Interactions among Nitrogen Input, Nitrification Inhibition, Edaphic and Environmental Conditions on N_2O Fluxes and associated Biological Processes in Central Alberta Soils

by

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Abstract

Increasing atmospheric concentration of nitrous oxide (N_2O) emissions due to intensified human activities is of concern, as N_2O is not only a precursor for stratospheric ozone destruction but also an important greenhouse gas. Agriculture accounted for about 82% of the anthropogenic N_2O production in the world and two-thirds came from soils receiving manures or synthetic N fertilizers. Therefore, it is important to gain new knowledge by investigating the N_2O production processes and their controlling factors. In the first study, field experiments were conducted from 2014 – 2017 in Edmonton and Lacombe, Alberta. We examined the effects of manure application timings (fall and spring) and nitrification inhibitors (NIs), namely nitrapyrin [2-chloro-6-(trichloromethyl) pyridine] and 3, 4-dimethylpyrazole phosphate (DMPP) on N_2O emissions and soil mineral N and investigated the potential residual effects of repeated manure additions. The annual N_2O emission factor due to fall manure injection without NIs was about four times greater than that due to spring injection without NIs in 2014-2015, whilst this comparison was reversed in 2015-2016. This was mainly owing to the contrasting precipitations between the two experimental years. Residual effects became evident by the increased, divergent N_2O emissions and soil NO_3^- concentration in the spring of 2017. The second study was a controlled laboratory study established to elucidate the impact of several soil moisture contents and NI application rate on the efficacy of two NIs [i.e., nitrapyrin and 3,4-dimethylpyrazole succinic acid isomeric mixture (DMPSA)] in reducing N_2O emissions and inhibiting nitrification in Black Chernozamic (BC) and Gray Luvisolic (GL) soils. Regardless of NI application rates and soils, the emission reductions due to NIs were obvious at high moisture contents as compared to the lowest moisture content in which there was no significantly different N_2O emission between the manured soils with versus without NI additions. With higher soil moisture content, greater NI application rate was required to efficiently inhibit nitrification and reduce N_2O emissions for both NIs in both soils. Moreover, it seemed that the BC soil required higher NI application rate than the GL soil under the same condition, likely because higher clay and organic matter contents would cause increased surface adsorption and microbial degradation. In the third study, a mesocosm experiment was conducted to investigate the N_2O production and sources from soils with different N management history (soil with (SW) and without (CT) legacy of manure additions) and three different water contents over a simulated fall-freeze-thaw cycle. Compared to the untreated control treatment, the urea-amended treatment showed greater soil-derived N_2O emissions during thawing, indicating a net positive priming effect. This positive priming effect was greater in the SW soil than the CT soil. Above 83% of the N_2O fluxes on the 2nd and 3rd days of thawing were produced by denitrification. In the fourth study, a process-based *ecosys* model was used to represent agroecosystems and predict the resultant N_2O emissions from soils receiving manure (as per our first study mentioned above) and also to simulate de-watered manure additions in fall and spring seasons. The model was able to simulate well the magnitude and timing of N_2O fluxes except for when the soil was very moist. The model could be further improved by refining the representation of soil water retention by implementing a simulation of snowmelt filtration and runoff in *ecosys*. Overall, this dissertation makes a contribution to better understand N_2O production sources and drivers and provides insights into N_2O emission mitigation.

Preface

This dissertation is an original work by Sisi Lin. With the exceptions explained below, all experiments were carried out and the data was analyzed by Sisi Lin under the supervision of Dr. Guillermo Hernandez Ramirez.

Chapter 2, 3, 4 and 5 are intended for publication. In Chapter 2 entitled “Nitrous Oxide Emissions in Barley Croplands over 2.5 Years: Effects of Liquid Manure Timing and Nitrification Inhibitor Use”, Dr. Len Kryzanowski, Trevor Wallace, Dr. Robert Grant, Dr. Rory Degenhardt, Dr. Nils Berger, Germar Lohstraeter and Leigh-Anne Powers made contributions to the experiment design and provided comments and suggestions to the research and writing. Germar Lohstraeter and Leigh-Anne Powers also made contributions to the data collection.

In Chapter 5 entitled “Mathematical Modeling of Nitrous Oxide Emissions from Fall versus Spring Manure Injection Timings and from De-watered Slurry”, Dr. Symon Mezbahuddin and Dr. Robert Grant made contributions to model establishment and performance and provided suggestions to writing.

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1. Introduction

1.1 Background information

Mitigating global climate change and reducing anthropogenic greenhouse gases (GHGs) have received increasing attention in recent decades. Nitrous oxide (N_2O) is a potent anthropogenic GHG with a long atmospheric lifetime of 114 years and a high 100-year global warming potential, which is about 310 times greater than CO_2 on a mass basis (Forster et al., 2007). It is also an active substance for ozone depletion (Ravishankara et al., 2009). The atmospheric concentration of N_2O increased by 19% from 1750 to 2011 (Myhre et al., 2013). Due to this concentration increase and the decreases in other GHGs such as chlorofluorocarbons (CFCs), N_2O is now the third largest radiative forcing anthropogenic GHG (Myhre et al., 2013). Agriculture is a major source of N_2O emissions, accounting for approximately 58% of total anthropogenic N2O emissions, and about 67% of these emissions are from agricultural soils receiving organic or synthetic fertilizers (Intergovernmental Panel on Climate Change, 2007; US-EPA, 2011). Given the expanding cultivated land and increasing fertilizer use, N_2O emissions from agricultural soils are expected to continue increasing (Reay et al., 2012). Therefore, it is necessary to investigate effective strategies to mitigate N2O emissions from fertilized soils. To do so, we need to identify the underlying N_2O-generating processes and factors impacting the magnitude of N_2O production.

1.2 Processes related to nitrous oxide production/emissions

N_2O is primarily generated by microbiological processes within the soil nitrogen cycle (Fig. 1-1). Microbial nitrification and denitrification are generally considered as the key N_2O-generating processes, contributing to 70% of global N_2O emissions (Wrage et al., 2001; Barnard et al., 2005; Braker and Conrad, 2011; Butterbach-Bahl et al., 2013). However, a few other biological processes also contribute to N_2O production, such as co-denitrification (Tanimoto et al., 1992; Kumon et al., 2002; Spott et al., 2011) and dissimilatory nitrate reduction to ammonia (DNRA)/nitrate ammonification (Bleakley and Tiedje, 1982; Smith, 1982; Giblin et al., 2013).

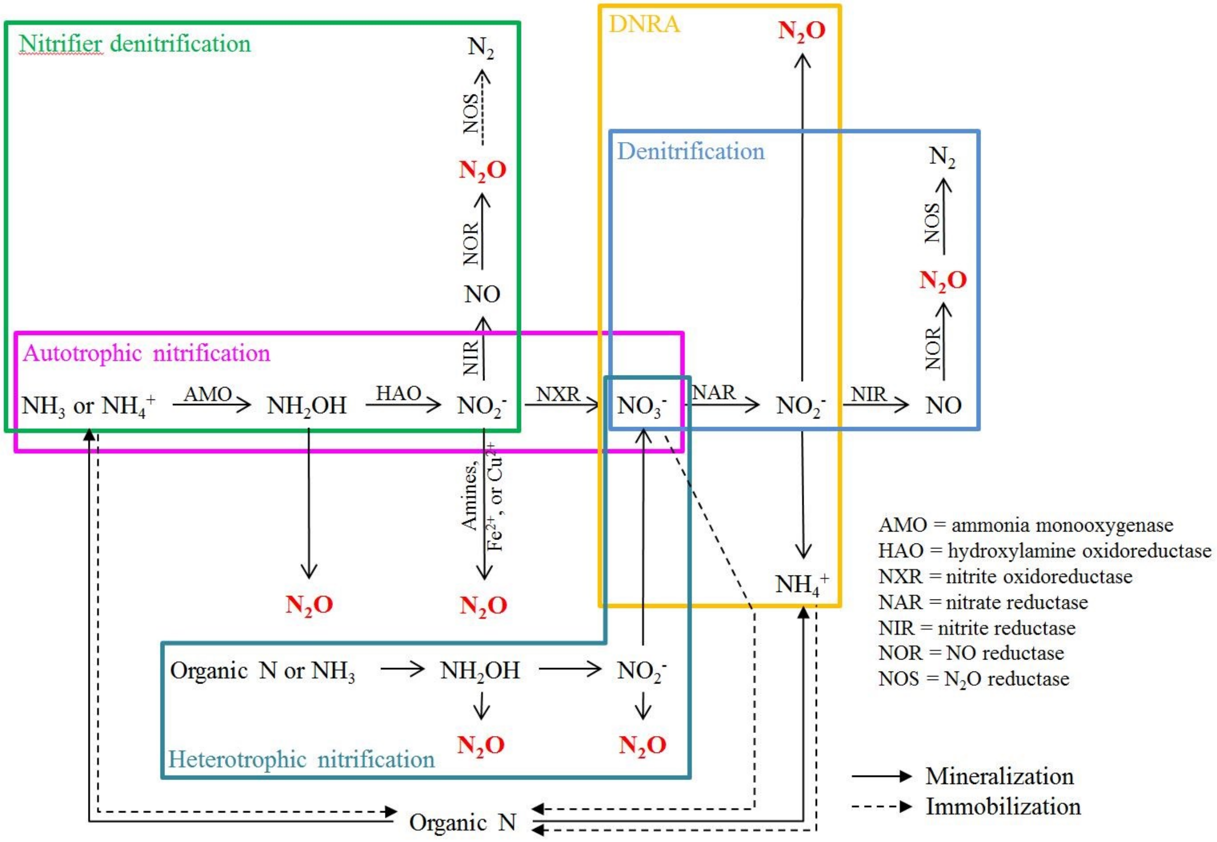

Fig. 1-1. Processes related to nitrous oxide production in soil. Adapted from Zhang et al. (2015).

1.2.1 Mineralization and immobilization

Mineralization and immobilization play important roles in regulating the substrate pools for N_2O production processes in the N-cycle, such as nitrification and denitrification. Mineralization is defined as a transformation of organic N to inorganic ammonium (NH_4^+) and it is performed by heterotrophic soil organisms that utilize organic N as the energy source (Jansson and Persson, 1982; Hart et al., 1994) (Fig. 1-1). Immobilization is a conversion of inorganic N forms [NH_4^+ and nitrate (NO_3^-)] to organic N (Fig. 1-1). If the N content in decomposing residues is low, soil microorganisms will assimilate inorganic N (NH_4^+ and NO_3^-) in their biomass as organic N for the development and growth (Hart et al., 1994; Havlin et al., 2014). Whether mineralization or immobilization dominates in soils is mainly controlled by the C/N ratio in the animal or plant residues being decomposed by soil organisms (Robertson and Groffman, 2007; Havlin et al., 2014). When the decomposing organic material has a C/N ratio below 20, net mineralization usually occurs (Hagemann et al., 2016). When the C/N ratio increases above 30, net immobilization typically takes place in the short- or long-term (Stevenson and Cole, 1999).

1.2.2 Nitrification

1.2.2.1 Autotrophic nitrification

Autotrophic nitrification is a two-step pathway, in which ammonia (NH_3) or NH_4^+ is firstly oxidized to nitrite (NO_2^-) via hydroxylamine (NH_2OH^-) by ammonia oxidizers and further oxidized to nitrate (NO_3^-) by nitrite oxidizers under aerobic conditions (Fig. 1-1). During this pathway, N_2O can be generated through either chemical decomposition of NH_2OH^- and NO_2^- or NO_2^- reduction in the presence of organic (e.g., amines) or inorganic (e.g., Fe^{2+} or Cu^{2+}) substances (Wrage et al., 2001; Braker and Conrad, 2011; Butterbach-Bahl et al., 2013). The latter non-biological chemical reduction is known as chemo-denitrification.

The main aerobic chemoautotrophic ammonia and nitrite oxidizers in the autotrophic nitrification are genera *Nitrosomonas* and *Nitrobacter* respectively (Koops et al., 1991; Shaw et al., 2006). They obtain C from CO_2 and energy from the N oxidation for their metabolism

(Braker and Conrad, 2011). Enzymes involved in these reactions are ammonia monooxygenase (AMO), hydroxylamine oxidoreductase (HAO) and nitrite oxidoreductase (NXR) (Fig. 1-1). Ammonia oxidation has been long thought to be executed by ammonia oxidizing bacterium (AOB) (Kowalchuk and Stephen, 2001). However, ammonia oxidizing archaea (AOA) were recently recognized as a new potential contributor in both marine and terrestrial habitats (Könneke et al., 2005; Treusch et al., 2005). Although AOA are ubiquitous in soils and also have the genetic potential for aerobic ammonia oxidation (Leininger et al., 2006; Hansel et al., 2008), it is still unknown if AOA can generate N_2O emissions since the gene encoding NO reductase was not observed in the AOA genome sequence (Walker et al., 2010; Braker and Conrad, 2011). This point was also supported by other studies, in which AOA were found in six N-rich grassland soils in New Zealand, but their abundance and ammonia oxidation activity were not connected with either nitrification or N_2O production (Di et al., 2009b; Di et al., 2010).

1.2.2.2 Heterotrophic nitrification

While it is generally accepted that autotrophic nitrification is a predominant nitrification pathway under most soil conditions, heterotrophic nitrification may become dominant in acidic forest soils (Schimel et al., 1984; Pedersen et al., 1999; Brierley and Wood, 2001; Zhang et al., 2011a), where the autotrophic nitrifiers are inhibited (Weber and Gainey, 1962; De Boer and Kowalchuk, 2001; Zhao et al., 2018). During heterotrophic nitrification, N_2O can be generated from the intermediates of the oxidation of organic N or NH_3 to NO_3^- (Papen et al., 1989; Zhang et al., 2015) by fungi or bacteria (Odu and Adeoye, 1970; Castignetti and Hollocher, 1984) (Fig. 1-1). In contrast to autotrophic nitrifiers, heterotrophic fungi and bacteria obtain C and energy sources from organic compounds for their metabolism (Castignetti, 1990; Brierley and Wood, 2001; Wrage et al., 2001; Braker and Conrad, 2011). The NH_3 oxidation by heterotrophic nitrifiers is not in conjunction with energy conservation, and the enzymes involving in this pathway are distinct from those in autotrophic nitrification (Braker and Conrad, 2011).

1.2.2.3 Nitrifier-denitrification

Nitrifier-denitrification is an alternative pathway of nitrification, which includes an oxidation of NH_3 or NH_4^+ to NO_2^- and a consecutive reduction to NO, N_2O and N_2 by the same autotrophic ammonia bacteria (Wrage et al., 2001; Shaw et al., 2006; Zhu et al., 2013) (Fig. 1-1). Limiting oxygen coupled with low organic C availability lead to the occurrence of nitrifier-denitrification, possibly as well as low pH (Wrage et al., 2001). This pathway is distinct from coupled nitrification-denitrification, in which nitrification and denitrification are carried out by different microbial groups (Wrage et al., 2001; Butterbach-Bahl et al., 2013). As the genes encoding N_2O reductase were not observed in the AOB genome (Beaumont et al., 2004; Beaumont et al., 2005; Casciotti and Ward, 2005; Shaw et al., 2006; Garbeva et al., 2006; Cantera and Stein, 2007), the most common end product of NO_2^- reduction via nitrifier-denitrification is considered as N_2O (Braker and Conrad, 2011; Law et al., 2012; Zhu et al., 2013) rather than N_2 that was only observed in *Nitrosomonas spp* (Poth, 1986).

1.2.3 Denitrification

Denitrification plays an important role in soil N cycling as it is the main pathway to return fixed N back to the atmosphere, thereby accomplishing the closure of N cycle (Philippot et al., 2009). In this biological pathway, NO_3^- is gradually reduced to NO_2^-, NO, N_2O and consequently N_2 under anaerobic conditions (Fig. 1-1). This process is carried out by a large variety of heterotrophic denitrifying microorganisms, including bacteria, archaea, fungi and other eukaryotes (Bremner, 1997; Laughlin and Stevens, 2002; Risgaard-Petersen et al., 2006; Philippot et al., 2007; Ma et al., 2008; Pina-Ochoa et al., 2010). The most dominant are

Pseudomonas, *Bacillus*, *Paracoccus* and *Thiobacillus* (Havlin et al., 2014). The denitrifiers are basically facultative anaerobic bacteria that can utilize NO_3^- as an electron acceptor when O_2 is limiting, and most denitrifiers are heterotrophic organisms that use organic compounds as C source (Bremner, 1997; Wrage et al., 2001). The end product of fungal denitrification is N_2O since N_2O reductase is generally absent in most fungi (Shoun and Tanimoto, 1991; Kim et al., 2009).

1.2.4 Other biological processes generating N_2O emissions

1.2.4.1 Co-denitrification

Co-denitrification, a unique microbial pathway, was discovered by Shoun et al. (1992) and Tanimoto et al. (1992). Through co-denitrification, N_2O or N_2 can be produced as hybrids, where a combination of denitrified NO or NO_2^- with an N atom provided by a co-substrate takes place (Su et al., 2004; Baggs, 2011; Spott et al., 2011). Both inorganic and organic N compounds, such as NH_4^+, NH_2OH^-, amino acids, azide, aniline, and salicylhydroxamic acid, can be used as co-substrates (Spott et al., 2011). Both bacteria and fungi have been identified to carry out co-denitrification (Shoun et al., 1992; Su et al., 2004; Spott et al., 2011; Baggs, 2011).

1.2.4.2 Dissimilatory nitrate reduction to ammonia (DNRA)/nitrate ammonification

Dissimilatory nitrate reduction to ammonia (DNRA) or nitrate ammonification is a biological pathway under anaerobic conditions, in which NO_3^- is reduced to NO_2^- and then NH_4^+ (Smith, 1982; Silver et al., 2001) (Fig. 1-1). Within this process, N_2O production takes place through the reduction of NO_2^- by the bacteria responsible for DNRA (Rütting et al., 2011; Baggs and Philippot, 2011; Butterbach-Bahl et al., 2013). DNRA occurs mainly through the activity of facultative and obligatory fermentative bacteria (Tiedje, 1988; Nijburg et al., 1997; Silver et al., 2001). Despite both denitrification and DNRA occur under similar conditions (e.g., low O_2, NO_3^- as substrate, available C), DNRA is considered to be dominant under an environment with a high ratio between available C (electron donor) and NO_3^- (electron acceptor) (Tiedje et al., 1983; Tiedje, 1988; Nijburg et al., 1997; Silver et al., 2001). Although denitrification is a major source of N_2O in most terrestrial soils, the importance of DNRA over denitrification has been recognized in tropical and paddy soils, as well as in some coastal sites (Silver et al., 2001; Yin et al., 2002; Giblin et al., 2013).

1.3 Factors impacting N_2O-generating processes and N_2O production

1.3.1 Oxygen availability [and redox potential (Eh)]

Oxygen (O_2) availability is regarded as a substantial factor in regulating the sources of N_2O production, as it is a key determinant of prevailing biological processes. Under well-aerated and dry conditions, nitrification becomes dominant because both autotrophic and heterotrophic nitrifiers obligatorily need O_2 (Schaufler et al., 2010; Butterbach-Bahl et al., 2013). When the O_2 concentration is limited or strictly deficient, denitrification gains an advantage through the activities of facultative heterotrophic denitrifiers that can transit aerobic to anaerobic respiration by using NO_3^- as an electron acceptor instead of O_2 (Wrage et al., 2001; Hagemann et al., 2016). In addition, moderate aerobic conditions with a redox potential (Eh) higher than 300 mV generally lead to quite significant rates of heterotrophic decomposition, N mineralization and immobilization (Mengel, 1996; Robertson and Groffman, 2007; Hagemann et al., 2016). In saturated soils, significant N_2O emissions were observed with a redox potential in the range of 120-250 mV (Hou et al., 2000b; Yu et al., 2001).

1.3.2 Moisture

In soils, O_2 availability is usually coupled directly with soil moisture content as both air and moisture (ice, water, or vapor) typically co-occupy soil pores (Hagemann et al., 2016). Nitrification usually dominates in producing N_2O below 60% WFPS, while denitrification becomes more important above 60% WFPS (Davidson and Schimel, 1995; Ruser et al., 2006). Maximum N_2O emissions were postulated to occur within a range of 60-80% water-filled pore space (WFPS) across different soil types (Davidson et al., 1991; Davidson et al., 2000; Schaufler et al., 2010; Butterbach-Bahl et al., 2013). In soils with WFPS higher than 80%, the amount of N_2O emissions gradually decline due to further reduction of N_2O to N_2 through complete denitrification (Bowman and Steltzer, 1998; Meixner and Yang, 2006; Butterbach-Bahl et al., 2013).

Apart from regulating O_2 supply, soil moisture serves as a transport medium of substrates for soil microbes, such as NH_4^+ for nitrifiers and NO_3^- for denitrifiers (Schaufler et al., 2010). Low moisture content limits the substrate diffusion through the soil profile (Meixner and Yang, 2006) and hence cuts down the effective interacting surfaces and volume for microbes, thereby retarding their activities and reducing related gaseous production.

Both the change in soil water content and the change in soil water states (e.g., ice vs. liquid) can control the magnitude of N_2O production. Short-lived N_2O pulses have been observed as a result of freeze-thaw (FT) or dry-wet (DW) cycles in many field and laboratory researches (Davidson, 1992; Nyborg et al., 1997; Teepe et al., 2004; Koponen et al., 2006; Harrison-Kirk et al., 2013). In agroecosystems, soil FT cycling contributes considerably to global annual N_2O budget, and this contribution varies from 30 to 90% (Wagner-Riddle et al., 2007; Yanai et al., 2011; Abalos et al., 2016; Lin et al., 2017). However, soil DW cycling may only account for a small source of total N_2O emissions (Muhr et al., 2008; Goldberg et al., 2010), accounting for only 2% of total annual N_2O emissions (Davidson, 1992).

The mechanisms leading to N_2O pulses from FT and DW cycling have similarities and differences (Congreves et al., 2018). An increase in soil 'liquid' water content is expected to take place during thawing of a frozen soil, which is theoretically similar to rewetting a dry soil (Spaans and Baker, 1996; Congreves et al., 2018). The increased soil 'liquid' water content in such situations can lead to an incremental substrate supply, oxygen-deficient environment, and stimulated microbial activities (Davidson, 1992; Priemé and Christensen, 2001; Borken and Matzner, 2009), which are all responsible for short-lived significant N_2O fluxes. A unique fact of soil FT cycling is the ice, which blocks the gas diffusion pathways (e.g., O_2 coming into the soil, N_2O and CO_2 escape from the soil); consequently, a mass of N_2O accumulated underneath the ice during freezing will escape from the soil in the course of thawing (Risk et al., 2014).

1.3.3 Temperature

Soil temperature is another important factor influencing N_2O production through its impacts on microbial activity and gas transfer. The change in rates of N_2O production as affected by temperature can be explained by Q_{10}, which is the change in microbial activity rate with a change in temperature of 10°C (i.e., process rate at (T+10°C)/process rate at T) (Smith, 1997; Smith et al., 2003). It was found that the apparent Q_{10} relationship was greater in a higher temperature interval than a lower interval (Castaldi, 2000; Jianwen et al., 2004; Bagherzadeh et al., 2008), indicating an exponential increase in microbial activity and hence potential increases in N_2O production with a rising temperature (Dobbie and Smith, 2001; Schindlbacher et al., 2004). This positive feedback can be attributed to the stimulated N_2O-related enzymatic process and enhanced denitrification by O_2 deficiency arisen from accelerated respiration (Butterbach-

Bahl et al., 2013). In addition, the decreases in both N_2O solubility and diffusion caused by increasing soil temperature contribute partly to the temperature-induced N_2O production (Moraghan and Buresh, 1977; Schmidt et al., 2000). After reaching to the optimal temperature point for biological N_2O-producing processes, N_2O production decreases with any further temperature increases (Schmidt et al., 2000). This optimal soil temperature for N_2O production is expected around 35-40°C (Granli, 1994; Schmidt et al., 2000).

1.3.4 Substrates

The availability of substrates for N_2O-generating processes, such as inorganic forms of N (e.g., NH_4^+ and NO_3^-), reduced organic N and organic C, could have direct impacts on the rate of microbial reactions and hence N_2O emissions when other factors are not limiting. The NH_4^+ pool is an essential factor controlling nitrification in most soils, similarly to the role of NO_3^- in denitrification (Firestone and Davidson, 1989; Chapuis - Lardy et al., 2007). High soil decomposition and N mineralization rates, low immobilization rate, and low plant NH_4^+ uptake, will all result in an increase in NH_4^+ supply for nitrifiers and consequently accelerate nitrification rate and promote N_2O emissions (Robertson and Groffman, 2007). Likewise, the NO_3^- availability is a result of a balance among various processes, for instance, immobilization, nitrification, plant uptake and leaching. Organic C is of importance in regulating denitrification, because a majority of denitrifiers are heterotrophic microbes that use organic C as an electron donor (Bremner, 1997; Wrage et al., 2001). Thus, stimulated denitrification caused by a combination of increased NO_3^- and organic C availability consequently may lead to significant N_2O fluxes (Barnard et al., 2005).

1.3.5 pH effects

The effects of pH on N_2O emissions are thought to be primarily connected with its influence on soil nitrifier and denitrifier communities, as well as on the NH_3 substrate for ammonia oxidation. Nitrifiers prefer living at moderate pH and denitrifiers favor a neutral environment (Barnard et al., 2005), implicating potential changes in N_2O emissions as a result of soil acidification or soil liming. A positive relationship between near neutral pH and nitrification rate has been observed in a long-term field study (Kemmitt et al., 2006). This is in line with other results showing that soil acidification could result in a decreasing rate of nitrification (Bäckman and Klemedtsson, 2003; Cheng et al., 2013; Jiang et al., 2015). This can be partially explained by the decreased availability of NH_3, a substrate for AMO in nitrification (Suzuki et al., 1974). This phenomenon, however, was not observed in subtropical acid soils (Zhao et al., 2007), possibly owing to the contributions of a group of nitrifiers that are capable of living at low pH, including AOA (He et al., 2012; Stempfhuber et al., 2015), heterotrophic nitrifiers (De Boer et al., 1992; Zhang et al., 2011b), and certain AOB tolerant to low pH (De Boer et al., 1992; Jiang et al., 2015). In the case of denitrification, decreasing pH has been reported to bring about greater ratios of N_2O to N_2 (Šimek and Cooper, 2002; Dannenmann et al., 2008), due to the inhibition of N_2O reductase at a low pH value (Thomsen et al., 1994).

1.3.6 Interplay of other soil properties

With respect to the importance of soil moisture content and O_2 on N_2O production, soil properties that can alter the distribution of air- and water-filled pores are considered to be tightly relevant to the soil N cycling and significantly affect N_2O production rate. The effect of soil texture on N_2O emissions has been emphasized in many studies (Weier et al., 1993; Parton et al., 1996; Weitz et al., 2001; Goossens et al., 2001; Bouwman et al., 2002; Rochette et al., 2008b). In an examination of 13 United Kingdom soils with contrasting textures, Skiba and Ball (2002) found that the N_2O production in May was generally higher in poorly drained clay soils than well-drained sandy soils (Skiba and Ball, 2002). It was likely due to the fact that smaller pore sizes in fine texture soils (e.g., clay soils) limited water drainage and hence increased anaerobic microsites, which in turn trigged denitrification and subsequent gas emissions (Pihlatie et al., 2004). This is consistent with the finding of greater N_2O production in compacted soils than less-compacted or uncompacted soils, as the compaction also results in decreased soil porosity and pore connectivity as suggested by increased bulk density (Ruser et al., 2006; Bessou et al., 2010) and changes in pore-size fractions. The soil aggregate size, in addition, is another factor influencing the magnitude of N_2O production. Uchida et al. (2008) found that the highest N_2O production occurred from in smaller soil aggregates (<1.0 mm) which had relatively higher bulk density than bigger aggregates. This observation was attributed to increased denitrification rate caused by decreased O_2 diffusivity and the build-up of an anaerobic environment within the smaller, denser aggregates (Uchida et al., 2008).

1.3.7 Nitrogen Management

1.3.7.1 N application methods

With the purpose of improving N use efficiency, ammonium-containing manures and fertilizers have been widely incorporated or injected into the soil rather than broadcasted on the surface, because such incorporation and injection application techniques successfully reduced substantial NH_3 losses through volatilization (Wulf et al., 2002; Nyord et al., 2008; Webb et al., 2010; Powell et al., 2011; Maguire et al., 2011). However, these N conservation application techniques could potentially lead to increased N_2O emissions, particularly in liquid manures (Flessa and Beese, 2000; Chadwick et al., 2000a; Nyord et al., 2008; Engel et al., 2010; Duncan et al., 2017). In contrast to the broadcasting of liquid manure, the incorporation and injection applications provide a soil environment favorable to producing N_2O, as they lead to abundant available N substrates for nitrifiers and denitrifers, increased anaerobic microsites and substantial available C (Flessa and Beese, 2000; Duncan et al., 2017).

1.3.7.2 N input

Fertilizer-induced N_2O emissions can be estimated based on the N_2O emission factor (EF), which is the percentage of emissions generated directly from the total N fertilizer applied to the soil. The current global default N_2O EF value recommended by Intergovernmental Panel on Climate Change (IPCC) is 1%, which assumes that the response of N_2O emission to increasing N input is linear (Smith et al., 2007). However, a global meta-analysis showed that this emission response is exponential for synthetic fertilizers and a majority of crops (Shcherbak et al., 2014).

Use of enhanced-efficiency N fertilizers, such as fertilizers containing nitrification inhibitors (NIs) and those with polymer-coated (PCFs), is considered to be an efficient N_2O mitigation option, with an IPCC estimation of 30% reduction in the global annual N_2O budget (Moomaw et al., 2001). According to a global meta-analysis, both NIs and PCFs are reported to significantly decrease N_2O emissions by 38% and 35% respectively compared to the conventional fertilizers (Akiyama et al., 2010). The mechanism of NIs is to block the autotrophic nitrification pathway by inactivating the proteins of related nitrifiers for a certain time (Vannelli and Hooper, 1992; Chaves et al., 2006; Benckiser et al., 2013). In this way, most inorganic nitrogen from manures or fertilizers can be kept in the soil as NH_4^+, delaying nitrifier and denitrifier activities and thus reducing N_2O emissions. In contrast, PCFs have a different mode of action. The function of the coating material in PCFs is to physically control N release at a slow rate that is in synchrony with plant metabolic requirements (Halvorson et al., 2014; Azeem et al., 2014).

1.3.7.3 N input timing

Fertilizer-induced N_2O pulses can be controlled by the right timing of application (e.g., fall versus spring). Several studies have quantified N_2O losses from fall versus spring applications, but the conclusions were inconsistent. In some cases, higher cumulative N_2O emissions were observed in the fall application compared to the spring, and this was likely due to the significant contribution of N_2O emissions produced during the spring thawing (Weslien et al., 1998; Thorman et al., 2007; Lin et al., 2017). The contrasting results, that greater N_2O emissions occurred in the spring than the fall application, seemed to be in part due to overriding N_2O emissions released during the growing season which is typically warm and moist than those released during thawing (Rochette et al., 2004; Hernandez-Ramirez et al., 2009; Cambareri et al., 2017).

1.3.8 Other management effects

Although environmental factors play an important role in regulating N_2O emissions, improved agricultural management practices provide potential opportunities to mitigate or moderate such emissions. Numerous studies have demonstrated that no-tillage is prone to soil C sequestration and implicated its potential to mitigate agricultural GHG emissions by means of long-term adoption of this improved practice (West and Post, 2002; Six et al., 2004; VandenBygaart et al., 2008). However, reported results with respect to the response of N_2O emissions to no-tillage are not consistent. Some studies indicated higher N_2O emissions under no-tillage than conventional tillage (Ball et al., 1999; Baggs et al., 2003; Rochette et al., 2008a), whilst others showed lower emissions in zero tilled soils relative to tilled soils (Chatskikh and

Olesen, 2007; Gregorich et al., 2008). This suggests that the impact of no-tillage practice on N_2O emissions is dependent on other factors, such as soil texture, drainage, crop choice, plant residues and climatic factors (Baggs et al., 2003; Desjardins et al., 2005; Rochette, 2008; Mutegi et al., 2010) --- all of which regulate soil aeration and thus influence N_2O emissions. Likewise, the magnitude of N_2O emissions induced by irrigation and drainage results from the combined effect of soil texture, microbial community, C and N availability and fertilizer application (Cai et al., 1997; Calderon and Jackson, 2002; Towprayoon et al., 2005; Sainju et al., 2010; Sainju et al., 2012). While the effect of crop rotation on N_2O emissions has been rarely examined and documented, the increased soil organic C caused by enhanced rotation complexity may have an opportunity to influence the soil N pools through microbial decomposition and immobilization processes (Campbell et al., 1996; West and Post, 2002), and hence possibly change the net N_2O emissions (Halvorson et al., 2014).

1.4 Knowledge gaps, research hypotheses and general aims

According to previous studies, timing of liquid manure application provides a possibility to reduce N_2O emissions in cropping ecosystems (Weslien et al., 1998; Rochette et al., 2004; Thorman et al., 2007; Hernandez-Ramirez et al., 2009; Lin et al., 2017); however, the right application timing (i.e., fall versus spring) is very likely to be affected by local soil and climatic conditions (Cambareri et al., 2017). Given that using NIs with fertilizers successfully reduced gaseous N losses (Di et al., 2014; Bell et al., 2015; Duan et al., 2016), it is an interesting research question to examine whether the addition of NIs could narrow or even close the N_2O emission gap between the fall and the spring application. Our previous one-year field study showed that the addition of NIs is able to close the N_2O-emission gap between the two application timings during a dry year (Lin et al., 2017), but this was not observed in a year with normal precipitation.

Thus, the treatments were continued in the very same research plots to examine the effects of manure application timings coupled with NIs on N_2O production and soil N dynamics in a year with normal weather conditions and to further evaluate the residual or carry over effects after two years of repeated liquid manure and NI amendments. Accordingly, we hypothesized that (1) the same amount of NIs would be less effective in closing the emission gap between different application timings in a normal precipitation year and that (2) there would be a residual effect of repeated manure additions in terms of more N_2O emissions.

Not only is soil moisture content a driving factor for N_2O production, it simultaneously may also impact the degradation of the NIs (Menéndez et al., 2012). To elucidate the impact of soil moisture content on the effectiveness of NIs, a controlled laboratory study is required. We therefore conducted a laboratory incubation with field soils collected in 2016 to investigate the interactions of NIs rates and soil moisture contents on the effectiveness of the NIs as evidenced by N_2O production responses in a Luvisolic and a Black Chernozemic soil. We expected higher N_2O emissions in Black Chernozemic than Luvisolic soil, because Black Chernozem has a higher organic matter content and a fine texture relative to Luvisol, which provide higher possibility of creating an anaerobic environment conducive to denitrification. Our hypotheses were (1) the efficiency of NIs in reducing N_2O emissions increased with an increase in NIs rates, (2) the efficiency of NIs in reducing N_2O emissions decreased with an increase in soil water content, and (3) the optimal rate of NIs in reducing N_2O emissions from Black Chernozemic soil was higher than that from Luvisolic soil under the same soil moisture condition.

While a number of studies have emphasized the significant contribution (30-90%) of N_2O produced during the spring thawing to the annual N_2O budget (Wagner-Riddle et al., 2007; Goldberg et al., 2008; Wolf et al., 2010; Wu et al., 2010; Yanai et al., 2011; Abalos et al., 2016;

Lin et al., 2017), the contribution of various microbiological processes to these N_2O hot momenta during spring thawing is not very well understood. Thus, a controlled mesocosm study was established using soils from a manure field experiment to distinguish the proportion of N_2O emissions derived from different N_2O-generating processes by identifying ^{15}N intramolecular positions within N_2O using a quantum cascade laser spectroscopy and to investigate the priming effect of N_2O production. We hypothesized that denitrification would dominate as a main source of N_2O emissions compared to nitrification in the course of thawing after freezing. This hypothesis is based on the knowledge that increased soil water content and temperature in the course of soil thawing typically result in enhanced microbial denitrification activity and hence increased N_2O production as described above. In addition, it is hypothesized that urea addition would result in a positive priming effect of N_2O emissions because urea additions can accelerate soil microbial activities related to the N cycle.

It is evident that the factors affecting the magnitude of N_2O production are complex and interrelated as reflected in the temporal and spatial variations in N_2O production rate. Despite the existence of numerous field studies involving the quantification of N_2O production, a modelling study can further contribute to develop a more accurate estimation for total N_2O emissions at a large scale and a better understanding of the N_2O-generating processes as well as their controlling factors. As such, a modelling study using the *Ecosys* model was conducted based on the dataset collected from a 2-year field project in central Alberta.

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2. Nitrous Oxide Emissions in Barley Croplands over 2.5 Years: Effects of Liquid Manure Timing and Nitrification Inhibitor Use

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2.1 Abstract

Increasing atmospheric N_2O concentrations from agriculture are a concern. Two-year field studies were established in Lacombe and Edmonton, Canada, to investigate the effects of liquid manure injection timing (fall vs. spring) with and without nitrification inhibitors (NIs), namely nitrapyrin [2-chloro-6-(trichloromethyl) pyridine] and 3, 4-dimethylpyrazole phosphate (DMPP), on N_2O production and soil inorganic N. Effects on the N use efficiency and N uptake efficiency of barley (*Hordeum vulgare* L.) and the residual effects on N dynamics were also investigated. The N_2O fluxes were measured in static manual chambers for 2.5 yr, except for months when the soil was frozen. Across all treatments receiving manure, annual N_2O emissions were 0.3 to 12.6 kg N_2O – N ha^{-1}, equivalent to a direct emission factor (EF_d) of 0.10 to 2.41%. Without NIs, the annual N_2O EF_d for the fall injection was fourfold greater than the spring injection in 2014 – 2015, whereas the reverse was seen in 2015 – 2016. This was probably caused by the extreme rainfall events in the spring of 2016. With the application of NIs in 2014–2015, the annual N_2O emissions for the fall application was consistently as low as that of the spring application. This was attributed to a reduction of the proportionally dominant spring thaw emissions from soils receiving manure in fall. Elevated N_2O emissions were observed in the spring of 2017. Denitrification was probably the major process contributing to N_2O production, as indicated by the increased NO_3 – N substrates at the end of the experiment and the springtime moisture conditions.

2.2 Introduction

Nitrous oxide has become the third largest anthropogenic greenhouse gas, with an increase of 6% in radiative forcing (0.17 ± 0.03 W m^{-2}) since 2005 (Myhre et al., 2013) and is also a reactant for the ozone depletion (Ravishankara et al., 2009). Atmospheric N_2O concentrations have increased markedly from ~270 parts per billion in 1750 to 324 parts per billion in 2011, with a constant growth rate of approximately 0.26% yr^{-1} over the past few decades (Myhre et al., 2013). This increased concentration is mainly attributed to human activity, including increased use of N fertilizers, intensification of agriculture, and burning fossil fuels (Intergovernmental Panel on Climate Change, 2007). It has been estimated that ~30 to 45% of the global N_2O emissions are anthropogenic, with agriculture being the largest contributor (Fowler et al., 2009). These anthropogenic emissions are projected to continue increasing in the next decades through expanded agricultural land and increased fertilizer use, which are mainly driven by the need to feed a growing human population (Reay et al., 2012). Effective N_2O mitigation strategies in agricultural systems are needed to cut global greenhouse gas emissions and retard the effect of climate change.

Soil N_2O production is primarily linked to microbial autotrophic nitrification and denitrification processes (Wrage et al., 2001; Braker and Conrad, 2011). In aerobic nitrification, N_2O is generated as a byproduct during the oxidization of ammonium (NH_4 – N) to nitrate (NO_3 – N) and it is formed as an intermediate product of anaerobic denitrification (Hayatsu et al., 2008). Factors influencing the nitrification and denitrification processes are thought to regulate the production and consumption of N_2O. Soil moisture content, as it interacts with O_2 availability, is a major factor impacting N_2O production (Butterbach-Bahl et al., 2013; Hagemann et al., 2016). Nitrification is dominant in well-aerated soils, whereas denitrification is more important in wet soils (Ruser et al., 2006). It has been hypothesized that the greatest N_2O emissions occur in water-filled pore space values ranging from 70 to 80%, where air-filled and water-filled pores exist simultaneously and where nitrification and denitrification enhance each other considerably (Davidson et al., 1991; Davidson and Schimel, 1995). Beyond this range, the N_2O emissions decrease, as the soil tends to become saturated and N_2 becomes the main product of denitrification (Bowman and Steltzer, 1998; Meixner and Yang, 2006). This can also occur when soils are very wet or saturated for extended periods (Rubol et al., 2012). Increased temperatures generally cause elevated N_2O production, as they enhance denitrification caused by decreased O_2 through respiration and stimulate the enzymes involved in N_2O generation (Dobbie and Smith, 2001; Butterbach-Bahl et al., 2013). Increased availability of NH_4 – N promotes nitrification and hence N_2O production when other factors are not limiting (Robertson and Groffman, 2007). When sufficient organic C and NO_3 – N are present in soils, N_2O fluxes can occur as a result of facilitated denitrification (Barnard et al., 2005). A recent study by Leytem et al. (2019) further established the responses of field N_2O emissions to increases in moisture and temperature in nitrate-enriched temperate manured soils.

The addition of NIs to manures or fertilizers has been documented to be an effective N_2O mitigation strategy (Pasda et al., 2001; Irigoyen et al., 2003; Cui et al., 2012; Burzaco et al., 2013; Zhang et al., 2018). The mechanism of nitrification inhibitors is to block the autotrophic nitrification pathway by inactivating the proteins of the related nitrifiers for a certain time (Vannelli and Hooper, 1992; Chaves et al., 2006; Benckiser et al., 2013). In this way, most inorganic N from manures or fertilizers can be kept in the soil in the form of NH_4–N, consequently delaying nitrifier and denitrifier activity and mitigating N_2O emissions.

