in a 2.5 year field experiment, a laboratory incubation, a modelling experiment and a greenhouse experiment. The field study revealed that the beneficial role of EENFs on N_2O emissions and crop yield were limited under N rich, fertile soils. Irrespective of the timing of N fertilization (fall vs spring), weather and soil conditions drove N_2O fluxes. In our incubation study, we used ^{15}N-N_2O isotopic composition to identify the soil N_2O sources following N additions and under five moisture contents. Results from ^{15}N-N_2O site preference indicated that denitrification was the major process contributing to N_2O flux. The N_2O fluxes from soil N were primed by both urea additions and high moisture. To improve quantifications of N_2O fluxes, a simulation experiment was conducted using *ecosys* model. Both modelled and measured data showed that environmentally smart nitrogen (ESN) reduced N_2O emissions relative to conventional urea and anhydrous ammonia fertilizers. Finally, a controlled greenhouse study was conducted to evaluate the differential effects of contrasting cropping management histories including continuous annual cropping, a complex crop rotation and a perennial forage as well as urea addition with and without nitrification inhibitors. This study revealed that long-term cropping systems that lead to greater soil C and N can enhance N_2O production via denitrification. Nitrification inhibitors used in our study were effective in reducing the effect of priming on N_2O emission and soils received long term fertilization enhanced the primed N_2O emissions over cumulative total N_2O emissions. Overall, the findings of these studies collectively enabled an improved understanding of soil N dynamics and associated N_2O production which can aid in developing N management strategies for sustainable agriculture.

Preface

The four research experiments were the original work by Shakila Thilakarathna. She was responsible for planning the experiments, conducting experiment, data collection, analyzing, interpretation and organizing of each chapter in the dissertation. All the work was done under the close supervision of Dr. Guillermo Hernandez Ramirez. Dr. Guillermo Hernandez Ramirez involved in manuscript editing of all four chapters. Drs. Robert Grant and Symon Mezbahuddin provided guidance and manuscript editing of the chapter 4.

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1 General Introduction

Nitrous oxide (N_2O) is a major contributor to global warming, and its global warming potential is 298 times greater than of CO_2. N_2O has a longer lifetime (114 years) compared to other major greenhouse gases and its contribution to global warming is 8.1% (Myhre et al., 2013). Moreover, nitrous oxide reacts with the ozone layer in the stratosphere thereby reduces the ability of atmosphere to filter out incoming harmful ultraviolet radiation (Ravishankara et al., 2009). N_2O emission mitigation measures are sought to prevent the risk of harmful effects of global warming and solar ultraviolet radiation emissions on the terrestrial biosphere.

Application of inorganic N fertilizers in croplands increases emissions of N_2O (Rudaz et al., 1999; Grant and Pattey, 2003; Grant et al., 2006). Synchronization of N release from fertilizers and the N demand of crops ensures optimum nutrient use efficiency and can reduce the availability of N substrate for N_2O production through microbial nitrification and denitrification (Li et al., 2018). One potential approach to achieve the synchronicity between N release and crop uptake is through the use of enhanced efficiency N fertilizers (EENFs). EENFs are defined as "fertilizer products with characteristics that allow increased plant uptake and reduce the potential of nutrient losses to the environment (e.g., gaseous losses, leaching, or runoff) when compared to an appropriate reference product" (AAPFCO, 2013).

Most commercial stabilized EENFs are nitrification inhibitors (NIs) and urease inhibitors (UIs). According to Ruser and Schulz (2015), NIs can inhibit ammonium mono-oxygenase (AMO) via different mechanisms and delay the oxidation of NH_4^+ to NO_2^- for 4 to 7 weeks (Nelson and Huber, 2001; Halvorson et al., 2014). The inhibition of NIs can be achieved via binding the substrate to the active site of AMO (competitive inhibition) or binding to the second site of the enzyme, which is not used for NH_3 binding (non-competitive inhibition). Adding Cu chelating compounds to remove co-factor Cu (that AMO is bound to in the membrane of microorganisms) can inhibit AMO. The inhibitory effect is also performed by the inactivation of the enzyme via covalent modification of proteins in microorganisms (Ruser and Schulz, 2015). The active ingredients (e.g., dicyandiamide (DCD), 2-chloro-6-[trichloromethy] pyrideine (nitrapyrin), 3,4-dimethylpyrazole phosphate (DMPP), and 2-(N-3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (DMPSA)) of NIs receive great commercial use. Urease inhibitors such as NBPT (N-(N-butyl) thiophosphoric triamide) and NPPT (N-(2-nitrophenyl) phosphoric triamide) slow the activity of the urease enzyme and delay the formation of ammonium from urea for 1 to 2 weeks depending on soil temperature and moisture (Trenkel, 2010). Coated water-soluble nitrogen fertilizers are manufactured with polymer coatings or elemental S. In coated water-soluble nitrogen fertilizers, water is diffused through the coating and the urea inside the coating is dissolved. Depending on soil temperature, moisture, and characteristics of the coating, dissolved urea diffuses to the soil solution and becomes available for plants and microbes (Halvorson et al., 2014).

Previous studies have investigated the impact of EENFs on reducing N_2O emissions from croplands. In one study, Maharjan and Venterea (2013) used a cornfield in Minnesota to determine that ESN (environmental smart N polymer-coated urea, PCU) and Super U reduced N_2O emissions by 53% and 70% when compared to urea. Another study, performed by Fernandez et al. (2015), used a continuous corn system in Illinois and found greater N_2O emissions from anhydrous ammonia (73%) and urea (44%) when compared to ESN. Through monitoring N_2O emissions in irrigated corn-based rotations, Halvorson et al. (2010) observed N_2O emission reductions from ESN and Super U in no-till tillage systems. In northeastern Colorado, N_2O emission reductions were shown for irrigated spring barley through the application of DCD (82%) and PCU (71%) (Delgado and Mosier, 1996). Representing Southern, Central, and Northern Alberta, Li et al. (2016) conducted field studies in three sites and observed an average of 15% less N_2O emissions than urea. In comparing the impacts of NIs and conventional fertilizers, Akiyama et al. (2010) used a meta-analysis and found N_2O emissions were reduced by 31-44% from NIs. In saying so, N_2O emission reduction is dependent on the inhibitor type (e.g., nitrapyrin-50%, DCD-30%, and DMPP-50%) (Akiyama et al., 2010). Ruser and Schulz (2015) also analyzed the effect of inhibitors and reported that DCD, DMPP, Nitrapyrin, and DMPSA have a N_2O reduction potential of 40%, 34%, 32%, and 28%, respectively. Some studies showed no effect or small effect of EENFs on N_2O emissions. In a study performed by Venterea et al. (2011), using a rainfed corn system in Minnesota, they found no significant N_2O emission reductions by inhibitors or polymer-coated urea. Similarly, in a high fertility cornfield located in Iowa, Parkin and Hatfield (2014) found that EENFs did not reduce N_2O emissions. As well, Sistani et al. (2011) conducted a study in Kentucky on a no-till corn production system to evaluate greenhouse gas emissions from different fertilizer sources and found inconsistent effectiveness of EENFs on N_2O emission reduction.

Moreover, the timing of fertilizer application (spring or fall application) influences N_2O emissions. Farmers in the Canadian prairies prefer fall application of fertilizer compared to spring application due to the low cost of fertilizer in the fall and the reduction of workloads in the following spring. Fall-applied fertilizer use efficiency is generally lower and may result in greater emissions of N_2O than spring application (Malhi and Nyborg, 1990; Nyborg et al., 1997). In fall addition, ammonium can be oxidized to nitrate via nitrification prior to soil freezing. During snow thawing, the soil is saturated and becomes anaerobic which favors N_2O production via denitrification (Nyborg et al., 1997). Although there is a risk of N_2O emission due to the application of conventional fertilizer in fall, systematic studies on this issue in Central Alberta are very limited.

Previous studies show that the efficacy of the EENFs in reducing N_2O emissions varies widely according to soil and climatic conditions (N substrate, soil moisture, soil organic C, aeration, pH, temperature, rainfall), cropping systems (dryland, grassland, paddy) and management practices (N fertilizer source, rate, placement, timing, type of crop, irrigation, tillage, management legacy) (Soares et al., 2015; Li et al., 2018). For example, the meta-analysis conducted by Li et al. (2018) reported that NI reduced N_2O emissions by 77% in fine-textured soils and 44% in coarse-textured soils. NI were effective in reducing N_2O emissions in acidic soils with a pH<6 but less effective in neutral or alkaline pH soils. Further, their meta-analysis showed that reduction of N_2O emissions was greater (63% N_2O reduction) in the soils with intermediate soil organic carbon (10-40 g kg^{-1}) than soils with low (<10 g kg^{-1}) and higher (>40 g kg^{-1}) soil organic carbon. In contrast to nitrification inhibitors, PCU was effective when soils contain soil organic carbon <10 g kg^{-1}, in coarse-textured soils or in acidic and alkaline soils. Crop type also has a considerable impact on EENFs. The response of N_2O emission reduction in wheat and maize to NI is marginal and moderate for paddy systems while PCU was more effective in paddy system and has less impact on wheat or maize.

In this context, the use of EENFs has become a practical management strategy in reducing N_2O emissions when appropriate EENF types are matched with the cropping system, soil and climatic conditions (Li et al., 2018). There is a need for more information about the effect of EENFs on N_2O reduction in Central Alberta. Given the potential interest in the further evaluation of the potential use of different EENFs to mitigate N_2O emissions in Central Alberta, a field experiment, a laboratory incubation, a modelling experiment and a greenhouse experiment was conducted. These 4 chapters aimed at improving the understanding of the combined effects of sources, processes, and biophysical conditions on soil N_2O dynamics. The specific objective of the dissertation, (i) to evaluate the effect of the interaction in N addition timing (fall N vs. spring N) and N fertilizer type (conventional vs. EENFs) on N_2O emissions and spring wheat productivity (Chapter 2), (ii) to determine the response of N_2O production to different WFPS and N sources under controlled conditions and the priming effects on soil N and associated N_2O emissions sourced by both N pools and processes (Chapter 3), (iii) to simulate N_2O emissions as a function of N fertilization options (conventional vs. EENFs) and application timing (fall N vs. spring N) using the process based *ecosys* model (Chapter 4), and (iv) to identify the effects of contrasting management legacy of cropping systems, associated land-use changes, and the addition of nitrification inhibitors with N fertilization on soil N_2O emissions (Chapter 5). Finally, general conclusions were elaborated with focus on recommendations for future research areas. The findings of these studies collectively enabled new mechanistic understanding of the underlying N cycling processes and associated N_2O production in cropland soils and this improved knowledge will be used to improve quantitative prediction ability and develop best management practices to mitigate N_2O emissions from cropping systems.

2 Nitrous Oxide Emissions and Nitrogen Use Efficiency in Wheat: N Fertilization Timing and Formulation, Soil N and Weather Effects

2.1 Abstract

Improved management of nitrogen fertilization in croplands could mitigate soil emissions of nitrous oxide (N_2O) which is a potent greenhouse gas and a precursor for the depletion of ozone in the stratosphere. This study investigated the effects of spring versus fall N applications of conventional vs. enhanced efficiency N fertilizers (EENFs) on N_2O emissions and N use-efficiency in spring wheat (*Triticum aestivum* L.) in replicated trials for 2.5-years in Central Alberta, Canada. The conventional fertilizers were anhydrous ammonia (AA) and granular urea. The EENFs were AA with a nitrification inhibitor as well as urea formulations with various urease and nitrification inhibitors and a polymer-coating. Emissions of N_2O were measured using manually-sampled, non-steady state chambers. Wheat dry matter (DM) and N in grain and straw at harvest, and soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were also determined. The fertilizer-N_2O emission factor (EF) derived from our study (0.31 ± 0.04%) supports the existing regional-specific EF of 0.33% (Tier 2). Irrespective of the choice of N fertilization, peak N_2O emissions were evident following major rainfalls and soil thawing, indicating that increasing soil moisture was a key trigger for a pulse of emissions. For the first study year, the cumulative annual emission of N_2O was 36% greater for spring- than fall-applied N ($P = 0.031$) as major rainfalls occurred after the spring N fertilization. Conversely, the opposite was observed for the second study year where spring-applied N emitted half the N_2O than fall N ($P < 0.001$), as most of the N_2O emissions were associated with soil thawing. The soil at the study site inherently had high availability of N (NH_4^+ and NO_3^-), and this likely hampered the effect of EENFs on N_2O emissions and grain yields. Only in the first study year, EENFs significantly reduced N_2O emissions (by 30% in average; $P = 0.019$), and with a tendency for higher grain yield across all spring EENFs as compared with spring conventional N addition. This implies

that EENFs may diminish N_2O emissions while supporting increased yields even under scenarios with high soil N availability.

2.2 Introduction

Inorganic nitrogen (N) fertilization in croplands can increase soil nitrous oxide (N_2O) emissions depending in part on management options and specific field conditions. The contribution of N_2O to global warming is significant due to its greater global warming potential compared to CO_2 as well as its contribution to ozone depletion in the stratosphere (Ravishankara et al., 2009; Myhre et al., 2013). Management practices that mitigate N_2O production from croplands can also result in enhanced nutrient use efficiency of cropping systems. Such improvements in agricultural efficiency are required to meet the growing global demand for food production.

Optimizing grain production while minimizing N_2O emissions using improved N fertilization is a continuous challenge for producers in agroecosystems worldwide. Due to the lower cost of fertilizer in the fall and the reduction of workloads in the following spring, many farmers in temperate agricultural regions such as the Canadian Prairies apply N fertilizers in fall. Producers often undertake N fertilization in the fall after harvesting when the soil conditions are still favorable for field operations. Fall-applied fertilizer N use-efficiency can putatively be lower and may result in greater emissions of N_2O than spring application (Malhi and Nyborg, 1990; Malhi et al., 2001; Tiessen et al., 2005). Following an early fall N addition (e.g., September), ammonium can be oxidized to nitrate via nitrification under relatively warm temperatures prior to soil freezing. Consequently, there can be a high likelihood for fall-applied N fertilizers to experience detrimental gaseous or leaching losses to the environment.

In addition to the timing of N fertilization, N formulation can play a significant role in minimizing N_2O production. Granular urea (46-0-0) and anhydrous ammonia (AA; 86-0-0) are the main conventional N sources in western Canada. Upon application of these conventional fertilizers, concentrations of plant-available soil N is increased due to their quick nutrient releasing characteristics. When N availability does not coincide with crop demand, microorganisms can utilize this N in processes such as nitrification, nitrifier-denitrification, and denitrification; all of which contribute to the production of N_2O (Venterea et al., 2012). Synchronization of N release from fertilizers and the N demand of crops ensures optimum nutrient use efficiency and can reduce the availability of N substrate for N_2O production through microbial nitrification and denitrification (Li et al., 2018). One potential approach to achieve the synchrony between N release and crop uptake is through the use of enhanced efficiency N fertilizers (EENF) as these are considered to be "fertilizer products with characteristics that allow increased plant uptake and reduce the potential of nutrient losses to the environment (e.g., gaseous losses, leaching, or runoff) when compared to an appropriate reference product" (AAPFCO, 2013).

Of the many EENF options, stabilized fertilizers and coated water-soluble N fertilizers typically receive great attention (Trenkel, 1997; Halvorson et al., 2014; Dungan et al., 2017). Stabilized EENFs are fertilizers with chemical additives that can inhibit microbial or enzyme activity. Two major types of stabilized EENFs are nitrification inhibitors (NIs) and urease inhibitors (UIs). The active ingredients [e.g., dicyandiamide (DCD), 2-chloro-6-[trichloromethy] pyrideine (nitrapyrin) and 3, 4-dimethylpyrazole phosphate (DMPP)] of NIs, inhibit ammonium mono-oxygenase and delay the oxidation of NH_4^+ to NO_2^- for 4 to 7 weeks (Nelson and Huber, 2001; Halvorson et al., 2014). Due to the delay of N transformation in the nitrification process, soil N is retained in the form of NH_4^+ which is more stable in the soil than NO_3^-, denitrification sourced N_2O emissions are reduced, and there are less NO_3^- losses via leaching (Bronson, 1992; Franzen, 2011; Halvorson et al., 2014). Urease inhibitors slow the rate of urea hydrolysis for 1 to

2 wk by decreasing the activity of urease enzyme and reducing NH_3 volatilization, NO_3^- leaching and N_2O emission (Trenkel, 2010; Dungan et al., 2017). N-(N-butyl) thiophosphoric triamide (NBPT) and N-(2-nitrophenyl) phosphoric triamide (NPPT) are active ingredients of commonly available urease inhibitors. Coated water-soluble N fertilizers are manufactured with polymer coatings or elemental S. When soil water moves into the fertilizer granule through the semipermeable coating, the N fertilizer inside the coating is diffused gradually and can minimize the rapid rise of available N in the early season.

Numerous studies have been conducted to determine the effectiveness of EENFs on N_2O emissions. A study of irrigated spring barley in northeastern Colorado showed that the application of NIs (DCD) reduced N_2O emissions by 82%, whilst polyolefin-coated urea reduced N_2O emissions by 71% during the 21 d after the application of fertilizers (Delgado and Mosier, 1996). Evaluating a continuous corn (*Zea mays)* system in Illinois, Fernández et al. (2015) found greater N_2O emissions from anhydrous ammonia (73%) and urea (44%) compared to polymer-coated urea (PCU; commercially known as Environmental Smart Nitrogen®, ESN). Halvorson et al. (2014) reported that ESN and Super U reduced N_2O emissions in comparison to urea by 42% and 46%, respectively. Halvorson et al. (2010) monitored N_2O emissions from irrigated corn-based rotations and found that ESN and Super U also reduced N_2O emissions in no-till tillage systems. According to a meta-analysis by Akiyama et al. (2010), NIs can reduce N_2O emissions by 31-44% compared to conventional fertilizers. The role of reducing N_2O emissions varies with the inhibitor type; nitrapyrin-50%, DCD-30 % and DMPP-50% (Akiyama et al., 2010). Conversely, some studies showed no effect or small effect of EENFs on N_2O emissions. Venterea et al. (2011) reported no significant reductions of N_2O emissions by inhibitors and polymer-coated urea in a rainfed corn system in Minnesota. Similarly, Parkin and Hatfield

(2014) also observed that EENFs did not reduce N_2O emissions from a corn field in Iowa. Sistani et al. (2011) conducted a study to evaluate the greenhouse gas emissions from different fertilizer sources in a no-till corn production system in Kentucky, and they found that the effectiveness of EENFs on N_2O reduction was not consistent.

Despite the availability of several earlier reports focusing on the effects of EENFs on N_2O production, results are inconsistent due to variability of soil, weather, and cropping systems (Parkin and Hatfield., 2014; Li et al., 2018). Although there is a risk of increased N_2O emissions due to the application of conventional fertilizer in the fall, systematic studies focusing on this N timing aspect in fertile soils such as Black Chernozems in the Canadian Prairies are still limited. Tenuta et al. (2016) reported that fall application of AA in Manitoba just prior to soil freezing did not result in more N_2O emissions; they found emissions were lower in one of two study years with fall than spring timing. Application of ESN in Manitoba as a broadcast application followed by incorporation reduced the emission factor by half compared to conventional urea and SuperU (Asgedom et al., 2014). When banded, ESN and SuperU similarly reduced the emission of N_2O by half compared to conventional urea (Gao et al., 2015). Across the Dark Brown, Black and Dark Grey Soil Zones in Alberta, Li et al. (2012 and 2016) reported a 15 to 20% reduction in N_2O emissions with banded ESN compared to conventional urea. Examination of a nitrification inhibitor with AA (e.g. N-serve) or newly available UIs (e.g. LIMUS) and NIs (e.g. eNtrench) have not been previously reported for Black Chernozems or elsewhere on the Canadian Prairies.

There is also a need for more studies examining the agronomic performance of spring wheat, the second most grown crop on the Canadian Prairies, as a function of EENFs under a wide range of soil and climates. Our study aimed at improving the understanding of the combined effects of N sources, addition timing and biophysical conditions on soil N_2O dynamics for Black Chernozem soils. As fertile soils enriched with N and C are common in certain temperate agricultural regions such as the Black soils of Central Alberta, our study intended to improve the understanding of N dynamics and to aid in the development of best management practices for mitigating N_2O emissions from such croplands, which by nature can typically experience high available soil N. Using field experiments, the objectives of this study were: (i) to quantify changes in N_2O emissions and emission factors as a function of using EENFs compared with conventional urea and AA fertilizers, (ii) to determine the N_2O emission reduction potential of spring versus fall N application timings as affected by variations in weather and soil conditions, and (iii) to evaluate the effect of the interaction in N addition timing (fall N vs. spring N) and N fertilizer type (conventional vs. EENFs) on spring wheat productivity, fertilizer-N use efficiency, and associated canopy greenness. For this study, we hypothesize that EENFs can gradually release the added N, and hence, reduce the availability of N substrates for soil N_2O production. Furthermore, greater losses of N_2O are anticipated with fall-applied N in comparison to spring-applied N fertilizer, presumably because of differential magnitude of emissions following soil thawing.

2.3 Materials and Methods

2.3.1 Site Description

The experiment was conducted at the St. Albert Research Station (53° 41′ 45.1″ N, 113° 37′ 33.6″ W) in Central Alberta, Canada. The experimental site is characterized by a cold, semi-humid, temperate climate. According to Canadian soil classification system, the predominant soils at the site are Black Chernozem (U.S. Soil Taxonomy equivalent: Typic Cryoboroll). Based on a composite sampling prior to treatment establishment, soils of the experimental site have a silty clay texture (86 g kg^{-1} sand, 494 g kg^{-1} silt, 420 g kg^{-1} clay) by hydrometer method (Bouyoucos, 1962), a bulk density of 1.25 ± 0.09 g cm^{-3}, pH of 6.2 ± 0.1 (1:10 soil:water), and organic carbon content of 45 ± 1.2 g C kg^{-1} and total N of 3.9 ± 0.2 g N kg^{-1} based on dry combustion method.

2.3.2 Experimental Design

Fifteen treatments were applied encompassing fertilizer types (conventional vs. EENFs) and application timings (fall N vs. spring N; Table 2-1). All treatments were established in a completely randomized block design. Four experimental block replicates (9.2 m × 46. 5 m) were established and each block replicate consisted of 15 individual plots (3.1 m × 9.2 m). Blocks were separated from each other by an 18 m wide buffer zone. In the fall, eight N treatments were applied 1) control (no N was added but did received a soil disturbance resembling fertilizer banding at the time of spring N application); conventional fertilizers: 2) urea and 3) anhydrous ammonia (AA); and, as EENFs: 4) polymer-coated urea (environmentally smart N - ESN®, 45-0-0), 5) urea constituted with N-(N-butyl) thiophosphoric triamide (NBPT) and dicyandiamide additives (Super U®, 46-0-0), 6) urea coated with N-(N-butyl) thiophosphoric triamide (NBPT) and N-(2-nitrophenyl) phosphoric triamide (NPPT) (LIMUS®, 0.69 L ha^{-1}), 7) urea coated with nitrapyrin additive (eNtrench®, 2.69 L ha^{-1}) and 8) AA with nitrapyrin additive (N-Serve®, 2.35 L ha^{-1}). Moreover, in the subsequent spring, experimental treatments 2) through 8) were also applied in the spring N plots (Table 2-1).

This study ran for two and a half years from Oct 2015 to May 2018 encompassing two full cropping cycles (fall application-growing season-subsequent spring thaw; Table 2-2). The first crop year began on 23 October 2015 with the fall N application followed by seeding and spring N application on 10 May 2016 and harvesting on 12 September 2016. The second crop year began on 24 October 2016 with the fall N application followed by seeding and spring N application on 23 May 2017. After harvesting on 11 September 2017, post seasonal N_2O emissions were monitored until 22 May 2018 to examine any potential carry-over effects of any residual N on thaw emissions.

In both years, the N fertilizer treatments were added in spring or fall at a rate of 100 kg N ha^{-1}. The fertilizer rate of 100 kg N $ha^{-1}$was calculated using the Alberta Farm Fertilizer Information and Recommendation Manager (AFFIRM) which is a decision support tool designed to develop nutrient management plans for crop production using the location, soil, previous crop, soil test laboratory results, expected crop price, farm fertilizer budget and so on. Experimental plots for each crop year were located in two adjacent sites. Fertilizer was double midrow banded (placed between every second set of plant rows) to a depth of 7.5 cm and 50 cm band spacing. Canada Western Red Spring wheat (*Triticum aestivum* cv. CDC Go) was seeded in all plots at the same time as spring N application each year. Wheat was seeded to a depth of 2.5 cm and 25 cm row spacing at the rate of 118 kg ha^{-1}. P_2O_5 was applied at the rate of 40 kg ha^{-1} with seeding in the spring as triple super phosphate (0-45-0). Herbicides, fungicides, and insecticides were applied to control weeds and crop diseases as needed.

2.3.3 Flux Measurements

The N_2O emission measurements were performed with a non-steady state, closed-manual chamber methodology (Hernandez-Ramirez et al., 2009; Lin et al., 2017). To capture and estimate more representative flux values, two chambers were deployed in each experimental plot (total number of chambers= 120). Chambers were rectangular (15 × 64.1 × 15.6 cm, height × length × width) with a detachable lid having a rubber septum. Chamber bases were installed in the soil to a depth of 5 cm and perpendicular to fertilizer midrows and crop rows. Chambers of control and fall-N treatment plots were installed in the fall every year and only removed for seeding in the following spring. Soon after seeding and spring N fertilization, chambers were installed in the spring treatment plots and the chambers used for the control and fall treatment plots were reinstalled in their same original locations. As two chambers were deployed in each experimental plot, upon chamber deployment, gas samples from the first chamber were collected at 16, 32, and 48 min, while only one gas sample was collected from the second chamber at 48 min after enclosure. Gas samples were collected through the rubber septum of the chamber lid using a 20 mL syringe and transferred into a 10 mL pre-evacuated glass vial. Additionally, ambient samples were collected 10 cm from ground surface during gas sampling at time zero (six samples). Gas sampling frequency was once per week. To capture periods of potentially high N_2O flux, gas sampling frequency was increased to twice per week during the week following N fertilizer application or high rainfall events. To ensure consistency of gas flux measurements and to represent daily mean temperature, gas samples were consistently collected between 1130 and

1530 hours. Gas samples were analyzed for N_2O concentration using an electron capture detector in a Varian 3800 gas chromatograph (GC) system (Varian Inc., Walnut Creek, CA) calibrated with certified reference gases (Praxair Specialty gases, Edmonton, AB) including five N_2O concentrations ranging from 0.25 to 4.84 $\mu L\ L^{-1}$ plus ultra pure dinitrogen as zero concentration.

Based on the change in N_2O concentrations over time (using the ambient N_2O measurements at time zero and the 16, 32, and 48-min gas samples collected from the first chamber), linear or quadratic relationships were used to calculate N_2O flux. Significant fluxes were determined using an alpha value of 0.20. When there was no significant relationship, N_2O flux was considered to equal zero (zero order). The modified ideal gas law (Eq. [1]) was used to determine the flux for significant relationships.

$$N_2O\ Flux = \frac{S \times P \times V}{R \times T \times A} \qquad [1]$$

where N_2O flux is the flux rate of N_2O ($\mu mol\ m^{-2}\ min^{-1}$); S is the slope of the line from either the simple linear regression or the first-order derivative at time zero from the quadratic curve ($\mu L\ L^{-1}\ min^{-1}$); P is the gas pressure (atm); V is the volume of the chamber (L); A is the surface area of the chamber (m^2); R is the gas constant ($atm\ \mu L\ K^{-1}\ \mu mol^{-1}$) and T is the temperature of the gas (K) (Yates et al., 2006). The N_2O flux for the second chamber in each plot was determined by plotting the linear relationship for the change of N_2O concentration at time 0 and 48 min. Combining the measured analytical detection limit for the GC system (Lin et al., 2017) and the time of chamber enclosure, we estimated a minimum detectable N_2O flux of 2.84 $g\ N\ ha^{-1}\ d^{-1}$. For the first cropping year (Oct. 2015 to Sep. 2016), 64, 7 and 29% of the fluxes were estimated using linear-, quadratic- and zero-order relationships, respectively. Fluxes during the second cropping year (Oct. 2016 to Sep. 2017) were 63, 12 and 25% for linear-, quadratic- and zero-order relationships, respectively. The cumulative N_2O fluxes from each treatment were calculated using trapezoidal-linear interpolation between the two consecutive sampling events. The N_2O emissions during the winter period were assumed to be negligible. N_2O emissions from spring N treatment plots prior to fertilizer application were assumed to be similar to the control plots.

Area-based emission factors (EF_{area}, Eq. [2]) were calculated as the difference of cumulative annual N_2O emissions between a fertilizer treatment and the corresponding control plot (for each separate block replicate) divided by the total applied N rate as follows:

$$Area\ based\ N_2O\ EF\ (\%) = \frac{N_2O\ emission_{treatment} - N_2O\ emissions_{control}}{N\ fertilizer\ input} * 100 \qquad [2]$$

2.3.4 Soil Sampling and Laboratory Analysis

To determine soil properties, samples were collected before fertilizer applications each year. To determine the temporal variations of soil NO_3^- and NH_4^+, soil samples were also repeatedly collected (0-15 cm in depth) following fertilizer applications on five ocassions. During the first study year, samples were collected in March, April, June, and July. During the second study year, samples were collected in May, June (twice), July, and August. Soil samples were also collected once in May 2018. After the spring fertilizer application, soil samples were collected from both fertilizer bands and interbands (non-banding area) to determine soil mineral N content in bands and interbands (Tables 2-3 and 2-4). Post-harvest soil samples from 0-15, 15-30, 30-60 and 60-90 cm in depth were collected in October 2016 and September 2017. Soils were air dried, ground, passed through 2-mm sieve and extracted with 50 mL 2 mol L-1 KCl (1:10 soil: extractant). The NH_4^+-N and NO_3^--N contents were measured colourimetrically using a SmartChem discrete wet chemistry analyzer (Westco Scientific Instruments, Inc., Brookfield, CT) (Maynard et al, 2008).

2.3.5 Plant Measurements and Sampling

To capture the N status of the plants within the growing season, we conducted repeated measurements of canopy greenness as normalized difference vegetation index (NDVI). The NDVI was taken 60 cm above the plant canopy from seven selected treatments: control; and, for both fall and spring N application of urea, urea with LIMUS, and urea with eNtrench. The measurements were taken between June and August 2017 using a handheld HCS-100 GreenSeeker® (Trimble® Inc., Sunnyvale, CA, USA). The NDVI measurements were taken once per week between 1130 and 1530 hours. Five sets of measurements conducted per plot were averaged to obtain a NDVI value representative for each plot.

For plant sampling, a 1 m^2 quadrat was placed in two representative spots in each plot, and physiologically mature wheat plants inside the quadrat were cut close to the soil surface by hand using a sharp sickle. Straw and grain yields were oven-dried to determine dry matter (DM), and total N contents were analyzed by near-infrared spectroscopy (FOSS™ DS2500; range 400 – 2500 nm, 0.5 nm spectral resolution). We calculated wheat aboveground N multiplying straw DM by straw N content plus grain DM by grain N content. Plant data were used in calculations of the yield-based emission factor (EF_{yield}), fertilizer N use efficiency (NUE), uptake efficiency (UE), physiological efficiency (PE), harvest index (HI) and N harvest index (NHI) as follows:

$$Yield\ based\ N_2O\ EF\ (\%) = \frac{Cumulative\ N_2O\ emission_{treatment}}{grain\ yeild} * 100 \qquad [3]$$

$$Fertilizer\ N\ use\ efficiency = \frac{Grain\ DM_{trt} - Grain\ DM_{control}}{N\ rate} * 100 \quad [4]$$

$$Fertilizer\ N\ uptake\ efficiency = \frac{Aboveground\ N_{trt} - Aboveground\ N_{control}}{N\ rate} * 100 \quad [5]$$

$$Physiological\ efficiency = \frac{Grain\ DM_{trt} - Grain\ DM_{control}}{Aboveground\ N_{trt} - Aboveground\ N_{control}} * 100 \quad [6]$$

$$Harvest\ Index = \frac{Grain\ yield\ DM}{Grain\ yield\ DM + straw\ yield\ DM} \quad [7]$$

$$Nitrogen\ Harvest\ Index = \frac{N\ in\ grain}{N\ in\ grain + N\ in\ straw} \quad [8]$$

2.3.6 Ancillary Measurements

Two digital data loggers with five soil sensors (5TM, aMeter™) each were installed in each experimental site. These sensors provided soil volumetric water content (VWC) and soil temperature measurements at the depth of 10 and 20 cm every 30 min. Soil VWC measurements were reported only for the unfrozen periods. Air temperature and precipitation data were measured in the nearest climate station (St. Albert Research Weather Station located approximately 600 m from the experimental sites) and retrieved via the Alberta Climate Information Service (ACIS).

2.3.7 Statistical Analysis

Normal distribution and homogeneous variance of data were examined using the Shapiro-Wilk and Bartlett tests, respectively. A random mixed model was used to determine the effect of fertilization timing and type and their interaction. When the blocking factor was insignificant, the effect of variables was determined using a generalized least squares (GLS) fixed model. One-way analysis of variance (ANOVA) and post-hoc comparisons (using Tukey's Honest Significant Difference (HSD)) were used to assess the differences across N treatments and to compare the treatment means. Unless stated otherwise, all statistical analysis was performed using R software (version 3.3.1) and an alpha critical value of 0.05.

2.4 Results

2.4.1 Weather characteristics

According to the 30-year climate normal (1981-2010), total annual precipitation at the experimental site is 456 mm and the annual mean temperature is 4.2 ˚C (Fig. 2-1a, b). The mean annual precipitation was near normal being 439 mm in 2016 and 422 mm in 2017. Monthly precipitation distributions differed between 2016 and 2017 with the greatest precipitations occuring in May, July and October in 2016 and April, June and September in 2017 (Fig. 2-1a). The average winter air temperature (November through April) in 2017 was lower than in 2016 (Fig. 2-1b).

2.4.2 Specific conditions leading to high nitrous oxide emissions

Small precipitation events (< 5mm) and low temperatures (average 1 ºC) during fall 2015 resulted in small daily N_2O emissions (0.34 g N_2O-N ha^{-1} day^{-1}; Fig. 2-2). Large N_2O peaks were observed after soil thawing and large rainfall events in spring. On 30 March 2016, a considerable emission of N_2O was observed from the control and fall N treatments in response to soil thawing. Even though treatment means were not significantly different, fall urea treatment exhibited the largest numeric flux (27 g N_2O-N ha^{-1} day^{-1}). After application of spring N fertilization treatments, the largest N_2O emission captured by our sampling in 2016 occurred on 25 May as a result of continuous rain for four days from 20 May to 23 May (22, 8, 33, 6 mm, respectively). As part of this large emission event, the spring AA treatment exhibited the numerically largest flux (102 g N_2O-N ha^{-1} day^{-1}) of the 15 treatments in this study.

Following soil thaw in spring 2017, VWC increased two-fold from 0.20 to 0.43 m^3 m^{-3} and resulted in the largest N_2O flux on 6 April 2017. Several small rainfall events (18 mm cumulative precipitation) occurred at the end of April 2017 and likely contributed to an increase in N_2O flux on 3 May 2017. And the greatest flux during this emission event was observed for the fall-urea (59 g N_2O-N ha^{-1} day^{-1}) treatment. On 20 June 2017, a 32 mm rainfall resulted in water-logged soil conditions sharply increasing the VWC two-fold (from 0.26 to 0.53 m^3 m^{-3}; Fig. 2-2B). The wet conditions prevented field gas sampling until 26 June, and hence, it is likely that the emission event was even larger than captured. Despite this delay in collecting flux data, elevated N_2O fluxes were still observed on 26 June 2017 when the VWC was still relatively high (0.38 m^3 m^{-3}), and the spring ESN treatment emitted had the highest N_2O flux (29 g N_2O-N ha^{-1} day^{-1}).

Our analyses of cumulative emissions calculated for three periods: fall, early spring (ES), and growing seasons (GS) as well as the aggregated of these three periods as annual cumulative revealed important seasonal and inter-annual variations (Table 2-2). There was a significant effect of N fertilization timing on N_2O emissions within GS 2016; spring N was on average 55% higher than fall N (537 vs. 345 g N ha^{-1}, respectively; $P = 0.044$). The fall 2016 period and the subsequent soil thaw within ES 2017 contributed to numerically higher N_2O emissions for the fall N treatments than for spring N. Even though fluxes remained low during the fall for all 3 years, cumulative N_2O emissions from the treatment of fall-Super U were significantly higher than both fall-AA and the control treatment in fall 2016 ($P = 0.005$).

