Nitrogen transformations and loss over winter in manure-amended soils with cover crops

Leanne Ejack

Department of Natural Resource Sciences
McGill University, Montreal

July 2019

A thesis submitted to McGill University in partial fulfillment
of the requirements of the degree of
MASTER OF SCIENCE

Abstract

M.Sc. Leanne Ejack Natural Resource Sciences

Many farmers apply manure in the fall (autumn season), but without an actively growing crop in the ground, the nitrogen (N) in the manure is susceptible to over-winter losses. Periods of freeze-thaw cycling can exacerbate N losses by stimulating soil microbes to transform reactive substrates like soil mineral N into nitrous oxide (N_2O), a potent greenhouse gas. The uptake of reactive N from fall-applied manure by a fall-sown cover crop may reduce over-winter N losses. The objective of my research was to investigate the effect of combining fall manure application with cover cropping on soil N dynamics over winter and during periods of freeze-thaw cycling under field and laboratory conditions. I also examined the relationship between N_2O production and reactive soil substrate concentrations. The field experiment was a full factorial in a randomized complete block design with three manure treatments (none, liquid, solid) and four cover crop types (no cover crop, 100% ryegrass [*Lolium multiflorum* Lam.], a 75% ryegrass/25% hairy vetch [*Vicia villosa* Roth] mixture and a 50% ryegrass/50% vetch mixture). The experiment was established at two field sites in Québec, Canada. A partial N mass balance (g N m^{-2}) was calculated in fall (sum of the fall soil N stock to 0.15 m depth, N in fall-applied manure, and N in cover crop biomass) and in spring (sum of the spring soil N stock to 0.15 m depth and N in the winter-killed cover crop) for each treatment combination. After terminating the cover crop, spring wheat (*Triticum aestivum* L.) was planted, and each main plot was split into two subplots that received either 100 kg N ha^{-1} urea fertilizer or no fertilizer. Wheat samples were taken at tillering, flowering, and maturity to determine N content. Final yield was also measured. Cover crops were not effective at retaining manure N (≤7% uptake) and there was no difference in the fall and spring N balance among the manure and non-manure plots. Residual N was not

supplied from fall-applied manure to the spring wheat in the next growing season, and average wheat yields were 11–14% less in the subplots that received no spring N fertilizer than those that received 100 kg N ha^{-1}. In the laboratory, pots with 280–285 g soil received four N fertilizer treatments (none, liquid manure, solid manure, urea), with or without an annual ryegrass cover crop. The pots were exposed to 0, 1, 2, or 3 simulated freeze-thaw cycles (FTCs) at -4 to +4°C. The N_2O production was measured at 0, 3, 6 and 9 h for each FTC, then pots were destructively sampled to determine the soil mineral N concentration. There was no difference in N_2O production among the treatment combinations across all FTCs, but the pots that received urea or liquid manure had the highest soil mineral N concentration. The cover crop had minimal effect on the soil mineral N concentration. Soil mineral N explained approximately 14% of the variation in N_2O production. Pots that underwent FTCs had a remarkable 937–1000% increase in N_2O production compared to unfrozen pots. This suggests that N_2O-producing microbial activity occurred in the frozen soils at -4ºC, causing N_2O to accumulate under ice and be released when the soils thawed at 4ºC, mostly within the first 3 h. The results of both the field and laboratory studies suggests that microbial N transformations do not stop during the winter months, leading to substantial losses of N in fertilized soils during the non-growing season in cold humid temperate regions.

Résumé

M.Sc. Leanne Ejack Natural Resource Sciences

De nombreux agriculteurs épandent du fumier à l'automne, mais sans une culture en pleine croissance, l'azote (N) dans le fumier est susceptible de subir des pertes pendant l'hiver. Les cycles de gel-dégel peuvent exacerber les pertes de N par la stimulation des microbes du sol, transformant ainsi les substrats réactifs tels que N minéral du sol en oxyde nitreux (N_2O). La culture de couverture pourrait absorber N réactif relâché par le fumier épandu pendant l'automne et pouvant réduire les pertes de N en hiver. L'objectif de ma recherche était d'étudier l'effet d'association du fumier épandue à l'automne et des cultures de couvertures sur les réactions de N du sol en hiver, et durant les cycles de gel-dégel, dans le terrain et au laboratoire. J'étudiais également la relation entre la production de N_2O et la concentration de substrat réactif présent dans le sol. L'expérience de terrain consistait en une conception factorielle complète en bloc aléatoire complet avec trois traitements de lisier et quatre types de cultures de couverture. L'expérience a eu lieu sur deux sites au Québec, Canada. Le total de N dans le sol en automne (0,15 m de profondeur), N du fumier et N de la culture de couverture ont été comparés au N total du sol au printemps (0,15 m de profondeur) et N des tissus végétaux de la culture de couverture tuée par l'hiver pour chaque combinaison de traitement. Après la fin de la culture de couverture, le blé (*Triticum aestivum* L.) a été ensemencée et chaque parcelle principale a été séparée en deux sous-parcelles recevant soit a) 100 kg d'engrais à base d'urée, ou b) aucun engrais. Des échantillons de blé ont été prélevés au tallage, à la floraison et à la maturité pour déterminer la teneur en N. Les rendements finaux des récoltes ont également été mesurés. Les cultures de couvertures ne retiennent pas efficacement N du fumier (absorption ≤ 7%) et il n'y avait pas de différence entre les niveaux de N d'automne et du printemps entre les parcelles recevant du

fumier et les parcelles sans fumier. Lors de la prochaine saison de culture, le fumier épandu à l'automne ne fournissait pas de N résiduel et les rendements moyens de blé étaient inférieurs de 11 à 14% dans les sous-parcelles sans engrais au printemps, par rapport à celles de 100 kg N ha^{-1}. Au laboratoire, des pots contenant de 280 à 285 g de terre agricole ont reçu quatre traitements d'engrais N (aucun engrais, fumier liquide, fumier solide, urée), avec ou sans culture de couverture de ray-grass. Les pots ont été exposés à 0, 1, 2 ou 3 cycles simulés de gel-dégel entre -4 et + 4°C. La production de N_2O a été mesurée par intervalle de 3 h à 0, 3, 6 et 9 h pour chaque cycle de gel-dégel, puis des pots ont été échantillonnés de manière destructive pour déterminer la concentration en N réactif. Il n'y avait pas de différence de production de N_2O entre les combinaisons de traitement parmi tous les cycles de gel-dégel, mais les pots contenant de l'urée ou de l'engrais à base de lisier avaient la concentration la plus élevée de N minéral dans le sol. La culture de couverture a eu un effet minimal sur la concentration de N minéral dans le sol. N minéral du sol explique environ 14% de la variation de la production de N_2O. La production de N_2O des pots sous cycle de gel-dégel a connu une augmentation de 937 à 1000% par rapport aux pots non congelées. Cela suggère qu'une activité microbienne qui produit du N_2O a eu lieu dans les sols gelés à -4°C, provoquant une accumulation de N_2O sous la glace et a été émis après la décongélation du sol (à 4°C), principalement durant les 3 premières heures. Les résultats de ces études de terrain et de laboratoire suggèrent que les transformations microbiennes de N ne s'arrêtent pas pendant les mois d'hiver, et entraîne des pertes importantes de N dans les sols fertilisés en dehors de la saison de croissance dans les régions tempérées froides et humides.

Contribution of Authors

My thesis consists of a general introduction outlining the context of my research project, three chapters written in manuscript format according to the guidelines of the McGill Graduate and Postdoctoral Studies Office, and a general conclusion that highlights key findings and suggestions for improvements and future advances.

Chapter 1 is a comprehensive literature review that explores the research and current knowledge on my thesis field of study. The four objectives of my project are presented at the end of this chapter.

Chapter 2 presents a field experiment that achieves the first two project objectives and Chapter 3 present a laboratory incubation study that addresses my third and fourth project objectives. Chapter 2 is followed by a connecting paragraph that explains the progression between the second and third chapters.

The Chapter 1 literature review was written by the candidate and edited by her supervisor, Dr. Joann K. Whalen. The Chapter 2 manuscript was co-authored by the candidate, Dr. Whalen, and PhD candidate Chih-Yu Hung. The Chapter 3 manuscript was co-authored by the candidate and Dr. Whalen.

The field experiment was designed by the candidate and Chih-Yu Hung with the guidance of Dr. Whalen. The field set-up and sampling were carried out by the candidate and Chih-Yu Hung, with the help of field assistants. The candidate was responsible for sample processing and statistical analysis. The laboratory experiment was designed by the candidate, and the candidate conducted all experiments, sampling, sample processing, and statistical analysis for the experiment. The data interpretation and preparation of both manuscripts was done by the candidate under the supervision of Dr. Whalen.

Acknowledgements

I first and foremost want to thank my supervisor, Dr. Joann Whalen, for her unwavering support and dedication to making me a better writer, thinker, researcher, and scientist. Without her ongoing guidance, commitment, and invaluable advice, I would not have completed this master's degree as smoothly and timely as I did. Dr. Whalen provided numerous opportunities for me to succeed in my time at McGill. She will continue to be an important professional role model and someone I highly look up to in my life and career moving forward.

I also want to thank the Soil Ecology Research Group. We were a large, diverse group of students, but everyone was always willing to come together to lend a hand during busy field times or provide supportive feedback and encouragement on each other's research. A special thank you to my research partner, Chih-Yu Hung, who I have been lucky to work and collaborate with from day one of my master's. Chih-Yu is gentleman and a scholar in the truest sense of the words, and I am grateful that we were able to work together so closely these past two years.

I am grateful to Dr. Ian Strachan, for providing me with many pearls of wisdom and for his assistance with the preliminary testing of the incubation study, to Mr. Marc Samoisette for always going above and beyond to support us with our field study, to Dr. Pierre Dutilleul for his guidance with statistical analysis and generosity with his time, to Ms. Hélène Lalande for her valuable scientific expertise, and to Mr. Hicham Benslim for his help in the lab. Antoine Mounier and Alexia Bertholon assisted with the French translation of the general abstract. Thank you to Luciano Vendittelli for generously providing the use of his land. I also want to express my gratitude for the scholarships and funding I received from the Schulich Fellowship, Centre SÈVE, and the Natural Sciences and Engineering Research Council (NSERC).

Lastly, I thank my family. To my mom for the love, comfort, and support that only a mother could offer, to my dad for always, always believing in me, and to Stephen for keeping me humble and honest. And to Justin, my best friend, soulmate, and constant companion. You were by my side more than anyone during this master's, and you saw me through everything – the challenges, the disappointments, the successes, and the triumphs. Thank you for being an occasional lab and field assistant, and most importantly, a constant support.

Table of Contents

List of Tables

Chapter 1

Chapter 2

Chapter 3

List of Figures

Chapter 1

Chapter 2

Chapter 3

List of Appendices

General Introduction

Nitrogen (N) is a key nutrient for plant growth and development, and to meet food demands of the world's growing population, N fertilizers are essential to crop production. Animal manure is an abundant N fertilizer that can support sustainable farming systems by increasing soil organic matter content and recycling locally-available nutrients. The N fertilizer value of manure comes from the ammonium (NH_4^+) that is immediately plant-available, and the organic N compounds, which are a mixture of proteins and urea that are slowly plant-available after being mineralized by soil microbes. The rate of organic N mineralization from manure is influenced by manure physico-chemical properties as well as soil and environmental conditions after manure application (Gagnon et al. 1999; Yang et al. 2011). This makes it difficult to synchronize the plant-available N supply from manure with crop N uptake and can result in significant losses of N from the soil system (Chadwick et al. 2000). Nitrogen losses occur when nitrate (NO_3^-) and other reactive N compounds leach or are transported through surface run-off, which can pollute nearby water sources (Chambers et al. 2001). Losses also occur when reactive N compounds are transformed into gaseous dinitrogen (N_2) and ammonia (NH_3), and nitrous oxide (N_2O) that are emitted to the atmosphere (Han et al. 2017).

For practical reasons, many farmers apply manure in the fall after harvesting the main crop. The N input from the fall-applied manure is susceptible to over-winter losses, especially during freeze-thaw events (Henry 2008; Risk et al. 2013; Tatti et al. 2014). In Southern Québec, there is expected to be wider fluctuations in winter temperatures and more frequent winter freeze-thaw events due to global climate warming (Logan et al. 2011). Soil freeze-thaw events affect soil N dynamics by increasing microbial cell lysis and root mortality, resulting in greater accumulation of reactive substrates such as nitrate (NO_3^-), ammonia (NH_4^+), and dissolved

organic carbon (DOC), and less N retention by microorganisms and crops (Henry 2008; Joseph and Henry 2008; Lange et al. 2017). Higher concentrations of reactive N compounds and DOC during periods of soil thawing are expected to stimulate microbially-mediated N transformations, such as heterotrophic denitrification, ammonia oxidation, and nitrifier denitrification. These metabolic processes result in soil N losses through gaseous forms, including relatively benign N_2, but also N_2O, a greenhouse gas with a 100-year global warming potential roughly 300 times that of carbon dioxide (Wagner-Riddle et al. 2008; Butterbach-Bahl et al. 2013; Congreves et al. 2018). In temperate regions, between 30–90% of annual N_2O emissions from agricultural soils occur during the non-growing season, especially during freeze-thaw events (Wertz et al. 2016; Han et al. 2017; Wagner-Riddle et al. 2017). Due to the challenges associated with studying N dynamics during winter months, how much of the N from fall-applied manure that is lost during this period is poorly documented.

A fall-sown cover crop that absorbs reactive N from fall-applied manure may prevent over-winter N losses (Drinkwater et al. 1998), but the magnitude of N retention in cover crops is unclear. In some cases, the presence of cover crops may increase N losses because actively-growing crops release labile C and N exudates through their roots, which stimulates microbially-mediated N transformations (Morkved et al. 2006; Thomas et al. 2017). However, senescent cover crops and their residues may be an effective N sink. For example, Pelster et al. (2013) found up to 80% reduction in N_2O emissions due to cover crop residue after eight laboratory-simulated freeze-thaw cycles. The N retention capacity of cover crops depends on whether they are leguminous or grass-based. Legume cover crops are selected to supplement N fertility through biological N_2 fixation whereas grass cover crops can sequester residual soil N in their tissues (Seman-Varner et al. 2017). In most studies, grass cover crops were more effective at

retaining fall-applied N fertilizer and less likely to stimulate N losses than legume cover crops (Singer et al. 2008; Jarecki et al. 2009; Cambardella et al. 2010; Adeli et al. 2011; Seman-Varner et al. 2017). Growing a bi-culture of legumes and grasses could optimize the N_2 fixation and N retention functions of the cover crop, though this ability is highly dependent on the species selected and botanical composition of the bi-culture (Kuo and Sainju 1998).

As freeze-thaw events become more frequent, we can expect more N loss from fall-applied manure and must adopt strategies to retain N within agroecosystems for agronomic, environmental, and economic benefits on farms. The overall objective of my thesis research was to better understand how fall manure application together with fall-sown cover crops would affect soil N dynamics over winter and during freeze-thaw events in a cold humid temperate agroecosystem. Specifically, the research objectives were to: (1) investigate how N from fall-applied manure is retained in the soil-plant system over winter in the presence of a cover crop, (2) determine if fall-applied manure can supply N in the next growing season, resulting in greater N uptake and yield in a spring wheat crop, (3) examine the effect of planting a cover crop after N fertilizer or manure application on N_2O production during freeze-thaw cycles, and (4) study the relationship between reactive N and DOC concentrations on N_2O-N loss during freeze-thaw cycles.

Chapter 1: The potential of cover cropping to reduce nitrogen loss from fall-applied manure over winter and through multiple freeze-thaw events: a review

Nitrogen (N) is one of the most critical nutrients required for plant growth and the dominant element in most agricultural fertilizer regimes. Animal manure can provide an organic, locally-produced source of N to crops and contribute to the formation of soil organic matter. Canada produces over 100 million tonnes of manure every year, with much of this manure production concentrated in Eastern Canada (Yang et al. 2011, Fig. 1-1). In these regions, over 40% of farmers apply manure fertilizer in the fall (autumn season) following the main harvest (Beaulieu 2004; Yang et al. 2011). However, with no actively growing crop in the ground, there can be significant losses of N in the form of gaseous emissions such as nitrous oxide (N_2O), as well as leaching or surface run-off of nitrate (NO_3^-) and other reactive N species (Fig. 1-2). These N species have the potential to contaminate the atmosphere or surrounding aquatic and terrestrial ecosystems. In cold humid temperate regions of Eastern Canada, such as southwestern Québec, the loss of N may be exacerbated by increased temperature fluctuations leading to freeze-thaw events in fall, winter, and during early spring snowmelt. Soil freeze-thaw events can modify soil N dynamics by increasing microbial cell lysis and root mortality, resulting in less N retention by microorganisms and crops (Henry 2008; Song et al. 2017). A cover crop that is sown after manure is applied in the fall provides a potential source of plant uptake for the reactive manure N leading to potential reductions in over-winter N losses (Fig. 1-2). The value of using cover crops to preserve the N in fall-applied manure will be explored in this literature review by addressing four key research areas: i) the use of manure as a fertilizer source, ii) N losses associated with applying manure in the fall, iii) how soil freeze-thaw cycles modify soil N dynamics and increase the risk of N loss from fall-applied manure, iv) the use of cover cropping

to help reduce N loss over winter by immobilizing available N in plant tissues until the following spring. This review focuses on cold humid temperate climates with maximum summer air temperatures as high as 30°C and winter temperatures as low as -30°C.

1.1 Factors that influence the nitrogen fertilizer value of manure

The ability of manure to supply plant-available N to crops compared to the amount of N applied is an indicator of its N fertilizer value (Chadwick et al. 2000). Due to the rising global demand for animal-sourced protein, there is an abundance of manure produced in many regions of the world (Bouwman et al. 2013). Projected use of manure N in agriculture is expected to rise to 130-153 Tg N per year by 2050, compared to 83-109 Tg N per year for inorganic N fertilizer over the same time period (Bouwman et al. 2013). In Canada over one million tonnes of manure N is applied to farmland every year (Yang et al. 2011). Manure can be a cost-effective alternative to synthetic-based fertilizers and is a particularly valuable for organic farmers who cannot rely on inorganic fertilizers (Watson et al. 2002). Livestock farmers also benefit from using manure-based fertilization to supply N and other nutrients to crops as well as to avoid the inconvenience and expense of storing or transporting manure off-farm. Manure supplies organic matter to the soil which contributes to the maintenance of soil health and quality by improving soil structure and feeding the soil food web.

Despite its benefits, there are challenges to using manure as a fertilizer source. Manure contains plant-available ammonium (NH_4^+) as well as organic N compounds that must be mineralized to be taken up by plant roots (Van Kessel and Reeves 2002; Sørensen et al. 2003). It is difficult to predict the amount and timing of organic N mineralization from manure due to the its heterogeneous physico-chemical characteristics as well as the influence of soil and

environmental conditions on N mineralization (Chadwick et al. 2000; Thomas et al. 2015). Manure is a continuum of materials with varied physical forms and chemical characteristics based on factors such as animal species, diet, storage conditions, application procedures, stage of decomposition (fresh vs. composted), and bedding material (Eghball 2000; Van Kessel and Reeves 2002; Sharifi et al. 2014; Whalen et al. 2019). All these factors influence the mineralizable N pool and overall N fertilizer value of manure. A comparison of the average chemical compositions of a few common liquid/slurry and solid manures is shown in Table 1-1.

1.1.1 Diet and crude protein content

Animal diet has a direct influence on the N content of manure in the form of NH_4^+ in urine and organic N (mainly undigested proteins) in feces. Diets higher in protein and lower in fibre tend to produce fresh manure with higher mineralizable N content (Sørensen et al. 2003; Morvan et al. 2006). Sørensen et al. (2003) found that the nitrogen fertilizer value of dairy cattle manure was positively correlated with dietary protein and negatively correlated with crude fibre content in the cattle's diet. Reductions in dietary crude protein content also decreased total excreted N by 27% in broiler chickens after six weeks of study (Blair et al. 1999) and by 40% in pigs fed diets reduced in crude protein by four percentage units for 15 days (Shriver et al. 2003).

1.1.2 Moisture content during storage

The moisture content of manure has a large influence on the amount and rate of N losses in stored manure. If mixed with wastewater, manure may be a liquid (up to 4% solid content), slurry (5-10% solid content), or semi-solid (10-20% solid content) (Lorimer et al. 2000). Swine manure is generally kept as a liquid or slurry whereas dairy manure is usually mixed with

milkhouse wastewater and bedding and kept as a slurry or semi-solid. Liquid, slurry, and semi-solid manures are often stored in uncovered outdoor concrete pits or lagoons where nutrients are diluted by precipitation events and N losses can range from 50-85% depending on storage conditions, length of storage, and the season (Lorimer et al. 2000; Crouse et al. 2018). Liquid manure stored in this manner is particularly susceptible to N losses from ammonia (NH_3) volatilization due to the absence of nitrification in the storage environment (Perazzolo et al. 2017). Seasonality also plays a significant role in N loss from liquid manure storage facilities. Blunden and Aneja (2008) found fluxes of NH_3-N from anaerobic liquid swine manure lagoons to be greater than 4200 μg N m^{-2} min^{-1} in the summer, with spring and fall fluxes at 1634±505 and >2495 μg N m^{-2} min^{-1} respectively, and winter fluxes the lowest at 1290±246 μg N m^{-2} min^{-1}. This is consistent with Perazzolo et al. (2017) who found N losses from unseparated dairy slurry above 38% in summer months and <7% during the winter. Mixing tends to increase N emissions from liquid manure so N losses can be mitigated by reducing agitation or installing liquid-solid separation procedures (Perazzolo et al. 2017). Sommer et al. (2017) also found that acidifying liquid cattle manure from pH 7.3 to 5.5 with sulfuric acid was effective at reducing $NH_{3\,(g)}$ emissions by up to 63% during storage.

1.1.3 Carbon content and overall C/N ratio

Beef cattle and poultry manure is typically mixed with lignocellulosic bedding materials (straw, wood chips, etc.) and stored as a solid with less than 80% moisture content on large concrete sheds or paddocks (Lorimer et al. 2000). During storage, solid manure may be semi-composted in a passive manner or actively composted using aeration technology. Active composting of manure can help reduce moisture content for easier storage and handling as well

as reduce odours and eliminate weeds and pathogens (Bernal et al. 2009). However, N losses that occur during composting, mainly through gaseous emissions (NH_3, N_2O and N_2), reduce the agronomic value of composted manure and contribute to greenhouse gas emissions (Larney et al. 2006; Bernal et al. 2009). Eghball et al. (1997) found up to 42% loss of N from beef cattle manure during composting, while Larney et al. (2006) found 46% more N loss in composted beef manure compared to fresh manure.

The presence of carbonaceous bedding material in solid manure increases the carbon/nitrogen (C/N) ratio of manure and decreases the NH_4^+/total N ratio, meaning that less N is prone to mineralization during storage (Morvan et al. 2006). In the process of decomposing materials with high amounts of C relative to N, microbes will immobilize N in order to maintain their internal C/N stoichiometry (Griffiths et al. 2012). However, solid manure with higher C/N ratios (15-25) tends to have less plant-available N and a lower N fertilizer value than manure with a lower C/N ratio (<10) (Whalen et al. 2019, Table 1-2). Though C/N ratio is only one of many factors that determine the N mineralization rate of manure, up to 40% of the differences in mineralization between manure types may be explained by manure C/N ratio (Chadwick et al. 2000). In addition to C/N composition of manure, Antil et al. (2011) found that hot water extractable N can predict the short-term N mineralization in solid composted manure and Gordillo and Cabrera (1997) found uric acid-N content of poultry litter to be strongly correlated with total N mineralization. While traditional laboratory chemical analysis may help approximate field N availability (Gale et al. 2006), N mineralization is highly variable among manures and depends largely on the interactions between manure, soil, and environmental conditions. Therefore, laboratory-based tests of N mineralization and plant-available N may not accurately reflect manure reactions in field conditions.

1.1.4 Interaction between manure and soil properties

Although the chemical nature and pre-application storage and handling of manure provides a baseline for its N fertilizer value, the ability of crop plants to uptake manure N is affected by the interaction between manure and site-specific soil properties. In general, solid manure tends to improve soil physical properties due to its high organic matter content and low bulk density. Solid manure has been found to increase macropore development, bulk density, water infiltration, soil water holding capacity, and aggregate formation, mostly at depths <10 cm (Barzegar et al. 2002; Shirani et al. 2002; Zhang et al. 2014; Whalen et al. 2019). For example, Zhang et al. (2014) found that applications of composted chicken manure with wheat straw resulted in significantly higher capillary water holding capacity (37.51%) and saturated water content (38.96%) in a silty loam with high salinity and low organic matter content compared to plots receiving no amendments (36.16% water holding capacity and 37.68% saturated water content). Solid cattle manure has been found to raise soil pH in acidic soils due to the buffering effect of bicarbonates and organic acids in the manure (Eghball 1999; Whalen et al. 2000). The organic matter addition from solid manure also tends to support food web cycling by increasing soil microbial activity and supporting larger populations of macrofauna such as earthworms (Haynes and Naidu 1998; Whalen et al. 1998).

The benefits of liquid and slurry manures on soil physical properties are less clear. Liquid and slurry manures can also raise soil pH, though the effect is more short-lived with pH often declining within a few days following application (Loria and Sawyer 2005). However, due to a high moisture content, liquid and slurry manures can saturate fine-textured soils and temporarily reduce water infiltration (Fares et al. 2008). Liquid and slurry manures are also more susceptible to leaching and gaseous losses of N than solid manure following application due to a higher

NH_4^+/total N ratio (Rasouli et al. 2014). Further details of N loss from manure are discussed in the following section.

1.2 Nitrogen losses from fall application of manure

Ideally, N mineralization should occur in synchrony with crop N uptake, which usually means applying manure in the spring (CRAAQ 2010). Agricultural operations regulation in Québec strongly discourages the application of fertilizers, including manure, on ground that is frozen or snow-covered (*Environment Quality Act* 2018). However, many farmers, especially in cold temperate humid regions like Québec, choose to apply manure in the post-harvest fall months. The wet, bulky nature of manure makes it difficult to handle and it must be stored in an approved facility with impermeable floor, walls and runoff control to avoid transferring nutrients, pathogens and other contaminants into the environment (*Environment Quality Act* 2018; Whalen et al. 2019). Since manure storages have finite capacity, many farmers apply manure in the post-harvest fall months to empty the storage and because they have more time for manure spreading in fall than in spring. In most parts of Québec, soil is drier in fall than in spring so there may be less risk of soil compaction with fall-applied manure. In Canada, 15-54% of manure application occurs during the post-harvest fall period (Yang et al. 2011), with up to 42% of farmers in Québec applying manure in the fall (Beaulieu 2004).