Optimal manure application timing could be a N_2O mitigation strategy but the best timing choice is likely to be determined by local edaphic and climatic conditions. A few studies have compared N_2O losses between two application seasons but these have shown contrasting results. Cambareri et al. (2017) observed higher N_2O emissions from soils receiving dairy manure in spring than in fall when planted to corn (*Zea mays* L.) in a dry year in Elora, ON, Canada, whereas Thorman et al. (2007) found higher emissions when cattle slurry was applied in fall–winter than in spring in a grassland soil in England. In Canada, about half of the manure is typically applied in spring and half is applied in fall (Beaulieu, 2004) because both have their own advantages. Compared with the fall timing, spring manure application after snow has thawed can be an efficient way to enable plants to use nutrients and achieve optimal crop productivity, because this timing may reduce the potential nutrient losses that can occur through leaching or runoff during thawing and snowmelt periods because of the seasonal nature of the water budget in temperate regions (Dick, 2006). Fall manure applications may make more efficient use of the available human labor and equipment (Dick, 2006; Cambareri et al., 2017) but fall N applications result in increased N_2O losses when the soil thaws in the following spring (Lin et al., 2017).

A recent study suggested that application of liquid manure amended with NIs in fall could reduce the N_2O emissions associated with the spring thaw, thus reducing annual N_2O emissions compared with the corresponding spring applications (Lin et al., 2017). However, these results were found under pronounced freezing winter conditions with abundant snowfall, which were then followed by very dry spring and summer periods. As the magnitude of N_2O production is largely regulated by transient environmental factors, a contrasting outcome would be expected in a year with normal precipitation. Therefore, we undertook a multiyear study to evaluate the effects of liquid manure injections in the fall or spring with and without NIs to gain an insight into how N_2O production and soil N respond to variations in weather, as well as the residual effects after 2 yr of repeated manure and NI applications in the same experimental fields. This research approach addresses the cumulative effects of repeated manure and NIs addition.

2.3 Methods

2.3.1 Site description

The study was carried out from October 2014 to May 2017 in two agricultural fields in Alberta, Canada: the Field Crop Development Center in Lacombe (52°27′17″N, 113°44′20″W) and the South Campus Research Farm in Edmonton (53°29′30″N, 113°31′53″W). The physical and chemical soil properties in both locations are shown in Table 2-1. Both locations have a humid continental climate, with warm summers and typically extremely cold and snowy winters. According to historical weather records (1981–2010), the annual mean air temperature is 2.9 and 3.5°C, and the annual cumulative precipitation is 436.1 and 452.8 mm in Lacombe and Edmonton, respectively (Table 2-2).

2.3.2 Experimental design

The field plots (2.4 by 6.1 m) were arranged in an incomplete split-plot design replicated four and three times in Lacombe and Edmonton, respectively. There were two control treatments, including a control without disturbance and the control disturbed by the liquid manure injector. The other plots were assigned to one of two manure application timings for the whole plot (i.e., fall and spring). Each plot was divided into three split-plots for the NI treatments, which were manure application without a NI, application with with eNtrench Nitrogen Stabilizer (Corteva Agriscience, Agriculture Division of DowDupont, Calgary, AB, Canada) (200 g nitrapyrin L^{-1}), and with ENTEC (Eurochem Agro, Mannheim, BW, Germany) (50 g DMPP L^{-1}).

Swine and dairy liquid manures were applied in Lacombe and Edmonton, respectively. The manure was applied once a year in fall or spring for 2 yr (i.e., 2014–2015 and 2015–2016) in the same experimental plots. The application rate of liquid manure was uniform across all injections at 56.17 m^3 manure ha^{-1}. Over both years, the liquid manure was incorporated into the same soil to a depth of 12.7 to 15.2 cm with an Avenger coulter manure injector (Yetter Manufacturing Inc., Colchester, IL). To ensure homogeneous distribution, the liquid manure was mechanically stirred for 20 mins in the injector tank prior to application. The NI was added into the manure tank prior to stirring at a rate of 0.4 kg a.i. ha^{-1} for both timings in both years. This NI application rate was the rate recommended by the manufacturer of DMPP but is only part of the rate recommended by the manufacturer of nitrapyrin (i.e., 40 and 80% for the fall and spring liquid manure applications, respectively). Keeping the same inhibitor rate for both fall and spring and for the two NIs meant that the results were directly comparable across all timing–inhibitor combinations. The injector created five injection bands of 2.5 cm wide each. Adjacent manure injection bands were spaced about 28 cm apart. Manure was applied after sowing and the direction of injection was perpendicular to the seeding row to reduce potential damage to the barley seeds.

2.3.3 Gas sampling, analysis, and calculation

In situ soil N_2O flux measurements were obtained via the nonsteady-state static chamber method. Plexiglass rectangular chambers were installed immediately after manure injection, removed during the plant harvest and seeding, and reinstalled to the same position shortly afterwards. Each chamber was placed near the middle of each plot, consistently crossing two manure injection rows and lying between two seed rows. The cross-sectional area of each chamber was 1113.5 cm^2 (65.5 cm by 17.0 cm), and its height was 15 cm (5 cm below and 10 cm above the ground). To avoid temperature perturbations, the external faces of the chambers were covered with the reflective tape and the chamber lids had reflective foil bubble insulation attached in place with silicone (Parkin et al., 2003). A polyvinyl chloride vent tube (0.48 cm i.d.

and 40 cm in length) was connected to the chamber lid to reduce pressure and sampling perturbations (Hutchinson et al., 2000; Parkin et al., 2003; Xu et al., 2006). Each chamber lid had a sampling port equipped with a halobutyl septum.

Gas inside the chambers was sampled throughout the experimental period (October 2014–May 2017) except for the frozen months, typically from November to March. Samples were taken twice per week after manure injections or intensive rainfalls and during the early spring thaw; otherwise, sampling was undertaken once weekly. The headspace gas samples (20 mL) were taken at 16, 32 and 48 min with a BD syringe with a Luer-Lok tip connected to a BD 23-gauge needle (Franklin Lakes, NJ). Gas samples were then transferred into a 12-mL pre-evacuated Exetainer vial with a pierceable chlorobutyl septum cap (Labco, Lampeter, Wales, UK). At the beginning, in the middle, and at the end of the sampling event, three to six ambient gas samples were taken at 10 cm above the soil surface. These ambient samples were used to determine the gas concentration at the point of chamber closure (t_0). The gas samples were stored at 4 to 5°C until analysis to prevent potential gas leakage.

All gas samples were analyzed via gas chromatography. Nitrous oxide concentrations were determined with a Varian Model 3800 gas chromatograph equipped with a Combi-Pal autosampler and electron capture detector (Varian Inc., Walnut Creek, CA). The vertical flux of N_2O at the soil–atmosphere interface was determined by first-order or second-order polynomial regression (Yates et al., 2006; Braun et al., 2013). For this regression, the flux (F) was calculated as the slope of a simple linear regression or as the first derivative of a quadratic regression at t_0 (Eq. [1]). Nonsignificant regressions ($P > 0.2$) of N_2O concentration versus time were considered as zero flux. In Lacombe and Edmonton, 1696 and 1272 N_2O flux measurements, respectively, were taken throughout the whole experiment. About 62, 27, and 11% of the measurements were determined by first-order regression, second-order regression, and zero flux, respectively, at Lacombe; 72, 15, and 13% were determined at Edmonton.

$$F = (\frac{dC}{dt}) \times (\frac{V}{A}) \times (\frac{P}{R \times T}) \times 2M \times k, \quad [1]$$

where F is the N_2O flux (μg N_2O–N ha^{-2} d^{-1}), $\frac{dC}{dt}$ is the slope of a simple linear regression or the first derivative of a quadratic regression at t_0 (μL L^{-1} min^{-1}), V is the headspace volume of the gas chamber (L), A is the cross-sectional area of the gas chamber (m^2), P is the pressure of the gas (Pa), R is the gas constant (Pa μL K^{-1} $μmol^{-1}$), T is the temperature of the gas (K), M is the molar mass of N (g mol^{-1}), and k is a conversion factor for the flux unit (from μg N_2O – N m^{-2} min^{-1} to g N_2O – N ha^{-2} d^{-1}).

Cumulative N_2O emissions were obtained by linear interpolation of N_2O flux between two adjacent measurements and numerically integration of the area below the curve in a given time interval. Because of the varying N concentrations in the liquid manures (Table 2-3), the manure N rates varied across application timings, even though the application volumes were kept constant. For instance, the manure N application rate was 1.65 times higher in the spring than in the fall timing for the Lacombe site. In an attempt to account for these varying manure N rates, a direct N_2O emission factor (EF_d), which was the amount of N_2O released from the N added) was calculated to make cumulative N_2O emissions among different manure applications comparable (Eq. [2]).

$$EF_d = \frac{(E_m - E_c)}{Ninp} \times 100,$$

[2]

where EF_d is the direct N_2O emission factor (%), E_c is the N_2O emissions from the control soil without manure (kg N ha^{-1} yr^{-1}), E_m is the N_2O emissions from the soil with manure (kg N ha^{-1} yr^{-1}), and Ninp is the rate of total N input in the liquid manure (kg N ha^{-1} yr^{-1}).

2.3.4 Soil sampling and analysis

Three or more composite topsoil samples (0–15 cm) were taken from each plot with a push probe (2.5 cm i.d.) or an auger (3.5 cm i.d.) throughout the experiment in Edmonton and in 2015–2016 in Lacombe (Fig. 2-1, Fig. 2-2). Because of the uneven nutrient distribution created by manure injection, soil samples for each fall plot were collected separately from the injection bands and the noninjection areas when the soil sampling took place between manure injection in the fall and the subsequent spring thaw. Likewise, soil samples from each spring plot were collected separately when soil samples were taken between the manure injection in the spring and the following harvest. The edges of the plots were avoided during sample collection. The NH_4–N and NO_3–N concentrations in the plots from separate soil samples were aggregated and presented on the basis of specific percentage weights for the injection bands (8.2%) and the noninjection area (91.8%).

Fresh soil samples were stored at 5°C before air drying. Air-dried soils were sieved (2 mm mesh) for further analyses. Soil texture, bulk density, pH (1:2 soil/water ratio), and 2 M KCl-extractable NH_4–N and NO_3–N were determined according to the standard methods (McKeague, 1978; Carter and Gregorich, 2008). Total organic C and total N were determined via dry combustion with a Costech Model EA 4010 Elemental analyzer (Costech International Strumatzione, Florence, Italy) (Cunniff, 1996; Sparks et al., 1996). Extractable NH_4–N and NO_3–

N in the filtrates were determined colorimetrically on a SmartChem 200 Discrete Wet Chemistry Analyzer (Westco Scientific Instruments, Inc., Brookfield, CT).

2.3.5 Plant sampling, analysis, and calculations

Barley (*Hordeum vulgare* L.) for silage was planted at a rate 300 seeds m^{-2} on 24 May 2015 and 3 June 2016 at Lacombe, and 11 May 2015 and 14 May 2016 at Edmonton. Adjacent seeding rows were spaced about 18 and 30 cm apart in Lacombe and Edmonton, respectively. The barley variety Muskwa (Markert Sees Ltd., Alberta, Canada) and variety CDC Austenson (SeCan, Ontario, Canada) were planted in Lacombe and Edmonton, respectively.

The aboveground biomass was removed on 11 Aug. 2015 and 19 Aug. 2016 in Lacombe and 28 July 2015 and 15 Aug. 2016 in Edmonton with a forage harvester after the dough stage. The harvested area in each experimental plot had a width of 0.6 m and a length of 2.0 to 4.5 m. A subsample was taken from each plot then oven-dried at 60°C until a constant weight was reached. The aboveground dry matter biomass was based on the oven-dry weight. The protein content of the silage barley was assessed via a near-infrared reflectance spectroscopy (Roberts et al., 2004).

Similar to EF$_d$, the N use efficiency (NUE) and N uptake efficiency (NUEff) of barley were calculated to make the aboveground biomass and barley N content comparable among different manure injections (Eq. [3] and Eq. [4]).

$$NUE = \frac{DM_m - DM_c}{Ninp}; \quad [3]$$

$$NUEff = \frac{PN_m - PN_c}{Ninp}, \qquad [4]$$

where NUE is the N use efficiency of barley (kg plant dry matter kg^{-1} N), DM_m is the aboveground dry matter of barley from the manured soil (kg ha^{-1}), DM_c is the aboveground dry matter of barley from the nonmanured soil (kg ha^{-1}), Ninp is the amount of total N in the liquid manure (kg N ha^{-1}), NUEff is the N uptake efficiency of barley (kg plant N kg^{-1} N), PN_m is the N uptake of barley for each treatment receiving manure (kg N ha^{-1}), and PN_c is the N uptake of barley for the controls (kg N ha^{-1}).

2.3.6 Manure sampling and analysis

During manure injection, three samples of liquid manure were taken from the manure without NI addition, the manure with DMPP, and the manure with nitrapyrin. A 2-L subsample of each manure sample was then collected to quantify the total N, NH_4–N, and water content in the liquid manures (Peters et al., 2003). The samples were heated at 110°C until they had dried and the water content was determined as the gravimetric weight lost. Ammonium was determined by steam distillation into boric acid, followed by titration with a standard acid. Samples were combusted at 900°C in the presence of O_2 and the combustion gases were then reduced to N_2, which was subsequently separated in a gas chromatographic column and detected by thermal conductivity to determine the total N (organic N and inorganic N) in a vario MACRO instrument (Elementar, Langenselbold, Germany).

Half-hourly values of soil volumetric water content and soil temperature at depths of 10 and 20 cm were recorded during the experiment by Decagon 5TM sensors and EM50 data loggers (Decagon Devices, Inc., Pullman, WA). The average water-filled pore space (WPFS) at

10- to 20-cm depth was calculated on the basis of the measured daily average volumetric water content (VWC) and bulk density (Eq. [5]). The average WFPS values were displayed in Fig. 2-1a and Fig. 2-2a only if they met the criterion that the daily average soil temperature was above 0°C.

$$WFPS = \frac{VWC}{1 - \frac{\rho_b}{\rho_s}} \times 100, \qquad [5]$$

where WFPS is the water-filled pore space at 10- to 20-cm depth (%), VWC is the daily average soil volumetric water content (cm cm^{-3}), ρ_b is the soil bulk density (g cm^{-3}) (Table 2-1), and ρ_s is the soil particle density, with a value of 2.65 (g cm^{-3}).

2.3.7 Statistical analysis

Statistical analyses were carried out in R version 3.1.3 (R Core Team, 2014). The data were transformed to meet the assumptions of normality and homoscedasticity if necessary. Significant differences in EF_d, NUE, NUEff, annual cumulative N_2O emissions, plant aboveground dry matter yield, plant N uptake, soil NH_4–N and NO_3–N among the treatments were determined via one-way ANOVA ($P < 0.05$) for a random effect model, as well as the differences in total N, NH_4–N, and water content in liquid manure among four injection times. The interactive effect of year (first vs. second experimental year) × timing on EF_d, NUE, and NUEff, and the effect of timing (fall vs. spring) × additives (manure only, DMPP, and nitrapyrin) were examined via two-way ANOVA with interaction analysis ($P < 0.05$).

2.4 Results

2.4.1 Precipitation and temperature

In Lacombe and Edmonton, the 30-yr average monthly temperatures from November to March (defined as frozen months) were below 0°C and the temperatures from April to October (defined as unfrozen months) were above 0°C (Table 2-2). During the experimental period, the average frozen and unfrozen month temperatures in both locations increased by 0.6 to 4.0°C compared with the corresponding average temperatures over 30 yr (Table 2-2). These increases in temperature were greater in Edmonton than in Lacombe (Table 2-2). In the first experimental year (2014 – 2015), the net cumulative snowfall was at least 56% higher than average in both locations and the rainfall in the following unfrozen months was 15% and 43% lower than average in Lacombe and Edmonton, respectively (Table 2-2). In the second experimental year (2015 – 2016), snowfall was 20% lower and rainfall was 20% greater than average in Edmonton, whereas precipitation varied only slightly in Lacombe (Table 2-2).

2.4.2 Manure characteristics

Total N (organic and inorganic N), NH_4-N, and water content in the liquid manure were significantly different among the four injection timings (Table 2-3). At least 92% (w/w) of the applied manures was water (Table 2-3). The total N (organic and inorganic N) varied from 245.2 to 521.1 kg N ha^{-1} at Lacombe; 56.8 to 76.5% of the total N was in NH_4-N form (Table 2-3). The total N (organic and inorganic N) ranged from 380.7 to 458.1 kg N ha^{-1} at Edmonton; 48.5 to 59.4% of the total N was NH_4–N (Table 2-3).

2.4.3 N_2O production

Throughout the experimental period (October 2014–May 2017), increased N_2O fluxes typically occurred during the spring thaw and after liquid manure application in both locations (Fig. 2-1d, Fig. 2-2d). In the late fall of 2016 (after harvest), there was another recognizable N_2O flux in Edmonton (daily N_2O for 20 Oct. 2016 was 0.09 kg N ha^{-1} across all treatments), which was as pronounced as the N_2O peaks observed during the spring thaw periods (N_2O was 0.12 kg N ha^{-1} for 7 Apr. 2015, 0.07 kg N ha^{-1} for 15 Mar. 2016, and 0.10 kg N ha^{-1} for 4 May 2017; Fig. 2-2d).

The two experimental years showed significantly different N_2O EF_d results in Lacombe and Edmonton (Table 2-4). In the first experimental year, the annual N_2O EF_d for the fall manure treatment without NI (FMW) was about four times greater than that of the spring manure treatment without NIs (SMW) in both locations (Fig. 2-3a). The largest contribution to the total annual N_2O budget for the fall treatment came from the emissions occurring during the spring thaw (~21-d duration), but the largest contribution to the spring treatments came from the emissions in spring and summer (Fig. 2-3a). In the second experimental year, the annual N_2O EF_d for the SMW treatment in Lacombe was about four times greater than that of FMW but there was no significant difference among the treatments in Edmonton (Fig. 2-3b). The emissions cumulated during the spring thaw still made up the majority of total N_2O emissions for the fall treatments in Lacombe but not in Edmonton (Fig. 2-3b, Table 2-5). Relative to the substantial N_2O emissions under SMW during the spring and summer 2016 in Lacombe, the spring application of DMPP significantly reduced cumulative emissions, though this inhibitory effect was not observed with nitrapyrin (Fig. 2-3b). During the spring of 2017 in Lacombe, the cumulative N_2O emissions from spring-manured soils were numerically higher than those of the controls (Fig. 2-4). Likewise, higher emissions than the controls were also recorded for both fall- and spring-manured soils in Edmonton, as expected. It is noticeable the overall emissions of the spring-manured soils were higher than those of the fall-manured fields (Fig. 2-4).

2.4.4 Soil ammonium and nitrate

Before the field experiment (30 Sept. 2014), both soil NH_4 – N and NO_3–N (at 0–15 cm) were higher in Edmonton than Lacombe (i.e., NH_4–N: 6.5 vs. 4.3 mg kg^{-1}; NO_3–N: 21.0 vs. 9.9 mg kg^{-1}; Fig. 2-1b – c, Fig. 2-2b–c, Fig. 2-4a–b). At the end of the 2-yr experiment (12 Oct. 2016 in Lacombe and 30 Sept. 2016 in Edmonton), the NH_4–N and NO_3–N at 0- to 15-cm depth decreased for the two control treatments, as did the average NH_4–N for all manure treatments at both locations; however, the NO_3–N for all manure treatments increased to 27.5 and 14.5 mg kg^{-1} on average in Edmonton and Lacombe, respectively (Fig. 2-1b–c, Fig. 2-2b–c, Fig. 2-4a–d). Notably, from 12 Oct. 2016 to 16 May 2017, the soil NH_4–N for the fall manure treatments in Lacombe dramatically increased by more than 58.8% (Fig. 2-1b).

2.4.5 Plant yield and N uptake

Manure application largely increased the dry matter yield and aboveground N uptake of barley compared with the controls, regardless of the location and year (Table 2-6). The effect of injection timing and NIs on plant NUE and NUEff was not clear in either location in both experimental years (Fig. 2-3c–f). However, the results for NUE and NUEff were significantly different between the two experimental years (2015 vs. 2016) at both sites (Lacombe and Edmonton) (Table 2-4). More specifically, both NUE and NUEff at both locations in the first year were higher than in the second year on average across all treatments (e.g., N use efficiency in Lacombe was 12.5 and 6.2 kg dry matter dry kg^{-1} total N in the first and second years, respectively; Fig. 2-3c–f), showing a potential residual effect across the two years of the study.

2.5 Discussion

2.5.1 Effects of manure timing on annual N_2O production and seasonal changes

The contrasting results for annual EF_d in response to manure timing in the two experimental years reflects the different contributions of seasonal N_2O emissions. This demonstrates the impact of environmental conditions and their temporal changes on N_2O production. In the first experimental year (2014 – 2015), the significantly higher annual EF_d of FMW relative to SMW in both locations was a result of increased N_2O emissions during the spring thaw for FMW, combined with limited N_2O emissions during spring and summer for FMW and SMW (Fig. 2-3a). The spring-thaw-induced N_2O emissions were more dominant in the first year than in the second year for soils receiving the fall manure injection, which could be ascribed to the wetter soil conditions at the onset of spring in 2015 than in 2016 (Fig. 2-1a, Fig. 2-2a). During the frozen months (November–March), the first year's snowfall exceeded the 30-yr average in both locations but it was slightly lower than average in the second year (Table 2-2). As the soil temperature gradually increased in spring, the accumulated snowpack started to melt and the melting water infiltrated the thawing soil, leading to an increase in soil moisture content. The magnitude of moisture increase was related to the snowfall over the previous months. In Edmonton, the soil moisture content in early spring increased to ~60 to 80% WFPS, though it only increased to ~25 to 40% WFPS in the second year (Fig. 2-2). Previous studies have found that N_2O production generally increases with increasing soil WFPS (Dobbie and Smith, 2003; Schaufler et al., 2010) and the highest level of production was postulated to occur in the range of 70 to 80% WFPS, depending on the soil type (Davidson et al., 1991; Davidson et al., 2000; Butterbach-Bahl et al., 2013). Therefore, although the N substrates were sufficient for microbes in the fall-manured soils at the beginning of the spring in both years, the N_2O response to the spring thaw was more evident in the first year than the second owing to the more pronounced increase in soil moisture. A controlled study by Adair et al. (2019) further substantiate the suggestion that thawing of soils that had received manure injection led to increased N_2O production.

In addition to the higher N_2O emissions observed during the spring soil thaw, higher emissions were also discovered in soils shortly after the manure was applied, provided thT temperature and moisture were favorable (Fig. 2-1, Fig. 2-2), as reported in previous studies (Ruser et al., 2006; Cui et al., 2012; Weller et al., 2019). The substantial amount of N_2O emitted following the spring manure application in 2016 largely contributed to the higher annual EF_d under SMW than under FMW regardless of the location in the second year (2015 – 2016) (Fig. 2-3b). On one hand, the increased emissions in the second year were probably caused by the occurrence of heavy rainfall, in contrast to the lack of rainfall in the first year (April–October; Table 2-2). On the other hand, these high emissions were possibly caused by the increased NH_4–N and NO_3–N availability for microbial activity, as suggested by the results for the second year of liquid manure injection. Assuming that barley's N uptake was consistent across growing seasons, the higher NH_4–N content of the liquid manure applied in spring 2016 (relative to spring 2015, Table 2-3) would have provided more substrates for nitrification and denitrification. As a result, excess NH_4–N and NO_3–N that was not used by the barley would be available for soil microbes, ultimately producing more N_2O emissions in moist soils after the spring manure application in the second year (Fig. 2-1d, Fig. 2-2d, Fig. 2-3b).

As aerobic and anaerobic microsites coexist in unsaturated soils (<100% WFPS), it is highly possible that both nitrification and denitrification contributed to the intensive N_2O emission pulses observed in our study (Bateman and Baggs, 2005). As evidence of this, when

WFPS was above 60% during the spring thaw in 2015 (Fig. 2-2a), denitrification seemed to become more significant as a result of decreases in the O_2 supply (Davidson and Schimel, 1995; Ruser et al., 2006). In specific situations where the soil WFPS was above 80% (6 d had >80% WFPS in Edmonton, Fig. 2-2a), the probability of complete denitrification might increase with an increasing proportion of anaerobic microsites, enlarging the fraction of N_2O reduced to N_2, hence decreasing N_2O emissions (Bowman and Steltzer, 1998; Schindlbacher et al., 2004; Meixner and Yang, 2006; Butterbach-Bahl et al., 2013). This might be an explanation for why the N_2O emissions produced during the very wet spring thaw in the first year of the study under the FMW treatment in Edmonton was quantitatively equivalent to that produced during the moderately wet spring thaw in the second year (i.e., 1.6 kg N_2O–N ha^{-1} in the first year vs. 1.8 kg N_2O–N ha^{-1} in the second year; Table 2-5, Fig. 2-3). However, this phenomenon was not observed in Lacombe, possibly because the soil texture in Lacombe is sandier than that of Edmonton (Table 2-1). With a higher fraction of sand, the soil in Lacombe provides better drainage and aeration under wet conditions, thus reducing the proportion of anaerobic microsites and the duration of anaerobic conditions, which, in turn, limited the occurrence of complete denitrification. Thus, greater N_2O emissions were generated during the wetter spring thaw in Lacombe in the first year (Table 2-5, Fig. 2-3). Despite the fact that the dominant process of N_2O production could be conjectured simply on the basis of the circumstances at the time (i.e., WFPS), there is still little quantitative information about the proportions of concurrent contributions of nitrification and denitrification to total N_2O emissions.

In addition to the N_2O flux generated during the spring thaw, the release of accumulated N_2O was considered as another source contributing to thaw-related emissions. Microbe-facilitated nitrification and denitrification processes can be sustained at temperatures below or near 0°C in soils (Dorland and Beauchamp, 1991; Schimel et al., 2004; Clark et al., 2009; Wertz et al., 2013), resulting in N_2O formation (Teepe et al., 2001a; Gregorich et al., 2006). Once the soil temperature started to increase in spring, N_2O emissions accumulated below and within the frozen layer during the winter consequently escaped from the soil profile (Risk et al., 2013). Though the winter-generated N_2O emissions have been recognized, their contribution to the elevated N_2O emissions in the early spring might be small, as supported by the low nitrification and denitrification transformation rates observed in previous studies (Dorland and Beauchamp, 1991; Clark et al., 2009).

2.5.2 Effects of NIs on N_2O emission reduction

Because of degradation, the ability of NIs to retard nitrification and thus eliminate potential NO_3–N losses (e.g., N_2O) can persist for only a certain time. Therefore, the effect of NIs on N_2O reduction was discussed in the context of the related seasonal N_2O reductions. No matter what the level of soil moisture was (saturated or moderately moist), a numerical reduction in total spring thaw N_2O emissions through the application of NIs was consistently observed for all fall manure applications (Fig. 2-3). This confirmed the long-term effectiveness of fall-applied NIs subjected to a cold fall and a 6-mo freezing winter, showing evidence of limited degradation under cool temperatures. In addition, similar spring thaw N_2O emissions under FMW in Edmonton were detected in both years but the magnitude of the N_2O reduction induced by NIs was greater in the second year, in which the soil was moderately moist in the early spring (Fig. 2-2, Fig. 2-3). The difference in N_2O reduction might be caused by the difference in the degradation rate of NIs, which may have been affected by different environmental conditions, in particular the contrasting soil moisture contents (Keeney, 1980; Woodward et al., 2016). To the best of our knowledge, it is still unclear how the degradation rates of different NI products respond to various soil moisture levels, particularly in fields with high nutrient availability and near N saturation resulting from repeated manured additions.

Likewise, the numerical reductions in spring–summer N_2O caused by NIs were detected in the first year's spring application at Lacombe and the second year's spring application in Edmonton (Fig. 2-3). However, this was not observed in the first year at Edmonton, when there was an extremely dry growing season (spring ‑ summer in Fig. 2-2) and most fluxes were consistently low. Nitrification and denitrification were probably limited by the soil dryness (Maag and Vinther, 1996; Del Prado et al., 2006), resulting in lower spring ‑ summer N_2O emissions and an annual EF_d of 0.1 ± 0.05 %, which was one order of magnitude lower than the default EF_d value (1%, with an uncertainty range of 0.3–3%) proposed by the Intergovernmental Panel on Climate Change (De Klein et al., 2006). In other words, even though NIs' active principles can be functional in a dry year, there is no need to slow down nitrification, as this process is already slow because of the soil dryness; these dry conditions are not conducive to N losses anyway. However, relative to the positive control (SMW), the spring application with DMPP and even the spring application with a reduced rate of nitrapyrin (i.e., lower than the rate recommended on the commercial label) showed a tendency to reduce N_2O emissions in the spring and summer of 2015 at the Lacombe site (Fig. 2-3) under relatively dry conditions (Table 2-2), suggesting the effectiveness of NIs across a wide range of environmental conditions. We suggest that the recommendations for NI application rates should be developed further and more comprehensively by accounting for specific soil characteristics and varying weather conditions.

Given the consistent results of low annual N_2O EF_d seen for certain treatments with NI additions (e.g., fall application with DMPP vs. spring application with DMPP), the use of NIs offers the opportunity to minimize the differences and variations in N_2O emissions induced by different manure timings. The failure to narrow down the annual N_2O difference between FMW and SMW in the second year at Lacombe can be mainly attributed to the reduced rate of spring-applied NI, as discussed above. This may also partly explain why the reduction in annual N_2O by NIs in the second year at Edmonton was not evident. Therefore, we hypothesize that the application of NIs will result in reduced emissions and consistently low N_2O emissions between the fall and spring manure injections, as long as sufficient NIs are added into the soil. Further investigation of these uncertainties is required under broader environmental and edaphic conditions and with different NI formulations and rates.

2.5.3 Effect of 2 yr of repeated liquid manure injections on subsequent N dynamics

After the 2 yr of repeated additions of liquid manure to the same soil, residual effects were discovered in terms of N_2O emissions and soil mineral N (NH_4–N and NO_3–N) concentrations. The soils with obviously higher NO_3–N concentrations (at 0–30 cm) at the end of the 2-yr experiment generated correspondingly larger N_2O emissions during the following spring thaw in 2017 (Fig. 2-4). These observations indicate the dominant role of denitrification for spring thaw N_2O emissions over nitrification. This is further supported by the soil moisture conditions being favorable to denitrification during the same period, fluctuating between 60 and 80% WFPS (Fig. 2-1, Fig. 2-2) (Davidson et al., 1991; Davidson et al., 2000). Our hypothesis is also supported by an earlier study, in which denitrification was shown to be the main process generating N_2O in a fertilized sandy loam soil subjected to 76% WFPS and evaluated via the ^{15}N-labelling technique (Senbayram et al., 2009).

The increased NO_3–N concentrations observed in the upper soil layers at the end of experiment could be a direct or indirect result of previously manure additions. It is noteworthy that the amount of liquid manure applied in our study was the maximum allowed by local regulations. The total N and NH_4–N loads applied via manure injections during the study consistently far exceeded the crop N removed (Table 2-3, Table 2-6). With such high manure N rates, the increased NO_3–N in the manured soils could be the residual N that originated from the inorganic N in liquid manure that was not used by plants or lost to the environment via other pathways. Likewise, the organic N present in the liquid manure additions could be another N supply for a increased NO_3–N through mineralization and ammonification, as considerable amounts of organic N were applied to the soils: ~124 and 186 kg organic N ha^{-1} on average in Lacombe and Edmonton, respectively. Chadwick et al. (2000b) measured the N mineralization rates of various animal manures in an incubation experiment and reported that 12 and 27% of organic N in dairy and swine slurry, respectively was mineralized after 199 d. Higher NO_3–N concentrations were not observed in the soils receiving fall manure at Lacombe (Fig. 2-4) but an apparently increased NH_4–N level at a depth of 0 to 15 cm was discovered in the same field in the next spring (Fig. 2-1). This delay in N availability and transformation might be evidence that the organic N in the liquid manure was a source of the higher mineral N concentrations, as different manure timings would result in mineralization taking place at different times. Furthermore, a priming effect induced by the repeated manure additions might also be responsible for the increased soil NH_4–N and NO_3–N. Earlier studies have found that the addition of synthetic N fertilizers to soils caused priming effects, accelerating mineralization (Westerman and Kurtz, 1973; Azam et al., 1993; Blagodatskaya et al., 2007; Chen et al., 2014) and thus increasing the soil NH_4–N and NO_3–N availability. A recent report indicates that the native soil N mineralization was ultimately increased by the addition of urea with or without NIs

(Ma et al., 2015). More research is necessary to elucidate and address the priming effects on N dynamics in soils receiving manure additions as a nutrient source.

2.5.4 Cumulative responses of plant NUE and NUEff

The N restricted from loss to the environment by NIs or by manure timing did not seem to be beneficial for barley growth, according to the differences in plant NUE and NUEff (Table 2-7, Fig. 2-3). Our findings for NI treatments were in agreement with the results reported in two meta-analysis studies, in which DMPP did not lead to a significant increase in crop yield when used in conjunction with different fertilizer forms (Abalos et al., 2014; Yang et al., 2016). It should be acknowledge that any N preserved in the soil system may easily be lost through other pathways (instead of being taken up by plants) such as nitrate leaching, NH_3 volatilization, complete denitrification, and immobilization (Högberg et al., 2006; Senbayram et al., 2012). As mentioned above, the manure N application rates were substantial and much higher than the recommended rate for agronomic N (i.e., 45–112 kg N $ha–^1$) (Alberta Agriculture, Food and Rural Development, 2004); therefore, the added N was more than enough to meet crop requirements in all manure treatments, leading to nonsignificant differences in plant NUE and NUEff among the treatments. In addition, we infer that the amount of N saved from loss to the environment was too small to deliver a significant gain in crop productivity. For instance, across all treatments, the proportion of N_2O loss from the manure N (calculated as annual N_2O EF_d) varied from 0.1 to only 2.4 % (Fig. 2-3). These annual N_2O EF_d values in our study were somewhat in line with the value reported by a meta-analysis, in which the EF_d for liquid manure was about 1.12 ± 0.18% for 149 observations, with a median N rate of 148 kg N ha^{-1} (Charles et al., 2017).

In our study, the NUE for the FMW treatment was numerically higher than that for the SMW treatment in all cases (Fig. 2-3), possibly indicating that barley seeds were damaged by the manure injection operation in the spring. Thus, an alternative could be to apply manure application prior to sowing to prevent any potential crop seed damages that would impact productivity. In addition, the greater NUE seen under FMW than under SMW may also be related to the amount of manure applied. In general, less manure N was applied in fall than in spring (Table 2-3). This would have resulted in greater NUE under FMW than under the SMW treatment if the crop N demand was satisfied and surpassed by both manure timing treatments, which appears to have been the case.

Although there was no consistently significant difference in plant NUE and NUEff across all scenarios, the NUE and NUEff in the first year were significantly higher than in the second year of manure application (Fig. 2-3). This could be explained, in part, by the detrimental impact of excessive N input in the second year (Table 2-3). Previous studies have found a quadratic relationship between N input and plant yield response (Schwenke and Haigh, 2016; Schlegel and Havlin, 2017; Hijbeek et al., 2017). More specifically, plant yield increases with increasing N input until it reaches a maximal yield response; after this, the yield reaches a plateau or decreases with any further increase in N input. In our study, the plant-available NH_4–N present in the liquid manure applied in the second year was higher than in the first year for each manure timing treatment in both locations (Table 2-3). The amount of manure NH_4–N in the second year seemed to be too great to optimize yield; hence, the general tendency of NUE and NUEff to decrease from the first to the second year was seen in our study. Furthermore, the increased soil N availability during the second year also corresponded to the cumulative effects of recurring manure additions on accelerated N dynamics in these soils with a known high fertility baseline.

2.6 Conclusions

The responses of annual and seasonal N_2O emissions to the timing of manure injection combined with NI additions differed between the two consecutive experimental years. In the absence of NIs, the fall manure application showed significantly greater annual N_2O emissions than the spring application in the first year, whereas higher N_2O emissions were observed for the spring application in the second year. This can mostly be attributed to differences in the weather between the two experimental years. Based on our observations, three out of four cases (two locations × 2 yr) experienced abnormal yearly weather, potentially altering the annual and seasonal distribution of N_2O losses. This implies the possibility of a strong feedback loop between escalating climate change and global N_2O sources and sinks, suggesting that further efforts to explore effective N_2O mitigation strategies adapted to future climate change are necessary.

Obvious N_2O reductions resulting from by the addition of NIs was only discovered in circumstances where the edaphic conditions facilitated accelerated nitrification and denitrification, such as moist conditions combined with above-zero soil temperatures. because of the effective reduction in the early-season N_2O emissions, which were the major contributors to the annual budget, the annual N_2O emissions from the fall manure applications were consistently low as those from the corresponding spring application. Different levels of spring ‑ summer N_2O reduction by nitrapyrin were observed in Lacombe between the two experimental years, indicating the influence of environmental factors on NI degradation and the importance of using an appropriate NI application rate. Accordingly, further efforts to better understand the optimal NI rates under various soil conditions are needed.

Residual effects during the spring period after2 yr of repeated manure injections were evident in the form of higher elevated N_2O emissions and abundant recovered soil NO_3–N, suggesting a possible soil priming effect caused by earlier manure additions.

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2.9 Tables

Table 2-1. Soil physical and chemical properties (0-15cm) prior to the treatment establishment in Lacombe and Edmonton.

Properties	Lacombe	Edmonton
Classification (U.S.)	Udic Boroll subgroups	Udic Boroll subgroups
Classification (Canada)	Black Chernozem	Black Chernozem
Texture (0–15 cm)	Clay loam	Clay
Clay (%)	32.0±0.5†	47.0±1.2
Silt (%)	36.4±1.3	36.0±0.3
Sand (%)	31.7±1.4	17.0±1.0
Organic C (g kg^{-1})	58.3±2.1	64.7±2.8
Total N (g kg^{-1})	5.1±0.2	6.1±0.2
Ammonium (mg N kg^{-1})	4.3±0.3	6.5±0.2
Nitrate (mg N kg^{-1})	9.9±0.5	21.0±1.5
Bulk density (g cm^{-3})	1.22±0.03	1.11±0.06
pH	7.0±0.1	6.1±0.2

† The number following the ± symbol indicates the standard error.

Table 2-2. Monthly air temperature and monthly cumulative precipitation in Lacombe and Edmonton during the experimental period (October 2014–May 2017) with average monthly values (1981-2010).

	Lacombe				**Edmonton**			
Months	30-yr values (1981–2010)†	2014–2015‡	2015–2016	2016–2017	30-yr values (1981–2010)	2014–2015	2015–2016	2016–2017
	Average temperature							
	°C							
Oct.	4.1	5.9(+)§			4.2	7.5(+)		
Nov.	–4.5	–7.3(–)	–4.1(+)	0.5(+)	–4.4	–6.7(–)	–1.7(+)	1.4(+)
Dec.	–10.1	–6.9(+)	8.0(+)	–13.3(–)	–8.8	–6.1(+)	8.0(+)	–11.1(–)
Jan.	–10.9	–8.5(+)	–10.3(+)	–10(+)	–11.7	–6.7(+)	–8.2(+)	–7.9(+)
Feb.	–8.7	–9.1(–)	–4.1(+)	–8.8(–)	–8.5	–8.5(–)	–1.5(+)	–6.6(+)
Mar.	–3.5	–0.1(+)	2.0(+)	–5.2(–)	–4	1.1(+)	2.0(+)	–4.8(–)
Frozen month average¶ (changes)#	–7.5	–6.4 (1.2)	–5.5 (2.1)	–7.3 (0.2)	–7.5	–5.4 (2.1)	–3.5 (4.0)	–5.8 (1.7)
Apr.	4.2	5(+)	7.9(+)	2.9(–)	5	6.5(+)	8.9(+)	3.4(–)
May	9.7	10.3(+)	10.6(+)	12.5(+)	10.8	12.1(+)	12.8(+)	14.0(+)
June	13.7	15.4(+)	15.7(+)	–	15.1	17.2(+)	17.2(+)	–
July	15.9	16.9(+)	16.3(+)	–	17.5	19.2(+)	18.2(+)	–
Aug.	15.0	15.6(+)	15.3(+)	–	15.9	17.9(+)	17.2(+)	–
Sept.	9.9	9.3(–)	9.9(–)	–	11	10.7(–)	11.6(+)	–
Oct.	4.1	5.8(+)	1.1(–)	–	4.2	7.8(+)	2.3(–)	–
Unfrozen month average¶ (changes)#	10.4	11.2 (0.8)	11.0 (0.6)	7.7†† (0.7)	11.4	13.0 (1.7)	12.6 (1.2)	8.7 (0.8)
	Cumulative precipitation							
	mm							
Oct.	19.7	10.2(–)			20.4	9.4(–)		
Nov.	14.6	29(+)	8.8(–)	12.0(+)	16.8	29.6(+)	12.5(–)	12.0(-)
Dec.	10.8	6.1(–)	12.2(+)	16.8(+)	9.3	3.8(–)	8.5(–)	11.1(+)
Jan.	14.8	21.0(+)	13.5(–)	7(–)	14.5	21.0(+)	8.6(–)	7.4(-)
Feb.	9.5	18.7(+)	9.5(–)	17.5(+)	9.9	24.4(+)	7.8(–)	14.2(+)
Mar.	14.4	23.0(+)	16.4(+)	19.5(+)	13.5	23.0(+)	13.6(+)	19.1(+)
Frozen month average¶ (changes)#	64.0	99.8 (35.8)	60.4 (–3.6)	76.6 (12.6)	64	101.8 (37.8)	51.0 (–13.0)	63.8 (–0.2)
Apr.	22.0	11.8(–)	14.6(–)	24.7(+)	27	6.9(–)	10.2(–)	73.3(+)
May	54.9	23.3(–)	78.3(+)	45.5(–)	53.7	19.4(–)	112.6(+)	53.8(+)
June	78.4	71(–)	26.8(–)	–	73.8	24.9(–)	61.2(–)	–
July	94.9	108.5(+)	119.6(+)	–	101.1	67.4(–)	112.7(+)	–
Aug.	61.9	48.2(–)	67.3(+)	–	73.7	26.5(–)	105.2(+)	–
Sept.	40.3	47.3(+)	31.4(–)	–	39.1	63.4(+)	23.6(–)	–
Oct.	19.7	13.0(–)	35.3(+)	–	20.4	13.0(–)	39.9(+)	–
Unfrozen month average¶ (changes)#	372.0	316.1 (–55.9)	373.3 (1.3)	70.2 (–3.3)	388.8	221.4 (–167.4)	465.4 (76.6)	127.1 (23.2)

† The 30-yr average values are from Government of Canada (2018).