For the first study year (2015-2016), cumulative annual N_2O emissions from spring N treatments were significantly 36% greater than fall N treatments (951 vs. 699 g N ha^{-1}, respectively; $P = 0.031$; Table 2-2) mainly due to a 33 mm rainfall 12 days after N fertilization and crop seeding in the spring, when very little to none soil N uptake by wheat seedlings was occurring. The opposite result (fall N > spring N) was observed in the second study year (2016-

2017) when emissions from across fall N treatments were double the emissions from spring N treatments (1187 vs. 599 g N ha^{-1}, respectively; *P* = 0.001). With respect to the N sources, even though cumulative annual N_2O emissions in the first study year were not significantly different between any of the specific N fertilization treatments, emissions from EENFs as a whole were significantly lower than from the conventional fertilizers by 30 % (*P*= 0.019). Conversely, in the second study, there was no statistical difference in cumulative emissions between EENFs and conventional fertilizer formulations.

2.4.3 Crop response to N fertilization timing and fertilizer types

Average grain yield across treatments in 2016 was higher than in 2017 (Table 2-5) by 1130kg DM ha^{-1}. Although the treatment means were not statistically different in 2016, the average grain yield of all spring EENFs tended to be 11% greater than with spring conventional N fertilization (urea and AA) (4682 vs. 4217 kg DM grain ha^{-1}) (*P*= 0.058), suggesting the potential of using EENFs for enhancing grain yield when used with spring N fertilization. In 2017 harvest, AA showed the influence of timing of fertilizer application numerically, with the lowest yield (2569 kg ha^{-1}) from fall N application while the highest (3870 kg ha^{-1}) was spring N application. In contrast to AA, grain yields of ESN were similar in both years and did not show a numerical response to the timing of application (Table 2-5). In both years the lowest grain yield was obtained from conventional N treatments. Similar to grain yields, straw yields in 2017 were lower than 2016 (Table 2-5). In 2017, the straw yield of fall-applied AA was significantly lower than the spring-applied AA, Urea + Limus and AA+N-Serve (*P*= 0.025). Straw N uptake in 2017 showed similar results (*P*=0.03). In general, N uptake pattern was consistent with grain and straw yield, although greater straw N uptake was observed in 2017 with lower straw yield relative to 2016 (Table 2-5).

In the current study, our estimations of NUE, uptake efficiency (UE), physiological efficiency (PE), harvest index (HI) and N harvest index (NHI) (Table 2-6) indicated no significant effects of fertilizer treatment, timing of N application or type of fertilizers on these N efficiency factors, with the sole exception of UE. The timing of N application had a significant effect on the UE in 2017 (spring N > fall N; $P=0.023$).

The NDVI measurements (canopy greenness) conducted in the 2017 growing season also captured that the spring N treatments had plants generally greater in N status than fall N treatments and significant differences were observed for 26 June and 10 July (spring N > fall N; $Ps < 0.05$; Fig. 2-3). Furthermore, we evaluated the relationship between UE and NDVI measurements as both showed responses to the N fertilization timings. Significant relationships were observed between UE and NDVI for fall N treatments on 10 July and for spring N treatments later in the season on 03 August (Fig. 2-4). These findings indicate that mid-season NDVI measurements can inform the outcome of N uptake efficiency at the end of the growing season. As anticipated, the NDVI values in all treatments gradually increased during the vegetative stages of the wheat until the middle of July (data not shown). In the reproductive stages, due to the translocation of N to grain and overall physiological senescence, NDVI values declined with time (Fig. 2-3). Likewise, the control treatment had the lowest NDVI values for all days of measurements (data not shown).

2.4.4 Area- and yield-based nitrous oxide emission factors

The 2-year treatment means of area- and yield-based EF in our study ranged from 0.16 to 0.58 % kg N_2O-N kg^{-1} N fertilizer and 0.14 to 0.29 g N_2O-N kg^{-1} grain DM, respectively (Table 2-7). There was a significant effect of N application timing on EF_{area} and EF_{yield} in both study years. The effect of fertilizer type was statistically significant only for the EF_{yield} in the second year of the study. We examined relationships between annual, seasonal cumulative N_2O emissions, EF_{area}, EF_{yield} with yield, N uptake, and N efficiencies. Significant relationships were observed for EF_{area} vs. NUE (P=0.001) and grain N uptake (P=0.001) in the second year of the study (Fig. 2-5). These positive relationships indicate that greater availability of soil mineral N in the study site serves as a substrate for detrimental N_2O production in addition to N for beneficial crop uptake.

2.5 Discussion

2.5.1 Weather drives most of nitrous oxide emissions from an N-rich soil

Our results indicate that weather and soil conditions following fertilizer applications had more of an influence on N_2O fluxes than the timing of N fertilization (fall N vs. spring N). The inconsistent effect of N fertilization timing on N_2O emissions in our study does not support our initial, broad hypothesis that greater N_2O emissions were expected from fall-applied N than from spring N. Despite high extractable soil NH_4^+ and NO_3^- contents, the lower N_2O emissions observed during the fall (Table 2-2) can be attributed to lower enzyme activity rates of nitrifiers and denitrifiers under low soil temperature. In early spring, snow melting and thaw events likely provided favorable warmer and nearly water-saturated soil conditions for denitrification while the competition with plants for NH_4^+ and NO_3^- substrates was absent. In both experimental years, the large N_2O emissions during early spring strikingly contributed with more than half of total annual emissions. Earlier studies conducted in the Canadian Prairies (Lemke et al., 1998, Tenuta et al., 2016) and in Eastern Canada (Wagner-Riddle and Thurtell, 1998; Grant and Pattey, 1999; Rochette et al., 2004) have found similar results.

In our study, post-growing season emissions were also evaluated (from Sep 2017 to May 2018) to examine any effects of residual N applied in fall 2016 or spring 2017. As no N treatments were applied in fall 2017, average emissions of N_2O during the subsequent early spring (ES) 2018 were much lower when compared to the ESs in both 2016 and 2017 (Table 2-2). Our results suggest that the N fertilizer additions at late fall (e.g., October) may detrimentally contribute to high spring N_2O emissions. Therefore, producers in temperate continental regions such as Central Alberta can consider application of fertilizer in mid or late spring to decrease the N substrate availability and the risk of denitrification during soil thawing in the early spring. We postulate that minimizing gaseous losses of N during soil thawing can be realized with mid-spring N fertilization followed by early spring and late fall N fertilization (mid-spring < early spring < late fall).

The occurrence of major N_2O fluxes following spring rainfall events and soil thawing substantiates that increased soil moisture under warming conditions is the key triggering factor for pulse N_2O emissions (Table 2-2). Small increases in soil water-filled pore space can result in a considerable decline in gas diffusivity (Grant and Pattey, 2003). As a result, diffusion of O_2 can become limited hindering its ability to serve microbes as an electron acceptor. This enhances the demand for alternative electron acceptors which make rapid transitions of oxidation-reduction reactions, accelerating N_2O production when there is no limitation of N substrate (Grant and Pattey, 1999; Rochette et al, 2004). Similarly, increases in temperature enhance the rate of microbial-mediated O_2 reduction reactions and increase the demand for electron acceptors in biologically-active sites (Schindlbacher et al., 2004). The accumulation of recently-produced N_2O within the soil profile shortly following soil thawing and rainfall (warm and wet soils) is subsequently emitted as peak fluxes as water is being lost from soil pores. Notably, there were no important emissions in late summer even after major rainfalls; this may be caused by the relatively lower N availability (a substrate limitation) due to an uptake of NO_3^- by actively growing wheat plants. Soil N measurements after N fertilization showed greater concentrations of soil NO_3^- in comparison to NH_4^+ (Tables 2-3 and 2-4), indicating the predominantly aerobic nature of soils as NO_3^- is produced via nitrification under aerobic conditions. As expected, N fertilization bands were much enriched in both ammonium and nitrate than the interband areas by up to one order of magnitude higher concentrations (Tables 2-3 and 2-4).

The role of EENFs in reducing N_2O emissions was not consistent across the two years of the study. Our observation that these emissions were highly influenced by weather conditions agrees with previous findings (Burton et al., 2008; Jumadi et al, 2008; Asgedom et al., 2014; Parkin, and Hatfield, 2014; Gao et al. 2015). Together it shows that the efficacy of the EENFs in reducing N_2O emissions varies widely according to soil and weather condition (N substrate, soil moisture, soil organic C, aeration, pH, temperature, rainfall), cropping systems (dryland, grassland, paddy), and management practices (N fertilizer source, rate, placement, timing, type of crop, irrigation, tillage) (Dungan et al., 2017; Li et al., 2018).

In our study, there was no statistical difference in N_2O emission between control and fertilizer treatments. Notably, certain cumulative fluxes for the control treatment emitted even more than the fertilized treatments in numerical terms (Table 2-2), and according to the post-harvest soil sampling conducted in fall, soil NH_4^+ concentration in the control treatment was in some instances numerically higher than both fall N and spring N treatments (Fig. 2-6). These findings can be attributed to the high fertility and mineralization characteristics of the Black Chernozem soil on site, which have by nature high soil organic matter content (> 10 %). Emissions from our control treatment (Table 2-2) showed that large amounts of naturally present organic N can be readily mineralized to provide a substrate for N_2O production. Adding N fertilizer to soils with pre-existing high N availability and already high N_2O production can contribute little or no further increase in N_2O production, as other controlling factors (different than N substrate availability) such as limited heat or moisture availabilities can act as the main drivers. On the other hand, N input and availability of C source are not likely the key limiting factors that determine the magnitude of N_2O fluxes in our experimental site. This supports that baseline fertility condition of the soil needs to be strongly considered prior to use of EENFs.

Moreover, these soils high in organic matter are easily primed in enzymatic and microbial activities, leading to further feedbacks on increasing nutrient availability and causing N gaseous losses during the spring and early summer months under wet-warming conditions. Further research can evaluate such soil priming effects on N_2O production.

2.5.2 N management effects on crop performance

The rationale behind the use of EENFs is to synchronize N release and crop uptake. However, the assessed EENFs, as well as the conventional N fertilizations in our study, exhibited no significant crop response (Table 2-5). According to the Alberta Crop production statistics, the 10-year average grain yield for spring wheat in Alberta is 3188 kg ha^{-1}. In our study, grain yields for both experimental years (4691 and 3330 kg DM ha^{-1}) are greater than this typical grain yield in Alberta, indicating that the soils in the experimental site are very fertile and likely approaching N saturation. High nutrient status of the soils in the experimental site is further confirmed by the mineral N contents in soil (Tables 2-3 and 2-4). When exogenous N enters the soils, this N may be initially immobile. Such immobilized N can subsequently undergo re-mineralization in the short-term (~ weeks) provided favorable warm and wet conditions become prevalent, and this series of shifts in N processes and availability may deliver sufficient N for plant growth. As N fertilizer addition generated null responses in terms of grain productivity, the need for N fertilization could perhaps be questioned; however, our study quantified an N output from the system of 94 ± 6 kg N ha^{-1} via grain-N at harvest (Table 2-5), and hence, an equivalent N fertilization is needed for maintaining and replenishing this grain N removal, enabling a sustainable agroecosystem.

The magnitude of crop N efficiency factors varied between the two experimental years due to differences in growing conditions such as timing and intensity of precipitation and temperature (~ heat availability) (Table 2-6). This notion is supported by the dependency of NUE on the ability of the plants to take up N from soil and to subsequently use the absorbed N efficiently during reproductive stages to produce harvested grain. As the underlying fertile and N rich soil increased the overall plant productivity, calculated fertilizer NUE values were low (15.6 and 4.4 %; Table 2-6). It is noteworthy that as customary, our NUEs were estimated by relating the treatments receiving N fertilization to the control treatment which received no N fertilization (e.g., Eq. 4), and hence, the low NUE values can be attributed to the high N supply available for plant growth in the control, leading to high grain productivity even in the control treatment as mentioned above.

The anticipated role of N fertilization in translating N input into a greater yield can be negligible in an N rich soil such as our study site, which exhibited high soil N availability, and N status near saturation. Future studies are required to evaluate the role of EENFs in crop performance and N_2O emissions with an application of lower rates of N for a soil where native available N is limiting. Based on our observations, we postulate that EENFs may be best utilized in terms of crop production and N_2O emissions in soils with medium soil N levels (in between surplus and deficient N conditions).

In the second year of the study (2016-2017), the efficiency of the AA fertilization depended on the timing of application. The crop N use efficiency seems much higher for the spring AA timing than for the fall-applied AA (Table 2-6), while larger N_2O losses were observed with fall-applied AA during the soil thawing than its spring treatment counterpart [note that emissions during the early spring (ES) in the spring N treatments are assumed to be similar as control] (Table 2-2). Overall, these results reflect the potential benefit of spring timing of conventional fertilizers for enhancing crop yield while minimizing N_2O production.

During 2016 growing season, ESN increased aboveground crop biomass while minimizing N_2O emissions when compared to the conventional N fertilizers, indicating that ESN provided better protection against N losses under these growing conditions (Table 2-5). In coated EENFs such as ESN, when soil water moves into the fertilizer granule through the semipermeable coating, the N fertilizer inside the coating is dissolved, and N diffuses into the soil gradually depending on the characteristics of the coating, soil moisture, and temperature (Halvorson et al., 2014). By contrast, based on previous studies in cropping systems, the use of ESN can also turn out to be inefficient due to the rapid loss of N in response to extreme, sudden increase in temperature and moisture content in the early season when plant uptake capacity is still insufficient to utilize this rapidly-available N. Additionally, N losses from ESN can release N late in the growing season when no or little crop N uptake is occurring. A few earlier studies have been conducted to determine the effectiveness of ESN on N_2O emissions and crop yield (Fernández, 2015; Li et al., 2016). However, most of the available studies to date have been conducted only until harvest. Only a few studies have assessed the fall emissions after harvest in Black Chernozem soils (Asgedom et al., 2014; Gao et al., 2015), reporting similar to as our study low N_2O losses during the fall post-growing season periods. However, there are no reports available for N_2O emissions during the subsequent spring. Taking this into account, we examined the N_2O losses during the post growing season into the subsequent spring from all the experimental plots (Table 2-2). Even though no delay in emissions from ESN was observed compared to other N fertilization treatments, we were able to confirm that the efficacy of ESN was likely determined by the sudden change of soil biophysical conditions. Water-logged conditions following a major rainfall event may have contributed to the highest N_2O fluxes observed for the spring Spring ESN treatment on 26 June 2017 (Fig. 2-2). Hence, future research could further evaluate the mechanistic responses of coated N fertilizers to such sharp moisture increases to further continue filling this knowledge gap.

2.5.3 Comparing our measured N_2O emission factors with IPCC Tiers 1 and 2

The magnitudes of EF_{area} in our study (Table 2-7) were typically lower than the default EF_{area} used in the Intergovernmental Panel on Climate Change (IPCC) worldwide Tier 1 methodology, which is 1 % N_2O-N for synthetic N fertilizers (Roche et al., 2016). As pointed by numerous studies, EF values lower than those predicted by the IPCC underscores the bias and uncertainty associated with the Tier 1. Emission factors that are specific to soil and weather conditions are indeed required to accurately estimate emissions at the national and regional levels. Based on available data, Rochette et al. (2018) recently reported a Tier 2 EF_{area} of 0.33% for synthetic N fertilization taking place specifically in Black soils within the Canadian Prairies. The overall mean of our N_2O EF_{area} (0.31 ± 0.04%; Table 2-7) closely agrees with Rochette's regional-specific Tier 2 EF_{area}. Moreover, a Tier 3 EF approach can provide an even higher certainty. The implementation of detailed processed-based N_2O mathematical models such as *Ecosys* (Grant and Pattey, 2003) can aid to further develop and adapt Tier 3 methodology for the purpose of informing regional and national emission inventories.

In addition to area-based emissions, N_2O emissions were also determined per kilogram of grain yield, as this EF_{yield} provides information about efficient use of N and environmental footprint. Similar to mean cumulative N_2O emissions, the timing of application had a significant influence on EF_{yield} in both study years. A significant effect of fertilizer type was observed only in the first year of our study (Table 2-7). This data reflects that EF_{yield} values follow the pattern of N_2O emissions since the variability of grain yields was narrower.

2.6 Conclusion

Our results suggest that soil moisture is the key driver for increased N_2O emissions in these soils rich in plant-available N. As our results showed that high N_2O losses occurred during soil thaw, application of N fertilizers in mid or late spring in comparison to fall N application can help to sustain high grain productivity while reducing N_2O losses during soil thawing. Since N_2O emissions associated with spring N addition were linked to major rainfall events taking place following such spring N fertilization, using weather forecast and planning the spring N fertilization to prevent such effects of excessive moisture could potentially minimize N_2O emissions. Although EENFs reduced N_2O emissions and spring-applied EENFs increased grain yield in one of our two experimental years, grain yields and N_2O emissions were not consistently affected by specific EENFs in these N-rich soils. Therefore, comparable studies can be conducted in soils with medium or deficient N levels or applying lower rates of N when comparing EENFs to the conventional fertilizers.

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2.8 Tables

Table 2-1. Treatment description implemented during this study.

Treatment - N source	Fertilizer type	Additive type	Active ingredient	N timing and placement
Control	-	-	-	None
Urea	Conventional	-	-	Fall Banded
Anhydrous Ammonia	Conventional	-	-	Fall Banded
ESN (polymer coated urea)	EENF	-	-	Fall Banded
Super U	EENF	Urease and nitrification inhibitor	NBPT and DCD	Fall Banded
Urea + LIMUS	EENF	Urease inhibitor	NBPT and NPPT	Fall Banded
Urea + eNtrench	EENF	Nitrification inhibitor	nitrapyrin	Fall Banded
Anhydrous Ammonia + N-Serve	EENF	Nitrification inhibitor	nitrapyrin	Fall Banded
Urea	Conventional	-	-	Spring Banded
Anhydrous Ammonia	Conventional	-	-	Spring Banded
ESN(polymer coated urea)	EENF	-	-	Spring Banded
Super U	EENF	Urease and nitrification inhibitor	NBPT DCD	Spring Banded
Urea + LIMUS	EENF	Urease inhibitor	NBPT, NPPT	Spring Banded
Urea + eNtrench	EENF	Nitrification inhibitor	nitrapyrin	Spring Banded
Anhydrous Ammonia + N-Serve	EENF	Nitrification inhibitor	nitrapyrin	Spring Banded

EENFs; Enhanced efficiency N fertilizers
NBPT; N-(N-butyl) thiophosphorictriamide
NPPT; N-(2-nitrophenyl) phosphoric triamide
DCD; Dicyandiamide

Table 2-2. Cumulative N_2O emissions (g N ha^{-1}) during fall, early spring, growing season periods, and annual N_2O emissions (g N ha^{-1}) during the study.

N Treatment	Fall 2015 (26-Oct-15 to 13-Nov-15)	ES 2016 (18-Mar-16 to 5-May-16)	GS 2016 (11-May-16 to 29-Aug-16)	AnCumN_2O 2015-2016 (26-Oct-15 to 29-Aug-16)	Fall 2016 (25-Oct-16 to 8-Nov-16)	ES 2017 (3-Apr-17 to 19-May-17)	GS 2017 (26-May-17 to 1-Sep-17)	AnCumN_2O 2016-2017 (25-Oct-16 to 1-Sep-17)	Fall 2017 (26-Sep-17 to 27-Oct-17)	ES 2018_noN (23-Apr-18 to 22-May-18)	Fall 2 year mean	ES 2 year mean	GS 2 year mean	AnCumN_2O 2 year mean
Control	7	406	208	622	9b	214	196	425	31	245	8	310	202	524
Fall-Urea	7	362	393	770	19ab	1113	278	1434	33	104	13	738	336	1102
Fall-Anhydrous Ammonia	9	416	317	746	8b	429	287	733	30	73	9	422	302	739
Fall-ESN	4	290	299	607	15ab	1013	293	1347	27	172	10	652	296	977
Fall-Super U	8	340	264	615	29a	1005	240	1293	21	145	18	672	252	954
Fall-Urea + LIMUS	8	378	259	652	16ab	1213	242	1501	21	126	12	796	250	1077
Fall-Urea + eNtrench	8	334	535	880	20ab	719	292	1044	36	76	14	527	414	962
Fall-Anhydrous Ammonia + N-Serve	8	256	353	624	20ab	751	180	959	18	168	14	504	267	792
Spring-Urea	7	406	630	1045	9b	214	232	460	13	183	8	310	431	752
Spring-Anhydrous Ammonia	7	406	1079	1493	9b	214	327	555	20	61	8	310	703	1024
Spring-ESN	7	406	365	779	9b	214	506	734	27	153	8	310	435	757
Spring-Super U	7	406	528	942	9b	214	233	461	32	125	8	310	380	702
Spring-Urea + LIMUS	7	406	407	821	9b	214	413	641	18	233	8	310	410	731
Spring-Urea + eNtrench	7	406	478	892	9b	214	428	656	37	132	8	310	453	774
Spring-Anhydrous Ammonia + N-Serve	7	406	270	684	9b	214	455	683	15	63	8	310	362	684
Treatment mean ± SE	7±1	375±13	426±56	811±60	13±2	530±101	307±26	862±97	25±2	137±15	10±1	453±47	366±31	837±43
ANOVA P values														
Treatment	0.969	0.166	0.300	0.415	0.005	0.061	0.323	0.652	0.601	0.559	0.090	0.056	0.103	0.549
N Timing (Fall vs Spring)	0.488	0.533	0.044	0.031	0.005	<0.001	0.172	<0.001	0.21	0.569	0.015	0.001	0.012	0.203
Fertilizer type (Conv. vs EENFs) §	0.672	0.675	0.102	0.019	0.232	0.542	0.401	0.438	0.831	0.303	0.47	0.772	0.197	0.523
N Timing X Fertilizer type	0.672	0.675	0.122	0.199	0.232	0.542	0.156	0.952	0.14	0.643	0.47	0.772	0.334	0.34

The periods of fall 2015, early spring (ES) 2016, Growing season (GS) 2016, annual cumulative N2O (AnCumN2O) 2015-2016 , fall 2016, ES 2017, GS 2017, AnCumN2O 2016-2017, fall 2017 and ES 2018 correspond to the time period from 26-Oct-15 to 13-Nov-15, 18-Mar-16 to 5-May-16, 11-May-16 to 29-Aug-16, 26-Oct-15 to 29-Aug-16, 25-Oct-16 to 8-Nov-16, 3-Apr-17 to 19-May-17, 26-May-17 to 1-Sep-17, 25-Oct-16 to 1-Sep-17, 26-Sep-17 to 27-Oct-17 and 23-Apr-18 to 22-May-18, respectively.

N_2O emissions during winter period were assumed to be negligible.

The differences across N treatments were determined using Tukey's Honest Significant Difference (HSD).

§ Conv, conventional N fertilizers; EENFs, enhanced efficiency N fertilizers.

Table 2-3. Temporal changes of soil ammonium (mg NH_4-N kg^{-1} soil) concentrations during the two experimental years.

N treatments	30 Mar 2016	29 Apr 2016	1 June 2016	1 June 2016	1 June 2016	6 July 2016	6 July 2016	6 July 2016	8 May 2017	4 June 2017	4 June 2017	26 June 2017	26 June 2017	10 July 2017	10 July 2017	3 Aug 2017	3 Aug 2017	11 May 2018
	Whole plot	Whole plot	Band*	Interband*	Weighted average**	Band*	Interband*	Weighted average**	Whole plot	Band	Interband	Band	Interband	Band	Interband	Band	Interband	Whole plot
Control	5.70	9.38			7.91			5.78	2.93	3.86	5.61	4.66	6.14	3.54	3.29	9.23	9.54	4.17
Fall-Urea	8.12	11.44			6.91			4.55	3.25	4.52	4.44	3.95	4.65	3.43	3.79	10.07	8.59	4.17
Fall-Anhydrous Ammonia	15.32	7.53			5.99			5.23	3.46	3.70	4.17	3.96	6.00	3.74	3.47	8.79	9.32	4.70
Fall-ESN	33.53	15.45			5.57			4.35	3.37	7.58	4.32	4.49	3.60	6.41	3.72	11.22	9.55	4.80
Fall-Super U	17.68	15.06			7.06			2.74	7.46	4.62	3.87	5.21	4.33	2.92	3.45	10.60	9.98	4.20
Fall-Urea + LIMUS	10.93	13.12			6.67			6.49	2.86	4.22	3.92	4.20	6.98	2.88	2.91	10.30	8.46	4.51
Fall-Urea + eNtrench	10.82	6.13			3.89			4.66	5.68	6.66	4.14	4.36	4.01	3.67	3.17	9.33	9.63	4.72
Fall-Anhydrous Ammonia + N-Serve	18.78	6.30			7.84			6.31	2.82	4.13	3.22	5.20	5.37	3.69	2.93	9.50	8.56	3.94
Spring-Urea	4.51	5.85	25.77	7.91	9.70	4.10	4.63	4.58		31.70	4.11	5.92	8.45	3.55	3.11	11.13	9.79	4.07
Spring-Anhydrous Ammonia	3.95	3.82	11.15	5.94	6.46	6.36	4.12	4.34		29.44	3.80	4.53	4.66	3.16	3.32	10.71	8.93	3.65
Spring-ESN	4.11	5.98	53.77	6.67	11.38	19.87	4.95	6.44		19.40	4.45	26.82	4.25	11.15	3.82	20.96	11.24	4.30
Spring-Super U	5.12	4.61	13.31	6.66	7.33	6.85	7.35	7.30		70.96	3.62	5.88	9.41	6.93	3.44	9.37	7.92	3.74
Spring-Urea + LIMUS	6.40	5.77	10.47	5.20	5.72	5.05	6.00	5.91		21.01	3.63	5.07	4.18	4.16	3.55	9.57	9.32	4.12
Spring-Urea + eNtrench	4.77	6.48	109.61	4.58	15.08	8.50	6.43	6.64		34.35	4.00	29.62	3.73	12.16	3.58	9.39	10.20	4.67
Spring-Anhydrous Ammonia + N-Serve	4.75	7.42	30.38	7.99	10.23	8.04	5.82	6.04		14.48	4.03	5.31	4.12	4.31	3.18	9.32	9.02	4.50
Treatment mean ± SE	10.30±2.11	8.29±0.97	36.35±13.50	6.42±0.33	7.85±0.12	8.40±2.00	5.61±0.29	5.42±0.31	3.98±0.44	17.38±4.78	4.09±0.14	7.95±2.14	5.33±0.45	5.05±0.76	3.38±0.07	10.63±0.76	9.34±0.21	4.28±0.09
ANOVA P values																		
N treatment	0.007	0.009								0.001	0.529	0.001	0.227	0.002	0.05	0.138	0.527	0.182
N timing (Fall vs spring)	0.001	0.004			0.112			0.385		0.001	0.44	0.084	0.458	0.154	0.584	0.363	0.481	0.052
Fertilizer type (Conv vs EENFS) §	0.214	0.369			0.610			0.133		0.835	0.276	0.117	0.355	0.064	0.711	0.638	0.652	0.282
N timing X fertilizer type	0.93	0.877			0.510			0.141		0.992	0.304	0.18	0.64	0.109	0.009	0.99	0.921	0.276

* Spring N plots have results separately for both band and interband on both 1 June and 6 July in 2016.

** Spatially aggregated N concentration were calculated using the spatial weights of bands (10%) and interbands (90%) for the spring N plots. Results for control and fall N plots are for whole plot on both 1 June and 6 July in 2016.

§ Conv, conventional N fertilizers; EENFs, enhanced efficiency N fertilizers.

Table 2-4. Temporal changes of soil nitrate (mg NO3-N kg-1 soil) concentrations during the two experimental years.

N treatments	30 Mar 2016	29 Apr 2016	1 June 2016	1 June 2016	1 June 2016	6 July 2016	6 July 2016	6 July 2016	8 May 2017	4 June 2017	4 June 2017	26 June 2017	26 June 2017	10 July 2017	10 July 2017	3 Aug 2017	3 Aug 2017	11 May 2018
	Whole plot	Whole plot	Band*	Interband*	Weighted average**	Band*	Interband*	Weighted average**	Whole plot	Band	Interband	Band	Interband	Band	Interband	Band	Interband	Whole plot
Control	38.11	62.54			24.72			15.06	13.47	27.61	29.29	20.07	20.88	7.46	8.05	10.71	11.77	16.08
Fall-Urea	144.96	89.12			29.43			15.47	41.36	52.99	40.11	60.86	32.30	17.97	10.61	14.22	9.73	18.31
Fall-Anhydrous Ammonia	138.72	134.03			35.62			16.60	24.21	29.45	29.10	18.29	19.31	11.83	10.09	11.03	9.97	17.93
Fall-ESN	190.49	139.33			25.65			15.63	14.92	69.79	25.52	30.25	17.65	17.97	7.93	14.58	11.26	14.22
Fall-Super U	126.64	169.88			48.53			40.46	51.12	74.65	40.10	25.18	22.19	10.30	12.31	10.42	13.20	18.27
Fall-Urea + LIMUS	144.41	149.41			35.31			26.26	26.52	43.33	51.04	27.38	25.32	8.43	8.85	13.52	10.16	21.43
Fall-Urea + eNtrench	79.62	73.28			82.71			16.84	50.36	88.64	29.01	24.23	22.17	10.58	10.02	10.44	10.54	17.96
Fall-Anhydrous Ammonia + N-Serve	148.80	111.92			29.69			17.54	24.77	29.36	24.51	23.46	23.52	9.79	8.46	11.87	10.92	18.71
Spring-Urea	26.46	27.25	313.87	20.83	50.13	96.26	13.42	21.70		218.27	38.37	117.92	29.28	18.72	9.31	16.86	11.49	19.45
Spring-Anhydrous Ammonia	29.47	41.02	238.80	75.57	91.90	106.99	34.77	41.99		138.34	69.85	54.20	62.69	11.09	18.73	15.73	10.94	17.08
Spring-ESN	26.98	28.37	228.30	30.28	50.08	107.42	16.25	25.37		58.22	19.76	123.44	16.46	46.17	7.92	36.79	11.23	14.34
Spring-Super U	26.04	46.02	243.61	25.60	47.40	53.38	16.44	20.14		136.38	25.41	109.51	21.69	49.39	11.45	17.15	13.29	15.66
Spring-Urea + LIMUS	33.71	71.75	266.91	20.43	45.08	50.38	25.20	27.72		229.77	24.19	129.02	23.99	10.96	8.18	13.40	13.27	16.01
Spring-Urea + eNtrench	31.07	62.83	286.04	39.05	63.75	108.69	16.77	25.96		152.82	25.07	148.32	21.08	72.86	9.50	10.45	11.79	15.64
Spring-Anhydrous Ammonia + N-Serve	31.54	44.33	288.98	27.99	54.09	75.74	29.66	34.26		198.92	31.43	90.15	21.77	18.61	8.91	11.83	10.86	17.99
Treatment mean ± SE	84.68±15.7	86.20±12.14	266.64±11.83	34.25±5.40	47.61±5.10	85.55±9.71	21.79±3.08		30.84±3.85	96.40±17.58	33.67±3.48	65.15±12.64	25.61±2.96	21.68±5.12	10.10±0.73	14.80±1.75	11.40±0.31	17.22±0.52
ANOVA P values																		
N treatment	0.012	0.001								0.001	0.193	0.001	0.320	0.001	0.301	0.314	0.986	0.814
N timing (Fall vs spring)	0.001	0.001			0.055			0.091		0.001	0.386	0.001	0.095	0.073	0.245	0.245	0.317	0.427
Fertilizer type (Conv vs EENFS) §	0.747	0.448			0.746			0.837		0.936	0.020	0.356	0.014	0.173	0.053	0.884	0.317	0.366
N timing X fertilizer type	0.605	0.543			0.179			0.251		0.278	0.024	0.037	0.062	0.073	0.167	0.795	0.323	0.368

* Spring N plots have results separately for both band and interband on both 1 June and 6 July in 2016.

** Spatially aggregated N concentration were calculated using the spatial weights of bands (10%) and interbands (90%) for the spring N plots. Results for control and fall N plots are for whole plot on both 1 June and 6 July in 2016.

§ Conv, conventional N fertilizers; EENFs, enhanced efficiency N fertilizers.

Table 2-5. Wheat dry matter (DM) grain and straw yield (kg ha^{-1}), grain and straw N uptake (kg N ha^{-1}), total aboveground yield (kg ha^{-1}) and N uptake (kg N ha^{-1}) for two experimental year.

N treatment	Grain yield			Grain N uptake			Straw yield			Straw N uptake			Total aboveground yield			Total aboveground N uptake		
	2015-2016	2016-2017	2-year mean	2015-2016	2016-2017	2-year mean	2015-2016	2016-2017	2-year mean	2015-2016	2016-2017	2-year mean	2015-2016	2016-2017	2-year mean	2015-2016	2016-2017	2-year mean
Control	4434	2960	3697	109	64	86	4691	3647ab	4169	19	30ab	24	9126	6608	7867	128	94	111
Fall-Urea	4316	3354	3835	108	79	93	5006	3514ab	4260	21	34ab	28	9322	6868	8095	129	113	121
Fall-Anhydrous Ammonia	4611	2569	3590	113	57	85	5265	2845a	4055	25	25a	25	9876	5414	7645	138	82	110
Fall-ESN	4764	3282	4023	119	73	96	4624	3655ab	4139	28	34ab	31	9388	6937	8162	147	106	127
Fall-Super U	4215	3854	4034	107	85	96	4473	4115ab	4294	21	38b	29	8688	7969	8328	128	123	125
Fall-Urea + LIMUS	4249	3534	3891	110	79	95	4625	3817ab	4221	22	35ab	29	8874	7351	8113	132	115	123
Fall-Urea + eNtrench	4670	3286	3978	116	77	97	5006	3662ab	4334	18	36ab	27	9676	6948	8312	134	113	123
Fall-Anhydrous Ammonia + N-Serve	4615	3147	3881	118	67	92	5040	3586ab	4313	16	28ab	22	9655	6733	8194	134	95	114
Spring-Urea	4067	3607	3837	100	83	91	4450	4084ab	4267	21	36ab	28	8517	7691	8104	121	119	120
Spring-Anhydrous Ammonia	4366	3870	4118	109	90	99	4748	4380b	4564	24	40b	32	9113	8249	8681	132	130	131
Spring-ESN	4773	3230	4001	116	74	95	5024	3850ab	4437	19	37ab	28	9797	7080	8439	136	110	123
Spring-Super U	4531	3730	4131	114	87	100	4884	3814ab	4349	21	36ab	28	9416	7544	8480	134	123	129
Spring-Urea + LIMUS	4618	3449	4033	116	73	94	4951	4179b	4565	18	34ab	26	9569	7627	8598	133	107	120
Spring-Urea + eNtrench	4797	3573	4185	121	81	101	5036	4162b	4599	27	38b	32	9833	7735	8784	147	119	133
Spring-Anhydrous Ammonia + N-Serve	4691	3330	3697	117	76	86	5107	4229b	4169	20	37b	24	9798	7559	8679	137	113	125
Treatment mean ± SE	4515±59	3385±88	3945±266	113±1	76±2	94±6	4862±63	3836±99	4326±291	21±1	34±1	28±2	9377±112	7221±178	8299±82	134±2	111±3	122±2
ANOVA P values																		
Treatment	0.8424	0.230	0.671	0.875	0.147	0.340	0.887	0.025	0.831	0.784	0.003	0.198	0.818	0.105	0.751	0.903	0.051	0.229
N Timing (Fall vs Spring)	0.6258	0.126	0.100	0.984	0.095	0.140	0.884	0.008	0.030	0.908	0.072	0.123	0.650	0.012	0.039	0.969	0.042	0.096
Fertilizer type (Conv. vs EENFs) §	0.0583	0.611	0.099	0.016	0.997	0.122	0.954	0.198	0.418	0.396	0.357	0.954	0.357	0.364	0.207	0.095	0.816	0.263
N Timing X Fertilizer type	0.1078	0.057	0.455	0.201	0.065	0.416	0.028	0.063	0.983	0.831	0.041	0.282	0.065	0.068	0.734	0.237	0.048	0.316

The differences across N treatments were determined using Tukey's Honest Significant Difference (HSD).

§ Conv, conventional N fertilizers; EENFs, enhanced efficiency N fertilizers.