1.2.1 Run off and leaching losses

Eutrophication of lakes in Canada from nitrogen enrichment is a growing concern and largely attributed to N management in the agriculture sector. Run-off and leaching from N fertilized soils and livestock operations are believed to be responsible for up to 49% of the total N load of surface waters in Canada (Rasouli et al. 2014). Soluble N species such as NH_4^+, NO_3^-, nitrite (NO_2^-), and dissolved organic N compounds all contribute to run-off and leaching losses of N from the soil (De Jong et al. 2009). Large amounts of these soluble N species are discharged into Eastern Canadian streams, rivers, subsurface aquifers, and other bodies of water, especially during colder months (Rasouli et al. 2014). Over-winter total N losses in Québec from agricultural fields ranged from 15.1 – 22.9 kg N ha^{-1} from 1981 – 2006 (De Jong et al. 2009). Fall applications of manure increase the residual soil mineral N pool, mainly in the form of NO_3^-, remaining at the end of the growing season. This residual soil N is prone to leaching losses due to a low biological demand for soil nutrients combined with conditions of high precipitation and low evapotranspiration. A comparison of ^{15}N-labelled pig slurry applied at different times (spring pre-plant, spring side-dress, and fall) over a 2-year period in Ontario found leaching losses of N ranging from 30–43 kg N ha^{-1} yr^{-1} in the manured plots compared to 27 kg N ha^{-1} yr^{-1} for the unfertilized control (Jayasundara et al. 2010).

1.2.2 Gaseous emissions of nitrous oxide (N_2O)

Over-winter emissions of N_2O from cultivated soils in temperate regions can be very high, with estimates ranging from 30-90% of total annual emissions (Röver et al. 1998; Wagner-Riddle et al. 2007; Congreves et al. 2018). In contrast, emissions of NH_3 from applied N are generally very low over the winter (<7%) compared to N_2O (Jayasundara et al. 2010).

Agriculture management, including manure application, contributes to over 60% of total global anthropogenic N_2O emissions (Reay et al. 2012; Han et al. 2017). Agriculture-derived N_2O emissions are expected to rise from 6.4 Tg N_2O-N yr^{-1} in 2010 to 7.6 Tg N_2O-N yr^{-1} by 2030 as nitrogen fertilizer use, including manure, continues to increase due to global pressures on food production (Cavigelli and Parkin 2012; Reay et al. 2012). Emissions of N_2O have considerable environmental implications as it is an extremely potent greenhouse gas with a global warming potential almost 300 times higher than CO_2 on a 100-year time scale (Zhang et al. 2015; Han et al. 2017). Heterotrophic denitrification and nitrification pathways (both ammonia oxidation and nitrifier denitrification) are the main sources of microbial-induced production of N_2O emissions from soil environments (Fig. 1-3). As denitrification is an anaerobic process, soil N_2O emissions are highly correlated with soil moisture content (Kool et al. 2011). Under soil moisture conditions of 80-90% water-filled pore space N_2O tends to dominate over N_2 as the final product of denitrification reactions (Cavigelli and Parkin 2012; Liu et al. 2016). Low soil temperatures (<5°C) also tend to decrease the activity of N_2O reductase, coded for by the *nosZ* genes, more than enzymes that produce N_2O (nitrite and nitric oxide reductase) resulting in the predominance of N_2O over N_2 production during colder months (Kariyapperuma et al. 2011; Cavigelli and Parkin 2012; Tatti et al. 2014).

1.3 Influence of freeze-thaw cycling on N_2O emissions

In northern temperate regions, including Québec, winter conditions are expected to become more unpredictable with wider fluctuations in temperatures, decreases in snow cover, and increases in freeze-thaw cycling (Henry 2008; Logan et al. 2011). Potential decreases in the presence, thickness, and duration of snow cover will also have an impact on the intensity and

frequency of winter freeze-thaw events as snow cover provides an insulating and moderating effect on soil temperatures (Gao et al. 2018). Although losses of N through NO_3-N leaching during the non-growing season can be substantial (Owens et al. 1995; Adeli et al. 2011; Shelton et al. 2018), recent research has indicated that N_2O flux may account for the largest source of N loss over winter, particularly in regions that experience frequent or intense freeze-thaw cycling (Jayasundara et al. 2010; Han et al. 2017; Wagner-Riddle et al. 2017). Over half of the increased N_2O emissions in temperate agroecosystems during the non-growing season are a result of soil thawing-related fluxes (Wagner-Riddle et al. 2007; Wagner-Riddle et al. 2017). Emission peaks of N_2O do occur during soil freezing, but the magnitude and extent is much less than what is observed during soil thawing (Koponen et al. 2004). Failing to account for freeze-thaw-related N_2O emissions from croplands could cause global N_2O emissions to be underestimated by up to 30% (Wagner-Riddle et al. 2017).

1.3.1 Mechanisms of N_2O release during freeze-thaw events

Liquid water can coexist with ice in frozen soils and enable the survival of nitrifying and denitrifying microbes in liquid water films at very low temperatures (Koponen et al. 2004; Congreves et al. 2018). This amount of liquid water in frozen soils is largely dependent on soil type and pore size distribution. Liquid water content is negligible in sandy soils below -5°C, but can be as high as 0.15 cm^3 cm^{-3} in clay and organic soil in temperatures between -5 and -10°C (Congreves et al. 2018). Even with extremely cold air temperatures as low as -30°C, soil temperatures rarely reach below -10°C, especially with an insulating layer of snow (Henry 2007).

Early hypotheses of the mechanism for N_2O emissions during freeze-thaw cycling focused on the physical release of previously-produced N_2O trapped under frozen soil layers at depth (Li et al. 2000; Teepe et al. 2001). During the early stages of soil freezing, anaerobic conditions build up which activates denitrifying microbes to consume residual substrates in the soil such as NO_3^-, NH_4^+, and dissolved organic carbon (DOC) and produce N_2O. Most of this N_2O is trapped in deep, unfrozen soil layers or in liquid water films present in the frozen soil, and is only released upon thawing of the topsoil (Li et al. 2000). The solubility of N_2O is inversely related to soil temperature, so the potential for degassing of N_2O increases during each thawing event (Li et al. 2000; Risk et al. 2013).

Current advances in research using ^{15}N tracer techniques have shown that the physical release of previously-produced N_2O trapped under frozen topsoil is a minor contributor to the overall quantity of thaw-induced N_2O emissions (Kariyapperuma et al. 2011; Wagner-Riddle et al. 2017; Congreves et al. 2018). Research involving the use of ^{15}N tracers have demonstrated that biological *de novo* denitrification contributes to the majority of N_2O emissions during major thaw events (Morkved et al. 2006; Wagner-Riddle et al. 2008). Wagner-Riddle et al. (2008) used labelled ^{15}N to show that N_2O originating from deep soil layers (12-17 cm depth) were 1.5 to 5 times lower than N_2O originating from surface layers (0-5 cm) during thawing. Most of the N_2O from the deeper soil layers was converted to N_2 before being released from the soil (Wagner-Riddle et al. 2008).

Anaerobic conditions and the accumulation of substrates in soil solution during thaw events induce denitrification and the *de novo* microbial production of N_2O in upper soil layers (Wagner-Riddle et al. 2008; Kariyapperuma et al. 2011; Congreves et al. 2018). Substrate accumulation tends to increase in the soil during thawing as microbial cells lyse and release

cytoplasmic contents (Wagner-Riddle et al. 2008; Congreves et al. 2018). Residual NO_3^- and DOC from the previous growing season as well as NH_4^+ and NO_3^- pools accumulated from nitrification over the winter may also contribute to increase substrate availability during thawing (Kariyapperuma et al. 2011). In addition, denitrifying microbes tend to take advantage of additional substrates present in thawing soil more than other heterotrophic microbes (Koponen et al. 2004).

1.3.2 Influence of repeated freeze-thaw events on N_2O emissions

Despite the deeper understanding of the mechanisms that influence N loss from freeze-thaw cycling, the influence of repeated freeze-thaw events on N loss is still unclear. Emissions of N_2O trapped in deep soil layers should decline as more gas is liberated during successive freeze-thaw events. The pool of susceptible freeze-thaw material and the microbial biomass of denitrifiers may also decline with increasing number of events (Yanai et al. 2004; Henry 2007). Conversely, some laboratory freeze-thaw simulations have indicated that N loss tends to increase with increasing freeze-thaw cycles (Chen et al. 1995; Joseph and Henry 2008), possibly due to increases in substrate availability after each successive thaw. However, many laboratory freeze-thaw experiments have employed unrealistic temperature extremes and durations or failed to account for moisture content in the soil (Henry 2007; Song et al. 2017). Such experiments are unlikely to capture the relationship between freeze-thaw frequency and N_2O release in field settings complicated by factors such as soil type, nutrient regime, moisture content, and snow cover.

1.3.3 Fall-applied manure and freeze-thaw events

Sustained nitrification, N mineralization, and denitrification over the winter, especially during freeze-thaw events, can have a significant effect on the fate of N in fall-applied manure. Clark et al. (2009) predicted significant amounts of N loss from fall-applied pig slurry during the winter, especially in fine-textured clay soils. Immobilization of N could not be detected at temperatures below 2°C, but net mineralization and nitrification of the organic N in manure occurred at significant rates in frozen soil (Clark et al. 2009). Accumulated pools of NH_4^+ and NO_3^- from manure are susceptible to loss from denitrification or leaching during spring thaw and other intermittent freeze-thaw events in cold, temperate agroecosystems (Jayasundara et al. 2010; Kariyapperuma et al. 2011). Chantigny et al. (2014) found that losses of N from pig slurry applied in spring were substantial (between 20 and 32.5 kg N ha^{-1}) due to frequent freeze-thaw cycling over the winter and subsequent loss through gaseous N emissions. Most of the N loss was from soil residual N present in the organic and clay-fixed pools (Chantigny et al. 2014).

1.4 Influence of cover crops on nitrogen loss over winter

It is particularly challenging to time the release of plant-available N with crop demand when using manure as a fertilizer source. The challenge is amplified when manure is applied in the post-harvest fall months and left on bare ground over winter where it is susceptible to considerable losses from freeze-thaw cycling (Adeli et al. 2011). Combining manure application with cover cropping may help reduce N losses by immobilizing available N in plant tissues until incorporation the following spring. Cover crops add both C and N inputs to the soil system and thereby increase the potential for plant and microbial N sinks over winter (Drinkwater et al. 1998). Cover crops are generally sown in fall after the main crop has been harvested and left in

the soil over winter. They are rarely harvested for economic benefits and instead provide ecosystem services such preventing soil erosion, enhancing soil structure and organic matter, suppressing weeds, and mitigating nutrient losses via leaching, run-off, and gaseous emissions (Cavigelli and Parkin 2012; Shelton et al. 2018).

1.4.1 Physico-chemical characteristics of cover crops

Physico-chemical characteristics have a major influence on the N retention properties of winter cover crops. Perennial crops are more adapted to freeze-thaw stress and release less of their cell contents than annual crops but are less practical as cover crops in annual cropping systems (Liu et al. 2013). Cover crops that are mature or senesced tend to retain nutrients more effectively than young or actively-growing cover crops (Øgaard 2015). This highlights the importance of planting cover crops early and selecting fast-growing species that can germinate at low temperatures. While the practicalities of early sowing of cover crops is often limiting in cold, temperate climates, low dry matter yield is not always indicative of an ineffective cover crop (Baggs et al. 2000). Cover crop N uptake is often limited more by soil N availability than N uptake capacity. Many cover crops are able to take up the majority of soil N available to them in only the first few weeks of growth (Thorup-Kristensen et al. 2003). Adding a source of N fertilizer, such as manure, with a cover crops tends to increase the plant N content and biomass production of cover crops (Thorup-Kristensen et al. 2003; Singer et al. 2008). Singer et al. (2008) found that N uptake by a winter rye-oat cover crop was 60.1 kg ha^{-1} when combined with injected liquid swine manure compared to N uptake of 35.6 kg ha^{-1} when the cover crop was grown without manure injection.

1.4.2 Interactions between cover crops and soil properties

The potential for field-grown cover crops to either release or retain nutrients depends largely on the environmental conditions and soil properties. Periods of freezing and thawing can cause direct changes in soil physical structure and microbial community, which in turn influences the leaching of nutrients from plant tissues into the soil (Bechmann et al. 2005). However, cover crops may also indirectly reduce nutrient losses by increasing organic matter content and improving the structure, aeration, infiltration, and water holding capacity of the soil (Lozier and Macrae 2017). Contrary to this, cover crops maintain high soil moisture levels and may contribute to anaerobic conditions that favour the loss of N through denitrification (Basche et al. 2014). Therefore retention of nutrients in cover crops can be optimized by promoting good drainage in the field (Lozier and Macrae 2017).

1.4.3 Nitrogen dynamics in legume vs. non-legume cover crops

Research has shown that the N dynamics can differ widely between legume- and non-legume-based cover crops. Legume cover crops are often grown as "green manure" with the purpose of providing N credit to the soil due to their ability to biologically fix N (Shelton et al. 2018). Legume residues generally have lower C/N ratios and higher proportions of labile fractions (cellulose, hemi-cellulose) than non-legumes, and therefore release plant-available N more readily upon incorporation (Baggs et al. 2000; N'Dayegamiye et al. 2015). Combining manure with a legume cover crop may also increase the N fertilizer value of both. Seman-Varner et al. (2017) found that combining fall-applied poultry litter with a legume cover crop in the Southern U.S. resulted in a significantly higher N credit to the following spring corn crop compared to poultry litter applied without a cover crop.

Despite their potential advantages as green manures, legumes are generally less effective at capturing and retaining excess N in their tissues than non-legumes and may contribute to N losses (Thorup-Kristensen et al. 2003). Zhou et al. (2017) found significantly higher rates of N leaching losses over winter from mesocosms amended with leguminous cover crop residue compared to mesocosms without legume residue. A similar result was found by Shelton et al. (2018) after comparing N losses from legume, cereal, and mixed legume/cereal cover crops. The legume cover crop treatment had the greatest losses of NO_3-N via leaching during the non-growing season as well as significantly higher fluxes of N_2O fluxes following cover crop termination compared to the non-legume and mixed cover crops (Shelton et al. 2018). In another study, soil cores containing legume cover crop residues resulted in 41-56% net N mineralization of residue N added, compared to nearly 0% for non-legume (grass) residues (Li et al. 2016). In this same study by Li et al. (2016), the soil cores with legume residues resulted in cumulative emissions of 701-1517 μg N_2O-N kg^{-1} soil compared to only 159-185 μg N_2O-N kg^{-1} soil for the ryegrass residue treatments. In a meta-analysis study, Basche et al. (2014) also reported that soils amended with low C/N ratio leguminous cover crops released significantly higher N_2O emissions after incorporation than non-legume cover crops with a high C/N ratio (Fig. 1-4). Therefore, the advantages of legume crops for their N credit must be balanced with their potential contribution to over-winter N losses.

Non-legume (grass-based) cover crops are rarely used as green manures due to their high C/N ratio and inability to biologically fix N. Non-legume cover crops release N slowly to the soil making it difficult to synchronize the N release with spring crop N uptake (Baggs et al. 2000). Despite the lack of benefits to increasing spring crop yields, the high C/N ratio of grass-based cover crops often results in decreases in N_2O emissions due to induced N immobilization during

incorporation, especially compared to legume crops (Basche et al. 2014; Shelton et al. 2018). Numerous studies have reported that grass-based cover crops can be particularly effective at reducing over-winter N losses from fall-applied manures (Singer et al. 2008; Jarecki et al. 2009; Cambardella et al. 2010; Adeli et al. 2011; Seman-Varner et al. 2017). Jarecki et al. (2009) found that when liquid swine manure was applied at a high rate of 195 kg N ha^{-1} the percentage of manure-N equivalents lost as N_2O was 0.64% in the presence of a winter rye (*Secale cereale* L.) cover crop compared to 1.45% when the same amount of manure was applied to the fallow plots. Adeli et al. (2011) compared over-winter leaching losses of NO_3-N when broiler litter was applied at a rate of 13.4 Mg ha^{-1} to plots cover-cropped with winter rye and plots with no cover crop. During the two years of the study mean NO_3-N leaching losses were 16.8 and 26.8 mg L^{-1} in Year 1 and 2 for the cover crop plots compared with NO_3-N leaching losses of 36.4 and 64.6 mg L^{-1} in the plots with no cover crop (Adeli et al. 2011).

Bi-cultures of legume and grass cover crops are often grown to optimize both N fixation and N retention, though this ability is highly dependent on the species and relative ratio of each component in the bi-culture (Kuo and Sainju 1998; Thorup-Kristensen et al. 2003). Shelton et al. (2018) found increased biomass production and lower NO_3-N leachate when hairy vetch (*Vicia villosa* Roth) was grown in a bi-culture with winter wheat (*Triticum aestivum* L.) compared to the vetch crop alone. However, the NO_3-N leaching losses of the bi-culture were higher than the pure winter wheat cover crop (Shelton et al. 2018). This was similar to results from Seman-Varner et al. (2017) who found the N retention ability of bi-cultures were intermediate between pure legume and pure cereal monocultures. The effects of bi-cultures on biological N fixation are also diverse. The fertilizer N equivalence of a legume-rye bi-culture was found to be highly variable over the course of three field seasons, ranging from a deficit of -12 to a credit of 75 kg

N ha^{-1} (Seman-Varner et al. 2017). On average these values were intermediate between the highest fertilizer N equivalence values found with the pure legume cover crop and the lowest fertilizer N equivalence values of the pure rye cover crops (Seman-Varner et al. 2017).

1.4.4 Influence of cover crops on nitrogen losses from freeze-thaw events

While studies have looked at the benefits of cover crops on leaching and gaseous losses over winter, less is known about the specific mechanisms of N retention in cover crops during multiple freeze-thaw cycles. During periods of freeze-thaw, cover crops may contribute to N_2O-N loss by releasing labile C and N exudates through their roots, especially if the plants have undergone a winterkill and the tissues are decaying, which provide substrates for N_2O-production microbial reactions (Morkved et al. 2006; Thomas et al. 2017). In a two-year field study, Thomas et al. (2017) found that deep-rooted cover crops (fall rye [*Secale cereale* L.] and oilseed radish [*Raphanus sativus* L.]) increased cumulative N_2O emissions during the non-growing season by 76% for the fall rye and 154% for the oilseed radish, compared to the no-cover crop control. This increased loss of N_2O-N could not be directly correlated with periods of freeze-thaw due to the lack of controlled conditions in the field system, but the authors found that peak fluxes of N_2O occurred during major thawing events during the growing season (Thomas et al. 2017). However, this was result was only observed in the second non-growing season and there was no difference between the cover crop and no-cover crop treatments in the first non-growing season. Additionally, winter rye was found to significantly decrease soil mineral N levels compared to the oilseed radish and no-cover crop treatments. It is possible that the extensive root system of the perennial winter rye plant enhanced root exudates of reactive N and provided favorable microsites for microbial denitrification (Thomas et al. 2017). Thus, more

shallow-rooted cover crops, such as annual ryegrass (*Lolium multiflorum* Lam.), may not contribute to N_2O emissions as much as deeper-rooted perennials.

Alternatively, fall-planted cover crops may accumulate mineral N in their tissues, and therefore reduce the amount of reactive N substrates in the soil that are prone loss through nitrification and denitrification pathways during freeze-thaw cycles. Pelster et al. (2013) did not specifically look at cover crops but performed a laboratory simulation of eight 10-day freeze-thaw cycles at either moderate (+1/-3°C) or high (+1/-7°C) temperature ranges in silty-clay soil cores amended with either soybean *(Glycine max* L.) or corn *(Zea mays* L.) residue or left bare. Soil NO_3^- concentrations and cumulative N_2O emissions were both greater in the un-amended treatments compared to corn or soybean residue treatments (Pelster et al. 2013). The effect was more pronounced in the higher freeze-thaw cycle temperature range which may prove the importance of vegetation covers if temperate climates begin experiencing less insulating snow cover and more intense freeze-thaw cycles. Wagner-Riddle and Thurtell (1998) compared in-field mean N_2O fluxes over the course of multiple freeze-thaw cycles from fields subjected to different agricultural practices in Ontario, Canada. The only fields that experienced negligible N losses from N_2O emissions were those that had a grass-based vegetative cover, indicating the potential to reduce N_2O emissions through the use of winter cover crops (Wagner-Riddle and Thurtell 1998). Similarly, Dietzel et al. (2011) found reductions in N_2O emissions of up to 65% in the presence of a winter rye cover crop compared to bare soil.

1.5 Conclusions and future directions

Manure is a valuable fertilizer source for sustainable farming systems because it is a rich source of N that can replace conventional N fertilizers, which are manufactured in energy-intensive fossil fuel-based factories. Manure also prevents soil acidification, recycles locally-available resources, and builds soil organic matter to support healthy soil food webs. However, manure must be properly applied and managed, or its deleterious environmental impacts can far outweigh its benefits as a soil nutrient source. Many farmers apply manure in fall because they generally have more time after the harvest and may find it convenient to empty their storage before winter soil conditions make it impossible to enter the fields. However, with no actively-growing crop in the ground to retain N from the applied manure, the risk of N loss from gaseous emissions and leaching of soluble N increases. This risk is amplified by freeze-thaw events during winter months and early spring thaw. Planting a winter cover crop with fall-applied manure may be one way to retain N for the next spring. However, there are few studies that have investigated the interactions between fall manure application and cover crops on N cycling over winter, especially in areas where freeze-thaw events occur. The cold humid temperate region of southwestern Québec is an area of concern due to the high levels of residual soil nitrogen and the fact that soil moisture remains close to saturation during the non-growing season.

My thesis research was based on a field experiment conducted in the Montéregie Ouest region of Québec, Canada. The objectives of the field study were to determine if N from fall-applied manure is retained in the soil-plant system over winter in the presence of a cover crop, and if fall-applied manure can supply N in the next growing season to a spring wheat crop. A complementary laboratory experiment investigated the N_2O production during freeze-thaw cycles in soil that had a cover crop planted after N fertilizer or manure application. The

laboratory experiment also examined the relationship between reactive N and DOC concentrations on N_2O-N loss during freeze-thaw cycles. Together, the results from these studies will contribute to better understanding the extent of N transformations and loss from manure during the non-growing season. This information will also provide practical advice to farmers on how to most effectively use manure as a N fertilizer source in cold humid temperate regions.

Table 1-1. Average characteristics of liquid/slurry and solid animal manures. Adapted from Bernal et al. (2009) and CRAAQ (2010).

Manure Type	Dry Matter (g kg^{-1} fresh weight)	Organic-C (g kg^{-1} fresh weight)	Total-N (g kg^{-1} fresh weight)	NH_4-N (g kg^{-1} fresh weight)	C/N ratio	pH
Liquid/Slurry						
Cattle (dairy)	15-123	3.8-36	2.0-7.0	1.0-4.9	10	7.1-8.4
Swine	4.9-152	1.0-65	0.6-7.8	0.3-6.6	3	6.7-8.9
Solid						
Cattle (dairy)	140-300	65-126	4.2-8.1	0.3-2.0	15	8.6
Poultry	220-700	103-597	10-58	2.4-18	11	7.6

Table 1-2. Estimations of N fertilizer value (kg N ha^{-1}) of solid manure applied at a rate of 100 kg total N ha^{-1} to annual crops based on manure C/N ratio and soil type. Adapted from CRAAQ (2010) and Whalen et al. (2019).

		Manure Application Season	
Manure C/N Ratio	**Soil Type**	**Spring/Summer**	**Fall/Post-Harvest**
<10	Clay	75	65
10-12	Clay	65	45
13-15	Clay	50	25
15-25	Clay	45	20
<10	Sand and loam	80	70
10-12	Sand and loam	75	55
13-15	Sand and loam	55	35
15-25	Sand and loam	50	30

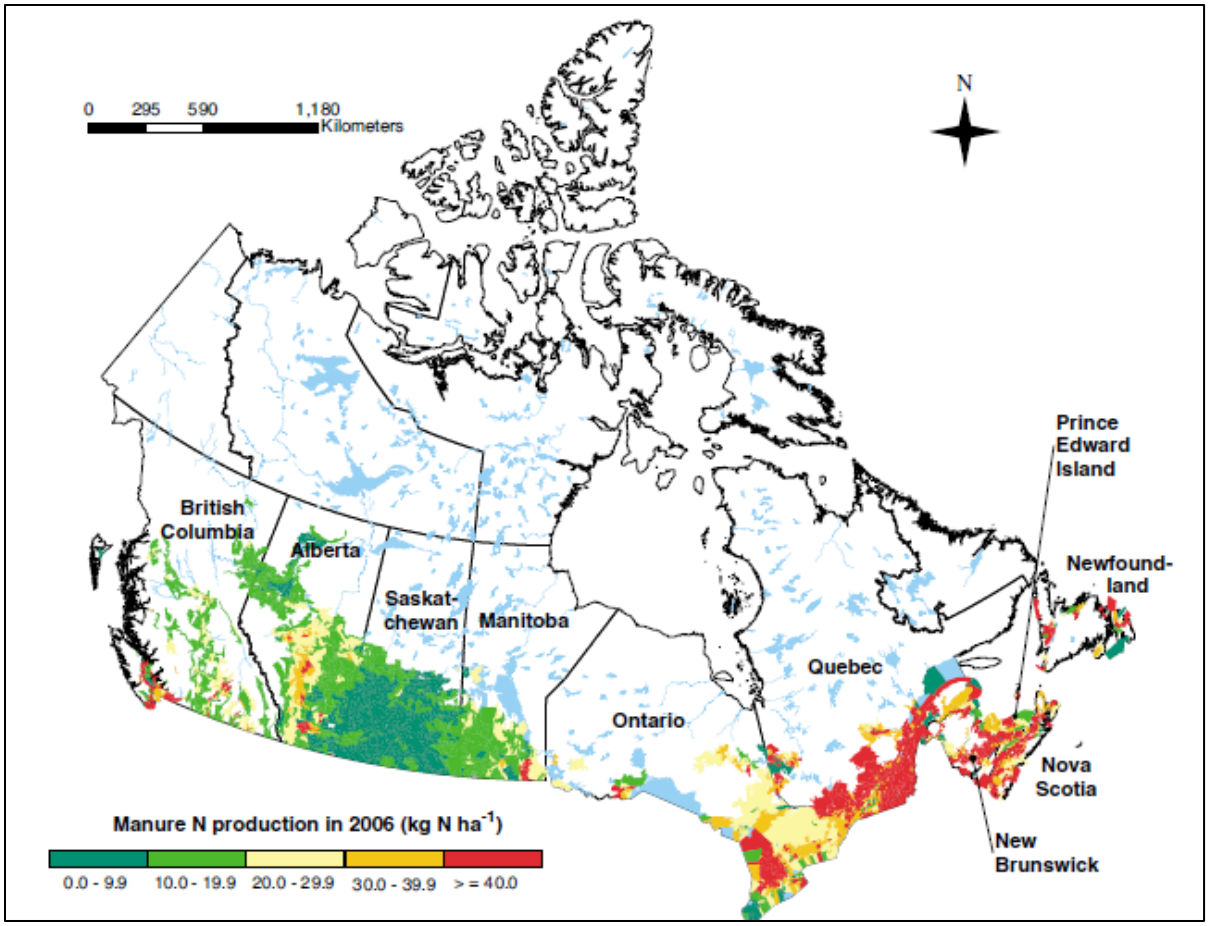


Figure 1-1. Total manure production in Canada in 2006. Adapted from Yang et al. 2011.