‡ The monthly air temperature and precipitation data are from Alberta Agriculture and Forestry (2018a).

§ +, increase compared with the corresponding 30-yr average value; –, decrease compared with the corresponding 30-yr average value.

¶ The months were split into frozen (November–March) and unfrozen (April ‒ October) months according to the 30-yr temperature values.
# Numbers in brackets represent the changes in average temperature or cumulative precipitation over the frozen and unfrozen months compared with the corresponding 30-yr values.
†† These values are the average temperature or cumulative precipitation for April and May 2017.

Table 2-3. Mean total N (organic and inorganic N), NH_4–N, and water content (with standard errors) in the liquid manure applied at four different times in 2014–2016.

Injection timing	Lacombe	Edmonton
	Total N	
	———kg ha^{-1}———	
Fall 2014	245.2 ± 34.8c†	380.7 ± 6.7b
Spring 2015	405.8 ± 5.4b	399.1 ± 11.4b
Fall 2015	315.6 ± 54.2bc	402.7 ± 21.4b
Spring 2016	521.1 ± 7.1a	458.1 ± 9.5a
	NH_4–N	
	———kg ha^{-1}———	
Fall 2014	161.9 ± 0.1d	216.7 ± 0.7b
Spring 2015	230.6 ± 0.1b	193.6 ± 1.5c
Fall 2015	199.8 ± 2.8c	212.9 ± 0.8b
Spring 2016	398.8 ± 2.5a	272.1 ± 5.6a
	H_2O	
	———% w/w———	
Fall 2014	98.7 ± 0.1a	91.8 ± 0.8b
Spring 2015	97.7 ± 0.2ab	92.9 ± 0.1ab
Fall 2015	97.9 ± 0.7a	93.6 ± 0.1a
Spring 2016	96.5 ± 0.1b	91.9 ± 0.2b

† Different letters indicate significant differences among the four manure application timings at each location ($P < 0.05$).

Table 2-4. ANOVA results for the year and treatment effects on N_2O emission factors, N use efficiency, and N uptake efficiency in Lacombe and Edmonton.

	Lacombe		**Edmonton**	
	F-value	*p*-value	*F*-value	*p*-value
	N_2O emission factors			
Year†	6.282	0.017*	18.193	<0.001**
Treatment‡	3.898	0.007*	0.664	0.655
Year × treatment	7.659	<0.001**	0.599	0.701
	N use efficiency			
Year	43.926	<0.001**	13.334	0.001*
Treatment	3.038	0.023*	2.161	0.096
Year × treatment	1.602	0.187	0.446	0.811
	N uptake efficiency			
Year	9.751	0.004*	43.502	<0.001**
Treatment	1.582	0.193	0.663	0.655
Year × treatment	2.827	0.031*	0.711	0.621

* $P < 0.05$; ** $P < 0.001$;

† Year indicates the first (2014–2015) and second (2015–2016) experimental years.

‡ Treatments included six manured treatments, including fall application of liquid manure with the inhibitor 3, 4-dimethylpyrazole phosphate (DMPP), fall application of liquid manure with the inhibitor nitrapyrin, fall application of liquid manure without inhibitors, spring application of liquid manure with the inhibitor DMPP, spring application of liquid manure with the inhibitor nitrapyrin, and spring application of liquid manure without inhibitors.

Table 2-5. Annual cumulative N_2O emissions ($AnCumN_2O$, in kg N ha^{-1}) and its seasonal proportions (in %) in Lacombe and Edmonton during the two experimental years.

Treatment	**Lacombe**				**Edmonton**			
	Proportion of $AnCumN_2O$			$AnCumN_2O$	Proportion of $AnCumN_2O$			$AnCumN_2O$
	Fall†	Spring thaw	Spring–summer		Fall	Spring thaw	Spring–summer	
2014–2015								
Start date	7 Oct. 2014	27 Mar. 2015	18 Apr. 2015	7 Oct. 2014	1 Oct. 2014	27 Mar. 2015	17 Apr. 2015	1 Oct. 2014
End date	31 Oct. 2014	17 Apr. 2015	6 Oct. 2015	6 Oct. 2015	31 Oct. 2014	16 Apr. 2015	30 Sept. 2015	30 Sept. 2015
Duration (d)	25	22	172		31	21	167	
CZ‡	0.5	61.5	38.0	0.3 ± 0.1b§	4.3	60.1	35.5	0.6 ± 0.4b
FMD	19.2	70.4	10.4	1.2 ± 0.2b	13.0	65.9	21.1	1.8 ± 0.6ab
FMN	13.9	80.4	5.7	2.6 ± 0.7b	21.1	63.9	15.0	2.2 ± 1.0a
FMW	11.0	84.4	4.6	6.2 ± 3.2a	17.9	68.9	13.2	2.3 ± 0.7a
CT	0.5	69.4	30.1	0.3 ± 0.1b	3.0	80.3	16.7	0.6 ± 0.1ab
SMD	0.2	31.5	68.3	0.9 ± 0.2b	1.8	49.1	49.1	1.0 ± 0.1ab
SMN	0.1	15.6	84.3	1.8 ± 0.4b	1.3	35.0	63.7	1.4 ± 0.2ab
SMW	0.1	10.7	89.2	2.7 ± 0.7b	1.9	51.2	46.9	1.0 ± 0.2b
2015–2016								
Start date	29 Sept. 2015	29 Mar. 2016	21 Apr. 2016	29 Sept. 2015	8 Oct. 2015	15 Mar. 2016	9 Apr. 2016	8 Oct. 2015
End date	4 Nov. 2015	20 Apr. 2016	3 Nov. 2016	3 Nov. 2016	12 Nov. 2015	8 Apr. 2016	20 Oct. 2016	20 Oct. 2016
Duration (d)	37	23	197		36	25	195	
CZ	1.5	70.2	28.3	0.6 ± 0.2c	3.9	16.4	79.7	1.9 ± 0.5b
FMD	11.2	51.8	37.0	1.3 ± 0.3bc	7.9	24.0	68.1	5.2 ± 2.4ab
FMN	9.0	64.6	26.4	1.9 ± 0.2bc	3.5	18.2	78.4	8.1 ± 4.8ab
FMW	14.9	59.6	25.5	2.3 ± 0.8bc	6.5	28.8	64.7	6.1 ± 1.2ab
CT	-0.1	64.5	35.6	0.5 ± 0.1c	1.0	13.3	85.8	2.3 ± 0.1b
SMD	0.1	5.5	94.4	5.5 ± 1.7b	0.7	13.1	86.3	5.5 ± 0.1ab
SMN	0.0	1.1	98.8	12.6 ± 1.8a	0.3	10.3	89.4	7.3 ± 1.4ab
SMW	0.0	2.8	97.2	11.2 ± 3.3a	0.6	4.7	94.6	9.9 ± 3.1a

† Fall, spring thaw, and spring–summer correspond to F, ST, and SS, respectively, in Fig. 2-1d and Fig. 2-2d.

‡ CT, control treatment where the soil was disturbed by liquid manure application; CZ, control without disturbance; FMD, fall application of liquid manure with the inhibitor 3, 4-dimethylpyrazole phosphate (DMPP); FMN, fall application of liquid manure with the inhibitor nitrapyrin; FMW, fall application of liquid manure without inhibitors; SMD, spring application of liquid manure with the inhibitor DMPP; SMN, spring application of liquid manure with the inhibitor nitrapyrin; SMW, spring application of liquid manure without inhibitors.

§ Different letters indicate significant differences among the eight treatments in each location in a year ($P < 0.05$).

Table 2-6. Barley aboveground dry matter and N uptake (with standard errors) in Lacombe and Edmonton in both years.

Treatment	Harvest 2015†		Harvest 2016	
	Lacombe	Edmonton	Lacombe	Edmonton
	Aboveground dry matter			
	kg ha^{-1}			
CZ‡	2946.4 ± 260.8d§	4410.6 ± 470.4cd	3360.3 ± 171.3d	2759.6 ± 213.8b
FMD	5865.5 ± 200.7c	7996.7 ± 559.5a	5626.3 ± 549.8ab	5675.7 ± 330.1a
FMN	6072.4 ± 393.2bc	7794.7 ± 891.2a	6121.6 ± 212.1a	4118.1 ± 805.3ab
FMW	6691.2 ± 387.5abc	7896.7 ± 99.5a	5401.1 ± 216.7ab	5366.6 ± 994.3a
CT	2416.5 ± 365.4d	3688.9 ± 189.5d	2086.2 ± 88.0e	3347.8 ± 434.5b
SMD	7414.4 ± 295.5a	7042.5 ± 378.9ab	4968.1 ± 284.5bc	4208.2 ± 720.9ab
SMN	7018.4 ± 401.1ab	6908.5 ± 893.0ab	4915.3 ± 639.8bc	4361.6 ± 482.6ab
SMW	7078.9 ± 452.9ab	5942.5 ± 256.5bc	4210.4 ± 470.5cd	4337.8 ± 730.9ab
	N uptake			
	kg ha^{-1}			
CZ	35.3 ± 1.4d	63.8 ± 8.8c	26.7 ± 3.2d	26.8 ± 2.6b
FMD	78.7 ± 3.2c	143.2 ± 8.7a	73.9 ± 7.4bc	47.2 ± 6.4ab
FMN	88.8 ± 6.1bc	134.0 ± 13.4ab	88.1 ± 4.3ab	52.4 ± 16.0ab
FMW	95.8 ± 2.8b	143.8 ± 2.8a	63.4 ± 6.0b	59.3 ± 11.3a
CT	33.1 ± 3.6d	56.3 ± 1.3c	24.2 ± 1.9d	28.3 ± 3.8b
SMD	138.6 ± 5.7a	132.7 ± 4.7ab	106.9 ± 5.8a	52.8 ± 9.6ab
SMN	126.6 ± 8.3a	126.8 ± 17.6ab	97 ± 10.6a	49.9 ± 4.5ab
SMW	133.1 ± 8.2a	110.4 ± 6.8bc	91.5 ± 8.3ab	61.8 ± 10.7a

† Data from the harvest 2015 (the first year) have previously been shown in Lin et al. (2017).

‡ CT, control treatment where the soil was disturbed by liquid manure application; CZ, control without disturbance; FMD, fall application of liquid manure with the inhibitor 3, 4-dimethylpyrazole phosphate (DMPP); FMN, fall application of liquid manure with the inhibitor nitrapyrin; FMW, fall application of liquid manure without inhibitors; SMD, spring application of liquid manure with the inhibitor DMPP; SMN, spring application of liquid manure with the inhibitor nitrapyrin; SMW, spring application of liquid manure without inhibitors.

§ Different letters indicate significant differences among the eight treatments in each location in a year ($P < 0.05$).

Table 2-7. ANOVA results of application timing and nitrification inhibitor (NI) effects on the emission factors, N use efficiency and N uptake efficiency in Lacombe and Edmonton in two experimental years.

	2014–2015				**2015–2016**			
	Lacombe		**Edmonton**		**Lacombe**		**Edmonton**	
	F-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
				Emission factors				
Timing†	2.870	0.189	7.515	0.111	15.110	0.030*	0.007	0.943
Addition‡	3.390	0.068	1.511	0.278	11.242	0.002*	1.998	0.198
Timing × addition	1.456	0.272	0.543	0.601	5.344	0.022*	1.408	0.299
				N use efficiency				
Timing	2.639	0.203	1.356	0.364	8.119	0.065	2.848	0.234
Addition	1.304	0.307	0.531	0.608	1.504	0.261	4.195	0.057
Timing × addition	2.542	0.120	0.494	0.628	0.315	0.736	5.471	0.032*
				N uptake efficiency				
Timing	3.237	0.170	3.400	0.207	0.498	0.531	0.198	0.700
Addition	1.414	0.281	0.811	0.478	2.880	0.095	1.010	0.406
Timing × addition	3.224	0.076	1.349	0.313	1.992	0.179	0.126	0.883

* $P < 0.05$

† Timing includes the fall and spring manure injections.

‡ Addition includes the manure without NI addition, manure with nitrapyrin, and manure with DMPP.

2.10 Figures

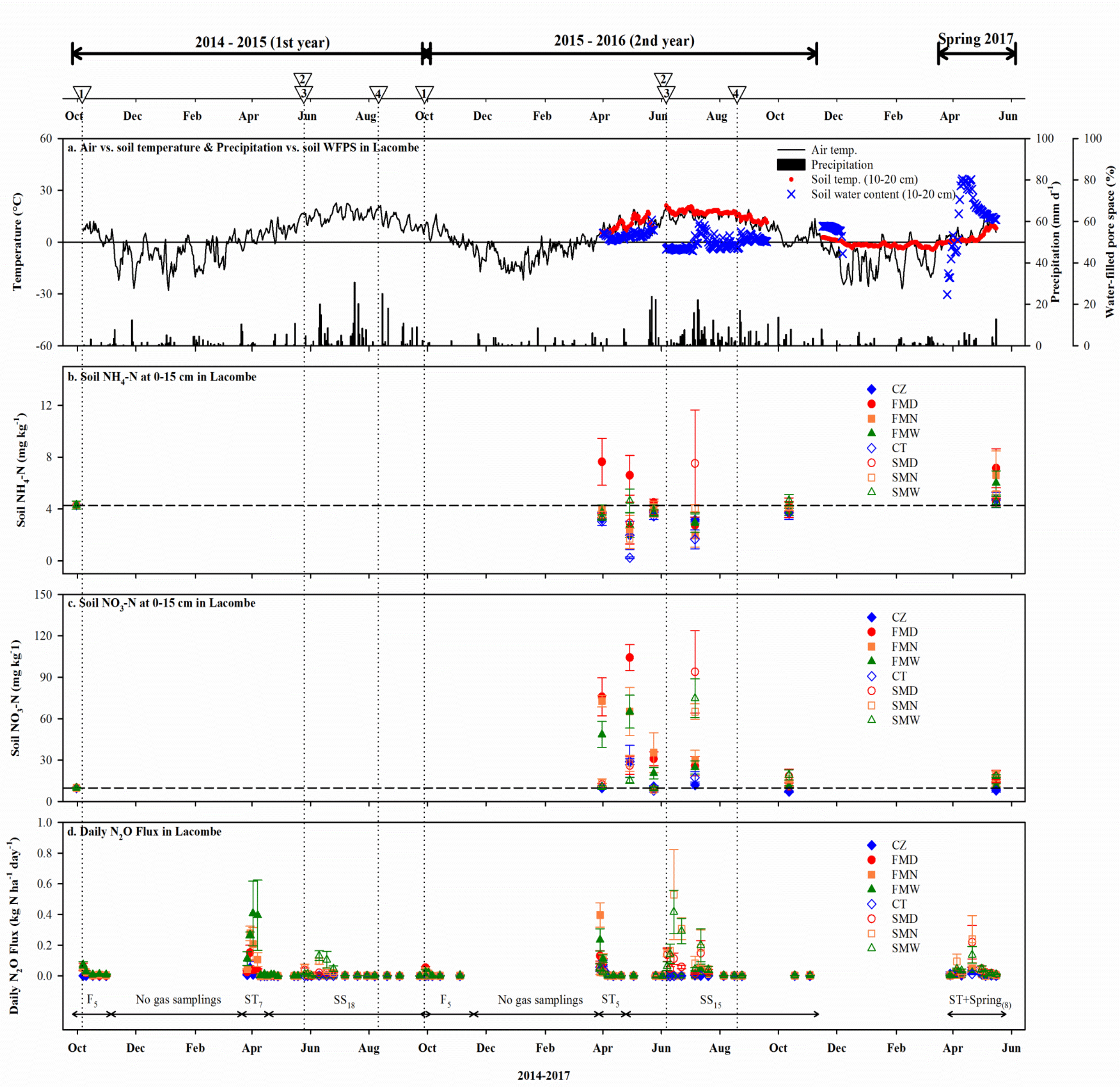

Fig. 2-1. (a) Daily average air temperature, daily cumulative precipitation, daily average soil temperature (10–20 cm), and daily average soil moisture content (10–20 cm); (b) soil ammonium at 0- to 15-cm depth; (c) soil nitrate at 0- to 15-cm depth; and (d) daily N2O flux in Lacombe from October 2014 to May 2017. Error bars correspond to one standard error (three replicates). the numbers 1, 2, 3 and 4 in the inverted triangles correspond to the time of fall manure application, seeding, spring manure application and harvest, respectively. Dashed horizontal lines represent the baseline levelsof soil NH_4–N and NO_3–N concentrations before the treatments were applied. F, fall; ST, spring thaw; SS, spring–summer. The subscripted numbers correspond to the frequency of flux measurements. CT, control treatment where the soil was disturbed during liquid manure application; CZ, control without disturbance; FMD, fall application of liquid manure with the inhibitor 3, 4-dimethylpyrazole phosphate (DMPP); FMN, fall application of liquid manure with the inhibitor nitrapyrin; FMW, fall application of liquid manure without inhibitors; SMD, spring application of liquid manure with the inhibitor DMPP; SMN, spring application of liquid manure with the inhibitor nitrapyrin; SMW, spring application of liquid manure without inhibitors. Data from October 2014 to September 2015 have previously been presented in Lin et al. (2017)

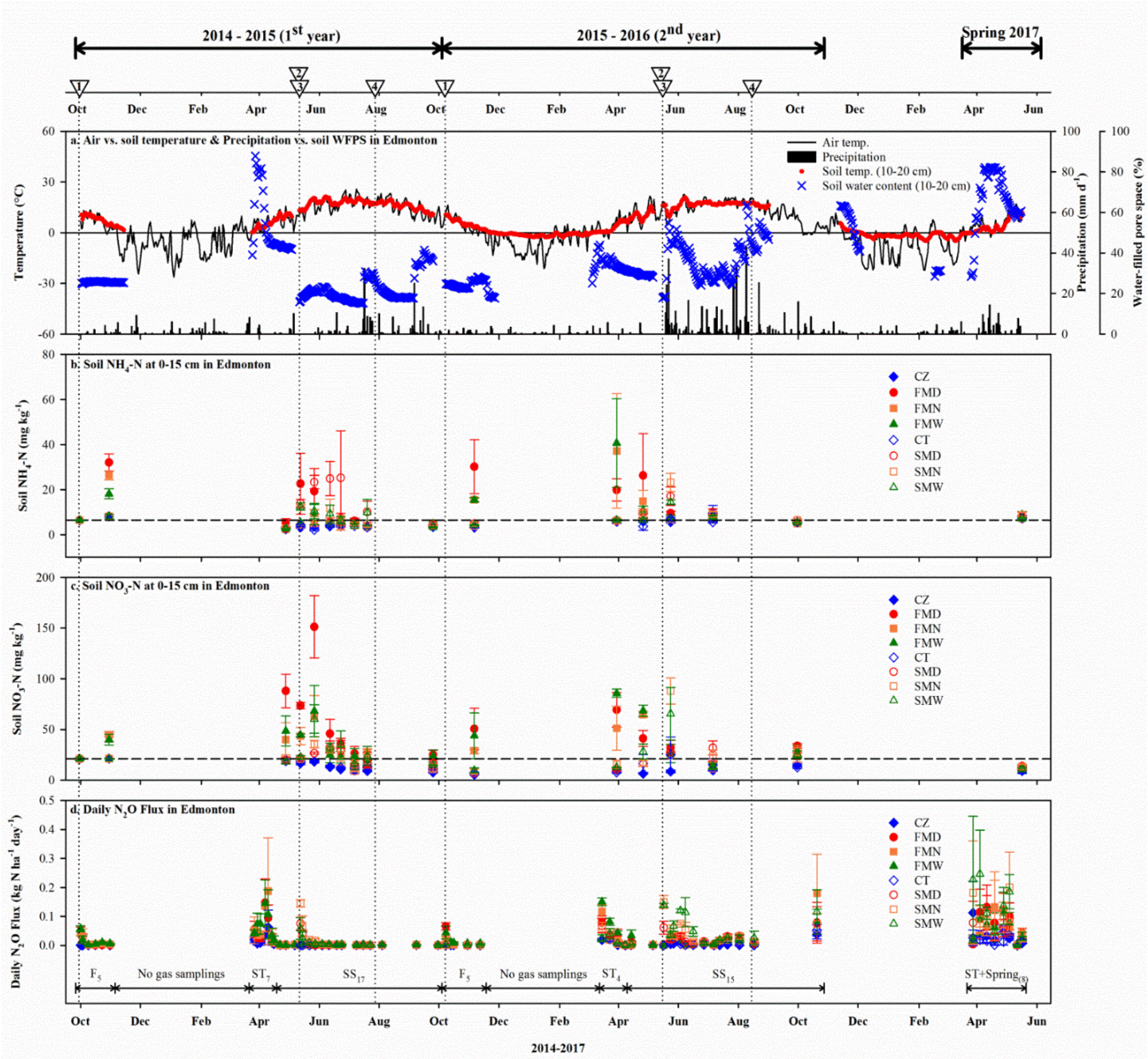

Fig. 2-2. (a) Daily average air temperature, daily cumulative precipitation, daily average soil temperature (10–20 cm), and daily average soil moisture content (10–20 cm); (b) soil ammonium at 0- to 15-cm depth; (c) soil nitrate at 0- to 15-cm depth; and (d) daily N2O flux in Edmonton from October 2014 to May 2017. Error bars correspond to one standard error (three replicates). The numbers 1, 2, 3 and 4 in the inverted triangles correspond to the time of fall manure application, seeding, spring manure application, and harvest, respectively. Dashed horizontal lines represent the baseline level of soil NH_4–N and NO_3–N concentrations before the treatments were applied. F, fall; ST, spring-thaw; SS, spring–summer. The subscripted numbers correspond to the frequency of flux measurements. CT, control treatment where the soil was disturbed by liquid manure application; CZ, control without disturbance; FMD, fall application of liquid manure with the inhibitor 3, 4-dimethylpyrazole phosphate (DMPP); FMN, fall application of liquid manure with the inhibitor nitrapyrin; FMW, fall application of liquid manure without inhibitors; SMD, spring application of liquid manure with the inhibitor DMPP; SMN, spring application of liquid manure with the inhibitor nitrapyrin; SMW, spring application of liquid manure without inhibitors. Data from October 2014 to September 2015 have previously been presented in Lin et al. (2017).

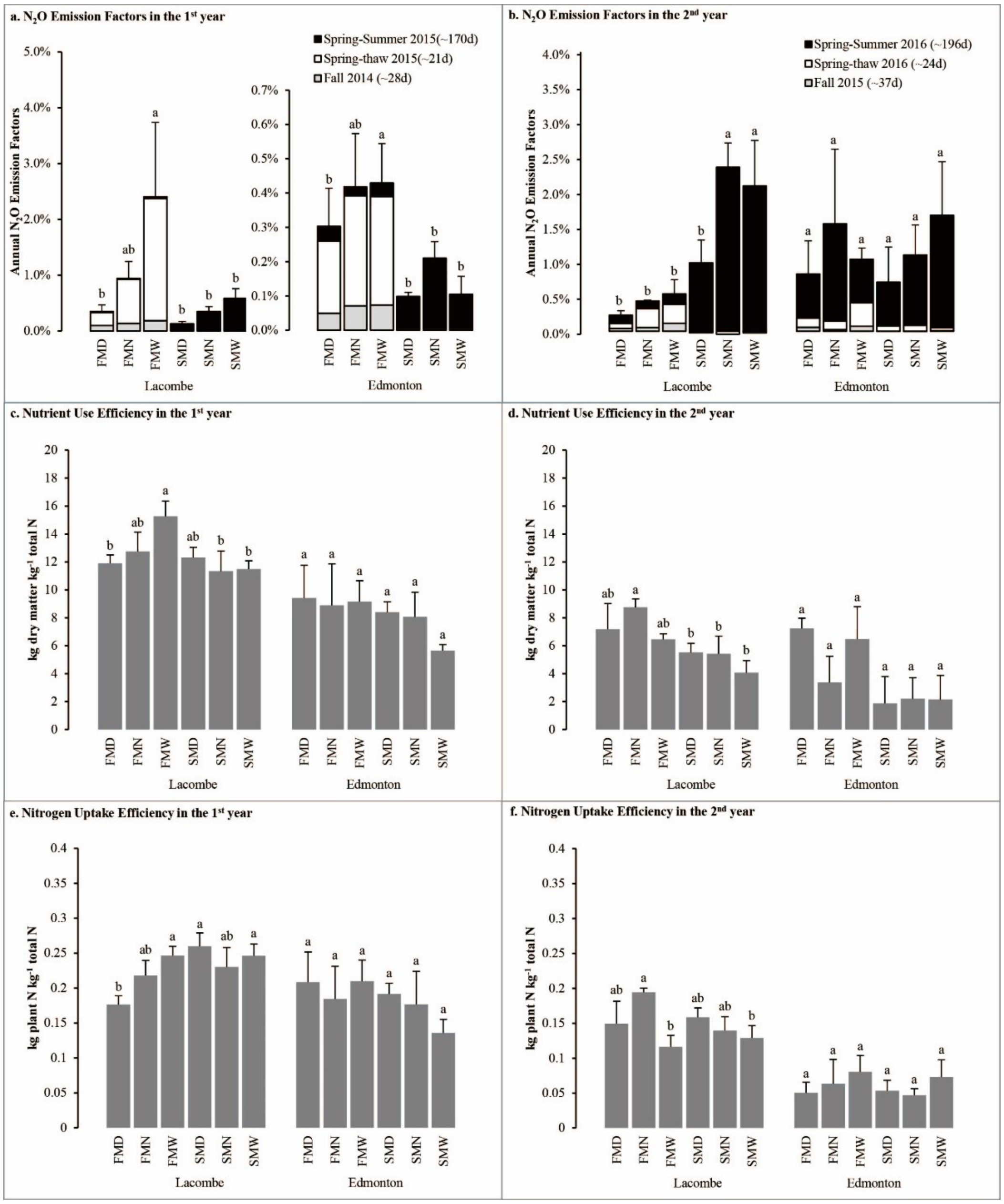

Fig. 2-3. Annual N_2O emission factors in the (a) first and (b) second experimental years, barley N use efficiency in the (c) first and (d) second experimental years, and plant N uptake efficiency in the (e) first and (f) second experimental year in Lacombe and Edmonton. Different letters indicate significant differences among the treatments in each location in a year ($P < 0.05$). Nitrogen use efficiency and uptake efficiency were calculated via Eq. [2], Eq. [3], and Eq. [4], respectively. CT, control treatment where the soil was disturbed by liquid manure application; CZ, control without disturbance; FMD, fall application of liquid manure with the inhibitor 3, 4-dimethylpyrazole phosphate (DMPP); FMN, fall application of liquid manure with the inhibitor nitrapyrin; FMW, fall application of liquid manure without inhibitors; SMD, spring application of liquid manure with the inhibitor DMPP; SMN, spring application of liquid manure with the inhibitor nitrapyrin; SMW, spring application of liquid manure without inhibitors.

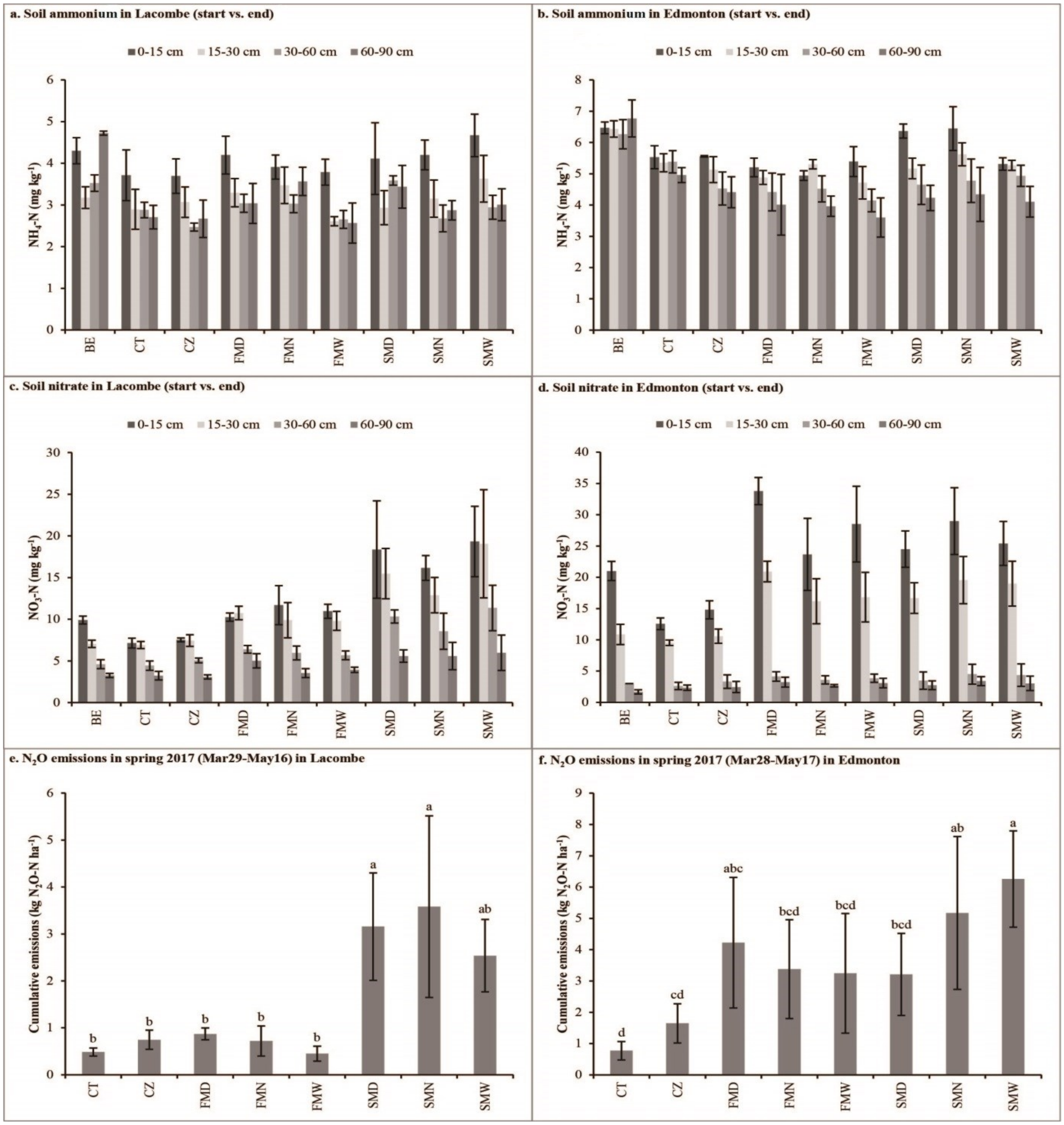

Fig. 2-4. Soil ammonium and nitrate concentrations at different depths prior to the experiment and at the end of the 2-yr experiment (a–d) and total N_2O emissions accumulated during the spring of 2017 after the end of the 2-yr experiment in (e) Lacombe and (f) Edmonton. The soil samples prior to the experiment were collected on 30 Sept. 2014 in both locations. The soil samples at the end of the 2-yr experiment were collected on 12 Oct. 2016 and 30 Sept. 2016 in Lacombe and Edmonton, respectively. Different letters indicate significant differences among the treatments in each location in a year ($P < 0.05$). The comparisons for soil NH_4-N and NO_3-N among treatments at different depths in both locations among treatments are in supplementary (S1). Note the different y-axis scales across panels. BE, before the experiment; CT, control treatment where the soil was disturbed by liquid manure application; CZ, control without disturbance; FMD, fall application of liquid manure with the inhibitor 3, 4-dimethylpyrazole phosphate (DMPP); FMN, fall application of liquid manure with the inhibitor nitrapyrin; FMW, fall application of liquid manure without inhibitors; SMD, spring application of liquid manure with the inhibitor DMPP; SMN, spring application of liquid manure with the inhibitor nitrapyrin; SMW, spring application of liquid manure without inhibitors.

3. Nitrous Oxide Emissions from Manured Soils as a function of various Nitrification Inhibitor Rates and Soil Moisture Contents

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3.1 Abstract

Application of nitrification inhibitors (NI) coupled with fertilizers can successfully reduce N losses. However, previous studies have found the NI effectiveness can be impacted by variations in edaphic and environmental conditions. Laboratorial incubations were conducted in Black Chernozemic (BC) and Gray Luvisolic (GL) soils to determine the effects of soil water content and NI application rates on the efficacy of two NIs – 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin) and 3,4-dimethylpyrazole succinic acid isomeric mixture (DMPSA) – by evaluating nitrous oxide (N_2O) production, soil NH_4-N and NO_3-N concentrations. The N_2O emission reduction caused by NIs was minimal at 40% WFPS, while the NI reduction effect became evident at higher water contents, with an averaged reduction of 60% and 56% at 60% and 80% WFPS of GL soil, respectively; an averaged reduction of 58% at 60% WFPS of BC soil was seen as well. The ineffective emission reduction at 80% WFPS of BC soil was very likely due to the rapid NI degradation under moist conditions in this carbon-rich soil. Focusing on the N_2O emission reduction and nitrification inhibition by NIs, as soil moisture content rose from 60 to 80% WFPS, the more efficient NI rate correspondingly increased from 0.25 to 1.0 kg a.i. ha^{-1} for nitrapyrin and from 0.25 to 0.75 kg a.i. ha^{-1} for DMPSA in both soils. Although texture differences between the two contrasting soils were not explicitly reflected on the most efficient NI rate, the faster NI degradation rate at 80% WFPS in the BC soil implies a greater NI rate required for soils with higher clay and organic matter contents. In sum, our study provided evidence of how soil moisture content, NI application rates and soil types influenced the efficacy and longevity of NIs and will help with improving strategies to reduce N losses from agricultural systems with NI implementation.

Key words: nitrous oxide (N_2O), nitrification inhibitors, soil water content, application rate, soil texture, manure

3.2 Introduction

Nitrous oxide (N_2O), as an important greenhouse gas, has a global warming potential that is 310 times higher than carbon dioxide and persists for more than 100 years in the atmosphere (Forster et al., 2007). It is also an important substance related to ozone destruction (Ravishankara et al., 2009). Given the facts of intensification in arable land use and N fertilizer additions, the atmospheric concentration of N_2O dramatically increased by 19% in 2011 on a concentration basis of 1750 and is expected to continue increasing (Reay et al., 2012; Myhre et al., 2013). Arable soils receiving manure and fertilizers are a major source of the global anthropogenic N_2O emissions, accounting for about 40% of the worldwide N_2O budget (Intergovernmental Panel on Climate Change, 2007). N_2O is mainly derived from both aerobic autotrophic nitrification as a byproduct and anaerobic denitrification as an intermediate product, and both processes are related to soil microbial metabolic activities (Butterbach-Bahl et al., 2013).

Adding NIs and fertilizers to the soil provides a technique to improve N fertilizer use efficiency. It has been well documented that NIs successfully inhibited nitrification, and consequently reduced N_2O emissions and NO_3-N leaching (Slangen and Kerkhoff, 1984; McCarty and Bremner, 1989; Dittert et al., 2001; Irigoyen et al., 2003; Di et al., 2009a; Lin et al., 2017; Huérfano et al., 2018; Guardia et al., 2018). 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin) has been a widely used commercial NI over the past years (Subbarao et al., 2006; Burzaco et al., 2013). 3,4-dimethylpyrazole succinic acid isomeric mixture (DMPSA) has been recently studied as a newly re-formulated, effective NI in reducing N_2O emissions (Huérfano et al., 2016; Pacholski et al., 2016; Guardia et al., 2018; Huérfano et al., 2018; Recio et al., 2019).

Both belong to copper-selective chelating compounds that aim to remove the co-factor of ammonia monooxygenase, an enzyme responsible for the first step of nitrification (NH_4^+ to NO_2^-), hence preventing the NH_4-N oxidation (Ruser and Schulz, 2015). In this manner, denitrification and NO_3-N leaching would be further indirectly suppressed by limiting the substrate NO_3-N. 3,4-dimethylpyrazole phosphate (DMPP), a DMP-based inhibitor, was reported to effectively inhibit nitrification at low concentrations of 0.5-1.0 kg active ingredient (a.i.) ha^{-1} (Zerulla et al., 2001), which was lower than the recommended rate of nitrapyrin (Lin et al., 2017). Pacholski et al. (2016) and Guardia et al. (2018) stated that DMPSA would have less volatilization and be prolonged in the soil due to the combination with succinic acid. Thus, DMPSA should hypothetically still be an efficient inhibitor at a lower concentration.

The NI inhibitory effect on N_2O production has been shown to greatly depend on environmental and edaphic factors such as soil temperature, moisture contents, and soil texture. Several studies have found that the persistence of NIs decreased with increasing temperature (Irigoyen et al., 2003; Kelliher et al., 2008). In an incubation study, Chen et al. (2010) found that the N_2O emission reduction due to the addition of DMPP was lower at 40% than at 60% WFPS, indicating the dependence of NI effectiveness on soil moisture content. Another study examined the effects of temperature and moisture on DMPP efficiency in reducing N_2O emissions (Menéndez et al., 2012). It found that the NI efficacy decreased with temperature at 40% WFPS, while it completely reversed at 80% WFPS. Besides environmental factors, McGeough et al. (2016) provided evidence that the efficacy of NI dicyandiamide (DCD) was greatly impacted by soil texture, with a negative correlation to soil clay content, total N and organic matter. Most studies focused on the effect of a single factor on NI efficacy; however, there is little information available about the combined effect of multiple factors such as texture and moisture contents. Additionally, increasing the rate of NI itself showed increases in the half-life of the inhibitor (Singh et al., 2008). Based on this information, it could be speculated that increasing the NI rate would be a beneficial technique to improve the NI efficacy; nevertheless, there is still a lack of knowledge about to what extent the increased NI rate would successfully inhibit nitrification and reduce N_2O emissions.

The main objectives of this study were to investigate the performance of NIs under a wide range of soil moisture contents in two contrasting soils, and to examine how different NI application rates influence the NI efficacy and the longevity of their effectiveness under various soil moisture contents. Moreover, since the NIs have been shown to be impacted by soil moisture content (Chen et al., 2010; Menéndez et al., 2012), in this work we also explored the hypothesis that the lowest NI application rate that efficiently inhibits nitrification and reduces N_2O emissions would increase with increasing soil water content.

3.3 Materials and methods

This study was implemented in two laboratory incubation experiments. With the aim of gaining general insights about NIs effectiveness, incubation A addressed whether the two NIs (i.e., nitrapyrin and DMPSA) could efficiently reduce N_2O emissions at both low and high application rates relative to control protocols and among three distinct water contents in two contrasting soils. Moreover, incubation B further focused on the performance of NIs at a more specific range of multiple application rates with the aim of identifying the optimum NI rates under the same combinations of moisture contents × soils (3 × 2) as in incubation A.

3.3.1 Collection and preparation of soil and manure

Two contrasting soils were collected in June 2016 from two agricultural experimental sites in Alberta, Canada: the South Campus Research Farm in Edmonton (53°29'30''N, 113°31'53''W) and the Classical Breton Plots (53°05'24''N, 114°26'39''W). The typical management for the Black Chernozemic soil (BC) in Edmonton was annual barley (*Hordeum vulgare* L.) without historic manure or fertilizer additions for at least five years prior to the collection. The Gray Luvisolic soil (GL) was from a control barley/hay plot within the Classical Breton site that had, since 1930, been following a five-year rotation cycle of wheat (*Triticum aestivum*), oats (*Avena sativa* L.), barley/hay, hay and hay (Kiani et al., 2017). Based on the historical records from the local weather stations (Government of Canada, 2018), the long-term mean annual air temperature was 3.5 and 4.2 °C, and the mean annual precipitation was 452.8 and 473.6 mm in Edmonton (1981-2010) and Breton (2002-2018), respectively.

Top soils (0-15 cm) were randomly taken from 5-6 sampling spots (surface area of each spot: ~20 × 20 cm^2) within the selected field plots. Each soil was mixed well and passed through an 8-mm mesh to remove large plant residuals and rocks, resulting in a homogeneous soil. Subsamples from each soil were dried at 105 °C for at least 24 h to check if the remaining soil moisture was below 0.17 g g^{-1}, which was equivalent to about 32% water-filled pore space (WFPS) for a bulk density of 1.1 g cm^{-3}. The BC soil was spread out (5-6 cm in thickness) to be air dried at room temperature (~20 °C) until it satisfied the moisture requirement. After both soils attained the moisture requirement, a subsample from each soil was then air dried and sieved (2-mm mesh) for further chemical and physical analyses (Table). Before the pre-incubation, the soils were stored at 5°C, except during the period of necessary air drying mentioned above. Two baskets (of about 4-5 L each) of liquid dairy manure were collected from the Dairy Research & Technology Center in Edmonton, AB, Canada in June 2016. These two baskets of manure were mixed in a big, clean container and stirred by a spoon to ensure homogeneity. Then the manure was redistributed into the two baskets and stored at 5 °C. Prior to the additions in both incubations, the liquid manure was completely mixed and subsamples were taken for further property analyses (Table 3-2).