Table 2-6. Nitrogen use efficiency (NUE) (kg dry matter (DM) kg-1 N), uptake efficiency (UE) (kg N kg-1 N), physiological efficiency (PE) (kg DM kg-1 N), harvest index (HI) (kg grain DM kg-1 grain and straw DM) and N harvest index (NHI) (kg grain N kg-1 grain and straw N) of wheat in each year.

N treatment	NUE			UE			PE			HI			NHI		
	2015-2016	2016-2017	2-year mean	2015-2016	2016-2017	2-year mean	2015-2016	2016-2017	2-year mean	2015-2016	2016-2017	2-year mean	2015-2016	2016-2017	2-year mean
Control										0.444	0.484	0.464	0.865	0.678	0.772
Fall-Urea	13.56	3.94	8.75	0.34	0.18	0.26	44.62	10.17	27.40	0.487	0.463	0.475	0.839	0.695	0.767
Fall-Anhydrous Ammonia	18.04	-3.91	6.29	0.53	-0.12	0.16	35.61	33.43	35.98	0.468	0.521	0.468	0.821	0.686	0.754
Fall-ESN	12.54	3.21	10.63	0.34	0.12	0.33	37.41	39.11	37.36	0.468	0.484	0.494	0.813	0.676	0.744
Fall-Super U	17.10	8.93	10.74	0.40	0.28	0.31	45.98	32.97	35.19	0.483	0.482	0.483	0.836	0.694	0.765
Fall-Urea + LIMUS	12.88	5.74	9.31	0.38	0.20	0.29	35.92	27.12	31.52	0.482	0.479	0.48	0.836	0.691	0.763
Fall-Urea + eNtrench	11.07	3.26	10.18	0.27	0.19	0.29	44.77	13.91	29.94	0.472	0.478	0.477	0.868	0.681	0.774
Fall-Anhydrous Ammonia + N-Serve	18.13	1.87	9.21	0.41	0.01	0.20	49.32	-20.03	11.56	0.468	0.487	0.473	0.88	0.702	0.791
Spring-Urea	15.71	6.46	8.76	0.40	0.25	0.26	41.79	19.44	32.10	0.472	0.482	0.475	0.822	0.697	0.76
Spring-Anhydrous Ammonia	18.37	9.09	11.57	0.53	0.36	0.37	34.50	24.49	31.14	0.469	0.488	0.474	0.821	0.693	0.757
Spring-ESN	16.58	2.70	10.41	0.39	0.16	0.29	43.94	16.25	32.79	0.452	0.483	0.47	0.859	0.662	0.761
Spring-Super U	16.50	7.70	11.70	0.43	0.29	0.34	38.53	24.68	33.23	0.493	0.467	0.488	0.848	0.707	0.777
Spring-Urea + LIMUS	16.54	4.88	10.73	0.40	0.13	0.26	43.16	37.00	40.47	0.450	0.478	0.466	0.867	0.681	0.774
Spring-Urea + eNtrench	14.05	6.13	12.25	0.38	0.25	0.39	37.79	-73.76	-19.63	0.460	0.480	0.474	0.818	0.681	0.749
Spring-Anhydrous Ammonia + N-Serve	17.31	1.87	10.50	0.43	0.19	0.31	40.96	27.82	34.39	0.439	0.479	0.459	0.851	0.667	0.759
Treatment mean ± SE	15.60±0.61	4.42±0.90	10.07±0.41	0.4±0.02	0.18±0.03	0.29±0.02	41.02±1.16	15.19±7.43	28.10±4.08	0.482±0.01	0.467±0.01	0.474±0.002	0.843±0.005	0.686±0.003	0.764±0.003
ANOVA P values															
Treatment	0.399	0.333	0.775	0.306	0.107	0.398	0.300	0.505	0.455	0.865	0.109	0.528	0.432	0.761	0.464
N Timing (Fall vs Spring)	0.815	0.062	0.088	0.657	0.023	0.056	0.705	0.741	0.792	0.672	0.155	0.562	0.780	0.796	0.677
Fertilizer type (Conv. vs EENFs) §	0.084	0.671	0.117	0.17	0.831	0.263	0.844	0.607	0.596	0.159	0.358	0.588	0.069	0.317	0.325
N Timing X Fertilizer type	0.143	0.093	0.479	0.334	0.092	0.318	0.63	0.734	0.798	0.359	0.583	0.281	0.642	0.428	0.918

§ Conv, conventional N fertilizers; EENFs, enhanced efficiency N fertilizers.

Table 2-7. Annual area-based N_2O emission factors (EFarea) (% kg N_2O-N kg^{-1} N fertilizer) and yield-based emission factors (EF yield) (g N_2O-N kg^{-1} grain DM) of wheat in each year.

N treatment	EF_{area} % 2015-2016	2016-2017	2-year mean	EF_{yield} 2015-2016	2016-2017	2-year mean
Control				0.137	0.149	0.143
Fall-Urea	0.147	1.009	0.578	0.177	0.412	0.295
Fall-Anhydrous Ammonia	0.123	0.308	0.216	0.161	0.26	0.211
Fall-ESN	-0.016	0.922	0.453	0.133	0.397	0.265
Fall-Super U	-0.008	0.868	0.430	0.153	0.332	0.243
Fall-Urea + LIMUS	0.030	1.076	0.553	0.158	0.436	0.297
Fall-Urea + eNtrench	0.257	0.619	0.438	0.186	0.329	0.258
Fall-Anhydrous Ammonia + N-Serve	0.001	0.534	0.268	0.142	0.3	0.221
Spring-Urea	0.422	0.035	0.229	0.254	0.129	0.192
Spring-Anhydrous Ammonia	0.870	0.130	0.500	0.327	0.153	0.240
Spring-ESN	0.157	0.309	0.233	0.168	0.242	0.205
Spring-Super U	0.320	0.037	0.178	0.207	0.123	0.165
Spring-Urea + LIMUS	0.199	0.216	0.207	0.179	0.203	0.191
Spring-Urea + eNtrench	0.270	0.231	0.251	0.182	0.193	0.188
Spring-Anhydrous Ammonia + N-Serve	0.062	0.258	0.160	0.148	0.224	0.186
Treatment mean ± SE	0.189±0.062	0.437±0.100	0.313±0.043	0.181±0.001	0.259±0.003	0.220±0.001
ANOVA P values						
Treatment	0.389	0.524	0.549	0.344	0.097	0.219
N Timing (Fall vs Spring)	0.022	<0.001	0.203	0.016	<0.001	0.021
Fertilizer type (Conv. vs EENFs) §	0.064	0.366	0.523	0.031	0.336	0.58
N Timing X Fertilizer type	0.199	0.952	0.34	0.094	0.686	0.466

§ Conv, conventional N fertilizers; EENFs, enhanced efficiency N fertilizers.

2.9 Figures

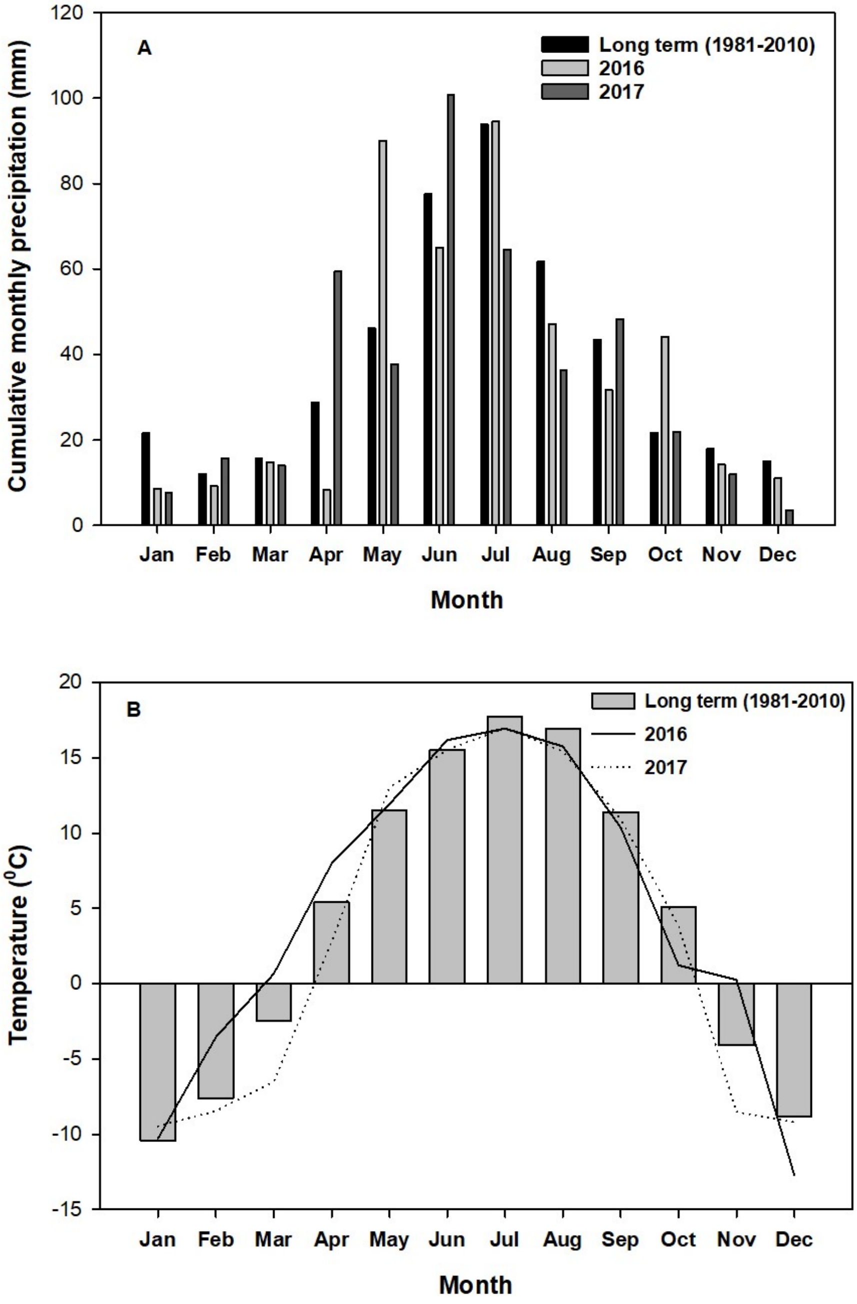

Figure 2-1. (A) Cumulative precipitation and (B) monthly average air temperature at the St. Albert site for year 2016, 2017 and the 30-year normal monthly data

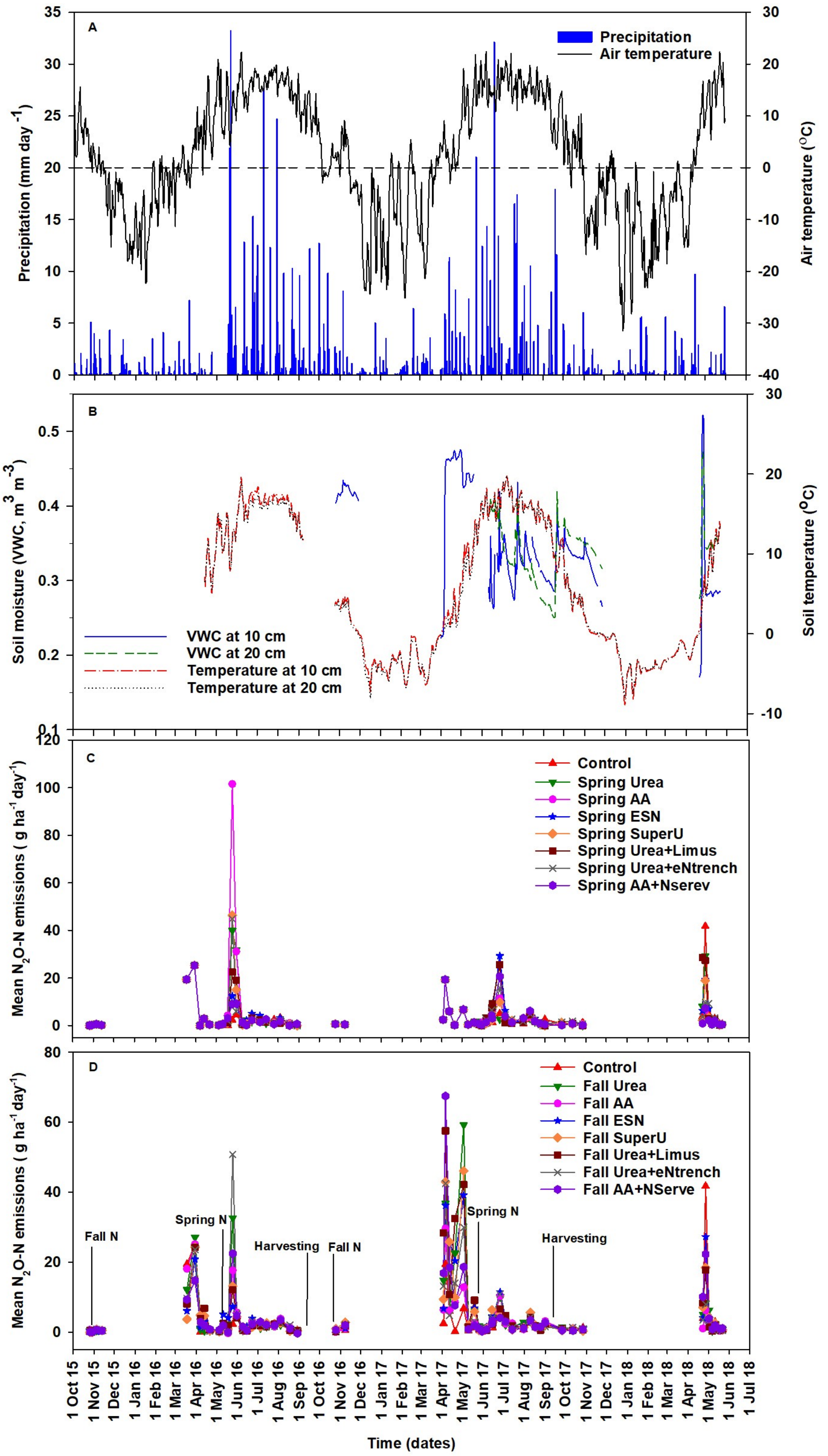

Figure 2-2. (A) Daily average air temperature and precipitation, (B) soil moisture and soil temperature at the depth of 10 cm and 20 cm, (C) daily N_2O fluxes for spring N treatments and (D) fall N treatments from Oct 2015 to May 2018.

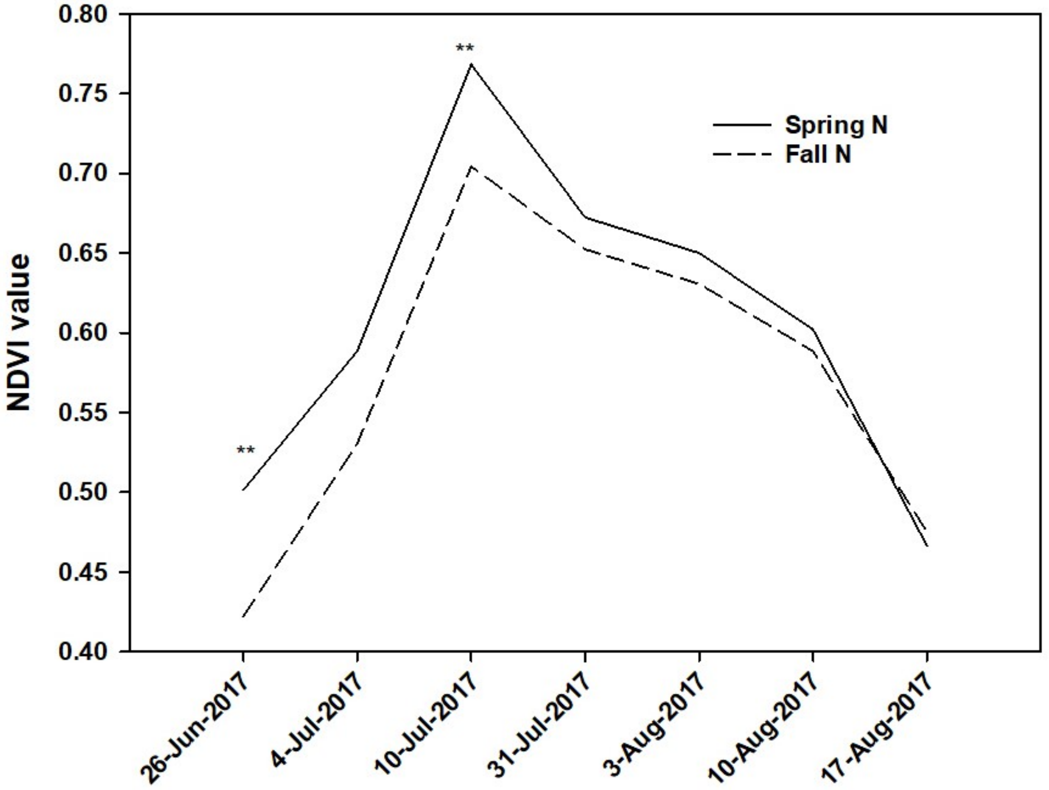

Figure 2-3. Normalized difference vegetation index (NDVI) in response to spring and fall N application in wheat. ** Statistically significant differences in 26 June and 10 July (spring N > fall N; *P*s< 0.05)

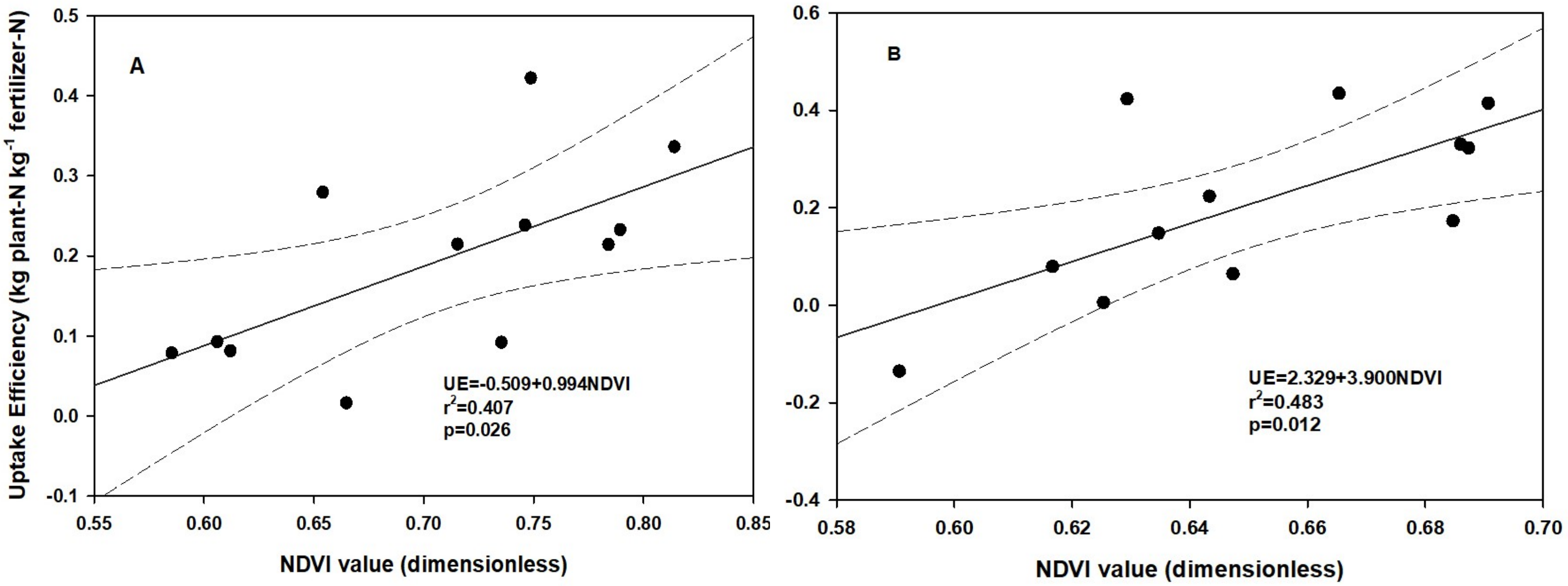

Figure 2-4. Nitrogen uptake efficiency as a function of normalized difference vegetation index (NDVI) canopy measurements (A) fall N treatments on 10 July 2017, and (B) spring N treatments on 03 August 2017

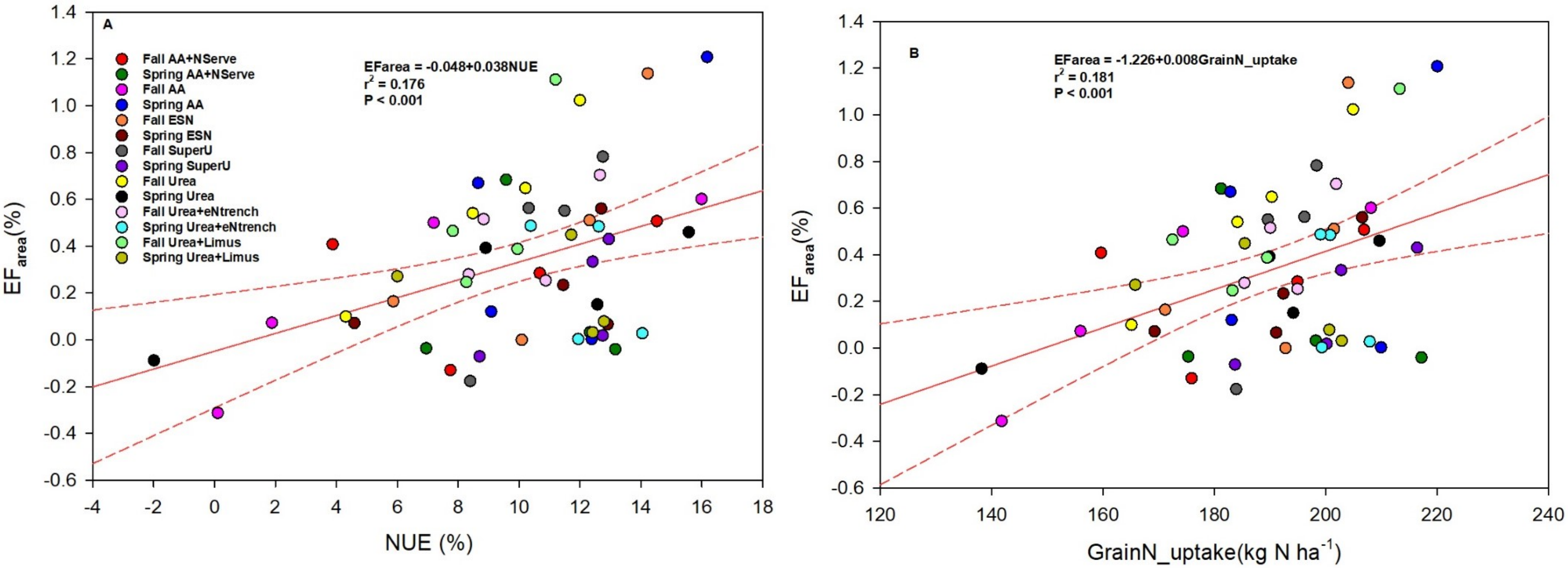

Figure 2-5. Relationship between nitrous oxide emission factor (EF_{area}) and (A) mean nitrogen use efficiency (NUE) (%) and (B) mean grain N uptake (kg N ha^{-1}).

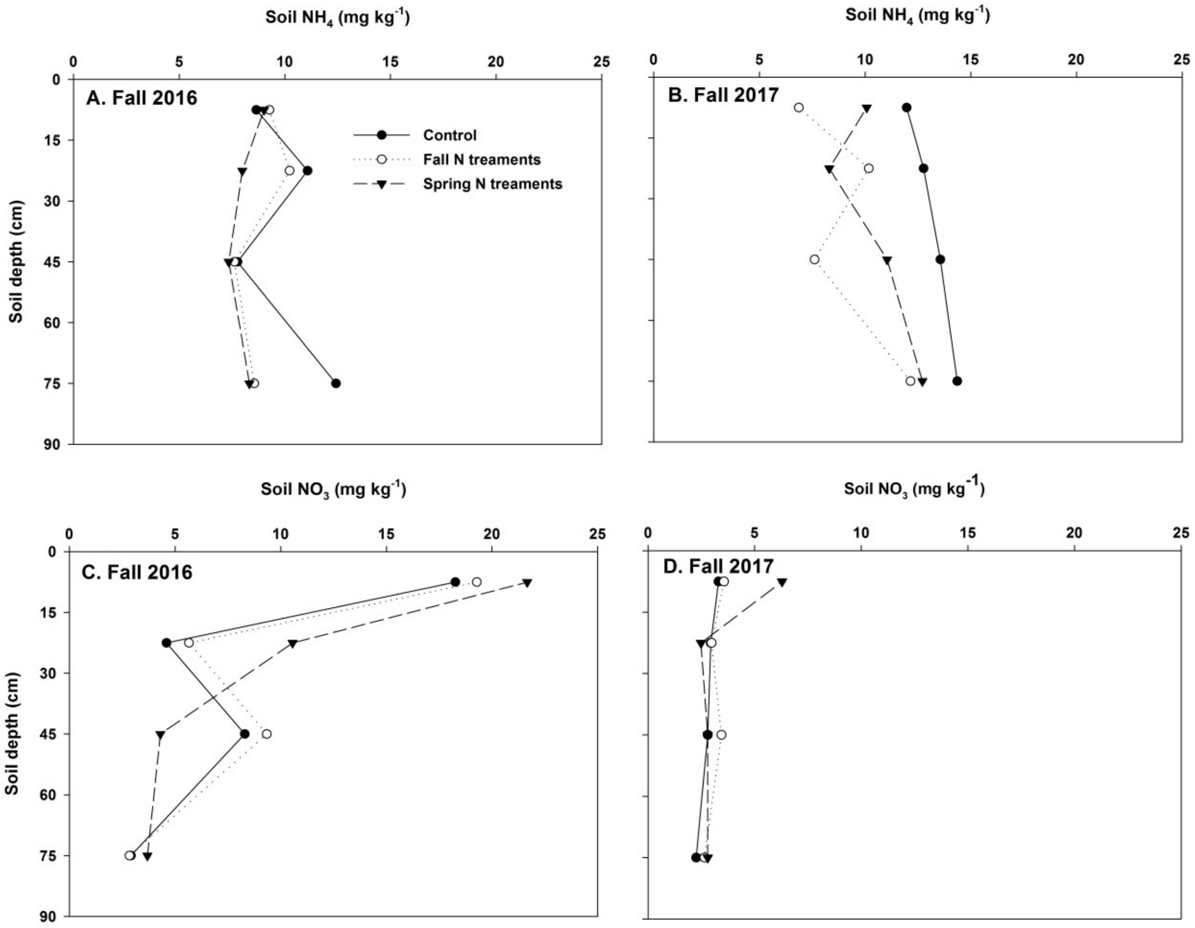

Figure 2-6. Soil residual NH_4-N and NO_3-N concentrations (mg kg $^{-1}$) with soil depth in control, all fall N and all spring N treatments for experimental years of 2015-2016 (A, C) and 2016-2017 (B, D). These post-harvest soil samples were collected at the soil depth increments of 0-15, 15-30, 30-60 and 60-90 cm in October 2016 and September 2017.

3 Nitrous Oxide Fluxes, Sources and Priming in a Temperate Agricultural Soil: Moisture and N Addition Effects

3.1 Abstract

Fertilized agricultural soils are major emitters of nitrous oxide (N_2O) to the atmosphere, yet the relative contributions of sources and processes to N_2O production remain uncertain. A laboratory incubation was conducted to investigate the effects of nitrogen additions on N_2O production, priming, and sources at multiple soil moisture contents. Soils were collected from wheat (*Triticum aestivum L.)* experimental fields located in St. Albert, Alberta, Canada, in mid-spring. Soils were incubated under five N addition treatments: 1) untreated control, 2) isotopically-unlabelled urea, 3) ^{15}N labelled urea at 5 atom%, 4) ^{15}N urea admixed with nitrification inhibitor (nitrapyrin), and 5) ^{15}N urea with two urease inhibitors [N-(N-butyl) thiophosphoric triamide (NBPT) and N-(2-nitrophenyl) phosphoric triamide (NPPT)]. Urea was added at a rate of 62.34 mg N kg^{-1} soil (equivalent to 100 kg N ha^{-1}). We also established five water-filled pore spaces (WFPS: 31%, 41%, 53%, 65%, and 78%). The fluxes and source-partitioning were derived from concentrations and ^{15}N isotopic compositions of N_2O that were measured using a quantum cascade laser spectroscopy analyzer with flow-through recirculation. Cumulative N_2O emissions increased substantially with the highest soil moisture; emissions at 78 % WFPS were three times larger than at all lower moisture contents ($P< 0.05$), supporting soil moisture as a key trigger of N_2O production. Most ^{15}N-N_2O site preference (SP) values were close to zero (0‰), in particular during the peak fluxes, indicating that denitrification was the major contributing process to N_2O fluxes. Although N_2O fluxes did not differ across N addition treatments ($P> 0.05$), the N_2O emission derived specifically from the native soil N pool was strongly primed by the urea addition, and this substantial positive priming was even larger with increasing moisture content.

3.2 Introduction

Nitrous oxide (N_2O) produced from fertilized agricultural soils has a significant role in the atmosphere as a greenhouse gas and as a stratosphere ozone-depleting substance. Explaining and differentiating the production processes and sources of N_2O under varying soil conditions can contribute to the development of better quantification and prediction of N_2O emissions. When nitrogen (N) is added to soil as fertilizer, N can cycle and undergo different N_2O production processes, such as nitrification, nitrifier-denitrification, chemo-denitrification, and denitrification (Wrage et al., 2001; Venterea et al., 2012). In the process of nitrification, N_2O is produced from hydroxylamine (NH_2OH) decomposition as a byproduct formed during ammonium (NH_4^+) oxidation. Under low oxygen concentrations, nitrite (NO_2^-) acts as an alternative electron accepter and produces N_2O, which is known as nitrifier-denitrification (Wrage et al., 2001). Denitrification produces N_2O as an intermediate product of nitrate (NO_3^-) reduction, and in chemo-denitrification, NO_2^- reacts with soil organic matter to chemically produce N_2O (Venterea et al., 2012).

Although many processes generate N_2O emissions, nitrification and denitrification are typically considered more quantitatively important in leading N_2O fluxes in agroecosystems (Braker and Conrad 2011; ButterbachBahl et al. 2013). Nitrification and denitrification can occur simultaneously under a wide range of soil conditions. Within many factors, soil moisture content is a major driver of N_2O emissions that determines the dominance of nitrification and denitrification processes at a given time (Butterbach-Bahl, 2013). Earlier literature indicates that the largest emissions of N_2O commonly occur within 70-80% of water-filled pore spaces (WFPS) (Davidson et al., 2000) depending on the soil properties (Butterbach-Bahl, 2013). When WFPS is between 35-60%, nitrification is assumed to play a major role in N_2O production, whereas when WFPS is greater than 70%, all N_2O could potentially be produced via denitrification (Bateman and Baggs, 2005). Complete denitrification is likely to become the dominant process with even wetter conditions, and some studies provide evidence of reduction of N_2O emissions under the wettest conditions (>90%) as shown by the formation and release of N_2 rather than N_2O (Butterbach-Bahl, 2013).

Previous studies have shown that the relative efficacy across N fertilization options for reducing of N_2O emissions can largely vary depending on the soil moisture content. Li et al. (2018) reported that synthetic N fertilizers admixed with nitrification inhibitors (NI) have greater positive effects in irrigated cropping systems compared to rainfed systems. Using NI and urease inhibitors (UI) with urea applications, Sanz-Cobena et al. (2012) evaluated N_2O emissions using a controlled and increased irrigation setting in a corn cropping system. They found a significant reduction of N_2O under controlled irrigation (58% WFPS) and no significant effect under highly irrigated conditions (70% WFPS). Sanz-Cobena et al. (2016) reported that thiophosphoric triamide (NBPT) applied with urea fertilizer was effective at reducing N_2O at 60% WFPS, but was not effective at 80% WFPS. Collectively, these contrasting reports suggest that across different soil moistures, the effects of N fertilization options on the relative contributions of sources and processes associated with N_2O emissions are still uncertain.

Stable isotope techniques can enable the examination of underlying processes of N_2O production. A ^{15}N labelled addition can be used to differentiate fertilizer-N- versus soil-N-derived N_2O emissions, enabling the determination of priming effects across various substrate N additions (Phillips et al., 2013). Within the linear N_2O molecule, the difference between the central and peripheral $\delta^{15}N$ (^{15}N-N_2O site preference, SP) provides information of the N_2O production processes as well as the relative contributions of nitrification vs. denitrification to the

N_2O emissions (Yoshida and Toyoda, 2000; Ding et al., 2019) and the priming responses of such processes. However, the contributions of soil processes to N_2O production and the priming effects caused by labile N additions under a wide range of water contents have rarely been reported. To gain a comprehensive insight into the explaining, quantifying, modeling and mitigation of N_2O emissions, all of these parameters need to be measured concurrently. Therefore, using ^{15}N isotope techniques, a laboratory incubation study was performed to evaluate N_2O emissions as a response to the addition of urea fertilization alone or admixed with inhibitors (NI and UI) at multiple soil moisture levels. The objectives of the study were to 1) determine the response of soil N_2O production and sources across a common range of WFPSs and N additions, 2) identify the interactive contributions of nitrification vs. denitrification processes to N_2O production under a broad range of conditions (N additions × WFPSs), 3) use these various N_2O partitions to quantify the priming effects on labile N addition on N_2O productions as associated with native soil N pool, nitrification, and denitrification sources.

3.3 Materials and methods

3.3.1 Study site

Black Chernozem soil (U.S. Soil Taxonomy equivalent: Typic Cryoboroll) was collected from a wheat experimental field at the St. Albert Research Station (53° 41′ 45.1″ N, 113° 37′ 33.6″ W) in Central Alberta, Canada. In mid-spring (May 10, 2017), soils were collected at 0 to 15 cm soil depth increments in buffer zones of the field experimental site (2nd chapter). The study site consisted of a cold, semi-humid, and temperate climate. Soil particle size, which was determined by the hydrometer method, was 86 g kg^{-1} sand, 494 g kg^{-1} silt, and 420 g kg^{-1} clay, translating into a silty clay texture. The bulk density of the soil was 1.25 ± 0.09 g cm^{-3} with a pH of 6.2 ± 0.1 (1:10 soil to water). When using the dry combustion method on untreated soils prior to the incubation study, total organic carbon content was 45 ± 1.2 g C kg^{-1}, and the total N was 3.9 ± 0.2 g N kg^{-1}.

3.3.2 Incubation set-up

Soil samples were mixed; coarse fragments were removed by sieving with an 8 mm mesh. Soils were stored at 5°C from field sampling until pre-incubation of two days under room temperature. About 107 g of dry soil were packed into 100 cm^3 graduated plastic containers.

The soil incubation containers were amended with 62.34 mg N kg^{-1} soil (equivalent to a field fertilization rate of 100 kg N ha^{-1}) with regular urea or ^{15}N labelled urea (5 atom%) to establish five N addition treatments: 1) untreated control, 2) isotopically unlabelled urea, 3) ^{15}N labelled urea (5 atom%), 4) ^{15}N urea + nitrification inhibitor with a nitrapyrin additive (eNtrench®) ,12.39 mL kg^{-1} urea), and 5) ^{15}N urea with two urease inhibitors [i.e., N-(N-butyl) thiophosphoric triamide (NBPT) and N-(2-nitrophenyl) phosphoric triamide (NPPT)] (LIMUS®, 3.3 mL kg^{-1} urea). Deionized room-temperature water was added to each incubation container to establish five different WFPSs at 31%, 41%, 53%, 65%, and 78%, which represented a typical range of soil moistures in Central Alberta. The plastic containers were capped with a lid containing four holes (0.5 cm diameter) to allow air exchange and, at the same time, moderate the rate of drying. Each treatment was replicated three times for measurements of N_2O fluxes and isotopic composition and an additional six times for destructive soil sampling for soil inorganic N (ammonium and nitrate concentration) determination [five WFPS x five fertilizers x (three for gaseous flux measurements + six destructive soil samplings); 5 x 5 x 9 = 225 incubation containers]. Soils were incubated under room temperature for thirty days, and the WFPS of each treatment was maintained at a target value based on daily weights. Destructive soil samplings were conducted on incubation days 0, 3, 7, 13, 21, and 30. Soils were extracted with 50 mL 2M KCl (1:10 soil:extractant), and the NH_4^+-N and NO_3^--N contents were measured colourimetrically using a SmartChem discrete wet chemistry analyzer (Westco Scientific Instruments, Inc., Brookfield, CT) (Maynard et al.,2008).