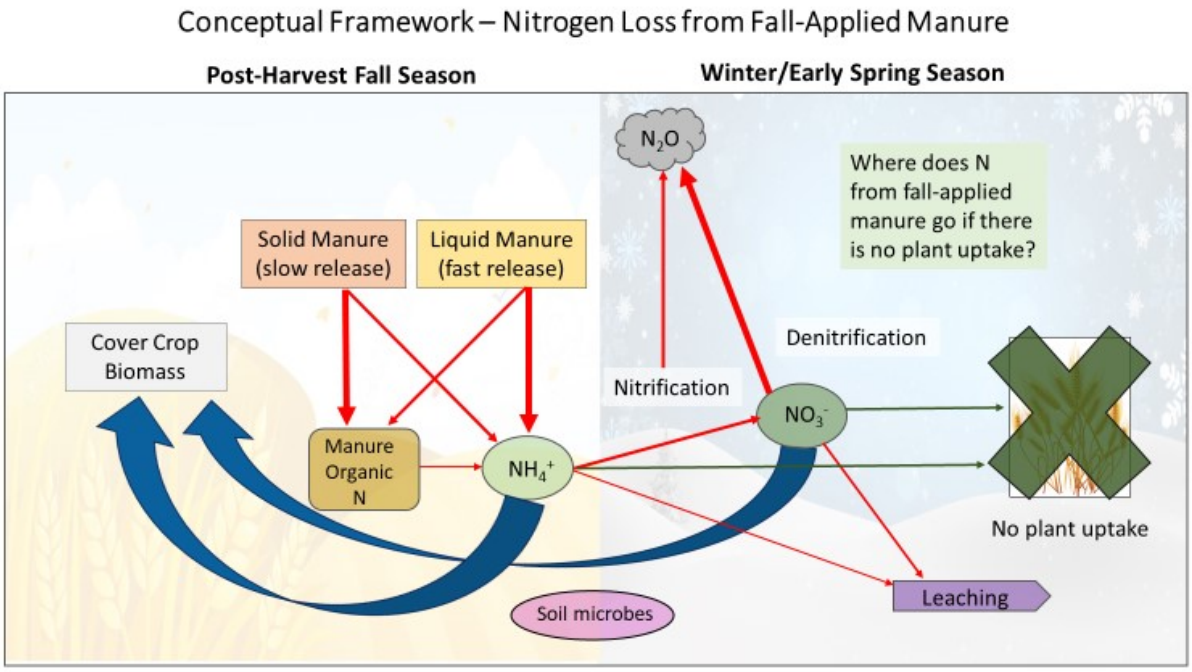


Figure 1-2. Simplified conceptual framework outlining the pathways of nitrogen (N) loss (leaching, nitrification, denitrification) from fall-applied manure (solid and liquid) during the non-growing seasons. These N transformations are primarily mediated by soil microbes. The main issue with applying manure in the fall is that the reactive N (NH_4^+ and NO_3^-) is susceptible to loss if there is no plant uptake. Gaseous losses of N often occur through N_2O production during the winter months due to cold temperatures and optimal water-filled pore space, but may also be lost as N_2 (not shown in diagram). The retention of reactive N in cover crop biomass may help reduce N loss in the non-growing seasons when there is no crop uptake.

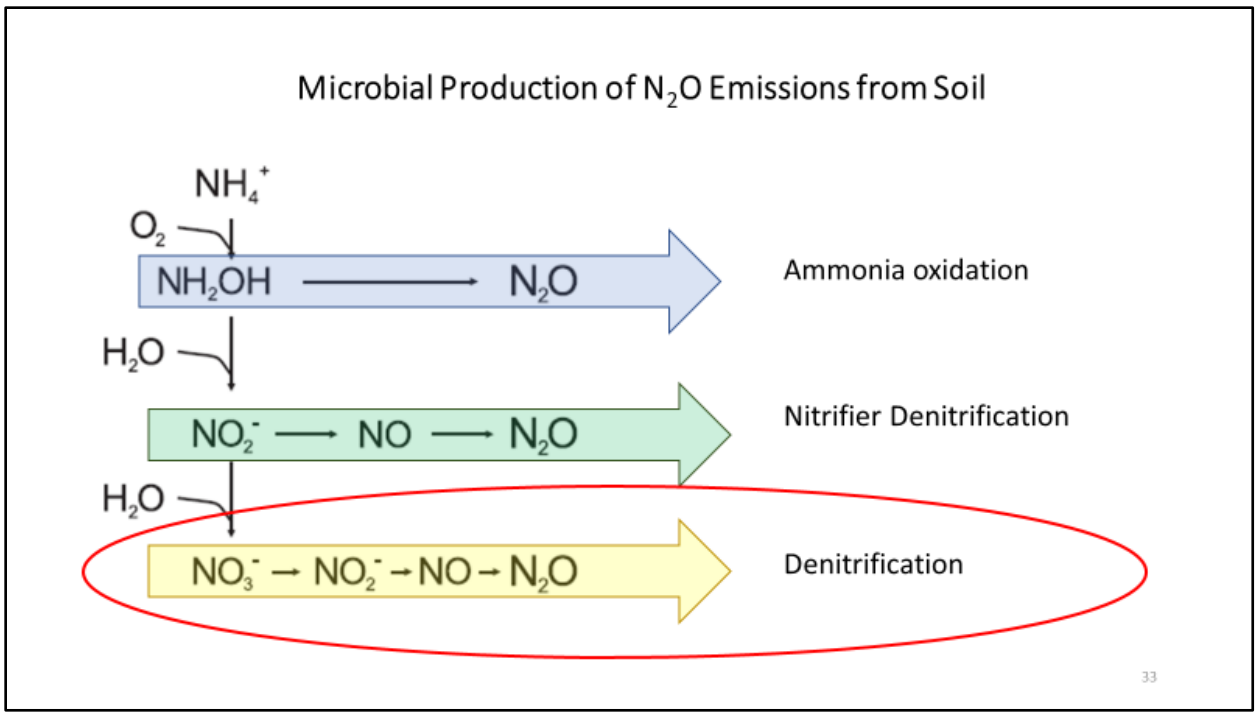


Figure 1-3. Three main pathways of N_2O emissions from soil microbial activity. Denitrification (circled) is the dominant source of N_2O production during winter. Adapted from Kool et al. (2011).

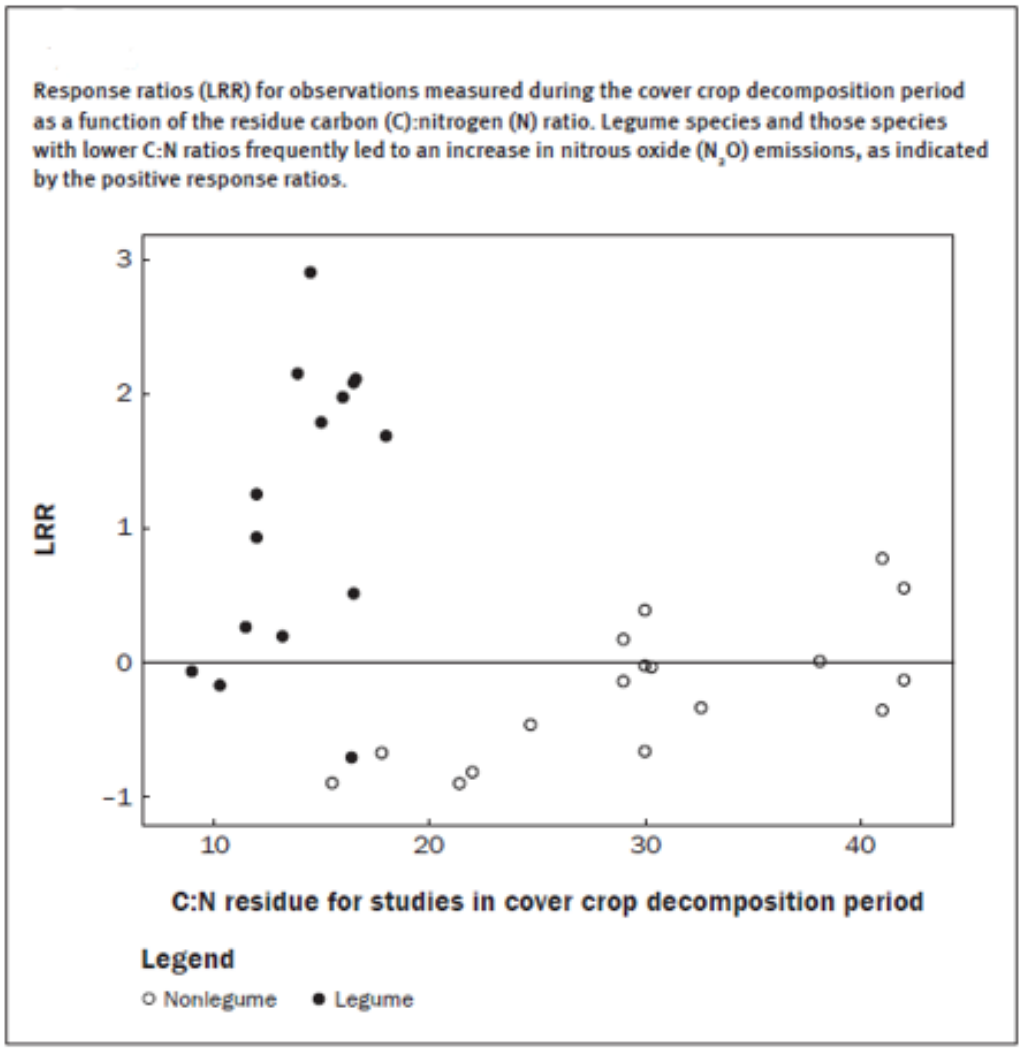


Figure 1-4. Response ratios (LRR) and C/N ratios of legume vs. non-legume cover crop residues. LRR refers to the natural log of the ratio between the N_2O flux with a cover crop and the N_2O flux without a cover crop. Positive LRR indicates an increase in N_2O emissions relative to no cover crop and a negative LRR indicates a decrease in N_2O emissions relative to no cover crop. Legume crops tend to have a lower C/N ratio and a higher LRR. This figure is taken from a meta-analysis performed by Basche et al. (2014). Twenty-six peer-reviewed articles with 106 observations of cover crop effects on N_2O emissions were used to generate the LRR data.

Chapter 2: Over-winter nitrogen loss from manure-amended soils with cover crops in a cold humid temperate region

Abstract

Applying animal manure to farmland in fall is a common practice on livestock operations in Canada. Nitrogen (N) in fall-applied manure is susceptible to over-winter losses, which may be reduced by fall-seeded cover crops that absorb some of this N input. The objective of my study was to determine how much N from fall-applied manure was retained in soil with and without cover crops, and subsequently used by a spring-sown wheat crop. The field experiment was a full factorial with three manure treatments and four cover crop types established at two field sites in southwestern Québec, Canada. A partial N mass balance (g N m^{-2}) was calculated in fall and in spring. After incorporating the cover crop, the main plots were split into two subplots that received no additional N fertilizer or 100 kg N ha^{-1} as urea, then spring wheat was planted. Above-ground wheat biomass and N content were measured at tillering, flowering, and maturity, and yield was determined. The cover crop retained ≤7% of the N from fall-applied manure. There was no significant difference ($p>0.05$) in fall or spring N balance in plots with and without manure. Fall-applied manure contributed to a numerical gain of 5–7% in the N balance of the field with good cover crop production (0.8–16.7 g dry matter m^{-2}), but the N balance was 2–7% less in the manure-amended plots than the control plots when the cover crop was grazed heavily by migrating birds. There was no residual N supplied by the fall-applied manure to the wheat crop, and the average wheat yields were 11–14% less in the subplots that received no additional N fertilizer than those with 100 kg N ha^{-1} in spring. I conclude that fall-applied manure is to be avoided and that fall-sown cover crops do not necessarily improve the N balance in sandy loam soils in cold temperate regions such as southwestern Québec.

2.1 Introduction

Animal manure is a nutrient-rich amendment that is used worldwide to improve soil fertility. In Canada, more than one million tonnes of nitrogen (N) is applied to farmland from manure (Yang et al. 2011). Much of the manure production is concentrated in Eastern Canada (Yang et al. 2011). Despite its suitability as an organic N fertilizer source, improper manure application practices can contribute significantly to environmental contamination by accelerating N losses through gaseous emissions, run-off and leaching. The best way to prevent N loss from manure-amended soils is to apply manure in synchrony with the crop demand for N (Whalen et al. 2019). In Canada, that usually means applying manure in the spring before a crop is sown (CRAAQ 2010). However, up to half of Canadian farmers apply manure in fall (autumn season), about 6 months before the spring crop is planted (Yang et al. 2011). For many Canadian farmers, fall is a more convenient time to spread manure because they have more time in the post-harvest period for manuring than during the busy spring months, and fields are often drier in fall than in spring. In addition, farmers prefer to empty their manure storage in fall, which increases their capacity to stockpile manure during winter months when they cannot spread manure in the field due to regulations and logistic constraints (Adeli et al. 2011; *Environment Quality Act* 2018). Without an actively-growing crop, the reactive N present in manure and released into manure-amended soils through decomposition and N mineralization processes is likely to be lost from the agroecosystem. In Eastern Canada, up to one-third of the N from fall-applied manure can be lost over the winter, with the majority of N loss (over 20% of applied N) occurring through gaseous emissions, and 3-5% losses of applied N occurring through leaching (Jayasundara et al. 2010; Chantigny et al. 2014). Agricultural practices that increase N retention in agroecosystems are

expected to reduce the magnitude of N losses from manure-amended soils, thereby preserving more N fertilizer value for crops planted in the next growing season.

Some farmers choose to plant cover crops in fall to prevent soil erosion, suppress pests and retain nutrients in the agroecosystem (Drinkwater et al. 1998; Cavigelli and Parkin 2012; Shelton et al. 2018). Cover crops planted in manured fields have potential to accumulate reactive N from fall-applied manure and retain N in their biomass during the winter months. The N content of cover crops tends to increase when combined with a source of N fertilizer such as manure, indicating the ability of cover crops to scavenge available soil N (Thorup-Kristensen et al. 2003; Singer et al. 2008). For example, the N uptake by a winter rye-oat cover crop was almost 70% higher (60.1 kg ha^{-1}) when combined with injected liquid swine manure than the N uptake of the same cover crop grown without manure injection (35.6 kg ha^{-1}) (Singer et al. 2008). In another study, the average N accumulation among three field seasons was 60 kg N ha^{-1} in winter rye (*Secale cereale* L.) and 40 kg ha^{-1} in oat (*Avena sativa* L.) grown on fields with simulated liquid manure application in the Eastern U.S. (Hashemi et al. 2013). After these cover crops were incorporated in the spring, they released N for the next corn silage crop, resulting in 41% greater silage yield with oat cover crop and 34% more silage yield with the winter rye cover crop (Hashemi et al. 2013). Thus, there is growing interest in the ability of cover crops to absorb reactive N from soils that receive fall-applied manure.

There are two important considerations in selecting a cover crop that can retain N over winter: (1) whether the cover crop is a legume or grass, and (2) the manure source applied to the field. Legume cover crops can supplement N fertility through biological N fixation but are generally less effective at scavenging residual N than grass-based cover crops. Numerous studies have reported increased levels of N loss either through leaching or gaseous emissions from

treatments receiving leguminous residues or cover crop, compared to either the control or a grass-based treatment (Thorup-Kristensen et al. 2003; Basche et al. 2014; Li et al. 2016; Zhou et al. 2017; Shelton et al. 2018). In contrast, non-legume (grass-based) cover crops are often more effective at sequestering residual nutrients, such as N, in their tissues (Basche et al. 2014; Seman-Varner et al. 2017). Adeli et al. (2011) found that leaching losses of NO_3-N in plots receiving 13.4 Mg ha^{-1} of broiler manure were reduced by over 50% in the presence of a winter rye cover crop compared to no cover crop. This reduction in leaching losses was attributed to the high root density of winter rye and its ability to scavenge NO_3-N into the organic compounds of the plant (Adeli et al. 2011). Legume and grass-based cover crops may be grown in a bi-culture to optimize both N fixation and N retention (Shelton et al. 2018), though results are variable and highly dependent on the species and relative ratio of each component in the bi-culture (Kuo and Sainju 1998; Thorup-Kristensen et al. 2003).

Planting a cover crop after manure is applied in the fall may be an effective strategy for reducing N losses over winter, but this needs to be confirmed with different manure types and cover crop mixes, as well as in field crop systems that are harvested in late fall. The purpose of this study was to investigate how fall-applied N from manure is retained in the soil-plant system over winter, with and without fall-sown cover crops, and subsequently used by a spring crop. Manure treatments were fall-applied semi-composted solid cattle manure (solid manure), liquid dairy cattle slurry (liquid manure), and an unfertilized control (no manure). The cover crop mixes were a 100% pure stand of annual ryegrass (*Lolium multiflorum* Lam.), a bi-culture of 75% annual ryegrass and 25% hairy vetch (*Vicia villosa* Roth), a bi-culture of 50% ryegrass and 50% vetch, and no cover crop. We expected that more N would be retained in the soil over winter in plots amended with solid manure compared to liquid manure, and that the pure ryegrass cover

crop would be more effective at retaining N from fall to spring than ryegrass-legume bi-culture. The treatments that resulted in the most N retention over winter were expected to show the highest wheat yields the following spring.

2.2 Materials and Methods

2.2.1 Field sites

The study was conducted from September 2017 to August 2018 at two agricultural fields in the Montéregie Ouest region of Québec, Canada. In this region, mean monthly temperature is between -9.7°C in January and 21.2°C in July, with an annual precipitation of 1000 mm, based on 30-year historical averages from 1980-2010 (Environment Canada 2018).

The Lods site is located at the Emile A. Lods Agronomy Research Centre in Sainte-Anne-de-Bellevue, Québec (N 45° 25' 34" W 73° 55' 43", elevation 39 m above sea level). It is on a Humic Gleysol of the St. Amable series containing 620 g sand kg^{-1} and 60 g clay kg^{-1} with 48 g organic C kg^{-1} and pH 6.3. Before treatments were applied for this study, the average bulk density was 1110 kg m^{-3}. The field was conventionally tilled for grain corn (*Zea mays* L.) in 2013 and 2015 and soybeans (*Glycine max* L.) in 2014, 2016, and 2017. Grain corn was fertilized with 175 kg N ha^{-1} from mixed urea [$(NH_2)_2CO$, 46-0-0] and ammonium sulfate [$(NH_4)_2SO_4$, 21-0-0] fertilizer, and corn also received liquid dairy manure at rates of 38,000 kg ha^{-1} (May 2013) and 29,800 kg ha^{-1} (May 2015). No fertilizer was applied to the soybean crops grown in 2014, 2016 or 2017.

The Laval site (N 45°39' 14", W 73° 42' 10", elevation 48 m above sea level) is on a gently sloping (2%) Gray Brown Luvisol of the well-drained Farmington-Châteauguay association overlaying calcareous till. This gravelly-loam soil contains 610 g sand kg^{-1} sand and

50 g clay kg^{-1} with 28 g organic C kg^{-1} and pH 7.6. The site had an initial bulk density of 1376 kg m^{-3}. The site was conventionally tilled for grain corn (2016) and soybeans (2017), which were fertilized with inorganic N-P-K fertilizers applied according to CRAAQ (2010) guidelines. No manure was applied to the site for at least ten years prior to the study.

2.2.2 Experimental design and treatments

In September 2017, soybeans were harvested, the fields were machine ploughed, and crop residues (above-and below-ground) were removed. Following soybean crop removal at the Lods site, the field was disked to 15.2 cm and smoothed with a 3.8 cm field cultivator. The Laval site was cultivated to 3-4 cm depth after soybean removal.

The field experiment, established at both field sites, was a full factorial design with two factors: manure (three levels) and cover crop (four levels). The twelve factorial treatments were applied to plots (5 x 5 m), randomly assigned in four replicate blocks that were separated by 3 m buffers, for a total of 48 plots per site (Appendix A).

At both sites, manure treatments were no manure, dairy cattle slurry (liquid manure) and solid composted cattle manure (solid manure) obtained from the McGill Macdonald Campus Farm in Sainte-Anne-de-Bellevue, Québec. Liquid manure was mixed with milk house wastewater and stored in a large, open, outdoor concrete tank. Solid manure was mixed with straw bedding and kept on a partially-covered concrete pad. The physico-chemical properties of both manure types are shown in Table 2-1. Manure was surface applied by hand at a rate of 28,000 L ha^{-1} (25 kg ha^{-1} NH_4-N) for the liquid manure and 30,000 kg ha^{-1} (42 kg ha^{-1} NH_4-N) for the solid manure, which are typical rates for fall-applied manure in Québec. Manure was

incorporated to a depth of 5 cm within two hours of application at both sites on 25 September 2017 (Lods) and 3-4 October 2017 (Laval).

Cover crops were seeded on 26 September 2017 (Lods) and 4 October 2017 (Laval). Cover crop treatments were no cover crop, a 100% pure stand of annual ryegrass (*Lolium multiflorum* Lam.) seeded at 34 kg ha^{-1}, a bi-culture of 75% annual ryegrass and 25% hairy vetch (*Vicia villosa* Roth) seeded at 25 kg ha^{-1} and 9 kg ha^{-1} respectively, a bi-culture of 50% ryegrass and 50% vetch seeded at 17 kg ha^{-1} each. At the Lods site, cover crops were broadcast seeded by a tractor-pulled seeder and the tractor passed over all plots, including those with no cover crop, to ensure comparable levels of compaction. At the Laval site, cover crops were hand-broadcast after manure application was complete and all plots were rolled with a sod roller to achieve the same level of compaction. A portable weather station (WatchDog 2000 Series, Spectrum Technologies Inc, Aurora, IL, USA) was installed at each field site to monitor soil temperature and moisture at 5 and 15 cm depths.

The cover crop was terminated at Lods on 9 May 2018 by tractor cultivation (0.05 m depth). All plots received a mixture of 10.8 kg of monoammonium phosphate ($NH_4H_2PO_4$, 11-52-0) and 9.3 kg muriate of potash (KCl, 0–0 -60) broadcast at a rate of 56 kg ha^{-1}. Spring wheat (*Triticum aestivum* L., cv. AC Walton) was planted in each plot at a seeding rate of 155 kg ha^{-1}. Urea fertilizer (46-0-0) was broadcast by hand to one half of each plot (subplot) at a split rate of 30 kg ha^{-1} applied immediately after seeding and 70 kg ha^{-1} applied at tillering stage (Appendix B). Chemical pesticides were not applied to the plots, but plots were hand-weeded as needed from planting to harvest. The cover crop was terminated at Laval on 30 May 2018 by tractor cultivation (0.05 m depth). However, due to difficulties with wheat crop establishment and weed control, spring wheat data was not collected for the Laval site.

2.2.3 Sampling procedures and calculations

2.2.3.1 Partial N mass balance

Fall N stock was calculated based on the fall soil N stock, the manure N input, and the cover crop N (Equation 1).

$$Fall\ N\ stock = M_{Nmanure} + M_{Nsoil} + M_{Ncover} \quad (1)$$

where:

M_{Nsoil} = elemental mass of N per unit area in soil N stock (g N m^{-2})

$M_{Nmanure}$ = elemental mass of N per unit area in applied manure (g N m^{-2})

M_{Ncover} = elemental mass of N per unit area retained in cover crop (g N m^{-2})

Soil N stock was determined in soil samples (0.15 m depth) collected prior to manure application and cover crop planting. Soil was oven-dried (55°C for 48 h) and finely ground (<1 mm mesh), and the total N content was analyzed by combustion at 900° C with a Thermo Finnigan Flash EA 1112 series C/N analyzer (Carlo Erba, Milan, Italy). The soil N stock was based on the elemental mass of N (g N m^{-2}, Equation 2) determined according to the methods of Ellert and Bettany (1995).

$$M_N = conc\ x\ \rho_B\ x\ T \quad (2)$$

Where:

M_N = element mass of N per unit area (g N m^{-2})

$conc$ = element concentration (g N kg^{-1} soil)

ρ_B = average pre-treatment soil bulk density (kg soil m^{-3})

T = thickness of soil layer (m)

The manure N input (g N m^{-2}) in solid and liquid manure was the total N content in manure multiplied by the manure application rate. Cover crop N (g N m^{-2}) was the N content in the standing plant biomass determined just before soil freeze-up at the Lods site on 8 November 2017 and at the Laval site on 14 November 2017. Shoot samples were collected by removing all above-ground vegetative material from a 0.25 m^2 area in each plot. No shoot samples were available at the Lods site because it was consumed by migrating Canada geese (*Branta canadensis* L.) six days prior to sampling. Roots were collected from two soil cores (0.15 m long, 0.05 m diameter) per plot and washed according to the methods of Böhm (1979). A subsample (~0.5 g) was oven-dried (55ºC for 48 h), weighed and ground (<1 mm mesh) for N analysis, and the remaining shoots and roots were oven-dried (105°C for 48 h) and weighed to determine above-and below-ground dry mass.

Spring N stock was calculated based on the spring soil N stock and the residue collected in the spring (Equation 3).

$$Spring\ N\ stock = M_{Nsoil} + M_{Nresidue} \quad (3)$$

where:

M_{Nsoil} = elemental mass of N per unit area in soil N stock (g N m^{-2})

$M_{Nresidue}$ = elemental mass of N per unit area retained in plant residue (g N m^{-2})

Soil samples (two samples per plot, 0.15 m depth, field sieved <4 mm) were collected from each experimental plot on 7 May 2018 at Lods and 16 May 2018 at Laval prior to cover crop termination. Spring soil N stock was determined using the same methods described for the fall N stock. The quantity of N recovered was assumed to include the soil N stock as well as any residual manure N from the fall application. Cover crop biomass was too small to collect in the

field so plant residue (roots and shoots) was separated from the soil by hand after sampling, oven-dried (55°C for 48 h), finely ground on a Wiley mill (1 mm), and analyzed for total N content. An adjusted residue N value (residue N of each cover crop treatment plot minus the residue N of the corresponding no-cover crop plot) was used to account for collected spring residue that was not due to cover crop biomass.

The fall N stock and spring N stock were used to assess the amount of N retention over winter by calculating the percent change in total N content (Equation 4).

$$\frac{(Spring\ N\ Stock - Fall\ N\ Stock)}{Fall\ N\ Stock}\ x\ 100\% = \%\ change\ in\ total\ N\ content \quad (4)$$

2.2.3.2 Potentially mineralizable nitrogen (PMN)

In spring 2018, soil samples (0-15 cm) were collected from all plots at Lods and analyzed for potentially mineralizable N (PMN) following the anaerobic incubation method by Drinkwater et al. (1996). Briefly, this involved rewetting of 6 g aliquots of dried, sieved soil with 2 mL of deionized water for 5 days at 24ºC to reactivate microbial activity. The NH_4 concentration after the reactivation phase was compared to the NH_4 concentration of saturated anaerobic soil (headspace purged with N_2 gas) after a 7-day incubation at 37ºC. The NH_4-N concentration in 2 M KCl extracts of the initial soil and incubated soil was determined colorimetrically at 650 nm using the modified indophenol blue method (Sims et al. 1995). The PMN (mg NH_4 kg dry soil^{-1} week^{-1}) was the difference in NH_4-N concentration of the initial soil and the incubated soil.

