

The Impact of Cover Cropping on Soil Nitrogen Availability, Crop Nitrogen Use, and Nitrous Oxide Emissions in a Prairie Potato—Grain Crop Rotation

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ABSTRACT

In prairie regions of Canada, growers are beginning to adopt cover cropping. Yet several questions remain unanswered on the viability, impact, and benefits associated with cover cropping in a short growing season region like the prairies. To address this gap, a four-year fully phased crop rotation trial consisting of wheat (*Triticum aestivum*)- canola (*Brassica napus*)- potato (*Solanum tuberosum*)- pea (*Pisum sativum*) grown with vs. without shoulder-season cover crops, red clover (*Trifolium pratense*), berseem clover/oat (*Trifolium alexandrinum*/*Avena sativa*), rye (*Secale cereale*), and mustard/tillage radish (*Brassica juncea*/*Raphanus sativus*) respectively, was set up in Saskatoon to determine cover crop influence on N availability, crop nitrogen use efficiency (NUE), and N₂O emissions. A short rotation (wheat-canola) and a perennial alfalfa were included as treatment checks. Results showed that cover crops are viable, but success depends on species, management, and weather conditions. It was evident that cover crops influenced N cycling during the non-growing season (in the fall or spring). Cover crops had potential to reduce post-season N losses through plant N uptake and subsequent N release through mineralization. However, there was no cover crop effect on main crop yields and NUEs. When cover crop impact on N₂O emissions were evaluated, several trends pointed towards higher N₂O emissions with vs. without cover crops especially if a legume appeared in the phase studied. However, there were exceptions where cover crops significantly increased emissions. Emission events were observed post-fertilization and at spring thaw for all crop rotations. The growing season, post-harvest and spring thaw contributed 51, 7 and 42%, respectively to the cumulative annual emissions. Overall, it can be concluded that N dynamics were not markedly influenced by cover crops in the crop rotations studied. Longer-term studies are recommended to detect any possible impacts on N cycling, as cover crop effects may gradually build overtime. Future research should investigate methods of supporting rapid cover crop establishment and biomass accumulation (i.e., cold hardy varieties, early-seeding or under-seeding techniques). To sufficiently benefit main crop NUEs and reduce the risk of N losses, cover crop management may need to be bundled with other practices such as N fertilizer rate adjustments.

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DEDICATION

This thesis is dedicated to my family who have been my source of inspiration and hope in pursuing this dream. You have supported me with your continual prayers, words of encouragement to defy all odds and been emotionally present with me though far away to keep me going. A family I could always count on, thank you for always being there for me.

Finally, I dedicate this work to the Almighty God for his amazing love towards me and for the strength, wisdom and protection granted me to complete this program.

“For I know the plans I have for you, saith the Lord

plans to prosper you and not to harm you

plans to give you hope and a future”

Jeremiah 29:11

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LIST OF ABBREVIATIONS

g cm^{-3}	Gram per cubic centimeter
$\text{g N}_2\text{O Mg}^{-1}$	gram nitrous oxide per megagram
kg ha^{-1}	Kilogram per hectare
meq	Milli equilibrium
mg m^{-2}	Milligram per square meter
Mg g^{-1}	Megagram per gram
Mg ha^{-1}	Megagram per hectare
mL	Milliliter
mL ha^{-1}	Milliliter per hectare
Tg	Teragram
%C	Percentage carbon
%N	Percentage nitrogen
AAFC	Agriculture and Agri-food Canada
ANR	Apparent nitrogen recovery
ATP	Adenosine Triphosphate
BNF	Biological nitrogen fixation
CC	Cover crop rotation
CCCR	Canada's Changing Climate Report
CN	Cumulative nitrous oxide
C:N	Carbon to nitrogen ratio
FAO	Food and Agriculture Organization
FN	Nitrous oxide fluxes
IPCC	Intergovernmental Panel on Climate Change
Kg N ha^{-1}	Kilogram nitrogen per hectare
LR	Long rotation
MAC	Maximum acceptable concentration
N_2	Dinitrogen gas
NO	Nitric oxide
NO_2^-	Nitrite
N_2O	Nitrous oxide
NHI	Nitrogen harvest index
NH_4^+	Ammonium
NO_3^-	Nitrate
NUE	Nitrogen use efficiency
NUpE	Nitrogen uptake efficiency
$\text{NUE}_{\text{yield}}$	Yield nitrogen use efficiency
pH	Potential of Hydrogen
P_n	Plant Nitrogen
PR	Perennial rotation
SARE	Sustainable Agriculture Research Education

SAS	Statistical Analysis software
SIN	Soil Inorganic Nitrogen
Sn	Soil nitrogen
SR	Short rotation
WHO	World Health Organization
Y_n	Yield nitrogen
YN_2O	Yield-scaled nitrous oxide
YSE_B	Yield-scaled emission for biomass
YSE_N	Yield-scaled emission for yield nitrogen
Y_w	Yield weight