3.3.3 Soil N_2O emission measurements

The N_2O concentration and isotope ratios were measured using a 1 Hz quantum cascade laser spectroscopy analyzer (Aerodyne™) with flow-through recirculation. To create a chamber headspace (0.5 L volume), each incubation container was placed into a Mason jar and connected to the Aerodyne. During the incubation, the N_2O concentration change with time was measured over four minutes for each soil microcosm. Flux and isotopic measurements were performed on days 0, 2, 5, 7, 9, 12, 14, 16, 19, 21, 23, 27, and 30. Temperature and pressure were recorded during flux measurements.

The measured N_2O concentrations were plotted vs. time to determine N_2O fluxes (Fig. 3-1A), and Keeling plots were obtained from plotting atom% ^{15}N-N_2O against the corresponding inverse of total N_2O concentrations (Fig. 3-1B).

The partition of source-pools was determined using a mass balance with two end members: measured atom% ^{15}N in the N_2O emitted from soils receiving zero N addition (control microcosms) and 5 atom% ^{15}N in added urea as follows:

$$Fraction\ N_2O_{urea} + Fraction\ N_2O_{soil} = 1 \qquad Eq.\,[1]$$

$$atom\%^{15}N_{urea} \times\ Fraction\ N_2O_{urea} +\ atom\%^{15}N_{soil} \times\ Fraction\ N_2O_{soil} =\ atom\%^{15}N_2O_{urea\ treatment} \qquad Eq.[2]$$

$$N_2O_{urea} = Fraction\ N_2O_{urea} \times N_2O_{urea\ treatment} \qquad Eq.[3]$$

$$N_2O_{soil\ at\ urea\ treatment} = N_2O_{urea\ treatment} - N_2O_{urea} \qquad Eq.[4]$$

$$N_2O_{priming} = N_2O_{soil\ at\ urea\ treatment} - N_2O_{control} \qquad Eq.[5]$$

Generally, the SP reference signatures for nitrification and denitrification are between 27 to 33‰ and -13 to 0‰, respectively (Sutka et al., 2003, Sutka et al., 2006; Frame and Casciotti, 2010; Decock and Six, 2013). For mass balance purposes, the values used in this study as SP isotopic signatures were 33‰ for nitrification and 0‰ for denitrification, which enabled determining the contribution of source-processes and priming of processes.

$$\delta\ 15N\alpha - \delta 15N\beta = Site\ Preference\ (SP) \qquad Eq.[6]$$

$$N_2O_{denitrification} = (33 - SP_{treatment}) * 100 \qquad Eq.[7]$$

$$N_2O_{nitrification} = 100 - N_2O_{denitrification} \qquad Eq.[8]$$

$$N_2O_{priming_{denitrification}} = N_2O_{denitrification_{soil} at\ urea\ treatment} - N_2O_{denitrification_{control}} \qquad Eq.[9]$$

$$N_2O_{priming_{nitrification}} = N_2O_{nitrification_{soil} at\ urea\ treatment} - N_2O_{nitrification_{control}} \qquad Eq.[10]$$

Soil $\delta^{15}N$ was measured using an Elemental Analyzer (EA-Isolink, manufactured by Thermo Fisher Scientific, Bremen, Germany) following the dry combustion method. The resulting N_2 gas was run through an isotopic ratio mass spectrometer (Finnigan Delta V Advantage IRMS, manufactured by Thermo Fisher Scientific, Bremen, Germany) which detects the ratio of ^{15}N:^{14}N. The isotopic fractionation (α) and enrichment/depletion factor (ε) in ‰ were calculated as follows.

$$\alpha = \frac{\frac{15N}{14N}\ isotopic\ ratio\ of\ emitted\ N2O}{\frac{15N}{14N}\ isotopic\ ratio\ of\ soil\ N} \qquad Eq.[11$$

$$\varepsilon = (\alpha - 1) * 1000 \qquad Eq.[12]$$

3.3.4 Statistical Analysis

A two-way analysis of variance (ANOVA) was used to determine the treatment effects of N addition, WFPS, and their interaction. Tukey's honest significant difference (HSD) was used for post-hoc comparisons of treatment means. The Shapiro-Wilk and Bartlett tests were used to determine the normality and homoscedasticity and data was Box-Cox transformed when needed Unless stated otherwise, all statistical analyses were performed using SigmaPlot software (version 13.0) with an alpha critical value of 0.05.

3.4 Results

3.4.1 Nitrous oxide fluxes in response to soil moisture and N addition

The peak N_2O emissions from all treatment combinations occurred on the second day of incubation (Fig. 3-2). After the peak emissions, fluxes gradually decreased, and after seven days of incubation, no considerable emission were observed. During the peak emissions, the maximum N_2O emission was observed in the urea treatment with 78% WFPS (2551 ng N_2O-N kg^{-1} soil hr^{-1}), while the control treatment with 31% WFPS had the lowest N_2O emissions (46 N_2O-N ng kg^{-1} hr^{-1}) (Fig. 3-2). The N_2O emissions clearly increased with increasing soil moisture content during peak emissions (Fig. 3-3A); likewise, the cumulative N_2O emissions of the 78 % WFPS treatment (198 µg N_2O-N kg^{-1} soil) was significantly greater than for other moisture contents ($P = 0.01$), confirming that soil moisture content has a triggering effect on N_2O fluxes. Across our N addition treatments, the cumulative mean N_2O emissions of the urea treatment was the highest with 110 µg N_2O-N kg^{-1} soil, which was significantly greater than the control treatment (63 N_2O-N µg kg^{-1}) ($P = 0.03$) (Fig. 3-3B). Even though N fertilization admixed with inhibitors did not significantly differ from soils receiving only urea, they showed a numerically small decrease in N_2O emissions.

3.4.2 Temporal changes in inorganic N concentration in the soil

Prior to treatment establishment, the mean NH_4^+ and NO_3^-concentrations were 3 and 11 mg N kg^{-1} soil, respectively. After the establishment of treatments, ammonium concentration rapidly decreased with time, likely due to oxidation (nitrification) (Fig. 3-4). Concomitantly, as NO_3^- was being formed, its concentration increased. Both moisture content and N addition influenced the changes in inorganic N. On incubation days 3 and 7, NH_4^+ concentration at the 31 % WFPS was significantly lower than in the wet soils (53, 65 and 78 % WFPSs; Fig. 3-4A).

Likewise, on days 7 and 13, NO_3^- concentration in the 31 % WFPS soil was significantly lower than all other moisture contents (Fig. 3-4B). On day 21, NO_3^- concentrations at both 31% and 41% WFPSs were lower than at 65 and 78% WFPS. Regarding N addition, NO_3^- concentrations were significantly higher in all soils receiving N fertilization than in the untreated control treatment during the entire incubation (Fig. 3-4D), while on day 0, NH_4^+ concentration of untreated control treatment was significant lower than NH_4^+ concentration of ^{15}N urea + NBPT/NPPT treatment (Fig. 3-4C).

3.4.3 Contributions of nitrification and denitrification pathways to the total of N_2O emissions

Denitrification was the major contributor (averaging 86% contribution) to N_2O fluxes during peak emissions, ranging from 71% contribution in drier soil (31% WFPS) up to 97% contribution under the wettest condition (78% WFPS). In fact, the N_2O flux derived from denitrification under the wettest condition was significantly higher than under all other moisture treatments ($P = 0.01$) (Fig. 3-5A). Similar to when comparing across moisture levels, denitrification was also the dominant source-process of N_2O emissions consistently across all five N addition treatments during the peak flux event (Fig. 3-5B). Across N addition treatments, the contribution of denitrification exhibited a much narrower range from 89% in the control N treatment to 95% in the urea treatment. During peak flux time, N_2O emissions from the untreated control treatment were significantly lower than from all other N addition treatments ($P = 0.009$) (Fig. 3-5B).

3.4.4 ^{15}N-N_2O site preference (SP) with N_2O flux and across WFPS

Most ^{15}N-N_2O SP data ranged within 0 to 33‰. When plotting SP against the corresponding N_2O fluxes (entire dataset), SP clustered mainly within a range from 0 to 10‰, predominantly near 0‰, and in association with the largest N_2O fluxes (Fig. 3-6A). Furthermore, plotting SP measured during the peak emissions (on incubation day 2) across WFPSs revealed that SP was negatively correlated with an increasing WFPS (Fig. 3-6B). The highest SP mean was 9.6‰ for the 31% WFPS, while the lowest SP mean was 1.2‰ for the 78% WFPS.

3.4.5 Priming effects on soil N_2O flux

We identified the priming effects of N addition (i.e., treatments: ^{15}N urea, ^{15}N urea + nitrapyrin and ^{15}N urea + NBPT/NPPT) on N_2O fluxes derived from pre-existing native soil N. Primed N_2O emissions temporally shifted between positive and negative primings and with strong dependency on WFPS (Fig. 3-7). During peak emission (incubation day 2), primed N_2O emissions switched to be strongly positive and peaked simultaneously with the total N_2O fluxes (Fig. 3-2). The greatest positive primed N_2O emissions were observed with the wettest moisture (WFPS of 78%). After the short peak emission period, all treatments returned to negative priming , and continued until the end of the incubation (Fig. 3-7).

To further examine the pathways behind N_2O production, ^{15}N-N_2O SP results were utilized to allocate the whole N_2O flux into nitrification and denitrification, and to further interpret the contributions of these source-processes. A substantial positive priming effect was short-lived and occurred only during the peak flux, and denitrification was the main contributor to this N_2O priming (Fig. 3-8). Following the peak flux, the positive denitrification priming also ceased and shifted to relatively neutral low rates. In contrast, nitrification primed N_2O emissions tended to be much lower in magnitude and were continual with generally positive contributions, with the exception of the drier soils, which exhibited slightly negative priming near the end of the experiment.

3.4.6 Isotopic fractionation showed ^{15}N depletion during N_2O production

When estimating the ^{15}N isotopic fractionation of the emitted N_2O in comparison to soil N, it was assumed that this was a one-step unidirectional process as the soil system is the source of N_2O and the transformation of soil N to N_2O followed by the N_2O transfer to the atmosphere can be considered to be irreversible. Upon calculating the ^{15}N-N_2O enrichment or depletion factor (ε; relating emitted N_2O vs. soil N) across different WFPSs, all ε results showed ^{15}N depletion and with a slight tendency of an even, more negative ^{15}N depletion with decreasing soil moisture (Fig. 3-9).

3.5 Discussion

3.5.1 Large contribution of denitrification to the total N_2O production

We had initially hypothesized that with high moisture (WFPS > 70%), denitrification would be the predominant N_2O source, while nitrification would be the dominant N_2O-producing process in intermediate and drier soils. Nevertheless, across all assessed combinations of WFPS and N addition, denitrification was consistently the dominant N_2O source-process in this study. The consistent dominance of denitrification may suggest that the soil from the study site promotes anaerobiosis due to presence of micropores (Sey et al., 2008). This soil is a Black Chernozem with more than 10% organic matter content. Microbial respiration, decomposition, and mineralization of this naturally abundant organic matter can consume a large amount of oxygen, creating anoxic zones where denitrification occurs (Ostrom et al., 2010) even at relatively low water content. Furthermore, the soil in our study had a texture dominated by fine-sized particles (92% of soil particles were clay + silt), also favouring the formation of microenvironments for N_2O production via denitrification (e.g., micropores within soil aggregates). Moreover, the measurements of soil inorganic N concentrations (Fig. 3-4) indicate that at the time of peak fluxes and N dynamics in our study (incubation days ~2-3), there was greater availability of NO_3^- (and likely also associated NO_2^-) in comparison to the initially available NH_4^+, and with NO_3^- being a direct substrate for N_2O production via denitrification. Therefore, in addition to the putative existence of anaerobic microsites, the high abundance of NO_3^- substrate for denitrification further supports the predominance of denitrification in N_2O production. Our findings lined up with earlier reports; Uchida et al. (2013) evaluated the contributions of denitrification and nitrification from Andosol and Fluvisol at a constant WFPS of 55% and found denitrification-derived N_2O emissions were dominant in Fluvisol due to their small pore size and low air permeability. Also, Pihlatie at al. (2004) showed that at 70% of WFPS, N_2O production was mainly due to nitrification in sandy soils, while denitrification was the dominant process that contributed to N_2O production in organic soils.

The measured ^{15}N-N_2O site preference (SP) provided information on N_2O production mechanisms as this parameter is independent from the isotopic composition of the substrates (Toyoda et al., 2005). In our study, the majority of N_2O fluxes were associated with SP values close to 0‰, confirming denitrification as the dominant process contributing to N_2O production (Fig. 3-6A). Moreover, the greatest N_2O fluxes were consistently found when SP values were close to 0‰, revealing also that the large N_2O emitted from denitrification was intense and episodic (Fig. 3-6A).

Overall ^{15}N depletion in N_2O relative to the soil N further suggests denitrification as the main contributing N_2O production process (Fig. 3-9). Nitrifiers and denitrifies have different affinities to the heavier N stable isotope (^{15}N). Yoshida (1988) reported that isotopic enrichment of ^{15}N can range from -13 to -27‰ when N_2O is produced via denitrification, while nitrification leads to a much more depleted N_2O with even -60‰. Generally, nitrification is a slow continuous process that prefers to select the isotopically light ^{14}N over ^{15}N during isotopic fractionation. Therefore, as influenced by slight increases in the relative contribution of nitrification to the overall N_2O flux, the depletion in emitted N_2O was more pronounced with the lowest moisture content in the study (i.e., -17.4 ± 5.5‰ at 31% WFPS). Comparatively, denitrification is a much quicker process than nitrification, leading to lesser kinetic isotope fractionation, and hence, the emitted N_2O becomes less ^{15}N depleted. In fact, less ^{15}N depletion in the emitted N_2O (-10.84 ± 0.1‰) was found with the greatest moisture content (78% WFPS) when denitrification was also found to be the source of the highest contribution to the overall flux based on SP results (Figs. 3-6A and 3-6B).

3.5.2 Shifts in N_2O source-processes as a function of soil moisture

Across a wide range of WFPSs, the ^{15}N-N_2O SP results on the day of peak emissions were inversely proportional to the WFPS (Fig. 3-6B). As initially hypothesized, the contribution of denitrification was vastly dominant in the wettest soil (SP of 1.2‰ with 78% WFPS). Concurrently, SP mean was higher in the drier soil (9.6‰ with 31% WFPS) where the N_2O production from denitrification was therefore relatively lower, but denitrification remained the main source-process, still accounting for a 71% portion of the whole flux. Moreover, it can be further noted that more precise SP means (with small standard errors) were found with an increasing denitrification contribution to N_2O production in the wetter soils (Bateman and Baggs, 2005; Ostrom et al., 2010).

3.5.3 N_2O flux due to soil priming of native soil N pool and N_2O-producing processes

Supporting our initial hypothesis that the addition of N substrate would increase N_2O emissions by an indirect contribution via the priming effect (in addition to the direct contribution of N fertilizers to N_2O emissions), priming of N_2O emissions was evident on the second day of incubation during the peak flux. A reason for the increased positive priming effect could be the stimulated microbial turnover of soil organic matter resultant from the addition of N fertilizers (Schleusner et al., 2018). On the other hand, after the rapid microbial growth during the peak emissions, the death of microbes and the acceleration of microbial turnover creates a readily available source of nutrients for new microbial utilization. The use of this labile source of nutrients from microbial turnover, instead of the use of native soil organic matter, can lead to the slight negative priming observed following the peak emissions (Kuzyakov et al., 2000).

Similarly, a temporal immobilization of added N, fixation of NH_4^+ to the clay minerals, or sorption of NH_4^+ to humic substances (Kuzyakov et al., 2000) may have caused the very short-term small negative priming.

In our study, in addition to the priming caused by inorganic N fertilization, soil moisture also was observed to have a great influence on the quantified priming effects. Similar to the magnitude of the total N_2O emissions, the magnitude of the primed N_2O flux increased profoundly with increasing WFPS, indicating that during an optimum microbial activity with moistures ranging from 60 to 80% WFPS (Dobbie et al., 1999; Bateman and Baggs, 2005), microbes mined pre-existing soil N, creating further positive priming.

According to Kuzyakov et al. (2000), priming effects can be ascertained as apparent and real. Apparent priming occurs immediately before the peak activity of microbes, while real priming takes place in concurrence with the maximum activity of microorganisms (Kuzyakov et al., 2000). A real priming with a lag phase was ascertained in this study (Fig. 3-7) as this priming was observed during the period of peak fluxes, which also reveals the time of maximum activity of microorganisms and associated N turnover.

In terms of N_2O-producing processes, an episodic contribution of denitrification to the priming effect was found in our study (Fig. 3-8), likely showing that denitrifiers utilized the available resources [nitrate substrate as electron acceptor, soil organic C sources as electron donors, and moisture] at once. By utilizing all of these resources simultaneously, a much greater N_2O emission was generated for an intense, short period. In contrast, the contribution of nitrification to N_2O priming seemed to be continuous and minor, suggesting the slow and constant utilization rate of resources by nitrifiers.

3.6 Conclusion

This study confirmed that differences in soil moisture significantly drive microbial N_2O production. Furthermore, across all soil moisture contents and urea-N additions, denitrification was the main contributing process for N_2O production. During the peak fluxes, N_2O produced from the native soil N was strongly primed by the labile N addition (urea) also with clear dependency on soil moisture content. Priming contributed to short-lived, vigorous N_2O production from denitrification, while the contribution of primed nitrification was minor yet continuous.

3.7 References

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3.8 Figures

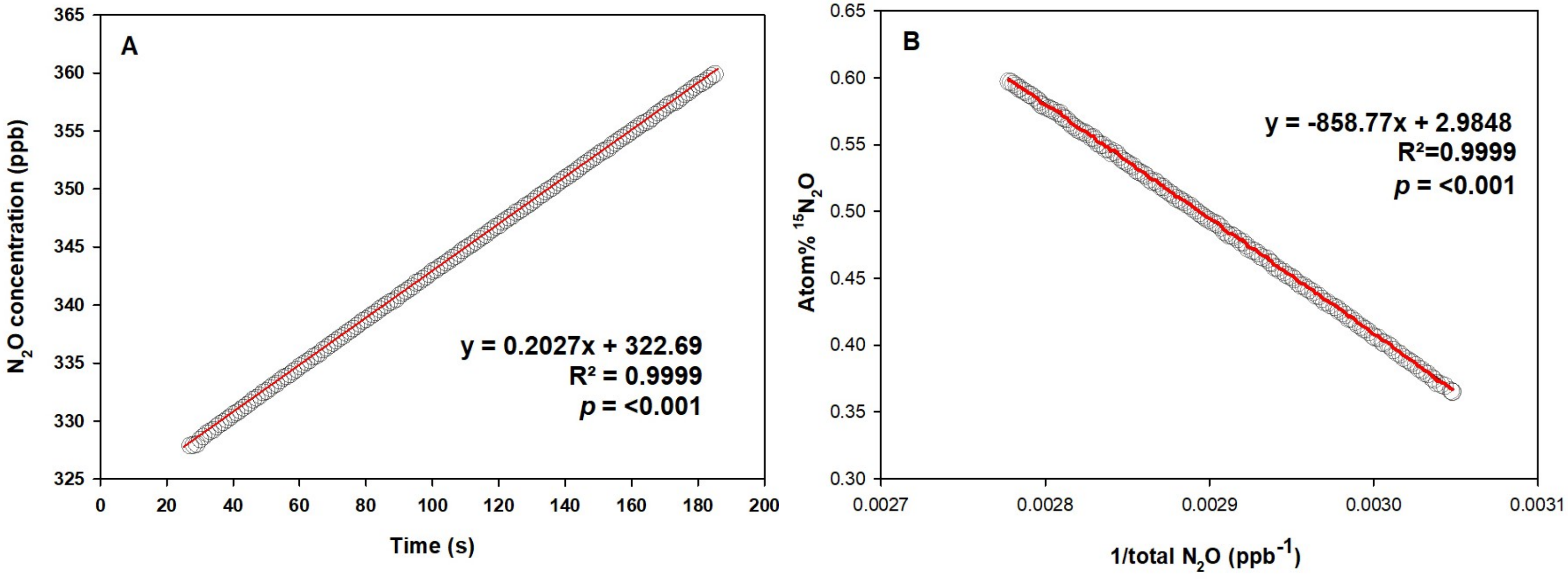

Figure 3-1. An example of (A) a flux measurement plotting total N_2O concentration (ppb) as a function of time, and (B) the Keeling plot for the same flux measurement with Atom % ^{15}N-N_2O as a function of 1/total N_2O concentration (ppb^{-1}).

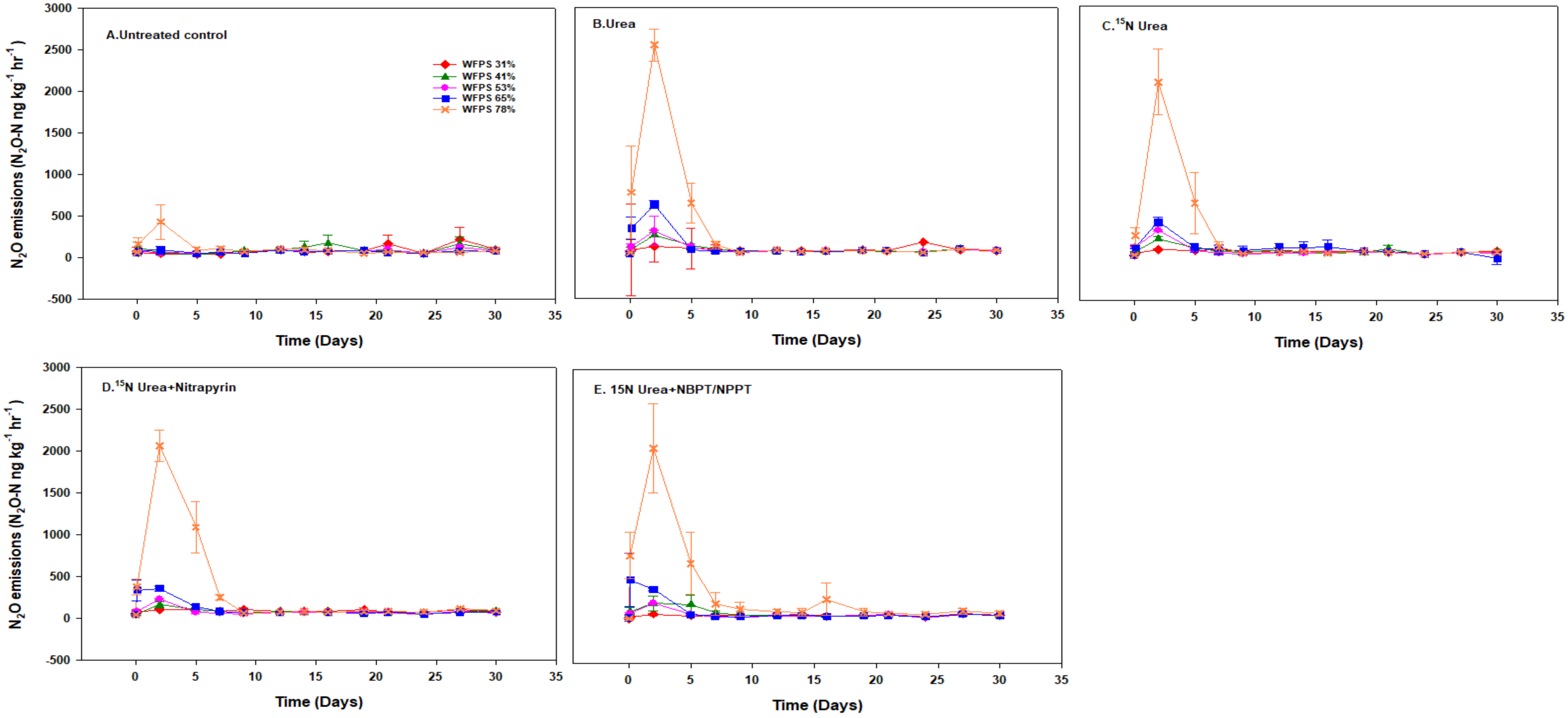

Figure 3-2. Mean daily N_2O emissions with incubation time under five water-filled pore spaces (WFPSs) for the A) untreated control, B) isotopically-unlabeled urea, C) ^{15}N-labelled urea, D) urea + nitrapyrin, and E) urea + NBPT/NPPT treatments.

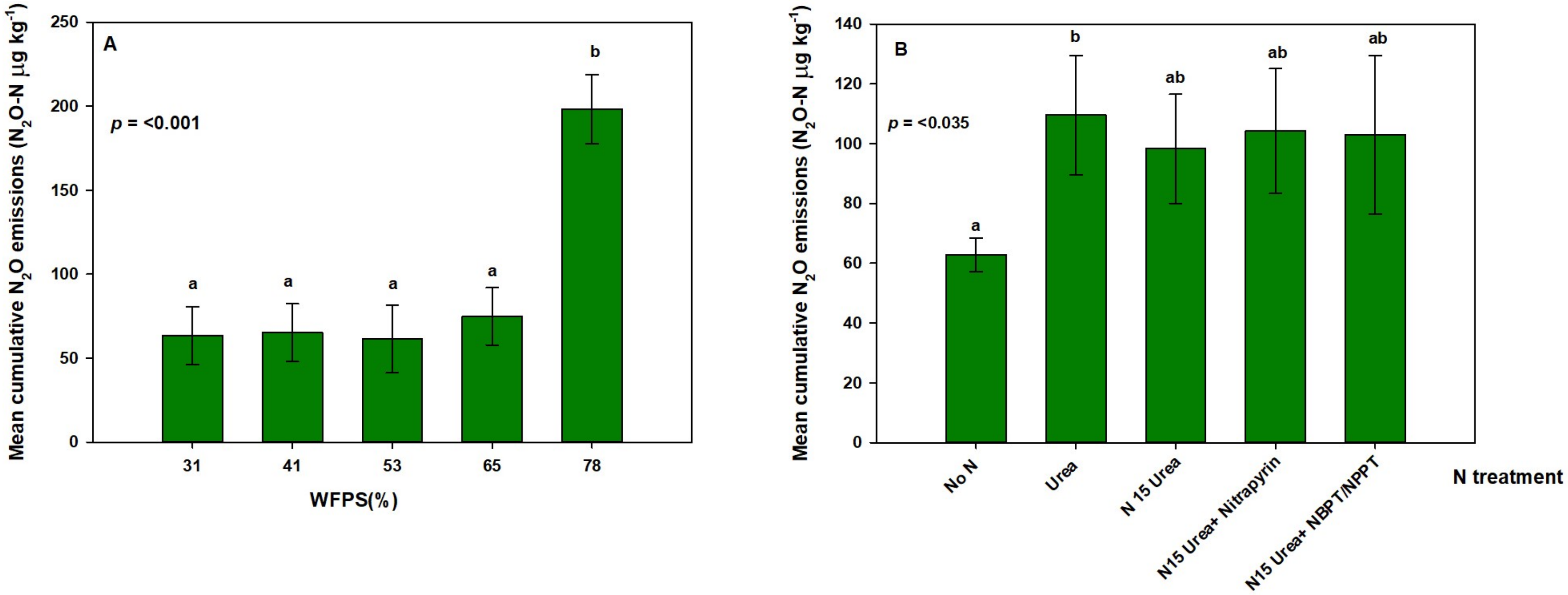

Figure 3-3. Cumulative N_2O emissions for the entire incubation period as a function of (A) five WFPSs and (B) five N addition treatments. Different letters indicate statistically significant differences between the treatments ($p<0.05$) according to Tukey's honest significant difference test.

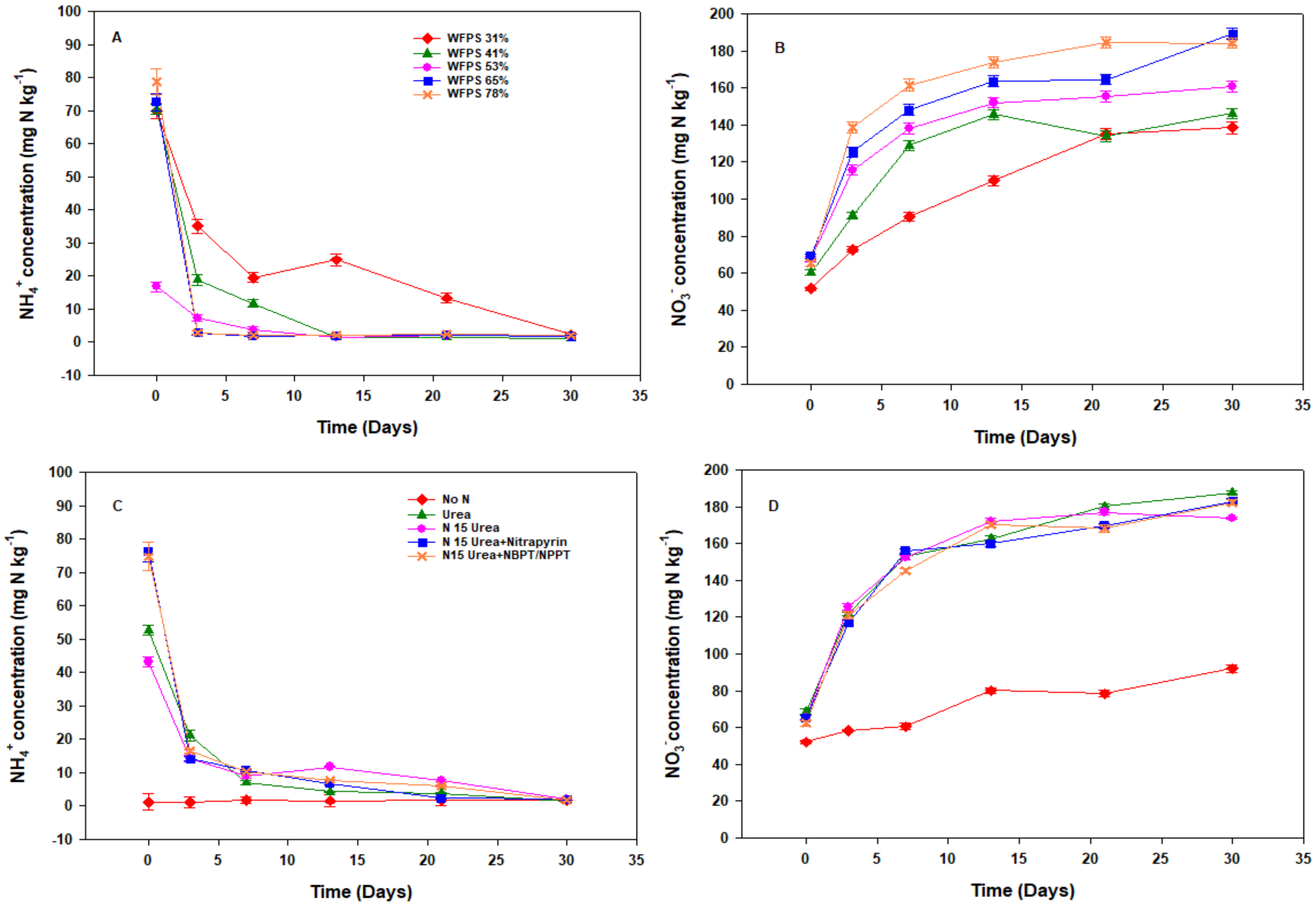

Figure 3-4. Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations (mg N kg^{-1}) with time as a function of (A and B) five water-filled pore space (WFPS) and (C and D) five N addition treatments.

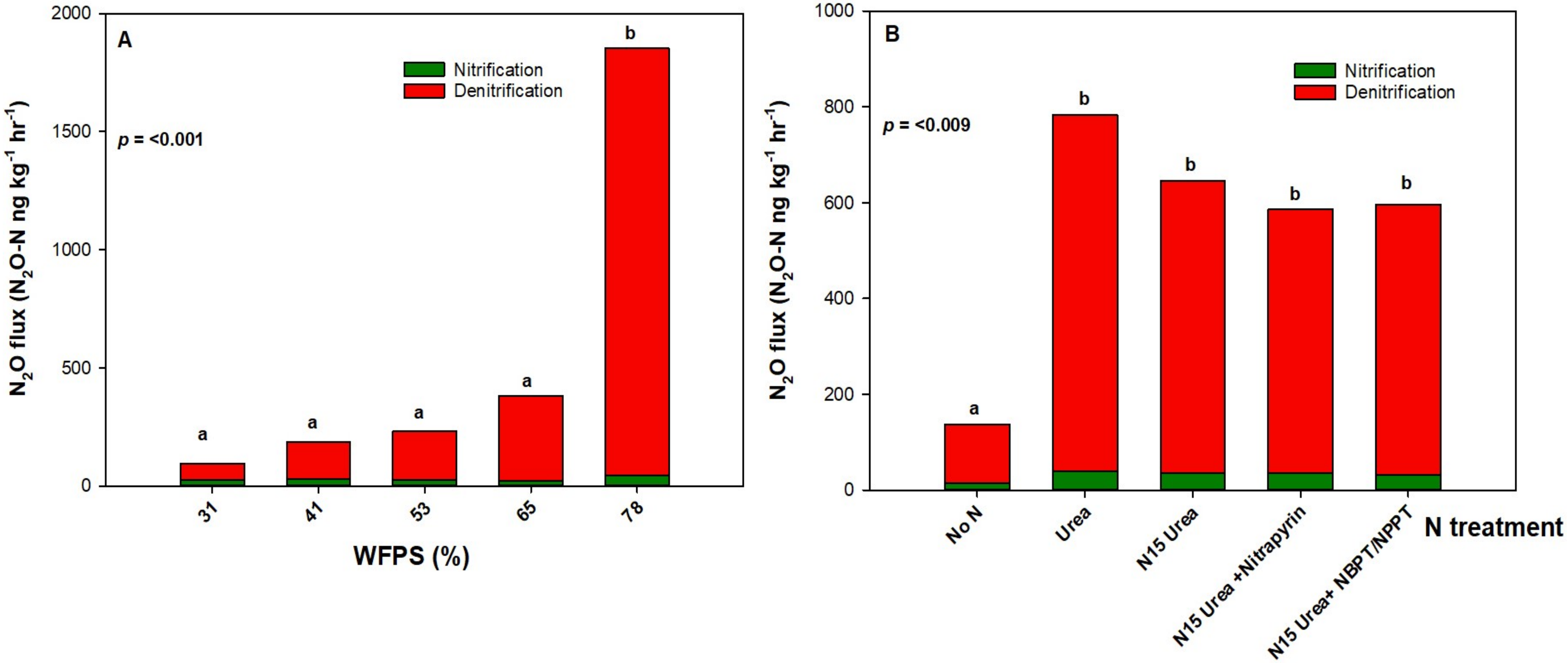

Figure 3-5. Emissions and source-processes partitioning (nitrification vs. denitrification) of N_2O as a function of (A) five water-filled pore space (WFPS) and (B) five N addition treatments during the peak emission time (incubation day 2). Different letters indicate statistically significant differences in N_2O emissions between the treatments ($p<0.05$) according to Tukey's Honest Significant Difference test.