2.2.3.3 Spring wheat growth and nitrogen uptake

Due to difficulties with cover crop termination and wheat establishment at the Laval site, N uptake and yield data were only determined at the Lods site. Spring wheat populations were

assessed by counting the number of plants per m^2 in each sub-plot, 60 days after emergence. To determine dry matter and N concentration, above-ground (shoot) wheat samples were taken at three key growth stages based on Zadoks principal growth stages for cereals (Zadoks et al. 1974): i) 2 - tillering ii) 5 - inflorescence emergence ("flowering"), and iii) 9 - ripening ("physiological maturity"). Tillering samples were collected on 5 June 2018 and flowering samples were collected on 3 July 2018 by cutting five plants of comparable health and maturity plants at the soil surface. Shoot biomass was dried (55°C for 48 h), weighed, and a subsample was ground (<1 mm mesh) for total N analysis. Wheat N uptake (g N m^{-2}) at tillering and flowering was extrapolated to a plot scale by multiplying the N concentration (g N kg^{-1}) and shoot biomass (kg) by the plant population per m^2. Final samples were collected on 20 August 2018 once the crop had reached physiological maturity and just prior to harvesting the plots. The above-ground biomass was removed from 0.25 m^2 within each subplot and separated into grain and straw components before weighing (55°C for 48 h), grinding, and analyzing the total N concentration. The wheat N uptake (g N m^{-2}) at maturity was based on the N concentration (g N kg^{-1}) x biomass (kg) of the grain plus straw, divided by the sample area (m^2). The final wheat yield was determined from the weight of the dried grain and converted to a tonnes per hectare basis.

2.2.4 Statistical analysis

Statistical analysis was done with SAS version 9.4 (SAS Institute 2013). The residuals of the data were assessed for normality using the PROC UNIVARIATE function. Data that were not normally distributed were log transformed to meet the assumption of normality of the residuals. To compare the total cover crop biomass collected in the fall between Lods and Laval,

a one-factor analysis of variance (ANOVA) was used in the PROC GLM function. The main and interaction effects of manure and cover crop type on the percent change in N content from fall to spring (partial N mass balance, Equation 4) were assessed using a two-factor ANOVA in PROC GLM. All values were analyzed as the means of the four replicates for each field site. The combined effect of all cover crop types with the different manure treatments and the controls was compared with a pre-planned orthogonal contrast analysis using PROC GLM. There was no effect of cover crop at either site, so the cover crop treatments were pooled (n=16) within each manure treatment and only the effect of manure type was analyzed for the PMN values of the spring soil at Lods using a one-factor ANOVA in PROC GLM. Cover crop treatments remained pooled within manure treatments (n=16) for subsequent analysis of the N uptake data of the spring wheat at tillering, flowering, and maturity, as well as yield. The main effect of manure type and split plot effect of urea application was assessed using a split-plot ANOVA in PROC GLM. When treatments were different ($p<0.05$), mean comparison were done with a post-hoc Tukey's Honest Significant Difference test.

2.3 Results

2.3.1 Environmental conditions

Conditions were unseasonably wet and mild for the first month following cover crop seeding. The average monthly temperature was 13.3°C during October 2017 and total monthly precipitation was 113.6 mm, both of which were above the 30-year historical average from 1980-2010 (Environment Canada 2018). These conditions were considered to be favorable for cover crop growth. Temperatures cooled in November 2017, resulting in an average monthly

temperature of 0.4°C and total precipitation of 67.4 mm, which were below the month historical average (Environment Canada 2018).

Soil temperature at 5 cm depth fell below 0°C on 10 November 2017 at both Lods and Laval sites. There were three to four periods of freezing-thawing between 10 November and 8 December 2017, based on soil temperature at 5 cm depth. End-of-month snow cover accumulation was approximately 15 cm in December 2017, 13 cm in January 2018, and 8 cm in February 2018 at both sites. At the Lods site, the soil temperature at 5 cm depth first rose above 0°C on 30 March 2018 and underwent freezing and thawing three to four times from 30 March to 17 April 2018, then remained unfrozen for the rest of the growing season. At the Laval site, the soil at 5 cm depth was below 0°C until 12 April 2018 and thereafter above 0°C, suggesting no freeze-thaw cycles in the spring. The growing season (May to August 2018) was characterized by temperatures that were slightly above normal and less than average precipitation (Environment Canada 2018). See Appendix C for average monthly air temperature, soil temperature, and soil moisture from October 2017 – August 2018 for the Lods and Laval sites.

2.3.2 Cover crop biomass and partial N mass balance

The average cover crop biomass in November 2017 did not differ among treatments at either Lods or Laval sites. Much of the above-ground biomass at Lods was removed by migrating Canada geese, so the cover crop biomass was considerably less at Lods compared to Laval.

The partial N mass balance in soil and crop components between November 2017 and May 2018 was similar among manure and cover crop treatments at both sites. The data were pooled to compare “cover crop” (n=12) and “no cover crop” (n=4) in manure-amended and

control treatments with a pre-planned orthogonal contrast analysis. Overall, there was approximately 4–14% less total N at Lods (Fig. 2-2) and 2–11% less total N at Laval (Fig. 2-3) in the spring than in the fall. Similar N losses occurred with and without cover crops, or with and without manure at both sites. Since the cover crop treatment was not significant, due to the loss of cover crop at Lods and similar cover crop growth among plots at Laval, the cover crop treatments were pooled (n=16) within each manure treatment. There was no significant difference between manure treatments at Lods (Fig. 2-4) or Laval (Fig. 2-5). Cover crop treatments remained pooled within manure treatments (n=16) for all subsequent analysis (PMN, N uptake, and yield).

2.3.3 Potentially mineralizable nitrogen (PMN)

In May 2018, the PMN was greater in plots that received solid manure application than in plots that received no manure application ($p=0.001$) and liquid manure application ($p=0.048$, Fig. 2-6).

2.3.4 Nitrogen uptake by spring wheat and final yield

Nitrogen uptake of the spring wheat was evaluated at tillering, flowering and physiological maturity, and was significantly higher in the plots that received spring urea application at the flowering and maturity stages ($p<0.001$, Table 2-2). Wheat yield was also significantly higher in the plots that received urea ($p=0.019$, Table 2-2). Fall manure application had no effect on wheat N uptake or yield.

2.4 Discussion

2.4.1 Partial N mass balance revealed a net loss of manure N over winter

The partial N mass balance revealed a net N loss from sandy-loam to loam soils in the cold humid temperate agroecosystems of southwestern Québec during the winter period. Since net N loss was not alleviated by adding up to 8.1 g N m^{-2} from liquid manure or between 21.0–36.6 g N m^{-2} from solid manure, it suggests that most or all the fall-applied N was part of the over-winter N losses. The no manure control plots at the Laval retained 92% of the total soil N from fall to spring, compared to 97% for liquid manure and 99% for solid manure plots, indicating that the manure application contributed to the over-winter retention of total soil N at Laval. However, the no manure control plots at Lods retained 95% of the total soil N from fall to spring, compared to 93% for liquid manure plots and 88% for solid manure plots.

These results are consistent with the observation that microbially-mediated N cycling processes in agricultural soils occur in winter conditions and lead to significant losses of fall-applied N before crop uptake the following spring. Using ^{15}N-labelled pig or dairy slurry, (Chantigny et al. 2019) investigated the loss of fall-applied NH_4-N from multiple regional sites in Canada under contrasting winter conditions and found that over-winter losses ranged from 47-94% before seeding the next spring. Most of the ^{15}N recovered in the spring was in the organic form, with small amounts as NO_3-N (Chantigny et al. 2019). Jayasundara et al. (2010) also used ^{15}N-labelled pig slurry and found that only 71% of fall-applied manure N in a silt loam and 84% of fall-applied manure in sandy loam was recovered the following spring. Only a small percentage (<5%) was measured as leaching loss, indicating that most of the unaccounted for manure N was lost in the gaseous form (Jayasundara et al. 2010). Other studies have confirmed that a considerable portion of N loss over winter in temperate agroecosystems is from microbial-

mediated production of nitrous oxide (N_2O), with the highest pulses of N_2O production occurring after freeze-thaw events (Kariyapperuma et al. 2011; Wagner-Riddle et al. 2017; Congreves et al. 2018). Losses of N in the form of N_2O have particularly deleterious environmental impacts since N_2O is a potent greenhouse gas with an almost 300 times greater global warming potential than carbon dioxide on a per molecule basis (Butterbach-Bahl et al. 2013; Hu et al. 2015; Wagner-Riddle et al. 2017).

I could not quantify the N mass balance in our study without using ^{15}N-labelled manure, but the numerical differences between the total soil N in no manure and manure-amended soils suggests that adding manure at Lods resulted in even more loss during winter than if no manure was applied. It is possible that there was a "positive priming" effect of manure where adding N substrates stimulated microbial activity and accelerated N mineralization (Kuzyakov et al. 2000). This priming effect appeared to be amplified at Lods where the cover crop was grazed heavily by geese prior to soil freeze-up. Grazing of young vegetation releases root exudate carbon (C) into the soil, which can further stimulate microbial activity and release of reactive forms of C and N into the soil that are prone to loss (Sun et al. 2017). CRAAQ (2010) guidelines for fertilization in Québec suggest that between 20–70% of the total N from fall-applied solid manure, depending on soil type and manure C/N ratio, will remain in the soil the following spring as a "N credit" for the next crop. Our results challenge this assumption since there was a negligible N credit in our field, and possibly more total soil N was lost over winter due to a positive priming effect of manure.

Fall-applied manure did not increase the system-level N balance, and the cover crop did not accumulate enough biomass to be effective at capturing a significant amount of the manure N input before cover crop growth ceased at the Lods and Laval sites. The cover crop at Lods

captured only 7% of the total N input from liquid manure and 2% of the total N input from solid manure, and the cover crop at Laval captured only 4% of the total N input from liquid manure and 1% of the total N input from the solid. This is likely because the cover crop biomass was so small, especially at Lods, or because the cover was not mature enough to be effective at retaining N. Cover crops that are mature and have more biomass tend to retain nutrients more effectively than young or actively-growing cover crops (Øgaard 2015), highlighting the importance of planting cover crops early and selecting fast-growing species that can germinate at low temperatures. Hashemi et al. (2013) found that oat and winter rye cover crops that were planted between September 1 and 15 in the Eastern U.S. had a higher biomass and retained more N in their tissues over winter (average of 60-48 kg N ha^{-1} over three years of data) than the same cover crops planted in late September and mid-October. Due to the shorter growing season in Eastern Canada where our study occurred, it may be difficult to achieve adequate vegetative mass for nutrient retention when cover crops are planted in late September. The reason that I attempted such a late cover crop planting was to determine if this practice might help conserve manure N that is spread in fall after harvesting corn silage or soybean. Based on our results, late-fall cover crop plantings appear to not be a beneficial solution.

Cover crops planted in fall are also susceptible to herbivory, especially when fields are located in common migratory bird flyways. McKay et al. (2001) recommends alternate feeding areas (AFAs) as a strategy to reduce the risk of herbivory by dark-bellied brent geese (*Branta bernicla* L.), a close relative to Canada geese (*Branta canadensis* L.), on agricultural land. Geese tended to prefer species like clover (*Trifolium repens* L.) over grass species such as ryegrass, so setting aside AFAs planted with more palatable crops like clover near cover crop fields may be one way to prevent geese damage (McKay et al. 2001). Alternatively, choosing a cover crop that

is less attractive to geese than ryegrass and legumes, such as tall fescue (*Festuca arundinacea* Schreb.), may also reduce the incidence of geese herbivory on cover crop fields. Additionally, installing visual deterrents such as white plastic flags or netting in a cover crop field can be an effective means to repel geese (Mason et al. 1993).

2.4.2 Potentially mineralizable nitrogen (PMN)

Organic N was present in spring soil samples collected from Lods based on PMN analysis. Mineralizable N could be released from the soil organic N pool, which is the primary source of NH_4^+ in the no manure treatment. Another source of PMN is the organic N from the fall-applied manure (liquid manure and solid manure treatments). Although senesced tissues from the cover crops may decompose and release NH_4^+, the soil samples for PMN analysis were collected prior to cover crop termination, and there was little cover crop biomass at Lods, so I assumed a negligible contribution to PMN from cover crop decomposition. Plots receiving solid manure had more PMN than the control and liquid manure plots, indicating that some of the organic N from fall-applied solid manure was retained over winter. This is consistent with other reports that semi-composted solid manure increased PMN compared to soils that received only inorganic fertilizer or no organic inputs in diversified cropping systems (Osterholz et al. 2018).

2.4.3 Differences in N uptake and yield by spring wheat

To assess residual N fertility from manure applied in the fall, spring wheat growth of plots that received 100 kg N ha^{-1} of urea fertilizer in the spring were compared with plots that received no spring urea application. The significantly higher N uptake at flowering and maturity in the urea vs. no-urea subplots further substantiated our claim that most of the fall-applied N

was lost over winter. The soil N supply was insufficient in the no-urea subplots, even those that received manure in the previous fall, to support wheat N demands from flowering to maturity. Wheat accumulates approximately 94% of its N in the later stages of vegetative and reproductive growth (30–230 days) compared to the early vegetative growth to tillering (0–30 days) stages (Chen et al. 2014).

The lower N uptake of wheat in the no-urea subplots resulted in significantly lower wheat yield compared to the urea subplots. The average wheat yield across urea subplots ranged from 1.9–2.1 t ha^{-1}, compared to 1.7–1.8 t ha^{-1} in the no-urea subplots. Both values were below the average wheat yield of 3.5 t ha^{-1} in Québec in 2018 (Institut de la statistique du Québec 2019), likely due to the unseasonably hot and dry conditions at Lods, a late planting date compared to commercial farms in this area, and insufficient weed control.

Although the PMN suggested that solid manure-treated plots had a slightly greater N supply than the unfertilized and liquid manure-treated plots before cover crop termination, this did not contribute extra plant-available N for spring wheat. It may be that PMN was susceptible to loss during or shortly after cultivation of the cover crop through leaching or gaseous emissions and left the agroecosystem before the wheat was planted. I suggest that PMN is not a reliable predictor of plant-available N in humid environments, consistent with results from Osterholz et al. (2018) and a meta-analysis by Mahal et al. (2018). The major challenge to interpolate plant-available N from PMN is that the measurement is done in a static laboratory test without plants, whereas actual N mineralization in the field is controlled by dynamic factors such as crop N demands, crop-microbial interactions, field management, weather, and soil moisture and temperature conditions (Mahal et al. 2018).

2.5 Conclusion

There is a belief that cover crops can be effective in retaining N from fall-applied manure in the cold humid temperate regions of Québec, Canada. This may be true if the cover crop is planted in late summer, allowed to accumulate a sufficient amount of biomass, and adequate deterrents to herbivory are implemented. However, if the cover crop is planted in mid to late fall, it cannot alleviate the considerable loss of N that can occur from fall manure application. The biological and physical processes that lead to N loss are stronger than "best management practices" such as cover cropping. Thus, fall-applied manuring should be discouraged in light-textured (sandy loam/loam) soils in cold humid temperate regions. Farmers are instead advised to spread manure in the spring to reduce the amount of money they spend on inorganic N fertilizers and prevent the N loss and environmental contamination that is inevitable with fall-applied manure in this climate.

Table 2-1. Selected physical and chemical properties of the manure types used in the field experiment

Physico-Chemical Properties*	Liquid Manure†	Solid Manure‡
Dry matter (%)	5.4	54.6
C/N Ratio	11.5	17.8
Total N (kg t^{-1})	2.0	9.8
NH_4-N (kg N t^{-1})	1.0	2.0
Total P_2O_5 (kg t^{-1})	1.1	6.1
Total K_2O (kg t^{-1})	2.1	10.1

** Units are expressed per wet weight of manure*
†Liquid manure = dairy cattle slurry
‡Solid manure = semi-composted cattle manure

Table 2-2. Average N content per m^2 of spring wheat at tillering, flowering and physiological maturity, and final wheat grain yield, as affected by fall-applied manure (no manure, liquid manure, solid manure) and in-season N fertilization (no urea or 100 kg N ha^{-1} split application of urea).

Manure	Urea	N Content at Tillering (g N m^{-2})	N Content at Flowering (g N m^{-2})	N Content at Maturity (g N m^{-2})	Grain Yield (t ha^{-1})
No manure	In-season application	1.52 ± 0.12* a†	13.48 ± 1.28 a	8.69 ± 0.62 a	2.09 ± 0.15 a
Liquid manure	In-season application	1.85 ± 0.17 a	18.42 ± 1.74 a	8.30 ± 0.59 a	1.98 ± 0.16 a
Solid manure	In-season application	1.44 ± 0.09 a	15.06 ± 1.60 a	8.02 ± 0.73 a	1.87 ± 0.19 a
No manure	No application	1.68 ± 0.09 a	6.89 ± 0.94 b	5.70 ± 0.50 b	1.67 ± 0.17 b
Liquid manure	No application	1.65 ± 0.12 a	6.91 ± 0.93 b	5.96 ± 0.33 b	1.72 ± 0.13 b
Solid manure	No application	1.81 ± 0.15 a	6.49 ± 0.82 b	6.23 ± 0.43 b	1.77 ± 0.13 b
ANOVA results					
Manure (main plot)		NS‡	NS	NS	NS
Urea (split plot)		NS	<0.001	<0.001	0.019
Manure x Urea		NS	NS	NS	NS

**Mean ± standard error of 16 replicates.*

†Values with different letters within a column are significantly different ($p<0.05$)

‡ NS = not significant ($p>0.05$)

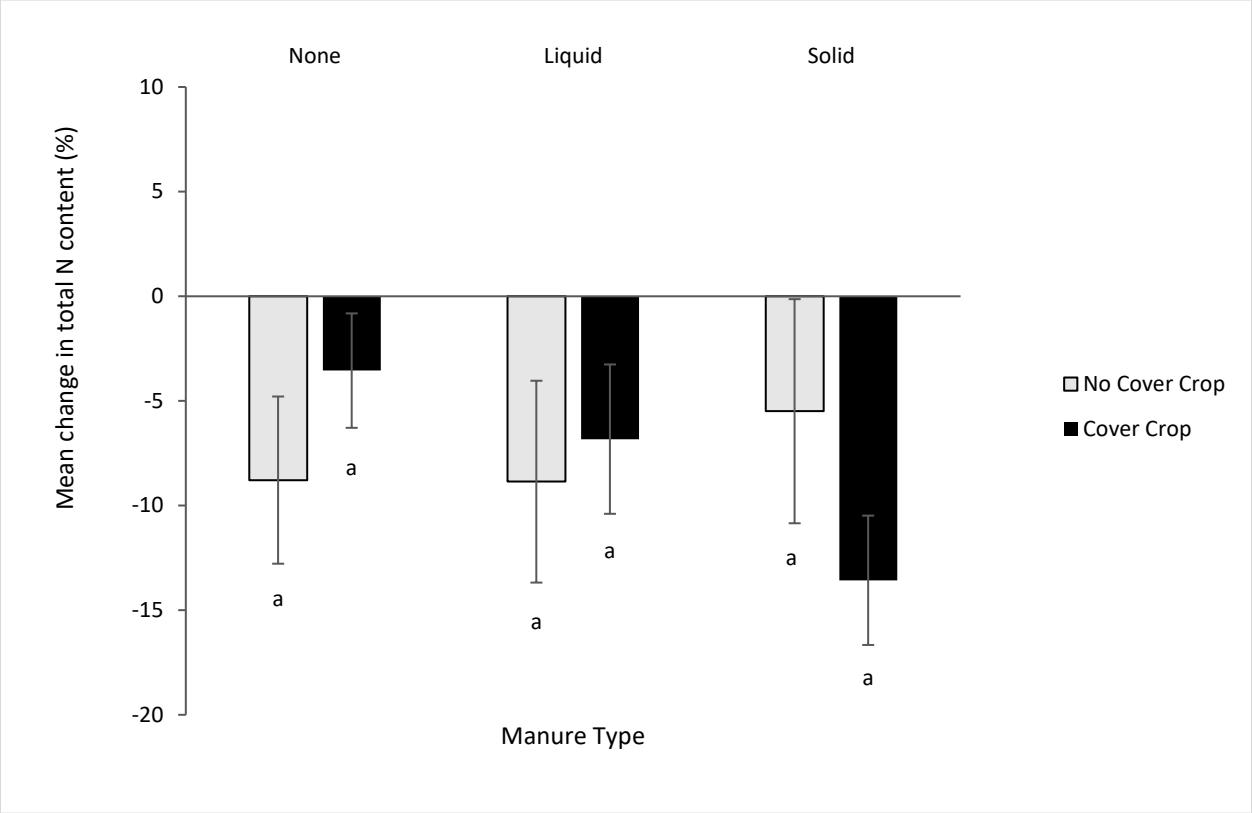


Figure 2-1. Change in total N content in the soil-plant system, between November 2017 and May 2018, for cover crop and manure treatments at the **Lods site**. Data were pooled among no cover crop (n=4) and cover crop (n=12) treatments. Negative values indicate a net loss of total N in soil from fall to spring. Bars on the columns are the standard error of the mean. Means were compared with a pre-planned orthogonal contrast analysis and those with the same letter are not significantly different ($p>0.05$).

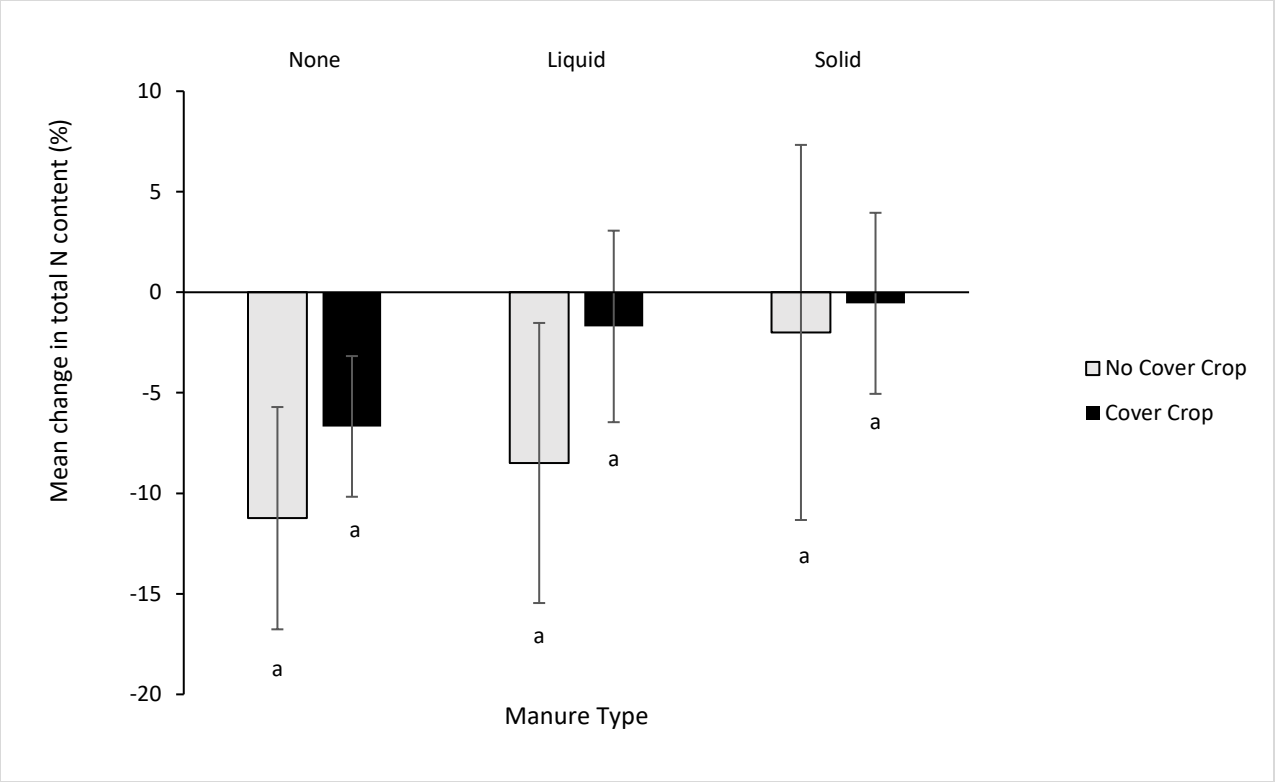


Figure 2-2. Change in total N content in the soil-plant system, between November 2017 and May 2018, for cover crop and manure treatments at the **Laval site**. Data were pooled among no cover crop (n=4) and cover crop (n=12) treatments. Negative values indicate a net loss of total N in soil from fall to spring. Bars on the columns are the standard error of the mean. Means were compared with a pre-planned orthogonal contrast analysis and those with the same letter are not significantly different ($p>0.05$).

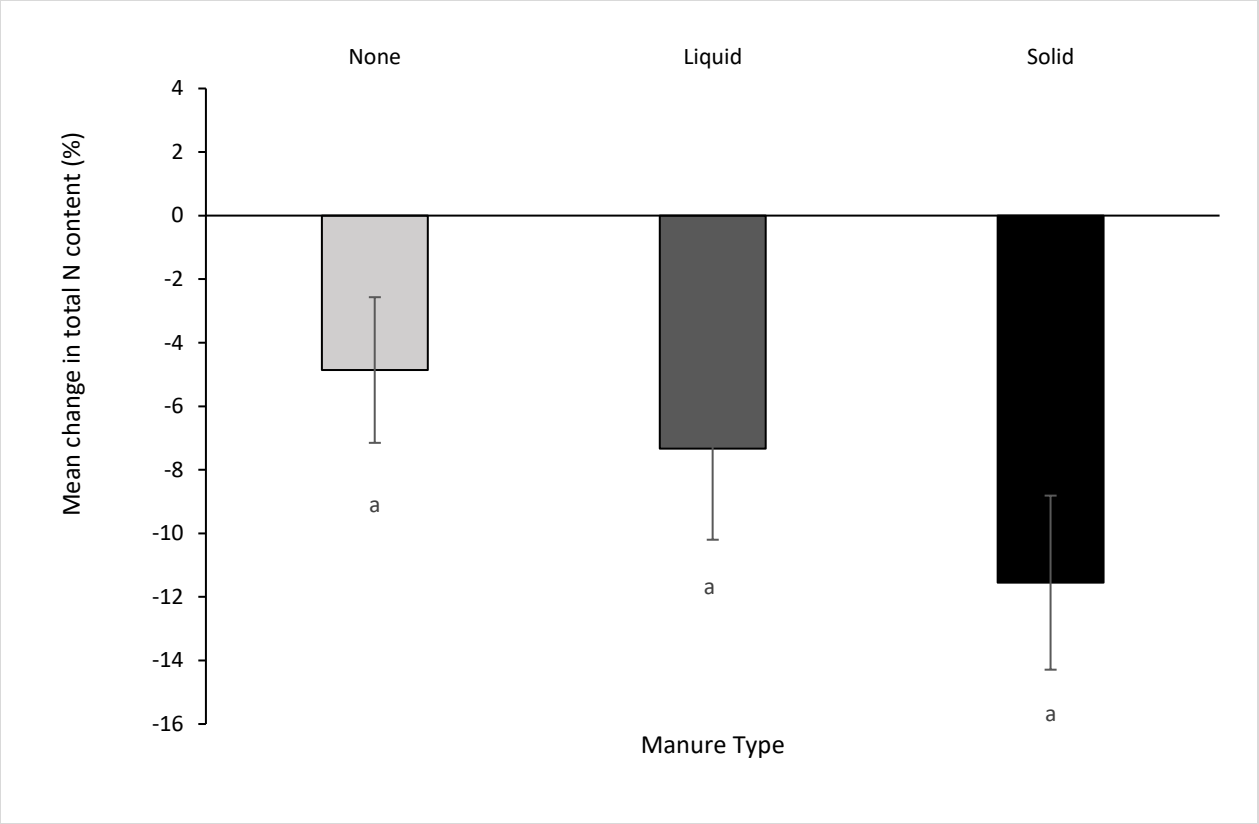


Figure 2-3. Change in total N content in the soil-plant system, between November 2017 and May 2018, for manure treatments at the **Lods site**. Cover crop treatments were pooled by manure type (n=16). Negative values indicate a net loss of total N in soil from fall to spring. Bars on the columns are the standard error of the mean. Means were compared with a one-way analysis of variance (ANOVA) and those with the same letter are not significantly different ($p>0.05$).