1.0 INTRODUCTION

Sustainable agriculture is envisioned as a food system in which food is nutritious and accessible to everyone and that natural resources are managed in a way that maintains ecosystem functions, supporting current and future human needs. Simultaneously supporting environmental quality, promoting social and economic development are key characteristics of sustainable agriculture (Food and Agriculture Organization of the United Nations 2018). Achieving sustainable agriculture is a complex challenge for many reasons but especially the pressure to intensify food production to meet the demands of a growing population. The global population is projected to increase beyond nine billion by 2050, which will require an increase in agricultural productivity of about 60% to meet the growing food demand (FAO 2012). Consequently, there is an urgent need to enhance the sustainability of agricultural systems. Meeting this ever increasing demand for food will result in a heavy reliance on N inputs to agricultural lands (Baligar et al. 2015; Heffer and Prud'homme 2016; Naeem et al. 2017; Sharma and Bali 2017). Nitrogen additions to soil are long known to increase food production, and without it, crop yields will decrease as soil N reserves decline (Janzen et al. 2003). However, N additions to soil comes with their own risk, such as the potential for soil N to leach below the root zone causing groundwater pollution, and its escape into the atmosphere which increases the accumulation of greenhouse gases thereby accelerating climate change and posing severe environmental and health risk to plants, animals, humans and the agroecosystem (Janzen et al. 2003; Fu et al. 2019). As a result, better understanding and managing N dynamics is central to achieving a balance between safeguarding the environment and increasing crop production, and it is necessary for improving agricultural sustainability (Janzen et al. 2003; Martinez-Feria et al. 2018; Fu et al. 2019).

To better manage N in cropping systems, it is important to improve the timing of soil N availability for crop use and to reduce N losses. Cover crops, recognized as an essential component of sustainable agriculture, can be a key strategy for meeting this challenge (Basche et al. 2016). Cover crops can improve crop nitrogen use efficiency (NUE)—as shown for vegetable crops on the Canadian prairies (Farzadfar et al. 2021), grain and tuber crops such as wheat

(Habbib et al. 2017), maize (Gabriel et al. 2016) and potato (Jahanzad et al. 2017a) under different N management practices. By increasing crop NUE, improving soil N storage and reducing excessive N fertilizer rates, N losses can be minimized (Fu et al. 2019). Furthermore, diversifying plants in crop rotations has potential to improve crop, nutrient and water use efficiencies (Arcand et al. 2013; Taveira et al. 2020). Therefore, integrating cover crops within crop rotations can increase nutrient and water use efficiencies and contribute additional benefits which can improve and strengthen the agroecosystem (Dabney et al. 2001; Finney et al. 2016).

The practice of cover cropping in Canada is increasing, but the practice is noticeably more dominant in Eastern Canada than it is in Western Canada (Statistics Canada, 2016)). This reflects the short growing season, coupled with dry periods in the semi-arid regions of Prairie Canada, which hinders the adoption of cover cropping. Nonetheless, the area under cover cropping in Prairie Canada is gradually increasing (Statistics Canada, 2016). A survey conducted across Prairie Canada in 2020 found there were 281 farmers who are early adopters of cover cropping and a relatively even distribution of the survey respondents came from each of the three provinces, 37% from Manitoba, 32% from Saskatchewan, and 31% from Alberta (Morrison and Lawley 2021). Yet, in semi-arid regions of the prairies, there is still a concern that cover crops use up soil water and reduce the available water content required for subsequent crops during the growing season. But even in the driest regions of Alberta and Saskatchewan, farmers are engaging in cover cropping (Morrison and Lawley 2021); thus, increasing the possibility of farmers to integrate cover crops into their rotations regardless of the limitations. From other regions, there are conflicting reports where some studies show positive effects of cover cropping on soil moisture (Basche et al. 2016) and others show negative effects (Qi et al. 2011; Vujić et al. 2021). Because cover cropping on the prairies is relatively new, many questions remain as to how cover crops influence crop production and N dynamics. Prolonged periods of little to no rainfall and short growing seasons are the major factors that control production in the semi-arid Prairies and may present barriers to the possibility of successfully incorporating cover crops into rotations. For cover crops to influence soil N dynamics, they must establish quickly and produce sufficient biomass, but there are limited studies in Prairie Canada and insufficient information on how cover crops may influence N dynamics in this region. More research is necessary to address this gap in knowledge and understanding.

The province of Saskatchewan has announced plans to expand irrigation, presenting a promising opportunity that may enable farmers to incorporate cover crops into rotations and to better manage N. Irrigation has been shown to help ensure sufficient cover crop germination and biomass production—a factor that is vital for successful cover cropping (Blackshaw et al. 2010; Roesch-McNally et al. 2018). Hence, by focusing on an irrigated cropping system in the semi-arid prairies, this study investigates the viability of establishing cover crops in rotation with cash crops and quantifies how cover crops impact N dynamics. Ultimately, my project ascertains if cover crops can provide agronomic and environmental benefits by improving soil N supply and crop NUE, and if they can minimize the risks associated with N₂O emissions.