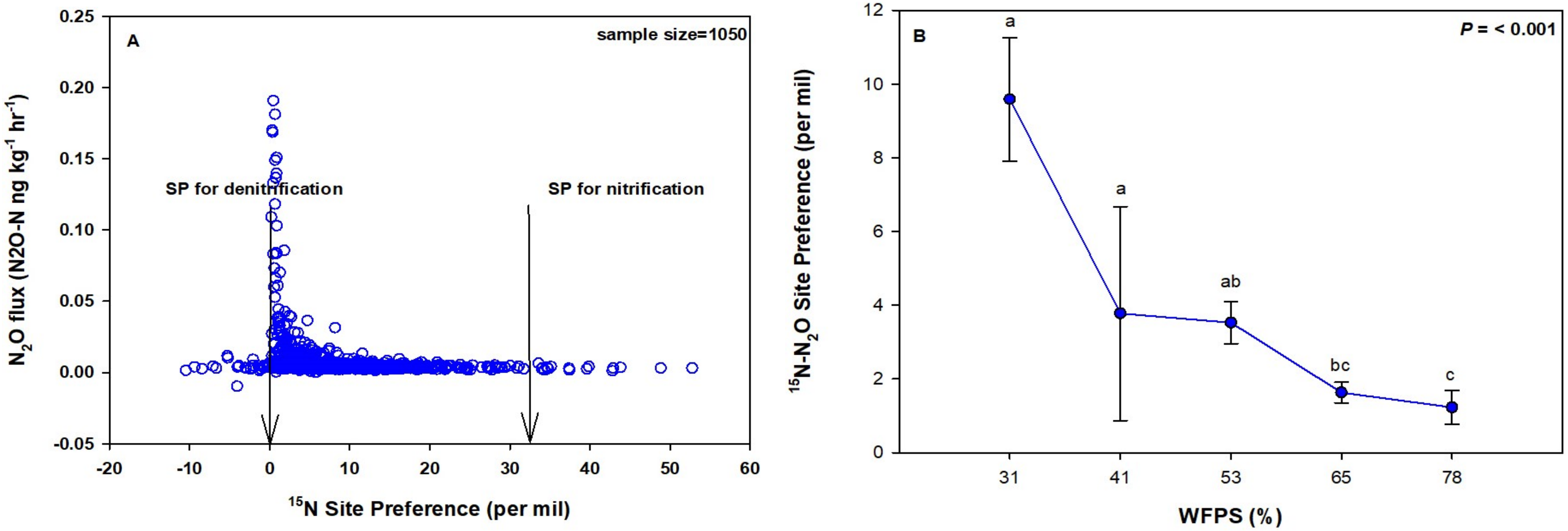

Figure 3-6. (A) N_2O fluxes versus ^{15}N-N_2O site preference (SP) from measurements taken during the entire incubation period, and (B) ^{15}N-N_2O SP as a function of WFPS on the day of peak flux (incubation day 2). Different letters indicate statistically significant differences between the treatments ($p<0.05$) according to Tukey's Honest Significant Difference test.

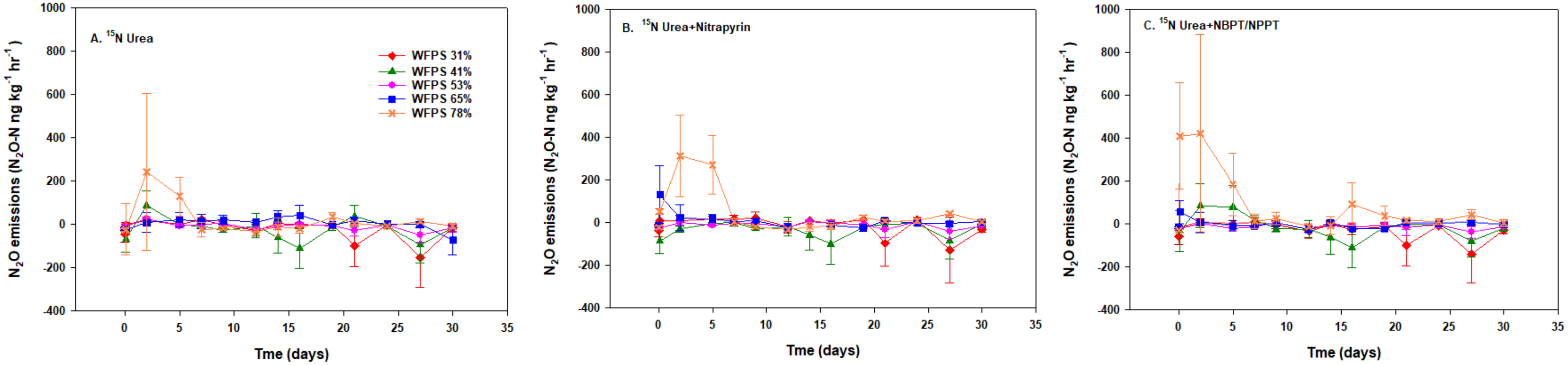

Figure 3-7. Priming of the N_2O flux derived from soil N pool as induced by initial urea addition across five different WFPS (calculated with Eq. [5]) for A) ^{15}N-labelled urea, B) urea + nitrapyrin, and C) urea + NBPT/NPPT treatments.

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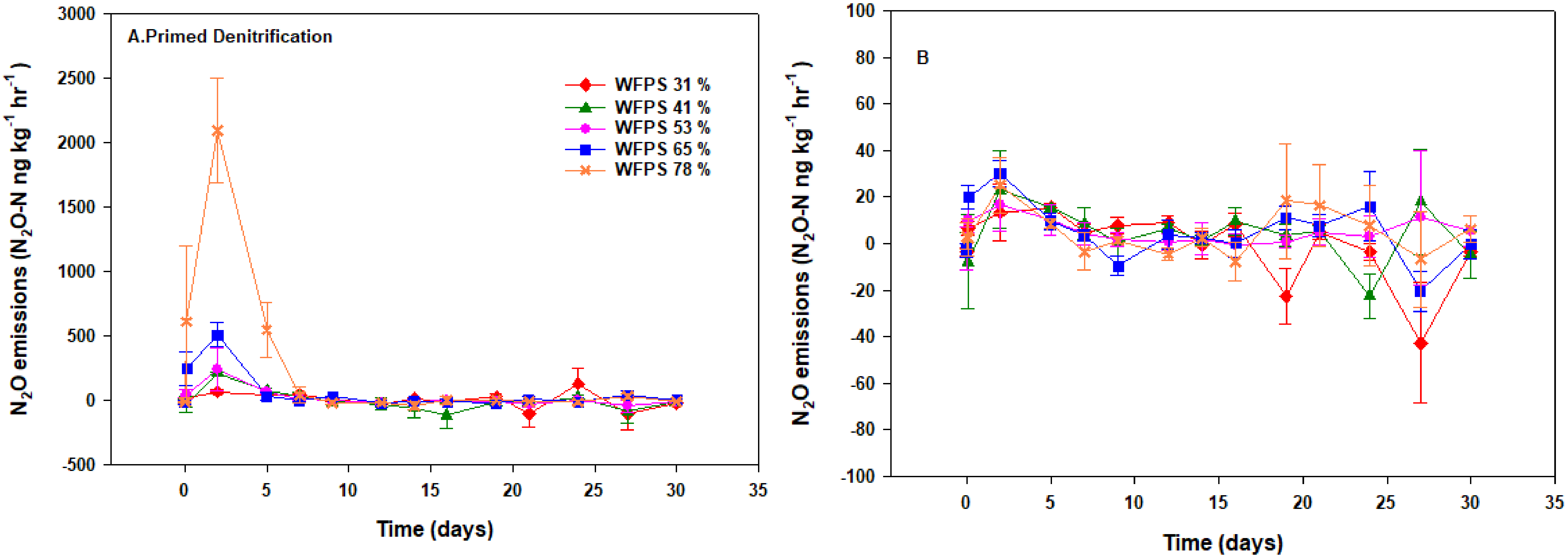

Figure 3-8. Priming of N_2O produced during (A) denitrification and (B) nitrification under 5 different WFPS treatments (calculated with Eqs. [9] and [10]).

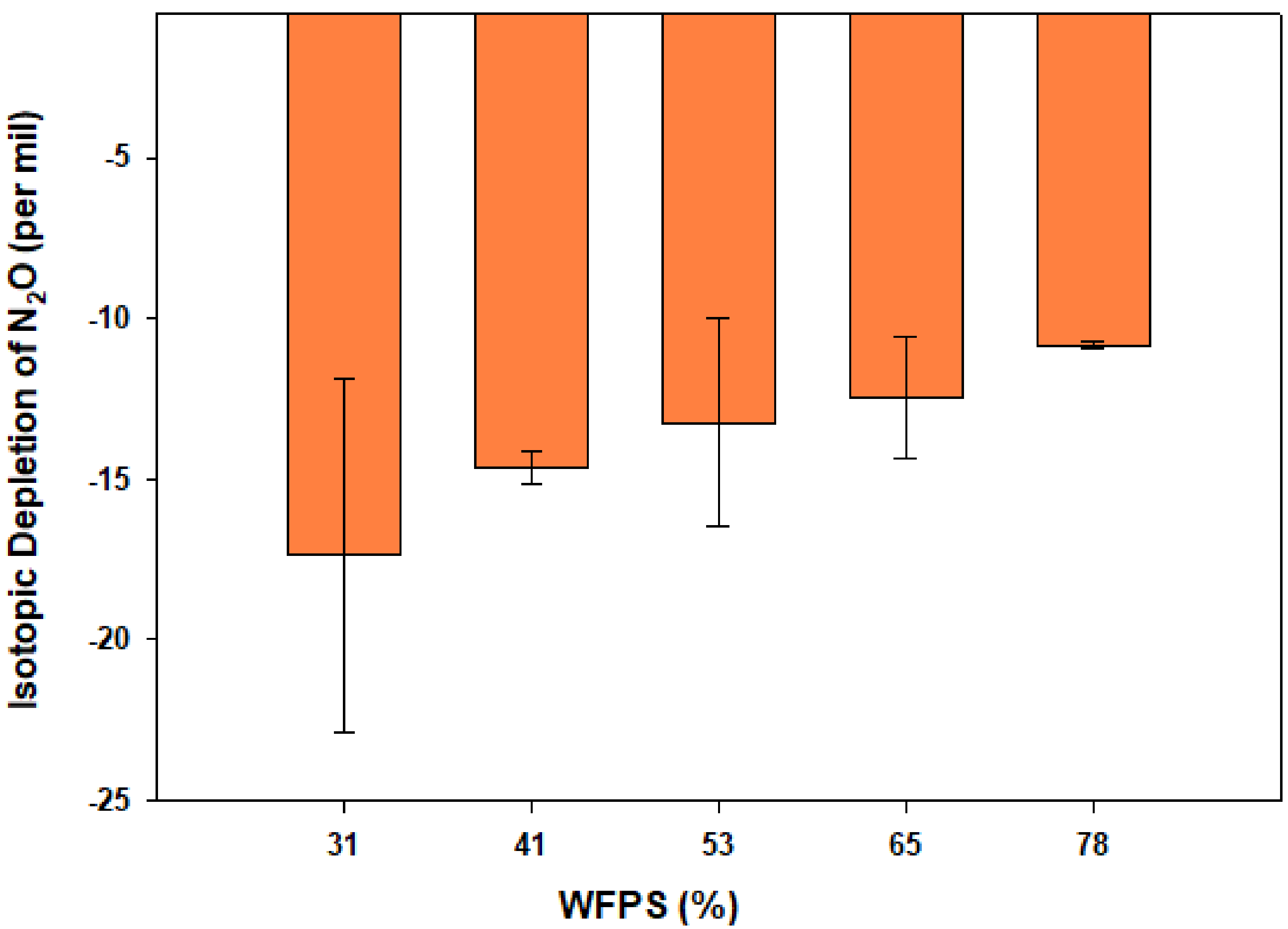

Figure 3-9. Isotopic fractionation (ε, depletion) of emitted N_2O relative to the soil N pool as incubated under different WFPS treatments (calculated with Eq. [12]).

4 Process-based Modelling of N_2O Emissions from Wheat Croplands: Nitrogen Fertilizer Formulation and Timing of Application

4.1 Abstract

Application of right types of nitrogen (N) fertilizer with the right timing can enhance N use efficiency while minimizing N losses such as those through nitrous oxide (N_2O) emissions. Quantification of temporal variability of N_2O emissions and emission factors (EFs) is challenging as N_2O emissions are variable and discontinuous. To contribute towards improving these quantifications, a simulation experiment was conducted with the *ecosys* model, which is a process-based mathematical model. The objectives of the study were to simulate N_2O emissions as a function of the addition of three fertilizer formulations [anhydrous ammonia (AA), conventional urea, and polymer-coated urea (environmentally smart N®, ESN)] at two application timings (fall N vs. spring N) at the rate of 100 kg N ha^{-1} and, subsequently, to model N_2O emissions with simulated lower rates of N fertilization (i.e., 25, 50 and 75 kg N ha^{-1}). Model performance was verified by comparing modelled data with measured soil temperature, soil moisture, grain yield, and grain N uptake, all of which were generated during a two-year (2015-2016 and 2016-2017) field experiment conducted in Central Alberta, Canada. Our study showed that both modelling and field measurements were able to describe temporal variability of N_2O emissions due to changes in both moisture and N availability; however, modelling was even more sensitive than measurements when depicting the changes in N availability due to N fertilizer additions, while measurements appeared to be more responsive than modelling in the capturing of moisture changes and effects. After gaining confidence in modelled daily flux results for the study year 2015-2016, cumulative N_2O emissions for each type of N management at the experimental fertilization rate of 100 kg N ha^{-1} were determined. The N_2O emission reduction caused by environmental smart nitrogen (ESN) was consistent both in the measured data and modelled outputs, while the direction of the effect of the N application timing on measured N_2O emissions (spring > fall) trended opposite to the modelled data (fall > spring).

Our modelled EFarea (average of 0.34%) closely agreed with the existing regional-specific EF of 0.33%, which is also similar to our measured EFarea of 0.28%. Using the model, the yield-based N_2O EF that was determined for reduced rates of N fertilizers suggested an optimum N rate of 25 kg N ha^{-1} to maintain grain yield in the short-term while reducing N_2O emissions.

4.2 Introduction

The application of inorganic N fertilizers in the fall is preferred in some temperate regions such as the Canadian Prairies because of the low cost and reduced workload the following spring. During the fall addition of N fertilizers, when soil conditions are still favourable before the soil freezes, ammoniacal fertilizers are subjected to nitrification (Nyborg et al., 1997; Tenuta et al., 2016), leading to nitrate (NO_3) accumulation. When the soils start to thaw in the early spring, under oxygen-limited and relatively high moisture and temperature conditions, NO_3 serves as an electron acceptor. As a result, there is a rapid transition in oxidation and reduction reactions, and N_2O is produced via denitrification (Grant and Pattey, 1999; Rochette et al., 2004**)**. When soil water drains or evaporates, gas diffusivity increases, resulting in peaks of N_2O release after the spring thaw (Wagner-Riddle et al., 2008; Tenuta and Sparling et al., 2011).

Enhanced efficiency N fertilizers (EENFs) have been developed to improve nutrient delivery to crops and also to minimize N_2O emissions relative to conventional N fertilizers (e.g., urea and anhydrous ammonia (AA)). Within many EENF products, environmentally smart N (ESN) is a controlled-release fertilizer that consists of a polymer coating around the urea granule (Trenkel, 1997; Trenkel, 2010). The release of N from ESN is determined by soil moisture and temperature, and the characteristics of the polymer coating (Li et al., 2012). When water moves inside the granule through the semipermeable polymer coating, N is diffused through the coating and into the soil solution.

Due to significant variability caused by the complex interaction of biological and physical controls on microbial N_2O-producing processes (Rudaz et al., 1999; Grant and Pattey, 2008; Grant et al., 2016), the quantification and prediction of N_2O emissions from croplands are still uncertain. Currently, N_2O fluxes are commonly measured manually using static chambers.

The use of such static chambers can limit the accurate assessments of temporal variability of N_2O due to low sampling frequency (1 to 2 times per week) (Molodovskaya et al., 2011) because large episodic fluxes can be missed. Using linear interpolation to calculate N_2O fluxes between consecutive dates leads to further uncertainty in the measured data. These limitations of estimating N_2O emissions can be minimized using N_2O flux models that accurately account for temporal fluctuations (Grant and Pattey, 2003).

Out of several existing N_2O models, such as DNDC and DayCent, we selected the process-based *ecosys* model, which is a detailed ecosystem terrestrial model that has been extensively tested against actual experimental results and has been used to represent the temporal variability of N_2O fluxes (e.g., in croplands in North America) (Grant et al., 2006; Grant et al., 2009). It is a three-dimensional mathematical model. The model encompasses existing theory that governs soil processes associated with N_2O emissions to provide predictions. The model represents the key processes of mineralization, immobilization, nitrification, denitrification, plant uptake, oxidation-reduction reactions, volatilization, convection, diffusion, and dissolution, among others (Grant et al., 2006; Grant and Pattey, 2006). *Ecosys* is capable of simulating N_2O emissions under different management practices, considering climate, soil profile properties, and soil and plant species. Even though previous studies have modelled N_2O emissions from conventional fertilizers, the simulation of N_2O emissions from an enhanced efficiency N fertilizer, such as the polymer-coated urea ESN using *ecosys,* has not been reported. Moreover, taking into consideration that fall-applied N inputs lead to greater N_2O emissions during the following spring thaw in comparison to spring-applied N (Lin et al., 2017), there is also a need to conduct modelling studies focusing on the timing effects of the fertilizer application (fall N vs. spring N). Therefore, *ecosys* was used to test and represent the seasonal variability of N_2O

fluxes, thereby predicting changes of emission patterns with contrasting fertilizer formulations (urea, AA, and polymer-coated urea ESN) and application timings (fall N vs. spring N). For our model testing, we used measured data from our recent two-year field study in Central Alberta (Chapter 2) for four fertilizer treatments: untreated control, urea, anhydrous ammonia (AA), and polymer-coated urea (environmentally smart N - ESN®), including both fall and spring N applications with a total of seven treatment combinations.

The magnitude of N_2O emissions from croplands typically increases with the amount of N fertilizer added (Ruser et al., 2001; Grant et al., 2006; Zebarth et al., 2008). When the soil mineral N concentration exceeds the plant needs, without any competition, microbes use the excess of N availability for N_2O production through nitrification and denitrification. Determining the optimum N rate for plant growth while minimizing N_2O emissions is a significant environmental challenge. The *ecosys* model has been tested against field studies for modelling N_2O emissions with a range of AA application rates (Grant et al., 2006), suggesting that N_2O emissions and crop yield as a function of varying fertilizer rates can be predicted accurately. Upon testing and constraining *ecosys* with field experimental data, one of our aims is to use the tested model to simulate and predict N_2O emissions and grain yield while gradually reducing the N fertilizer application rate to identify any optimal threshold of reductions in emissions versus grain yield from the model results. These findings can be used to adjust fertilizer rates to minimize N_2O emissions without significantly penalizing yields.

The ability of the mathematical model *ecosys* to estimate N_2O emissions accurately can provide the means to simulate effects of management, soil, and climatic factors on N_2O emissions. Subsequently, modelled flux data can be used as best estimates for the dates when measured data are unavailable instead of assuming linear interpolation between consecutive flux measurement dates. Site-specific N_2O emission values derived from *ecosys* can aid in minimizing the uncertainty associated with Tier I and Tier II emission factors (EF) and develop Tier III EF for use in regional and national emission inventories of greenhouse gases.

Our study aimed at improving the understanding of the underlying factors controlling the processes associated with N_2O production to provide opportunities to mitigate N_2O emissions. Our sequential objectives were (i) to test whether biogeochemical N cycle process algorithms in the process-based model *ecosys* can simulate N_2O emissions as affected by N fertilizer formulation (AA vs. urea vs. ESN) and application timing (fall N vs. spring N), along with testing the model for soil temperature, soil moisture, grain dry matter (DM), and grain N, and (ii) to evaluate whether reducing the rates of N fertilization in the simulations can minimize the modelled N_2O emissions without sustaining the grain yield.

We hypothesized that the higher soil moisture and lower soil aeration in the model during the springtime would force the modelled microbes to use NO_3 as an alternate electron acceptor because of a deficiency of oxygen for microbial activity. For fall- fertilized soils, there will be enough substrate (NO_3) available to be denitrified, which would cause the model to simulate higher N_2O emissions than the spring-applied fertilizer. Slower water diffusion into the ESN granules and hence lower dissolution rates of ESN compared to urea in the model would cause lower substrate availability in fall-applied ESN than in fall-applied urea, which would in turn cause the model to simulate lower N_2O in fall-applied ESN than in fall-applied urea.

4.3 Materials and methods

4.3.1 Model description

The *ecosys* model is constructed from algorithms representing physical, biological, and chemical processes of the soil-plant-atmosphere system that simulate the C, N, P transformations and soil water, solute, and heat transport (Grant et al., 2016; Grant et al., 2020). Equations representing these transformations are detailed and explained in previous literature (Grant and Pattey, 2003, 2008; Grant et al., 2006; Metivier et al., 2009; Grant et al., 2016). Briefly, all soil microbial functional types in each substrate-microbe complex (animal manure, coarse and fine woody plant residue, particulate organic matter, and humus) maintain the C:N ratio by mineralization or immobilization of NH_4^+ (Grant et al., 2016). Biological fixation is simulated in *ecosys* for both symbiotic and non-symbiotic relationships. Soil NH_4^+ from fixation and mineralization is oxidized, while the O_2 is reduced by nitrifiers to produce NO_2^-. Further oxidation of NO_2^- and reduction of O_2 by nitrifiers increases the soil NO_3^- (Grant et al., 2020). Under the O_2-limited condition, N_2O can be produced via nitrifier denitrification, which is the oxidation of NH_3 and the reduction of NO_2^- by nitrifiers. Moreover, the limitation of O_2 increases the demand for alternative electron accepters that can be met by NO_3^-, NO_2^- N_2O and produces NO_2^- N_2O, N_2 by denitrifiers. The transport of O_2 in gaseous and aqueous phases and through soil is simulated via convection, dispersion, dissolution, and uptake of O_2 (Grant et al., 2016). Volatilization of NH_3 is affected by the soil pH, moisture, and temperature, while the soil cation exchange capacity and iron paring govern the adsorption and desorption of NH_3. Soil NO_3^- can be lost from the ecosystem via surface runoff and percolation to the water table. The processes of active uptake, ion exchange, radial diffusion, and convection in *ecosys* governs the plant uptake of NO_3^- and NH_4^+ (Grant et al., 2016).

4.3.2 ESN modelling in *ecosys*:

ESN is a polymer-coated fertilizer that was developed to control N release. In the soil, when water diffuses through a semipermeable polymer coating, urea is hydrolyzed, and NH_4^+ is slowly released to the soil solution. The release of N from slow release fertilizers such as ESN can be simulated in *ecosys*. Hydrolysis of urea in the model is governed by the soil moisture and temperature. Soil temperature is calculated using the Arrhenius function and rate constant using Michaelis-Menten kinetics. The rate of granular urea hydrolysis is calculated using Eq. 1 and urease inhibition used in Eq. 1 is calculated using Eq. 2.

$$\begin{aligned}&\text{N release from ESN}\\&\quad = \text{specific rate constant of urea hydrolysis} * \text{total microbial activity}\\&\quad * \text{microbial concentration} * \text{temperature function}\\&\quad * (1.0 - \text{urease inhibition}) \qquad Eq.[1]\end{aligned}$$

$$\begin{aligned}\text{Urease inhibition} &= \text{inhibition at previous hour} - \text{rate constant for loss of inhibition}\\&* \text{inhibition at previous hour} * (1\\&- \text{inhibition at previous hour} / \text{inhibition at time of application}) \qquad Eq.[2]\end{aligned}$$

4.3.3 Field experiment

For testing and constraining the model, we used data generated during a field experiment (Chapter 2).

4.3.4 Site Description

A two-year field experiment was established at the St. Albert Research Station (53° 41′ 45.1″ N, 113° 37′ 33.6″ W) in central Alberta, Canada. In this field experiment, N_2O emissions, grain yield, grain N, soil moisture and temperature were measured and used to test the *ecosys* model. The site has a cold, semi-humid, temperate climate with a total annual precipitation of 456 mm and a mean annual temperature of 4.2 ˚C. The soil is classified as a Black Chernozem (U.S. Soil Taxonomy equivalent: Typic Cryoboroll).

4.3.5 Experimental Design

The field experiment was a completely randomized block design with four experimental block replicates with two general fertilizer types (conventional vs. EENFs) and two application timings (fall N vs. spring N) (Chapter 2). Of the total of fifteen treatments available in the field study, we selected seven experimental treatments to test the model hypotheses: 1) control, where no N was added, 2) fall urea and 3) fall anhydrous ammonia (AA), both as conventional fertilizers, and 4) fall polymer-coated urea (environmentally smart N - ESN®, 45-0-0) as an EENF, as well as (5) spring N applied urea, (6) spring AA, and (7) spring polymer-coated urea (Table 4-1).

In both experimental years, N fertilizers were double midrow banded (depth 7.5 cm, band spacing 50 cm) at a rate of 100 kg N ha^{-1} (Table 4-2). The fertilizer rate of 100 kg N ha^{-1}was calculated using the Alberta Farm Fertilizer Information and Recommendation Manager (AFFIRM) which is a decision support tool designed to develop nutrient management plans for crop production using the location, soil, previous crop, soil test laboratory results, expected crop price, farm fertilizer budget and so on. The experiment was conducted with Canada Western Red Spring wheat (*Triticum aestivum* cv. CDC Go). The first experimental year ran from Oct 2015 to Sep 2016. Experimental plots in the second year (Oct 2016 to Sep 2017) were located adjacent to the first-year experimental plots (Table 4-2).

4.3.6 Soil, plant, and meteorological measurements

Soil volumetric water content (VWC) for the unfrozen periods and soil temperature measurements at depths of 5, 10, and 15 cm over 2017 were recorded every 30 minutes using soil sensors (5TM, Meter™). Physiologically mature wheat grain was collected during harvest and oven-dried to determine dry matter (DM) grain yield and total N content through near-infrared spectroscopy (FOSS™ DS2500; range 400 – 2500 nm, 0.5 nm spectral resolution). Hourly precipitation, air temperature, humidity, radiation, and wind speed measurements were recorded in the St. Albert Research Weather Station located approximately 600 m from the experimental sites.

4.3.7 N_2O flux measurements

A non-steady state, closed manual chamber methodology, as described in Hernandez-Ramirez et al. (2009), was used for N_2O flux measurements with two rectangular chambers (15 × 64.1 × 15.6 cm, height × length × width) in one plot. Chambers were installed after the fall N fertilizer application. After the closure of chambers, three gas samples were collected at 16, 32, and 48 minutes from one chamber, and one gas sample at 48 minutes was collected from the second chamber. During flux measurements, a syringe needle was inserted through the rubber septum of the chamber lid to collect a 20 ml gas sample, which was injected into a 12 ml pre-evacuated glass vial. Gas samples were collected weekly, and more frequent sampling took place with the N fertilizer application and after rainfall events. Chambers in the fall and control plots stayed in the same locations except when they were removed for seeding in the spring. After seeding and spring N fertilization, chambers were installed in the spring N plots. The N_2O concentrations of collected gas samples were analyzed using an electron capture detector in a Varian 3800 gas chromatograph (GC) system (Varian Inc., Walnut Creek, CA) with a flux detection limit of 2.84 g N ha^{-1} d^{-1}. The rate change of measured N_2O concentration was used to calculate N_2O flux by using linear or quadratic relationships.

4.3.8 Model spin up and evaluation runs

The *ecosys* model was used to simulate N_2O emissions under different N fertilizer sources and timing of application. To attain stable site conditions before the evaluation period, a model spin-up was run from Jan 2000 to Dec 2014. Weather data (precipitation, air temperature, humidity, radiation, and wind speed) recorded at the St. Albert Research Weather Station was retrieved via the Alberta Climate Information Service (ACIS) for use as input to the model. Daily weather data from 2000 to 2009 and hourly data from 2010 to 2017 were used. During the spin-up, in addition to weather data, topographic characteristics of the site, soil properties of Black Chernozem (Table 4-3), and plant and management data were used to represent site history prior to the experiment.

When the modeled agroecosystem reached a steady state following an arbitrary initial condition, model runs were continued separately for two testing periods (2015-2016 and 2016-2017, corresponding to periods within which our experimental field data were available). For these two models runs simulating the periods 2015-2016 and 2016-2017, we used continuous hourly-recorded weather data and the field management practices as shown in Table 4-2. Field data collected for soil moisture and temperature, grain yield, and grain N uptake were used for testing the model runs prior to the simulation and prediction of N_2O emissions. All the parameters in *ecosys* were modelled hourly, and the output of the model-simulated soil moisture, soil temperature, and N_2O fluxes was recorded hourly, while grain yield and grain N uptake were recorded at daily intervals. As part of our model study, the fluctuations in modelled N_2O

emissions aggregated to daily and seasonal values as affected by soil moisture, temperature, and N management choices were evaluated.

The results from our earlier field experiment (Chapter 2) revealed that the type of N fertilizer used (e.g., urea vs. AA, or conventional vs. enhanced efficiency N fertilizers) did not significantly affect the measured N_2O emissions in these Black Chernozem soils with high organic matter (OM) and substantial natural fertility. Based on the observations emerging from our field study, we further postulated that N fertilizer formulation and timing may have significant beneficial effects on crop yield and N_2O emissions in soils with intermediate soil N availabilities. To examine this possible effect of N formulation and timing of application on N_2O emissions and grain yield with N application rates lower than 100 kg ha^{-1} yr^{-1}, the model was run simulating reduced N application rates (i.e., 25, 50, and 75 kg ha^{-1}).

4.4 Results

4.4.1 Model testing: Soil temperature and soil water

The *ecosys* model successfully simulated the hourly soil temperature in terms of timing and magnitude throughout the experiment. In the first experimental year, measured soil temperature was in clear agreement (R^2=0.63) with modelled soil temperature for the 10 cm soil depth (Fig. 4-1). During the second experimental year, soil temperature was measured for 5, 10, and 15 cm soil depths and modelled soil temperature was also highly correlated (5 cm, R^2=0.75; 10 cm, R^2=0.77;and 15 cm, R^2=0.81) with field measurements (Fig. 4-2A, 4-2B, and 4-2C).

Similar to soil temperature, soil volumetric water content (VWC) measurements were modelled for 5 (R^2=0.70), 10 (R^2=0.86) and 15 cm (R^2=0.84) depths in 2017 (Fig. 4-3A, 4-3B, and 4-3C). Both measured and modelled soil VWC increased with rainfall events as expected. The temporal pattern of modelled and measured soil VWC was similar. However, simulated soil VWC Oct in 2017 was greater in magnitude than measured soil VWC (Fig. 4-3A, 4-3B, and 4-3C).

4.4.2 Model testing: Grain yield and grain N

The model prediction of grain yield was satisfactory (Figs. 4-4A and 4-4C). Most modelled grain yields were within the range of measurements, while the grain yields of control, fall-applied AA and fall-applied ESN were underestimated by the model in a 2015-2016 experimental year. The model accurately simulated the magnitude of grain N uptake for the experimental year 2016-2017 (Fig. 4-4B and 4-4D); in contrast, modelled grain N uptake was slightly underestimated during the experimental year 2015-2016.

4.4.3 Temporal variability of N_2O fluxes

During the fall of 2015 and the fall of 2016, fall N fertilizer application did not elevate either measured or modelled N_2O emissions (Fig. 4-5 and Fig. 4-6). Minimal fluxes were observed in AA and urea N treatments, while null fluxes were recorded in the control and fall ESN treatments. During the winter (from November through March), field fluxes were not measured, assuming that N_2O emissions during winter were negligible, which was confirmed by the modelled results as the simulated N_2O fluxes were near zero during the winter until late March. In general, substantial agreement was found between measured and modelled fluxes during fall and winter seasons in our study. Measurements showed considerable N_2O peaks on March 30, 2016, and April 6, 2017, in response to soil thawing in the early spring. Even though *ecosys* was able to capture these major emissions associated with the thaw events (with the exception of the control treatment in spring 2016), the N_2O fluxes generated by the model were typically delayed by several days in comparison to measured N_2O fluxes. Across the modelled spring N types of management, spring AA resulted in the largest simulated peak of N_2O. The rainfall events following N fertilization caused increases in both measured and modeled N_2O fluxes (Fig. 4-5 and Fig. 4-6).

4.4.4 Effects of N fertilizer formulation and timing of application

Simulated emission patterns for the various fertilizer types were similar across the two study years (Fig. 4-5 and Fig. 4-6). Conventional fertilizers, AA and urea, exhibited greater emissions than ESN, and the lowest emissions occurred in the control treatment. Within the conventional fertilizers, AA had higher emissions than urea. Emissions from ESN treatment remained low, giving similar emissions as the control treatment receiving no N addition (Fig. 4-5 and Fig. 4-6).

After gaining confidence in modelled hourly flux results for the study year 2015-2016, cumulative N_2O emissions for each N fertilizer treatment were determined and compared with cumulative values derived from measurements (Table 4-4). The modelled results in our study highlighted that the spring N application reduced N_2O emissions by 13 % in comparison to fall N application, and a 50 % reduction in N_2O emissions was observed through the adoption of ESN rather than the use of AA or urea. This beneficial role of ESN in the modelled data, consistent with the field-measured data, resulted in 32% lower emissions from ESN than from conventional fertilizers urea and AA. In contrast, the direction of the effect of the N application timing (spring N vs. fall N) on measured N_2O emissions (spring N > fall N) trended opposite to the modelled data (fall N > spring N) (Table 4-4).

4.4.5 Effects of N fertilizer application rate: Model simulation runs

Upon verifying an adequate agreement between modelled and measured data (i.e., soil temperature, soil moisture, soil ammonium and nitrate, grain yield, grain N uptake, as well as daily and cumulative N_2O fluxes) for the study period 2015-2016, N_2O emissions and grain yields were simulated using the *ecosys* model for reduced N fertilizer rates. The field-applied N fertilizer rate for wheat production in our experimental site was 100 kg N ha^{-1}. Based on the field experiment results, we found that, in general, the different types of N fertilizers at this N application rate did not significantly affect grain yields or emissions in the Chernozemic soil enriched with available N. Therefore, in this modelling study, we further evaluated crop yield performance and soil N_2O emissions while using three reduced N fertilization rates (i.e., 25, 50, 75 kg N ha^{-1}), anticipating that N fertilizers may be utilized more efficiently in soils with gradually lower exogenous soil N. We found that N_2O emissions responded nonlinearly with changes in the application rate of N fertilizers (Fig. 4-7). On the basis of modelled emission and yield data, we estimated the yield-based N_2O EF as an indicator of environmental footprint (g N_2O-N kg^{-1} grain DM). These yield EFs suggested an optimum N rate of 25 kg N ha^{-1} to maintain grain yield in the short-term (at the same grain productivity as with the full N fertilization rate of 100 kg N ha^{-1}) while also reducing N2O emissions (Fig. 4-8). Overall, with both urea and AA, the magnitudes of N_2O emissions and grain yield increased with increasing N rate, while spring ESN showed also an increase in grain yield, but consistently minimizing N_2O emissions, indicating advantageous use of this EENF based on model results(Fig. 4-7).

4.4.6 Area and yield-based emission factors (EF) across N managements

EFarea and EFyield were calculated for the modelled and measured data for the study period 2015-2016 (Table 4-5). The highest modelled EFarea was observed in the fall N applied AA treatment (0.653 % kg N_2O-N kg^{-1} N fertilizer), while the lowest modelled EFarea was found in the spring N applied ESN treatment (-0.04 %). Similarly, the modelled EFyield ranged from 0.093 to 0.316 g N_2O-N kg^{-1} with the highest and lowest yield-based EF for fall N applied AA and spring N applied ESN, respectively (Table 4-5).

4.5 Discussion

4.5.1 Testing model performance

Our study is the first to report the performance of the *ecosys* model in modelling N_2O emissions from croplands receiving ESN, the effects of fall vs. spring application of inorganic N fertilizers, and also the prediction of the best rate of N fertilization. Earlier studies have successfully simulated N_2O emissions using *ecosys* (Grant et al., 2006; Grant et al., 2009; Metivier et al., 2009; Grant et al., 2016); however, we herein further constrained and ensured the reliability of model predictions and modelled outputs by testing with supplementary field measurements such as soil temperature, soil moisture, grain yield, and grain N uptake. In general, there was good agreement between measured and modelled data for most of the tested variables. Our study is consistent with previous studies. In our study, some discrepancies between measured and modelled soil moisture data in the year 2017 were observed. When the temporal variability of N_2O emissions was simulated from the fertilized agricultural soil, Metivier et al. (2009) reported a similar concern regarding the response of *ecosys* with respect to increases in soil moisture due to rainfall events. The greater sensitivity of the model to the small change in soil moisture in comparison to 5TM soil sensors may have caused the difference of soil VWC, and soil sensors cannot measure soil moisture accurately when soil is frozen. Similarly, Grant et al. (1999) and Metivier et al. (2009) reported model discrepancies with soil moisture readings from time-domain reflectometer probes in field experiments, specifically during snow thawing and major rainfall events. Another possible reason for the soil moisture discrepancies may be the hysteresis effect simulated in the model during wetting and drying, differing from the actual field conditions.

4.5.2 Temporal variability of N_2O emission

Fall N addition of urea or ammonium-based fertilizer increases the soil NO_3^- concentrations via nitrification. Relatively low measured and modelled N_2O emissions from these N-rich soils following the fall N applications may be due to limited microbial activity and N transformations in dry and cool soil conditions that are typical of the late fall in Central Alberta. With water freezing during the winter, soil pores are impeded by ice, leading to near-zero N_2O emissions due to null or minor gas transfer, minimal C oxidation, and limited biological activity.