3.3.2 Incubation A design

Incubation A was conducted using the following treatments under 40, 60 and 80% WFPS for each soil: (1) CT: control with no manure and NI additions; (2) MO: liquid manure only; (3) MN0.25: liquid manure + nitrapyrin (Corteva Agriscience™, Agriculture Division of DowDupont™, Canada) at a rate of 0.25 kg a.i. ha^{-1} (equivalent to 1.67×10^{-4} mg a.i. kg^{-1} soil); (4) MN1.5: liquid manure + nitrapyrin at a rate of 1.5 kg a.i. ha^{-1} (equivalent to 1.00×10^{-3} mg a.i. kg^{-1} soil); (5) MD0.25: liquid manure + DMPSA (Eurochem Group, Germany) at a rate of 0.25 kg a.i. ha^{-1}; (6) MD1.5: liquid manure + DMPSA at a rate of 1.5 kg a.i. ha^{-1}. The eighteen treatment combinations (6 addition treatments × 3 water contents) for each soil were replicated three times. The liquid dairy manure application rate was consistent at 0.037 mL manure cm^{-3} soil across all treatments. This rate corresponded to the field application rate of 56.17 m^3 manure ha^{-1} for a soil increment of 0-15 cm (Lin et al., 2017).

Prior to the 29-d incubation, a 3-d pre-incubation was conducted to stimulate soil microorganism activity. On the first day of pre-incubation, soils were treated with the preset rates of manure or manure with NI additions, while they were still kept at low moisture contents [the gravimetric water content for BC and GL soils was 0.10 (19% WFPS) and 0.11 g g^{-1} (20% WFPS), respectively]. Due to the small amount of manure and NI required for one cylindrical incubation microcosm (4.5 cm in the bottom diameter, 5.5 cm in the top diameter, and 7.2 cm in height), each addition treatment (e.g., MN0.25) was implemented for ten microcosms together (3 water contents × 3 replications + 1 in case). During this process, soils were transferred into a basket with the preset amount of liquid manure or manure plus inhibitors, and then mixed thoroughly by hand. Prior to mixing with soils, 5 or 10 ml of deionized (DI) water was pipetted into a separate container with the NI, and this mixture was then added to the liquid manure. In order to clean the residual NI from the inner walls of the container, an additional 5 or 10 mL of deionized water was used and mixed into the manure basket. The well-mixed soil was then distributed into nine microcosms and compacted by increments of up to 100 cm^3 with a bulk density of 1.1 g cm^{-3} (similar to the field bulk density of BC, as shown in Table 3-1). Excess soil was discarded and all tools were cleaned with DI water and dried with clean paper towels for the next treatment setup. Throughout the whole incubation (3-d pre-incubation + 29-d incubation), the microcosms were incubated at room temperature (20.1 ± 0.1 °C), which was recorded every minute by a UX100-001 data logger (Onset Computer Corp., Massachusetts, USA). The water levels were set up on the first day of the 29-d incubation and maintained by weight monitoring every day. Room-temperature DI water was carefully and evenly dropped on the soil surface by a syringe to minimize disturbances and to provide, as far as possible, evenly distributed moisture. During the 29-d incubation, cardboard was placed over the top of the microcosms at a distance which minimized evaporation and provided some space for air circulation.

3.3.3 Incubation B design

The experimental procedures in incubation B were mostly the same as those in incubation A; only the differences are described in this section. On the first day of the 3-d pre-incubation, the soils were moved from 5 °C to room temperature. In order to keep them at their original qualified moisture content, the soils continued to be stored in large containers (68 L) with closed lids. On the first day of the 29-d incubation, the additional treatments were established under 40, 60 and 80% WFPS for each soil with three replications: (1) CT; (2) MN0.25; (3) MN0.5; (4) MN0.75; (5) MN1.0; (6) MN1.5; (7) MD0.25: liquid manure + DMPSA at a rate of 0.22 kg a.i. ha^{-1}; (8) MD0.5: liquid manure + DMPSA at a rate of 0.44 kg a.i. ha^{-1}; (9) MD0.75: liquid manure + DMPSA at a rate of 0.65 kg a.i. ha^{-1}; (10) MD1.0: liquid manure + DMPSA at a rate of 0.87 kg a.i. ha^{-1}; (11) MD1.5: liquid manure + DMPSA at a rate of 1.31 kg a.i. ha^{-1}. The nitrapyrin rates of 0.25, 0.5, 0.75, 1 and 1.5 kg a.i. ha^{-1} were equivalent to 1.67×10^{-4}, 3.33×10^{-4}, 5.00×10^{-4}, 6.67×10^{-4} and 10^{-3} mg a.i. kg^{-1} of soil, respectively. The DMPSA rates of 0.22, 0.44, 0.65, 0.87 and 1.31 kg a.i. ha^{-1} were equivalent to 1.45×10^{-4}, 2.90×10^{-4}, 4.35×10^{-4}, 5.81×10^{-4} and 8.71×10^{-4} mg a.i. kg^{-1} of soil, respectively.

3.3.4 Gas sampling and analysis

Daily N_2O flux was determined on days 0, 3, 8, 11, 15, 22 and 29 of incubation A, and on days 1, 3, 7, 10, 15, 23 and 29 of incubation B. To do this, each microcosm was placed in a modified 500 mL mason jar. Through a drilled hole on the jar's lid, a Wheaton® sleeve stopper (DWK Life Sciences, New Jersey, USA) for multi-puncture applications was attached by a silicone sealant. A leakage test using water was carried out for each modified mason jar before the first gas sampling event. The mason jar was closed at 0 min and gas samples were taken at 27, 54, and 81 minutes in incubation A and at 33, 66 and 99 minutes in incubation B. Gas samples (20 mL) were collected using a BD™ syringe (Franklin Lakes, New Jersey, US) then injected into 12 mL pre-evacuated Exetainer® vials (Labco, Lampeter, Wales, UK). Two ambient gas samples were collected at the beginning, in the middle and at the end of a gas sampling event, respectively. The six ambient gas samples were considered as the N_2O concentration at 0 min. The gas samples were stored at 5 °C prior to the analysis to prevent potential leakage. The N_2O concentration was analyzed by a Varian Model 3800 gas chromatography equipped with a Combi-Pal autosampler and an electron capture detector (Varian Inc., Walnut Creek, California, US). The lower detection limitation for this instrument was 0.307 mg N_2O-N kg^{-1} soil. The N_2O production rate was calculated by developing a linear or quadratic regression regarding the concentrations at 0, 27, 54, and 81 min, or at 0, 33, 66, and 99 min (Eq. [1]). About 95.5 and 4.5% of measurements in incubation A were determined by linear and quadratic regressions, respectively. Similarly, about 94.7 and 5.3 % of measurements in incubation B were determined by linear and quadratic regressions, respectively. Cumulated N_2O emissions were calculated by linear interpolation between sampling dates.

$$F = (\frac{dC}{dt}) \times (\frac{V}{M}) \times (\frac{P}{R \times T}) \times 2M \times k \quad [1]$$

where F is the N_2O flux (mg N_2O-N kg^{-2} d^{-1}); dC/dt is the slope of a simple linear regression or as the first derivative of a quadratic regression at t_0 (μL L^{-1} min^{-1}); V is the headspace volume of the gas chamber headspace (L); M is the dry soil weight (kg); P is the pressure of the gas (atm); R is the gas constant (atm μL K^{-1} $μmol^{-1}$); T is the temperature of the gas (K); M is the molar mass of the N basis (g N mol^{-1}) and k is a conversion factor for the flux unit (from μg N_2O-N kg^{-1} min^{-1} to mg N_2O-N kg^{-1} d^{-1}).

3.3.5 Other sampling and analyses

The soil texture, organic C, total N, pH, electrical conductivity, soil NH_4-N and NO_3-N were determined based on standard methods (McKeague, 1978; Carter and Gregorich, 2008). More detailed methods were described in Lin et al. (2017). Two bottles of liquid manure (~1.5 L) were collected and sent to the PBR Laboratories Inc. in Edmonton, Alberta, Canada for the total N, NH_4-N, solids and moisture analyses. These manure samples were stored at 5°C before they were sent to the lab, and kept in a freezer during transportation to minimize microbial activities.

3.3.6 Statistical analyses

The data was analyzed by R 3.1.3 (R Core Team, 2014). Before the statistical analyses, the assumptions of normality and homoscedasticity were accessed by the Shapiro-Wilk and Levene tests, respectively. The soil moisture associated with the additional treatments or with the NI application rates and their interactions were tested using two-way ANOVA separately for each soil. The treatment effects on cumulated N_2O, soil NH_4-N and NO_3-N within each specific comparison (e.g. the effect in cumulated N_2O among CT, MO, MN0.25, MN1.5, MD0.25 and

MD1.5 treatments at 40%WFPS of BC soil) were tested using one-way ANOVA, and a post-hoc test was conducted using Tukey's Honest Significant Difference test (HSD) if the treatment effects were significant with a default alpha of 0.05.

3.4 Results

3.4.1 N_2O production

The magnitude of N_2O production largely varied among different soil water contents, soil types and NI application rates (Table 3-3). At 40% WFPS, the daily N_2O fluxes throughout the incubation were consistently low across all treatments in both soils, with an averaged flux of 0.31 ± 0.03 and 0.32 ± 0.05 mg N_2O-N kg^{-1} soil d^{-1} for the BC and GL soil, respectively (Fig. 3-1a and d). At higher WFPS, N_2O emission pulses clearly occurred in the manure only (MO) treatment of both soils and these pulses were obviously reduced by using NIs, except for the BC soil at 80% WFPS (Fig. 3-1 b, c, e and f). Moreover, the overall flux patterns were quite different between 60% and 80% WFPS, as well as between soil types (Fig. 3-1 b, c, e and f). For the BC soil, the flux from the MO treatment at 60% WFPS gradually increased to 1.46 mg N_2O-N kg^{-1} soil d^{-1} on day 11 then levelled off, but the fluxes from the NI treatments remained stable over the incubation with an average flux of 0.51 ± 0.04 mg N_2O-N kg^{-1} soil d^{-1} (Fig. 3-1 b). By contrast, the MO flux at 80% WFPS increased to 8.35 mg N_2O-N kg^{-1} soil d^{-1} on day 3 and decreased to 0.97 mg N_2O-N kg^{-1} soil d^{-1} on day 15; however, on the same day, much more substantial N_2O fluxes were emitted from all NI treatments (ranging from 47.93 to 334.16 mg N_2O-N kg^{-1} soil d^{-1}); this overwhelming increase of fluxes compared to MO continued, and decreased towards the end of incubation (Fig. 3-1 c). For the GL soils, fluxes from the manured treatments at 60% WFPS generally peaked on day 3, followed by a steady decline to the background level until day 15 (Fig. 3-1 e). Similar patterns were observed in the GL soil at 80% WFPS, but the peaks occurred five days later than at 60% WFPS (Fig. 3-1 f).

With regard to the cumulative N_2O emissions, the addition of liquid manure in the absence of NIs apparently increased the emissions compared to the unfertilized soils (CT) in all conditions (Fig. 3-2). Regarding the MO treatment, the lowest N_2O emission was found at 40% WFPS in both soils (Fig. 3-2). Under dry soil conditions (40% WFPS), there was limited emission reduction with the presence of NIs compared to the MO treatment in the GL soil (i.e. an average reduction of 10% across all NI treatments) and no reduction in the BC soil (Fig. 3-2). By contrast, at higher WFPS in both soils, NIs numerically or significantly decreased the cumulative N_2O emissions compared to the MO treatment (i.e. reduced by an average of 58% at 60% WFPS of BC, 60% at 60% WFPS of GL and 56% at 80% WFPS of GL across manured soil with NI additions), except for the BC soil at 80% WFPS where N_2O emissions were higher with NI additions regardless of NI types and application rates (Fig. 3-2).

Moisture contents exhibited an intricate interplay with the effectiveness of the various NI rates. Although at 40% and 60% WFPS of both soils there was in general no significant difference in cumulative N_2O emissions among various NI application rates–except for the nitrapyrin 1.5 treatment at 40% WFPS of the BC soil–there was still a tendency towards increased emission reduction with incremented NI rates at 60% WFPS of the BC soil (Fig. 3-3). Likewise, under moist soil conditions the reduction tendency became significant, excluding the 80% WFPS of the BC soil where no significant difference was observed among various NI rates (Fig. 3-3).

In order to interpret the results in our study, the economic efficient rate or optimal rate is defined as the minimal NI rate at which the cumulative N_2O emission was not significantly different, or at which the soil NH_4-N was significantly higher than the unfertilized control treatment. Regarding the emission reduction, the economic efficient rate for DMPSA in the GL soil was half of that for nitrapyrin with the GL soil at 80% WFPS (i.e., 0.5 kg DMPSA ha^{-1} versus 1.0 kg nitrapyrin ha^{-1}, Fig. 3-3). Conversely, when DMPSA was applied in the BC soil at 80% WFPS, the economic efficient rate was 0.75 kg a.i. ha^{-1} (Fig. 3-3).

3.4.2 Soil ammonium and nitrate

There was a significant soil moisture effect on the soil NH_4-N and NO_3-N concentrations in most cases (Table 3-3). In comparison with the initial concentrations prior to the treatment establishment, both NH_4-N and NO_3-N concentrations of the unfertilized control treatment collectively increased by the end of both incubations under all situations, except for the GL soil at 80% WFPS of incubation B (Fig. 3-4, Fig. 3-5 and Fig. 3-6). The NH_4-N increments in CT were similar among diverse moisture contents; nevertheless the NO_3-N increments were always greater at 60% WFPS than at both 40% and 80% WFPS (Fig. 3-4, Fig. 3-5 and Fig. 3-6).

The averaged NH_4-N concentration at 40% WFPS across all manure treatments with and without NIs in the BC soil was consistently 62.0 ± 1.4 mg N kg^{-1} soil, which was about six and seven times higher than the average values at 60% and 80% WFPS, respectively (Fig. 3-4; incubation A). By contrast, the highest averaged NH_4-N concentration of GL soil was found at 80% WFPS, with an average of 52.1 ± 7.6 mg N kg^{-1} soil that was five and four times larger than the averages at 40% and 60% WFPS, respectively (Fig. 3-4). Particularly, at 40% and 60% WFPS of GL soil, the NH_4-N concentration in the manured soil with a higher rate of DMPSA (MD1.5) was about four and five times greater than that in other manured soils (Fig. 3-4). At 80% WFPS of GL soil, the NH_4-N concentration evidently increased with higher rates in each NI product (Fig. 3-4). In terms of NO_3-N concentration across manured BC and GL soils, the amplitude of NH_4-N among distinct water contents was coherently opposite to the magnitude of NO_3-N (Fig. 3-4). As an illustration, the lowest averaged NO_3-N concentration across manured soils was observed at 40% WFPS of BC soil, where the averaged NH_4-N concentration was highest when compared with other water contents (Fig. 3-4). To summarize this relationship of soil N pools, higher NH_4-N accumulation coupled with lower NO_3-N indicated lower net nitrification rates.

On the basis of the changes in NH_4 and NO_3 concentrations with time, we further interpreted noticeable variants in nitrification across soils and moisture contents (Fig. 3-4, Fig. 3-5 and Fig. 3-6). For the BC soil, the averaged highest net nitrification occurred at 40% WFPS, not accounting for certain treatments with higher NI application rates at 80% WFPS (i.e., MN1, MN1.5, MD0.75 and MD1.5, Fig. 3-5 a and b). Conversely, for the GL soil, the averaged highest net nitrification was found at 80% WFPS (Fig. 3-6). Regarding NI application rates, there was no clear tendency of NH_4-N and NO_3-N concentrations among the various NI rates at 40% and 60% WFPS in any combination of soil × water content (Fig. 3-6). Specifically, higher DMPSA rates consistently led to slower net nitrification rates at all water contents in the GL soil (Fig. 3-6).

3.5 Discussion

3.5.1 Impact of soil moisture content on NI effectiveness

The performance of NIs on nitrification inhibition and N_2O emission reduction varied among different soil moisture contents. Under dry soil condition (40% WFPS), the cumulative N_2O emission of the MO treatment was the lowest, and since emissions were low, the N_2O emission reduction due to NIs was not as effective when compared to moist conditions (60% and 80% WFPS) (Fig. 3-2). Our results are partially consistent with an earlier controlled study, in which DMPP was appreciably effective in N_2O emission reduction at both 40% and 60% WFPS, but this reduction effect was less obvious at 40% than 60% WFPS (Chen et al., 2010). A much higher NI rate in Chen's study than in our study can explain an inhibitory effect even at the low soil water content of 40% WFPS. More specifically, in Chen's study the rate of active NI was about three orders of magnitude higher than the maximum rate used in our study. Moreover, two meta-analyses also revealed that the inhibitory effect of NIs became more powerful in mitigating N_2O when the emission was relatively high (Akiyama et al., 2010; Gilsanz et al., 2016). Therefore, from the perspective of the efficient use of NIs for N_2O emission reduction, it is unnecessary to apply NIs with manure or fertilizer where the N_2O production is continually low, such as under drought soil conditions. However, NIs could also prove beneficial by diminishing nitrate leaching losses (Di and Cameron, 2005; Hua et al., 2008; Di et al., 2009a), specifically for soils and weather conditions with a high risk of nitrate leaching. It has been found that soils with less clay content and less organic matter could leach substantial NO_3-N (Bergström and Johansson, 1991; Sogbedji et al., 2000; Beaudoin et al., 2005). In such soils, somewhat similar to the GL soil in our study, one possible solution to reduce potential NO_3-N losses could be the application of sufficient NI, as reduced nitrification and lower NO_3-N concentration were found with higher DMPSA application rates in our study (Fig. 3-4, Fig. 3-6).

As soil moisture increased, the effect of NIs on cumulative N_2O emission reduction became more evident in most cases but not in the BC soil at 80% WFPS (Fig. 3-2). This could be mainly due to the much greater N_2O fluxes from all manured soils with NIs than from the MO soil following incubation day 15 (Fig. 3-1). The robust fluxes from the NI treatments that took place on day 15 were very likely evidence of NI degradation unlocking nitrification and N_2O production. From incubation day 15, the soil NH_4-N previously stabilized by NIs was suddenly exposed to and consumed by soil microbial activity upon the degradation of NIs, and consequently emitted N_2O as a byproduct of nitrification and a product of denitrification. Furthermore, increasing rates of NIs were found to retain more NH_4-N. Once NI degraded, more N substrates became available for nitrifiers and denitrifiers, resulting in even stronger N_2O fluxes in the higher NIs rates, in particular in DMPSA (Fig. 3-1, Fig. 3-2).

Compared to the N_2O production in the BC soil at 80% WFPS, the NIs were still effective in reducing N_2O emissions at 60% WFPS (Fig. 3-1). This demonstrated the influence of soil moisture content on NI degradation, with a higher NI persistence at lower water contents. Considering the observation by Menéndez et al. (2012) as discussed above, the opposite finding in the current incubation indicated that the efficacy of NIs was not fully associated with oxidation and that enhanced microbial degradation stimulated by greater moisture might play a significant role.

In the GL soil, NIs showed a similar reduction in cumulated N_2O emissions between 60% and 80% WFPS (with an average reduction of 60% and 56% at 60% and 80% WFPS respectively, Fig. 2 2). Regarding the soil mineral N, nitrification was more constrained under 80% WFPS as evidenced by relatively higher NH_4-N together with lower NO_3-N compared to 60% WFPS (Fig. 2 3). Taking these observations into account, the inhibitory effect by NIs seemed to reduce more denitrification-derived N_2O than nitrification at 80% WPFS, when the denitrification was supposed to be the main source of N_2O as the soil environment gradually becomes anaerobic. Implementing NI addition has repeatedly been shown to reduce N_2O emissions by directly slowing down the nitrification rate and indirectly constraining the substrate (NO_3-N) for denitrification (Hatch et al., 2005; Migliorati et al., 2014; Ruser and Schulz, 2015; Wu et al., 2017); nevertheless, information about the proportional reduction by NIs of N_2O derived from nitrification vs. denitrification processes is still limited.

3.5.2 Effects of NI application rates on emission reduction

Focusing on both N_2O emission reduction and nitrification inhibitory effects, our results showed that the economic efficient rate of NIs varied among distinct soil moisture contents and NI products. Provided that N_2O emissions from manured soils with and without NIs were as low as the controls at 40% WFPS of both soils (Fig. 3-3), adding NIs into such dry soils was of minor importance in emission reduction.

At 60% WFPS, the addition of NIs significantly reduced N_2O emissions, although no significant differences in the cumulative emissions were found among various NI rates in the GL soils (Fig. 3-3). Based on these results, the economic efficient rate of NI for emission reduction should be around 0.25 kg a.i. ha^{-1}, regardless of soils and NI types. It is noticeable that the nitrification rate at 60% WFPS over 29 days was comparably faster than other moisture contents, as shown by the relatively lower NH_4-N coupled with much higher NO_3-N across all treatments (Fig. 3-4, Fig. 3-5 and Fig. 3-6). This also implies the soils at 60% WFPS had a higher potential risk of NO_3-N losses by leaching. To avoid potential major N losses from NO_3-N leaching, it would be necessary to increase the NIs rate when the soil moisture content was around 60% WFPS as mentioned above.

With increases in soil water content, the more efficient NI rate increased irrespective of soils. At 80% WFPS, the cumulative N_2O emissions generally tended to decrease with increasing NI application rates, except in the case of the BC soil with nitrapyrin (Fig. 3-3). This finding is in agreement with results from Ruser and Schulz (2015) who reported a decreasing N_2O due to an increased DMPP application along with 180 kg N ha^{-1} N-fertilizer. Similarly, Zaman and Blennerhassett (2010) discovered that the N_2O emissions significantly decreased by 25, 47 and 47% after urine application in the spring and by 28, 37, and 53% after urine application in the autumn with the addition of 5, 7 and 10 kg DCD ha^{-1}, respectively. Despite the fact that the declining trend in N_2O emissions was not observed in the BC soil with nitrapyrin, the soil mineral N results revealed that higher NI application rates (1 and 1.5 kg a.i. ha^{-1}) were still effective in further inhibiting nitrification compared to lower application rates (Fig. 3-5). This could be attributed to the longer NI persistence at higher NI rates, as supported by Singh et al. (2008). They found that the half-life of DCD prolonged at a higher application rate of DCD in a laboratory incubation at 25°C. Thus, with regard to a combination of N_2O emission reduction and soil mineral N results, the optimal application rates would be about 0.75 kg a.i. ha^{-1} of

DMPSA and 1 kg a.i. ha^{-1} of nitrapyrin, which are approximately 3-4 times higher than the NI optimum rate at 60% WPFS.

3.5.3 Effect of soil types on NI performance

The substantial N_2O fluxes from NI treatments at 80% WFPS due to NI degradation were found in the BC soil but to a lesser degree in the GL soil under comparable treatments. This indicated the influence of soil types on NI efficacy, with faster degradation in the BC soil which is much richer in organic matter and clay (Table 3-1). Other findings in our study further supported this postulate. For instance, the DMPSA at the highest rate (1.5 kg a.i. ha^{-1} in both incubations A and B) still remained effective in inhibiting nitrification at 60% WFPS in the GL soil as opposed to the corresponding treatment in the BC soil (Fig. 3-4 and Fig. 3-6), and this was consistently supported also by the effects of DMPSA at both 0.75 and 1 kg a.i. ha^{-1} rates (Fig. 3-6; incubation B). These findings were consistent with a recent study by McGeough et al. (2016) which found that the half-life of nitrification inhibitor DCD varied widely among nine UK soils, and the ability of DCD in suppressing net nitrate production was negatively related to the soil clay content, soil N and organic matter. Increased soil clay and organic matter contents could lead to more NIs being adsorbed on mineral surfaces, thereby accelerating the NI decomposition rate and decreasing its efficacy and longevity (Zhang et al., 2004; Singh et al., 2008; McGeough et al., 2016). In addition to the physio-chemical degradation, Singh et al. (2008) also pointed out that the higher DCD decomposition rate in the soil with higher organic matter content could be a consequence of more active microbial activities, possibly inducing faster bio-degradation of NIs.

The lowest net nitrification rates observed at 40% WFPS of BC soil and at 80% WFPS of the GL soil across the manured soils with and without NIs suggested a minor impact of NIs on inhibiting nitrification under these conditions, even though certain N_2O emission reductions in numerical terms were discovered in the latter case (Fig. 3-4, Fig. 3-5Fig. 4-6 and Fig. 3-6). Moreover, the contrasting net nitrate production rates between the two soils at both 40% and 80% WFPS and across all manured treatments also demonstrated the impact of soil clay and organic matter contents on soil structure, water availability and movement. When water was limited, the NH_4-N dissolved in the liquid phase became easier to deliver for nitrifiers in the GL soil, because the poor soil structure and reduced aggregate hierarchy associated with lower clay and organic matter contents favored the local water storage (Boyle et al., 1989). By contrast, at 80% WFPS, where soil aeration can become limited, soils with good structure and a greater aggregation hierarchy due to higher organic matter and clay contents, such as the BC soil, presumably had a greater capacity to sustain aerobic conditions favoring nitrification.

3.6 Conclusions

Our results showed that both nitrapyrin and DMPSA can be effective inhibitors for slowing down nitrification and reducing N_2O emissions. However, their effectiveness was interactively driven by soil moisture contents, soil types, and NI application rates. Overall, as soil water content increased, the optimal NI application rate that successfully reduced the N_2O emissions increased. Because variations in the soil properties could influence the physio-chemical and biological degradation of NIs, the optimal NI rate appeared to vary among different soils. Our findings can help to predict the appropriate timing and rate of NI additions under specific pedoclimatic scenarios. Nevertheless, certain processes–in particular plant uptake and nitrate leaching–that were not represented in our controlled study could also have critical interactions with NI rates under varying soil moisture contents as their interplay fills an essential role in altering N pools and transformations within the N cycle. This requires further investigation.

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3.8 References

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3.9 Tables

Table 3-1. Physical and chemical properties of soils collected in Edmonton and Breton (0-15 cm depth).

Properties	Edmonton			Breton		
Classification	Black Chernozem (BC)			Gray Luvisol (GL)		
Texture	Clay†			Silty clay loam		
Clay (%)	47.0	±	1.2†	31.1	±	2.0
Silt (%)	36.0	±	0.3†	49.1	±	2.4
Sand (%)	17.0	±	1.0†	19.8	±	0.3
Organic C (g kg^{-1})	63.1	±	1.0	19.8	±	0.2
Total N (g N kg^{-1})	6.1	±	0.1	2.0	±	0.1
Ammonium (mg N kg^{-1})	4.2	±	0.1	1.9	±	0.2
Nitrate (mg N kg^{-1})	16.1	±	0.6	10.6	±	0.1
Bulk density (g cm^{-3})	1.11	±	0.06†	1.28	±	0.02‡
pH	7.7	±	0.1	7.8	±	0.1
Electrical conductivity (µS cm^{-1})	174.0	±	17.0	234.5	±	28.5

† The data was derived from Lin et al. (2017) for the soils at 0-15 cm.
‡ The data was derived from Kiani et al. (2017).

Table 3-2. Means of characteristics of liquid dairy manure with one standard error (SE).

Properties	Mean ± SE (n=2)		
Total N (g N L^{-1})	5.4	±	0.1
Ammonium (g N L^{-1})	2.7	±	0.2
Water content (%)	93.2	±	0.1
Solids (%)	6.8	±	0.1
pH	7.3	±	0.1

Table 3-3. Significance of ANOVA for soil moisture and treatment effects on total N_2O emissions, soil ammonium and nitrate for two studied soils in incubations A and B.

Treatments	Black Chernozem (BC)			Orthic Gray Luvisol (GL)		
	Total N_2O emissions	NH_4-N	NO_3-N	Total N_2O emissions	NH_4-N	NO_3-N
				Incubation A		
Soil moisture	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
Addition trts†	0.054	n.s.	<0.001	<0.001	<0.001	<0.001
Soil moisture × Addition trts	n.s.	0.002	0.011	n.s.	<0.001	n.s.
				Incubation B		
Soil moisture	<0.001	0.007	0.009	<0.001	<0.001	<0.001
Addition trts‡	0.021	0.033	<0.001	0.004	<0.001	<0.001
Soil moisture × Addition trts	n.s.	0.002	0.007	0.035	0.055	n.s.
Soil moisture	<0.001	0.021	n.s.	<0.001	<0.001	<0.001
Nitrapyrin rates (0.25 to 1.5)	0.098	n.s.	n.s.	n.s.	n.s.	n.s.
Soil moisture × nitrapyrin rates	n.s.	<0.001	<0.001	n.s.	n.s.	n.s.
Soil moisture	<0.001	n.s.	0.063	<0.001	<0.001	<0.001
DMPSA rates (0.25 to 1.5)	0.056	n.s.	n.s.	0.001	0.010	n.s.
Soil moisture × DMPSA rates	0.021	0.030	n.s.	0.078	0.032	n.s.

n.s. = not significant ($P > 0.1$)

† The manure-inhibitor addition treatments in the incubation A include CT§, MO, MN0.25, MN1.5, MD0.25 and MD1.5.

‡ The manure-inhibitor addition treatments in the incubation B include CT, MN0.25, MN0.5, MN1.0, MN1.25, MN1.5, MD0.25, MD0.5, MD1.0, MD1.25 and MD1.5.

§ CT = control, MO = manure only, MN = manure with nitrapyrin additions, MD = manure with DMPSA additions. The numbers following the treatment acronyms in capital letters represent the nitrification inhibitor application rate.

3.10 Figures

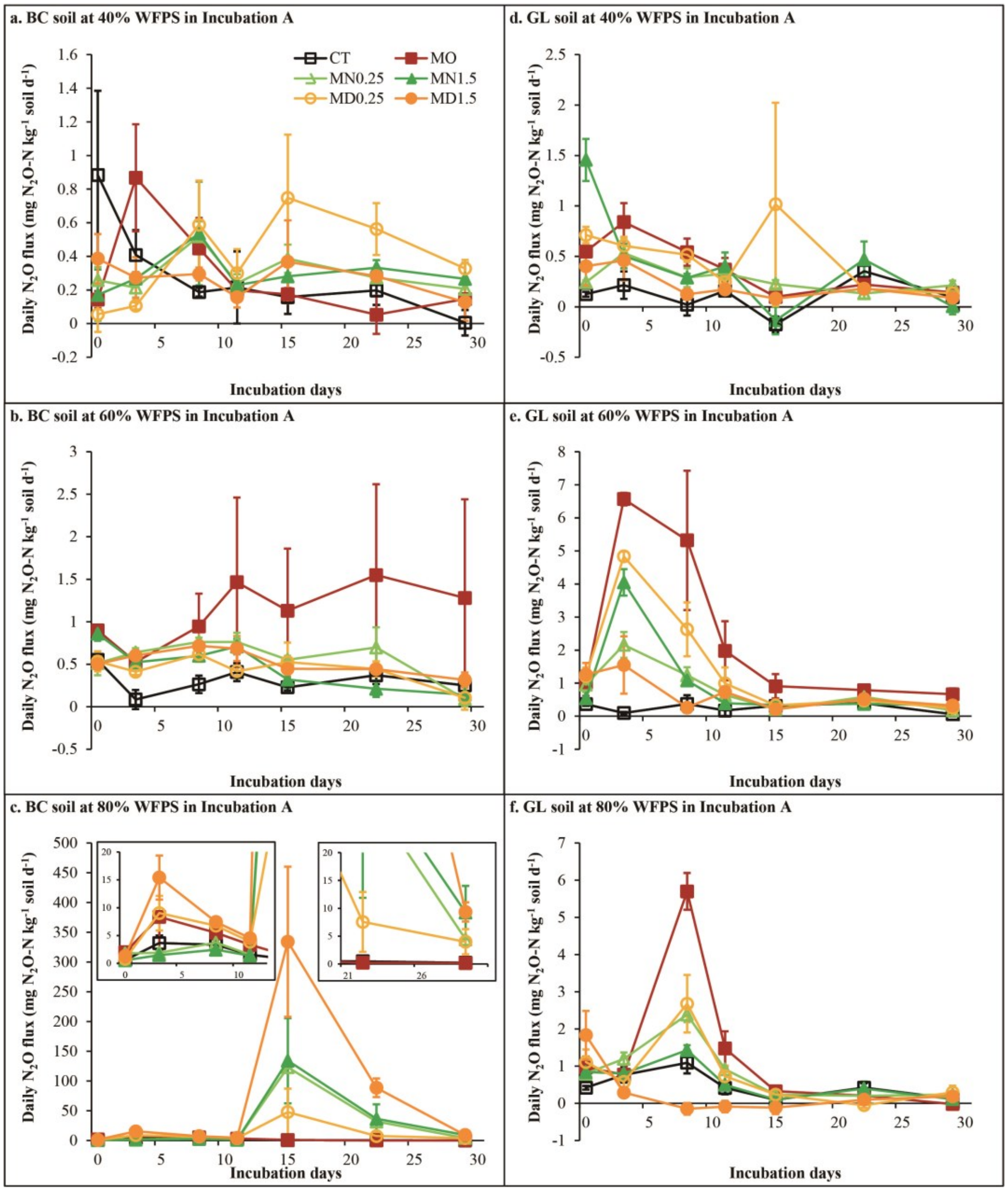

Fig. 3-1. Daily N$_2$O fluxes across the manure-inhibitor addition treatments for the Black Chernozem (BC) soils (a, b and c) and the Gray Luvisolic (GL) soils (d, e and f) at 40, 60 and 80% WFPS during incubation A. Error bars correspond to one standard error (3 replicates). The inside figures were the same as the large figures but they were in different y-axis scales. CT = control, MO = manure only, MN = manure with nitrapyrin additions, MD = manure with DMPSA additions. The numbers in the acronyms in the legend represent the nitrification inhibitor application rate (kg a.i. ha^{-1}).

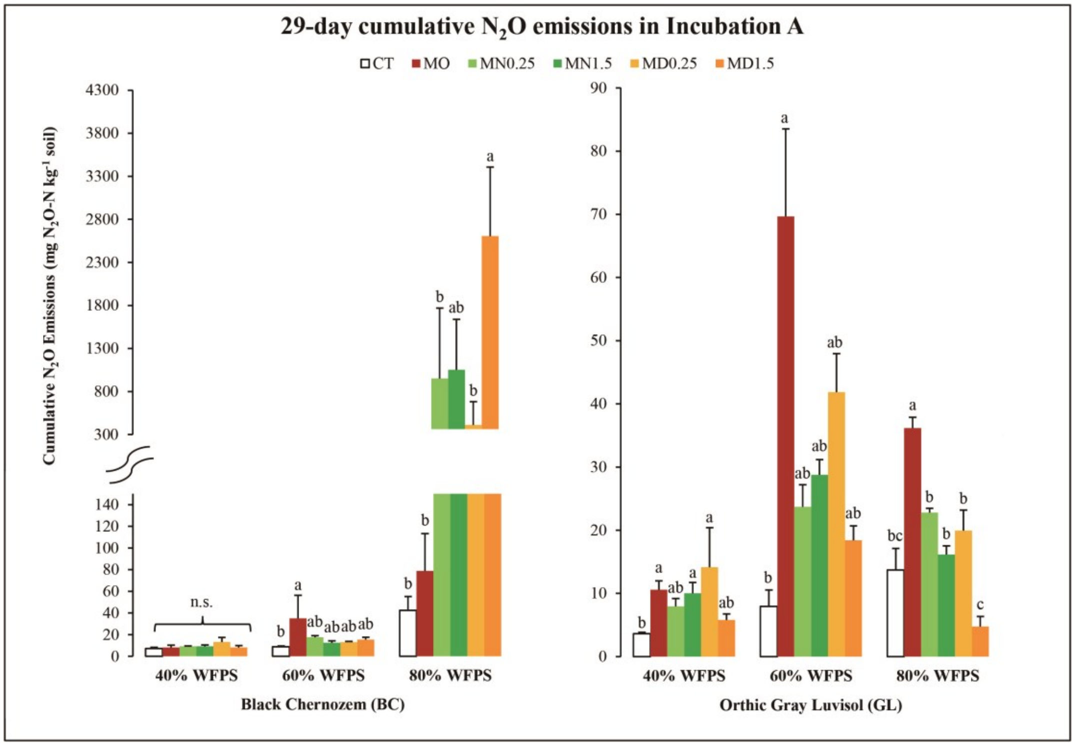

Fig. 3-2. Cumulative N_2O emissions across all treatment combinations (manure-inhibitor additions × moisture contents) in the Black Chernozem and Orthic Gray Luvisoil soils in incubation A. Error bars correspond to one standard error (3 replicates). Different letters indicate significant differences among the treatments for each soil based on HSD test ($P < 0.05$). CT = control, MO = manure only, MN = manure with nitrapyrin additions, MD = manure with DMPSA additions. The numbers in the acronyms in the legend represent the nitrification inhibitor application rate (kg a.i. ha^{-1}).

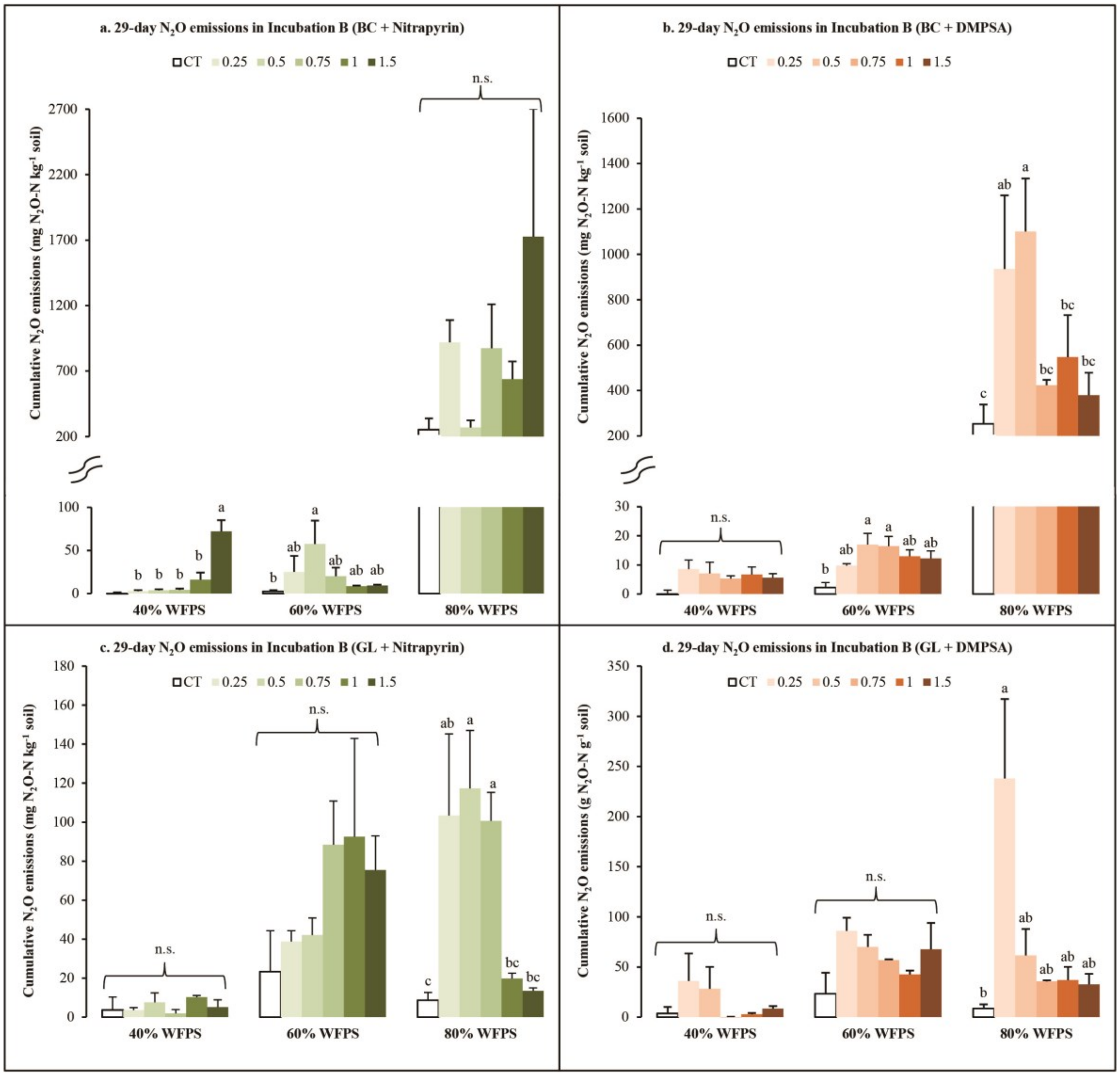

Fig. 3-3. Cumulative N$_2$O emissions from the Black Chernozem (BC) soil with different rates of (a) nitrapyrin and (b) DMPSA, and cumulative N$_2$O emissions from the Orthic Gray Luvisoil (GL) soil with different rates of (c) nitrapyrin and (d) DMPSA under different soil moisture levels during the incubation B. Error bars correspond to one standard error (3 replicates). Different letters indicate significant differences among the treatments within each panel ($P < 0.05$) based on HSD test. CT = control. The numbers in the legends represent the nitrification inhibitor application rate.