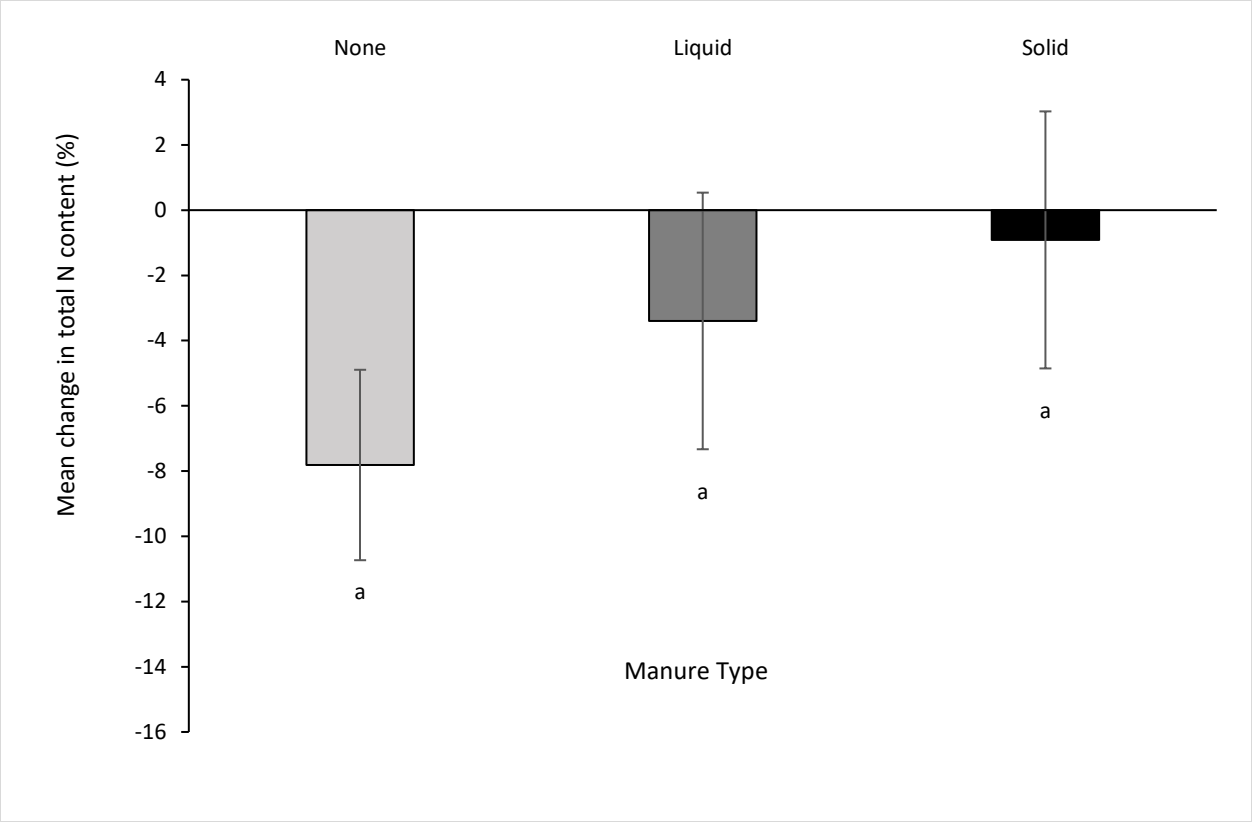


Figure 2-4. Change in total N content in the soil-plant system, between November 2017 and May 2018, for manure treatments at the **Laval site**. Cover crop treatments were pooled by manure type (n=16). Negative values indicate a net loss of total N in soil from fall to spring. Bars on the columns are the standard error of the mean. Means were compared with a one-way analysis of variance (ANOVA) and those with the same letter are not significantly different ($p>0.05$).

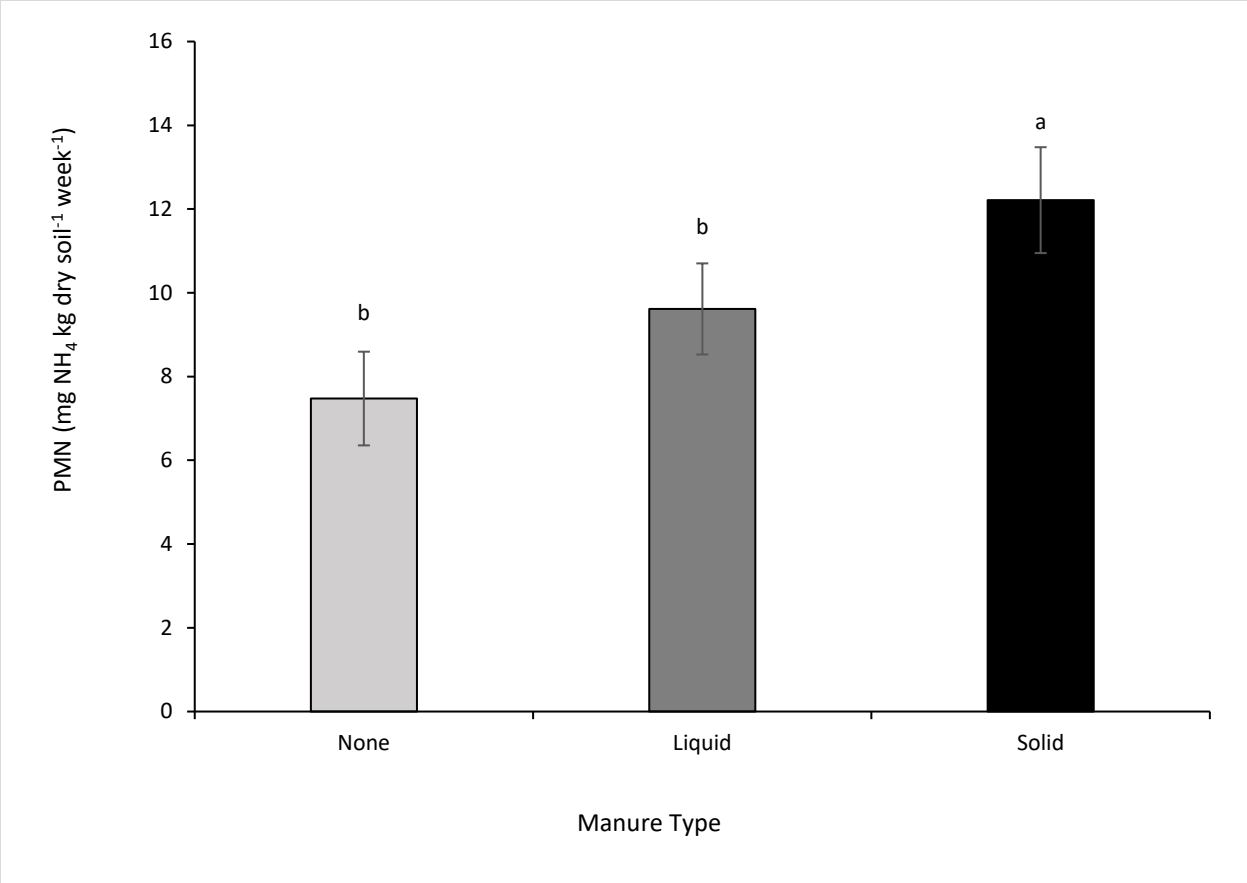


Figure 2-5. Potentially mineralizable nitrogen (PMN) concentration in manure-amended plots (cover crop pooled, n=16) at the Lods site. Soil was collected on May 7, 2018 prior to cover crop termination. Bars on the columns are the standard error of the mean. Mean values with different letters are significantly different ($p<0.05$).

Connecting Paragraph

The results from the field study in Chapter 2 indicate that most of the N from the fall-applied manure was lost over winter and not available to the subsequent spring crop. The fall-sown cover crops were not effective at capturing the manure N, largely due to the low cover crop biomass. Previous research indicates that most of the N loss over winter is from biologically-mediated production of N_2O (denitrification, nitrification) induced by freeze-thaw cycling. Chapter 3 describes a controlled incubation study that explores the mechanisms leading to the loss of N_2O-N from multiple freeze-thaw cycles and how these processes are influenced by different N fertilizer sources, with or without an annual ryegrass cover crop.

Chapter 3: Freeze-thaw effects on nitrous oxide production in cover-cropped soils fertilized with organic and inorganic nitrogen sources

Abstract

Nitrogen (N) in fall-applied manure is susceptible to over-winter loss through nitrous oxide (N_2O) emissions, especially during freeze-thaw events that occur frequently in cold humid temperate regions. A fall-sown cover crop may accumulate N from fall-applied manure, thereby reducing the amount of reactive mineral N (nitrate and ammonium) in soil that undergoes denitrification and releases N_2O during soil thawing. The objective of this study was to quantify the N_2O-N loss in relation to N fertilizer and cover crop combinations, as well as the soil mineral N concentration during three freeze-thaw cycles (FTCs). The soil incubation study was a full factorial with four N fertilizer treatments (none, liquid manure, solid manure, urea) combined with two cover crop treatments (annual ryegrass, no cover crop). Annual ryegrass (*Lolium multiflorum* Lam.) was seeded in pots and grown for three weeks in the greenhouse before pots were transferred to a freezer at -4°C. Pots were frozen at -4ºC and thawed at 4ºC. Production of N_2O was measured after 0, 3, 6 and 9 h of thawing before pots were destructively sampled to determine the soil mineral N concentration. Urea and liquid manure had the highest soil mineral N concentrations, and the effect of the cover crop was inconclusive. None of the treatment combinations affected N_2O production. Approximately 14% of the variation in N_2O production was explained by the soil mineral N concentration. There was a remarkable 937–1000% increase in N_2O production in pots that underwent FTCs compared to pots that were left unfrozen. It appears that N_2O was produced in frozen soils at -4ºC, trapped under ice, and subsequently released when the soils thawed at 4ºC, suggesting that denitrification does not stop during the winter months in cold humid temperate regions.

3.1 Introduction

In many cold temperate regions of the world, including Québec, increasing temperature fluctuation during winter months are predicted, which could lead to more frequent wintertime freeze-thaw events in agroecosystems (Henry 2008; Logan et al. 2011; Williams et al. 2011; Congreves et al. 2018). Soil microbes remain active over winter in frozen soil, especially in liquid water films that coexist with ice in frozen soil (Koponen et al. 2004; Congreves et al. 2018). More thawing periods over winter will increase the amount of liquid water in the soil and is expected to stimulate microbial activity and their metabolic processes such as soil nitrogen (N) transformations. Reactive or residual mineral N compounds resulting from microbial N transformations can escape from the soil, especially in the non-growing season when there is no plant uptake, by leaching into groundwater or entering surface waters from overland flow or tile drainage (Chambers et al. 2001; Drury et al. 2016). Reactive N can also be lost through gaseous emissions of dinitrogen (N_2), ammonia (NH_3), and nitrous oxide (N_2O) (Han et al. 2017).

In cold temperate climates, N_2O emissions tend to be the dominant source of N loss during the non-growing season, especially during freeze-thaw periods (Jayasundara et al. 2010; Han et al. 2017; Wagner-Riddle et al. 2017). Freeze-thaw events can contribute to more than 50% of annual N_2O emissions in temperate agroecosystems, which is highly problematic considering that N_2O is a potent greenhouse gas (Wagner-Riddle et al. 2007; Pattey et al. 2008; Wagner-Riddle et al. 2017). As soil freezes, the soil environment becomes increasingly anaerobic, which causes denitrifying microbes to consume reactive soil mineral N such as nitrate (NO_3^-) and ammonia (NH_4^+), along with dissolved organic carbon (DOC), and produce N_2O (Li et al. 2000). When soil thaws, the N_2O trapped in the deeper soil layers is released to the atmosphere. Soil freeze-thaw dynamics also stimulate microbial cell lysis and release of

substrates in the upper soil layers, as well as the breakdown of soil aggregates (Lange et al. 2017). Greater substrate availability and enhanced microbial activity during thawing leads to biological *de novo* production of N_2O (Wagner-Riddle et al. 2008; Kariyapperuma et al. 2011; Congreves et al. 2018), mainly from heterotrophic denitrification, but also from ammonia oxidation and nitrifier denitrification (Kool et al. 2011; Li et al. 2016; Song et al. 2017).

Clark et al. (2009) applied $^{15}NH_4$-labeled pig slurry to soils incubated at -6, -2, 2, 6, and 10 °C. Manure addition increased nitrification by 3-14 times across all temperatures and up to 30% of applied ^{15}N was present in microbial biomass N (MNB), indicating that net mineralization and nitrification of the organic N in manure occurred at significant rates even in frozen soil (Clark et al. 2009). Therefore, the extent of N_2O production during freeze-thaw events is expected to be highly correlated with the amount of reactive substrates (NH_4^+, NO_3^-, DOC) in soil (Pelster et al. 2013; Wertz et al. 2016).

Residual Soil Nitrogen (RSN) Indicator values are an estimate of the amount soil inorganic (mineral) N left in Canadian farm fields at the end of the growing season (Drury et al. 2016). RSN values nationwide increased by more than 150% in 30 years (9.4 kg N ha^{-1} in 1981 to 23.6 kg ha^{-1} in 2011), due to a greater increase in N inputs (fertilizer) compared to N outputs (crop uptake) (Drury et al. 2016). This was particularly evident in Québec, which had the highest percentage of farmland (72%) in Canada with RSN values in the *very high risk* category (>40 kg N ha^{-1}) (Drury et al. 2016). With frequent over-winter freeze-thaw events and high RSN values, Québec likely experiences considerable N losses during the non-growing season.

Québec also has some of the highest rates of manure production in Canada (Yang et al. 2011). Many farmers apply manure to their fields in fall for convenience and to avoid over-winter manure storage (Beaulieu 2004; Yang et al. 2011). Nitrogen (N) in fall-applied manure is

susceptible to over-winter losses, especially during freeze-thaw events that occur in late fall, winter, and during early spring snowmelt in this cold humid temperate region. This was confirmed in the field study of Chapter 2 and is consistent with other studies (Jayasundara et al. 2010; Chantigny et al. 2019).

A cover crop that is planted after manure or inorganic fertilizer is applied in fall may accumulate some of the available mineral N from the manure, and thereby reduce the amount of reactive N in the soil that is accessible for microbial production of N_2O during freeze-thaw cycling. Field studies have linked the presence of a vegetative cover on agricultural land to lower production of N_2O during spring thaw events (Wagner-Riddle and Thurtell 1998; Dietzel et al. 2011), including reductions in N_2O emissions of up to 65% during freeze-thaw cycles compared to bare soil (Dietzel et al. 2011).

In a laboratory simulation, Pelster et al. (2013) compared soil NO_3^- and N_2O emissions after eight freeze-thaw cycles from soil cores that were left bare or amended with corn *(Zea mays* L.) or soybean *(Glycine max* L.) residue. The incorporation of the high C/N ratio residues resulted in up to 80% decrease in N_2O production after the eight freeze-thaw cycles compared to the soils that were left bare in C-rich silty loam soil, possibly because the crop residues induced NO_3^- immobilization (Pelster et al. 2013). Cober et al. (2018) found that while freeze-thaw cycling stimulated the release of water-extractable phosphorus (WEP) from cover crops, it had minimal influence on the release of mineral N. However, these laboratory freeze-thaw experiments (Pelster et al. 2013; Cober et al. 2018) used plant residues as a proxy for cover crops, and not intact plants grown in soil. To my knowledge, few laboratory freeze-thaw incubation experiments have compared different types of manure, and none have investigated the influence of manure and intact cover crops on reducing N loss from repeated freeze-thaw cycles.

The purpose of my experiment was to determine the effect of an annual ryegrass (*Lolium multiflorum* Lam.) cover crop combined with different N fertilizer types (liquid manure, solid manure, urea) on the mitigation of N_2O-N loss during three freeze-thaw cycles (FTCs) in the upper soil layer (0-10 cm) at temperatures that would be experienced in field conditions (-4°C to 4°C). Due to the potential for the cover crop to uptake reactive N, the N fertilizer treatments that were combined with a cover crop were predicted to have lower N_2O emissions after each FTC than the corresponding N fertilizer treatment without a cover crop. I also investigated the relationship between N_2O-N loss from freeze-thaw cycling and the reactive soil N and DOC substrate concentrations. Production of N_2O is dependent on access to substrate concentrations, so I expected that N_2O emissions would be positively correlated with both soil mineral N and DOC, and these soil parameters would be related to N_2O emissions after each FTC.

3.2 Materials and Methods

3.2.1 Soil collection and site description

The soil used in this study was collected from the Chapter 2 field site at the Emile A. Lods Agronomy Research Centre in Sainte-Anne-de-Bellevue, Québec (N 45° 25' 34" W 73° 55' 43", elevation 39 m above sea level). The field was previously planted with soybeans (*Glycine max* L.) that were harvested and removed from the field in September 2017. As described in Chapter 2, manure (liquid, solid, and none) was applied and cover crop treatments (annual ryegrass or annual ryegrass/hairy vetch [*Vicia villosa* Roth] bi-cultures) were planted on 25 September 2017 in a full factorial randomized complete block design with four replicates (blocks). Soil for this study was collected from the buffer zones between the treatment blocks on 7 December 2017 just prior to freeze-up to represent the soil chemical and biological properties

of the late fall/early winter period. Soil was a sandy loam Humic Gleysol of the St. Amable series with approximately 620 g sand kg^{-1}, 60 g clay kg^{-1}, 48 g organic C kg^{-1}, an average pH of 6.3, and an average bulk density of 1.11 g cm^{-3}. Soil was sieved (<4 mm) and stored in plastic bins at 4°C for five months until the freeze-thaw study began.

3.2.2 Experimental design and treatments

The experiment was a full factorial with four N fertilizer treatments (no fertilizer, liquid manure, solid manure, or urea) and two cover crop treatments (no cover crop or annual ryegrass) applied in a completely randomized design. Liquid manure was dairy cattle slurry obtained from a large, open, outdoor concrete tank at the McGill Macdonald Campus Farm in Sainte-Anne-de-Bellevue, Québec (Table 3-1). Solid manure was a semi-composted cattle manure collected from a partially covered concrete pad at the Macdonald Farm where the manure was mixed with straw and other bedding material (Table 3-1).

Prior to treatment application, soil was warmed at approximately 25°C for at least 24 h. Soil (280-285 g) was then packed into 0.2 L pots to a bulk density of 1.11 g cm^{-3}. The soil in all pots was amended with triple super phosphate ($Ca[H_2PO_4]_2 \cdot H_2O$, 0-46-0) and muriate of potash (KCl, 0-0-60), both at a rate of 20 kg ha^{-1} (0.20 mg cm^{-2}) to ensure no phosphorous or potassium limitation to the cover crop. During the freeze-thaw incubation, triplicates of each factorial treatment underwent 0, 1, 2, or 3 freeze-thaw cycles (FTCs), so the total number of pots was 96 (twelve pots per eight factorial treatments). An additional eight pots (half with cover crop, half without, no N fertilizer applied to any) were prepared for the purpose of pre-testing the equipment used for the freeze-thaw incubation.

Urea fertilizer ($[NH_2]_2CO$, 46-0-0) was applied at a rate of 150 kg N ha^{-1} (approximately 152 mg of urea per pot). Application rates for liquid manure (approximately 30 g per pot) and solid manure (approximately 42 g per pot) were also 150 kg N ha^{-1}, based on the assumption of 40% plant-available N in solid manure and 60% plant-available N in liquid manure (Eghball 2000; Eghball et al. 2002). All N fertilizers were mixed with the top 5 cm of soil. Ryegrass seeds were pre-germinated on moist paper in sealed plastic petri dishes prior to planting. Immediately after N fertilizer application, six seedlings were planted in the cover cropped pots with spacing that represented a seeding rate of approximately 34 kg ha^{-1}, to cover the soil surface.

The pots were grown under a semi-controlled environment (between 5–25°C) in a greenhouse for three weeks until the ryegrass reached the 2–3 leaf stage. All pots (including the pots without ryegrass) were watered regularly during this period to keep the soil moist and facilitate plant growth. After three weeks, distilled water was used to adjust the moisture content of the soil in each pot to approximately 80% water-filled pore space (WFPS) and all pots were put in plastic Ziploc bags to reduce moisture loss. According to Ludwig et al. (2004) and Kool et al. (2011), maximum N_2O production through denitrification occurs at 80%. Pots were then transferred to a cooler at 4°C for four days to simulate a "hardening off" of the cover crop. Twenty-four control pots (0 FTC treatment) remained at 4°C for three months, and the other 72 pots in the 1, 2 and 3 FTC treatments were moved to a freezer at -4°C and stored for approximately three months until the freeze-thaw incubation began.

3.2.3 Pre-testing temperature dynamics for the freeze-thaw incubation

Before the freeze-thaw incubation, four of the non-treatment pots (two with cover crop, two without) underwent pre-testing of freezing (-4°C) and thawing (4°C) to document soil

temperature dynamics and the length of time required to completely freeze and thaw soil in the experimental pots. Two thermocouple sensors were placed in the upper and lower layers of soil in each pot and connected to a datalogger (CR23X Micrologger, Campbell Scientific, Inc.) prior to freezing. Temperature data was recorded every 5 min for at least 48 h of freezing and 48 h of thawing. Soil was frozen in less than 4 h, and temperatures stabilized at around -4 to -5°C within 12 h. During the thaw, soil temperature was above 0°C within 4 h and reached a relatively stable temperature of around 4ºC by 9 h. Pots were weighed before and after the thaw period, and the change in mass was minimal, indicating negligible moisture loss during the thaw. These findings were confirmed by pre-testing four additional non-treatment pots. Based on these pre-tests, 9 h was chosen as the length of the thaw period for the freeze-thaw incubation.

3.2.4 Freeze-thaw incubation

Two weeks before the freeze-thaw incubation, gas samples and soil samples were collected from the unfrozen control pots (FTC 0) to represent the baseline conditions. For the first freeze-thaw cycle (FTC 1), all treatment pots were transferred from the -4°C freezer to an incubator set at 4°C. Three replicates of each treatment were designated as "FTC 1" pots (24 in total), and gas samples were only taken from these pots during the course of the 9 h thaw period. After 9 h, the FTC 1 pots were removed from the experiment for soil analysis. The remaining pots (n=48) were placed back in the freezer at -4°C for one week. The following week, the remaining pots were put back into the 4°C incubator for 9 h, then the FTC 2 pots (n=24) were destructively sampled. The remaining FTC 3 pots (n=24) were returned to the -4ºC freezer for one more week, and then transferred to the 4°C incubator for the third and final FTC.

3.2.4.1 Gas sampling

Headspace gas was collected in a non-flow-through steady-state chamber (height = 10.5 cm, inner diameter = 10.0 cm) placed on top of each experimental pot and tightly sealed to prevent gas exchange. A 20 mL gas sample was taken from the chamber headspace using an air-tight polypropylene syringe and injected into a pre-evacuated 12 mL glass exetainer immediately following chamber deployment (0 h), and then every 3 h during the thaw period (3, 6 and 9 h). To keep a constant gas pressure in the chamber headspace, 20 mL of inert N_2 gas was injected into the headspace following the gas sample collection. Gas samples were analyzed for N_2O concentration by a Bruker 450 Gas Chromatograph (Bruker Corporation, Billerica, MA, USA). The concentration of N_2O was converted from ppm to μg L^{-1} using the ideal gas law, according to the equation (Equation 1) from Holland et al. (1999).

$$C_m = \frac{C_v M P}{R T} \qquad (1)$$

where:

C_m = mass/volume concentration in μg L^{-1} (μg N_2O-N L^{-1})

C_v = concentration (v/v) in ppm (μL L^{-1})

M = molecular weight of the trace species (N_2O-N = 28 μg N μmol^{-1} N_2O^{-1})

P = atmospheric pressure (1 atm)

R = universal gas constant (0.082 L atm mol^{-1} K^{-1})

T = incubation temperature (277 K)

The production of N_2O was calculated on a per dry soil basis (μg N_2O-N kg^{-1} soil) by dividing the quantity of N_2O-N (in μg, after multiplying the C_m value by the volume of headspace = 0.6247 L) by the dry mass of soil in each pot. Cumulative N_2O production was

based on the N_2O concentration measured at the end of the 9 h thaw period, adjusted for N_2O removal and N_2 addition to the headspace at 0, 3 and 6 h.

3.2.4.2 Soil sampling and laboratory analysis

After each 9 h thaw, experimental pots were selected at random for destructive sampling. Plant residues were removed manually and a soil subsample (about 10 g) was oven-dried at 105°C for 24 h for gravimetric moisture determination. Another subsample (about 15 g) was extracted with 0.5 M K_2SO_4 (1:4 soil:extractant) and the NH_4^+ and NO_3^- concentrations were determined colorimetrically at 650 nm using the modified indophenol blue method (Sims et al. 1995). Dissolved organic carbon (DOC) concentration in the K_2SO_4 extract was measured on a Sievers Innovox TOC analyzer (GE Analytical Instrument, Boulder, CO, USA).

3.2.5 Statistical Analysis

Statistical analyses was done with SAS version 9.4 (SAS Institute 2013). Two N_2O values in the control (FTC 0) pots and one N_2O value in the FTC 1 pots that were approximately one order of magnitude larger than the rest of the data were considered outliers and excluded from the statistical analysis. Residuals of the data were assessed for normality using the PROC UNIVARIATE function, and data that were not normally distributed according to the Shapiro-Wilk test was log-transformed to achieve normality. The main and interactive effects of N fertilizer and cover crop treatments on the cumulative N_2O production, soil mineral N (NH_4^+ + NO_3^-), and DOC after each FTC and across all FTCs were assessed using a two-factor ANOVA in the PROC GLM function. When N fertilizer, cover crop, or N fertilizer × cover crop treatments were different ($p<0.05$), mean comparisons were done with a post-hoc Tukey's Honest Significant Difference test. Pearson correlation coefficients (r) between cumulative N_2O

production, soil mineral N, and DOC concentrations were assessed using the PROC CORR function for the unfrozen control pots, each FTC, and across all FTCs (excluding the control). Linear relationships between cumulative N_2O production, soil mineral N and DOC concentrations were fitted using a stepwise regression procedure in the PROC REG function. Cumulative N_2O production after each FTC was compared in the treated pots and the unfrozen control pots with a one-factor ANOVA in PROC GLM.