The aforementioned delays (by several days) of N_2O peak emissions in the model output compared with the field measurements during the early spring suggest that the model takes more time to emit peak fluxes following soil thawing in comparison to field chamber measurements. Soil thawing creates water-saturated conditions and reduces the role of O_2 as an electron acceptor. In a soil environment that is rich in N and increasingly warm, NO_3^- becomes the next favourable electron acceptor (Metivier et al., 2009), and this accelerates N_2O production via denitrification. The emission of produced N_2O may have been suppressed by delayed thawing in the model simulation relative to the actual field conditions, therefore taking more time to lose water from soil pores and restore the pathway for gas transfer. Such delays in the peak N_2O emissions in the model are consistent with the experimental results of Grant and Pattey (2003) and Metivier et al. (2009). In addition to the difference in peak timing, larger N_2O emissions during the spring were modelled by *ecosys*. The greater inorganic N availability resulting from increased N mineralization due to shallow soil disturbance (e.g., from fertilizer banding or crop seeding operations) may have led to larger emissions modelled by *ecosys* compared to the field measurements. In sum, *ecosys* predicted strong N_2O fluxes following spring N fertilization, while the field chamber measurements registered the large fluxes as a result of major rainfall events. The field chamber measurement suggest that measured fluxes are particularly sensitive to moisture content fluctuations, whereas the modelled results seem to be even more responsive to N input and availability triggering asymmetrically increased emissions.

4.5.3 Effects of N fertilizer formulation and timing of application on N_2O emissions

The choice of the N fertilization source is one of the critical factors that determines the magnitude of N_2O emissions from croplands. Urea and AA are commonly used N fertilizers in Canada, collectively representing about three-fourths of fertilizer usage in the Canadian Prairies. Previous studies have shown that AA exhibits more N_2O emissions than urea (Bremner et al., 1981; Venterea et al., 2010). Venterea et al. (2010) compared the effects of AA and urea on N_2O emissions and found that higher NO_2^- levels in relatively low pH soils (the mean soil pH was 5.6 measured in 1 mol L^{-1} KCl extracts) promote nitrification-driven N_2O emissions with AA. Since the soils of our study site have a near-neutral pH of 6.2 (1:10 soil: water), the greater N_2O emissions from AA may not be related to the lower soil pH effect reported by Venterea et al. (2010). The AA can rapidly provide a readily available N as NH_4^+, urea upon application needs to be hydrolyzed by the urease enzyme, which converts urea into NH_4^+.

Many previous studies have determined the potential of ESN in reducing N_2O emissions. In a study of continuous corn in Illinois, greater N_2O emissions from AA (73%) and urea (44%) were reported compared to those from ESN (Fernández et al., 2015). Halvorson et al. (2010) found N_2O emission reductions by ESN in a no tillage system in comparison to the application of urea. Li et al. (2012 and 2016) reported that banded ESN in Alberta reduced N_2O emissions by 15 to 20% compared to urea. Even though many field studies have been conducted to determine the effectiveness of ESN in reducing N_2O emissions, to the best of our knowledge, our study is the first to simulate the effect of ESN addition on N_2O emissions, using the model *ecosys*. *Ecosys* was able to simulate emissions as initially hypothesized, and modelled emissions from ESN-treated fields remained similar to those from the control treatment, which is consistent with the measured data.

Reducing N_2O emission peaks associated with fall N addition remains a crucial management challenge (Parkin and Hatfield, 2010; Tenuta et al., 2016; Lin et al., 2017). The measured results from our field study suggest that the short-term weather and soil conditions strongly influence N_2O emissions irrespective of using fall vs. spring N application. Conversely, the N_2O emissions predicted by the model behaved according to our initial hypothesis that fall-applied N fertilizer leads to greater N_2O emissions occurring during the following spring thaw in comparison to the reduced emissions derived from spring N fertilization.

4.5.4 Optimum N fertilizer rates and their trade-off with environmental pollution

Both N_2O emissions and yield increased nonlinearly with increasing N fertilizer rates. Our results were consistent with previous studies (Ruser et al., 2001; Zebarth et al., 2008). When an ecosystem reaches the maximum N uptake capacity by the soil and plants, increases in N_2O emissions are observed. Thorburn et al. (2010) observed nonlinear increase in N_2O emissions with N fertilizer input for a sugarcane crop by using an APSIM model while a NZ-DNDC model was able to simulate the same nonlinear relationship for New Zealand grasslands (Saggar et al., 2007). Grant et al. (2006) modelled the effects of AA application rates on N_2O emissions using the *ecosys* model and also found a nonlinear rise in N_2O emissions with fertilizer application rates. Grant et al. (2006) concluded that N_2O emissions from the ecosystem are mostly determined by the current fertilizer inputs and the history of the fertilizer use. When soil residual N is low, N from current fertilizer inputs may be immobilized first and contribute little to N_2O emissions. When the residual soil N meets the immobilization capacity, additional N input can typically become available for N_2O production as an interactive function of plant N demand. The experimental site for our field study is highly fertile with soils containing high organic matter (>

10 %), likely amplifying greater soil residual N via mineralization of organic matter. Our model results indicated that upon fertilizer application, N_2O production might be further increased with excess N substrate, given that other driving factors (e.g., organic carbon) are also available. The direct influence of fertilizer application is the increase in the availability of N substrate for N_2O production via nitrification and denitrification. Increasing soil N due to fertilizer input can also narrow the soil C:N ratio which enhances the OM decomposition and creates hotspots of priming, leading to a further increase of mineral N availability and N_2O losses. This emphasizes the importance of conducting future research to evaluate priming effects on N_2O production due to single or recurrent N fertilizer additions. The identification of the optimum N fertilizer rate by accounting for past and current fertilizer use and priming effects can enhance nutrient use efficiency, minimize the fertilizer cost, and reduce N-related environmental pollution. However, additional field trials are required to further deepen these insights and recommendations.

4.5.5 Measured and modelled EFs in comparison to Tier I and Tier II IPCC EFs

National inventories of N_2O emissions are estimated as per the Intergovernmental Panel on Climate Change (IPCC) guidelines using various methodologies. Tier I methodology is currently used by 69% of the countries where limited emissions data are available, and it has a default value of 1% for all N inputs (Rochette et al., 2008; Metivier et al., 2009). Both measured and modelled EFarea in our study are much smaller than this default value implying the uncertainty and inaccuracy associated with the Tier I methodology most likely because of neglecting the soil, and weather factors, and accounting for only N input to estimate N_2O emissions in a linear mode. About 31% of the countries use Tier II approaches to determine country specific EFs when sufficient N_2O emission data is available. Tier II EF for Black soils in

Canadian Prairies is 0.33% (Rochette et al., 2008). The measured EFarea averaged across the selected fertilizer treatments in our study is 0.28 % (Table 4-5), which is very close to the EF value reported by Rochette et al. (2008). Furthermore, our modelled EFarea (average of 0.34%) is in an even closer agreement with the EF provided by Rochette et al. (2008) (Table 4-5). These findings support the notion that process-based mathematical models such as *ecosys* can capture long-term legacy effects of land management and simulate N_2O emissions based on complex biological, physical and chemical processes providing EFs data with higher certainty. Future work can undertake regional or national inventories of N_2O emissions using a modelling approach such as one that follows a Tier III IPPC methodology. Yet, certain differences in magnitudes between measured and modelled EF can be still noticed, and this uncertainty could be attributed to spatio-temporal variability of measured N_2O emissions as well as the lack of continuity of measured fluxes (with chamber measurements conducted only one or two times per week). Based on our study, it can be also acknowledged that the emission uncertainty seems to sharply reduce when hourly flux modelled results are integrated over larger temporal scales such as seasons or years.

4.6 Conclusion

Our modeling study showed that there was good agreement between measured and modelled results of soil temperature, soil moisture, grain yield and grain N uptake. N_2O emissions from both model and field measurements were numerically lower in ESN than conventional urea and AA fertilizers. Greater model responses were observed for N fertilizer additions, while fluxes from chamber measurements were more sensitive to soil moisture changes. This suggests that future work should undertake improvements in the model representation of soil moisture.

The model predicts a rate of 25 kg N ha^{-1} as the optimum N fertilization that can be applied to minimize N_2O emissions and sustain crop yields under highly fertile soils and environmental conditions similar to those in our study. Our modelled EFarea results closely agreed with the existing regional-specific EF, indicating that the process-based *ecosys* model is capable of providing EFs with high certainty and contributing to the national inventories of greenhouse gases.

When the *ecosys* is fed with soil, site, climate, plant and management data, the N_2O emissions simulated in our study can be scaled up to larger spatial scales (regional, continental and global) for use in more advanced IPCC methodology.

4.7 References

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4.8 Tables

Table 4-1. Description of treatments.

Treatment - N source	**N timing and placement**
Control	None
Anhydrous Ammonia	Fall Banded
Urea	Fall Banded
ESN (polymer coated urea)	Fall Banded
Anhydrous Ammonia	Spring Banded
Urea	Spring Banded
ESN (polymer coated urea)	Spring Banded

ESN: Environmental smart nitrogen

Table 4-2. Rates and dates of nitrogen fertilizer application and harvesting during the two experimental years.

Experimental year	Fertilization timing	Fertilization date	Date of harvesting
2015-2016	Fall banding	October 23, 2015	September 12, 2016
	Spring banding	May 10, 2016	
2016-2017	Fall banding	October 24, 2016	September 11, 2017
	Spring banding	May 23, 2017	

Table 4-3. Key soil properties of the Black Chernozem soil at the St. Albert Research Station used as input data in *ecosys*.

Soil depth (m)	**BD ($Mg\ m^{-3}$)**	**FC ($m^3\ m^{-3}$)**	**WP ($m^3\ m^{-3}$)**	**Sand ($g\ kg^{-1}$)**	**Silt ($g\ kg^{-1}$)**	**Clay ($g\ kg^{-1}$)**	**pH**	**TOC ($g\ kg^{-1}$)**	**TN ($g\ kg^{-1}$)**
0.01	1.25	0.33	0.15	86	494	420	6.2	45	3.9
0.03	1.25	0.33	0.15	86	494	420	6.2	45	3.9
0.07	1.25	0.33	0.15	86	494	420	6.2	45	3.9
0.13	1.25	0.33	0.15	86	494	420	6.2	45	3.9
0.17	1.25	0.33	0.15	86	494	420	6.2	45	3.9
0.23				83	326	591	7.88		
0.37				83	326	591	7.88		
0.60				38	237	725	8.08		
0.90				40	271	689	8.08		
1.30				40	271	689	8.08		

Abbreviations BD: bulk density, FC: field capacity, WP: wilting point, TOC: total organic carbon, TN: total N.

Values from laboratory measurements are shown in the table. When the measured values were not available, they were calculated in *ecosys*.

Table 4-4. Measured and modelled cumulative N_2O emissions (g N ha^{-1}) for the study year 1 Oct. 2015 to 30 Sept. 2016.

N Treatment	**Measured cumulative N_2O emissions (g N ha^{-1})**	**Modelled cumulative N_2O emissions (g N ha^{-1})**
Control	622	479
Fall N applied AA	746	1131
Spring N applied AA	1493	1122
Fall N applied Urea	770	987
Spring N applied Urea	1045	734
Fall N applied ESN	607	532
Spring N applied ESN	779	439

Table 4-5. Measured and modelled annual area-based N_2O emission factors (EFarea) (% kg N_2O-N kg^{-1} N fertilizer) and yield-based emission factors (EF yield) (g N_2O-N kg^{-1} grain DM) of wheat in the study year October 1,. 2015 to September 30, 2016.

N Treatment	**Measured EFarea %**	**Modelled EFarea %**	**Measured EF yield**	**Modelled EF yield**
Control			0.137	0.159
Fall N applied AA	0.123	0.653	0.161	0.316
Spring N applied AA	0.870	0.643	0.327	0.239
Fall N applied Urea	0.147	0.508	0.177	0.237
Spring N applied Urea	0.422	0.255	0.254	0.149
Fall N applied ESN	-0.016	0.053	0.133	0.173
Spring N applied ESN	0.157	-0.040	0.168	0.093
Average for all N treatments	0.28	0.34	0.19	0.19

4.9 Figures

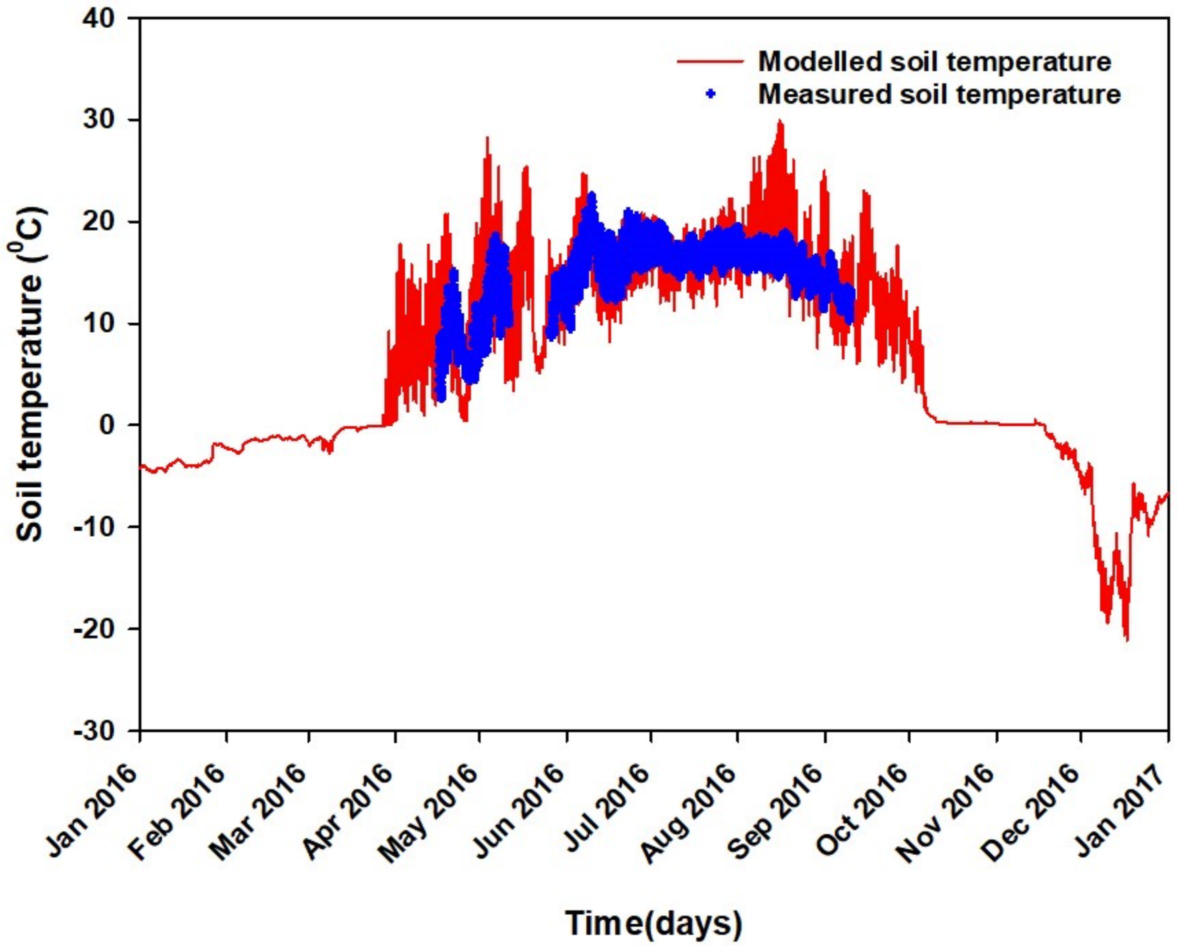

Figure 4-1. Modelled and measured hourly soil temperature at 10 cm soil depths over the year 2016

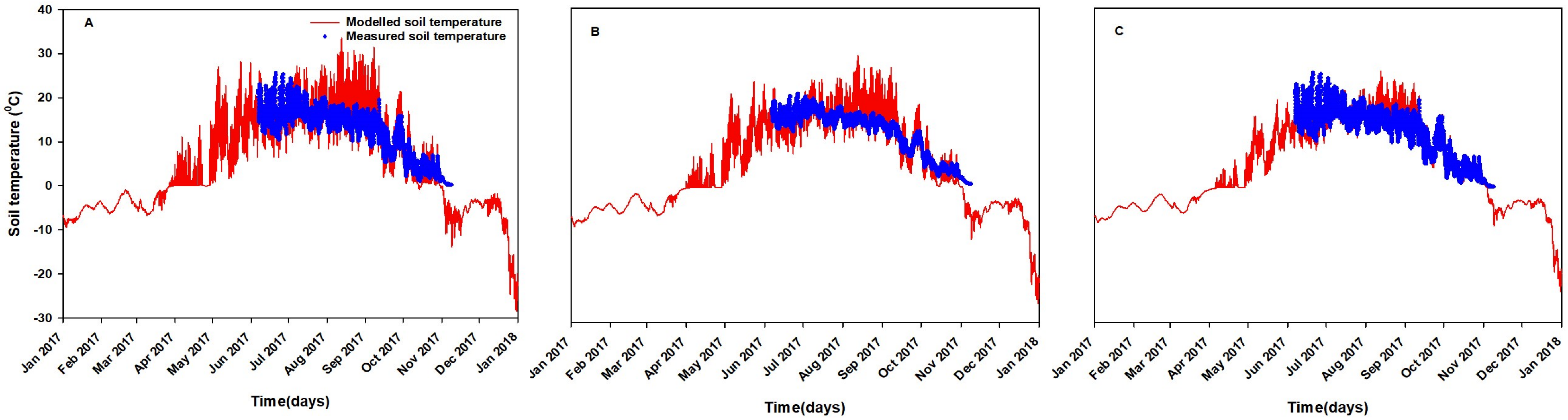

Figure 4-2. Modelled and measured hourly soil temperature at (A) 5 cm, (B) 10 cm and (C) 15 cm soil depths over the year 2017

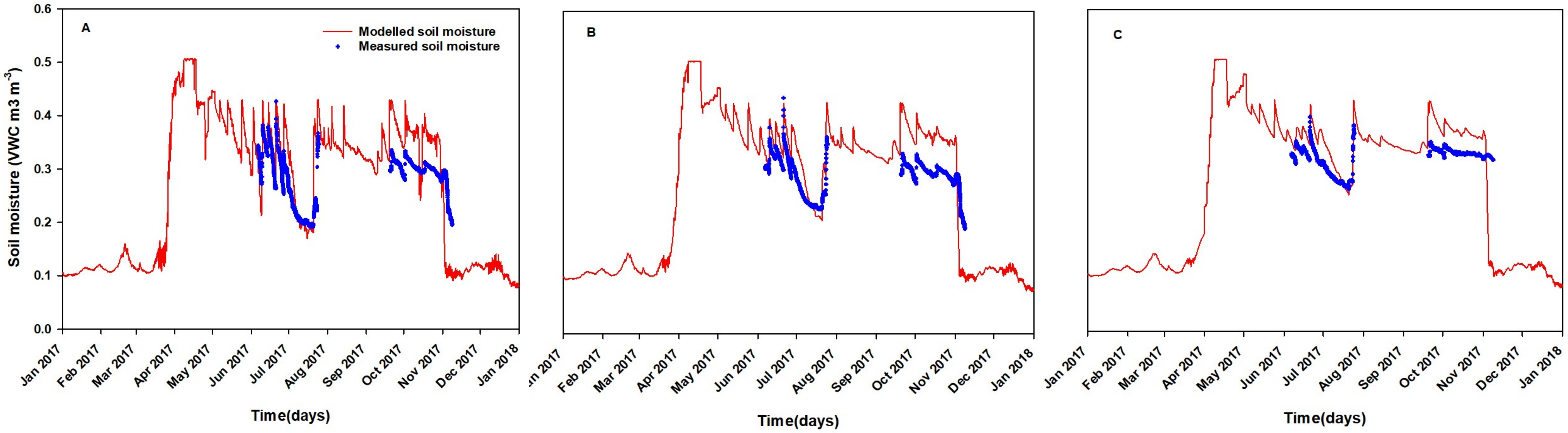

Figure 4-3. Modelled and measured hourly soil moisture at (A) 5 cm, (B) 10 cm and (C) 15 cm soil depths available over the year 2017

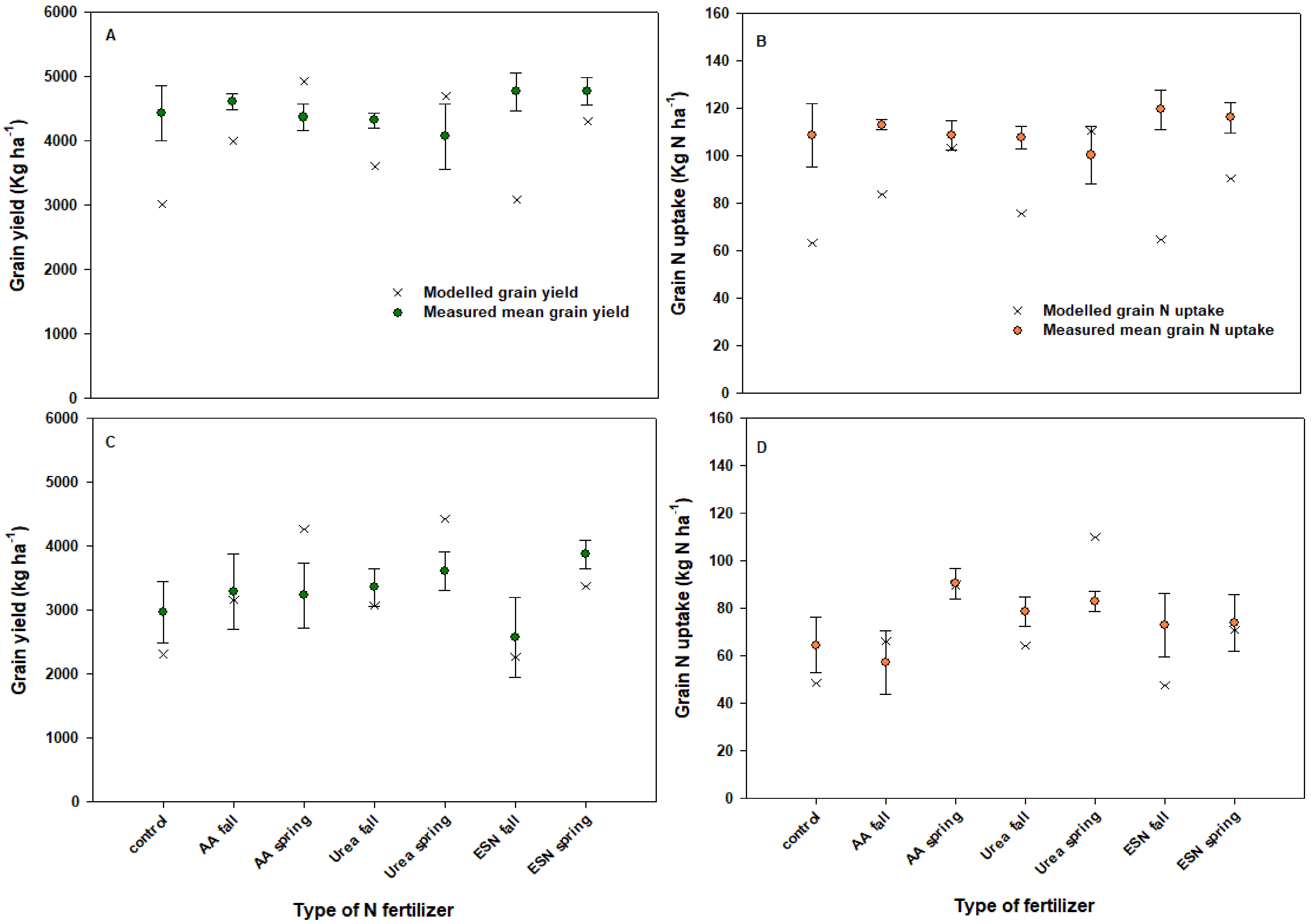

Figure 4-4. Modelled and measured mean grain yield for the study years 2016 (A) and 2017 (C), and grain N uptake for the study years 2016 (B) and 2017 (D).

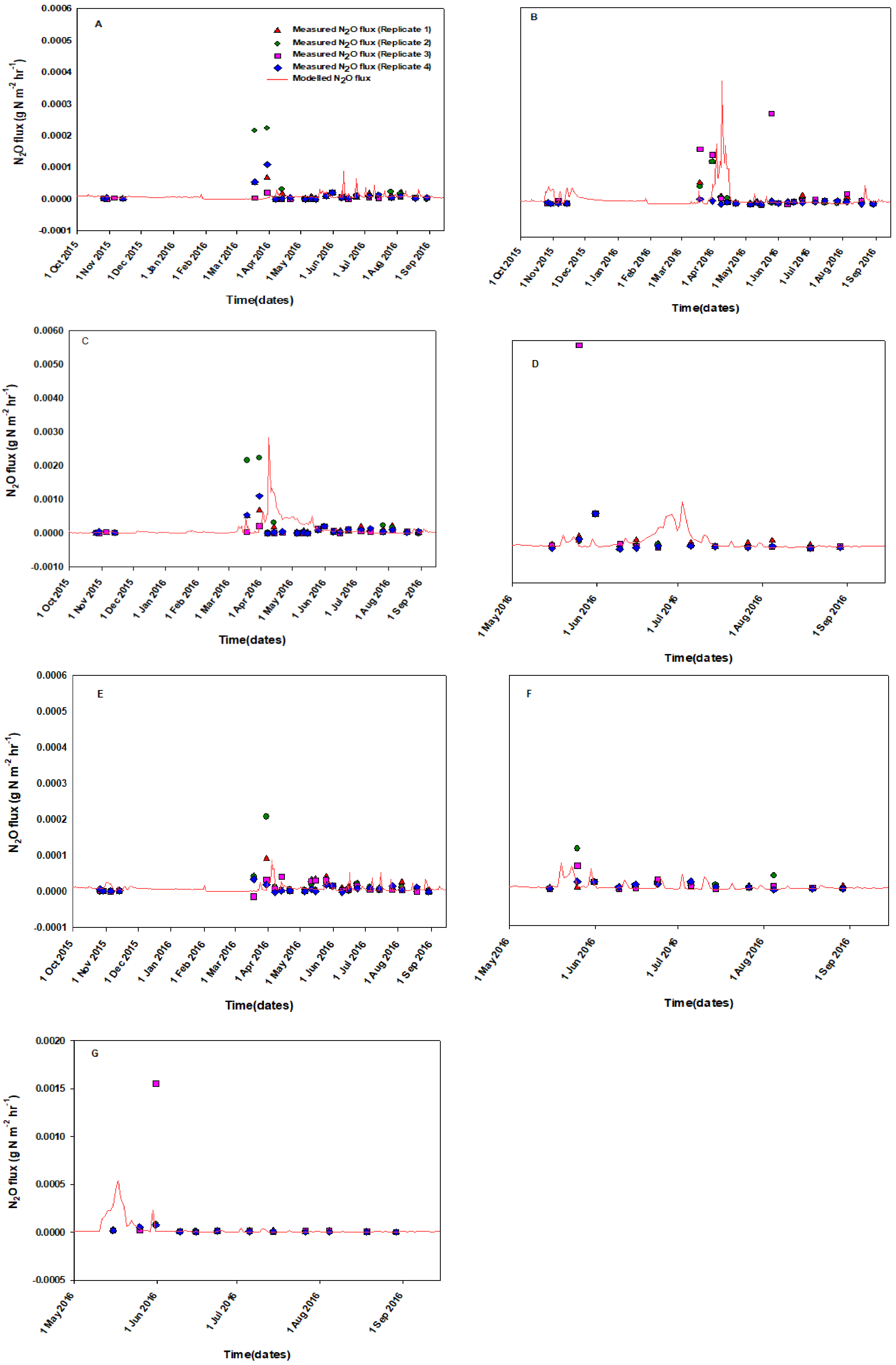

Figure 4-5. Modelled and measured N_2O emissions in A) control, B) fall AA, C) fall urea, D) spring urea, E) fall ESN, F) spring ESN G) spring AA for the study year 2015-2016. A, B, C D,E and F are plotted on the same y-axis scale and G is on a different y-axis scale.

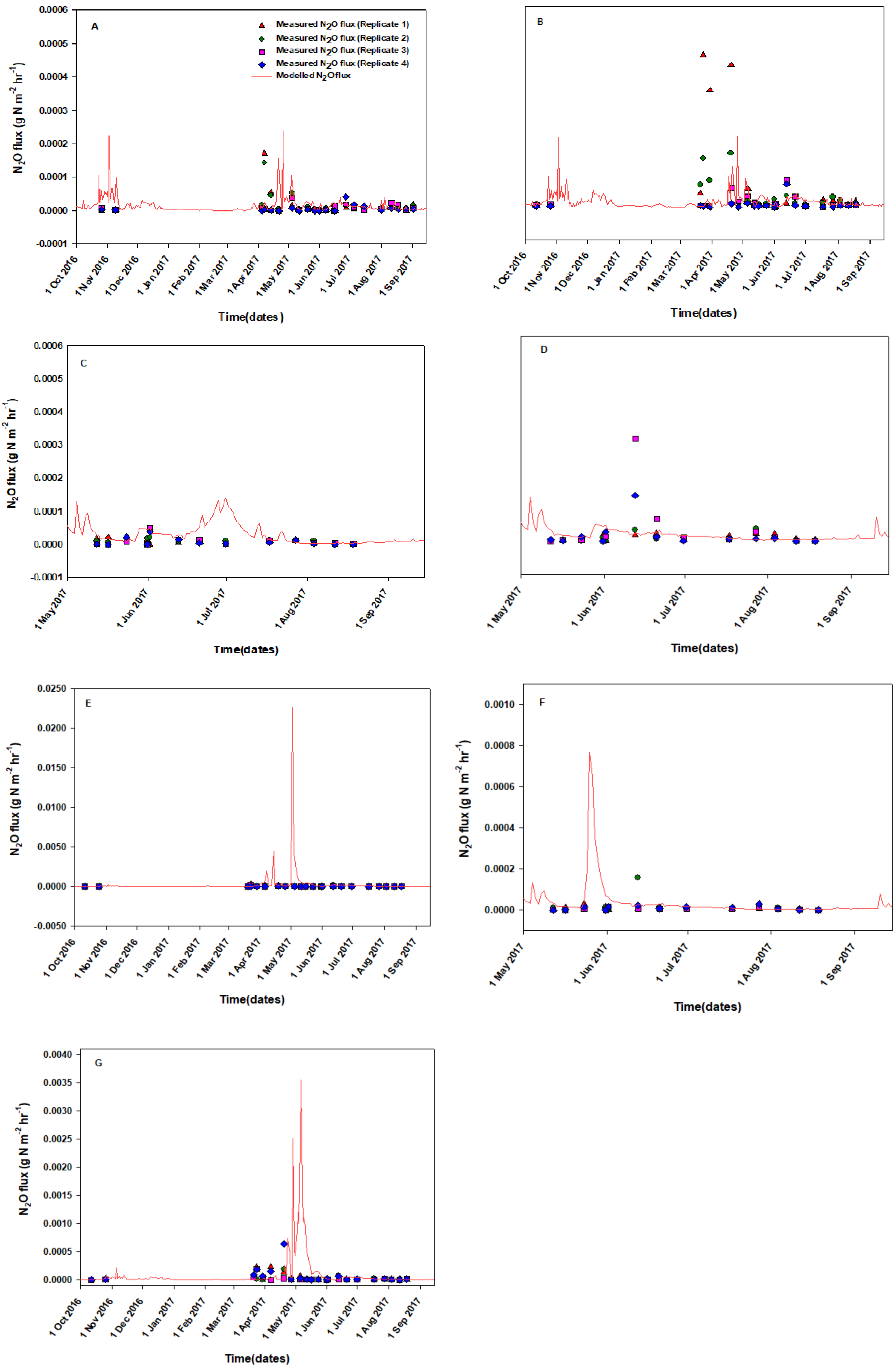

Figure 4-6. Modelled and measured N$_2$O emissions in (A) control, (B) fall ESN, (C) spring urea, (D) spring ESN, (E) fall AA, (F) spring AA and (G) fall urea for the study year 2016-2017. For better representation N$_2$O emissions of A, B, C and D are plotted on the same y-axis scale and E, F, and G are on a different y-axis scale.

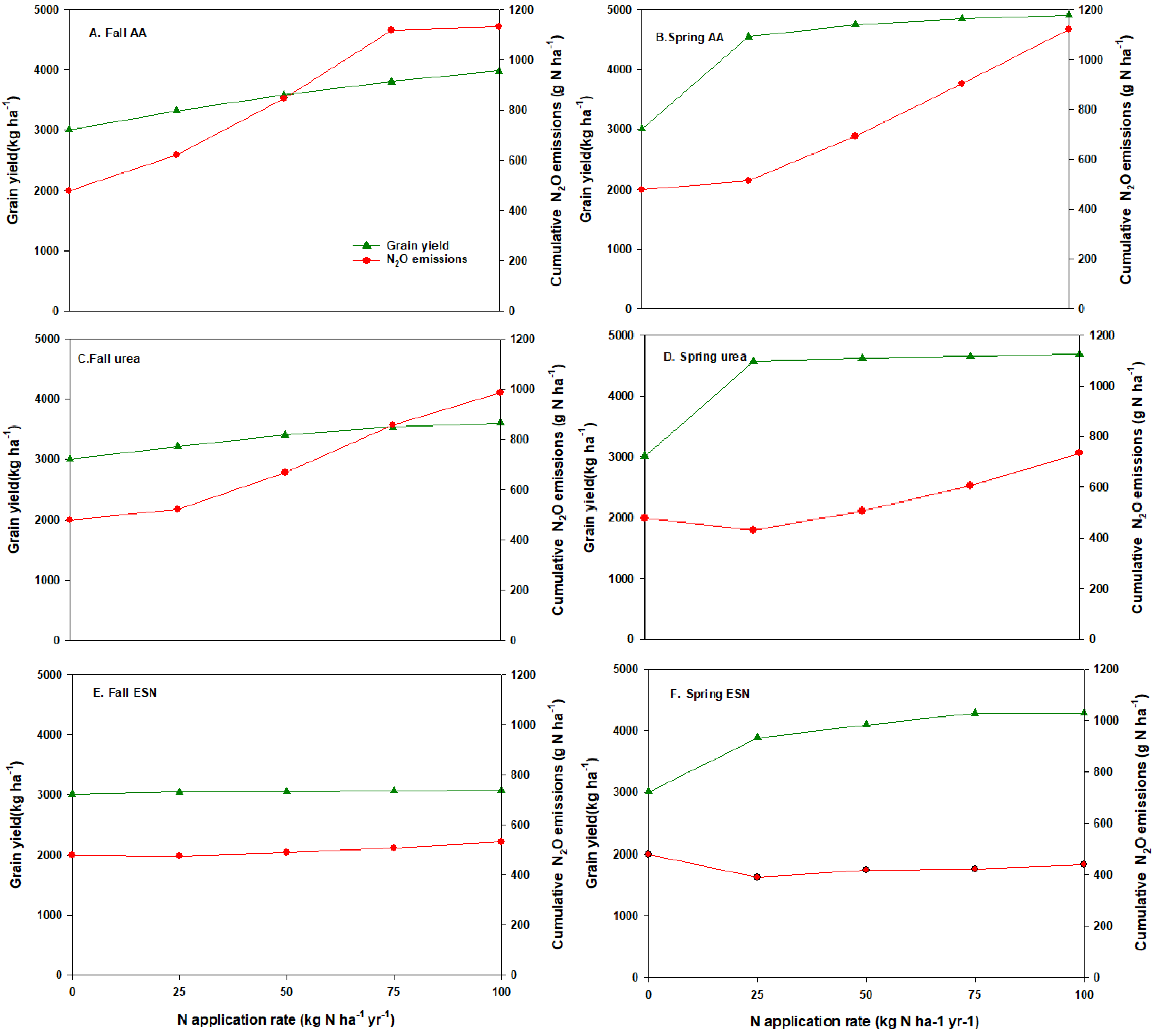

Figure 4-7. Predicted grain yield and N_2O emissions for (A) fall AA, (B) spring AA, (C) fall urea, (D) spring urea, (E) fall ESN, and (F) spring ESN with N fertilizer rates of 0, 25, 50, 75, and 100 for the year 2015-2016. Note that results for the N fertilization rates of 25, 50, and 75 were modelled and predicted with the aim of testing our reduced-N-rate modelling hypothesis through *ecosys* simulations. The model results for the N fertilization rate of 100 Kg N ha^{-1} yr^{-1} and the control with 0 Kg N ha^{-1} yr^{-1} were previously tested against our field experimental data. All the graph panels are plotted on the same y-scales.