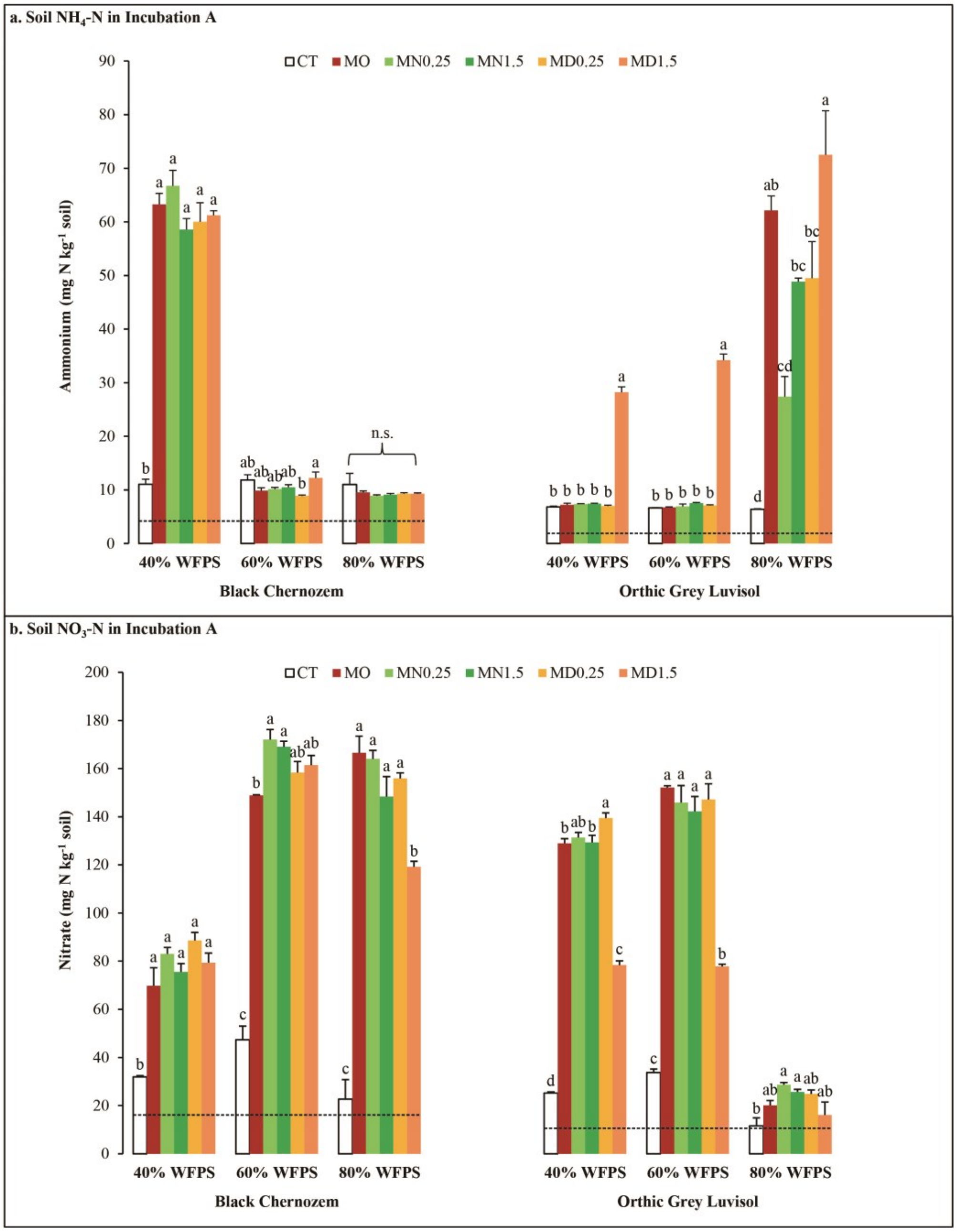

Fig. 3-4. Soil (a) ammonium and (b) nitrate concentrations across all treatment combinations (manure-inhibitor additions × moisture contents) in the Black Chernozem and Orthic Gray Luvisoil soils at the end of incubation A. Error bars correspond to one standard error (3 replicates). Different letters indicate significant differences among the treatments for each soil based on HSD test ($P < 0.05$). Dashed horizontal lines indicate the soil ammonium and nitrate concentrations at the beginning of the incubation (Table 3-1). CT = control, MO = manure only, MN = manure with nitrapyrin additions, MD = manure with DMPSA additions. The numbers in the legends represent the nitrification inhibitor application rate.

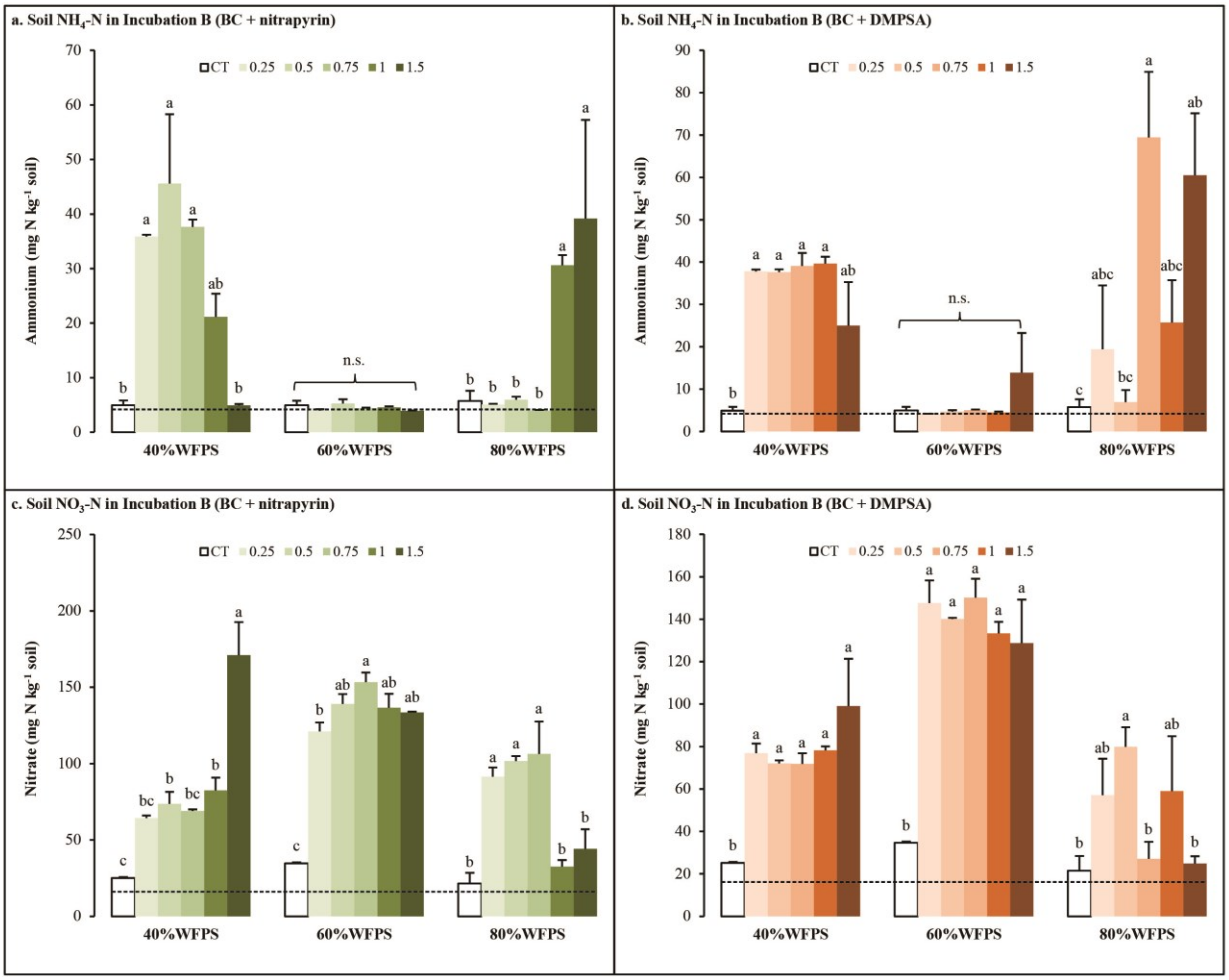

Fig. 3-5. Soil ammonium and nitrate concentrations for the Black Chernozem (BC) soil with different rates of (a, c) nitrapyrin and (b, d) DMPSA under different soil moisture contents at the end of incubation B. Error bars correspond to one standard error (3 replicates). Different letters indicate significant differences among the treatments within each panel based on HSD test ($P < 0.05$). Dashed lines indicate the soil ammonium and nitrate concentrations at the beginning of the incubation (Table 3-1). CT = control. The numbers in the legends represent the nitrification inhibitor application rate.

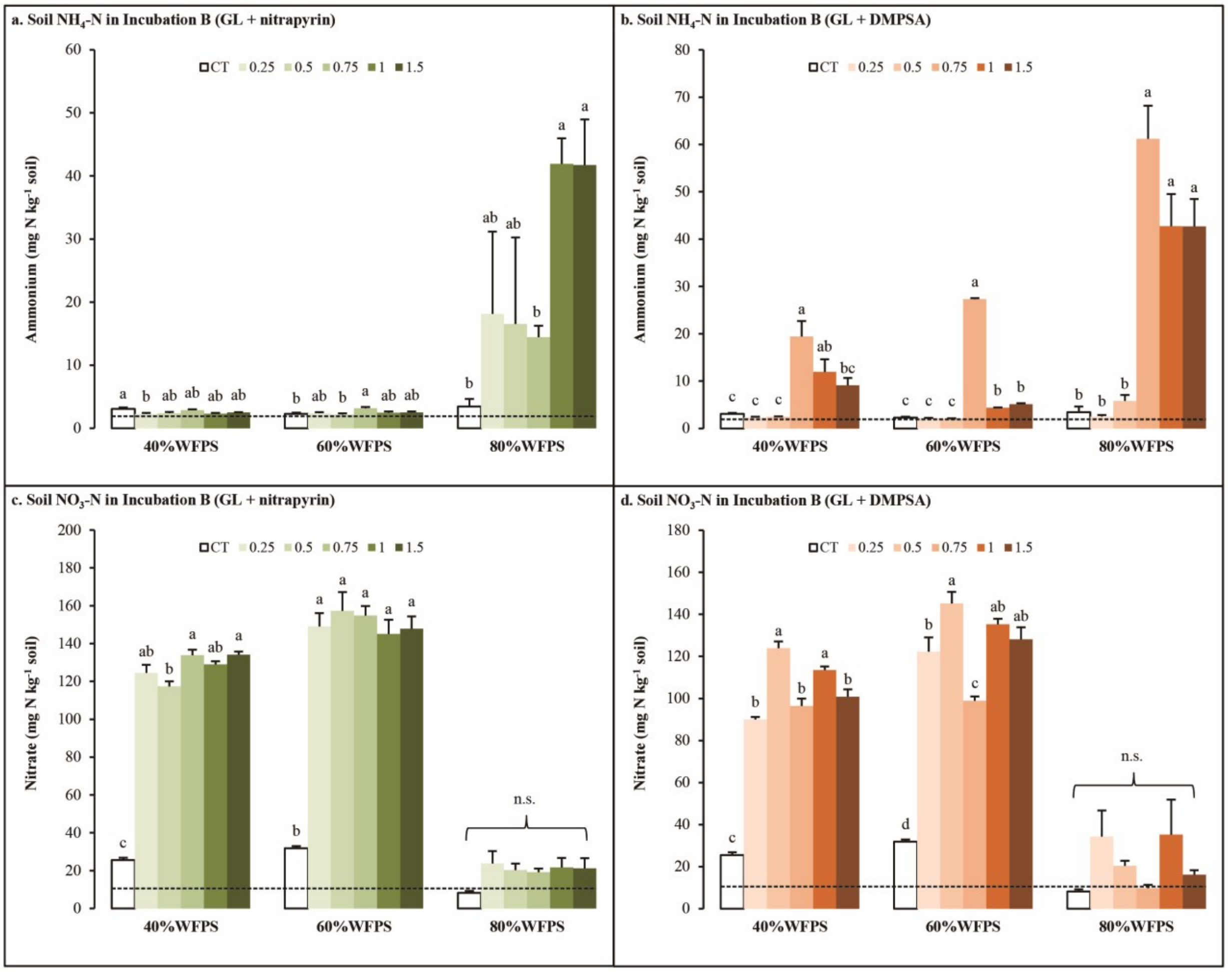

Fig. 3-6. Soil ammonium and nitrate concentrations for the Orthic Gray Luvisol (GL) soil with different rates of (a, c) nitrapyrin and (b, d) DMPSA under different soil moisture contents at the end of incubation B. Error bars correspond to one standard error (3 replicates). Different letters indicate significant differences among the treatments within each panel based on HSD test ($P < 0.05$). Dashed horizontal lines indicate the soil ammonium and nitrate concentrations at the beginning of the incubation (Table 3-1). CT = control. The numbers in the legends represent the nitrification inhibitor application rate.

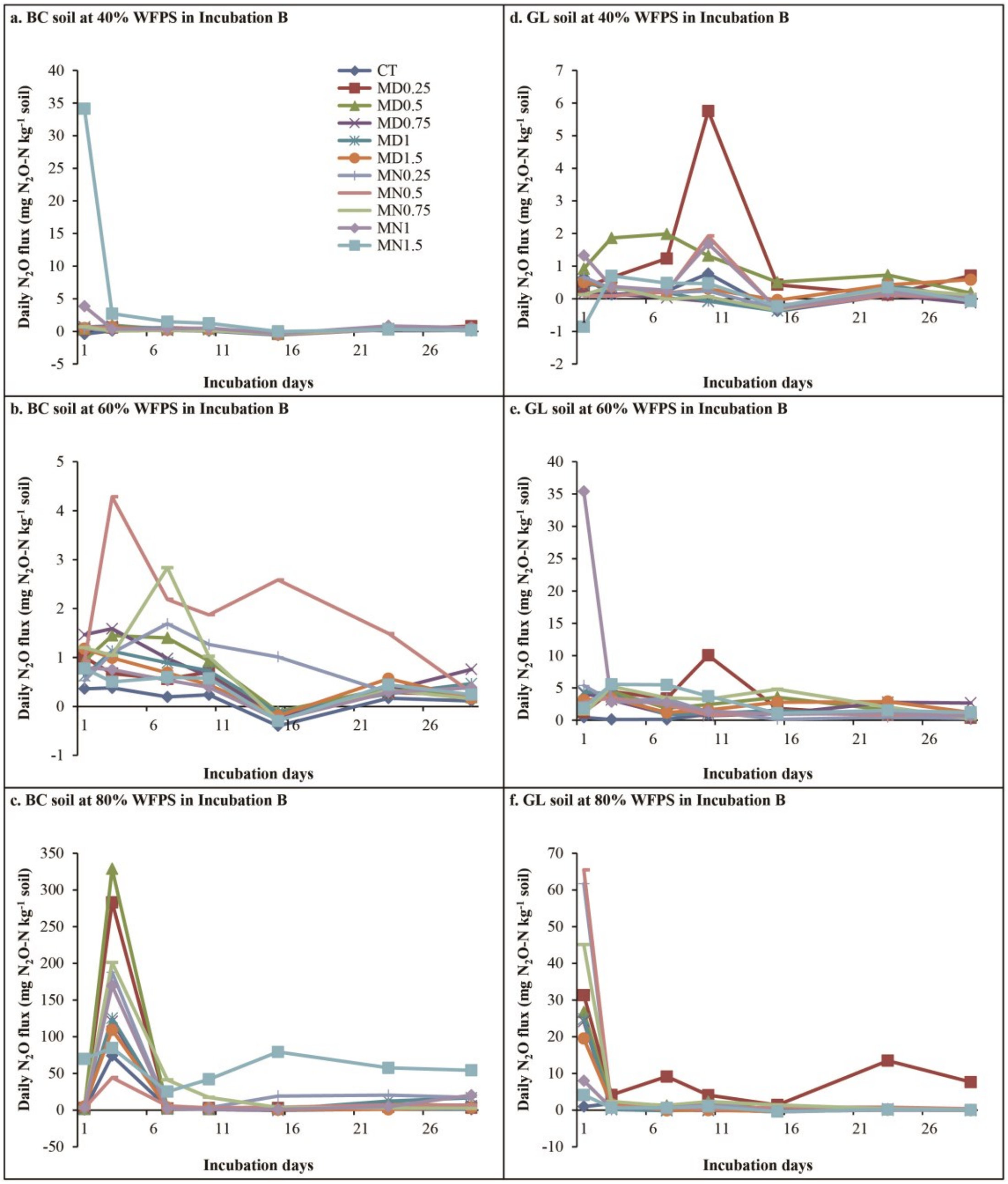

Fig. 3-7. Daily N_2O fluxes across the manure-inhibitor addition treatments for the Black Chernozem (BC) soils (a, b and c) and the Gray Luvisolic (GL) soils (d, e and f) at 40, 60 and 80% WFPS during incubation B. CT = control, MO = manure only, MN = manure with nitrapyrin additions, MD = manure with DMPSA additions. The numbers following the capital letters of legends represent the nitrification inhibitor application rate.

4. Soil N_2O Priming Effect of Urea Addition as a Function of Manure Management History and Water Content in a Simulated Fall-freeze-thaw Cycle - a Mesocosm Study

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4.1 Abstract

The addition of N substrates to soils has been found to induce short-term changes in soil organic matter transformations, which is called the “priming effect”. However, questions about the priming effect on N_2O production from N-amended soils following a freeze-thaw cycle and the factors impacting the dynamics of the priming effect are poorly understood. A mesocosm study with (a) two soil management histories (with (SW) and without (CT) manure applications) and (b) three water contents (low, medium and high) was established in simulated conditions consisting of a fall (with and without urea addition), followed by a winter freezing and subsequent spring thawing. Our results showed that urea added in the fall caused a positive priming effect on the cumulative N_2O emissions during spring soil thawing across all treatment combinations. Also, the SW soil showed higher soil-derived N_2O emissions produced during thawing when compared to the corresponding water content treatments in the CT soil. This might be due to the potentially greater amount of easily decomposable organic N in the SW soil. Two weeks after thawing, a negative priming effect on daily N_2O fluxes was observed only in the soils under high water content. This effect was likely due to N immobilization related to the decomposition of plant residues with a high C:N ratio. The total N_2O emissions increased with increasing water contents regardless of the soil management history and N addition, implying potentially increased N_2O emissions as a response to increased fall and winter precipitation in future climate conditions. Thaw-induced N_2O emissions accounted for more than 92% of total emissions, and at least 83% of the large N_2O fluxes on the 2nd and 3rd days of thawing were produced via denitrification. A shift in the dominant process of N_2O production was expected to occur as soil water gradually decreased during the soil thawing period; however, denitrification consistently remained the main source-process.

Key words: priming effect, residual effect, nitrous oxide, urea, depletion, ^{15}N.

4.2 Introduction

Soil freezing and thawing is a common phenomenon that regularly occurs in areas with relatively high latitude and altitude. Many laboratory and field experiments have observed increasing N_2O emissions taking place over soil freeze-thaw cycles, which can account for 30-90% of annual N_2O emissions (Wagner-Riddle et al., 2007; Goldberg et al., 2008; Wolf et al., 2010; Wu et al., 2010; Yanai et al., 2011; Abalos et al., 2016). A previous field study found that at least 67% of the annual N_2O emissions occurred during the spring thaw in soils receiving fall-applied liquid manure (Lin et al., 2017). In addition to the underlying driving effect of soil moisture on N_2O production (Davidson et al., 1991; Ruser et al., 2006), it is relevant to evaluate the N_2O release induced by soil thawing under various soil water contents. Moreover, we speculated that western Canada will experience wetter falls in the near future as a function of climate change, based on a recent climate change report projecting a large increase in precipitation in the fall season across the adjacent continental U.S. (Easterling et al., 2017). Therefore, in our study, we established a range of increasing soil moistures in a simulated fall season shortly before undertaking a freezing period to mimic winter temperatures, finally leading to re-creating a thawing phase of the soil (a sequence of seasonal events typical of western Canada).

Nitrification and denitrification are considered two major biological processes involved in N_2O production (Butterbach-Bahl et al., 2013). However, it is still unclear which of these processes is dominant in the intensive N_2O fluxes occurring during spring snow melting and soil thawing following a freezing winter (Lin et al., 2017). The ^{15}N isotope ratios at the central (α; ^{14}N-^{15}N-O) and terminal (β; ^{15}N-^{14}N-O) positions within the N_2O molecule (known as “isotopomers”) can reveal the dominant process contributing to N_2O production (Toyoda and Yoshida, 1999; Toyoda et al., 2011; Yamamoto et al., 2017). The difference between α and β can be expressed as the intramolecular site preference in the delta notation (SP= $\delta^{15}N^{\alpha}$ - $\delta^{15}N^{\beta}$) (Toyoda and Yoshida, 1999; Yamamoto et al., 2017). The bulk $\delta^{15}N$ in N_2O could provide another independent indicator of N dynamics, in particular when the ^{15}N isotopic composition of the soil subtract is also documented. These approaches enable recognizing and apportioning the contribution of various N_2O-source pathways can be recognized and apportioned, building a better knowledge about the N cycle under a wide range of diverse edaphic and climatic environments. In addition, our study also used an N fertilizer addition enriched with ^{15}N, and hence, a mass balance based on isotopic composition of the emitted N_2O (atom%) can be conducted to separate the contribution of two N pools (i.e., added N fertilizer versus pre-existing soil N) to the overall N_2O flux.

A priming effect has been discovered in earlier studies (Bingeman et al., 1953; Johnson et al., 2000; Conde et al., 2005; Blagodatskaya et al., 2007; Zimmerman et al., 2011), where a short-term acceleration (positive priming effect) or retardation (negative priming effect) of soil-derived CO_2 and mineral N occurred in a treatment receiving an addition of easily decomposable or utilizable substrate compared to a control treatment (Kuzyakov et al., 2000). An increase or decrease in soil-derived mineral N directly impacts soil N dynamics, potentially aggravating or mitigating environmental problems such as NO_3^- leaching and N gaseous losses. However, there are still many uncertainties about N_2O-related processes and the potential priming effects associated with soil freeze-thaw cycles.

To address these unknowns, we conducted a mesocosm study to investigate the N_2O production and sources under elevated soil moisture contents in response to a simulated fall N addition, followed by freezing and a subsequent thawing period. Specifically, this study aimed to distinguish the sources and contributions of N_2O-generating processes (nitrification vs. denitrification) for the major N_2O production, and to explore the dynamics of the priming effect regarding the N_2O emitted during thawing from soils with different N management history and water contents. The following hypotheses would be tested: 1) denitrification would dominate at the beginning of the thawing due to a typically high soil moisture content during thawing; 2) the labile N (urea) addition would lead to a positive priming effect on soil N as compared to soils without N addition (control); 3) larger N_2O emissions and priming effect would occur in soils that had received recurrent manure additions; 4) the priming effect would be amplified with increasing soil moisture due to accelerated mineralization caused by increased water content.

4.3 Materials and Methodology

4.3.1 Soil collection

Soils (0-15 cm depth) were collected from agricultural experimental plots with (spring manure, SW) and without (control disturbance, CT) a history of manure additions. The soil was collected in October 2016, from a site located at the Edmonton Research Farm (53°29’30’’N, 113°31’53’’W), Alberta, Canada. The site and field management were further described in Lin et al. (2017) and the physical and chemical properties of the soil are shown in Table 4-1. Due to the high moisture content at the time of field sample collection, the collected soils were air-dried for several days, then passed through an 8-mm sieve to remove large rocks and plant residuals. To accomplish sample homogeneity, each soil was mechanically mixed in several portions, after which all portions were laid on a sheet and manually mixed with shovels. After mixing, a small amount of each soil was collected to dry in an oven (105 °C) for 24 hours to measure the moisture content. Soils were stored at 2 °C until the experiment establishment.

4.3.2 Experimental setup

The whole experiment was separated into four phases: an initial conditioning phase in the greenhouse, a fall phase including N (^{15}N-urea) addition, a freezing phase and a thawing phase. The experiment was established in 5.5-L plastic pots measuring 21 cm in height and 19.8 cm in inner diameter at the top of the container. Soils (5.5 kg dry soil at 20% and 22% gravimetric water content for CT and SW soils, respectively) were weighted into each pot and compacted by increments up to 5 L (18.4 cm height) with a packing bulk density of 1.1 kg cm^{-3}.

4.3.3 Initializing phase in the greenhouse

To improve the soil structure regarding soil aggregate build-up and to simulate field plant growth, an initializing phase was conducted with wheat growth in a greenhouse receiving local daylight from August to October, 2017. The daylight progressively decreased from about 16 hours in August to 9 hours in October. The minimum, average, and maximum greenhouse temperatures were 12, 23 and 36 °C, respectively.

On August 1, 2017, 12 wheat seeds (AC® Muchmore, Canadian Western Red Spring cultivar) (FP Genetics, Regina, SK, Canada) were evenly distributed in a circle about 1.5 cm from the edge of the pot and 4 cm below the soil surface. After germination, the number of wheat plants was reduced to eight per pot as recommended (McKenzie et al., 2011). When the wheat grew to 8-10 cm above the ground, four bamboo sticks were installed around the plants in each pot to prevent tilting. Soil moisture was increased to 33% v/v (~57% water-filled pore space (WFPS)) on the day of planting and checked two or three times per week. From the tillering phase, all pots began receiving a 0.5 g L^{-1} solution of 20-8-20 fertilizer weekly [20% N, 8% P_2O_5, 20% K_2O, 0.5% Mg, 0.02% B, 0.05% Cu, 0.4% Fe, 0.05% Mn, 0.005% Mo, 0.05% Zn and 2.8% DTPA (ethylene diamine tetra-acetate, chelating agent)]. Throughout the growing season, each pot received the equivalent of a total amount of 50.85 kg N ha^{-1} fertilizer according to typical cropping recommendations (McKenzie et al., 2013). Due to a fungal disease (powdery mildew) occurring at the base of the plants, 2 L of Bumper® 418 EC fungicide (Adama Canada Ltd., Winnipeg, MB, Canada) was applied at a rate of 1.5 mL to 1 L of water on September 25, 2017. The aboveground plant biomass (> 5 cm height) was harvested and removed on October 30, 2017. To simulate the crop residue, 5 g of straw biomass was added to the soil surface. Two pots from each soil were randomly selected for destructive sampling to conduct soil mineral and isotopic N analyses. Six soil cores of 18.4 cm were taken from each pot by an auger with a diameter of 3.5 cm. The collected soils were air dried and passed through a 2-mm sieve. The other pots were sealed with caps and stored at 2 °C until the beginning of the next experimental phase.

4.3.3.1 Drying conditioning phase before the fall phase

Prior to implementing the N addition and moisture treatments, all pots were removed from 2 °C to room temperature for air drying to achieve a water content below the target WFPS. Chamber bases were installed in the soil to take CO_2 and N_2O flux measurements during drying. Detailed information about the chamber and flux measurement is provided in the section below. Based on the flux results collected during the drying phase, the pots were separated and stratified into three blocks for each soil; subsequently, they were randomly assigned to moisture and N treatments within a block. The treatments for each soil (CT and SW) were established at the end of this drying conditioning phase (day 0 of the measurement period) as follows: two levels of N additions (^{15}N-labelled urea (5 atom%^{15}N) and control without N) and three levels of water content (low (45-70-55 % WFPS), medium (55-80-65 % WFPS) and high (65-90-75 % WFPS)).

4.3.3.2 Simulated fall fertilizer addition phase

The treatments were established on day 0 and the fall phase (days 0-27) ended on day 27 of the experiment. With the assumption that the fall N application was for canola growing for the next season, 0.29 g ^{15}N-Urea (Sigma-Aldrich, St. Louis, MO, US) was applied into each pot, which was equivalent to 85 kg N ha^{-1} for the SW soil. The granular fertilizer was manually ground into powder in a grinding bowl, which had been washed with deionized (DI) water before use. The fertilizer powder was evenly placed 5 cm below the soil surface along the diameter of the pot area to simulate fertilizer banding. Then, room-temperature DI water was added to achieve an initial target of 45, 55 and 65% WFPS. These water contents were maintained by adding DI water every day throughout the fall phase. All pots and glass flasks with DI water were stored at 2°C. Cardboard was placed 3-5 cm above the top of the pots to prevent rapid evaporation while still allowing air circulation between the air and soil.

4.3.3.3 Simulated winter freezing phase

On day 28 of the experiment, all pots were moved from 2 °C to -18 °C and this freezing phase lasted for 27 days (days 28-55) to make sure the soils were fully frozen. To simulate typical water additions due to winter snow accumulation in central Alberta, 2 °C DI water was added to increase the soil water content by a total of 25% WFPS for each pot (i.e. from 45 to 70%, 55 to 80%, and 65 to 90%). This simulation of winter water additions was accomplished by increments of 8.3, 8.3 and 8.4% WFPS on days 31, 37 and 45, respectively.

4.3.3.4 Simulated spring thawing phase

On day 56, all pots were moved from -18 °C to room temperature to simulate a spring thawing for 30 days (days 56-86). The room temperature averaged 23 °C, ranging from 20.4 to 25.9 °C (recorded using a HOBO data logger at 1 Hz). Soil moisture content was allowed to decrease by 0.5% WFPS daily and this corresponded to a total decrease of 15% WFPS over the 30-day thawing period. Soil moisture content was adjusted by weighting the pots and adding room temperature DI water. On the last day, three cores of soils (18.4 cm in height and 3.5 cm in diameter) were taken from each pot using soil augers for further mineral and isotopic N analyses.

4.3.4 Measurements of CO_2 and NH_3 fluxes

During the drying, fall and freezing phases, CO_2 and NH_3 fluxes were determined by a simple system, which included a Picarro G2508 cavity ring-down spectroscopy (CRDS) with a 105 mL analytical cell at constant 140 Torr pressure and 45 °C temperature (Picarro, Santa Clara, CA, USA), a low-leak diaphragm A0702 pump (Picarro, Santa Clara, CA, USA) and a custom-made non-steady-state chamber. The vacuum pump enabled the re-circulation of gas sample flow through the chamber with a rate of 240 standard mL min^{-1}. The polyvinyl chloride artificial chamber contained a base and a top. The chamber base was installed 3 cm into and 7 cm above the soil surface at the center of each pot. The cross-sectional area of the chamber was 184 cm^2 (diameter of 15.3 cm). The chamber top (5 cm in height and 15.3 cm in inner diameter) consisted of two tubing connection ports for gas circulation (one for inlet and another one for outlet), a stainless capillary tubing (3/8 mm in inner diameter, 10 cm in length) on the wall for the purpose of pressure equilibration, and rubber seals fitted to the chamber top to ensure hermetic chamber closure. The total headspace of the chamber system was 2.2 L. The flux measurement duration was 3 minutes (300 data points). The CO_2 and NH_3 flux measurements took place on days 0, 1, 2, 3, 6, 9, 13, 17, 20, 24 and 27 during the fall phase, and on days 31, 37, 45, 52 and 55 during the freezing phase.

During the thawing phase, CO_2 and NH_3 fluxes were measured by an automated chamber system, which included the CRDS as described above, and an eosMX multiplexer connected with twelve eosAC automated chambers (Eosense Inc., Dartmouth, NS, Canada). The total headspace of the automated chamber system was 2.8 L. The measurement duration was about 10 minutes. The fluxes were measured every day during the 30-day thawing phase. The flux calculation for CO_2, NH_3 and N_2O was determined by equation [1].

$$F = (\frac{dC}{dt}) \times (\frac{V}{M}) \times (\frac{P}{R \times T}) \times 2M \times k \qquad [1]$$

where F is the gaseous flux (μg kg^{-1} d^{-1}); dC/dt is the slope of a simple linear regression or as the first derivative of a quadratic regression at t_0 (μL L^{-1} s^{-1}); V is the headspace volume of the gas chamber (L); M is the dry soil weight (kg); P is the pressure at chamber headspace during measurement (atm); R is the gas constant (atm μL K^{-1} μmol^{-1}); T is the temperature at chamber headspace during measurement (K); M is the molar mass of C or N (g mol^{-1}) and k is a conversion factor for the flux unit (from μg kg^{-1} s^{-1} to μg kg^{-1} d^{-1}).

4.3.5 Measurements of N_2O flux and N_2O isotopomers

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 (Aerodyne Research, Inc., Billerica, MA, USA) equipped with 200-m path length analytical cell (2 L volume at 30 Torr vacuum), Nafion^{TM} tubing (Perma Pure, Lakewood, New Jersey, USA) and coupled in recirculation with a non-steady-state chamber as further described below. Temperature (20 °C) and sample flow rate (1.5 standard L min^{-1}) were held constant in the instrumentation. The TDLWintel software provided system control as well as data acquisition and recording at 1 Hz resolution.

The α and β isotopic ratios were calibrated in a similar way as described by 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. 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 integration of five seconds).

The same chamber as described above (2.8 L headspace) was connected to the spectroscopy system and closed for 3 minutes. The isotopic composition of both site-preference and bulk ^{15}N in N_2O was expressed in δ (‰) notation. The calculation for $^{15\alpha}R$, $^{15\beta}R$, $\delta^{15\alpha}N_2O$, $\delta^{15\beta}N_2O$, $\delta^{15bulk}N_2O$ and SP was determined by the following equations [2-6].

$$^{15i}R = \frac{^{15i}N}{^{14}N}\ (i = \alpha\ or\ \beta) \qquad [2]$$

$$\delta^{15i}N_2O = \left(\frac{^{15i}R_{sample}}{^{15}R_{std}} - 1\right) \times 1000\ (i = \alpha\ or\ \beta) \qquad [3]$$

$$\delta^{15bulk}N_2O = \frac{\delta^{15\alpha}N + \delta^{15\beta}N}{2} \qquad [46]$$

$$Atom\%^{15}N_2O = \frac{\frac{^{15\alpha}N + {}^{15\beta}N}{2}}{^{15\alpha}N + {}^{15\beta}N + {}^{14}N} \times 100 \qquad [57]$$

$$SP = \delta^{15\alpha}N_2O - \delta^{15\beta}N_2O \qquad [68]$$

where $^{15\alpha}N$, $^{15\beta}N$ and ^{14}N represent the mixing ratio of $^{15\alpha}N$- N_2O, $^{15\beta}N$- N_2O and ^{14}N- N_2O in the sample, respectively (ppm); $^{15\alpha}R$ represents the isotopic ratios of $^{15\alpha}N$ to ^{14}N; $^{15\beta}R$ represents the ratios of $^{15\beta}N$ to ^{14}N; $^{15}R_{std}$ is the isotopic ratio in the atmospheric N_2 ($^{15}R_{std} = 0.003676$);

Atom%$^{15}N_2O$ represents the isotopic percentage of ^{15}N in N_2O; SP represents the intramolecular ^{15}N site preference (‰).

The atom%$^{15}N_2O$, $\delta^{15\alpha}N_2O$ and $\delta^{15\beta}N_2O$ emitted from each pot was obtained from the intercept of a Keeling plot (i.e. a linear regression of atom %$^{15}N_2O$, $\delta^{15\alpha}N_2O$ or $\delta^{15\beta}N_2O$ as y-axis vs. 1/total N_2O as x-axis). The partitioning-contribution of nitrification and denitrification to the N_2O production was calculated using equations [7-8], with an assumption that the SP of nitrification and denitrification are 0 and 33 ‰, respectively (Sutka et al., 2006). The priming effect of daily N_2O flux and cumulative N_2O emissions was calculated based on equations [9-12].

$$F_{ni}(\%) = \frac{SP}{33} \times 100 \qquad [7]$$

$$F_{deni}(\%) = \frac{33 - SP}{33} \times 100 \qquad [8]$$

$$FN_2O_{15N-urea}(\%) = \frac{Atom\%^{15}N_2O_{15N-urea} - Atom\%^{15}N_2O_{control}}{5\% - Atom\%^{15}N_2O_{control}} \qquad [9]$$

$$FN_2O_{soil}\ (\%) = \frac{5\% - Atom\%^{15}N_2O_{15N-urea}}{5\% - Atom\%^{15}N_2O_{control}} \qquad [10]$$

$$N_2O_{15N-urea} = FN_2O_{15N-urea} \times N_2O\ flux\ from\ soil\ with\ urea\ addition \qquad [119]$$

$$Priming\ effect\ of\ N_2O\ (PE) = N_2O_{15N-urea} - N_2O_{control} \qquad [1210]$$

where F_{ni} and F_{deni} represent the proportional contribution of nitrification and denitrification, respectively; $FN_2O_{15N-urea}$ and FN_2O_{soil} represent the fraction of N_2O from added ^{15}N-labelled urea and from the soil itself, respectively; Atom%$^{15}N_2O_{15N-urea}$ and Atom%$^{15}N_2O_{control}$ represent the isotopic percentage of ^{15}N in N_2O from the pot with and without urea addition, respectively;

$N_2O_{15N\text{-}urea}$ and $N_2O_{control}$ represent the N_2O production derived from the soil itself in the treatments with and without urea addition, respectively (μg N_2O-N kg^{-1} d^{-1} or μg N_2O-N kg^{-1}); PE >0 indicates positive priming effects; PE <0 indicates negative priming effects.

4.3.6 Other measurements

Soils were air dried and passed through a 2 mm mesh for further laboratorial analyses. Soil extractable NH_4^+ and NO_3^- in the filtrate (2M KCl) were determined by using a SmartChem 200 Discrete Wet Chemistry Analyzer (Westco Scientific Instruments, Inc., Brookfield, CT, US) based on the standard method (McKeague, 1978; Carter and Gregorich, 2008). The soil organic C and total N were determined by a dry combustion method using a Costech Model EA 4010 Elemental analyzer (Costech International Strumatzione, Florence, Italy). The soil clay, silt and sand percentages were determined by the rate of settling in solution using a hydrometer (McKeague, 1978; Carter and Gregorich, 2008). Soil pH was determined in a mixture with a soil to water ratio of 1:2 (McKeague, 1978; Carter and Gregorich, 2008).

With the aim of measuring ^{15}N isotopic composition in the soil without urea addition, soil samples were oven dried at 60 °C and ball-grounded to a fine consistency to ensure homogeneity for isotope analysis. The soil $\delta^{15}N$ was determined by using a Flash 2000 Elemental Analyzer (Thermo Fisher Scientific, Delft, the Netherlands) to dry combust the soil sample converting all N to N_2. Subsequently, this analyzer was interfaced online to a Finnigan Delta V Plus isotopic ratio mass spectrometer (Thermo Electron, Bremen, Germany) to detect the ^{15}N isotope composition. Based on the isotopic analyses of N_2O and soil N, the soil N isotope discrimination value (ε) was calculated based on the equation [13].

$$\varepsilon\ (‰) = (\frac{^{15}R_{N2O}}{^{15}R_{soilN}} - 1) \times 1000 \qquad [13]$$

where $^{15}R_{N2O}$ represents the isotopic ratio of emitted N_2O on day 57 (2nd day of thawing); $^{15}R_{soilN}$ represents the isotopic ratio of soil N; positive ε implies an enrichment of ^{15}N in the transformation processes of soil N to N_2O production; and negative ε implies a depletion of ^{15}N in the transformation processes.

During the flux measurement, air temperature and pressure were recorded by a HOBO UX100-001 data logger (Onset® Computer Corporation, Bourne, MA, USA) and a Testo 511 barometer (Testo Inc., Lenzkirch, Germany), respectively.

4.3.7 Statistical analyses

Statistical analyses were performed in R 3.1.3 (R Core Team, 2014). The data was transformed to meet the assumptions of normality and homoscedasticity as necessary. The difference in the priming effect of cumulative N_2O emissions during the thawing phase between the coupled soils (i.e. with and without urea addition) was examined by a two-sample t test. The significance of the differences in the contribution of nitrification and denitrification to the N_2O produced on the 2nd day of thawing was determined using two-way analysis of variance (ANOVA) analysis ($P < 0.05$) for a fixed-effect model. The effect of soil N management history (CT vs. SW soils), nitrogen (^{15}N-labelled urea vs. control) and soil water content (low, medium vs. high) treatments on soil NH_4^+, NO_3^-, cumulative N_2O, CO_2 and NH_3 emissions were examined using three-way ANOVA for a fixed-effect model with interaction analysis ($P < 0.05$). Tukey's Honest Significant Difference test (HSD) ($P < 0.05$) was used to further compare the difference if the treatment effects described above were significant.

4.4 Results

4.4.1 N_2O production and contributing N processes to significant N_2O fluxes

Throughout the whole experiment, the magnitude of N_2O production varied among the different phases. During the freezing phase, the average daily N_2O fluxes across all treatments were limited, with an average of 0.22 ± 0.05 μg N_2O-N kg^{-1} soil d^{-1} (Fig. 4-1c). After urea and water additions in the fall phase, the average daily N_2O flux rose up to about 0.48 to 0.75 μg N_2O-N kg^{-1} d^{-1} on days 1-3, then dropped to 0.17 ± 0.01 μg N_2O-N kg^{-1} d^{-1} on day 9 followed by a gradual increase to 1.23 ± 0.40 μg N_2O-N kg^{-1} d^{-1} until the last day of the fall phase (Fig. 4-1c). Robust fluxes occurred in the thawing phase. The average daily N_2O flux reached the peak of 71.44 ± 7.08 μg N_2O-N kg^{-1} d^{-1} on the 2nd day of thawing (day 57 after N addition) and quickly declined to 17.66 ± 3.90 μg N_2O-N kg^{-1} d^{-1} on the 6th day of thawing (day 61) (Fig. 4-1c). Following this decline, the average daily N_2O flux gradually decreased further to a relatively constant rate of 0.74 μg N_2O-N kg^{-1} d^{-1}, remaining at 0.76 ± 0.18 μg N_2O-N kg^{-1} d^{-1} on the last day of thawing (day 86) (Fig. 4-1c). Focusing on specific treatment comparisons, the N_2O peak occurred one day later in the soil with high moisture content compared to the soils with low and medium moisture contents (Fig. 4-2). Due to the robust fluxes emitted during the thawing phase, the N_2O emissions produced during thawing were responsible for at least 92% of total emissions across all treatments (Fig. 4-3).