3.3 Results

3.3.1 Influence of nitrogen fertilizer and cover crop treatments on N_2O production and soil parameters

Cumulative N_2O production did not differ significantly among N fertilizer or cover crop treatments for any of the FTCs or in the unfrozen control pots, due to the considerable variation (large standard errors) of the mean values for each treatment.

Soil mineral N concentration (NH_4-N + NO_3-N) was affected significantly ($p<0.05$) by the N fertilizer × cover crop interaction after FTC 1, 2, and 3. For FTC 1, pots receiving urea with cover crop had a significantly ($p<0.05$) greater soil mineral N concentration than other N fertilizer × cover crop combinations (Fig. 3-1). Soil mineral N concentration was similar in pots amended with urea and no cover crop, and the liquid manure and solid manure treatments, both with and without cover crop (Fig. 3-1). The lowest soil mineral N concentration was in the unfertilized control without cover crop (Fig. 3-1).

For FTC 2, the largest soil mineral N concentration was in the urea with cover crop and liquid manure without cover crop treatments (Fig. 3-2). Intermediate soil mineral N

concentrations were observed in the other pots that received N fertilizer, and the lowest soil mineral N concentration was in the unfertilized control without cover crop (Fig. 3-2).

For FTC 3, urea treatments, with and without cover crop, and liquid manure treatment without cover crop had the highest soil mineral N concentrations. There was no significant difference in soil mineral N between the other treatment combinations (Fig. 3-3).

On average, the soil mineral N concentration across all FTCs was affected significantly ($p<0.05$) by the N fertilizer × cover crop interaction. The urea treatments, with and without cover crop, and the liquid manure without cover crop had significantly ($p<0.05$) greater soil mineral N concentration than other treatments combinations (Fig. 3-4). The liquid manure with cover crop had a similar soil mineral N concentration as the solid manure pots with and without cover crop, as well as the no fertilizer pots with cover crop (Fig. 3-4). The lowest soil mineral N concentration was in the unfertilized control without cover crop (Fig. 3-4).

3.3.2 Influence of soil parameters on N_2O production after each freeze-thaw cycle

Cumulative N_2O production after the 9 h thaw period was positively correlated with soil mineral N concentration after FTC 1 and FTC 3, as well as across all FTCs (Fig. 3-5). These parameters were not correlated after FTC 2 or in the unfrozen control pots (FTC 0).

Cumulative N_2O production after the 9 h thaw period was positively correlated with DOC concentration after FTC 2, but not after FTC 1 or FTC 3, and there was no correlation across all FTCs (Fig. 3-6). The N_2O produced from the control pots (FTC 0) was not significantly correlated with DOC concentration.

The linear relationship between cumulative N_2O production, and soil mineral N and DOC concentrations, across all FTCs is shown in Equation 2 ($R^2=0.16$, $p=0.001$, $n=71$). Soil mineral N

explained 14% of the variation in cumulative N_2O production. The intercept was of the model was slightly less than zero.

$$\text{Log } N_2O = -0.27 + 0.58 \text{ (Log Mineral N)} + 9.4\text{x}10^{-4} \text{ (DOC)} \quad (2)$$

Standard Error = 0.34	Standard Error = 0.18	Standard Error = $7.5\text{x}10^{-4}$
	Partial R^2=0.14	Partial R^2=0.02
	p=0.001	p=0.211

3.3.3 Cumulative N_2O production across freeze-thaw cycles

All FTCs had significantly ($p<0.05$) more cumulative N_2O production after a 9 h thaw period compared to the unfrozen control, but there was no difference between FTCs (Fig. 3-7).

3.3.4 Production of N_2O during soil thawing

During FTC 1, the majority (61%) of N_2O was produced in the first 3 h and 30% occurred between 3–6 h (Fig. 3-8). In the 6–9 h period, the negative flux indicated that 9% of the N_2O was consumed (Fig. 3-8). Similar patterns of N_2O production occurred during FTC 2 and FTC 3, with approximately 30–40% of N_2O produced in each 3 h increment during the 9 h thaw period (Fig. 3-8). On average across all FTCs, 50% of N_2O was produced in the first 3 h, with 32% produced between 3 and 6 h, and 18% produced in the final 6–9 h (Fig. 3-8).

3.4 Discussion

3.4.1 Treatments affected soil mineral N concentration but not N_2O production

Nitrogen fertilizer sources had a greater impact on the soil mineral N concentration than cover crops, resulting in a significant N fertilizer × cover crop interaction. The cover crop did not reduce soil mineral N in the urea treatments, and possibly contributed to higher soil mineral N

concentrations after FTC 1 and 2. Liquid manure without cover crop had a similar soil mineral N concentration as the urea treatments, but liquid manure plus cover crop had lower soil mineral N concentrations after FTC 2 and 3. The no N fertilizer, with and without cover crop, had the lowest soil mineral N concentration, and were similar to the solid manure treatments with and without cover crop across all FTCs.

These results suggest that solid manure is less likely to be a source of reactive soil mineral N during freeze-thaw cycles compared to urea and liquid manure. Urea fertilizer is water-soluble and readily hydrolyzed to NH_4-N, and almost 40% of the total N in liquid manure was present as NH_4-N (Table 3-1), so it is not surprising that these treatments had the highest soil mineral N concentration. Solid manure contained less than 17% of the total N as NH_4-N (Table 3-1) and had a higher proportion of organic N, so it was expected that soil would have less mineral N when amended with solid manure than with liquid manure or urea.

Overall, the effects of the N fertilizer × cover crop combinations on soil mineral N concentration were inconsistent during sequential freeze-thaw cycles. This could indicate that an insignificant amount of N was taken up by the cover crop during the three weeks of growth in the greenhouse, or it could suggest that NH_4 assimilated by the cover crop was vulnerable to release into the soil mineral N pool during freeze-thaw cycles. It was not possible to recover and quantify the N remaining in the small amount of cover crop biomass after the freeze-thaw periods, so I do not know whether N uptake by the cover crop was affected by the N fertilizer treatments.

Despite the significant N fertilizer × cover crop effect on soil mineral N concentrations, there was no effect of N fertilizer or cover crop treatments on N_2O production. Given that N_2O production was measured in all soils, including the unfertilized controls, it does not appear that

denitrification was limited by a shortage of soil mineral N or DOC substrates. Soil mineral N and DOC concentrations at the time of soil collection were not measured, but the initial levels were not maintained because soil handling (sieving, cooling, warming, mixing) before and during the cover crop growth period likely stimulated decomposition and N mineralization before the freeze-thaw incubation began. The unfertilized soil that was never frozen contained 13–143 mg soil mineral N kg^{-1}. Soils with NO_3^- levels greater than 5–10 mg N kg^{-1} are not limiting to denitrification (Gillam et al. 2008; Thomas et al. 2017), indicating that there was ample mineral N for denitrification even when fertilizer was not added.

3.4.2 Relationship between N_2O production and soil mineral N

It was predicted that N_2O production would be related to the soil mineral N and soluble organic C sources, and this was partially substantiated by the significant correlation between N_2O production and soil mineral N. As N_2O production was unaffected by the DOC concentration, this indicates that there was enough organic C for denitrification (Pelster et al. 2013; Thomas et al. 2017). Reactive soil mineral N, mainly NO_3^-, is the key driver of N_2O production from soils in other freeze-thaw simulation studies (Pelster et al. 2013; Li et al. 2016; Thomas et al. 2017). However, I found that soil mineral N explained only approximately 14% of the variation in N_2O production across all FTC simulations, and the slightly negative intercept indicates that other factors contributed to the production of N_2O during the FTCs.

Other factors that led to the production or consumption of N_2O from soil that underwent freezing-thawing could be biological and physical in origin. Substrates that are metabolized by denitrifying bacteria, but were not measured in this study, include nitrite (NO_2^-) and dissolved organic N (Butterbach-Bahl et al. 2013). Soil moisture content could also be an important driver

of denitrification since it changes the redox conditions experienced by denitrifiers. The soils in my study were maintained at 80% WFPS on average, but this does not guarantee that the water was distributed uniformly through all macro- and micro-pores in the soil mass. It seems possible that denitrifiers experienced both anaerobic and micro-aerobic conditions, which may result in nitrifier-denitrification within the same population or coupled nitrification-denitrification by separate populations (Kool et al. 2011; Butterbach-Bahl et al. 2013). While chemical decomposition of nitrite via chemodenitrification is another process that produces N_2O, it is unlikely to be important in this study because chemodenitrification dominates in soils with low pH (pH<5) and produces nitric oxide gas (NO) preferentially (Li et al. 2000; Risk et al. 2013).

Another source of N_2O produced during the thaw period could be entrapped, previously-produced N_2O that was released as ice melted. Nitrifying and denitrifying activity still occurs in frozen soils, especially in liquid water films associated with ice-filled soil pores (Koponen et al. 2004; Congreves et al. 2018). Anaerobic conditions in frozen soils stimulate these microbes to consume residual soil substrates and produce N_2O that becomes trapped either in deep, unfrozen soil layers or in liquid water films (Li et al. 2000; Wagner-Riddle et al. 2008). Due to the small soil volume used in this study and the freezer temperature (-4^{o}C), I assume that most of the entrapped N_2O was in the liquid water films and not in deep unfrozen soil layers.

3.4.3 N_2O production stimulated by freeze-thaw events

Freezing and thawing produced 937–1000% more N_2O from the pots that underwent 1, 2, or 3 FTCs compared to pots with unfrozen soil. As mentioned, a large portion of this was probably previously-produced N_2O that built up during the frozen period and was released in a "burst" once the frozen soil barrier was removed during thawing. This is evidenced by the fact

that over 50% of N_2O production, averaged across all FTCs, occurred in the first 3 h of the total 9 h thaw. After 6 h there was a net consumption of N_2O during FTC 1, indicating that most of the soil mineral N was fully reduced to N_2. Gross consumption of N_2O may have also occurred in FTC 2 and 3, but was possibly masked by higher overall rates of N_2O production. Although the total soil water content remained constant, the water changed from solid (ice) to liquid during the thaw period, creating an increasingly anoxic soil environment as thawing progressed. More anaerobic conditions in the later stages of thawing, combined with a depletion of soil mineral N, would tend to favour N_2O consumption and full reduction to N_2 (Chapuis-Lardy et al. 2007). Temperature also influences the proportion of N_2 versus N_2O denitrification end product since the activity of the N_2O reductase enzyme increases with temperature (Henry 2007; Cavigelli and Parkin 2012; Tatti et al. 2014).

In meta-analyses of laboratory and field studies, freeze-thaw effects resulted in an increase of N_2O emissions by 95–145% (Song et al. 2017; Gao et al. 2018), which is high, but an order of magnitude less than the N_2O production in this study. Many previous laboratory-based freeze-thaw experiments used conventional freezers set at -20°C to freeze the soil, which is not representative of soil temperature during winter in temperate regions (DeLuca et al. 1992; Henry 2007; Song et al. 2017). Freezing soil at -20ºC seems likely to halt microbial activity and N_2O production more abruptly than at the -4ºC temperature I used, since virtually all the water should become ice within a few days of placing soil in a freezer at -20ºC. In addition, it is possible that the high rates of N input (150 kg N ha^{-1}) and small soil volume that I used resulted in more intense freeze-thaw effects than would be experienced in more normal field settings. Despite the artificial conditions, the strong contrast in N_2O production between the control and experimental

pots indicates that considerable amounts of N_2O accumulate in soils that are frozen to -4°C and then thawed.

The amount of N_2O produced from each successive FTC cycle was relatively uniform, which is contrary to other laboratory simulations. Chen et al. (1995) found a 60% increase in N_2O fluxes in the first FTC compared to the unfrozen control and increasing N_2O flux after the second and third FTCs, indicating a possible increase in substrate availability with each freeze-thaw event. However, the pool of susceptible freeze-thaw material and the microbial biomass of denitrifiers may also decline with increasing number of events, and emissions of N_2O entrapped in deep soil layers could decline as more gas is liberated during successive freeze-thaw events (Yanai et al. 2004; Henry 2007). Eventually, the entrapped N_2O within the soil will be depleted as a new equilibrium is reached between the soil air and external atmosphere. Pelster et al. (2013) performed eight freeze-thaw cycles and found that N_2O flux declined after five FTCs in a silty clay soil, but was highest in the sixth to eight FTCs in a sandy loam soil. This suggests that repeated thawing might increase the soluble substrates for denitrifiers in coarse-textured soils and stimulate more *de novo* N_2O production in sandy loam than silty clay soils. I did not observe a change in N_2O production with time, but would need to do more than three FTCs to confirm this.

3.4.4 Limitations of laboratory soil freeze-thaw experiments and future directions

Although my experiment provides an indication of N dynamics in the surface soil layers (0–10 cm depth), the freezing and thawing processes were more rapid in this study than under field conditions. This was mainly due to the small volume of soil that was required in my experimental design. Soil microbes tend to be more sensitive to fast rates of freeze or thaw that

occur under laboratory conditions, compared to field environments (Henry 2007; Risk et al. 2013). However, any volume of pots placed in freezers and refrigerators will freeze and thaw unevenly – first from the sides and bottom, and later from the center of the pots. Using larger soil volumes in laboratory freeze-thaw simulations could create more distinct regions of frozen and unfrozen soil in the pots. Installing insulating materials at the sides and bottom of the pots may help reduce artifacts (Henry 2007).

Additionally, the question of why there is such a strong stimulatory effect of freeze-thaw events on release of N_2O could not be fully explained in this study. To investigate both the biological and physical origins of N_2O production from the soil, N_2O loss pathways can be traced using ^{15}N-labelled NH_4^+ and NO_3^-, along with ^{18}O-labelled H_2O. Hu et al. (2015) also noted a research gap related to the functional microbial genes, enzymes, and regulatory mechanisms that drive production of N_2O from soil that undergoes freezing and thawing. Future experiments could monitor critical N_2O-relevant biomarker genes (eg. *nirK, nirS, narG, nosZ)* through quantitative PCR and next-generation sequencing methods. When linked with N_2O flux measurements, these techniques could provide insight into the temporal and spatial dynamics of biological N_2O production over the course of multiple freeze-thaw events.

3.5 Conclusion

The production of N_2O from freeze-thaw events appeared to be environmentally driven and it may be difficult to adapt management practices that will stop it. Regions like Québec, that historically have high amounts of mineral N left in the soil after the growing season (high RSN values) and also experience wintertime freeze-thaw cycling, are at particular risk of losing N through N_2O during the non-growing season. Applying N fertilizers that increase the soil mineral

N concentration could exacerbate the N_2O loss during freezing and thawing events. Although denitrification was not limited by C substrates in this study, it could occur under field conditions. I do not recommend fall application of manure containing reactive N and soluble C compounds because these could stimulate N_2O production in soils that have inherently low N and C concentrations in the non-growing season. Whether fertilizer management, alone or in combination with cover cropping, can be an effective way to control N_2O production still needs to be confirmed under field conditions. Future studies should also determine what proportion of the variation in N_2O production can be explained by substrate availability, compared to other factors that affect the N_2O released from soil through biological and physical processes.

Table 3-1. Selected physical and chemical properties of the manure types used in the laboratory freeze-thaw incubation experiment

Physico-Chemical Properties*	Liquid Manure†	Solid Manure‡
Dry matter (%)	11.6	21.4
C/N Ratio	13.6	22.4
Total N (kg t^{-1})	3.8	4.2
NH_4-N (kg N t^{-1})	1.5	0.7
Total P_2O_5 (kg t^{-1})	1.7	2.1
Total K_2O (kg t^{-1})	2.9	4.8

** Units are expressed per wet weight of manure*
†Liquid manure = dairy cattle slurry
‡Solid manure = semi-composted cattle manure

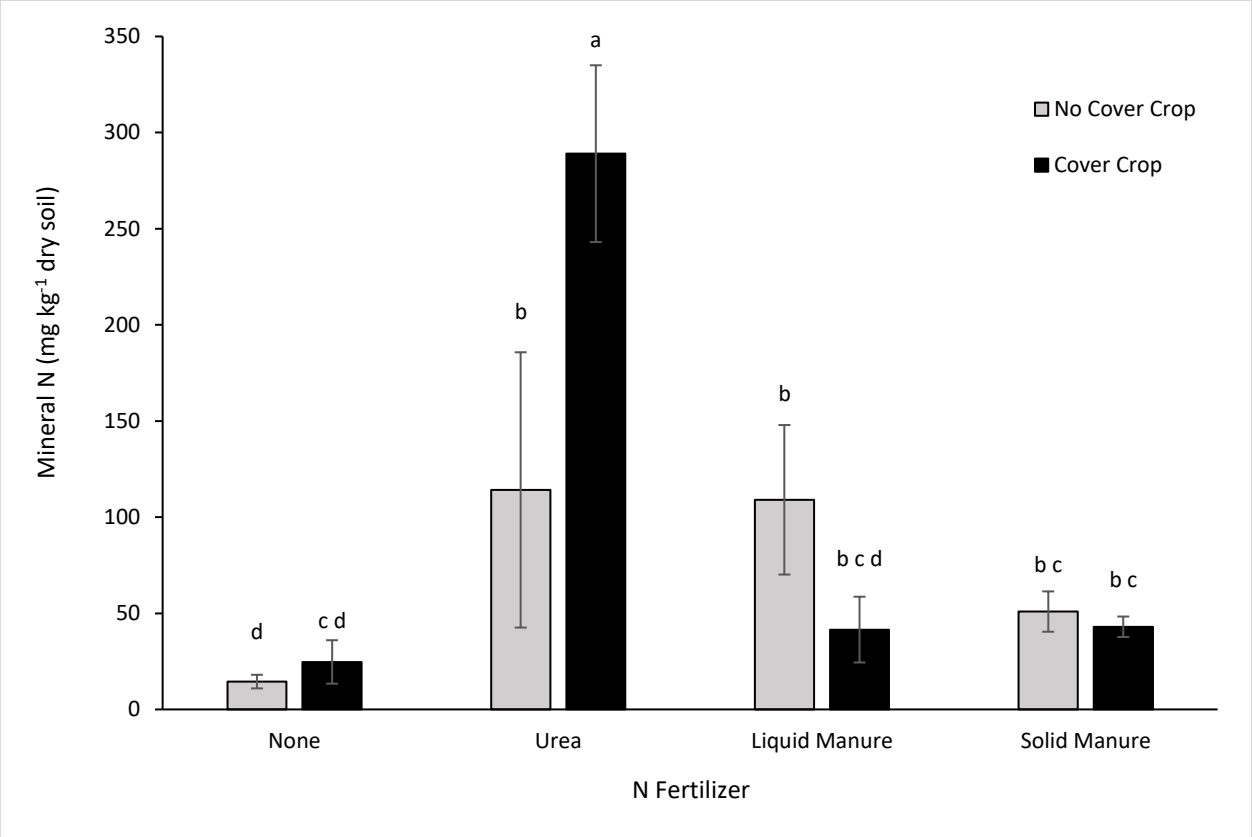


Figure 3-1. Soil mineral N concentration as affected by the interaction of N fertilizer × cover crop treatments after FTC 1. Data are the mean values (n=24) with standard error bars. Means with different letters are significantly different at $p<0.05$.

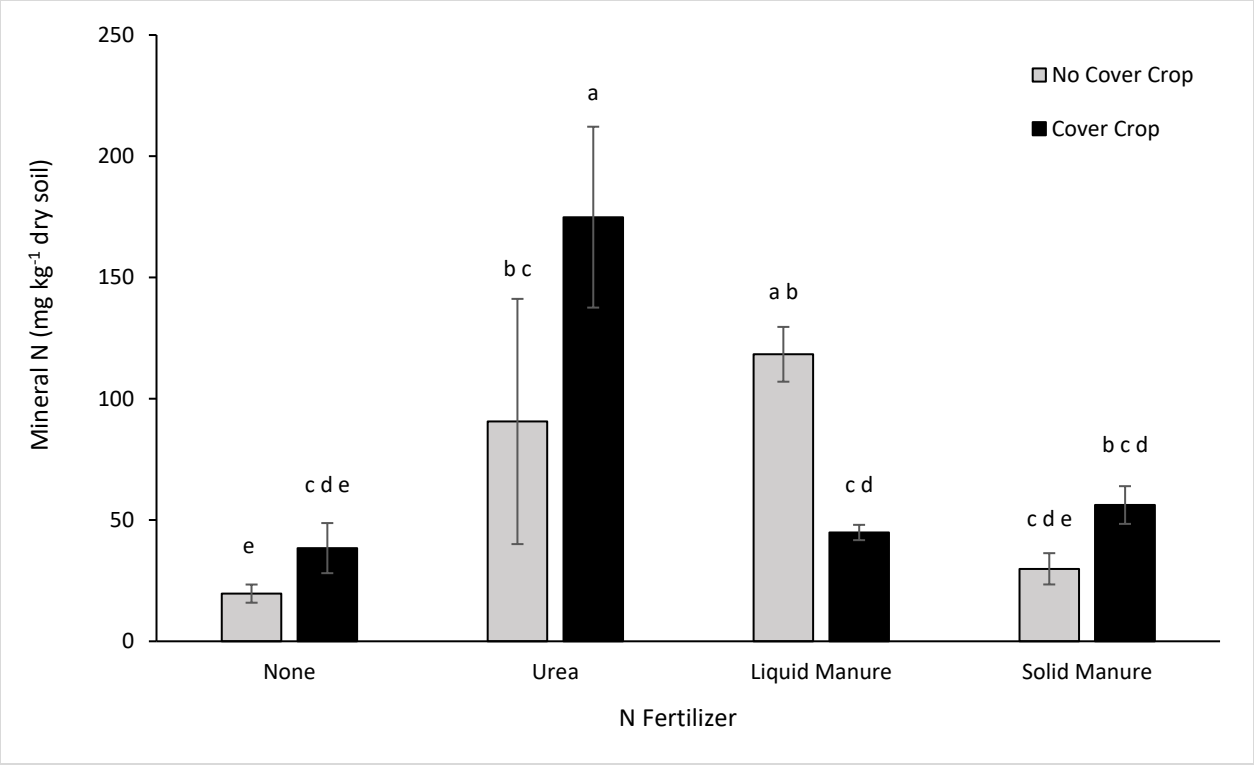


Figure 3-2. Soil mineral N concentration as affected by the interaction of N fertilizer × cover crop treatments after FTC 2. Data are the mean values (n=24) with standard error bars. Means with different letters are significantly different at $p<0.05$.

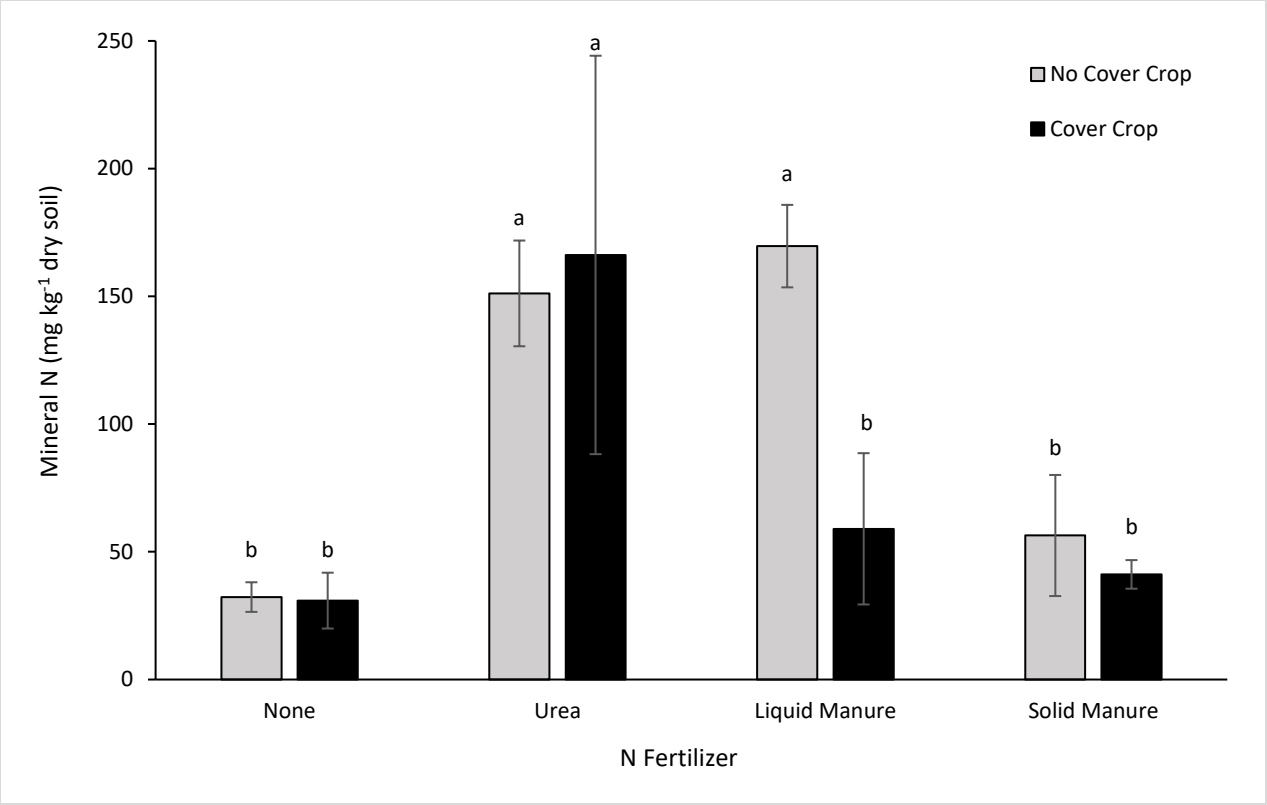


Figure 3-3. Soil mineral N concentration as affected by the interaction of N fertilizer × cover crop treatments after FTC 3. Data are the mean values (n=24) with standard error bars. Means with different letters are significantly different at $p<0.05$.