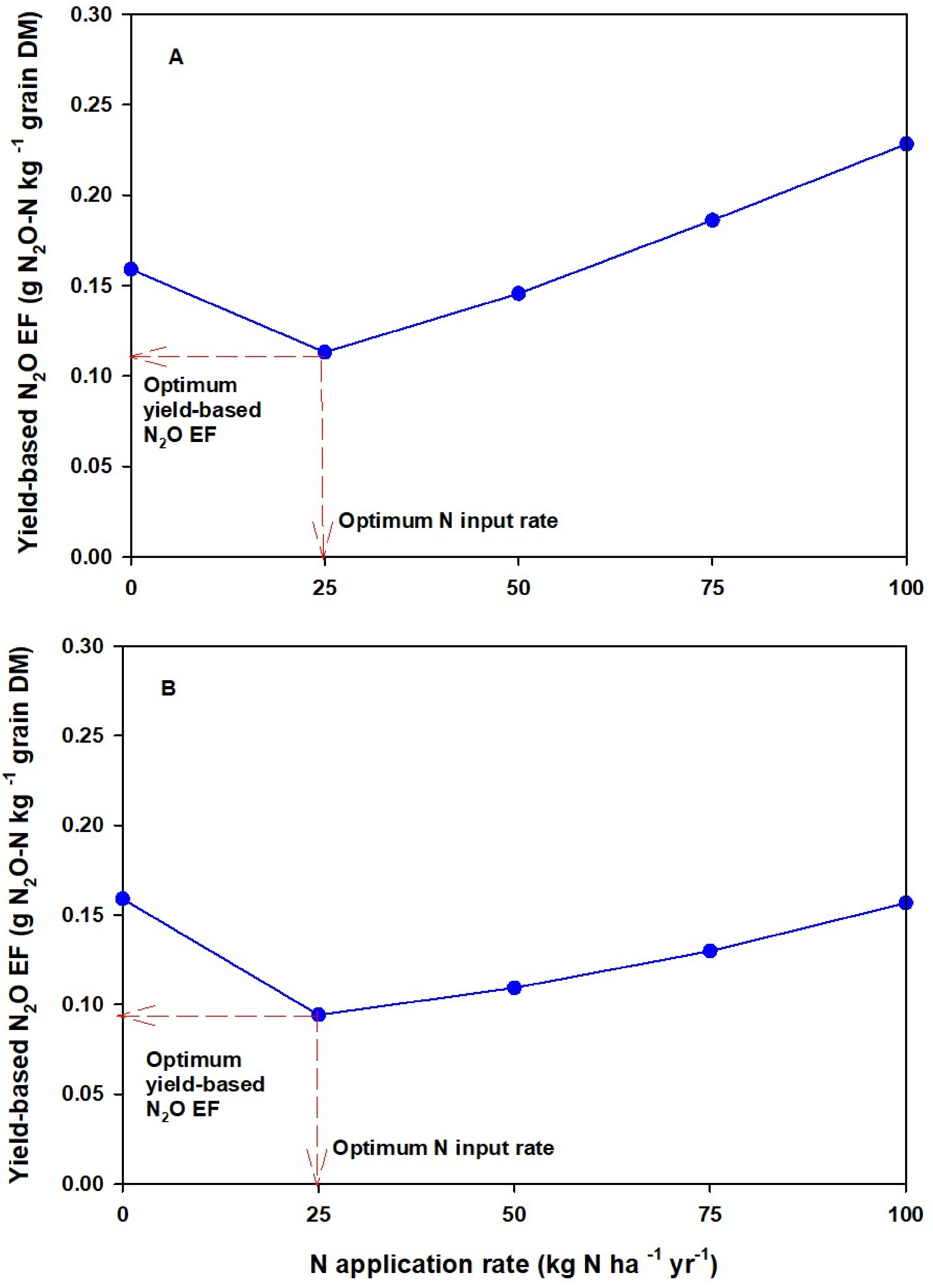

Figure 4-8. Predicted yield-based N_2O EF (g N_2O-N kg^{-1} grain DM) as a function of N fertilizer application rate (Kg N ha^{-1} yr^{-1}) for (A) spring AA and (B) spring urea for the year 2015-2016.

5 Agricultural Management History, Nitrogen Addition and Nitrification Inhibitors Effects on Soil N Priming and Nitrous Oxide Production.

5.1 Abstract

Long-term management of cropping systems and land-use changes can drive N_2O fluxes and sources. To examine this premise, soils were collected from four contrasting agricultural managements within the long-term Hendrigan experiment at Breton, Alberta. We assessed a continuous barley (CB) (*Hordeum vulgare*), a continuous fescue (CF) (*Festuca arundinacea*) forage, and two phases of a diversified eight-year rotation: a fababean (FB) (*Vicia faba L.*) and a hay phase. In a greenhouse replicated experiment, barley was grown as a test crop in these four soils which were treated with ^{15}N labeled urea only or admixed with nitrification inhibitors [i.e., nitrapyrin and 2-(N-3,4-dimethyl-1H-pyrazol-1-yl) succinic acid (DMPSA)]. Untreated controls were also evaluated. Measured ^{15}N-N_2O site preference (SP) identified denitrification as the major contributor (averaging 92%) to N_2O production. Relative to soils receiving only urea, N_2O emissions were beneficially reduced by adding nitrapyrin (16%) or DMPSA (25%) while plant biomass remained similar. Primed N_2O emissions derived from native soil N were triggered by the labile N addition (only urea), and the magnitude of this N_2O priming was strongly impacted by agricultural management histories. Upon measuring ^{15}N uptaken by plant canopies and the residual ^{15}N retained in the soils, the greatest estimated N losses and lower N recovery were associated with the long-term continuous barley. Conversely, the soils with hay management legacy had the greatest overall N_2O flux. Results suggest that the contents and availabilities of C and N in the soil, which were a reflection of the long-term management history, have critical influence on N_2O production by governing both denitrification and the priming effects.

5.2 Introduction

The global need to increase crop yields to meet the demand for food production while minimizing adverse effects on the environment is a crucial challenge. Agricultural soils contribute 60% of N_2O emissions to the atmosphere (Parry, et al., 2007). Agricultural management and cropping systems interact with and regulate biotic and abiotic factors within the agroecosystem, and consequently drive greenhouse gases fluxes (Abalos et al., 2016). The combined effect of utilization of water, nitrogen (N), carbon (C), oxygen availability and plant biomass allocation can impact N_2O emissions; however, this interactive role on N_2O production is just starting to be understood.

Perennial cropping systems tend to produce lower N_2O emissions compared to annual crops due to their greater water use efficiency and N retention capacity from a more extensive root system (Ross et al., 2008). Conversely, annual cropping systems typically have low water and soil N retention ability, leading to greater N losses to the environment (Ross et al., 2008). More specifically, perennial vegetation reduces the availability of N substrates and water for N_2O production by enhancing belowground biomass growth and at deeper layers as well as by utilizing nutrients and water in the early spring and late fall, effectively extending the active growing season in temperate, continental regions (Ferchaud et al., 2015; Abalos et al., 2016; Ferchaud et al., 2015).

Diversified crop rotation is a beneficial management practice that typically improves nutrient cycling as well as soil physical, chemical, and biological properties. Legumes are an important component in crop rotation due to their ability of biological N fixation (BNF). BNF can reduce the requirement of external N for the following crop, and hence, this could lead to reductions in N_2O production as compared with sole dependency on synthetic N fertilization

(Migliorati et al., 2015). Butterbach-Bahl et al. (2013) reported that N_2O serves as a substrate for BNF, and Hénault and Revellin (2011) also showed that N_2O can be consumed in legume nodules. Conversely, although these existing studies have associated legume growth with minimizing N_2O emissions, Zhong et al. (2009) stated that N_2O can be produced during BNF while increasing N substrate availability via rhizodeposition and the easy decomposition of legume residues, therefore leading to increasing net N_2O emissions.

Conversion of perennial cropping systems into annual croplands declines soil N and C stocks (Richter et al., 2007; Giweta et al., 2017; Hebb, et al., 2017; Kiani et al., 2017) and enhances N_2O emissions (Abraha et al., 2018). The influence of long-term management (e.g., crop rotation and fertilizer application) in cropping systems on N_2O emissions remains understudied. Thus, understanding the legacy effects of long-term cropping and nutrient management on N_2O production is needed to identify and develop land use systems that minimize N_2O emissions.

Within many management strategies that aim at enhancing plant N use efficiency while minimizing N_2O losses, the use of nitrification inhibitors such as nitrapyrin, dicyandiamide (DCD), and 3,4-dimethylpyrazole phosphate (DMPP) are receiving increasing attention (Huérfano et al., 2016). Nitrification inhibitors block the activity of ammonia monooxygenase (AMO) enzyme and delay oxidation of ammonium (Ruser and Schulz, 2015; Recio et al., 2019). Blocking the activity of AMO enables N from fertilizers to be kept in the NH4+ form, which is a more stable form of nitrogen when compared to the NO3- (Guardia et al., 2018). Previous studies have evaluated the reduction potential of N_2O emissions nitrification inhibitors (Huérfano et al., 2016; Guardia et al., 2017; Guardia et al., 2018). For example, Akiyama et al. (2010) addressed the efficiency of nitrification inhibitors in reducing N2O losses (i.e., with effectiveness at 30%

for DCD,, 50% for nitrapyrin and 50% for DMPP). Although many inhibitors have been widely studied, research on the performance of the newly reformulated 2-(N-3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (DMPSA) is scarce. An evaluation of the effectiveness of DMPSA in reducing N_2O emissions merits attention as it can lead to improvements compared with the earlier DMPP formulation.

A controlled greenhouse study was conducted to evaluate the effects of contrasting management histories of cropping systems (including annual crop, crop rotation and perennial forage) and N fertilizer addition with and without admixing nitrification inhibitors (i.e., nitrapyrin or DMPSA) on soil N cycling and N_2O emissions. In addition, we examined the fate of the added N fertilizer into the plant canopy N uptake, soil N retention, and N2O production. Moreover, we focused on partitioning the pools of N_2O sources (i.e., added fertilizer-N versus native soil N) as well as distinguishing the N_2O-producing processes (nitrification vs. denitrification). A further mechanistic understanding of the underlying soil N cycling was pursued by assessing the priming effects of labile N addition on the soil N-derived N_2O production across the contrasting agricultural management legacies.

5.3 Materials and Methods

5.3.1 Evaluated soils

The soils for this greenhouse study were collected from the long-term Hendrigan experimental site (1980 to present) located in Breton, Alberta (53°07′N, 114°28′W). The mean air temperature is 2.1°C and annual precipitation is 547 mm. Soils are Gray Luvisolic with moderate to poor drainage (Ross et al., 2008; Dyck et al., 2012). The Hendrigan experimental site consists of three cropping systems: 1) continuous barley (CB) (*Hordeum vulgare*), 2) continuous fescue (CF) (*Festuca arundinacea*) forage, and 3) an eight-year agro-ecological rotation. Fescue and white Dutch clover (*Trifolium repens*) are grown in the CF system for forage harvest purposes. Both CB and fescue are fertilized with N, P, K, and S; the N supply in the continuous fescue system is mainly based through N-fixing legumes. The rotation sequence of the agro-ecological Hendrigan experiment is barley-barley-fababeans (*Vicia faba*)-barley-barley/hay-hay-hay-hay; solid manure addition and biological fixation are the N sources for this agro-ecological rotation (Ross et al., 2008; Dyck et al., 2012). The mean rate of 46.6 kg N $ha^{-1}yr^{-1}$ manure N are applied to agro-ecological rotation. This rate of manure is calculated as 70% of the N removals in a given year (Ross et al., 2008). All the plots in agro-ecological rotation receive P, K and S at the rate of 22, 46, and 20 kg ha^{-1}, respectively. Hay plots are seeded with mixture of bromegrass (*Bromus inermis* Leyss) and alfalfa (*Medicago sativa* L.). Each of the eight rotation phases of this agro-ecological rotation are represented in every crop year. Barley is harvested for grain and straw is incorporated to soil. Hay plots are harvested as 2 cuts of hay per year. Whole plant biomass of FB was plough down from 1980 throughout 2000, and subsequently harvest as silage from 2001 to the present (Ross et al., 2008).

5.3.2 Greenhouse experiment

Soils were randomly collected from the Hendrigan experimental site in spring 2018 (early May) from 1) CB; 2) fescue, and two crop phases within the agroecological 8-year rotation; 3) fababean (FB) (*Vicia faba L.*); and (4) in the hay rotation phase just prior to a barley rotation phase. In addition, four N fertilizer treatments were established for each soil: 1) untreated control, 2) ^{15}N labeled urea (5 atom%), 3) ^{15}N urea with the nitrification inhibitor nitrapyrin (eNtrench™), and 4) ^{15}N urea with the nitrification inhibitor DMPSA. Each treatment protocol was replicated three times for flux measurements and one additional set for destructive, repeated measurements of soil ammonium and nitrate.

Soils were mixed; coarse fragments were removed by sieving with an eight mm mesh. Soil gravimetric water content was measured to account for moisture. The dry mass of soil per pots (1.867 kg) was determined using a pre-set bulk density (1.06 g cm^{-3}) consistently for all soils. Pots were cylindrical with a 13 cm height and a 15 cm inner diameter in polyvinyl chloride (PVC). Prior to initiating the experimental phase, soil columns were subjected to two wetting and drying cycles to precondition the soils.

The ^{15}N-labelled urea fertilizer was applied at a rate equivalent to 45 kg N ha^{-1}. Urea was banded to a soil depth of 3 cm and each pot received 53.01 mg N in powder form except the control treatment, which received no N. The nitrification inhibitors were admixed with urea prior to banding at rate of 12.39 mL kg^{-1} urea for eNtrench™ and 4.21 mL kg^{-1} urea for DMPSA. Phosphorous, potassium, and sulphur were applied according to the fertilization rates applied in the Hendrigan long-term site (Ross et al., 2008).

With the aim of determining the comparative effects of management history in the contrasting cropping systems on soil N cycling and N_2O emissions, barley was grown in all soils as a test crop. Here we were testing how barley and soil N dynamics responded to the legacy effects of the previous field season: a perennial grass-legume (continuous fescue), continuous barley for grain (CB), FB, or hay. Ten barley seeds were placed in a uniform circle near the edge of each pot. After germination of barley seeds, plants were thinned to four per pot. Plants were watered every day, and 44% WFPS was consistently maintained throughout the experiment. Pots were kept in the greenhouse under an average temperature of 26°C. Locations of the pots on the greenhouse bench were rotated within each replicate to minimize the microenvironmental effect.

The NH_4^+-N and NO_3^--N contents in destructive soil samples were measured colourimetrically using a SmartChem discrete wet chemistry analyzer (Westco Scientific Instruments, Inc., Brookfield, CT) (Maynard et al, 2008).

5.3.3 Soil N_2O emission measurements and flux calculations

The mixing ratios of ^{14}N-^{14}N-^{16}O, ^{14}N-^{15}N-^{16}O (α) and ^{15}N-^{14}N-^{16}O (β) were quantified in a continuous mode using direct absorption spectroscopy (wavenumber of 2188 cm^{-1}) with a thermoelectrically-cooled, mid-infrared quantum cascade laser (CWRT-QC laser). The 2 L analytical cell of the spectroscope, which used a 200-m pathlength, was under the 30 Torr vacuum. The temperature and sample flow rate in the instrumentation were 20°C and 2.5 standard L min^{-1}, respectively. Data was recorded at one Hz resolution. Data acquisition and the system were controlled by the TDL Wintel Software. The α and β isotopic ratios were calibrated similarly to Mohn et al. (2014) with primary gas standards A ($\delta^{15\alpha}N_2O$: 15.70‰, $\delta^{15\beta}N_2O$: -

3.21‰) and B ($\delta^{15\alpha}N_2O$: 5.55‰, $\delta^{15\beta}N_2O$: -12.9‰) at a concentration of 0.8 ppm. For routine calibration every 120 minutes, secondary gas standards included synthetic N_2O ($\delta^{15\alpha}N_2O$: -1.8‰, $\delta^{15\beta}N_2O$: 0.2‰) at concentrations of 0.5, 1.2, and 1.9 ppm; breathing air ($\delta^{15\alpha}N_2O$: 15.4‰, $\delta^{15\beta}N_2O$: -2.7‰, 342 ppb); and ultra-high purity dinitrogen for background absorption spectra subtraction. The analytical precision of $\delta^{15\alpha}N_2O$ and $\delta^{15\beta}N_2O$ at 1.2 ppm was 0.4‰ (standard deviation of thirty continuous measurements using an integration of five seconds).

The N_2O mixing ratios were measured in an Aerodyne system via flow-through recirculation and equipped with 12 automatic non-steady-state chambers (Eosense eosAC). Using the eosAnalyze-AC software each chamber ran for five minutes with a four-minute chamber closure and one minute of flushing. These N_2O measurements were performed on days 0 (1 hour after the addition of urea and inhibitors to the pots), 1, 2, 3, 4, 5, 6, 7, 8, 10, 13, 20, 22, 26, 29, 32, 36, 40, 43, 46, 50, 54, 56, and 64 of the study. Both air temperature (Onset HOBO® UX100 data logger) and pressure (Testo 511) were recorded during measurements.

The modified ideal gas law (Eq. [1]) was used to determine the flux.

$$N_2O\ Flux = \frac{S * P * V * 3600}{R * T * m} \qquad Eq. [1]$$

N_2O flux is the flux rate of N_2O (ng N_2O kg $^{-1}$ soil h^{-1}); S is the slope (regression coefficient) of the simple linear regression (µL L^{-1} sec^{-1}); P is the gas pressure (atm); V is the volume of the chamber headspace (L); m is the dry soil mass (kg); R is the gas constant (atm µL K^{-1} µmol^{-1}) and T is the temperature of the gas (K) (Yates et al., 2006).

The partition of source-pools was calculated to determine the priming of N_2O. Fraction N_2O_{urea} and fraction N_2O_{soil} were calculated using a mass balance with two end members. The addition of these fractions is equal to 1 (*Eq*. [2]).

$$Fraction\ N_2O_{urea} + Fraction\ N_2O_{soil} = 1 \qquad Eq.[2]$$

$$atom\%^{15}N_{urea} \times\ Fraction\ N_2O_{urea} +\ atom\%^{15}N_{soil} \times\ Fraction\ N_2O_{soil} =\ atom\%^{15}N_2O_{urea\ treatment} \qquad Eq.[3]$$

$atom\%^{15}N_{urea}$ is 5 atom%; $atom\%^{15}N_{soil}$ is the measured atom% ^{15}N in the N_2O emitted from soils receiving zero N addition; $atom\%\ ^{15}N_2O_{urea\ treatment}$ is the atom % in the N_2O emitted from the labelled urea treatment. The $atom\%\ ^{15}N_2O_{urea\ treatment}$ and $atom\%^{15}N_{soil}$ in (*Eq.3*) equations were determined as the Keeling plot intercepts.

The N_2O derived from urea and soil N were calculated via multiplication of N_2O flux of urea treatment by fractions of urea and soil, respectively *Eq*. [4] and *Eq*. [5].

$$N_2O_{urea} = Fraction\ N_2O_{urea} \times N_2O_{urea\ treatment} \qquad Eq.[4]$$

$$N_2O_{soil\ N} = Fraction\ N_2O_{soil} \times N_2O_{urea\ treatment} \qquad Eq.[5]$$

The subtraction of N_2O flux of unfertilized control treatment from the N_2O flux coming from soil N in the urea-only treatment resulted in the priming of N_2O due to the N source addition.

$$N_2O_{priming} = N_2O_{soil\ at\ urea\ treatment} - N_2O_{control} \qquad Eq.[6]$$

Generally, the ^{15}N-N_2O site preference (SP) values for N_2O derived during nitrification and denitrification are found to be between 27 to 33‰ and -13 to 0‰, respectively (Sutka et al., 2003; Sutka et al., 2006; Frame and Casciotti, 2010; Decock and Six., 2013). The values used in this study as SP isotopic signatures (end members) were 33‰ for nitrification and 0‰ for denitrification, which enabled estimating the contributions of N_2O-producing processes as well as the priming occurring in such processes.

$$\delta\,15N\alpha - \delta 15N\beta = Site\ Preference\ (SP) \qquad Eq.[7]$$

$$N_2O_{denitrification} = (33 - SP_{treatment}) * 100 \qquad Eq.[8]$$

$$N_2O_{nitrification} = 100 - N_2O_{denitrification} \qquad Eq.[9]$$

$$N_2O_{priming_{denitrification}} = N_2O_{denitrification_{soil} at\ urea\ treatment} - N_2O_{denitrification_{control}} \qquad Eq.[10]$$

$$N_2O_{priming_{nitrification}} = N_2O_{nitrification_{soil} at\ urea\ treatment} - N_2O_{nitrification_{control}} \qquad Eq.[11]$$

5.3.4 Plant sampling and analyses

At the end of the experiment, the barley plants of each treatment were cut close to the soil surface and oven-dried at 70°C to determine dry matter of the harvested aboveground biomass. The C and N concentrations and $\delta^{15}N$ isotopic composition in soil and plant samples were measured using a Costech 4010 Elemental Analyzer following the dry combustion method. The resulting N_2 gas was run through a Thermo Finnigan Delta Advantage isotopic ratio mass spectrometer (ThermoFisher Scientific, Waltham Massachusetts, USA), which detects the ratio of ^{15}N:^{14}N.

5.3.5 Statistical analysis

A two-way analysis of variance (ANOVA) was used to analyze the effect of management legacy, N fertilizer, and their interaction on N_2O, NH_4^+, NO_3^-, aboveground plant dry matter, plant N uptake and soil N retention. Tukey’s Honest Significant Difference test was subsequently performed for treatment comparisons. Normal distribution and homogeneous variance of data were examined using the Shapiro-Wilk and Bartlett tests, respectively and data was Box-Cox transformed when needed to meet the assumptions. All statistical analyses were produced using SigmaPlot Software (version 13.0) with an alpha critical level of 0.05.

5.4 Results

5.4.1 N_2O emissions

All treatment combinations showed increased N_2O emissions at the onset of the study period (Fig. 5-1) with the peak N_2O emissions taking place on day one. During this initial peak time, of the soils receiving urea only, the greatest daily N_2O fluxes occurred in the hay soil, while the lowest emissions were found from the fescue soil (13.28 and 0.29 μg N_2O-N kg^{-1} soil hr^{-1}, respectively). In fact, most of N_2O emissions occurred within the first five days of the study when noticeably divergent trends could be ascertained across soils: hay > FB > CB >fescue. Thereafter, emissions remained low and relatively stable throughout the rest of the experiment although smaller emission peaks (up to 3 μg N_2O-N kg^{-1} soil hr^{-1}) were observed mostly from the hay soils irrespective of N fertilization options, and also some minor peaks could be noted from the FB soil treated with urea only.

Both soil management history ($p < 0.001$) and N addition ($p < 0.006$) had significant effects on the cumulative N_2O emissions (Fig. 5-2A and 5-2B). Mean cumulative N_2O emissions from hay soils were significantly greater (2083 μg N_2O-N kg^{-1} soil) than all of the other three soils. Cumulative N_2O emissions from hay soils were approximately nine fold greater than those from the fescue soil (225 μg N_2O-N kg^{-1} soil), which had the lowest N_2O emissions. A Tukey test showed that cumulative N_2O significantly differed across four soil management legacies (fescue < CB < FB < hay) (Fig. 5-2A). A mean comparison between N sources revealed that soils amended only with urea emitted the greatest N_2O emissions, and emissions from these urea-amended soils were statistically greater than the control treatment (Fig. 5-2B). As expected, soils that received inhibitors with urea in particular DMPSA had reduced N_2O emissions in comparison to soils amended with urea without inhibitors, and the statistical difference between urea and urea+DMPSA was marginally significant (p= 0.054).

5.4.2 Plant aboveground biomass, N uptake, and ^{15}N recovery

Plant N uptake was significantly different across all agricultural management legacies ($p < 0.001$) with an increasing order as follows: CB < fescue < FB < hay (Fig. 5-2E). On the other hand, focusing on the effect across N addition treatments, plant N uptake across the three urea treatments (urea, urea + nitrapyrin, and urea + DMPSA) was consistent (averaging 68 mg N per experimental pot), and this N uptake was significantly greater than in the unfertilized control (49 mg N) ($p < 0.001$) (Fig. 5-2F). To determine the sources of plant N uptake, the N coming from added urea and native soil in the fertilized barley plants were allotted using a ^{15}N mass balance of two end members (urea-N and soil N). Plants grown in the hay and FB soils took up approximately four and three times more N from the soil than from added urea, respectively (Fig. 5-3). The partition of plant N uptake from both urea and soil N was affected by the management legacy ($p < 0.001$). Plants grown in CB soils had the lowest N uptake from urea, while the greatest urea-N uptake was found in hay soils. Barley aboveground biomass yield was proportional to plant N uptake across management legacies (CB < fescue < FB < hay) (Fig. 5-2C). This study hypothesized that greater plant yield would be caused by adding N fertilizer with inhibitor because of an enhanced synchrony of added N and plant demand would minimize N losses. Even though nitrification inhibitors did not cause a statistically significant effect on plant biomass productivity, combining urea with nitrapyrin resulted in numerically higher aboveground biomass (1.69 g per experimental pot) in comparison to the untreated urea, while the DMPSA produced a similar plant biomass as untreated urea (1.58 g) (Fig. 5-2D).

We also quantified plant N recoveries, soil N retention and estimated N losses across nitrification inhibitors and management legacies. In the fescue, hay and FB soils, the amounts and proportions of urea-N uptaken by barley plants were consistent (ranging from 33 to 40%); however, the recovery of urea-N in barley plants grown in the CB soil was significantly lower (21%) (Fig. 5-4). Furthermore, at the end of the study period, the amount of urea-N retained in the hay and CB soils (4% and 6%, respectively) was significantly lower than the soil N retention in the fescue and FB soils (35% and 51%, respectively). When accounting for soil N retention, N recovery by barley plants, and measured N_2O emissions, the proportion of applied ^{15}N-labelled fertilizer that was not recovered ranged from 16 to 72% (assumed to be unaccounted-for gaseous losses) (Fig. 5-4).

5.4.3 ^{15}N-N_2O site preference (SP), underlying N_2O production processes, and priming by source-pools

The SP values in this study widely ranged from -3.75‰ to 32.75‰ with an overall average of 2.62‰ (SP data calculated with Eq. [7]). Averaging over the entire study period, the mean SP for the four management histories were 5.14 ± 0.39‰ for the fescue soil, 0.83 ± 0.09‰ for hay soil, 2.77 ± 0.23‰ for CB and 1.88 ± 0.16‰ for FB, respectively. A visual examination of the measured SP against the corresponding daily N_2O fluxes showed that the majority of SP data were close to zero resulting also in the greatest N_2O fluxes, while the smaller fluxes resulted in a wider spread in the SP data range (Fig. 5-5).

Further analyses of SP data (Eqs. [8] and [9]) enabled distinguishing the relative contributions of N_2O production during nitrification or denitrification (Fig. 5-6). Irrespective of agricultural management history and N fertilizer treatment, denitrification was the major contributor to N_2O production (overall average of 92% denitrification). In comparison to the other three agricultural management histories, the fescue soil generated the greatest contribution to N_2O production from nitrification (16%), whereas the hay soil led to the lowest contribution of nitrification N_2O production (3%) (Fig. 5-6A). On the other hand, across all four N treatments (control, urea, urea+nitrapyrin, and urea+DMPSA), the average N_2O contribution from denitrification (92%) and nitrification (8%) was very consistent (Fig. 5-6B).

Priming effects on soil-derived N_2O production for urea, urea+nitrapyrin, and urea+DMPSA treatments were observed shortly after the N fertilizer application in the beginning of the experiment. Positive priming peaks took place on study days 2, 3 or 4 (Fig. 5-7 and Fig. 5-1) (priming calculations were based on source-pool partitioning of urea-N vs. pre-existing soil N using Eqs. [4], [5] and [6]). During peak emission of priming, the highest positive priming effect was observed in the soil under the hay crop rotation phase, while the lowest primed N_2O emissions occurred in the fescue soil (Fig. 5-7). After the positive priming peaks, hay soil switched by frequently showing negative priming episodes, while the other soils remained mostly close to null priming.

To further examine the priming effects on N_2O production as affected by N addition and soil history, both the cumulative primed N_2O emissions and the cumulative primed N_2O emissions over the cumulative total N_2O emissions (denoted as % priming) were calculated. Even though cumulative primed N_2O emissions did not statistically differ across the four soil histories, % priming showed to be marginally significant ($p= 0.051$). It is noteworthy that 24% of N_2O emitted from the CB soil was derived from the priming of native soil N, corresponding to a cumulative primed flux of 153 μg N_2O-N kg^{-1} soil. By contrast to this highest % priming by CB, the lowest priming was observed in hay soils with -9% (equivalent to a negative primed N_2O emission of -145 μg N_2O-N kg^{-1} soil). Moreover, across the three N formulation additions, urea admixed with inhibitors reduced the magnitudes of primed N_2O emissions in comparison to the untreated urea treatment (urea > urea+nitrapyrin > urea+DMPSA; $p > 0.05$). With urea only, the largest priming magnitude of 189 μg N_2O-N kg^{-1} translated into 22% of the total N_2O being produced from priming of the native soil N. And this priming due to addition of only urea was much larger than with urea+DMPSA which only generated 14 μg N_2O-N kg^{-1} as priming – equivalent to a 5% of its total N_2O flux.

5.5 Discussion

5.5.1 N_2O emissions reduction by nitrification inhibitors

The reduction potential of N_2O emissions by a range of nitrification inhibitors has been researched under different climatic and management conditions (Schwenke and McPherson, 2018). Still little is known about the N_2O reduction potential of the newly re-formulated nitrification inhibitor DMPSA. Throughout this study, differential effects of DMPSA on N_2O losses in comparison to nitrapyrin and untreated urea were observed. The results support that the adoption of nitrification inhibitors, such as nitrapyrin and DMPSA, are a promising mitigation strategy to minimize N_2O emissions from Orthic Gray Luvisols. As initially postulated, the addition of nitrification inhibitors decreased the N_2O emissions (Fig. 5-2B). The DMPSA inhibitor reduced 25% of the N_2O emissions, while the reduction potential of N_2O emissions from the nitrapyrin inhibitor was at a magnitude of 16%.

Nitrification inhibition can be achieved by different mechanisms. According to Ruser and Schulz (2015), there are three main mechanisms to inhibit the first enzymatic (enzyme ammonia monooxygenase (AMO)) step of nitrification: 1) direct binding of inhibitors to the active site of the enzyme; 2) chelation of copper, which is a co-factor that AMO is bound to in the membrane of microorganisms; and 3) inactivation of the enzyme via covalent modification of proteins in microorganisms. Amongst these three mechanisms, it is assumed that both nitrapyrin and DMPSA inhibit NH_4^+ oxidation via removal of cofactors (the second mechanism). Although these two inhibitors shared their active principle as a commonality, numerical differences (non-statistical) in N_2O emission reduction between them were observed in our study. The chemical formulation of DMPSA may have contributed to a result of enhanced effectiveness. The presence of the succinyl group makes DMPSA less volatile and prolongs availability and effectiveness, even at low concentrations (Guardia et al., 2017).

5.5.2 Impacts of management legacy on N_2O emissions

This study highlights that greater N_2O emissions can be driven by higher C and N availabilities, which jointly increase the activity of denitrifiers and, consequently, that denitrification rates. It is noted that among the assessed cropping systems, hay soils had accumulated more soil TOC and TN (Table 5-1). Addition of manure may have increased soil C and N levels in agro-ecological rotational plots. Greater N_2O emissions from hay soils indicated that naturally present organic matter can be readily mineralized and cycled into available C and N to provide substrates for N_2O production (Abraha et al., 2018), and oxidation of soil organic carbon also enhances the demand for electron acceptors. Since all four soils were fertilized at the same urea-N rate, our study supports that differences in existing soil C concentration and availability can play a critical role in N_2O production. In addition to higher soil organic C, hay soils initially had elevated inorganic N contents indicating substrate availability for N_2O production other than the fertilizer addition.

Cumulative N_2O emissions from FB soils were roughly fourfold greater than those from the fescue soil (Fig. 5-2A). The high N_2O fluxes from FB soil were likely due to the addition of soil N through manure addition, biological fixation and the increment of N substrate availability via rhizodeposition and the decomposition of recent FB plant residues (Xiong et al., 2002; Zhong et al., 2009). Additionally, the fact that fescue soil had the lowest cumulative N_2O emissions could be attributed in part to an intense N immobilization (Davidson et al., 1996) potential of the fescue soils as well as the N removal through biomass harvest.

5.5.3 Fate of added-N fertilizer: plant uptake, soil retention, and losses of N

We had initially hypothesized that plants grown in soils with a long-term history of annual cropping (CB) would rely more on added N than on existing native soil N to meet plant N demand. Contrary to this hypothesis, a lower reliance on fertilizer-N was observed in CB soils, as two times more N was taken up from the pre-existing soil N (24 mg N per experimental pot) than from recently added urea (11 mg N). These perplexing results can be attributed to the rapid production of readily available N in the CB soil (due to the mineralization of organic matter and decomposition of barley residues) and explained also as a consequence of the microbial adaptation, which has been stimulated by the long-term history of fertilization that consisted of annually recurring N additions over 38 years (90 kg N ha^{-1} year^{-1}). Moreover, CB soils showed the lowest aboveground biomass production, crop N recovery (21%), and soil N retention (6%) as well as the highest N losses (72%). A long-term continuous application of inorganic fertilizer (e.g., over nearly four decades) and continuous soil disturbances like tillage can cause detrimental changes in chemical, physical, and biological properties of the soil and consequently diminishes soil quality and the productive capacity of soil (Belay et al., 2002). Thus, the CB soils have little inherent fertility and can rapidly lose added and native soil N (Dalal and Mayer.; 1987; Migliorati et al., 2014) in comparison to the other three soils in our study. A low N fertility status in CB soils is further confirmed by the relatively low total N content (Table 5-1) and reduced initial mineral N during the study (Tables 5-2 and 5-3). Supporting this postulate, in our FB soils that had experienced a diversified rotation including a recent legume crop (FB), barley plants grown in the greenhouse were able to uptake much more N from the pre-existing soil N pool (60 mg per experimental pot) than from recently added N fertilizer (18 mg). In addition to the N contributions from manure addition, atmospheric N_2 fixation, the potential of legumes to enhance soil N availability (Rochester et al., 2001; Migliorati et al., 2014) via supplying easily decomposable plant residues further contributes to a greater reliance on the native soil N pool. Hay soils had high ammonium and nitrate at various stages of the study period indicating that the greater mineral N availability contributes higher plant N uptake and the N losses as well. The greatest organic C availability of hay soils have contributed to the highest denitrification to enhance N_2O emissions and N_2 losses. Since fescue soils does not typically receive high amount of inorganic fertilizers, with N fertilizer addition soil N in fescue soils can be immobilized short term which is indicated by the lower amounts of mineral N. These immobilized mineral N can be available later increasing plant N uptake and less losses due to better soil structure with the perennial root growth.

The overall mean plant recovery of added ^{15}N labelled fertilizer was 33 ± 1.6 % (Fig. 5-4). To the best of our knowledge, no previous studies have reported barley N recovery as a combined function of widely contrasting agricultural management histories and nitrification inhibitor additions. In this study, mean crop N recovery under four management histories ranged from 21% for the CB soil to 38% for hay soil. Plant recovery of added N can be influenced by inherent soil properties such as cation exchange capacity, availability of native soil N, moisture availability and environmental conditions such as temperature and rainfall (Dourado-Neto et al., 2010).

The unaccounted N losses were relatively high (with an estimated mean of 43%; Fig. 5-4) in this study compared to earlier field studies. Guardia et al. (2018) reported that unaccounted N losses of irrigated maize fields ranged from 10 to 17%. Due to less leaching and denitrification in Oxisols, low N losses were also found in a field study by Migliorati et al. (2014). In our controlled experiment, N losses via NO_3^- leaching were assumed as null as PVC pots were bottom sealed. Given the ideal biophysical conditions in our study (constantly optimal moisture and heat availabilities), we inferred that the urea-N likely had been lost through NH_3 volatilization and complete denitrification to N_2 (Pan et al., 2016), hence making up for the considerable amount of unaccounted N (assumed as estimated N losses). Even though the proportion of N lost as N_2O production (mean value of 0.5%) was a relatively small part of the estimated total N loss to the atmosphere, mitigation strategies should be implemented as such small increases in N_2O emissions have major impacts on environment quality and climate change.