The cumulative N_2O emissions were significantly impacted by the soil with contrasting historical managements (CT vs. SW), soil water content and N addition (urea vs. control) throughout the whole experiment (Table 4-2). Specifically, the cumulative N_2O emissions from the soil with historical N additions (SW) were higher compared to the corresponding soil without

N addition (CT) (Fig. 4-3). We observed that the cumulative N_2O emissions increased with increasing soil water content (Fig. 4-3). The addition of urea increased the cumulative N_2O emissions regardless of soil management history and water content (Fig. 4-3).

The N_2O production from nitrification and denitrification processes was examined for the very large N_2O fluxes on the 2nd and 3rd days of thawing (days 57 and 58, respectively) for the soils under ^{15}N natural abundance (without ^{15}N-labelled urea addition) (Fig. 4-4). Although no significant difference in N_2O fluxes was found between the soil management history (SW vs. CT) and among water contents, there was a decreasing tendency of the N_2O magnitude on day 57 and an increasing tendency on day 58 with larger soil water contents (Fig. 4-4). Across all treatments, denitrification was definitely the dominant process contributing largely to the vigorous N_2O fluxes on days 57 and 58, ranging from 83% to the entire N_2O production (Fig. 4-4). The important role of denitrification in N_2O production seemed to become stronger with an increase in the soil water content in the CT soil, as evidenced by an increased contributing proportion on day 57 (Fig. 4-4).

4.4.2 Priming effects on soil N_2O production

Irrespective of soil water content and management history, soils subjected to the urea addition were consistently higher in soil-derived N_2O emissions compared to control soils, clearly showing a net positive priming effect triggered by thawing (Fig. 4-5). Compared to the CT soil, the cumulative priming effect was higher in the SW soil, as shown by the larger soil-derived emission difference between the soil with and without urea additions in the SW soil (i.e. N vs. C, Fig. 4-5). However, with respect to the daily priming effect, the magnitude and direction of the priming effect changed not only across soils, but also with time and with moisture content

(Fig. 4-6). At low moisture content, the priming effect for both CT and SW soils reached the maximum magnitude on the 2nd day of thawing (day 57), sharply declined below zero on the next day, then sharply increased to being positive or around zero for the rest of thawing (Fig. 4-6a). By contrast, the positively pronounced priming effect achieved one day later (day 58) at the higher moisture contents (medium and high moisture contents), and then the priming effect gradually dropped back to approximately zero on day 61 (Fig. 4-6b and c). In addition, the magnitude of the priming effect peak was greater at higher moisture contents than lower moisture contents in the SW soils (29.44 μg N_2O-N kg^{-1} d^{-1} at low vs. 62.95 at medium and 52.82 at high moisture content), and it was also greater in the SW than in the CT soil at both medium and high soil moisture contents (Fig. 4-6). From day 65 to 72, the SW soil showed a slightly stronger positive priming effect compared to the CT soil at medium water content (Fig. 4-6b). Nevertheless, this phenomenon reversed at high water content from day 63 to 65, where a stronger positive priming effect was observed in the CT soil rather than in the SW soil (Fig. 4-6c). Interestingly, there was clearly a negative priming effect taking place during the late thawing phase when both soils had high moisture contents, but the negative priming effect occurred slightly earlier in the SW soil (day 66) than in the CT soil (day 70) (Fig. 4-6c). Overall, the daily priming effect across all treatments significantly increased to positive values within the first several days of thawing, then declined and fluctuated around zero, and finally reached a plateau near zero or neutral priming (Fig. 4-6). Moreover, the soils with higher water content required more time to reach the plateau, as the plateau occurred on approximately days 72, 74 and 78 at low, medium and high moisture content, respectively (Fig. 4-6).

4.4.3 Soil CO_2 and NH_3 exchange

The CO_2 fluxes throughout the experiment varied among different phases. Following N addition and from the early fall phase to the end of the experiment, the soil became a minor CO_2 source (Fig. 4-1b). Within the fall phase (excluding day 0), CO_2 fluxes were generally low and relatively stable across all treatments, with an average production rate of 1.29 ± 0.13 µg CO_2-C kg^{-1} d^{-1} (Fig. 4-1b). Afterwards, the CO_2 flux steadily decreased to 0.57 ± 0.14 µg CO_2-C kg^{-1} d^{-1} on the 6th day of the 24-day freezing, and finally became negligible (Fig. 4-1b). At the beginning of thawing, the average CO_2 flux across all treatments sharply increased to 8.65 ± 0.29 µg CO_2-C kg^{-1} d^{-1}, and thereafter slowly decreased over time, reaching 1.94 ± 0.16 µg CO_2-C kg^{-1} d^{-1} on the last day of the study (Fig. 4-1b). In addition, we noticed a coherent high correlation between CO_2 and N_2O fluxes during most of the simulated spring thaw phase, with the exception of the 2nd, 3rd and 4th days of thawing when CO_2 and N_2O fluxes appeared to be unrelated to each other (Fig. 4-7). Upon establishing a linear regression for N_2O flux as a function of CO_2 flux for most thawing data (Fig. 4-7), we used graphic extrapolation to separate contributions of *de novo* biology production versus physical release to the overall N_2O flux on the 2nd, 3rd and 4th days of thawing. Among these three days, the largest proportion of estimated N_2O production due to physical release was found on the 2nd day of thawing, with a value of 71.5% (Fig. 4-7). This estimated proportion decreased to 63% on the 3rd day of thawing, and then to 26.3% on the 4th day (Fig. 4-7).

The daily NH_3 efflux clearly peaked following urea addition and then gradually declined during the fall phase (Fig. 4-1d). At both freezing and thawing phases, the average daily NH_3 flux became negligible (Fig. 4-1d).

Regarding cumulative emissions, soil moisture content had a significant effect on cumulative CO_2 emissions (Table 4-2). Across treatment combinations (soil management history × N addition), the cumulative CO_2 emissions increased with increasing soil water content, except for the treatment of SW with urea addition, where the highest CO_2 emission was found at the medium water content (Fig. 4-8a), perhaps indicating an optimum for biology at intermediate water contents. Across all treatments, the majority of cumulative CO_2 emissions were produced during thawing, accounting for at least 65% of total cumulative emissions, while the dominant contributor to the net cumulative NH_3 emissions was the fluxes emitted during the fall phase following the N addition (Fig. 4-8b).

4.4.4 Mineral N patterns and ^{15}N-N_2O depletion from soil N transformations

Both soil NH_4^+ and NO_3^- concentrations at the end of the experiment were higher than the levels prior to the treatment establishment (Fig. 4-9). There was a significant effect on soil NO_3^- concentration in terms of the different soil management history (CT vs. SW), moisture water content, N addition (urea vs. control) and the interaction between the soil management history and water contents; however, there were no such significant effects on the soil NH_4^+ concentration (Table 4-2). The NO_3^- concentration was consistently greater in the soil with historical N addition (SW) compared to the corresponding control soil (CT) (Fig. 4-9b). The NO_3^- concentration apparently increased with increasing soil moisture content in CT soils, but this pattern was not found in SW soils (Fig. 4-9b). Soils receiving urea addition showed greater increments in the NO_3^- concentration in comparison to the soil without urea (i.e., CT + Urea vs. CT and SW + Urea vs. SW, Fig. 4-9b). Regardless of soil management history and water content, there was a consistent negative depletion of ^{15}N (ε) in the unilateral transformation processes from the soil N pool into the emitted N_2O pool (Table 4-3). Regardless of soil management history and water content, it seemed that the depletion of ^{15}N (ε) was stronger in the SW than the CT soil (Table 4-3).

4.5 Discussion

4.5.1 N_2O, CO_2 and NH_3 production through a simulated cycle of fall, freeze, spring thaw

The dynamics of N_2O, CO_2 and NH_3 fluxes varied among phases with different environmental conditions and substrate availability. After the urea addition in the fall phase, the urea was quickly hydrolyzed due to simultaneously increased soil water contents (Fig. 4-1). The abundant urea-derived NH_4^+ then became available for nitrifiers and transformed to NO_3^-. With the transformation, the soil NH_4^+ gradually declined, leading to a decrease in NH_3 volatilization. In the later period of the fall phase, the increased NO_3^- from nitrification became available for denitrifiers, and was specifically conducive for those under moist conditions (Smith et al., 2018). This could explain why gradually increasing N_2O fluxes were observed in moist treatments in the later fall phase (Fig. 4-1c and Fig. 4-2). The largest cumulative NH_3 during the fall phase could be largely due to the sufficient substrate of NH_4^+ , primarily owing to the urea hydrolysis.

Over the freezing period, negligible N_2O, CO_2 and NH_3 fluxes were a consequence of restricted soil microbial activities and diffusion pathways blocked by the ice layer at freezing temperatures (-18 °C) (Pietikäinen et al., 2005). Our results were consistent with previous studies, where limited N_2O and CO_2 emissions were observed under subzero temperatures (Teepe et al., 2004; Ludwig et al., 2006).

During the simulated spring thaw in this study, the NH_3 volatilization that was expected to be high under high temperature (20°C) was minor (Ernst and Massey, 1960). This might be mainly driven by the insufficient soil NH_4^+, which was possibly a result of a relatively completed transformation to NO_3^- by nitrifiers during the previous 27-day fall phase. This inference could be partially supported by the N_2O source-partitioning result which showed that the denitrification which uses NO_3^- as a substrate was the main process contributing to the major N_2O fluxes on the 2nd and 3rd day of thawing (Fig. 4-4). In addition, the high NH_4^+ adsorption to clay particles and high buffering capacity due to the high organic matter content of the soils could suppress NH_3 volatilization in our controlled study which used closed chambers (Sommer et al., 1991; Whitehead and Raistrick, 1993).

At the beginning of the simulated spring thaw, substantially elevated CO_2 and N_2O fluxes closely related with increased soil temperature and water content were triggered by thawing, in line with findings in other cold regions (Wagner-Riddle and Thurtell, 1998; Wang et al., 2013). Our results were also supported by an earlier field study with consecutive four-year flux measurements by Chantigny et al. (2016), who found that CO_2 and N_2O fluxes from the soils with fall-applied slurry peaked the highest when the snow melt occurred, and those flux bursts lasted for about 2-6 week periods. The suddenly increased soil temperature and water content due to thawing unblocked the gas diffusion pathway in the soil profile and triggered a rapid soil microbial recovery as evidenced by raised respiration rates. Besides the impact on CO_2 production rates, the increased soil water content coupled with low O_2 availability were conducive for N_2O production via denitrification, as was partially shown by the source-partitioning result of the significant N_2O fluxes on the 2nd and 3rd days of thawing in this study (Fig. 4-4). Apart from the altered microbe-favorable environment, earlier studies have discovered rapid increases in the availability of soil inorganic N and labile C compounds upon soil thawing (Schimel and Clein, 1996; Nielsen et al., 2001; Herrmann and Witter, 2002; Matzner and Borken, 2008), which could simultaneously activate further microbial activity and strengthen associated fluxes. Mineralization of soil organic matter, destruction of soil aggregates due to ice crystal expansion, and microbial lysis could be possible causes of the increased substrate availability (Congreves et al., 2018).

Although the sudden flux spikes occurred at the beginning of thawing, the magnitude of CO_2 and N_2O fluxes synchronously decreased with time as soil moisture content gradually declined. This result emphasized the driving role of soil moisture on soil microbial activity and N_2O emissions, in particular during the spring thaw when major changes in soil water content usually take place. By contrast, the daily N_2O production on the 2nd day of thawing tended to decrease with increasing water content (Fig. 4-4). This could be a result of much longer melting time and prolonged blocked gas diffusion pathways at higher soil water contents. This notion could also explain the occurrence of a very large but delayed N_2O priming effect during thawing exclusively at the higher moisture contents. In addition to the delaying melting effect, the high possibility of transformation from N_2O to N_2 through denitrification at higher moisture contents (i.e., ~90% WFPS in the high water content treatments) could minimize the total thaw-induced N_2O losses (Ruser et al., 2006).

The high coherence of most CO_2 and N_2O fluxes during the simulated spring thaw is in general agreement with previous field research by Furon et al. (2008) who found a close connection between these two fluxes produced during spring thawing based on measurements over 4 years in silt loam soil in Ontario, Canada. This coherence was evaluated in an earlier study in which there was a linear correlation with an average R^2 of 0.57 between the N_2O and CO_2 production rates from late January to mid-March in southern Germany (Dörsch et al., 2004). However, the early-thawing fluxes in our study were decoupled and outside of the major linear pattern (Fig. 4-7). This could suggest that the initial response of N_2O fluxes to the beginning of thawing is not only linked with the *de novo* microbial production during thawing but also with the physical release of trapped gases over the freezing period, which has been recognized in previous literature (Grant and Pattey, 1999; Teepe et al., 2001a; Gregorich et al., 2006). Within the 30-day spring thaw period, the short-lived duration for the initial flux pulses associated in part with the physical release indicated to a certain extent the less important role of physically released N_2O to the entire spring thaw emissions. This was denoted by Risk et al. (2013) who reported that only 25-37% of total N_2O emissions produced during the spring thaw were derived from the physical release. Moreover, the trapped N_2O emissions accumulated over the simulated winter freeze seemed to be of greater amplitude compared to the CO_2, but to our knowledge this has not been previously demonstrated.

The important role of spring thaw N_2O emissions as found in this study is consistent with other field research in agricultural systems (Wagner-Riddle et al., 2007; Yanai et al., 2011; Abalos et al., 2016; Lin et al., 2017). The freeze-thaw induced N_2O emissions from their croplands accounted for about 30-90% of the total annual N_2O budget. Despite the fact that our study encompassed part of the annual cycle, more than 90% of total N_2O emissions were produced during thawing. However, the substantial contribution of thawing could be influenced by our experimental mesocosm conditions. The soil in this study was subjected to one cycle of freeze-thaw for 30 days, whereas field conditions are likely to register even more frequent freeze-thaw cycles over a longer non-growing season in realistic and future climate scenarios. Increasing both freezing duration and freeze-thaw cycle frequency have been found to considerably increase the N_2O emissions during thawing (Chen et al., 1995; Teepe et al., 2004;

Singurindy et al., 2009). Therefore, additional laboratorial and field studies are recommended to further elucidate the important influence of freeze-thawing on soil C and N dynamics.

4.5.2 N_2O production and soil N pool as a function of N additions, water contents and soil histories

Cumulated N_2O emissions were significantly influenced by N addition, soil water content and soils with different management history. The soils amended with urea significantly increased the total N_2O emissions compared to the un-amended soils, in particular for the emissions produced during thawing, as reported by previous studies (Burton et al., 2008; Tenuta et al., 2016; Lin et al., 2017). The cumulated N_2O emissions increased with increasing water content in both soils with and without urea additions, which was consistent with Hou et al. (2000a). They found that N_2O emissions positively increased with soil water content from 40% to 100% WFPS in both control and urea-amended soils. The fact that the SW soil had greater cumulative N_2O emissions when compared with the CT soil could be because the SW soil had more easily decomposable organic N due to receiving recurrent manure additions.

Although there were no significant interacting effects on N_2O emissions, the responses of soil NO_3^- to the various water contents were differed significantly between the CT and SW soils (Table 4-2). This contrasting NO_3^- response indicated a residual effect from the preceding manure amendments in terms of the changes in soil nutrient pools and their dynamics. In the CT soil, increased NO_3^- concentration with increasing water content would be a consequence of enhanced mineralization and nitrification rates (Wang et al., 2006). In the SW soil, however, the repeated manure field applications would result in a greater amount of soil mineralizable N (Ginting et al., 2003), which in turn would improve the N supply, buffering the capacity of the soil. Taking this into consideration, the high soil NO_3^- in the SW soil would resist changing to lower status with the alteration in moisture content as shown by our results (Fig. 4-9). This could also explain why the contributing proportion of denitrification to the large thaw-N_2O production tended to increase with increasing water content in the CT soil, but not in the SW soil on the 2nd day of thawing (Fig. 4-4).

4.5.3 Priming effect of N addition on thaw-induced N_2O production as influenced by soil management history and water content

Our results showed that fall-applied N fertilizer would induce a net positive priming effect during the following spring thaw. Regardless of management history and water content, the consistent net positive priming effects across the treatments receiving fall-applied urea were evident by the positively primed daily N_2O at the beginning of thawing (Fig. 4-5, Fig. 4-6). These net positive priming effects indicated an excessive mineralization caused by the fall-applied urea, implying potential soil fertility degradation owing to NO_3^- leaching and N gaseous losses. The soils with urea amendment provided more microbe-available N compared to the soils without urea at the onset of thawing. These extra N substrates due to fall-applied urea in addition to the increased soil temperature and moisture content during thawing concurrently activated further microbial activity and hence accelerated a greater mineralization rate. Afterwards, the excess in mineralized inorganic N in the soil with urea additions became available for activated nitrifiers and denitrifiers, consequently producing extra native soil-derived N_2O. Blagodatskaya and Kuzyakov (2008) found that the levels of added substrate C (as the percentage of soil microbial biomass C) drove the direction of the priming effect. Accordingly, we speculated distinct responses of primed N_2O dynamics to fall-applied organic fertilizers due to their extra supply of organic C source. Future research focusing on this aspect would help to deepen our understanding of soil C and N turnovers, particularly in agricultural systems with frequent nutrient inputs and outputs.

The priming effect was clearly influenced by different soil management histories. Compared to the CT soil, the SW soil had more easily decomposable organic matter (OM) associated with historical manure amendments. This could explain the larger net positive priming effects in the SW compared to the CT soils. The question regarding the OM sources stimulating larger primed N_2O emissions in the soils with a history of organic amendments remains open.

Besides the soil management history, soil moisture content evidently impacted the dynamics of primed daily N_2O fluxes. The dissimilar observations regarding the difference in peak primed N_2O fluxes between the two soils across various water contents indicated that low water content was the key controlling factor. Furthermore, the apparent negative primed N_2O fluxes taking place in both soils at higher rather than lower water contents further demonstrated the influence of soil moisture content on priming.

The shift of daily priming effect from positive to negative and eventually back to zero or positive at the high water content in both soils could be explained by the mechanism of preferential substrate utilization. The preferential substrate utilization hypothesis states that microorganisms prefer easily available and highly accessible substrates compared to recalcitrant organic substrates when given a variety of nutrient supplies (Cheng, 1999; Cheng and Kuzyakov, 2005; Blagodatskaya and Kuzyakov, 2008). In this study, we assumed that the activated microorganisms initially used the easily available substrates at the onset of thawing, and then switched to consume more complex organic substrates (e.g. plant residuals), and eventually utilized the soil recalcitrant OM. When soil microorganisms switched to decompose plant residuals, they needed to uptake inorganic N due to a high C:N ratio of wheat straw and roots (Gan et al., 2011), thus inducing a net immobilization and accompanied negative primed N_2O production. With the decreases in the decomposition of more easily available substrates, soil microorganisms progressively utilized soil recalcitrant OM to sustain their metabolism, causing an accelerated N mineralization coupled with a diminishing negative priming N_2O production.

The reasons for explaining why the apparent primed N_2O fluxes were only observed at the high water content rather than the lower water content remain obscure. It might be a result of a greater N mineralization rate at higher water contents (Stanford and Epstein, 1974; Paul et al., 2003). As a result, the substrate pool substitution (i.e. the switch in the utilization of easily decomposed substrates to moderately decomposed substrates, such as plant residues) associated with the switch to a negative priming effect would occur earlier at the high moisture content compared to that at lower water contents. In addition, the more inorganic N available in the soils at the high water content would have a higher possibility of inducing obvious negative priming effects owing to net N immobilization. Blagodatskaya et al. (2007) found that apparent negative primed CO_2 fluxes were observed in the glucose-enriched soil with N addition compared to the treatment without N addition. The presence of more inorganic N at the high water content can be partially verified by the final NH_4^+ and NO_3^- concentrations in the CT soil at the end of the experiment (Fig. 4-9). The equivalent inorganic N in the SW soil could be a result of the residual effect of historical manure additions, which provides a high buffering capacity to regulate and continuously replenish soil nitrogen availability.

4.5.4 Sources and processes responsible for the significant N_2O fluxes produced during thawing

Irrespective of different soil management history and water content, the consistent negative isotope discrimination values (ε) indicated ^{15}N depletion in the transformation processes of the soil N pool to the major N_2O fluxes produced on the 2nd day of thawing. Alternatively, it means the soil N pool was the major source for this substantial N_2O production, because a number of soil N transformations fractionate against the heavier isotope (i.e., ^{15}N), resulting in ^{15}N depletion in the product relative to the remaining substrate (Högberg, 1997). Considering denitrification as the main source of this significant production, the soil NO_3^- pool was very likely to be the primary pool contributing to this large N_2O emission rather than the NH_4^+. As nitrification is considered a key source for N_2O emissions at lower water contents (Davidson et al., 1991; Ruser et al., 2006), the gradually decreasing soil water content during thawing is expected to be accompanied by a shift in processes (from denitrification to nitrification) primarily responsible for N_2O production; however, information available on this research field is limited. Moreover, compared to the CT soil, a more negative average ε in the SW soil indicated a greater fraction of the N pool in the SW soil seemed to be converted to the large N_2O production, which is consistent with our aforementioned supposition of more easily decomposable N in the SW soil. However, it is noted that the averages of ε were not significantly different between the two soils.

4.6 Conclusions

Urea addition in the simulated fall resulted in a positive priming effect regarding the cumulative thaw-N_2O emissions across all treatments. This implies that the fall-applied urea would potentially aggravate soil fertility degradation due to excessive mineralization and would intensify N losses in the form of NO_3^- leaching and N gaseous losses. The magnitude and direction of the priming effect in relation to daily N_2O production were driven by different soil management history and various water contents. The soil with historical manure additions apparently further enhanced the priming effect compared to the soil without manure addition. This could be due to the more easily decomposed organic N in the soils with preceding manure amendments. A negative priming effect in daily N_2O fluxes subsequently occurred in the soils at high moisture content, which might be owing to concurrent plant residual decomposition and N immobilization. Regardless of treatments, the N_2O produced during the simulated thaw was the largest contributor to the total emissions in this study. As compared to nitrification, denitrification was the major contributing process to the substantial N_2O fluxes produced on the 2nd and 3rd days of thawing, accounting for at least 83% of total N_2O production. Increasing soil water content resulted in increased cumulative N_2O emissions irrespective of different soil management history and N additions, indicating potential accelerated N losses as a response to prospective greater precipitation in the fall and winter as induced by escalating climate change.

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4.9 Tables

Table 4-1. Soil physical and chemical properties for the soils with (SW) and without (CT) historical manure applications (0-15 cm depth increment).

Properties	SW	CT
Organic C (g kg^{-1})	63.7 ± 5.0	61.6 ± 4.3
Total N (g kg^{-1})	5.9 ± 0.3	5.9 ± 0.4
Classification	Black Chernozem	
Texture	Clay	
Clay(%)	47.0 ± 1.2	
Silt(%)	36.0 ± 0.3	
Sand(%)	17.0 ± 1.0	
pH	6.1 ± 0.2	
Bulk density (g cm^{-3})	1.11 ± 0.06	

Table 4-2. P-values of three-way ANOVA analysis for soil NH_4^+, NO_3^-, N_2O, CO_2 and NH_3 emissions.

	NH_4^+	NO_3^-	N_2O	CO_2	NH_3
Soil management history (Soil)†	n.s.	0.002	0.007	n.s.	n.s.
Water content (Water) ‡	n.s.	0.035	<0.001	<0.001	n.s.
Nitrogen§	n.s.	<0.001	<0.001	n.s.	n.s.
Soil * Water	n.s.	0.042	n.s.	n.s.	n.s.
Soil * Nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.
Water * Nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.
Soil * Water * Nitrogen	n.s.	n.s.	n.s.	0.035	n.s.

† Soil management history included the soil with (SW) and without (CT) historical manure additions.
‡ Water content included low, medium and high water contents.
§ Nitrogen included the treatment with and without urea additions.

Table 4-3. Isotope discrimination value (ε) of ^{15}N at the N_2O produced on the 2nd day of thawing compared to the soil N pool in CT and SW soils at various water contents.

Soil	Water content	By Soil × Water Content	By Soil
		Mean ε ± SE (δ notation, ‰)	Mean ε ± SE (δ notation, ‰)
CT	Low	-10.19 ± 11.21	-11.73 ± 3.86
	Med	-7.68 ± 2.76	
	High	-17.34 ± 4.46	
SW	Low	-18.78 ± 3.60	-15.26 ± 2.30
	Med	-15.45 ± 4.94	
	High	-11.56 ± 3.65	

4.10 Figures

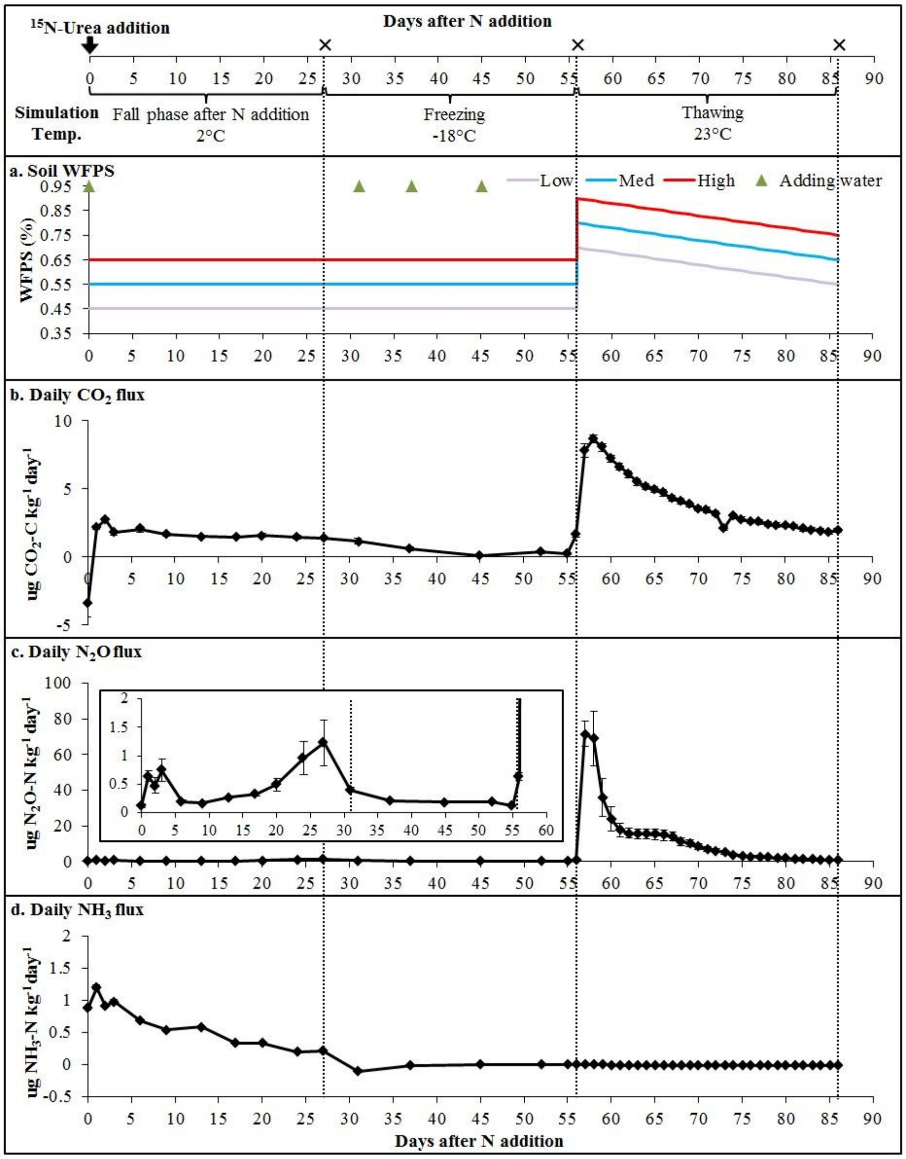

Fig. 4-1. (a) Changes of soil water-filled pore space in the treatments of low (Low), medium (Med) and high (High) water contents and average (b) daily CO_2, (c) N_2O and (d) NH_3 fluxes across all treatments during the whole experiment. Error bars correspond to one standard error.

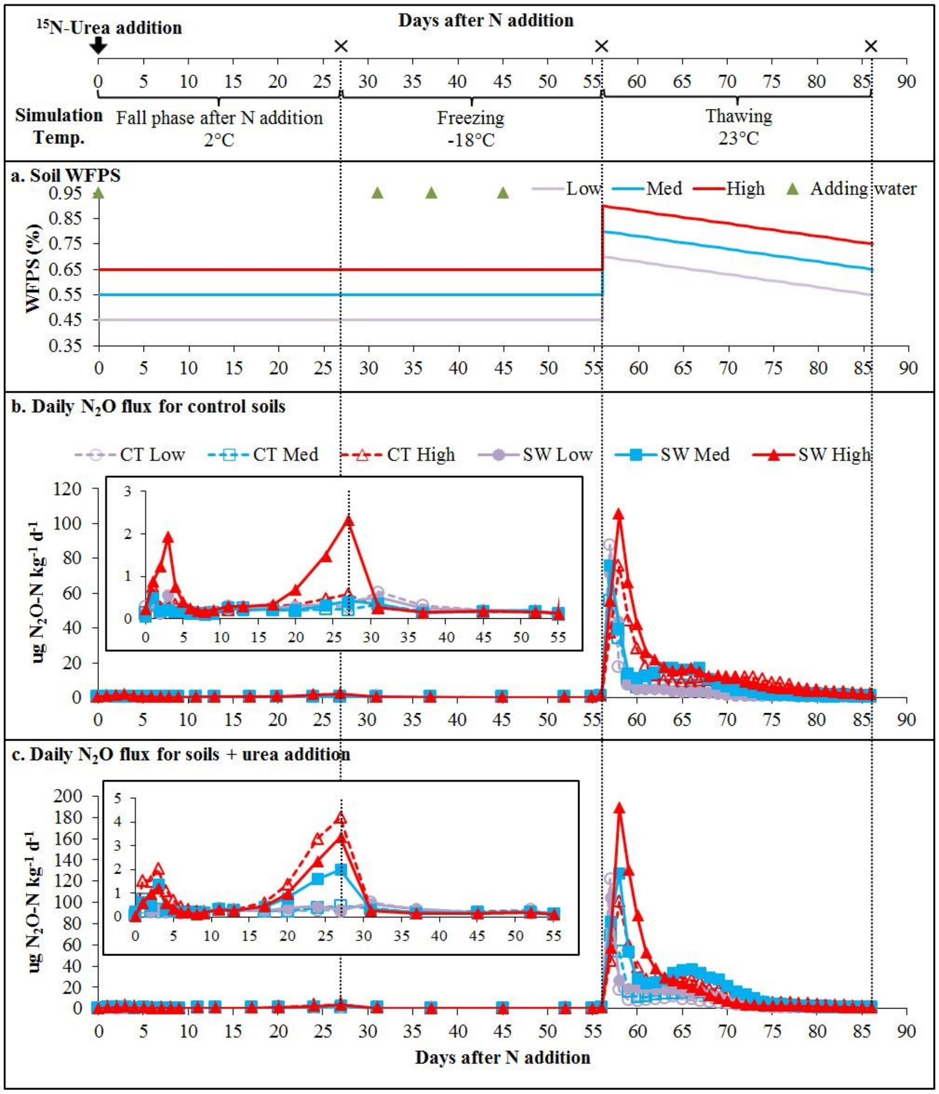

Fig. 4-2. Average daily N_2O fluxes for the soils (b) without and (c) with urea additions and (a) changes of soil water-filled pore space in the treatments of low (Low), medium (Med) and high (High) water contents during the whole experiment. SW and CT correspond to the soils with and without historical manure additions, respectively. Error bars correspond to one standard error.

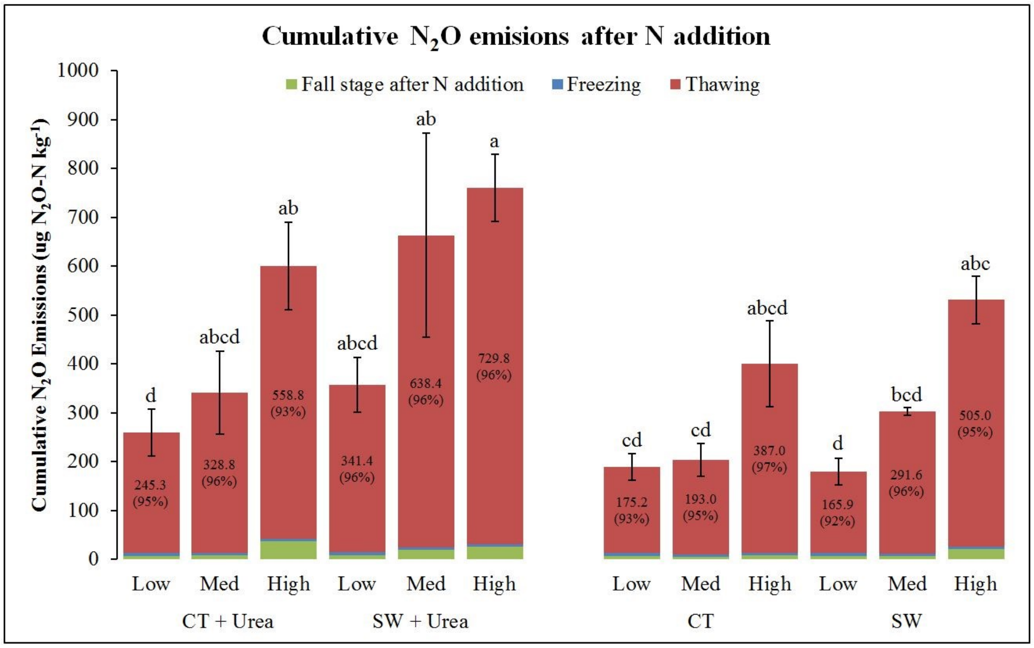

Fig. 4-3. Cumulative N_2O emissions during the fall, freezing and thawing phases for the soil with (SW) and without (CT) historical manure additions at low (Low), medium (Med) and high (High) water contents. Different letters indicate significant differences among the treatments ($P < 0.05$). Numbers above the brackets represent the thaw-induced emissions and numbers in the brackets represent the percentage of thaw-induced emissions to the total budget. Error bars correspond to one standard error.

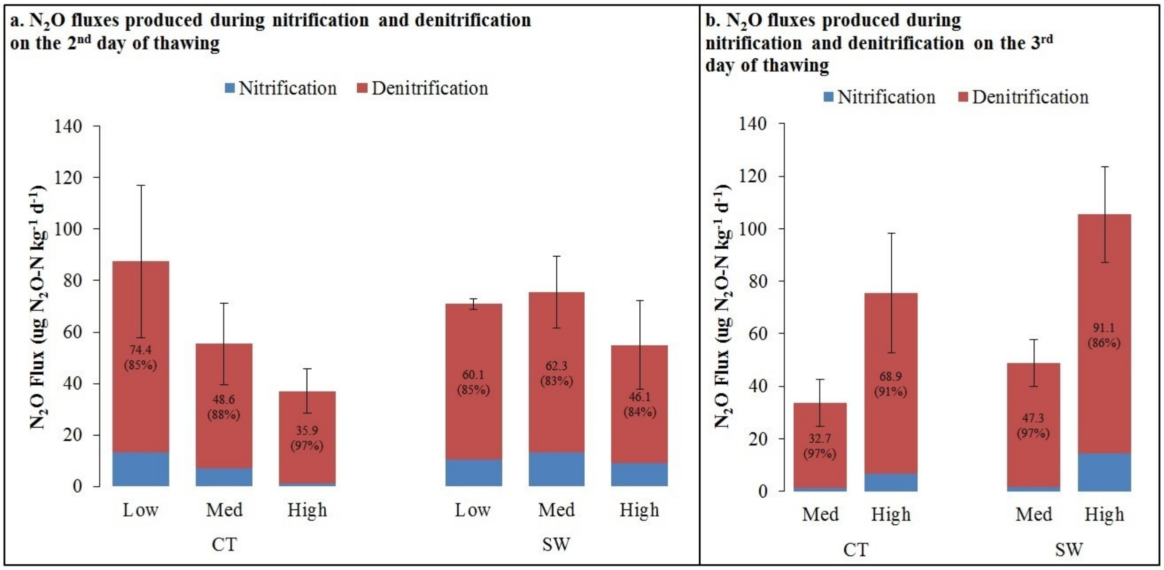

Fig. 4-4. N_2O fluxes produced during nitrification and denitrification (a) on the 2nd day (day 57) and (b) the 3rd day (day 58) of thawing for the soil with (SW) and without (CT) historical manure additions at low (Low), medium (Med) and high (High) water contents. It was unfeasible to detect the N_2O processes in the low water content on the 3rd day of thawing due to low N_2O production. Numbers inside the brackets represent the percentage of emissions produced during denitrification relative to the total emissions, while the numbers above the brackets represent the fluxes produced during denitrification. Error bars correspond to standard error.

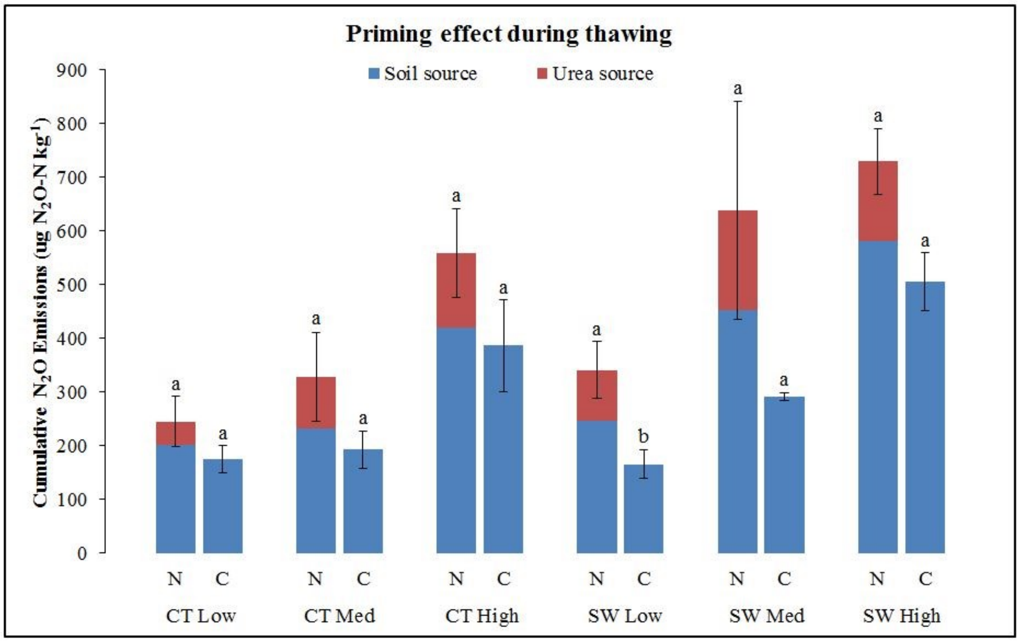

Fig. 4-5. Priming effect of N addition on N source-pools of cumulated N_2O emissions during thawing for the soil with (SW) and without (CT) historical manure additions at low (Low), medium (Med) and high (High) water contents. Different letters indicate significant difference between the soil with (N) and without (C) urea additions within each treatment group ($P < 0.05$). Error bars correspond to one standard error.