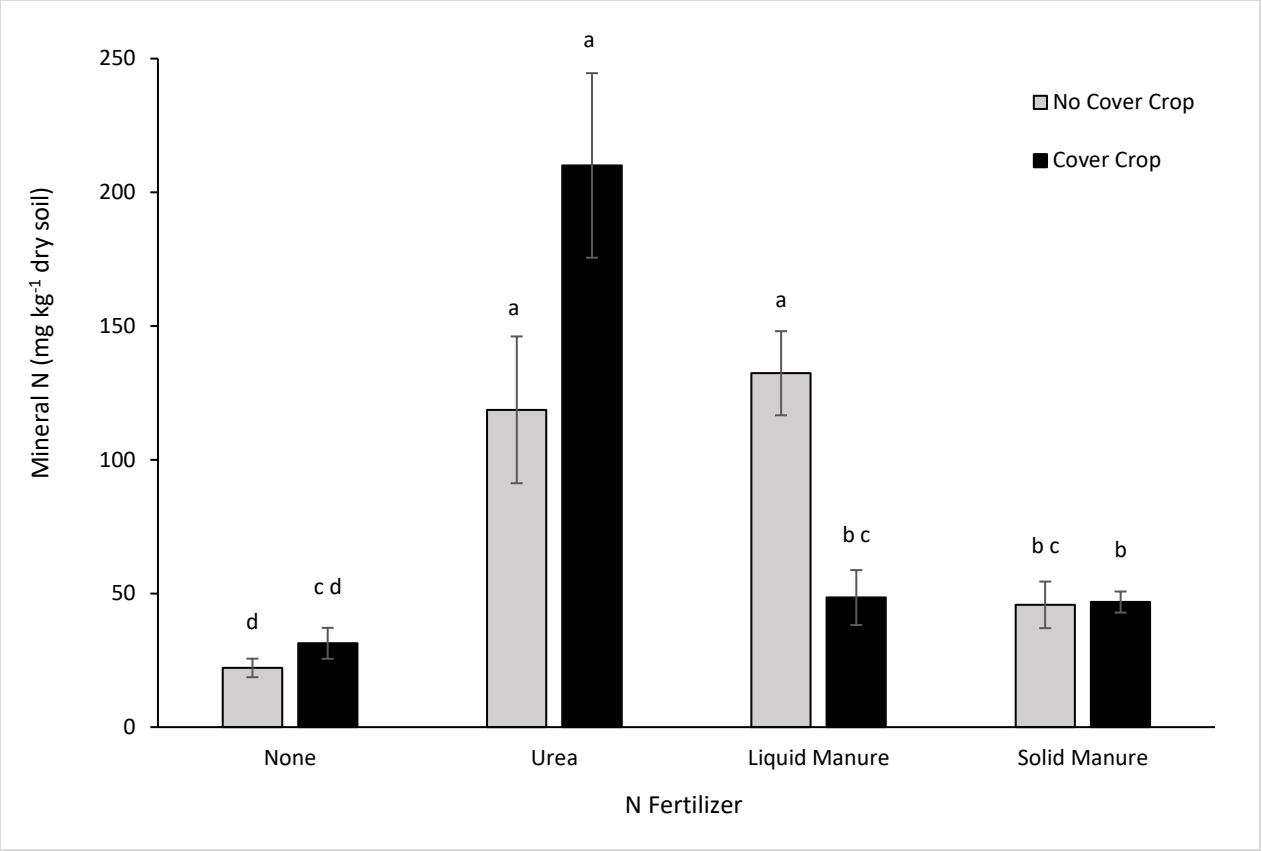


Figure 3-4. Soil mineral N concentration as affected by the interaction of N fertilizer × cover crop treatments averaged across all FTCs. Data are the mean values (n=72) with standard error bars. Means with different letters are significantly different at $p<0.05$.

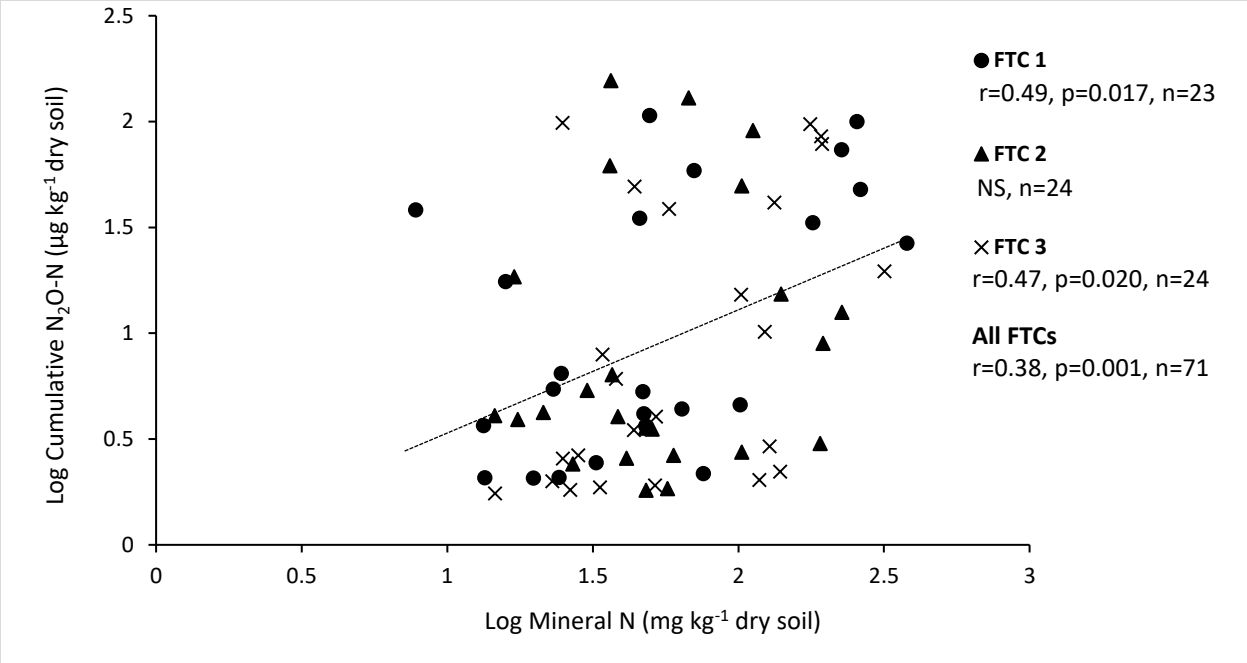


Figure 3-5. Linear relationship of cumulative N_2O production and soil mineral N concentration for each FTC after a 9 h thaw period. The dashed line represents the linear trend of three FTCs. Both N_2O and mineral N values were log-transformed. The Pearson correlation coefficients (r), the significance level (p), and the sample size (n) are shown for each FTC separately as well as the combined values across all FTCs. Non-significant r values ($p>0.05$) are denoted as NS.

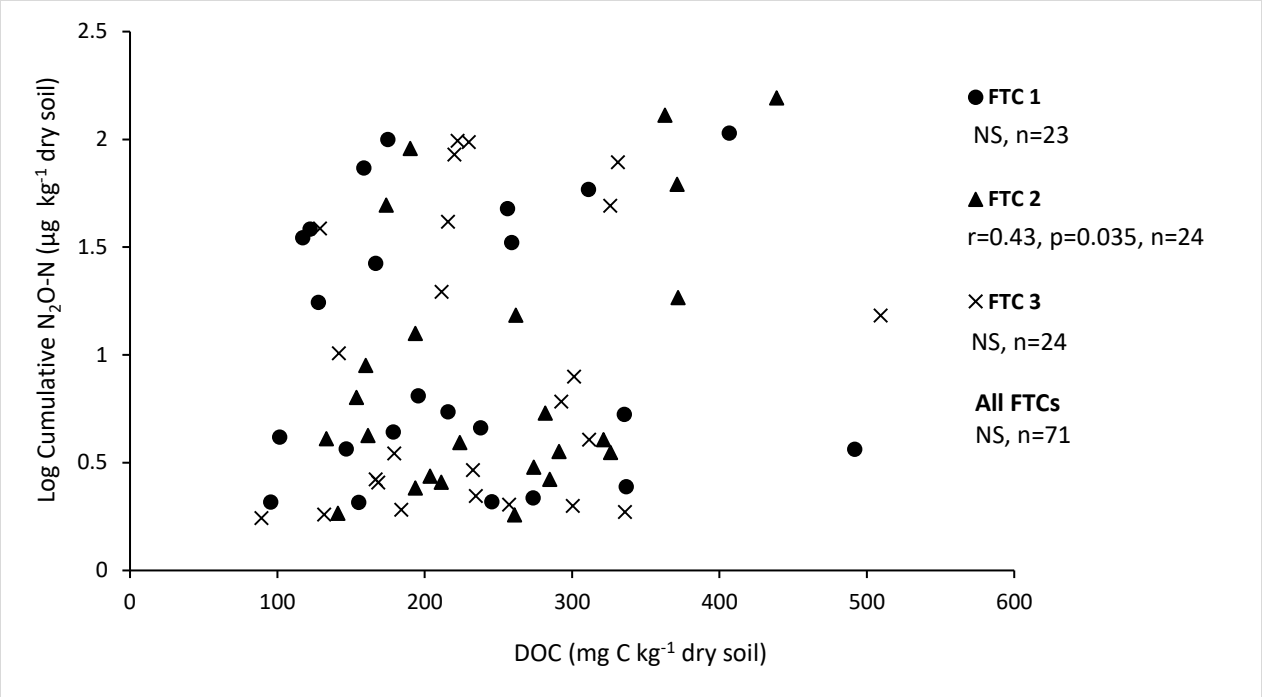


Figure 3-6. Linear relationship of cumulative N_2O production and soil DOC concentration for each FTC after a 9 h thaw period. Cumulative N_2O values were log-transformed. The Pearson correlation coefficients (r), the significance level (p), and the sample size (n) are shown for each FTC separately as well as the combined values across all FTCs. Non-significant r values ($p>0.05$) are denoted as NS.

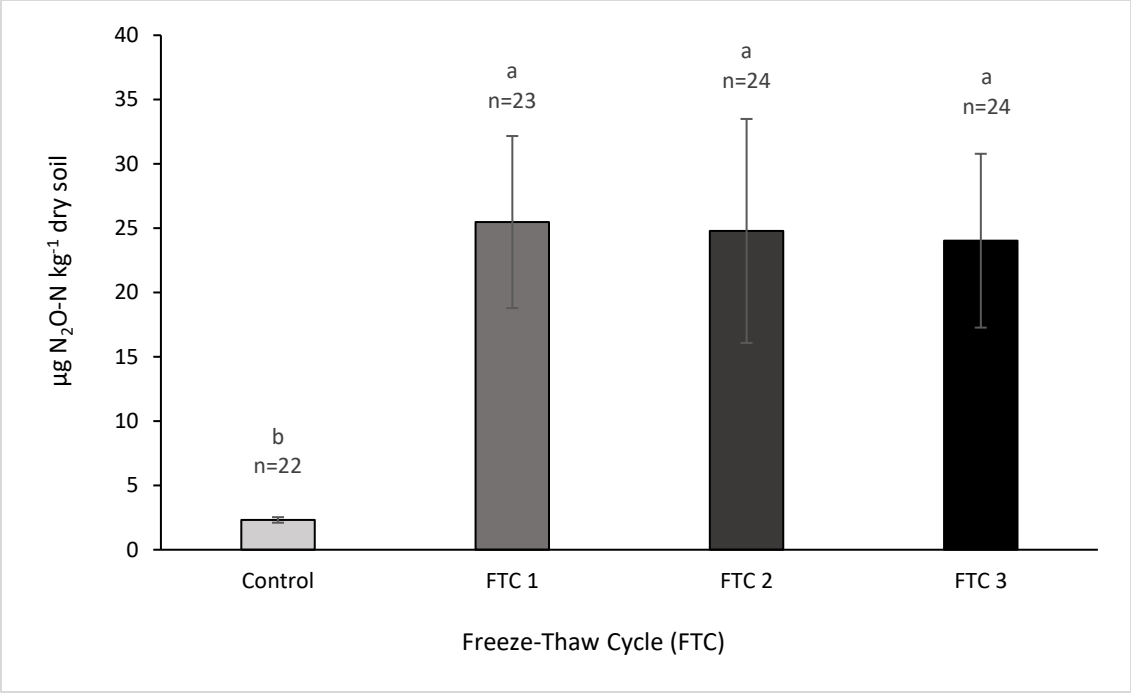


Figure 3-7. Cumulative N_2O production at the end of the 9 h thaw period for each FTC. Data are the mean values with standard error bars, pooled among N fertilizer and cover crop treatments. Sample sizes (n) are shown above each bar. Means with different letters are significantly different at $p<0.05$.

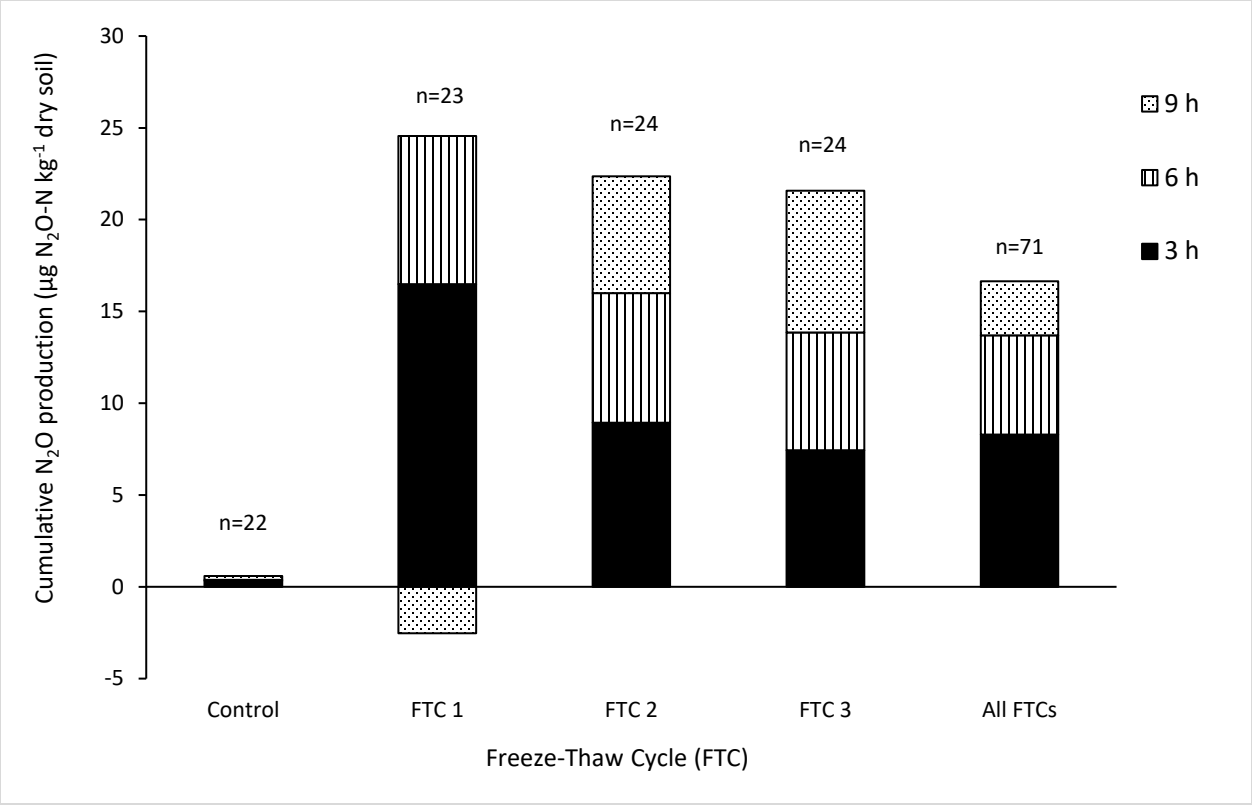


Figure 3-8. Incremental N_2O production after 3, 6, and 9 h during the 9 h thaw period for each FTC separately and on average across all FTCs (excluding the control). Data were pooled among N fertilizer and cover crop treatments, and sample sizes (n) are indicated above each bar.

General Conclusions and Future Research

Overview of conclusions and recommendations

The significant finding of the field experiment (Chapter 2) was that fall application of liquid and solid manure resulted in ±7% change to the system-level N balance from fall to spring. Planting a cover crop in soils with fall-applied manure did not alter the manure N losses incurred over winter. Average wheat yields were 11–14% less in the subplots that received no additional N fertilizer than those with 100 kg N ha^{-1} of urea in spring, indicating that there was insufficient residual N supplied by the fall-applied manure to the spring wheat crop. These results contradict the CRAAQ (2010) guidelines, which suggest that farmers will have between 20–70% of the total applied N from fall-applied solid manure left by the next spring as a "N credit" for the subsequent crop. From my work, it is clear that very little or no fall-applied manure N remains for the next crop. This finding has agronomic and economic implications for farmers who apply manure N in fall for convenience and practical reasons and expect that frozen winter conditions will conserve some of the N for the following spring crop.

Fall application of manure in cold humid temperate regions has a greater risk of losing than conserving its N fertilizer value. Since N is the most limiting nutrient for grain crops in cold humid temperate regions, it represents an economic loss for the farm because the producer will either lose yield or will need to purchase additional N fertilizer to reach a profitable yield. Applying manure in the fall is also environmentally risky, because the N loss occurs through gaseous emissions and leaching that pollute the atmosphere and surrounding bodies of water (De Jong et al. 2009; Rasouli et al. 2014). Therefore, producers should be discouraged from applying manure in fall to sandy-loam soils in cold humid temperate regions such as southwestern Québec. Instead, manure can be applied in the spring, preferably in split amounts during the

period of active crop growth, to ensure that most of the available N (NH_4^+) input is taken up by the crop. Government-enforced policies and non-compliance fines that deter fall manure application in at-risk regions may be the best way to reduce the practice of applying manure in fall.

The controlled experiment in Chapter 3 confirmed that well-fertilized soils are very likely to lose N as N_2O during freeze-thaw cycles that occur during the non-growing season. The freeze-thaw effect resulted in a remarkable 937–1000% increase in N_2O production compared to the unfrozen controls. Although N_2O production was not related to the experimental treatments (N fertilizer sources and cover crops) in this study, the sandy loam soil investigated was relatively rich in soil mineral N and DOC concentration, meaning there was no limitation to N_2O production by microorganisms even in soil at -4°C.

Soils in southwestern Québec have some of the highest levels of residual (reactive) soil N in all of Canada (Drury et al. 2016) and this area is also expected to have an increased number of freeze-thaw events during the winter months (Logan et al. 2011), resulting in more N_2O emissions during the non-growing season. While applying N fertilizers in fall, whether as urea or manure, increases the amount of reactive soil mineral N, most of the N_2O production resulting from freeze-thaw events is environmentally-driven, and management practices alone are not enough to prevent it.

Ideas for future experimental work

For future field experiments it would be valuable to include sites with contrasting winter climate conditions, soil types, and snow cover depths to compare the retention of fall-applied N combined with cover cropping under different winter conditions. In addition, my study only

looked at two types of manure (dairy slurry and semi-composted cattle manure). However, manure has diverse physico-chemical properties, based on factors such as animal species, diet, storage conditions, application procedures, and bedding material, that influence the mineralizable N pool and overall N fertilizer value of manure (Eghball 2000; Van Kessel and Reeves 2002; Sharifi et al. 2014). Future experiments should compare the N retention of other manures that are commonly applied to farm fields in fall, such as swine and poultry manure.

It was also difficult to fully assess the influence of the cover crop in the field study since almost all the above ground biomass at the Lods site was removed due to grazing migratory geese. At both sites, the cover crops were planted late (after mid-September), so the amount of time the cover crop had to accumulate biomass and uptake N before soil freeze-up in early December was minimal. Therefore, in temperate regions it may only be useful to plant a cover crop after wheat, barley, or other crops that are generally harvested in August, as opposed to crops such as soybeans and corn that are harvested in early to late fall. Future work could involve comparing earlier and later cover crop planting dates, and varying the time between manure application and cover crop planting.

The results from the laboratory freeze-thaw experiment indicated that mitigation of N_2O production from soils that undergo freeze-thaw events must involve a deeper understanding of physiology, genetics, and regulatory mechanisms of key N_2O-producing microbes, particularly nitrifiers and denitrifiers. Tools or soil conditions that enhance the expression and activity of the bacterial N_2O reductase enzymes, coded for by the *nosZ* genes, will result in more conversion of N_2O to N_2. Identifying novel microbes that are capable of reducing N_2O to N_2, and inoculating soils with these microbes, may also help prevent soil N_2O emissions. Future freeze-thaw experiments could use quantitative PCR and next-generation sequencing methods to monitor

N_2O-relevant biomarkers (eg. *nirK, nirS, narG, nosZ)*, and link these with N_2O flux measurements, to better understand the spatial and temporal conditions of N_2O-producing/consuming microbes. Upscaling microbial data to landscape-scale processes will be an important factor in future N_2O mitigation strategies on agricultural land.

References

Adeli A, Tewolde H, Jenkins JN, Rowe DE. 2011. Cover crop use for managing broiler litter applied in the fall. Agronomy Journal. 103(1):200-210.

Antil RS, Bar-Tal A, Fine P, Hadas A. 2011. Predicting nitrogen and carbon mineralization of composted manure and sewage sludge in soil. Compost Science & Utilization. 19(1):33-43.

Baggs EM, Watson CA, Rees RM. 2000. The fate of nitrogen from incorporated cover crop and green manure residues. Nutrient Cycling in Agroecosystems. 56(2):153-163.

Barzegar AR, Yousefi A, Daryashenas A. 2002. The effect of addition of different amounts and types of organic materials on soil physical properties and yield of wheat. Plant and Soil. 247(2):295-301.

Basche AD, Miguez FE, Kaspar TC, Castellano MJ. 2014. Do cover crops increase or decrease nitrous oxide emissions? A meta-analysis. Journal of Soil and Water Conservation. 69(6):471-482.

Beaulieu MS. 2004. Manure management in Canada. In: Division SC-A, editor. Ottawa, Ontario: Farm Environmental Management in Canada. p. 7-52.

Bechmann ME, Kleinman PJA, Sharpley AN, Saporito LS. 2005. Freeze–thaw effects on phosphorus loss in runoff from manured and catch-cropped soils. Journal of Environmental Quality. 34(6):2301-2309.

Bernal MP, Alburquerque JA, Moral R. 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresource Technology. 100(22):5444-5453.

Blair R, Jacob JP, Ibrahim S, Wang P. 1999. A quantitative assessment of reduced protein diets and supplements to improve nitrogen utilization. Journal of Applied Poultry Research. 8(1):25-47.

Blunden J, Aneja VP. 2008. Characterizing ammonia and hydrogen sulfide emissions from a swine waste treatment lagoon in North Carolina. Atmospheric Environment. 42(14):3277-3290.

Böhm W. 1979. Techniques of root washing. Methods of studying root systems. Berlin, Heidelberg: Springer Berlin Heidelberg. p. 115-124.

Bouwman L, Goldewijk KK, Van Der Hoek KW, Beusen AHW, Van Vuuren DP, Willems J, Rufino MC, Stehfest E. 2013. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. Proceedings of the National Academy of Sciences. 110(52):20882-20887.

Butterbach-Bahl K, Baggs L, Dannenmann M, Kiese R, Zechmeister-Boltenstern S. 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philosophical Transactions of the Royal Society B: Biological Sciences. 368:20130122.

Cambardella CA, Moorman TB, Singer JW. 2010. Soil nitrogen response to coupling cover crops with manure injection. Nutrient Cycling in Agroecosystems. 87(3):383-393.

Cavigelli MA, Parkin TB. 2012. Chapter 9 - Cropland management contributions to greenhouse gas flux: Central and Eastern U.S. . In: Franzluebbers AJ, Follett RF, editors. Managing Agricultural Greenhouse Gases. San Diego: Academic Press. p. 129-165.

Chadwick DR, John F, Pain BF, Chambers BJ, Williams J. 2000. Plant uptake of nitrogen from the organic nitrogen fraction of animal manures: a laboratory experiment. Journal of Agricultural Science. 134:159-168.

Chambers PA, Guy M, Roberts ES, Charlton MN, Kent R, Gagnon C, Grove G, Foster N. 2001. Nutrients and their impact on the Canadian environment. Agriculture and Agri-Food Canada, Environment Canada, Fisheries and Oceans Canada, Health Canada and Natural Resources Canada. 241 p.

Chantigny MH, Angers DA, Rochette P, Pomar C, Pelster DE. 2014. Evidencing overwinter loss of residual organic and clay-fixed nitrogen from spring-applied, ^{15}N-labelled pig slurry. Canadian Journal of Soil Science. 94(1):1-8.

Chantigny MH, Bittman S, Larney FJ, Lapen D, Hunt DE, Goyer C, Angers DA. 2019. A multi-region study reveals high overwinter loss of fall-applied reactive nitrogen in cold and frozen soils. Canadian Journal of Soil Science.1-10.

Chapuis-Lardy L, Wrage-Mönnig N, Metay A, Chotte J-L, Bernoux M. 2007. Soils, a sink for N_2O? A review. Global Change Biology. 13(1):1-17.

Chen BQ, Liu EK, Tian QZ, Yan CR, Zhang YQ. 2014. Soil nitrogen dynamics and crop residues. A review. Agronomy for Sustainable Development. 34(2):429-442.

Chen Y, Tessier S, MacKenzie AF, Laverdière MR. 1995. Nitrous oxide emission from an agricultural soil subjected to different freeze-thaw cycles. Agriculture, Ecosystems & Environment. 55(2):123-128.

Clark K, Chantigny MH, Angers DA, Rochette P, Parent L-É. 2009. Nitrogen transformations in cold and frozen agricultural soils following organic amendments. Soil Biology and Biochemistry. 41(2):348-356.

Cober JR, Macrae ML, Van Eerd LL. 2018. Nutrient release from living and terminated cover crops under variable freeze-thaw cycles. Agronomy Journal. 110(3):1036-1045.

Congreves KA, Wagner-Riddle C, Si BC, Clough TJ. 2018. Nitrous oxide emissions and biogeochemical responses to soil freezing-thawing and drying-wetting. Soil Biology and Biochemistry. 117:5-15.

CRAAQ. 2010. Centre de référence en Agriculture et Agroalimentaire du Québec. Guide de référence en fertilisation, (In French). 2nd ed. Québec, Canada: Centre de référence en Agriculture et Agroalimentaire du Québec. p. 519.

Crouse DA, Crozier CR, Smyth TJ, Hicks K. 2018. Livestock and poultry manure production rates and nutrient content. In: Watson W, editor. North Carolina Agricultural Chemicals Manual. Chapel Hill, North Carolina: The University of North Carolina Press. p. 1-70.

De Jong R, Drury CF, Yang JY, Campbell CA. 2009. Risk of water contamination by nitrogen in Canada as estimated by the IROWC-N model. Journal of Environmental Management. 90(10):3169-3181.

DeLuca TH, Keeney DR, McCarty GW. 1992. Effect of freeze-thaw events on mineralization of soil nitrogen. Biology and Fertility of Soils. 14(2):116-120.

Dietzel R, Wolfe D, Thies JE. 2011. The influence of winter soil cover on spring nitrous oxide emissions from an agricultural soil. Soil Biology & Biochemistry. 43(9):1989-1991.

Drinkwater LE, Cambardella CA, Reeder JD, Rice CW. 1996. Potentially mineralizable nitrogen as an indicator of biologically active soil nitrogen. Methods for Assessing Soil Quality. Madison, WI: Soil Science Society of America. p. 217-229.

Drinkwater LE, Wagoner P, Sarrantonio M. 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. Nature. 396:262-265.

Drury CF, Yang J, De Jong R, Huffman T, Reid K, Yang X, Bittman S, Desjardins R. 2016. Chapter 11: Nitrogen. In: Clearwater RL, Martin T, Hoppe T, editors. Environmental sustainability of Canadian agriculture: agri-environmental indicator report series – Report #4 Ottawa, ON: Agriculture and Agri-Food Canada. p. 113-120.

Eghball B. 1999. Liming effects of beef cattle feedlot manure or compost. Communications in Soil Science and Plant Analysis. 30(19-20):2563-2570.