Notably, no differences were observed among N fertilization management on plant N uptake, soil N retention, plant fertilizer-N recovery, and estimated N losses. Nevertheless, N fertilizer application induced an increased aboveground biomass of barley (Fig. 5-2D). Between the two nitrification inhibitors, DMPSA was highly effective in reducing N_2O emissions compared to similar emissions as the unfertilized control (Fig. 5-2B) as well as maintaining plant biomass productivity similar to the urea addition without an inhibitor, while nitrapyrin played a beneficial role mostly in consistently sustaining plant biomass productivity (Fig. 5-2D). These findings are comparable to results by Huérfano et al. (2016) and Guardia et al. (2018). As our controlled study represents ideal conditions for soil N_2O production, both a greater N_2O mitigation efficiency and sustained plant yield can support the DMPSA inhibitor as an environmentally sustainable N fertilization strategy.

5.5.4 N_2O source-partitioning and priming effects of N addition

We used measured ^{15}N-N_2O site preference (SP) data to interpret and partition the N_2O production mechanisms as nitrification and denitrification (Yoshida and Toyoda, 2000). The SP results for the larger N_2O fluxes in the study were consistently close to zero (~0 ‰) indicating that most of the N_2O emissions were derived from denitrification (Fig. 5-5). The presence of plants may have enhanced denitrification across all treatment combinations, creating anoxic conditions due to high microbial activity in the rhizosphere, consumption of oxygen by root respiration, and the provision of root exudates as C substrate. Comparing the four soils with contrasting agricultural management legacies, N_2O fluxes produced from hay and FB soils were nearly all attributed to the denitrification process (99.8%). The underlying driver for this pronounced N_2O source-partitioning towards denitrification could be the soil organic C content and availability. A supply of organic C compounds (electron donor) and also NO_3^- (electron acceptor) is needed for the occurrence of denitrification. The activity of denitrifiers is governed by the availability of C substrate, as denitrifiers oxidize C into CO_2 to obtain energy (Yamamoto et al., 2017). The hay and FB soils have elevated concentrations of both C and NO_3^- (Tables 5-1 and 5-2), providing favourable substrate conditions for the occurrence of denitrification. In contrast to hay and FB soils, the concentration of NH_4^+ was initially high (Table 5-3) in the fescue soil, resulting in the greatest contribution of the nitrification process to the overall N_2O flux (16%) across the four evaluated soils.

The inherent priming effects of contrasting long-term agricultural management histories on soil N_2O production are still not well documented. This study was able to identify such inherent priming effects on N_2O fluxes following the addition of labile N (only urea) to a soil under four contrasting management legacies. We hypothesized that N_2O production would increase and be strongly primed in the CB soil as this soil had received long-term (over a period of 38 years) fertilization of urea (90 kg N ha^{-1} $year^{-1}$). Such recurrent annual addition of labile N can train urease-producing microorganisms to efficiently decompose and utilize recently added urea, leading to a rapid increase in N_2O production (Giweta et al., 2017). Supporting our initial hypothesis, the CB soil showed the greatest % priming effect on N_2O production. After the peak emission period, the reason for negative primed N_2O emissions from hay soils could be a temporal switch to immobilization of N as well as the existence of recalcitrant organic matter in this soil (Kuzyakov et al., 2000). Furthermore, our study showed that addition of inhibitors can reduce primed N_2O emissions in comparison to uninhibited urea, and in particular DMPSA was highly effective in minimizing this priming of N_2O, confirming its beneficial role in mitigation of N_2O emissions.

5.5.5 Estimating N_2O emission factors

The overall mean N_2O emission factor (EF; calculated by subtracting the emission during the entire study period from the emission in the unfertilized control and dividing by the N fertilizer rate) was 0.61% across all soil management histories and N fertilizer formulations, which is lower magnitude than the default value of 1% of the Tier I IPCC methodology (Rochette et al., 2008; Metivier et al., 2009), but greater than the Tier II EF (0.02 %) specific for the Canadian Prairies (Rochette et al., 2008) as these Tiers I and II are conventionally applied to field inventories at regional and national scales. When comparing EFs across the three N formulations in our study, the EF for the only urea treatment was 1.02%, which closely agrees with the Tier I default EF. Interestingly, nitrification inhibitors largely reduced this EF, with 0.54% for nitrapyrin and even much lower for DMPSA (0.29%). These findings highlight the potential of nitrification inhibitors in mitigating N_2O production.

5.6 Conclusion

This study demonstrated that a diversified cropping system with a long-term management history that enriches the soil with C and N can exhibit high N_2O production via denitrification. Furthermore, results highlighted that priming of N_2O emissions is influenced profoundly by the previous agricultural management history via stimulation of microbial turnover of existing organic matter. Additionally, detrimentally high N losses and low N recovery capacity (soil retention + plant uptake) can be associated with continuous annual grain cropping perhaps in part due to long-term recurrent inorganic N fertilization stimulating consistently positive priming. Of the nitrification inhibitors assessed in this study, the newly reformulated inhibitor DMPSA was consistently effective in mitigating N_2O emissions while sustaining plant biomass productivity.

5.7 References

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5.8 Tables

Table 5-1. Key soil chemical and physical properties (0-15 cm depth increment) under four land management legacies at the long-term Hendrigan experiment at Breton, Alberta.

Soil Property	**Fescue**	**Hay**	**Continuous Barley**	**Fababean**
pH (H_2O)	5.90	5.92	6.23	6.23
TOC (g C kg^{-1})	16.43	31.48	16.01	25.61
TN (g N kg^{-1})	1.41	2.84	1.34	2.09
C:N ratio	11.68	11.07	11.98	12.26
Texture (USDA)	Loam	Loam	Loam	Loam
Clay (%)	16	15	13	12
Silt (%)	43	48	43	42
Sand (%)	41	37	44	45

Table 5-2. Soil nitrate (NO_3^-) concentration influenced by cropping management history and N application.

NO_3^- (mg N kg^{-1})		**Days after N treatment application**				
Cropping management history	N treatment	0	3	13	42	64
Fescue	Control	0.50	0.42	2.92	1.73	0.77
	Urea (U)	0.62	0.66	0.61	2.76	1.66
	U+Nitrapyrin	0.86	0.45	0.54	4.68	8.88
	U +DMPSA	0.50	0.38	0.68	0.46	0.77
Hay	Control	58.29	51.85	69.82	6.24	6.38
	U	57.60	67.36	98.84	100.11	10.28
	U +Nitrapyrin	58.65	71.93	92.93	9.66	10.9
	U +DMPSA	55.43	55.73	104.35	8.27	4.39
Continuous Barley	Control	0.98	1.64	2.16	11.22	4.26
	U	1.59	5.89	1.71	5.81	4.44
	U +Nitrapyrin	1.18	3.82	2.30	1.82	4.48
	U +DMPSA	1.05	0.93	6.28	2.18	3.13
Fababean	Control	23.24	24.48	32.12	4.46	6.18
	U	32.95	14.35	39.84	13.24	8.35
	U +Nitrapyrin	18.60	26.60	46.69	3.52	4.92
	U +DMPSA	34.11	27.78	35.42	27.67	4.50

Table 5-3. Soil ammonium (NH_4^+) concentration influenced by cropping management history and N application.

NH_4^+ (mg N kg^{-1})		**Days after N treatment application**				
Cropping management history	N treatment	0	3	13	42	64
Fescue	Control	18.31	50.78	21.86	16.80	10.79
	Urea (U)	16.60	115.49	26.52	56.20	24.53
	U+Nitrapyrin	13.95	86.15	17.93	22.53	18.22
	U +DMPSA	8.63	12.73	9.84	7.17	7.13
Hay	Control	8.10	104.73	11.10	11.02	7.18
	U	7.60	73.39	12.12	6.26	5.44
	U +Nitrapyrin	9.73	103.90	12.93	27.04	10.36
	U +DMPSA	7.23	4.22	6.44	10.14	2.02
Continuous Barley	Control	6.11	129.87	7.65	5.74	1.70
	U	8.40	105.02	11.35	3.92	2.86
	U +Nitrapyrin	5.88	188.49	5.75	5.80	3.94
	U +DMPSA	7.48	13.93	7.82	5.76	8.42
Fababean	Control	8.25	57.96	7.70	6.21	6.98
	U	8.89	160.75	9.64	7.09	7.44
	U +Nitrapyrin	8.96	128.41	6.75	5.80	8.49
	U +DMPSA	8.96	128.41	6.75	5.80	8.49

5.9 Figures

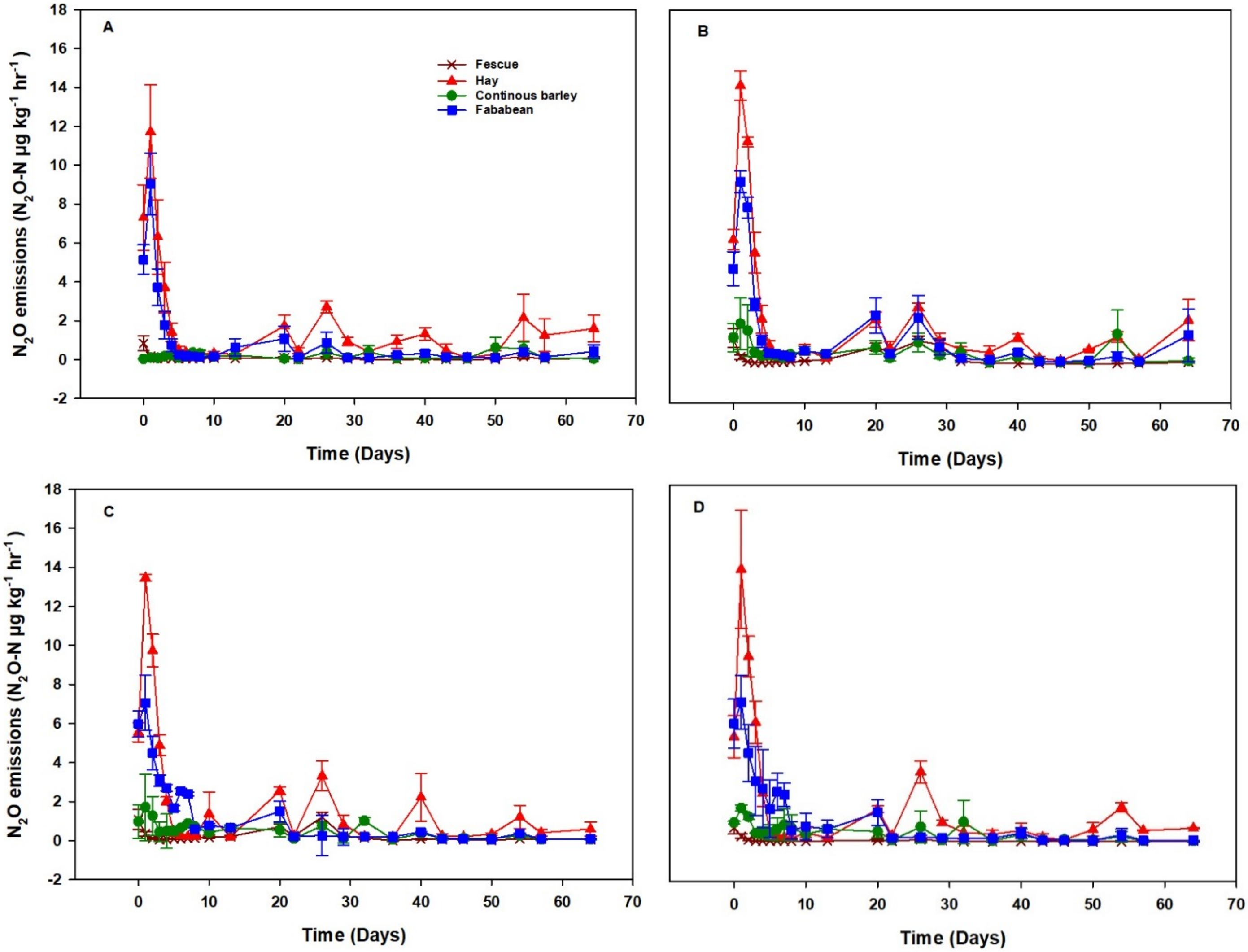

Figure 5-1. Mean daily N_2O emissions with time under four land management legacies for the A) unfertilized control, B) ^{15}N labeled urea, C) ^{15}N urea + nitrapyrin, and D) ^{15}N urea + DMPSA treatments.

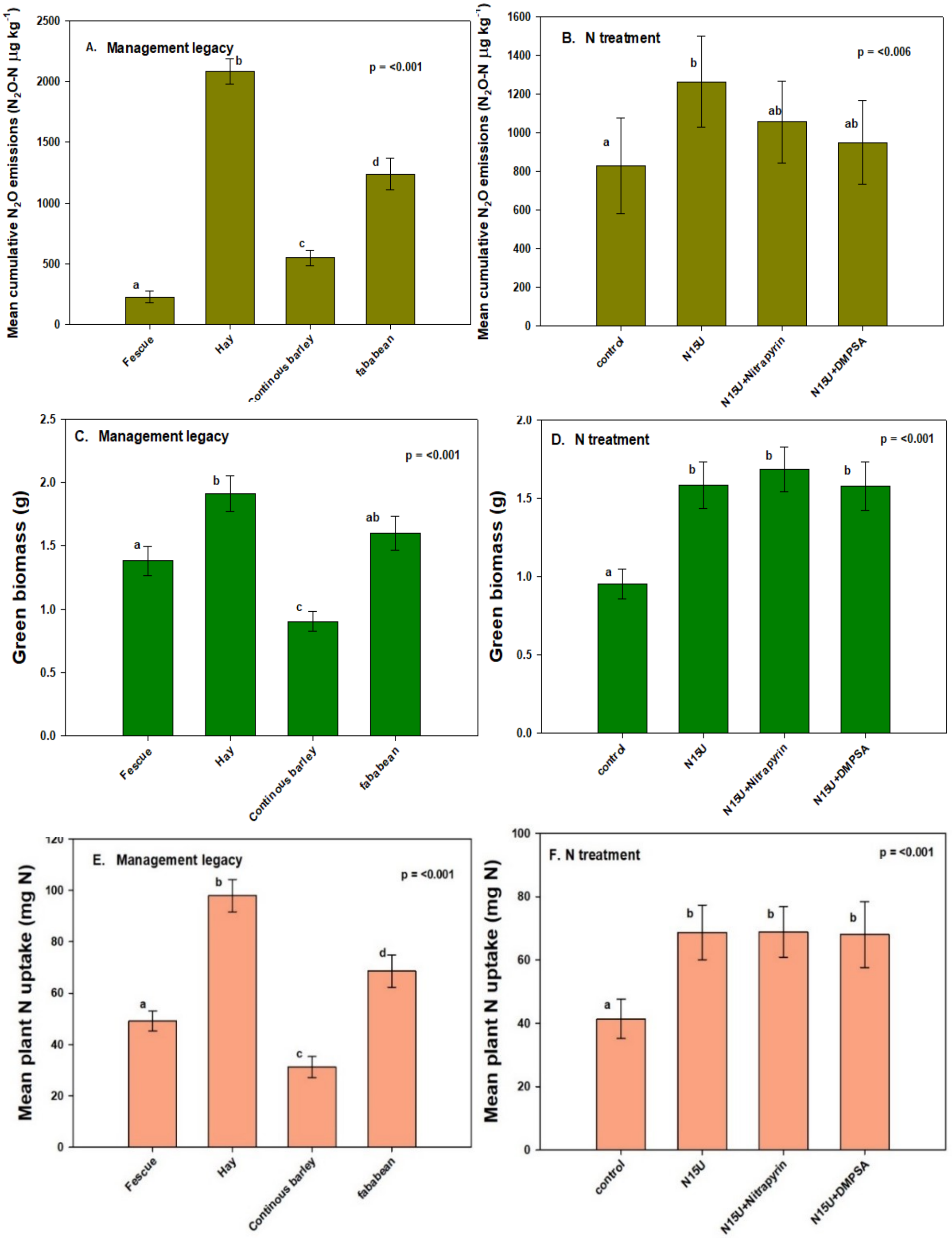

Figure 5-2. Mean cumulative N_2O emissions (A, B), aboveground dry matter biomass (g per experimental pot) (C, D) and mean uptake of N (mg N per experimental pot) (E, F) for the entire study period as a function of four management legacies and four N fertilizer source treatments. Different letters indicate statistically significant differences between the treatments ($p<0.05$) according to Tukey's Honest Significant Difference test. Error bars are standard error.

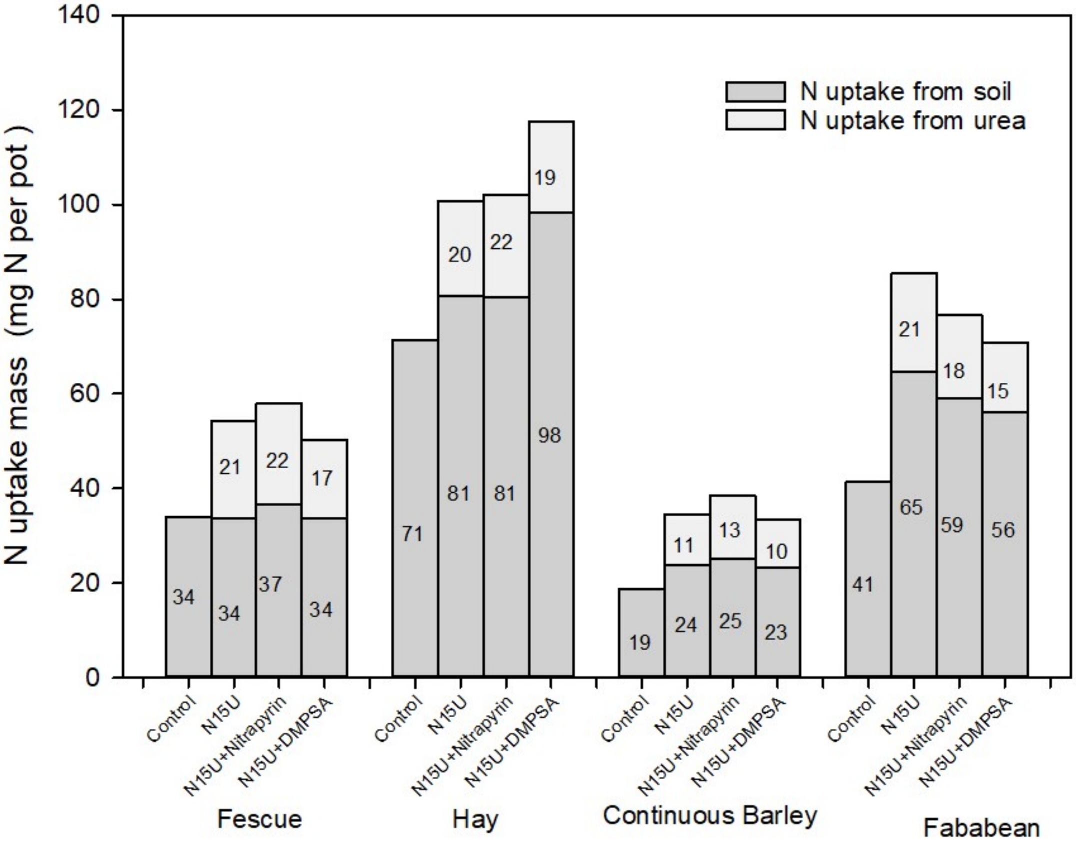

Figure 5-3. Plant N uptake (mg N per experimental pot) of four management legacies and four N fertilization derived from soil and urea.

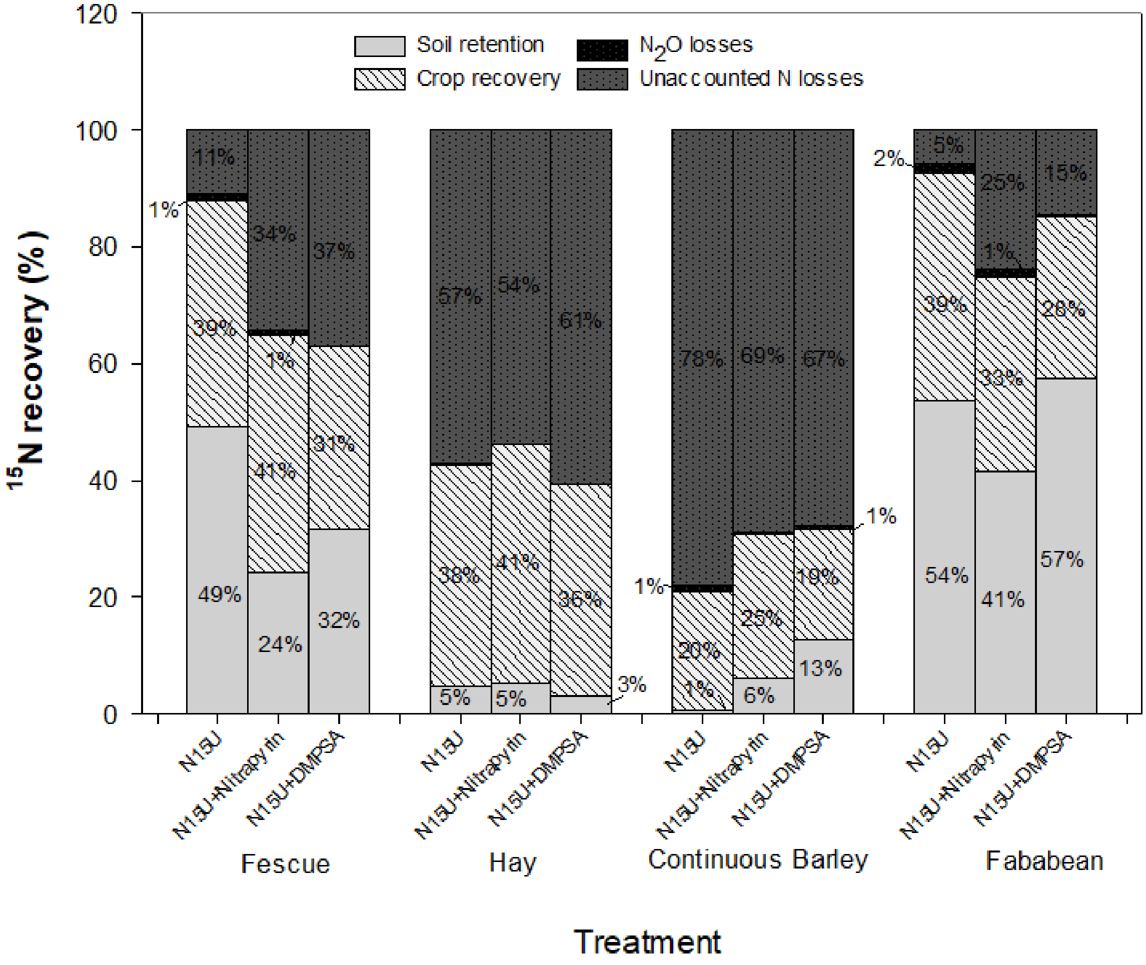

Figure 5-4. Partitioning of added urea-N into plant uptake, soil retention (assumed to be primarily due to immobilization), nitrous oxide production, and unaccounted N losses (e.g., dinitrogen, ammonia).

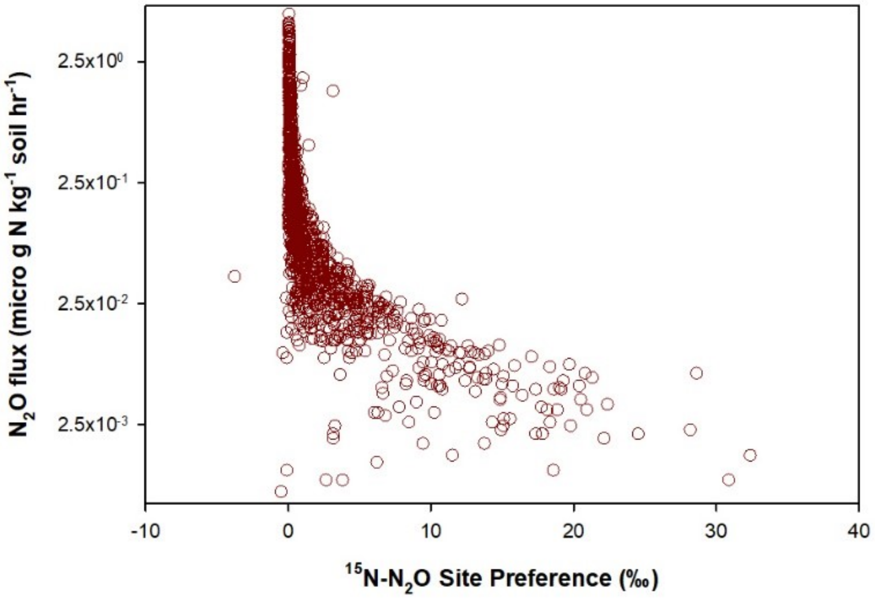

Figure 5-5. N_2O fluxes versus ^{15}N-N_2O site preference (SP) for the entire data collected during the study. The y axis is in log scale.

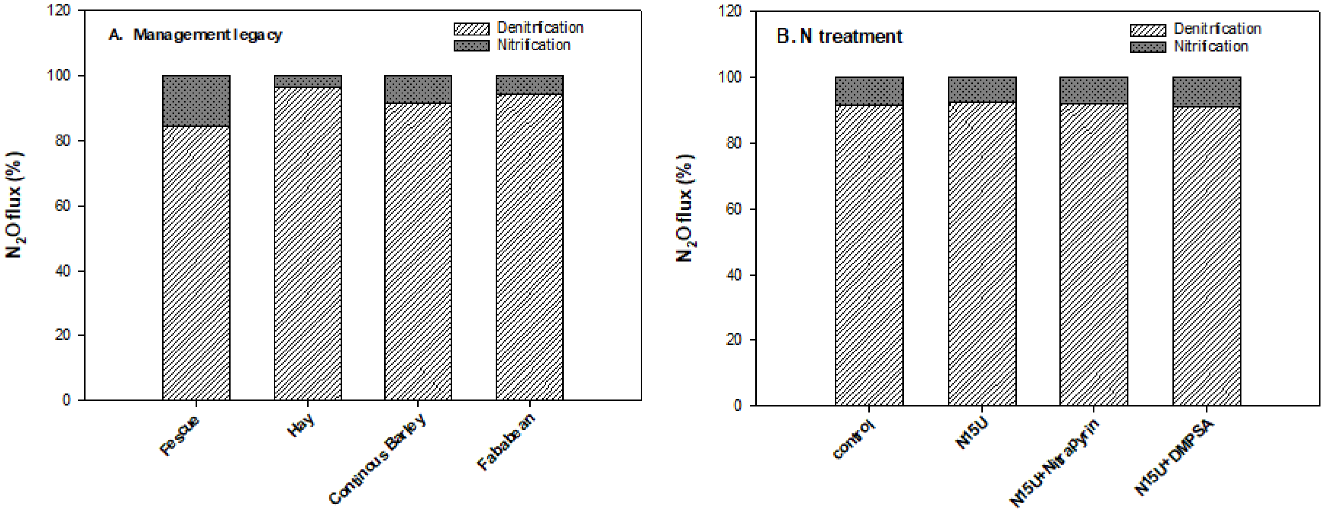

Figure 5-6. Emissions and source-processes partitioning (nitrification versus denitrification) of N_2O as a function of management legacy (A) and N addition treatments (B) during the entire period of the study.

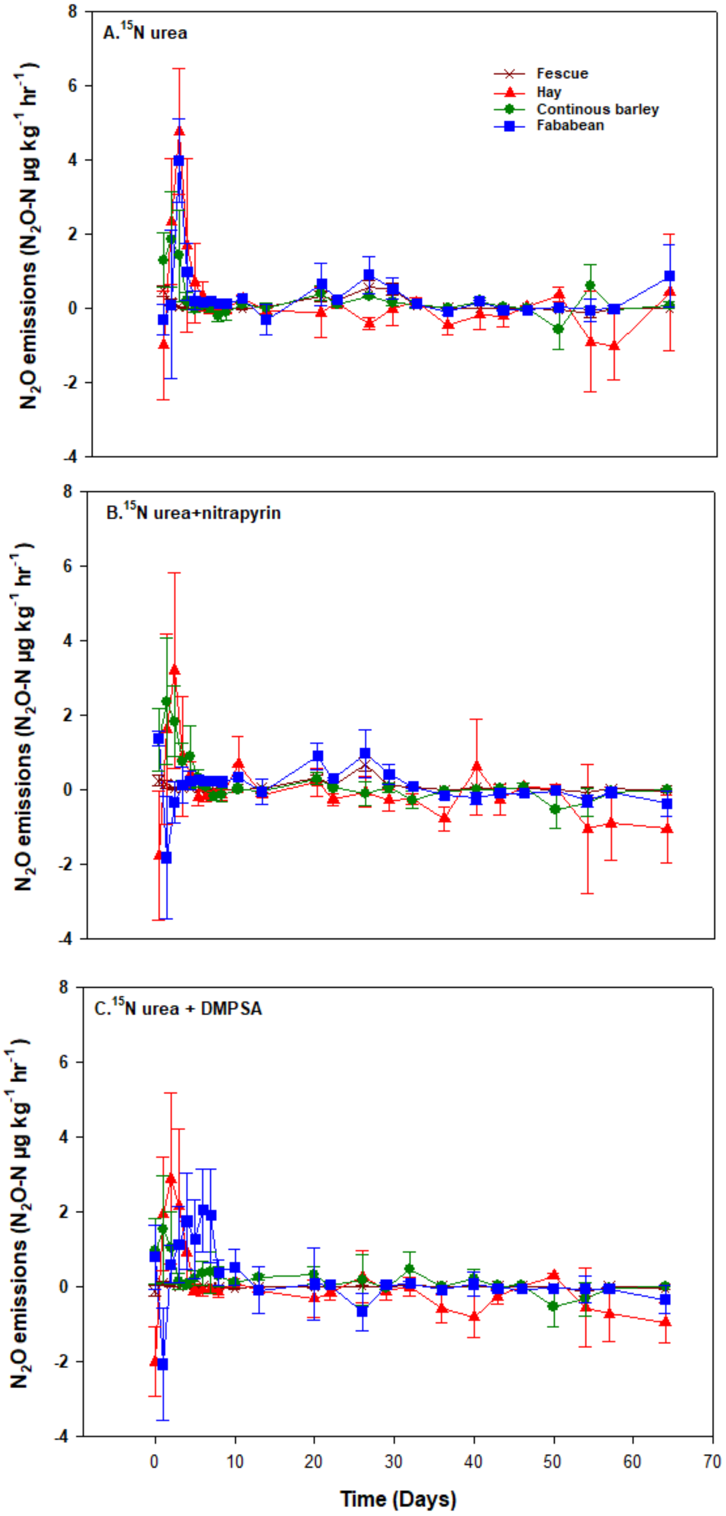

Figure 5-7. Priming effects of N addition on soil-derived N_2O emissions across four management legacies. Primed N_2O fluxes were calculated based on source-pool partitioning of urea-N vs. pre-existing soil N as derived using Eqs. [4], [5] and [6].

6 General conclusions and suggestions for further studies

The four studies compressed in this dissertation examined the implementation and effects of EENFs in agricultural systems. When appropriate fertilization options are matched with cropping systems, soil, and climatic conditions, EENFs can be beneficial in sustaining or attaining greater plant yield while reducing N_2O emissions. The N_2O peak emissions during the early spring and following major rainfalls revealed that N_2O emissions are responsive to a combination of timing and formulation of N fertilizer application as well as dependent upon the availability of soil moisture in N-rich, warm soils. Determining what causes N_2O emission sensitivity is environmentally beneficial. Land managers and producers can plan for N fertilization in mid or late spring while utilizing the weather forecast to reduce the risk of concurrence with major rainfall events following spring N addition. Since our studies indicated that fall N addition contributes to high N_2O emissions during spring thaw, future studies are encouraged to focus and evaluate the N_2O emission reductions of middle versus early spring N fertilization, where middle spring corresponds to in-crop N fertilization.

In our field study, there was little crop benefit when using EENFs; due to their incremental cost and lack of yield response in this high fertile soil, the economic prospects are near null. However, EENFs reduced N_2O emissions by 30% during one of the study year. From an environmental perspective, a 30% reduction becomes a considerable reduction when estimating a national inventory of greenhouse gas emissions because N_2O warming potential coefficient corresponds to 310 times CO_2 (equivalent on mass basis). Therefore, the adoption of EENFs could be environmentally cost-effective as the externality cost associated with N_2O emissions is reduced. Future research combination of EENFs and the 4R principles of nutrient management (right source, right rate, right place and right time) can enable improvements in both crop productivity and environmental outcomes. Our field study showed that greater availability of soil N can hamper the effects of EENFs, highlighting that findings of our study contributes to the scientific knowledge relevant to highly fertile soils. Since the N availability can vary in field scale, studies observing variable rates of EENF application could provide further insight onto best management practices.

Since the results of the field experiment showed soil moisture as a key driver for N_2O fluxes, a laboratory incubation study evaluating a range of five different WFPS was carried out using the same Black Chernozem soil (collected from the control wheat fields at the St. Albert research station where the two year field experiment was established). The incubation study confirmed that WFPS is an important controlling factor in regulating N_2O emissions as emissions increase with increasing soil moisture content. The study focused on a wide range of WFPS (i.e., from 31 to 78%).

The ^{15}N-N_2O SP data identified that N_2O emissions from wheat soils were predominately produced by the denitrification process across all combinations of moistures and N additions. This finding could be associated with high soil NO_3^- concentration as substrate, presence of clay that provides a large proportion of microspores within soil aggregates, and anoxic conditions created by the microbial respiration, decomposition and mineralization of naturally present organic matter. Analysis of stable isotopes of N_2O emerged as a promising approach to quantify the relative contribution of nitrification and denitrification. Therefore, future isotopic labelling studies can be combined with biomolecular techniques to further evaluate all N_2O production pathways of bacterial nitrification and denitrification as well as fungal denitrification and nitrifier denitrification.

Given the interest in the evaluation of different EENFs to mitigate N_2O emissions, a modeling experiment was conducted using selected N fertilizer treatments from the field study. The N_2O emissions from EENFs and the effect of fall vs. spring N fertilizer addition were successfully simulated by the process-based model *ecosys*. Modelled N_2O emissions from field amended with polymer-coated urea ESN (a EENF) were numerically lower than from fields receiving conventional fertilizers such AA and urea, which is a result also found in the measured data. This consistent result showcase the potential benefit of adopting EENFs. Moreover, the observed N_2O peaks in the modelling study suggested that the model is more sensitive to N fertilizer additions, while field measurements responded more to variations in soil moisture; this indicates the importance of further studies to improve the model representation of soil moisture temporal patterns in fined-textured soils. When the grain yield and N_2O emission were simulated in the tested model for reduced N application rates, the study identified that 25 kg N ha^{-1} is the optimum N fertilization rate to minimize N_2O emissions while maintaining crop grain yields in the short term in these soils with high natural fertility.

Finally, a greenhouse study tested the influence of different management legacies of cropping systems and the effects of land-use changes on soil N_2O emissions. This study found that the accumulation of soil C and N which reflects the land use history regulated N_2O emissions. A soil from a legume crop phase (fababean, FB) emitted high N_2O fluxes (much greater than the fertilized continuous barley) likely due to the increased availability of atmospheric-fixed N for microbial N_2O producers.

Comparing the nitrification inhibitors used in the greenhouse study, the use of new inhibitor DMPSA was effective in mitigating N_2O emissions and it delivering high crop biomass productivity, showing the beneficial role of DMPSA in comparison to other inhibitors. This controlled study determined the priming of soil-derived N_2O production due to labile N addition under the four contrasting long-term crop management histories. The study revealed that the primed N_2O emissions in soils that receive long term fertilization can contribute even more to fertilizer induced N_2O emissions.

Moreover, using the ^{15}N isotopic technique, the fate of added-N fertilizer was tracked into the plant N recovery, soil N retention, N_2O production and other gaseous N losses across the soils with contrasting management legacies. Greater N losses and low N recovery were associated with a continuous barley (CB) cropping, which had continuously received annual inorganic N fertilization over 38 years. These greater N losses emphasize the importance of management recommendations such as the addition of organic amendments, diverse crop rotations including perennials, and incorporation of legumes to improve soil quality.

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