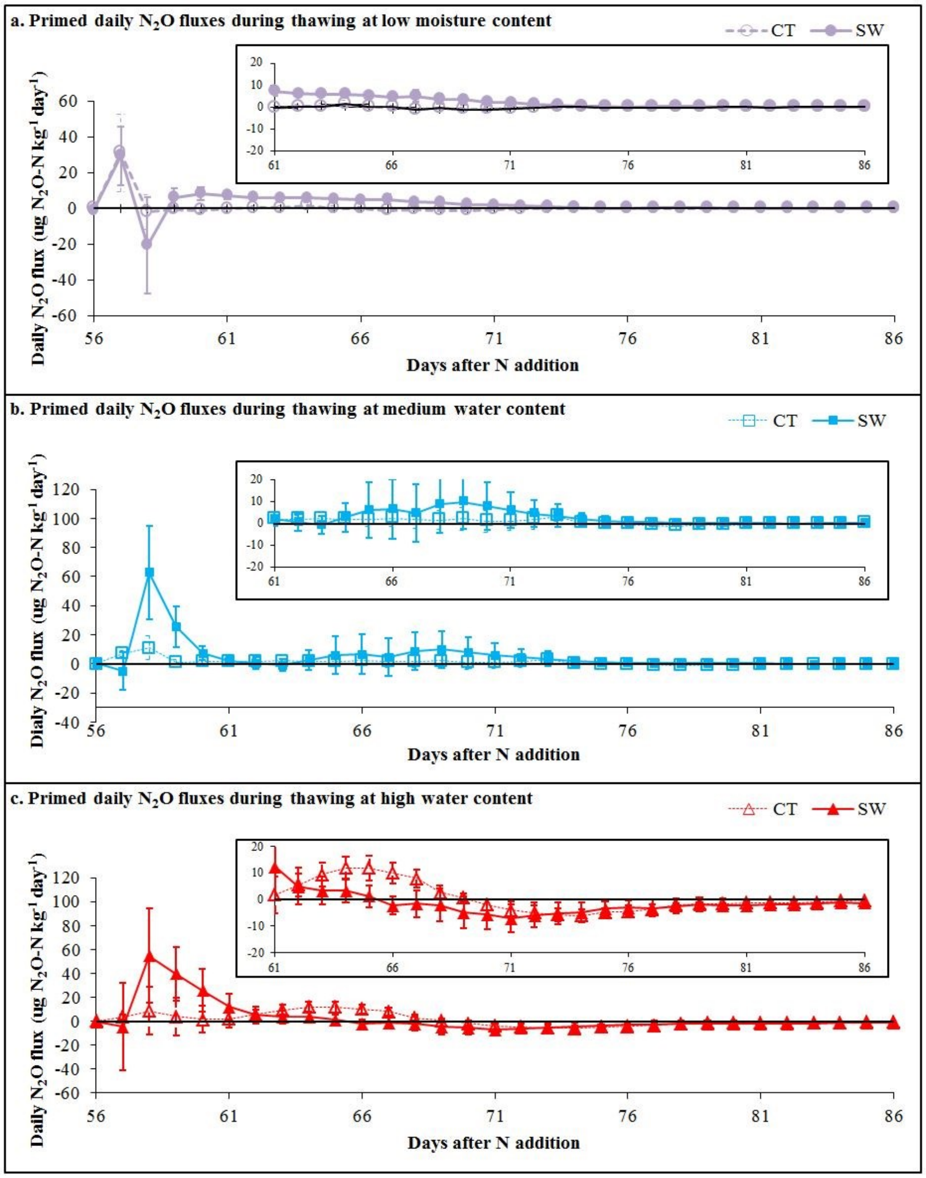

Fig. 4-6. Primed daily N_2O fluxes during thawing for the soil with (SW) and without (CT) historical manure additions at (a) low (Low), (b) medium (Med) and (c) high (High) water contents. Positive and negative primed daily N_2O fluxes represent a positive and negative daily priming effect, respectively. Error bars correspond to one standard error.

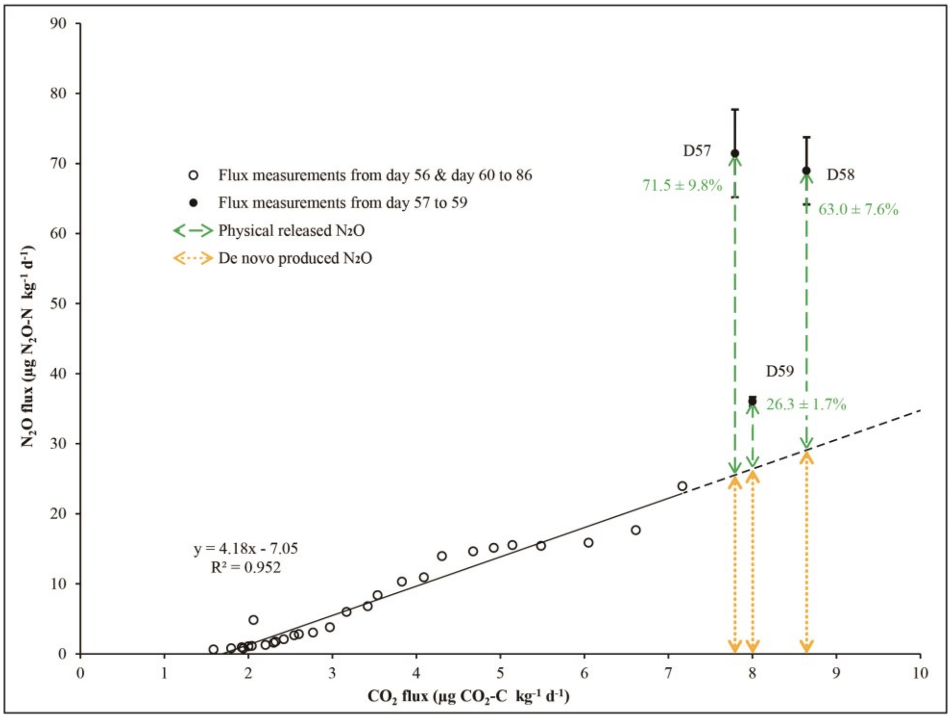

Fig. 4-7. Correlation between N_2O and CO_2 fluxes during the simulated spring thawing phase (days 56 to 86). The linear regression model was evaluated for most of the measurement days (hollow circles), with the exceptions of days 57, 58 and 59 (solid points) which corresponded to the 2nd, 3rd and 4th days of thawing. There is a decoupled response of N_2O and CO_2 productions to thawing in these three days with disproportionally higher N_2O production. The shown percentages with standard errors correspond to the N_2O produced from physical release (estimation derived from extrapolating the shown linear regression equation).

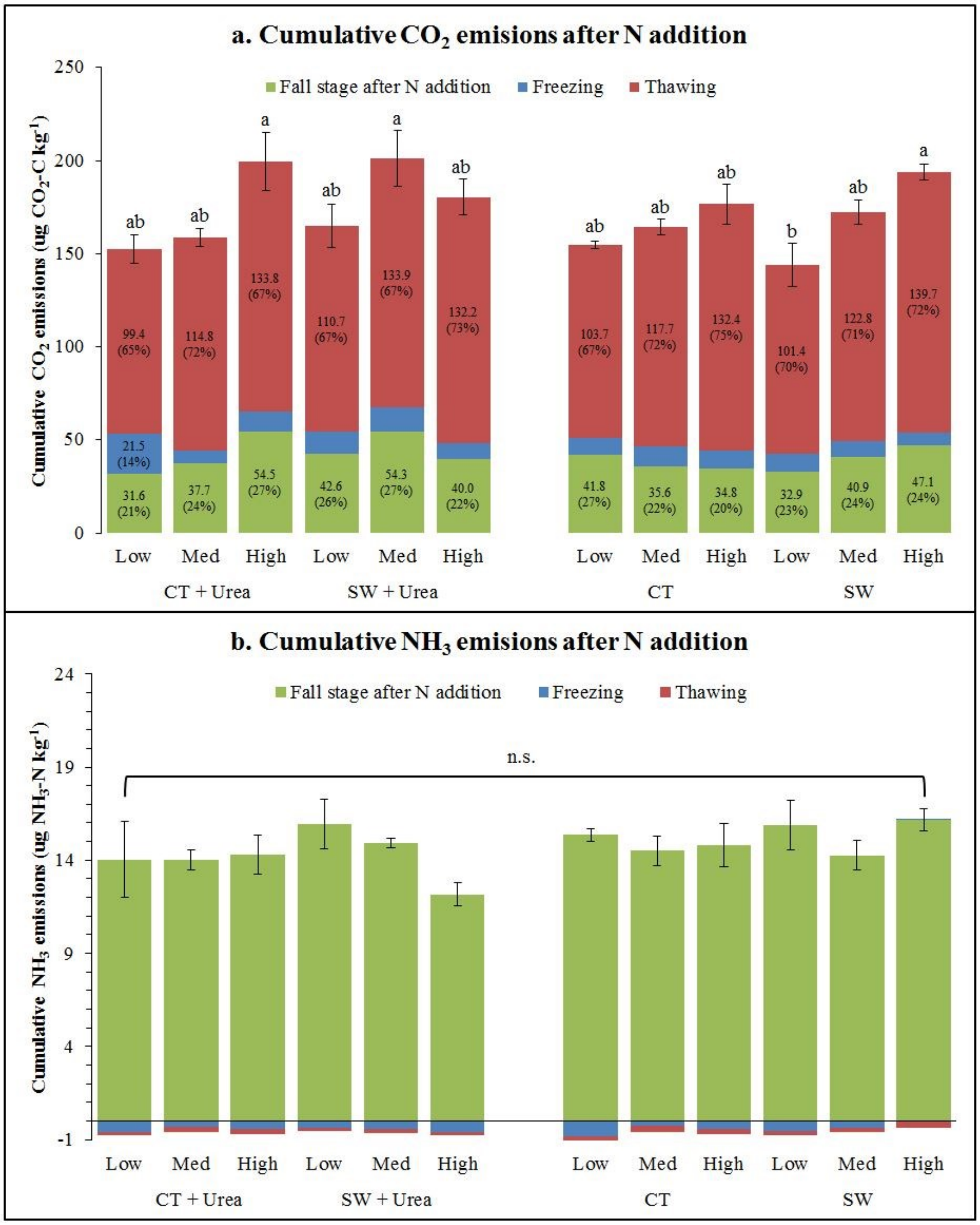

Fig. 4-8. Cumulative (a) CO_2 and (b) NH_3 emissions during the fall, freezing and thawing phases for the soil with (SW) and without (CT) historical manure additions at (a) low (Low), (b) medium (Med) and (c) high (High) water contents. Different letters indicate significant differences among the treatments ($P < 0.05$). Numbers above the brackets represent the phase-induced emissions and numbers in the brackets represent the percentage of phase-induced emissions to the total budget. Error bars correspond to one standard error. n.s. = not significant.

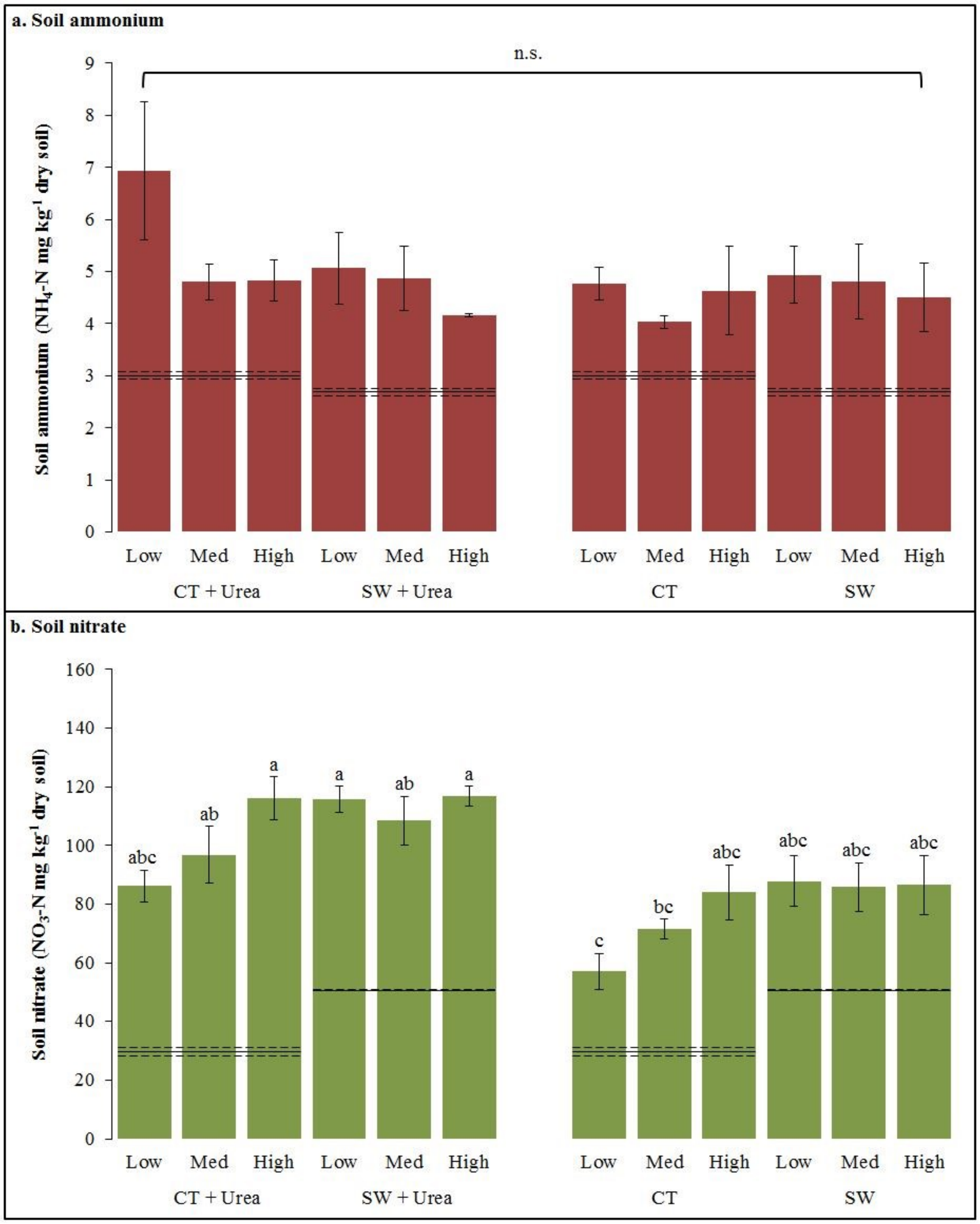

Fig. 4-9. Soil (a) ammonium and (b) nitrate concentrations at the end of the experiment for the soil with (SW) and without (CT) historical manure additions at (a) low (Low), (b) medium (Med) and (c) high (High) water contents. Different letters indicate significant differences among the treatments (*P* < 0.05). Error bars correspond to one standard error. n.s. = not significant. Horizontal solid lines represent the average soil ammonium or nitrate concentrations prior to the urea addition. Dashed lines represent the standard errors.

5. Mathematical Modeling of Nitrous Oxide Emissions from Fall versus Spring Manure Injection Timings and from De-watered Slurry

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Abstract

Process-based ecosystem models, such as *ecosys*, can be useful tools to gain insights about nitrous oxide (N_2O) emissions by testing scientific hypotheses, to accurately project N_2O inventories in national, regional and global scales, and to explore potential emission reduction strategies. The *ecosys* model was used to simulate hourly N_2O fluxes from 2014 to 2017 in a cropping system without and with slurry (fall and spring additions) in comparison with field measurements in Alberta, Canada. We also performed simulations of de-watered fall and spring slurry to investigate how de-watering would impact N_2O production. Our results showed that *ecosys*, in general, adequately simulated soil temperatures and moisture contents at 10 and 20 cm depths (correlation coefficients (r) of 0.94 and 0.996 for temperatures at 10 and 20 cm, respectively; r of 0.535 and 0.557 for soil moistures at 10 and 20 cm, respectively). The divergences of modeled and measured soil water contents during spring thaws could be attributed to uncertainties in model hydrological parameter inputs and uncertainties in field measurements. The model captured reasonably well the dynamics of N_2O fluxes from soils receiving fall and spring slurry (r = -0.425). However, the concurrent discrepancies of soil moisture content, N_2O fluxes and soil mineral N between modeled and measured values during the wetter spring thaw of 2017 could be a result of an unsatisfactory simulation of snowmelt infiltration and runoff. Simulated de-watered slurry resulted in considerable reductions in cumulated N_2O emissions between 17 and 21% compared to whole slurry. This result indicates that de-watering slurry would be an efficient emission mitigation strategy. Future research can further address this knowledge gap.

5.1 Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas (GHG), accounting for 6.2% (3.1±1.9 Gt CO_2 equivalent per year) of total anthropogenic GHG emissions (Intergovernmental Panel on Climate Change, 2014). It also plays a significant role in stratospheric ozone layer depletion (Ravishankara et al., 2009) and can stay much longer (~121 years) than CO_2 in the atmosphere (Forster et al., 2007). By 2010 the tropospheric N_2O emissions had increased by approximately 43% of the background emissions in 1970 (Intergovernmental Panel on Climate Change, 2014). Intensified use of nitrogen (N) fertilizers, which increased by 230% from 1970 to 2010, was one of the key drivers of this dramatic change (FAOSTAT, 2013; Intergovernmental Panel on Climate Change, 2014). The agricultural sector is one of the major contributors to the global anthropogenic N_2O emissions, two-thirds of which is emitted from fertilized soils (US-EPA, 2011). N_2O emissions from arable soils is not going to slow down in next decades if no additional strategies for N_2O emission reduction are put in place (Popp et al., 2010; US-EPA, 2011).

An effective strategy to reduce N_2O losses from agricultural soils can be appropriately combining sources, and application timing, placement and rates of nitrogen for specific crops, cultivated under specific soil and climatic conditions, which will improve N use efficiency and hence will reduce N losses in the forms of N_2O (Fujinuma et al., 2011; Hoben et al., 2011; Zhu et al., 2013; Kim et al., 2013; Roy et al., 2014; Nelissen et al., 2014; Soares et al., 2015; Duncan et al., 2017; Awale and Chatterjee, 2017; Millar et al., 2018). In addition to synthetic fertilizers, livestock manure has been applied to the farmlands as carbon and N inputs for hundreds of years. Adding animal manure into the soil is not only an efficient way to increase the productivity and quality of crops but also improves soil health (Steiner et al., 2007). However, this practice has also the potential to emit a considerable amount of N_2O from the arable soils (Amon et al., 2006). Fall-applied manure produced greater cumulative soil N_2O emissions than spring-applied manure in cold regions (Thorman et al., 2007; Lin et al., 2017). Higher emission from soils receiving fall manure addition was mainly contributed by substantial flushes of N_2O during the following spring thaw, which could be as high as 64% of the total annual N_2O emission (Lin et al., 2017). Shortly after the manure addition in the fall and prior to the frozen winter, most of the manure-derived easily decomposable C and mineral N could remain in the soil due to lack of plant uptake and limited microbial activities caused by the cool and dry soil conditions (Lin et al., 2017; Chantigny et al., 2019). Once the soil gradually became warmer and wetter in the following spring, soil microbial activities progressively accelerated, particularly in the soil with sufficient substrates of residual soluble C and mineral N from the manure applied in the preceding fall (Adair et al., 2019). Denitrification was the major process responsible for the robust N_2O fluxes during the spring thaw period (Müller et al., 2003; Ludwig et al., 2004; Wagner-Riddle et al., 2008), which was due to a combination of expanded anaerobic conditions, increased substrate supply and shifts in the composition of microbial community during thawing (Wagner-Riddle et al., 2017; Congreves et al., 2018). At the onset of soil, ice and snow thawing, the soil moisture content largely increased which reduced soil aeration and hence microbial O_2 availability. Reduced O_2 availability in the soil pore environment gave rise to the needs for alternative electron acceptors to sustain reduction oxidation reactions for microbial energy and growth (McKenney et al., 2001; Morley and Baggs, 2010). Abundance of NO_3^- from mineralization and nitrification of fall applied manure supplied the required alternate electron acceptors that promote denitrification (Wagner-Riddle et al., 2017; Congreves et al., 2018). However, anaerobic conditions caused by large precipitation events during spring and summer also resulted in large flushes of N_2O emissions provided that there was enough substrate (e.g. NO_3^-) available for denitrification. During extremely wet springs and summers, annual N_2O emissions from spring application sometimes offset large flushes of N_2O during the spring thaw period from fall application, thereby producing higher annual N_2O emission from spring application of manure (Rochette et al., 2004; Cambareri et al., 2017).

Selecting appropriate manure application timing for N_2O emission reduction would thus require a clear understanding of how effects of application timing on N_2O emission vary with weather conditions and soils. Field measurements of N_2O fluxes by using static chamber systems can provide valuable information on how manure application timing influences N_2O emissions from different soils under varying weather conditions (Rochette et al., 2004; Hernandez-Ramirez et al., 2009; Cambareri et al., 2017; Lin et al., 2017). However, these chamber measurements are usually temporally discrete and often require linear interpolations to fill the gaps in between consecutive measurement dates which can range from a day to a week in order to derive estimations of cumulative seasonal or annual emissions. These linear interpolations may impart substantial uncertainties into the annual N_2O emission estimates from different manure application timing due to the episodic nature of N_2O emissions which may be missed by weekly field samplings and linear interpolations. Process-based ecosystem models can improve these estimates once rigorously tested against actual field measurements, which would also largely increase our insights into the drivers of N_2O emissions and would improve our predictive capacity to help strategizing efficient manure application timing for N_2O emission reduction. To simulate N_2O emissions from agricultural soils receiving fall- or spring-applied manures, a process-based model should be capable of representing and integrating all important factors and interacting processes that regulate the magnitude of N_2O production, for instance, C and N cycles, soil thermal and hydraulic changes (simulation of freeze-thaw cycles), plant growth, management practices (e.g., fertilizer or manure additions, rotation) and physical and biogeochemical processes involved in C and N cycles (e.g., mineralization, immobilization, nitrification, denitrification). Such a model should be devised and implemented based on site-independent knowledge basic C and N transformation processes and soil biogeochemical cycles, instead of being calibrated by using site measurements which would reduce temporal and spatial scalability of the modelled outputs. As one of integrated process-based models, Denitrification-Decomposition (DNDC) has been used in predicting N_2O emissions from a range of regions and ecosystems (Li et al., 1996; Butterbach-Bahl et al., 2001; Wang and Wang, 2003; Saggar et al., 2004). However, the algorithms in DNDC needed to be parameterized and modified for specific locations, systems and purposes (Giltrap et al., 2010). Spatial and temporal parameterization led to diverse DNDC-based models for specific systems and purposes (Giltrap et al., 2010; Gilhespy et al., 2014), which could provide gaps among models and potentially reduce the reliability of temporally and spatially scaled up emission estimations. By contrast, another process-based ecosystem model *ecosys* does not require site specific tuning of model algorithms or site parameterization, rather it simulates N transformations from basic research theory and fed by site specific model inputs for weather, soil properties and management activities (Grant and Pattey, 1999; Grant and Pattey, 2003; Grant et al., 2006; Grant and Pattey, 2008; Metivier et al., 2009; Grant et al., 2016). This methodology in *ecosys* would minimize some of the limitations in models such as DNDC while temporally and spatially scaling up of N_2O emissions from different manure application timings.

This study aimed at testing whether the set of coupled algorithms in *ecosys* representing N transformation processes and the interaction between soil physics and biogeochemistry in a soil-plant-microbes-atmosphere C-N-phosphorus-water-energy scheme would be able to simulate the effects of fall versus spring manure application on N_2O emissions from a barley field in Alberta, Canada. We hypothesized that the model would simulate higher cumulative thaw-derived N_2O emissions from the fall manure application than that from the spring application due to enhanced denitrification triggered by higher NO_3^- accumulation from fall applied manure under anaerobic conditions during the following spring thaw, which would lead to microbial O_2 deficiency and eventual use of NO_3^- as an alternative electron acceptor to facilitate microbial energy yields and growth. Model performance would be tested against N_2O fluxes measured at the same site from 2014 to 2017 (Lin et al., 2017). In Lin's field study, liquid manure was injected down to ~12.7-15.2 cm depth to avoid losses of N through volatilization. However, the excess water applied with liquid manure could modify the magnitude of the effects of manure application timing on N_2O emissions. To numerically assess the potential effects of excess water in the liquid manure on N_2O emissions from fall vs spring application, we performed a parallel simulation by not adding water to the manure (dry basis equivalent) and compared these modelled N_2O results from simulated de-watered slurry with those from the modelled liquid manure.

5.2 Materials and methods

Ecosys is a process-based hourly time-step terrestrial ecosystem model that stacks up a number of canopy and soil layers, establishing a comprehensive framework of energy, water, C, N and P exchanges and transformations which are related to plants and microorganisms. The algorithms in *ecosys* are used to simulate fundamental physical, chemical and biological procedures and to assess their processing rates in complex biomes within a broad scope of spatial scale. A more detailed description of *ecosys* regarding redox reactions about N_2O production and related gaseous and soluble transport of inputs and outputs coupled with equations, variable definitions, parameters and references can be found in Grant et al. (2016) and associated supplementary materials.

Ecosys hypothesizes that i) the N_2O generation and its key controlling processes take place in a profile consisting of atmosphere, litter layer, soil surface and subsurface layers; ii) the substrates and products of these processes within each lithospheric layer exist in some or all of microbial, soluble and gaseous states in soil, water and air phases, respectively; and iii) these substrates and products can laterally exchange between phases within a layer and vertically exchange between layers within a phase. These hypotheses are portion of soil C, N and P transformations in a larger comprehensive model, which are associated with soil water, heat and solute transport in lithospheric layers (Grant, 2001). The key processes governing N_2O production are briefly described as below.

1) **Mineralization and immobilization of ammonium or nitrate by all functional soil microbes.** During growth, soil microbes gain energy from coupled oxidation-reduction reactions, comprising of dissolved organic C (DOC) oxidation by heterotrophs or mineral N (NH_4^+ and NO_2^-) oxidation by autotrophs, along with O_2 or nitrous oxides

(NO_x) reductions. The functional microbes pursue to maintain a defined C:N ratio for their growth by N mineralization (organic N to NH_4^+) or immobilization (NH_4^+ or NO_3^- to organic N). In the case of N limitation, the increased C:N ratio would return back to the defined ratio by immobilization with a cost of microbial function. The exchanges between the organic and inorganic N regulated by these transformations in turn influence the provision of electron acceptors involved in nitrification and denitrification and hence N_2O generation.

2) **Heterotrophic oxidation of DOC and reduction of O_2.** N_2O generation occurs once the supply of O_2 cannot meet its demand as electron acceptors from aerobic heterotrophic and autotrophic oxidations. This process supports explicit representation of O_2 transport and uptake in modeling.

3) **Oxidation of DOC and reduction of NO_3^-, NO_2^- and N_2O by denitrifiers.** When O_2 is limiting, alternative electron acceptors, such as NO_3^-, NO_2^- and N_2O can be reduced by denitrifiers, coupling with DOC oxidation and N_2O may be as a substrate or product.

4) **Oxidation of NH_3 and reduction of O_2 by nitrifiers.** When O_2 is non-limiting, N_2O generation may occur by reduction of NO_2^- associated with NH_4^+ oxidation.

5) **Oxidation of NO_2^- and reduction of O_2 by nitrifiers.** When O_2 is non-limiting, N_2O generation may occur by reduction of NO_2^-, which is regulated by NO_2^- oxidation. The energy for NO_2^- oxidation is associated with CO_2 fixation for constructing nitrifier biomass.

6) **Oxidation of NH_3 and reduction of NO_2^- by nitrifiers.** When O_2 is limiting, N_2O generation may occur by reduction of NO_2^-, which is regulated by NO_2^- oxidation.

7) **Uptake of NH_4^+ and reduction of O_2 by roots and mycorrhizae.** Other microbes, roots and mycorrhizae that demand NH_4^+ and O_2 are competitor with nitrifiers for the substrates, thus impacting the nitrifier population and N_2O generation.

8) **Cation exchange and ion pairing of NH_4^+.** NH_4^+ adsorption governs the substrate pool for nitrifiers and hence affects N_2O generation.

9) **Soil transport and surface – atmosphere exchange of substrates and products.** The exchange of soluble or gaseous N_2O and O_2 between the soil and atmosphere impacts the availability of substrates and products for the key processes governing N_2O production.

5.2.1 Field Datasets

The field data used as modelled inputs and for the subsequent modelled output testing were collected at the Field Crop Development Center in Lacombe, Alberta, Canada (52°27’17’’N, 113°44’20’’W). The study site had a humid continental climate with freezing winters typically occur from November to March (Mean annual precipitation: 436 mm water equivalent; mean annual temperature 2.9°C) (Government of Canada, 2018). Prior to the start of the field measurements, the field site was under continuous annual barley (*Hordeum vulgare* L.) cultivation. The barley variety Muskwa (Markert Sees Ltd., Alberta, Canada) was planted in Lacombe. The variety CDC Austenson (SeCan, Ontario, Canada) was planted in Edmonton. The experiment was established under annual barley silage system from October 2014 to May 2017 in an incomplete split-plot design with four replicates and eight treatments (Lin et al., 2017), four of which were simulated in this modeling study. The treatments that were simulated in this study were fall slurry application (FW), spring slurry application (SW), control without manure application (CZ) and control with disturbance of manure applicator (CT). The swine slurry was injected into 12.7-15.2 cm below the soil surface with 28-cm spacing at a rate of 56.17 m^3 ha-1. The fall slurry injections occurred in October 2014 and September 2015. The spring slurry injections and manure application disturbances occurred in May 2015 and June 2016. Slurry subsamples were taken in each application and measured properties were given in Table 5-2. Soil NH_4^+ and NO_3^- concentrations were measured for topsoil (0-15 cm) samples collected over the experiment. The barley was planted in May 2015 and June 2016. The aboveground biomass was removed in August 2015 and 2016 for further plant biomass and N content analyses.

N_2O fluxes were measured using non-steady-state static chamber method (Lin et al., 2017). Gas sampling activities took place throughout the experiment (October 2014 to May 2017), excluding soil frozen months (November to March) with a typical frequency of once or twice weekly according to the management practices and weather. Gas samplings were carried out as early as possible in each spring to capture spring-thaw N_2O emissions as much as possible depending on the snow melting and soil conditions. The cumulative N_2O emissions were determined by interpolating and integrating adjacent N_2O fluxes. Further detailed on the field experiments can be found in Lin et al. (2017).

5.2.2 Model experiment

Ecosys simulations in this study began with a spin-up run to allow the model to attain equilibrium under the site conditions ahead of the experiment. These simulations were initialized by the physical and chemical properties of the Black Chernozemic soils measured at the site (Table 5-1) and were forced by land use practices (Table 5-2). Upper boundary conditions of the simulations were driven by daily (from 2005 – 2007) and hourly (from 2008 – 2013) weather data i.e. temperature (T), precipitation, humidity, radiation, and wind speed (Government of Canada, 2018). These spin-up runs continued to the simulations (i.e., CT, CZ, FW or SW) with inputs of field management practices and schedules described in Table 5-2 and forced by hourly weather data from 1 January 2014 to 31 December 2017 (Government of Canada, 2018).

5.3 Results

5.3.1 Modelled vs. measured soil temperature and moisture

Given sufficient soil NH_4^+ and NO_3^- as substrates, the simulation of elevated N_2O fluxes only occurred when the simulated soil was warm and moist (Fig. 5-1 and Fig. 5-2), indicating the importance of soil temperature and moisture on N_2O production. The simulated soil temperatures at 10 and 20 cm below the surface were highly correlated with the averaged measurements from 2016 to 2017 (Table 5-3 and Fig. 5-2). This indicates the seasonal soil temperature patterns were adequately simulated by *ecosys*. The summer peak soil temperatures at 10 and 20 cm depth were 31.3 and 25.2 °C, and the lowest soil temperatures at 10 and 20 cm depth were -25.7 and -20.9 C, respectively (Fig. 5-1 and Fig. 5-2). The simulated sub-zero soil temperatures typically lasted from early November until late March (Fig. 5-1 and Fig. 5-2).

Significant agreements between measured and modelled soil moisture contents at 10 and 20 cm below the surface were obtained from 2016 to 2017 (Table 5-3). The seasonal dynamics of soil moisture contents at both depths and soil drying and rewetting during the summer and fall were successfully captured by the model (Fig. 5-2). However, some discrepancies were found between the measured and modelled soil moistures at 10 and 20 cm depths in terms of the processes of thawing frozen soil and drying-rewetting during the early spring of 2016 and 2017 (Fig. 5-2). In addition, there was a time lag of simulated soil moisture increment between the two depths during the spring thaw of 2017, which was not found in the measurements (Fig. 5-2g). The decrease in simulated soil moisture at 10 cm depth occurred on Mar 31 in 2017, whilst the drainage of melting snow took place 20 days later based on field observations (Fig. 5-2). Compared to the corresponding field records, the simulated soil thawing occurred three days earlier at 10 cm depth, and two days later and at the 20 cm depth (Fig. 5-2g).

5.3.2 Modelled vs. measured N_2O fluxes and seasonal cumulated N_2O emissions

The model simulated the dynamics and magnitude of daily N_2O fluxes reasonably well from 2014 to 2017 in most situations. However, there were some exceptions in terms of timing and magnitude (Table 5-3, Fig. 5-1 and Fig. 5-2). Simulated major fluxes from the SW treatment occurred immediately after the spring manure addition in 2015, which was earlier than the measurements where larger fluxes took place when there were major precipitations (i.e., >5 mm) following the manure addition (Fig. 5-1). In spring 2016, simulated N_2O fluxes captured the pattern of field measured fluxes from the SW treatment reasonably well, but the magnitude of large N_2O pulses after the manure addition were lower and shorter than the measurements (Fig. 5-2). In addition, the timing and magnitude of simulated spring-thaw N_2O pulses for all treatments in 2017 were different compared to the measurements (Fig. 5-2). The simulated N_2O peaks occurred on April 1st when the simulated soil temperatures just became above zero °C, whilst the measured N_2O peaks took place 20 days later (Fig. 5-2). The averaged simulated spring-thaw peaks across all treatments were 0.004 g N_2O-N m^{-2} h^{-1}, which was about eighteen times larger than the averaged measured N_2O peaks; however, the duration of large simulated N_2O fluxes were shorter compared to that of major measured fluxes (Fig. 5-2), and hence, magnitudes of seasonal fluxes converged.

Major simulated N_2O fluxes varied among seasons and treatments (Table 5-4). For the control treatments, simulated major N_2O pulses were observed during spring thaws (Fig. 5-1, Fig. 5-2). For the manured treatments (FW and SW), simulated major N_2O fluxes were found after the corresponding manure applications and the spring thaws (Fig. 5-1 and Fig. 5-2). Compared to the measurements, the model predication of seasonal N_2O emissions from manured treatments showed variability. For the FW treatment, the model overestimated the cumulated

N_2O emissions during the fall seasons (i.e., Oct 7 – Oct 31 in 2014 and Sep 29 – Nov 4 in 2015), while it correctly simulated the cumulated emissions during the spring thaws except for that in 2017 where there was an overestimation (Table 5-4). For the SW treatment, the model slightly underestimated the cumulated N_2O emissions from Apr 21 to Nov 3 in 2016 (Table 5-4). During other periods when major N_2O fluxes occurred from the SW treatment, the modelled cumulated emissions were higher than the measured values (Table 5-4).

5.3.3 Simulation of de-watered slurry

Throughout the whole experimental period, the timing and pattern of simulated N_2O fluxes from the de-watered slurry treatments (FW no water and SW no water) were highly coincident with those from the manured treatments (FW and SW); however, the magnitude of simulated fluxes from the de-watered slurry treatments were consistently lower than the fluxes from the corresponding treatment (Fig. 5-3). In terms of seasonal cumulated N_2O emissions, clear emission reductions due to the de-watered slurry were only observed in the substantial peak emissions that took place shortly following the manure additions (Table 5-4). The addition of de-watered slurry showed emission reductions of at least 17% and 21% for the FW and SW treatments, respectively (Table 5-4).

The dynamics of simulated soil NH_4^+ and NO_3^- concentrations at 0-15 cm from de-watered slurry treatments were highly coherence with that from corresponding slurry treatments from 2014-2016 (Fig. 5-4). There was only a small difference (about 1-2 mg N kg^{-1} soil) in NH_4^+ concentration between the slurry and its corresponding de-watered slurry simulations and this small difference only occurred about 6-8 days immediately following the additions; otherwise, there was very little difference most time (Fig. 5-4). By contrast, the NO_3^- concentration in the de-watered slurry simulation was generally higher than that in the corresponding slurry simulation (Fig. 5-4). This difference was much clear during the spring thaw for the fall manure simulation as well as immediately after the manure additions in the spring and fall simulations (Fig. 5-4).

5.3.4 Modelled vs. measured soil mineral N

Model simulated soil NH_4^+ and NO_3^- concentrations at 0-15 cm varied over time and among treatments (Fig. 5-1 and Fig. 5-2). Over the experimental period, the difference in simulated NH_4^+ concentration among treatments were not clear with an exception of certain instances for manured treatments (Fig. 5-1 and Fig. 5-2). From 2014 to 2016, the simulated NH_4^+ concentration in soils receiving liquid manures correspondingly peaked right after additions, and then gradually decreased back to its equilibrium level after about 1-2 months (Fig. 5-1 and Fig. 5-2). Over the winter in 2015 and 2016, the simulated NH_4^+ concentration across all treatments were similar and remained at its background level. However, this concentration on average gradually increased from 0.15 to 0.85 mg NH_4-N kg^{-1} soil over the winter in 2017, where the simulated soil temperature at both depths were below but close to zero °C (Fig. 5-2). Once the simulated soil temperature at both depths became above-zero °C, the simulated NH_4^+ concentration across all treatments rapidly decreased back to its background level (Fig. 5-2).

Similarly to NH_4^+, the simulated NO_3^- concentration in manured treatments rapidly and substantially increased to its peaks after the additions, but later than the peak of NH_4^+ (Fig. 5-1 and Fig. 5-2). The increased simulated NO_3^- concentration after fall manure additions remained high and stable over the winter until the following spring thaw (Fig. 5-1 and Fig. 5-2). By contrast, the increase in simulated NO_3^- concentration due to spring manure additions was followed by a quick decrease (Fig. 5-1 and Fig. 5-2). The simulated NO_3^- concentrations in all treatments slightly decreased at the beginning of spring thawing followed by a gradually increase and this change was more obvious in manured treatments (Fig. 5-1 and Fig. 5-2). Once the soil receiving manure additions, the simulated NO_3^- concentration would consequently equilibrate at a higher level than the control soils until late July in 2017 (Fig. 5-2).

The soil NH_4^+ and NO_3^- concentrations at the beginning of the field experiment were well achieved by the model simulation (Fig. 5-1). From 2016 - 2017, the model simulation in general captured well the dynamics of NO_3^- concentration and reasonably captured the concentration difference among treatments (Fig. 5-2 and Table 5-3). However, there was a divergence between measured and modeled soil NH_4^+ (Table 5-3), which was mainly attributed to the inconsistent timing of peak NH_4^+ concentrations in July of 2016 and in spring 2017 (Fig. 5-2). In 2016, the modeled NH_4^+ for the SW treatment increased due to spring slurry additions, while the measurement did not reveal such slurry effect (Fig. 5-2). In 2017, the measured NH_4^+ was much higher than the modeled NH_4^+ (Fig. 5-2).

5.3.5 Modelled vs. measured plant biomass and N uptake

There were no clear differences in both simulated plant biomass and N uptake between 2015 and 2016 for a certain treatment, but there was a difference among treatments (Fig. 5-5). The simulated plant biomass in manured treatments were slightly higher than that in the controls in both years, and this difference was much clear for the simulated aboveground plant N content (Fig. 5-5). Compared to the measurements, the model simulation of plant biomass and N uptake in general captured well the difference among treatments but not the magnitude. For the plant biomass, the model simulation was in agreement with the measurements for the manured treatments, but the model overestimated barley biomass for the controls (Fig. 5-5). Regarding aboveground plant N content, all model simulated values were higher than the corresponding measurements except for the SW treatment in 2015 (Fig. 5-5).

5.4 Discussion

5.4.1 Model simulation of fall and spring applications of slurry

The simulated burst of N_2O fluxes during spring thawing was related with a combination of increased soil temperature, increased soil moisture contents and associated O_2 depletion. The soil temperatures at 10 and 20 cm depths increased above zero °C at the beginning of spring thaw, which was concurrently with largely increases in soil moistures at both depths as simulated in the model (Fig. 5-1 and Fig. 5-2). The sudden increased soil moisture content due to snow-melting and soil-thawing creates a water-ice layer in the top soils, eventually preventing vertical O_2 diffusion (Teepe et al., 2001b; De Bruijn et al., 2009). During thawing frozen soil, microbes were stimulated by not only the increased temperature, but also by increased soil N and C compounds from organic matter mineralization, aggregate disruption and microbial lysis following spring thawing (Congreves et al., 2018). Because of enhanced soil microbial respiration during spring thawing, the O_2 availability further suppressed due to increased O_2 consumption rate (Giles et al., 2012). As a favorable electron acceptor when O_2 is restricted, NO_3^- was then reduced to meet the energy requirements of organic matter oxidation through denitrification, producing N_2O emissions (McKenney et al., 2001; Morley and Baggs, 2010). As more soil NO_3^- was available in the fall-applied slurry during the spring thaw, higher microbial respiration rate resulted in more restrict anaerobic conditions, thereby generating more N_2O compared to other treatments (Fig. 5-1 and Fig. 5-2). This can explain why there was a slightly decreased in simulated NO_3^- at the onset of soil thawing and the NO_3^- decline was more obvious in manured soils (Fig. 5-1 and Fig. 5-2). The gradually slight increase in simulated NO_3^- following the early-thawing decline could be a result of accelerated mineralization and retarded nitrification during the post-thawing period as reported in a field research using ^{15}N labeling approach (Müller et al., 2002).

Our results of simulated spring-thaw N_2O emissions from fall and spring applied slurry are in agreement with a DNDC model study by He et al. (2019). They found that bursts of both simulated and measured N_2O fluxes took place during spring thaw and the magnitude of these fluxes were generally larger in soils receiving fall dairy manure than soils with spring manure or even larger than the control soils. The concurrent simulation of soil anaerobic conditions and shortly declined in NO_3^- indicates the dominant process of denitrification to early spring-thaw N_2O emissions, which is widely reported in previous field and laboratory studies (Mørkved et al., 2006; Wagner-Riddle et al., 2008; Yanai et al., 2011; Wertz et al., 2016; Yin et al., 2019).

Similarly to the anaerobic conditions caused by spring thawing and snow melting, the applied slurry provided abundant substrates for soil microbes, such as inorganic N and labile organic C compounds. Due to the oxidation of labile organic C and oxidation of NH_4^+ or NH_3 in the slurry, the O_2 demand suddenly increased coupled with a formation of localized anaerobic microsites that are favorable for denitrification (Flessa and Beese, 2000; Giles et al., 2012; Van Nguyen et al., 2017). Therefore, elevated N_2O production was simulated following slurry additions given at proper soil temperatures (Fig. 5-1 and Fig. 5-2). The transient O_2 depletion following slurry additions was also measured in a recent field study, in which the soil O_2 concentrations at 5 and 10 cm below the surface decreased by about 2-4% soil O_2 within 24 hours after the cattle urine deposition (Owens et al., 2017).