Eghball B. 2000. Nitrogen mineralization from field-applied beef cattle feedlot manure or compost. Soil Science Society of America Journal. 64(6):2024-2030.

Eghball B, Power JF, Gilley JE, Doran JW. 1997. Nutrient, carbon, and mass loss during composting of beef cattle feedlot manure. Journal of Environmental Quality. 26(1):189-193.

Eghball B, Wienhold BJ, Gilley JE, Eigenberg RA. 2002. Mineralization of manure nutrients. Journal of Soil and Water Conservation. 57(6):470-473.

Ellert BH, Bettany JR. 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. Canadian Journal of Soil Science. 75(4):529-538.

Environment Canada. 2018. Monthly meterological summaries for Montreal/Dorval International Airport. Ottawa, ON, Canada: Atmospheric Environment Branch, Environment Canada.

Environment Quality Act, CQLR Q-2, r 26, s 31.

Fares A, Abbas F, Ahmad A, Deenik JL, Safeeq M. 2008. Response of selected soil physical and hydrological properties to manure amendment rates, levels, and types. Soil Science. 173(8):522-533.

Gagnon B, Robitaille R, Simard RR. 1999. Characterization of several on-farm and industrial composted materials. Canadian Journal of Soil Science. 79(1):201-210.

Gale ES, Sullivan DM, Cogger CG, Bary AI, Hemphill DD, Myhre EA. 2006. Estimating plant-available nitrogen release from manures, composts, and specialty products. J Environ Qual. 35(6):2321-2332.

Gao D, Zhang L, Liu J, Peng B, Fan Z, Dai W, Jiang P, Bai E. 2018. Responses of terrestrial nitrogen pools and dynamics to different patterns of freeze-thaw cycle: A meta-analysis. Global Change Biology.

Gillam KM, Zebarth BJ, Burton DL. 2008. Nitrous oxide emissions from denitrification and the partitioning of gaseous losses as affected by nitrate and carbon addition and soil aeration. Canadian Journal of Soil Science. 88(2):133-143.

Gordillo RM, Cabrera ML. 1997. Mineralizable nitrogen in broiler litter: I. effect of selected litter chemical characteristics. Journal of Environmental Quality. 26(6):1672-1679.

Griffiths BS, Spilles A, Bonkowski M. 2012. C:N:P stoichiometry and nutrient limitation of the soil microbial biomass in a grazed grassland site under experimental P limitation or excess. Ecological Processes. 1(1):6.

Han Z, Walter MT, Drinkwater LE. 2017. N_2O emissions from grain cropping systems: a meta-analysis of the impacts of fertilizer-based and ecologically-based nutrient management strategies. Nutrient Cycling in Agroecosystems. 107(3):335-355.

Hashemi M, Farsad A, Sadeghpour A, Weis SA, Herbert SJ. 2013. Cover-crop seeding-date influence on fall nitrogen recovery. Journal of Plant Nutrition and Soil Science. 176(1):69-75.

Haynes RJ, Naidu R. 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutrient Cycling in Agroecosystems. 51(2):123-137.

Henry HAL. 2007. Soil freeze–thaw cycle experiments: trends, methodological weaknesses and suggested improvements. Soil Biology and Biochemistry. 39(5):977-986.

Henry HAL. 2008. Climate change and soil freezing dynamics: historical trends and projected changes. Climatic Change. 87(3):421-434.

Holland EA, Robertson GP, Greenberg J, Groffman P, Boone R, Gosz J. 1999. Soil CO_2, N_2O, and CH_4 exchange. In: Robertson GP, Bledsoe CS, Coleman DC, Sollins P, editors. Standard soil methods for long-term ecological research. New York, New York, USA: Oxford University Press. p. 185-201.

Hu HW, Chen D, He JZ. 2015. Microbial regulation of terrestrial nitrous oxide formation: understanding the biological pathways for prediction of emission rates. FEMS Microbiology Reviews. 39(5):729-749.

Institut de la statistique du Québec. 2019. Area of field crops, yield per hectare and production, by administrative region, Québec, 2018. Québec, Québec: Gouvernement du Québec.

Jarecki MK, Parkin TB, Chan ASK, Kaspar TC, Moorman TB, Singer JW, Kerr BJ, Hatfield JL, Jones R. 2009. Cover crop effects on nitrous oxide emission from a manure-treated Mollisol. Agriculture Ecosystems & Environment. 134(1-2):29-35.

Jayasundara S, Wagner-Riddle C, Parkin G, Lauzon J, Fan MZ. 2010. Transformations and losses of swine manure ^{15}N as affected by application timing at two contrasting sites. Canadian Journal of Soil Science. 90(1):55-73.

Joseph G, Henry HAL. 2008. Soil nitrogen leaching losses in response to freeze–thaw cycles and pulsed warming in a temperate old field. Soil Biology and Biochemistry. 40(7):1947-1953.

Kariyapperuma KA, Wagner-Riddle C, Furon AC, Li CS. 2011. Assessing spring thaw nitrous oxide fluxes simulated by the DNDC model for agricultural soils. Soil Science Society of America Journal. 75(2):678-690.

Kool DM, Dolfing J, Wrage N, Van Groenigen JW. 2011. Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. Soil Biology and Biochemistry. 43(1):174-178.

Koponen HT, Flöjt L, Martikainen PJ. 2004. Nitrous oxide emissions from agricultural soils at low temperatures: a laboratory microcosm study. Soil Biology and Biochemistry. 36(5):757-766.

Kuo S, Sainju UM. 1998. Nitrogen mineralization and availability of mixed leguminous and non-leguminous cover crop residues in soil. Biology and Fertility of Soils. 26(4):346-353.

Kuzyakov Y, Friedel JK, Stahr K. 2000. Review of mechanisms and quantification of priming effects. Soil Biology & Biochemistry. 32(11-12):1485-1498.

Lange SF, Allaire SE, Castillo MAC, Dutilleul P. 2017. N_2O and CO_2 dynamics in a pasture soil across the frozen period. Canadian Journal of Soil Science. 97(3):497-511.

Larney FJ, Buckley KE, Hao XY, McCaughey WP. 2006. Fresh, stockpiled, and composted beef cattle feedlot manure: nutrient levels and mass balance estimates in Alberta and Manitoba. Journal of Environmental Quality. 35(6):2439-2439.

Li C, Aber J, Stange F, Butterbach-Bahl K, Papen H. 2000. A process-oriented model of N_2O and NO emissions from forest soils: 1. model development. Journal of Geophysical Research: Atmospheres. 105(D4):4369-4384.

Li X, Sørensen P, Olesen JE, Petersen SO. 2016. Evidence for denitrification as main source of N_2O emission from residue-amended soil. Soil Biology and Biochemistry. 92:153-160.

Liu J, Khalaf R, Ulen B, Bergkvist G. 2013. Potential phosphorus release from catch crop shoots and roots after freezing-thawing. Plant and Soil. 371(1-2):543-557.

Liu R, Hu H, Suter H, Hayden HL, He J, Mele P, Chen D. 2016. Nitrification is a primary driver of nitrous oxide production in laboratory microcosms from different land-use soils. Frontiers in Microbiology. 7(1373):1-10.

Logan T, Charron I, Chaumont D, Houle D. 2011. Atlas of climate scenarios for Québec forests. Ouranos; Ministère de ressources naturelles et de la faune du Québec (MRNF).1-132.

Loria ER, Sawyer JE. 2005. Extractable soil phosphorus and inorganic nitrogen following application of raw and anaerobically digested swine manure. Agronomy Journal. 97(3):879-885.

Lorimer J, Powers W, Sutton A. 2000. Manure management systems series: manure characteristics. In: University IS, editor. 2 ed. Ames, Iowa: MidWest Plan Service. p. 1-24.

Lozier TM, Macrae ML. 2017. Potential phosphorus mobilization from above-soil winter vegetation assessed from laboratory water extractions following freeze-thaw cycles. Canadian Water Resources Journal. 42(3):276-288.

Ludwig B, Wolf I, Teepe R. 2004. Contribution of nitrification and denitrification to the emission of N_2O in a freeze-thaw event in an agricultural soil. Journal of Plant Nutrition and Soil Science. 167(6):678-684.

Mahal NK, Castellano MJ, Miguez FE. 2018. Conservation agriculture practices increase potentially mineralizable nitrogen: a meta-analysis. Soil Science Society of America Journal. 82(5):1270-1278.

Mason JR, Clark L, Bean NJ. 1993. White plastic flags repel snow geese (*Chen caerulescens*). Crop Protection. 12(7):497-500.

McKay HV, Milsom TP, Feare CJ, Ennis DC, O'Connell DP, Haskell DJ. 2001. Selection of forage species and the creation of alternative feeding areas for dark-bellied brent geese *Branta bernicla bernicla* in southern UK coastal areas. Agriculture Ecosystems & Environment. 84(2):99-113.

Morkved PT, Dorsch P, Henriksen TM, Bakken LR. 2006. N_2O emissions and product ratios of nitrification and denitrification as affected by freezing and thawing. Soil Biology & Biochemistry. 38(12):3411-3420.

Morvan T, Nicolardot B, Pean L. 2006. Biochemical composition and kinetics of C and N mineralization of animal wastes: a typological approach. Biology and Fertility of Soils. 42(6):513-522.

N'Dayegamiye A, Whalen JK, Tremblay G, Nyiraneza J, Grenier M, Drapeau A, Bipfubusa M. 2015. The benefits of legume crops on corn and wheat yield, nitrogen nutrition, and soil properties improvement. Agronomy Journal. 107(5):1653-1665.

Øgaard AF. 2015. Freezing and thawing effects on phosphorus release from grass and cover crop species. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science. 65(6):529-536.

Osterholz WR, Liebman M, Castellano MJ. 2018. Can soil nitrogen dynamics explain the yield benefit of crop diversification? Field Crops Research. 219:33-42.

Owens LB, Edwards WM, Shipitalo MJ. 1995. Nitrate leaching through lysimeters in a corn-soybean rotation. Soil Science Society of America Journal. 59(3):902-907.

Pattey E, Blackburn LG, Strachan IB, Desjardins R, Dow D. 2008. Spring thaw and growing season N_2O emissions from a field planted with edible peas and a cover crop. Canadian Journal of Soil Science. 88(2):241-249.

Pelster DE, Chantigny MH, Rochette P, Angers DA, Laganière J, Zebarth B, Goyer C. 2013. Crop residue incorporation alters soil nitrous oxide emissions during freeze–thaw cycles. Canadian Journal of Soil Science. 93(4):415-425.

Perazzolo F, Mattachini G, Riva E, Provolo G. 2017. Nutrient losses during winter and summer storage of separated and unseparated digested cattle slurry. Journal of Environmental Quality. 46(4):879-888.

Rasouli S, Whalen JK, Madramootoo CA. 2014. Review: Reducing residual soil nitrogen losses from agroecosystems for surface water protection in Québec and Ontario, Canada: best management practices, policies and perspectives. Canadian Journal of Soil Science. 94(2):109-127.

Reay DS, Davidson EA, Smith KA, Smith P, Melillo JM, Dentener F, Crutzen PJ. 2012. Global agriculture and nitrous oxide emissions. Nature Climate Change. 2:410.

Risk N, Snider D, Wagner-Riddle C. 2013. Mechanisms leading to enhanced soil nitrous oxide fluxes induced by freeze-thaw cycles. Canadian Journal of Soil Science. 93(4):401-414.

Röver M, Heinemeyer O, Kaiser E-A. 1998. Microbial induced nitrous oxide emissions from an arable soil during winter. Soil Biology and Biochemistry. 30(14):1859-1865.

SAS Institute I. 2013. SAS/ACCESS 9.4. Cary, NC, USA.

Seman-Varner R, Varco J, O'Rourke M. 2017. Nitrogen benefits of winter cover crop and fall-applied poultry litter to corn. Agronomy Journal. 109(6):2881-2888.

Sharifi M, Zebarth BJ, Miller JJ, Burton DL, Grant CA. 2014. Soil nitrogen mineralization in a soil with long-term history of fresh and composted manure containing straw or wood-chip bedding. Nutrient Cycling in Agroecosystems. 99(1-3):63-78.

Shelton RE, Jacobsen KL, McCulley RL. 2018. Cover crops and fertilization alter nitrogen loss in organic and conventional conservation agriculture systems. Frontiers in Plant Science. 8(2260):1-14.

Shirani H, Hajabbasi MA, Afyuni M, Hemmat A. 2002. Effects of farmyard manure and tillage systems on soil physical properties and corn yield in central Iran. Soil and Tillage Research. 68(2):101-108.

Shriver JA, Carter SD, Sutton AL, Richert BT, Senne BW, Pettey LA. 2003. Effects of adding fiber sources to reduced-crude protein, amino acid-supplemented diets on nitrogen excretion, growth performance, and carcass traits of finishing pigs. Journal of Animal Science. 81(2):492-502.

Sims GK, Ellsworth TR, Mulvaney RL. 1995. Microscale determination of inorganic nitrogen in water and soil extracts. Communications in Soil Science and Plant Analysis. 26(1-2):303-316.

Singer JW, Cambardella CA, Moorman TB. 2008. Enhancing nutrient cycling by coupling cover crops with manure injection. Agronomy Journal. 100(6):1735-1739.

Sommer SG, Clough TJ, Balaine N, Hafner SD, Cameron KC. 2017. Transformation of organic matter and the emissions of methane and ammonia during storage of liquid manure as affected by acidification. Journal of Environmental Quality. 46(3):514-521.

Song Y, Zou YC, Wang GP, Yu XF. 2017. Altered soil carbon and nitrogen cycles due to the freeze-thaw effect: a meta-analysis. Soil Biology & Biochemistry. 109:35-49.

Sørensen P, Weisbjerg MR, Lund P. 2003. Dietary effects on the composition and plant utilization of nitrogen in dairy cattle manure. Journal of Agricultural Science. 141(1):79-91.

Sun G, Zhu-Barker X, Chen DM, Liu L, Zhang NN, Shi CG, He LP, Lei YB. 2017. Responses of root exudation and nutrient cycling to grazing intensities and recovery practices in an alpine meadow: an implication for pasture management. Plant and Soil. 416(1-2):515-525.

Tatti E, Goyer C, Chantigny M, Wertz S, Zebarth BJ, Burton DL, Filion M. 2014. Influences of over winter conditions on denitrification and nitrous oxide-producing microorganism abundance and structure in an agricultural soil amended with different nitrogen sources. Agriculture, Ecosystems & Environment. 183:47-59.

Teepe R, Brumme R, Beese F. 2001. Nitrous oxide emissions from soil during freezing and thawing periods. Soil Biology and Biochemistry. 33(9):1269-1275.

Thomas B, Sharifi M, Whalen JK, Chantigny M. 2015. Mineralizable nitrogen responds differently to manure type in contrasting soil textures. Soil Science Society of America Journal. 79(5):1396-1405.

Thomas BW, Hao XY, Larney FJ, Goyer C, Chantigny MH, Charles A. 2017. Non-legume cover crops can increase non-growing season nitrous oxide emissions. Soil Science Society of America Journal. 81(1):189-199.

Thorup-Kristensen K, Magid J, Jensen LS. 2003. Catch crops and green manures as biological tools in nitrogen management in temperate zones. Advances in Agronomy, Vol 79. 79:227-302.

Van Kessel J, Reeves J. 2002. Nitrogen mineralization potential of dairy manures and its relationship to composition. Biology and Fertility of Soils. 36(2):118-123.

Wagner-Riddle C, Congreves KA, Abalos D, Berg AA, Brown SE, Ambadan JT, Gao XP, Tenuta M. 2017. Globally important nitrous oxide emissions from croplands induced by freeze-thaw cycles. Nature Geoscience. 10(4):279-283.

Wagner-Riddle C, Furon A, McLaughlin NL, Lee I, Barbeau J, Jayasundara S, Parkin G, Von Bertoldi P, Warland J. 2007. Intensive measurement of nitrous oxide emissions from a

corn-soybean-wheat rotation under two contrasting management systems over 5 years. Global Change Biology. 13(8):1722-1736.

Wagner-Riddle C, Hu QC, van Bochove E, Jayasundara S. 2008. Linking nitrous oxide flux during spring thaw to nitrate denitrification in the soil profile. Soil Science Society of America Journal. 72(4):908-916.

Wagner-Riddle C, Thurtell GW. 1998. Nitrous oxide emissions from agricultural fields during winter and spring thaw as affected by management practices. Nutrient Cycling in Agroecosystems. 52(2):151-163.

Watson CA, Atkinson D, Gosling P, Jackson LR, Rayns FW. 2002. Managing soil fertility in organic farming systems. Soil Use and Management. 18(s1):239-247.

Wertz S, Goyer C, Zebarth BJ, Tatti E, Burton DL, Chantigny MH, Filion M. 2016. The amplitude of soil freeze-thaw cycles influences temporal dynamics of N_2O emissions and denitrifier transcriptional activity and community composition. Biology and Fertility of Soils. 52(8):1149-1162.

Whalen JK, Chang C, Clayton GW, Carefoot JP. 2000. Cattle manure amendments can increase the pH of acid soils. Soil Science Society of America Journal. 64(3):962-966.

Whalen JK, Parmelee RW, Edwards CA. 1998. Population dynamics of earthworm communities in corn agroecosystems receiving organic or inorganic fertilizer amendments. Biology and Fertility of Soils. 27(4):400-407.

Whalen JK, Thomas BW, Sharifi M. 2019. Novel practices and smart technologies to maximize the nitrogen fertilizer value of manure for crop production in cold humid temperate regions. Advances in Agronomy. 153:1-85.

Williams MR, Feyereisen GW, Beegle DB, Shannon RD, Folmar GJ, Bryant RB. 2011. Manure application under winter conditions: nutrient run-off and leaching losses. Transactions of the Asabe. 54(3):891-899.

Yanai Y, Toyota K, Okazaki M. 2004. Effects of successive soil freeze-thaw cycles on soil microbial biomass and organic matter decomposition potential of soils. Soil Science and Plant Nutrition. 50(6):821-829.

Yang JY, Huffman EC, Drury CF, Yang XM, De Jong R. 2011. Estimating the impact of manure nitrogen losses on total nitrogen application on agricultural land in Canada. Canadian Journal of Soil Science. 91(1):107-122.

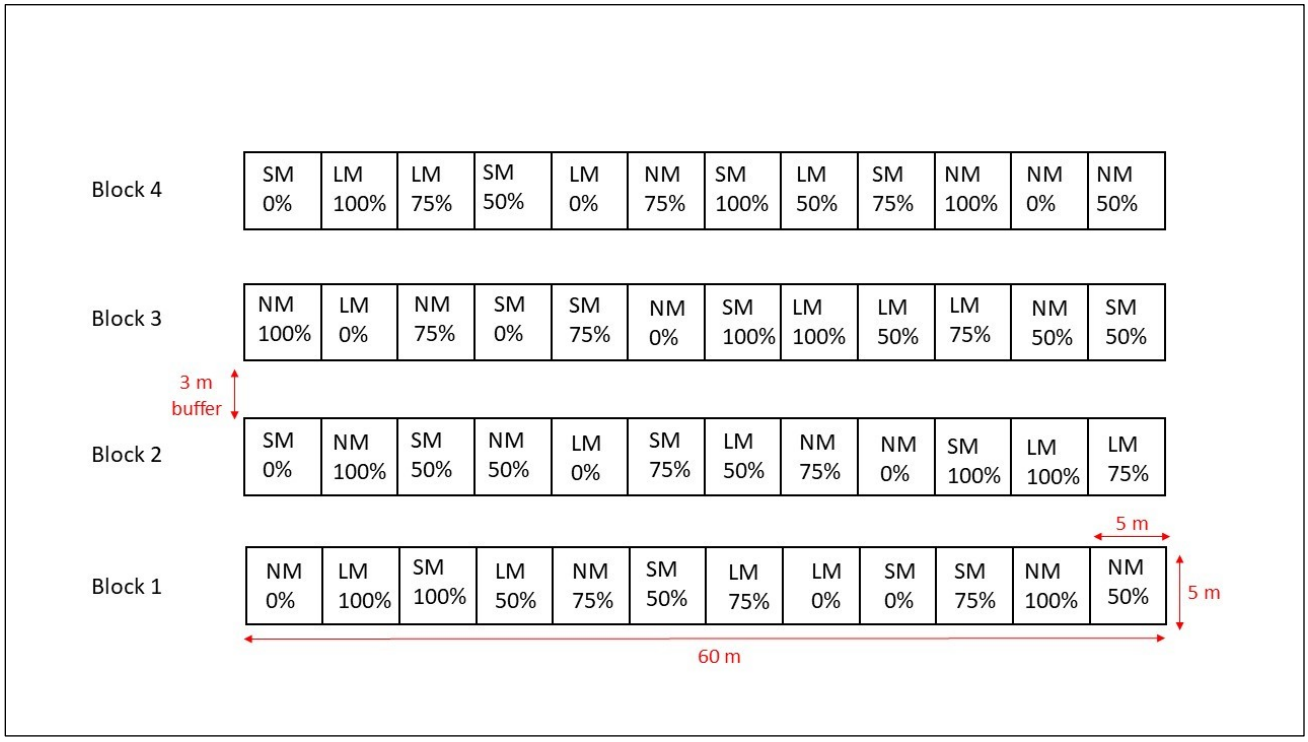
Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth stages of cereals. Weed Research. 14(6):415-421.

Zhang JB, Yang JS, Yao RJ, Yu SP, Li FR, Hou XJ. 2014. The effects of farmyard manure and mulch on soil physical properties in a reclaimed coastal tidal flat salt-affected soil. Journal of Integrative Agriculture. 13(8):1782-1790.

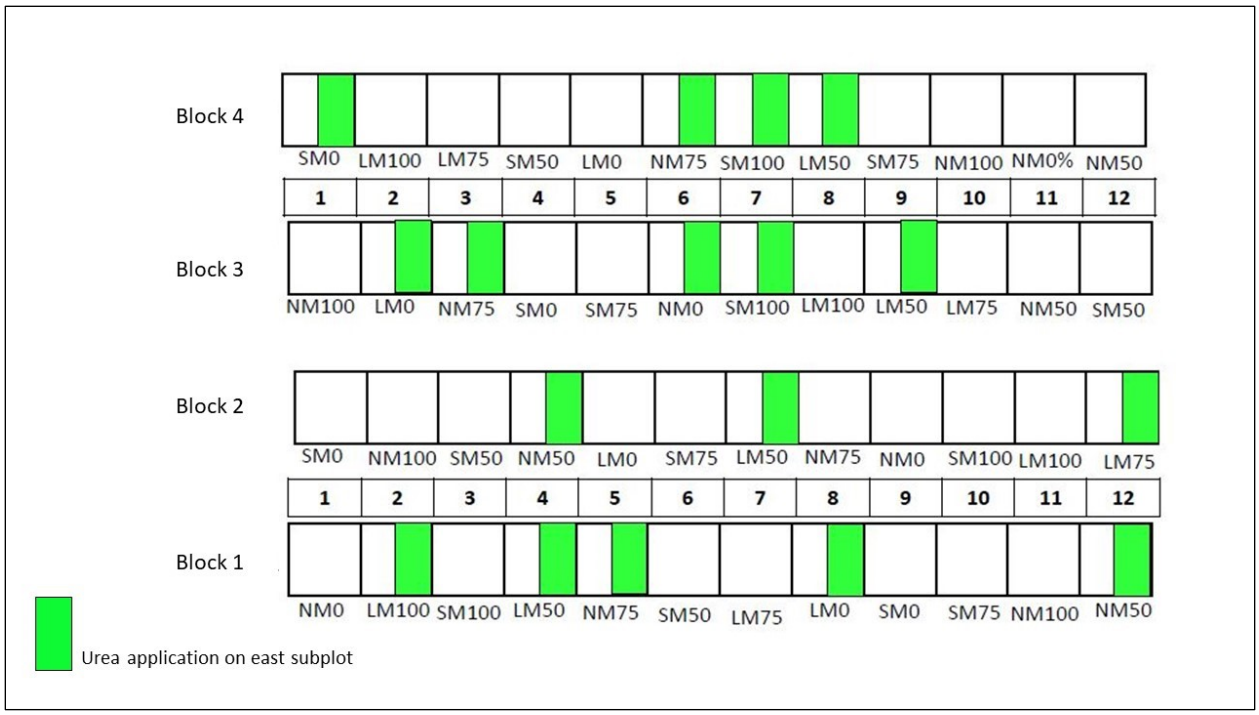
Zhang X, Davidson EA, Mauzerall DL, Searchinger TD, Dumas P, Shen Y. 2015. Managing nitrogen for sustainable development. Nature. 528:51-59.

Zhou YX, Berruti F, Greenhalf C, Henry HAL. 2017. Combined effects of biochar amendment, leguminous cover crop addition and snow removal on nitrogen leaching losses and nitrogen retention over winter and subsequent yield of a test crop (*Eruca sativa* L.). Soil Biology & Biochemistry. 114:220-228.

Appendix A: General plot set-up for the manure and cover crop treatments at the Lods and Laval sites. Manure treatments were liquid manure (LM) applied at 28,000 L ha^{-1}, solid manure (SM) applied at 30,000 kg ha^{-1}, and a no manure control (NM). Cover crop treatments were a 100% pure stand of annual ryegrass (*Lolium multiflorum* Lam.) seeded at 34 kg ha^{-1}, a bi-culture of 75% annual ryegrass and 25% hairy vetch (*Vicia villosa* Roth) seeded at 25 kg ha^{-1} and 9 kg ha^{-1} respectively, a bi-culture of 50% ryegrass and 50% vetch seeded at 17 kg ha^{-1} each, and no cover crop (0%).



Appendix B: The same plot set-up as Appendix A but showing the location of the subplots at the Lods site that received 100 kg N ha^{-1} split application of urea after seeding spring wheat (*Triticum aestivum* L., cv. AC Walton). The shaded subplots received the urea application on the east side of the plots, while the unshaded plots received the urea application on the west side.



Appendix C: Average monthly air temperature, soil temperature, and soil moisture for the Lods and Laval sites from October 2017 – August 2018.

Year	Month	Average Monthly Air Temperature (°C)*	Average Monthly Soil Temperature (°C, 5 cm depth)†		Average Monthly Soil Moisture (%VWC, 5 cm depth)†	
			Lods	Laval	Lods	Laval
2017	October	13.3	10.6	11.1	13.9	29.1
2017	November	0.4	1.7	1.8	13.0	28.2
2017	December	-8.6	-0.8	-0.6	3.6	14.4
2018	January	-9.7	-1.0	-0.4	4.0	21.5
2018	February	-4.6	-0.7	-0.2	7.6	30.3
2018	March	-0.9	-0.3	0.0	11.3	33.7
2018	April	3.8	3.6	4.2	3.1	32.6
2018	May	15.8	15.7	14.8	6.9	25.4
2018	June	19.2	19.7	21.8	7.8	9.8
2018	July	24.2	24.9	26.0	4.0	7.8
2018	August	23.0	22.3	22.0	5.0	8.3

**Based on Environment Canada monthly climate summary from Montreal Trudeau International Airport*
†Based on data from portable weather stations (WatchDog 2000 Series, Spectrum Technologies Inc, Aurora, IL, USA) installed at Lods and Laval