The simulated increase in N_2O fluxes was tightly linked with increases in soil mineral N (NH_4^+ and NO_3^-) after slurry additions (Fig. 5-1 and Fig. 5-2), confirming that both nitrification and denitrification contribute to these N_2O production (Wrage et al., 2001; Toyoda et al., 2017). Our results were in agreement with the simulation in a DailyDayCent model by Senapati et al. (2016), who found concurrent increased soil NH_4^+ and NO_3^- and enhanced N_2O production following fertilizer applications from both mown-grass and grain-cropping systems. After slurry applications, both NH_4^+ and NO_3^- reached its peak, but the NO_3^- peak was consistently delayed after the NH_4^+ peak (Fig. 5-1 and Fig. 5-2). This clearly shows that the increased in soil NO_3^- was a result of rapid transformation of NH_4^+ by nitrifiers. This result also implies that the contribution of nitrification to elevated N_2O fluxes at the early stage after slurry applications should take into account, although stimulated denitrification is usually considered as the main explanation to the burst of N_2O emissions after fertilizer or organic ammendments (Flessa and Beese, 2000; Wulf et al., 2002; Zebarth et al., 2008; Pfab et al., 2011). The knowledge of microbial processes responsible for N_2O emission after N additions is still limited and more field and modeling studies are recommended to address this topic.

The discrepancies of measured and modeled soil moisture content during the spring thaws of 2016 and 2017 could be a result of uncertainties of model inputs as well as the uncertainties in field measurements. Furthermore, modeled soil water contents in *ecosys* are derived as functions of saturated hydraulic conductivities, field capacity and wilting point, all of which in turn directly influence the simulated soil water retention. However, these unmeasured hydrological model inputs were set up in the model based on earlier published studies with similar edaphic and climatic conditions or field observations in soil water content. By doing this, there would be some uncertainties in the modeled soil water contents. In the field, the soil moisture contents were detected by frequency domain reflectometry (FDR) sensors (Decagon 5TM, Decagon Devices, Inc., Pullman, WA), which measures the dielectric constant of the soil-water-air mixture (Fares et al., 2016). Tian et al. (2013) found that there was a drift in soil moisture readings monitored by FDR sensor when the soil temperature below 0 ºC. This finding of uncertainties in sensor measurements can partially explain the divergence of modeled and measured soil moisture contents during spring thawing , because the soil water during thawing is typically a mixture of liquid water and ice with a temperature near 0 ºC.

The uncertainties in model soil water retention could also explain the early estimation of enhanced N_2O due to spring slurry application in 2015 and the lack of accurate representation of slurry-derived soil NH_4^+ increment in July 2016. In the dry spring of 2015, the model represented an immediately release of N_2O fluxes following the slurry application; however the measurements showed a postponed release until there were several large rainfalls (Fig. 5-1). This divergence implies overestimated soil retention by the model under dry conditions. In July 2016, the modeled soil water contents were generally higher than the measurements due to the hydrological parameter uncertainties (Fig. 5-2). This simulated more anaerobic conditions, which in turn impeded nitrification to a certain extent. Thus, the decrease in soil NH_4^+ after spring slurry application would be delayed compared to a more realistic situation.

In addition to discrepancies of soil moisture, there were differences in timing of soil inorganic N and the peak N_2O fluxes between the modeled and measured values during the wetter spring thaw of 2017 (Fig. 5-2). In both model simulation and field measurement, the peak N_2O production during the spring thaw of 2017 occurred one day after the major declined soil moisture content; both simulated flux peak and decreased soil moisture content consistently occurred 20 days ahead compared to the measurements (Fig. 5-2). Unlike 2016, the air temperature fluctuated around 0 ºC at the early stage of thawing in 2017 (Fig. 5-2), indicating several cycles of water freezing and melting at the onset of spring 2017 and a prolonged ice-water-snow layer during thawing. However, the model simulated an earlier decrease in soil moisture content at 10 cm depth, an earlier occurrence of peak N_2O flux and a potential earlier soil NH_4^+ peak compared to the measurements (Fig. 5-2). Besides the hydrological parameter uncertainties, an unsatisfactory simulation of snowmelt infiltration and runoff could be another explanation of these timing differences. The timing of snowmelt infiltration and runoff largely impacts the duration of freezing, and hence the magnitude of timing of N_2O fluxes. Teepe et al. (2004) found that the cumulated N_2O emissions increased with longer freezing duration from three agricultural soils with different textures. They explained that increased N_2O emissions caused by longer freezing duration were likely associated with increases in nutrient availability and increased number of N_2O-related microbes.

The timing lag of peak N_2O fluxes between the model simulation and field measurements during the spring thaw of 2017 could also be a result of a chamber effect. During snow melting and soil thawing, the snow accumulated preferentially inside the chamber requiring more time to melt compared to the snow outside the chamber based on anecdotal field observations. Also, we noticed that when the snow outside the chamber has melted, there was still a layer of snow-ice-water inside the chamber. This layer can be regarded as a sealing layer within the field chambers, impeding vertical gas diffusion. Thus, the N_2O produced or accumulated underneath the chamber area could be horizontally transported and then vertically released from the soil profile due to gas concentration gradients. This can also explain why the measured N_2O emissions were much lower than the simulated N_2O emissions on cumulative basis.

Although modeled plant biomass for both control treatments was consistently higher than the measurements, the modeled plant biomass in 2015 and 2016 were reasonably closed to the reported barley yields in central Alberta, which was about 4203 and 5185 kg ha^{-1}, respectively

(Alberta Agriculture and Forestry, 2018b). The explanation to the large difference between modeled and measured plant biomass in soils without slurry additions remains unclear. One potential explanation could be due to a certain nutrient deficiency in the control soils, such as potassium or a micronutrient, whilst the lack of this nutrient was supplied by adding slurries.

5.4.2 Model de-watered slurry reduces N_2O emissions

The success in reducing N_2O emissions by using de-watered slurry could be mainly attributed to the decrease in water added via slurry with the result that denitrification was limited due to decreased anaerobic microsites. Thus, more NO_3^- would be available in the soil as shown in the simulated results that consistently higher NO_3^- was present in the soil receiving de-watered slurry compared to the corresponding whole slurry (Fig. 5-4). These results demonstrate the driving role of soil moisture content on N_2O emissions, which has been previously recognized in laboratorial and field experiments (Davidson et al., 1991; Ruser et al., 2006). In addition, the amount of water excluded in the de-watered slurry model was equivalent to about 5 mm irrigation, and as a result, the strategy of applying de-watered slurry could reduce at least 17% N_2O emissions (Table 5-4).

The model simulations of soil NH_4^+ and NO_3^- concentrations in the slurry and de-watered slurry additions provided clues about the contribution of processes to the magnitude of the emission reduced due to de-watering. The little difference in the NH_4^+ and clear difference in the NO_3^- concentrations between de-watered and whole slurry simulations implied that greater contribution of N_2O emissions might be a result of denitrification rather than nitrification.

The comparison between the paired slurry simulations (de-watered vs. whole) indicated evident benefits of using de-watered slurry instead of whole slurry; nevertheless, unintended consequences should take into account. The field application of de-watered slurry may result in shallow soil penetration, which may favor ammonification and release of NH_3, and reductions in plant yield and quality. The knowledge about the effect of de-watered slurry on soil N dynamics is still limited. Therefore, more studies are required to carefully identify potential benefits and potential unintended consequences of using de-watered slurry.

5.5 Conclusions

Our results showed that *ecosys* can simulate reasonably well the dynamic of N_2O fluxes from soils receiving fall and spring slurry additions, although there were some discrepancies under certain conditions. The divergence of measured and modeled soil moisture content over spring thaws could be owing to uncertainties in both model hydrological inputs and field moisture measurements. During the wetter spring thaw of 2017, the differences in timing of soil NH_4^+ and peak N_2O fluxes between the model and measurements suggested the need for improving the snowmelt infiltration and runoff simulations in *ecosys*. Cumulated N_2O emissions were successfully reduced by de-watered slurry compared with whole slurry in model simulations, suggesting that dewatering liquid manure would potentially be an efficient technique not only for emission mitigation and global warming retardation but also for efficient use of manure N.

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5.8 Tables

Table 5-1. Physical and chemical properties of Black Chernozemic soil in Lacombe in the model.

Layer #	Depth	Range			Bulk Density	Sand	Silt	Clay	Field Capacity	Wilting point	Organic C	Organic N	pH
	m	m			kg cm^{-3}	g kg^{-1}			m^{3} m^{-3}		g C kg^{-1}	g N kg^{-1}	
1	0.01	0	-	0.01	1.22	316.5	364	319.5	0.25	0.15	58.3	5	7
2	0.03	0.01	-	0.03	1.22	316.5	364	319.5	0.25	0.15	58.3	5	7
3	0.07	0.03	-	0.07	1.22	316.5	364	319.5	0.25	0.15	58.3	2.5	7
4	0.13	0.07	-	0.13	1.22	316.5	364	319.5	0.25	0.15	58.3	2	7
5	0.15	0.13	-	0.15	1.22	316.5	364	319.5	0.25	0.15	58.3	2	7
6	0.25	0.15	-	0.25	1.22	339	353	308	0.25	0.15	41.8	1	8.13
7	0.3	0.25	-	0.3	1.22	339	353	308	0.25	0.15	41.8	1	8.13
8	0.6	0.3	-	0.6	1.44	386.5	284.5	329	-[†]	-	13.1	0	7.88
9	0.9	0.6	-	0.9	1.46	307.5	288.25	404.25	-	-	13.1	0	8.08
10	1.3	0.9	-	1.3	1.46	307.5	288.25	404.25	-	-	13.1	0	8.08

[†] The value is calculated from texture and organic matter by the model itself.

Table 5-2. Soil and plant management practices and schedules in Lacombe from 2005 to 2016.

Management			2005	2006	2007	2008	2009	2010	2011	2012	2013	2014		2015		2016
Disturbance	Date		-	-	May 01	-	-	-	-	-	-	-	Oct 06	May 25	Sep 28	Jun 06
	Disturbance agent				Sweep cultivator								Disk plow	Disk plow	Disk plow	Disk plow
	Depth (cm)				12								14	14	14	14
Fertilizer	Date		Jun 03	Jun 03	-	Jun 03	Jun 03	Jun 03	Jun 03	Jun 03	Jun 03	Jun 03	Oct 06	May 25	Sep 28	Jun 06
	Fertilizer (F) /Slurry (S)		F	F		F	F	F	F	F	F	F	S	S	S	S
	Amount (g m^{-2})	Urea-N	3.9	3.9		7.9	7.9	7.5	8.8	6.9	9.7	5.2				
		P	0.7	0.7		1.0	1.0	0.7	0.9	1.2	0.9	1.5	1.6	5.3	4.5	5.2
		NH_4^+-N	0	0		0	0	0	0	0	0	0	16.2	23.1	20	39.9
		Organic N	0	0		0	0	0	0	0	0	0	73.7	17.5	11.6	12.2
		Organic C	0	0		0	0	0	0	0	0	0	8.3	122	94.9	156.7
	Placement method (Broadcast or Banded)		banded	banded		banded	banded	banded	banded	banded	banded	banded		banded	banded	banded
	Placement depth if incorporated into the soil (subsurface) (cm)		8	8		8	8	8	8	8	8	8	14	14	14	14
	Placement width if banded fertilizer is applied (cm)		25	25		25	25	25	25	25	25	25	28	28	28	28
Irrigation	Date		-	-	-	-	-	-	-	-	-	-	Oct 06	May 25	Sep 28	Jun 06
	Amount (mm)												5.505	5.493	5.505	5.426
	Starting Hour (1-24)												10	10	10	10
	Finishing Hour (1-24)												12	12	12	12
	Depth (cm)												14	14	14	14
	Irrigation water quality - pH												7.8	7.8	7.8	7.8
Planting	Date		Jun 03	Jun 03	May 05	Jun 03	Jun 03	Jun 03	Jun 03	Jun 03	Jun 03	Jun 03		May 25		Jun 03
	Species		barley	barley	pea	barley	barley	barley	barley	barley	barley	barley		barley		barley
	Density(m-2)		300	300	88	300	300	300	300	300	300	300		300		300
	Depth (cm)		5	5	6.5	5	5	5	5	5	5	5		5		5
Harvesting	Date		Sep 15	Sep 15	Sep 15	Sep 15	Sep 15	Sep 15	Sep 15	Sep 15	Sep 15	Sep 15		Aug 11		Aug 19
	Type		Cutting	Cutting	Grain removal	Cutting	Cutting	Cutting	Cutting	Cutting	Cutting	Cutting		Cutting		Cutting
	Removal height above ground (m)		0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15		0.15		0.15
	Fraction of plant removed above removal height (0-100%)		95	95	60	95	95	95	95	95	95	95		95		95

Table 5-3. Correlation coefficient (r) between the modelled and measured values for soil temperature, moisture, ammonium (NH_4^+), nitrate (NO_3^-) and nitrous oxide (N_2O) flux during the experiment from 2014-2017.

Measured/simulated variables	r	n
Soil temperature at 10cm	0.94**	5581
Soil temperature at 20cm	0.996**	5560
Soil moisture at 10cm	0.535**	5581
Soil moisture at 20cm	0.557**	5560
Nitrous oxide (N_2O) flux (g N m^{-2} h^{-1})	-0.425**	228
Soil ammonium (NH_4^+) (mg N kg^{-1} soil) in top 15 cm soil layer	-0.142	28
Soil nitrate (NO_3^-) (mg N kg^{-1} soil) in top 15 cm soil layer	0.831**	28

n = number of samples.
**Significant correlation between modelled and measured values at p-value < 0.001.

Table 5-4. Cumulative N_2O emissions ($N_2O_{cum.}$ in g m^{-2}) based on field measurements and *ecosys* modeling runs from 2014-2017.

2014								
Treatments (estimated duration) [†]							FW (10.7-10.31)	
Measured $N_2O_{cum.}$							0.05±0.01	(0.04-0.06)
Modelled $N_2O_{cum.}$ [‡]							0.15	*19%*[‖]
Modelled $N_2O_{cum.}$ (de-watered slurry) [§]							0.13	
2015								
Treatments (estimated duration)	FW (1.1-4.17)		SW (1.1-4.17)		SW (4.18-10.6)		FW (9.29-11.4)	
Measured $N_2O_{cum.}$	0.56±0.32	(0.24-0.88)	0.02±0.01	(0.01-0.03)	0.25±0.07	(0.18-0.32)	0.04±0.02	(0.02-0.06)
Modelled $N_2O_{cum.}$	0.25	*19%*	0.16	*0%*	0.41	*27%*	0.28	*17%*
Modelled $N_2O_{cum.}$ (de-watered slurry)	0.20		0.16		0.30		0.23	
2016								
Treatments (estimated duration)	FW (1.1-4.20)		SW (1.1-4.20)		SW (4.21-11.3)			
Measured $N_2O_{cum.}$	0.12±0.03	(0.09-0.15)	0.02±0.00	(0.02-0.02)	1.10±0.34	(0.76-1.44)		
Modelled $N_2O_{cum.}$	0.15	*35%*	0.07	*-4%*	0.72	*21%*		
Modelled $N_2O_{cum.}$ (de-watered slurry)	0.10		0.08		0.57			
2017								
Treatments (estimated duration)	FW (1.1-5.16)		SW (1.1-5.16)					
Measured $N_2O_{cum.}$	0.05±0.02	(0.03-0.07)	0.25±0.08	(0.17-0.33)				
Modelled $N_2O_{cum.}$	0.47	*0%*	0.45	*0%*				
Modelled $N_2O_{cum.}$ (de-watered slurry)	0.47		0.45					

† The estimated duration was indicated in Fig. 5-1, Fig. 5-2and Fig. 5-3.

‡ The modeling run (model 1) was the one simulated with the exact field practices from 2014-2017.

§ The modeling run was the one same with the model 1, but with de-watered slurry applications.

‖ The underlined number represents the percentage of water in the slurry contributing to N_2O emissions.

5.9 Figures

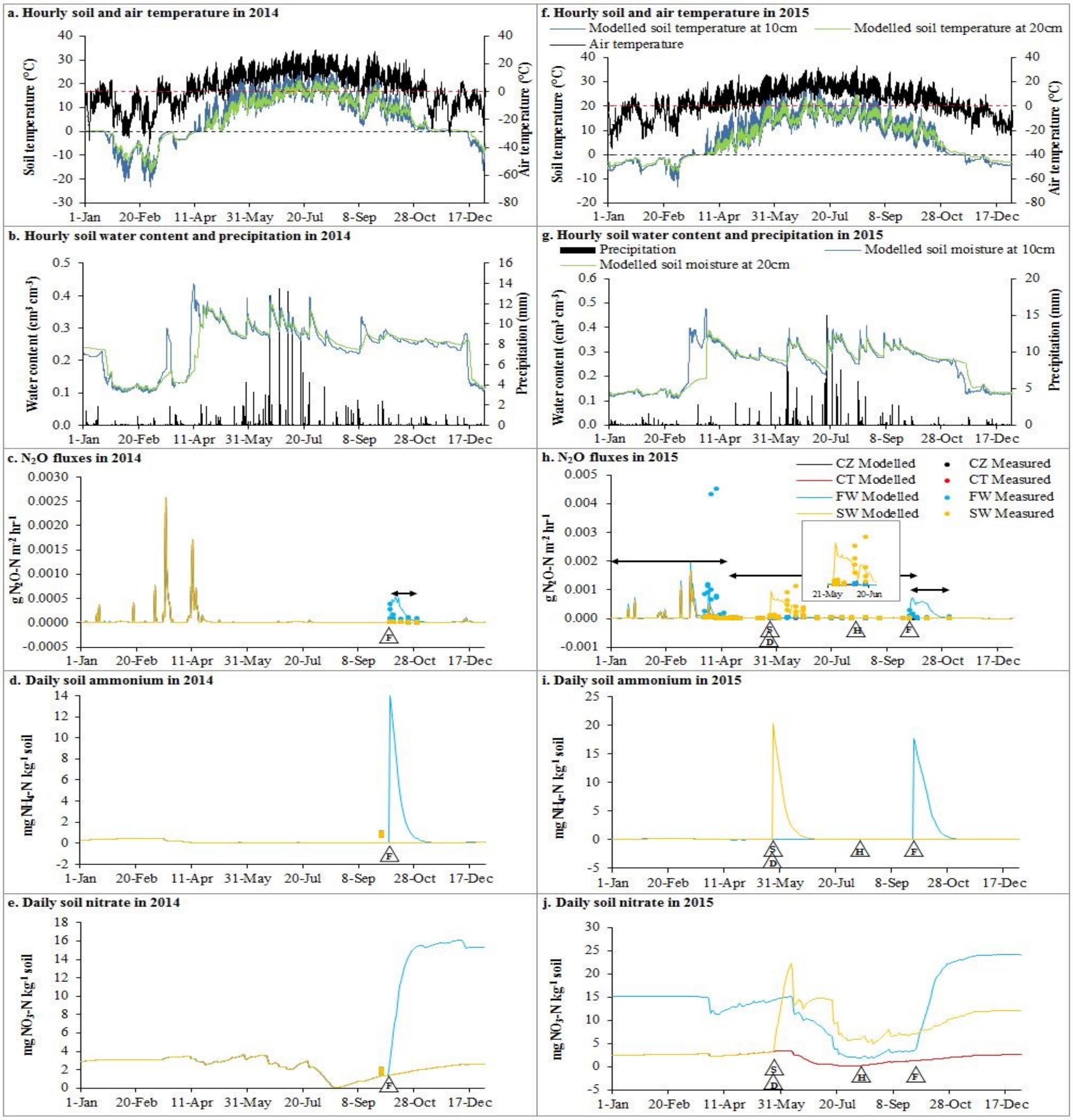

Fig. 5-1. (a, f) Hourly soil and air temperature, (b,g) hourly soil water content and precipitation, (c.h) measured (points) and modelled (lines) N_2O fluxes, (d,i) soil ammonium and (e.j) soil nitrate concentrations in top 15 cm soil layer in Lacombe from 2014-2015. The field measurements of soil ammonium and nitrate concentrations represent the top 20 cm soil layer. Panel a and f, b and g, plus c, d, e, h, i, and j share the same legend, respectively. Horizontal double arrow line indicates the duration of estimated cumulative N_2O emissions based on measurements in Table 5-3. Correlation coefficient (r) between the modelled and measured values for soil temperature, moisture, ammonium (NH_4^+), nitrate (NO_3^-) and nitrous oxide (N_2O) flux during the experiment from 2014-2017.

Measured/simulated variables	r	n

Soil temperature at 10cm	0.94**	5581
Soil temperature at 20cm	0.996**	5560
Soil moisture at 10cm	0.535**	5581
Soil moisture at 20cm	0.557**	5560
Nitrous oxide (N_2O) flux (g N m^{-2} h^{-1})	-0.425**	228
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n = number of samples.
**Significant correlation between modelled and measured values at p-value < 0.001.

Table 5-4. D, S, H and F in the triangle indicate seeding, spring slurry application, harvest and fall slurry application, respectively. The modelled N_2O fluxes were based on the average modelled hourly N_2O fluxes from 10 am to 3 pm every day. CZ = control zero (without disturbances); CT = control treatment where the soil was disturbed by the slurry applicator; FW = fall slurry treatment; SW = spring slurry treatment.

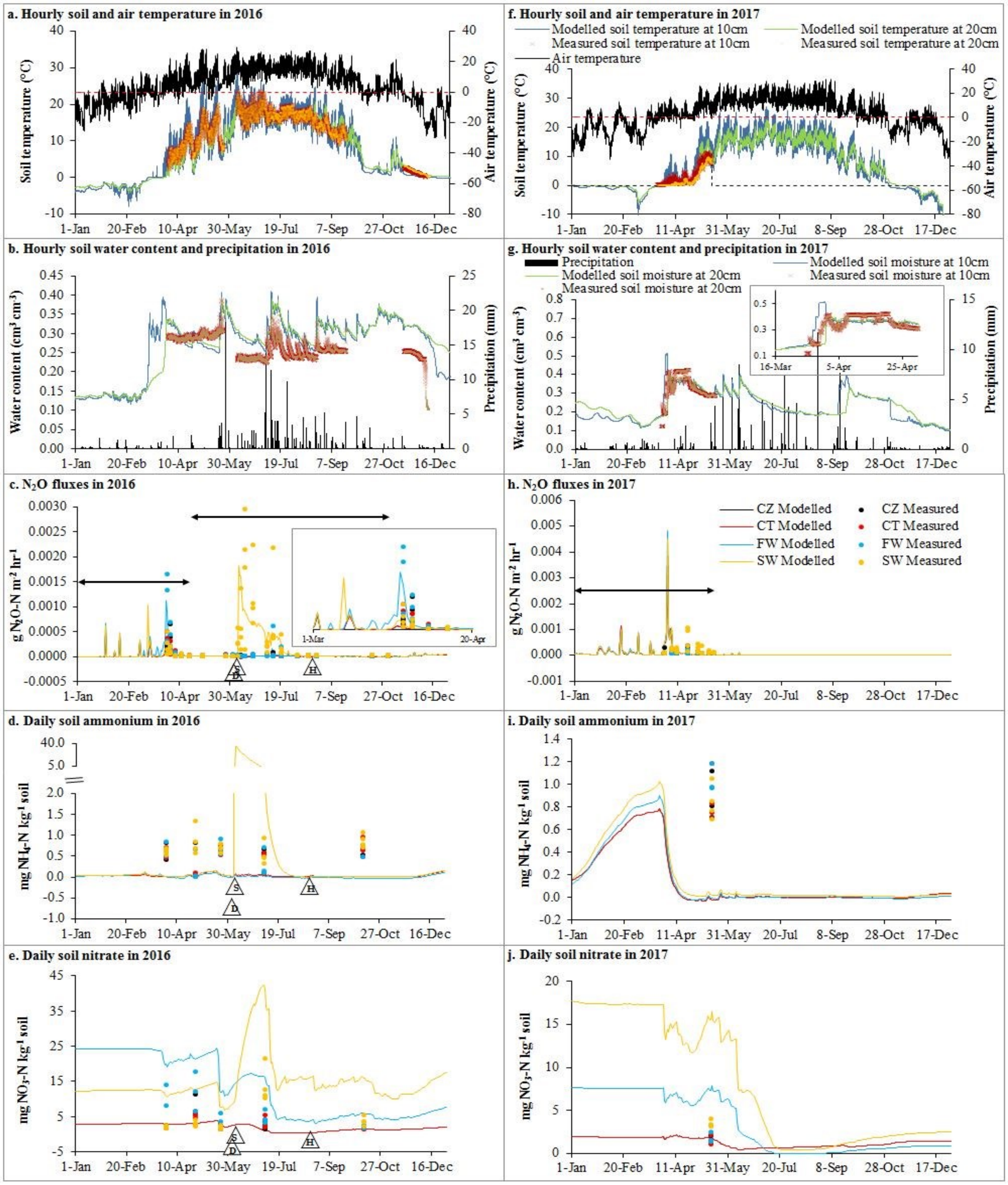

Fig. 5-2. (a, f) Hourly soil and air temperature, (b,g) hourly soil water content and precipitation, (c.h) measured (points) and modelled (lines) N_2O fluxes, (d,i) soil ammonium and (e.j) soil nitrate concentrations in top 15 cm soil layer in Lacombe from 2016-2017. Panel a and f, b and g, plus c, d, e, h, i, and j share the same legend, respectively. Horizontal double arrow line indicates the duration of estimated cumulative N_2O emissions based on measurements in Table 5-3.

Correlation coefficient (r) between the modelled and measured values for soil temperature, moisture, ammonium (NH_4^+), nitrate (NO_3^-) and nitrous oxide (N_2O) flux during the experiment from 2014-2017.

Measured/simulated variables	r	n

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Table 5-4. D, S, H and F in the triangle indicate seeding, spring slurry application, harvest and fall slurry application, respectively. The modelled N_2O fluxes were based on the average modelled hourly N_2O fluxes from 10 am to 3 pm every day. CZ = control zero (without disturbances); CT = control treatment where the soil was disturbed by the slurry applicator; FW = fall slurry treatment; SW = spring slurry treatment.

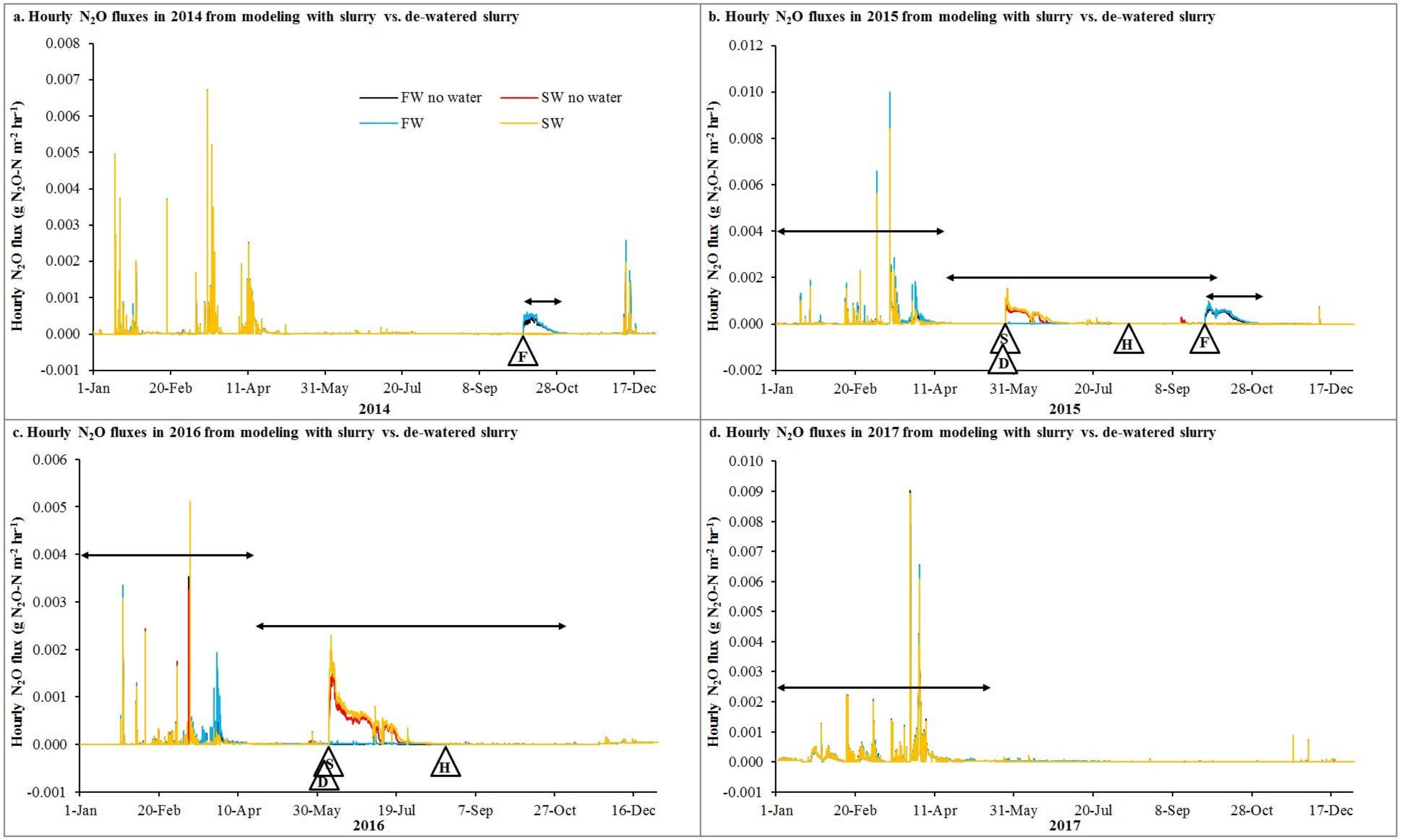

Fig. 5-3. Hourly N_2O fluxes from the modeling run with slurry applications (FW or SW) and de-watered slurry applications (FW no water or SW no water) in (a) 2014, (b) 2015, (c) 2016 and (d) 2017. Horizontal double arrow line indicates the duration of estimated cumulative N_2O emissions based on modelled values in Table 5-3.

Correlation coefficient (r) between the modelled and measured values for soil temperature, moisture, ammonium (NH_4^+), nitrate (NO_3^-) and nitrous oxide (N_2O) flux during the experiment from 2014-2017.

Measured/simulated variables	r	n

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n = number of samples.
**Significant correlation between modelled and measured values at p-value < 0.001.

Table 5-4. D, S, H and F in the triangle indicate seeding, spring slurry application, harvest and fall slurry application, respectively. The modelled N_2O fluxes (lines) were the hourly N_2O fluxes per day. CZ = control zero (without disturbances); CT = control treatment where the soil was disturbed by the slurry applicator; FW = fall slurry treatment; SW = spring slurry treatment.

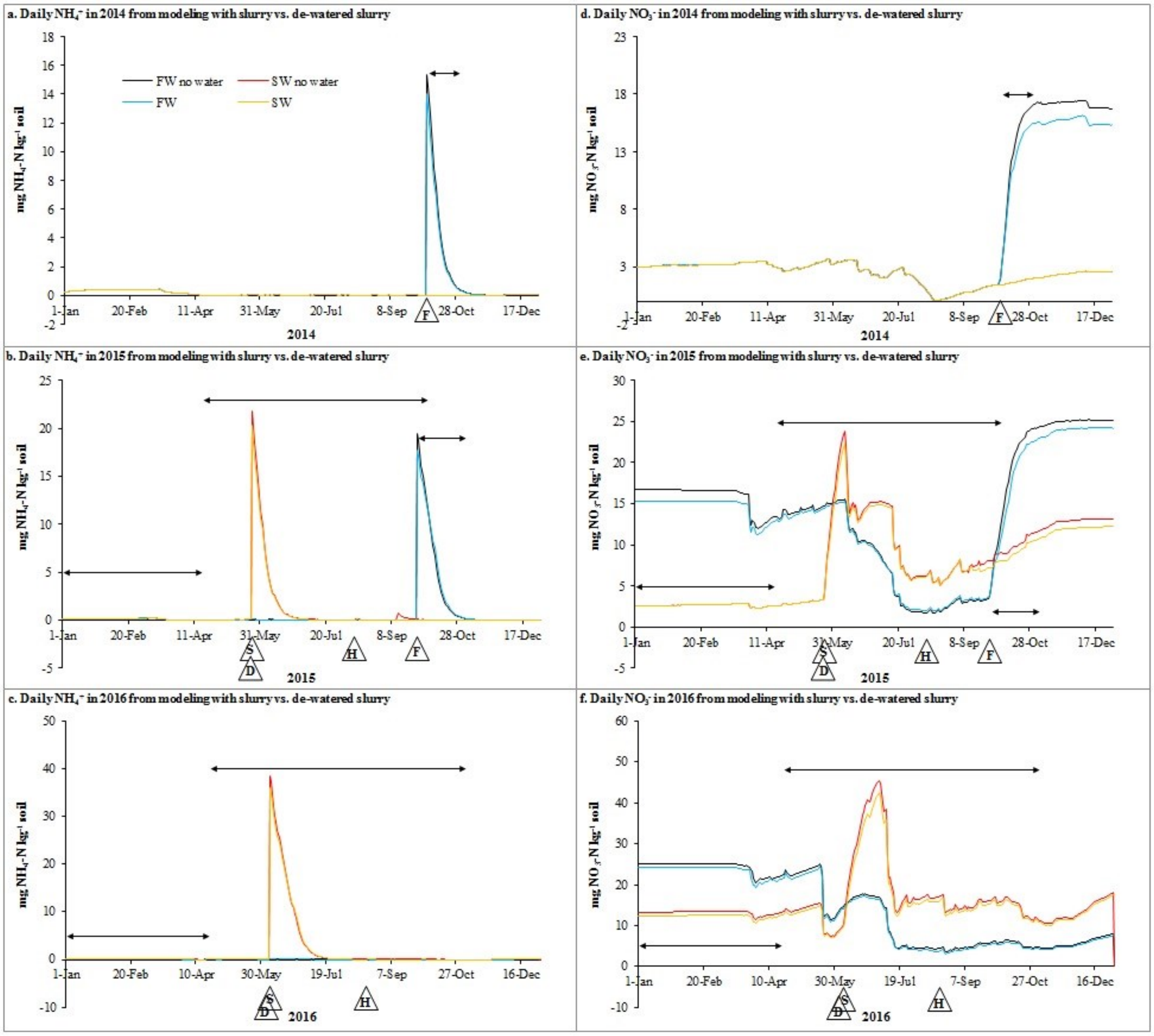

Fig. 5-4. Daily soil NH_4^+ (a-c) and NO_3^- (d-f) concentrations at 0-15 cm from the modeling run with slurry applications (FW or SW) and de-watered slurry applications (FW no water or SW no water) in 2014, 2015 and 2016. Horizontal double arrow line indicates the duration of estimated cumulative N_2O emissions based on modelled values in Table 5-3.

Correlation coefficient (r) between the modelled and measured values for soil temperature, moisture, ammonium (NH_4^+), nitrate (NO_3^-) and nitrous oxide (N_2O) flux during the experiment from 2014-2017.

Measured/simulated variables	r	n
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n = number of samples.
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Table 5-4. D, S, H and F in the triangle indicate seeding, spring slurry application, harvest and fall slurry application, respectively. The modelled N_2O fluxes (lines) were the hourly N_2O fluxes per day. CZ = control zero (without disturbances); CT = control treatment where the soil was disturbed by the slurry applicator; FW = fall slurry treatment; SW = spring slurry treatment.

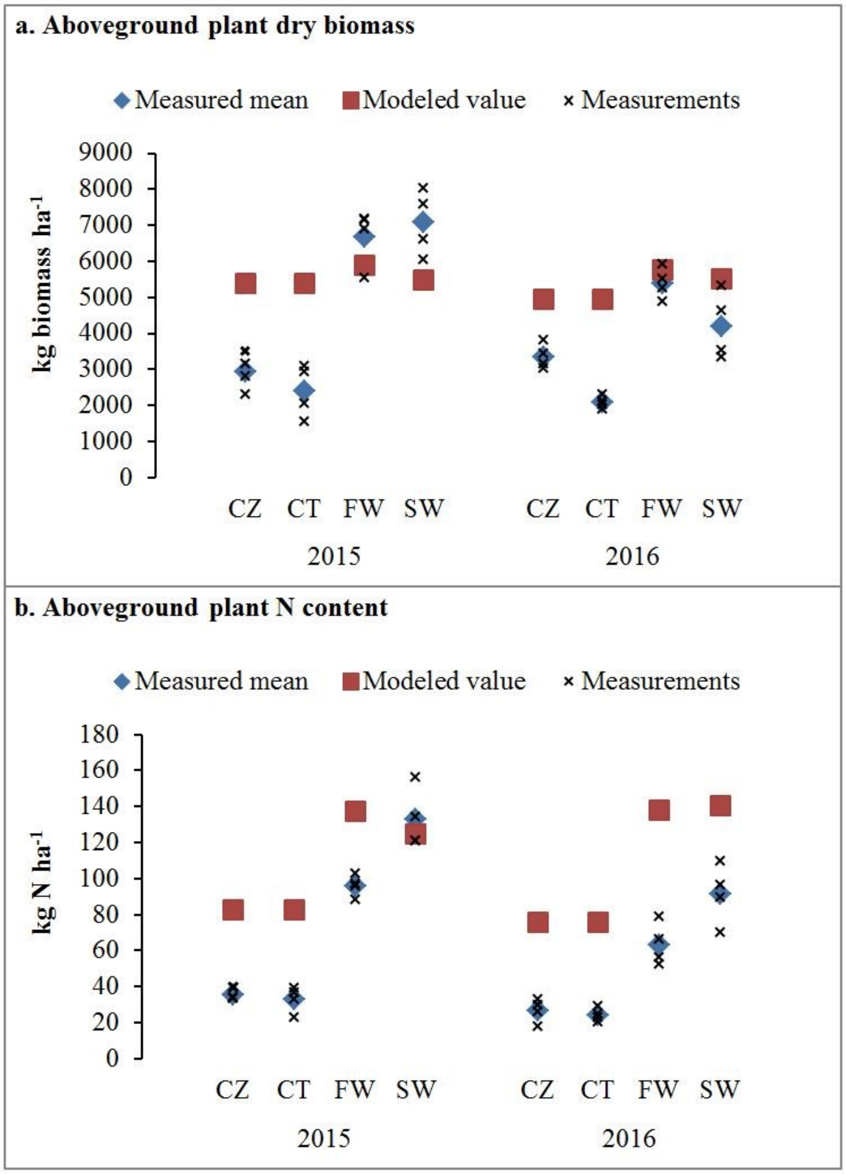

Fig. 5-5. Measured and modelled (a) aboveground plant dry biomass and (b) aboveground plant N content in 2015 and 2016. CZ = control zero (without disturbances); CT = control treatment where the soil was disturbed by the slurry applicator; FW = fall slurry treatment; SW = spring slurry treatment.

6. Conclusions

The studies in this dissertation can help to improve our understanding of the spatial and temporal dynamics of N_2O emissions from agricultural soils in association with edaphic and climatic conditions. In the field study, our results showed that whether fall or spring manure application without NIs would cause greater annual N_2O emissions in connection with precipitation occurrence and intensity. With the application of NIs, three out of four cases showed no significant difference in annual N_2O emissions between the fall and spring manure applications. The existence of one case with significantly different emissions can imply that the effectiveness of NIs in reducing N_2O emissions varied depending on the NI application rate. In the spring of 2017, residual effects of previous manure injections were evident as elevated N_2O emissions and NO_3^- concentrations in the topsoil, which indicating the important role of denitrification in the N_2O production during the springtime.

In the incubation study, we found that the efficacy of NIs was impacted by soil moisture content, NIs application rate and soil organic matter content. The N_2O emission reduction due to NIs was only found at high moisture contents rather than low moisture contents irrespective of soil types This contrasting result suggested that addition of NIs with N fertilizers is necessary when N_2O production is expected to be high, such as in moist soils. On the other hand, soils with higher organic matter are likely to require higher NI application rate to inhibit nitrification and N_2O production due to increased surface adsorption and bio-degradation. In addition, the positive relationship between the soil moisture content and efficient NI application rate in inhibiting nitrification indicated a greater NI degradation rate under higher soil moisture contents. It also implied that microbial NI degradation played a more important role than O_2 availability in moist soils; however, this has not been fully explored yet and more research is needed to gain further insights into this question.

In the mesocosm study, the total N_2O emissions increased with higher soil moisture content irrespective of soil or N treatments. This implied a potential risk of N losses in the form of N_2O emissions for the regions with increased precipitation in fall and winter. Our results also confirmed that thaw-induced N_2O emissions played an important role in the total budget. The positive priming effect of cumulated N_2O emissions was observed in all treatment combinations. Greater positive priming effect due to urea addition was found in the soil with historical manure additions than the control soil without manure additions. This could be related with more easily decomposable organic matter presented in the soil with historical manure additions. Due to the N immobilization by decomposing plant residues, negative priming effect of daily N_2O fluxes was also found under the highest soil water content. The largest N_2O fluxes were generated on the 2nd and 3rd day of thawing and more than 83% of these fluxes were produced through denitrification.

In the modeling study, our results showed that *ecosys* could simulate well the magnitude and timing of N_2O fluxes from soils that had received liquid manure additions; however, there were some discrepancies between the modeled and measured values when the soil was increasingly moist. This might be owing to uncertainty in the simulation of soil water retention and snow pack accumulation and melting in the model. The simulation of de-watered slurry addition in the model suggests that de-watering could successfully reduce cumulated N_2O emissions when compared with the simulation of regular slurry addition.

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