This dissertation is organized in manuscript format with a general introduction (this section, Chapter 1) followed by a literature review (Chapter 2) and two research chapters (Chapter 3 and 4) covering the study on N dynamics and N₂O emissions. Chapter 5 presents a synthesis of key findings, conclusions, and recommendations for future research.

1.1 Research objectives

The specific objectives of this study are to:

1. Test the viability of establishing cover crops (red clover, berseem clover/oat, fall rye, and tillage radish/mustard) into a wheat-canola-potato-pea rotation.
2. Quantify the impact of cover cropping on soil N availability in wheat-canola-potato-pea sequence.
3. Estimate crop NUE by assessing crop N uptake relative to soil N availability, as influenced by cover cropping
4. Determine the amount of N₂O emissions produced at spring thaw and after fertilization during the growing season, as influenced by cover cropping.

1.2 Research hypotheses

Based on above objectives, it was hypothesized that:

1. Cover crop biomass accumulation will differ by species and by how it is incorporated into the rotation—i.e., inter-seeded or early-seeded species will accumulate greater biomass.

2. Crop rotations with cover crops will have lower post-harvest season soil N levels due to plant N uptake but higher in-season soil N levels due to plant tissue N mineralization, compared to rotations without cover crops.
3. Crop rotations with cover crops will increase crop NUE by influencing soil N availability—possibly translating into improved crop yields—compared to rotations without cover crops.
4. Nitrous oxide emissions will be influenced by cover crops at spring thaw and at fertilization depending on cover crop species—i.e., legumes will increase thaw-induced N₂O emissions by increasing available soil N, whilst non-legumes will decrease N₂O emissions by reducing available soil N.

To address these objectives and hypotheses, this research focuses on a single field trial located in Saskatoon, Saskatchewan. This research is part of a larger network of trials with similar experimental designs located across Prairie Canada (Manitoba, Saskatchewan, and Alberta). These trials are on-going. For the purpose of my MSc thesis, I have focused on the Saskatoon site from May 2019 to May 2021 (Chapter 3, N cycling) and May 2020 to May 2021 (Chapter 4, N₂O dynamics).

2.0 LITERATURE REVIEW

2.1 Cover cropping as related to climate across Canada and the USA

The implementation of cover crops is a popular agricultural practice in Eastern Canada—where depending on the area, about 25 to 100% of the farmers report using cover crops (Statistics Canada, 2016). Noticeably, the practice of cover cropping is more dominant in Eastern Canada, than it is in Western Canada (Fig. 2.1). In Western Canada only about 1-15% of farmers use cover crops, however, the practice is gradually increasing (Statistics Canada, 2016). In dryland systems where adequate precipitation is a top concern, there is potential for cover crops to deplete available soil water, and this can have a detrimental impact on cash crop growth and development—risking yield penalties. A study conducted in Central Iowa to measure soil water at 0-30 cm depth in a maize-soybean rotation with a rye cover crop *vs.* no cover crop showed high soil water storage with cover crop rotations compared to no cover crop rotations (Basche et al. 2016). What works in one region of Canada may not work in another because Canada’s climate is highly variable, ranging from region to region, and fluctuating with time (CCCR, 2019). The annual average precipitation in the prairie region is about 454 mm which is much less than the Eastern region of Canada which receives the annual average of 800 mm. Saskatchewan receives the lowest amount of annual precipitation (395 mm) of the three prairie provinces, followed by Alberta (482 mm) and Manitoba (486 mm) (Floate and McGinn 2010). Temperature and precipitation have significant impact on agriculture largely by determining the suitable crops for a particular region (CCCR, 2019)—and this is also true for cover crops.

Rainfall amount and timing and the duration of growing season are the dominant climate characteristics that regulate the stability of prairie ecosystems, and inadequate water supply clearly constrains production (Floate and McGinn 2010; Basche et al. 2016). The Canadian prairies are typically classified under semi-arid conditions, where the periodic extremes fluctuate between long, cold winters and short, hot summers. The challenge of growing cover crops on the semi-arid prairies is that cover crops may not be viable due to the relatively short growing window, and that limited water availability may constrain their usefulness. These factors limit the range of cover

crop species that can be established here successfully. The performance of cover crop is enhanced under irrigated systems where there is significant reduction in water and nutrient competition thereby increasing cover crop returns (Snapp et al. 2005a). Snapp reviewed different studies under cover crop management and concluded that an intricate network of complexity exists between cover crops and water relations as found in cropping systems. A study conducted in Ohio showed that a cover crop mixture of pea-rye improved the yield of tomato crops when cover crop biomass exceeded 4 Mg ha^{-1} with adequate supply of moisture (Snapp et al. 2005a). Additionally, mustard and flax yields declined considerably when intercropped with sweet clover under dry conditions, whilst wheat yields were increased by 47-75% when intercropped with sweet clover (Snapp et al. 2005a). It may be challenging but nonetheless possible to establish cover crops successfully even in dry regions when proper selection and management are implemented.

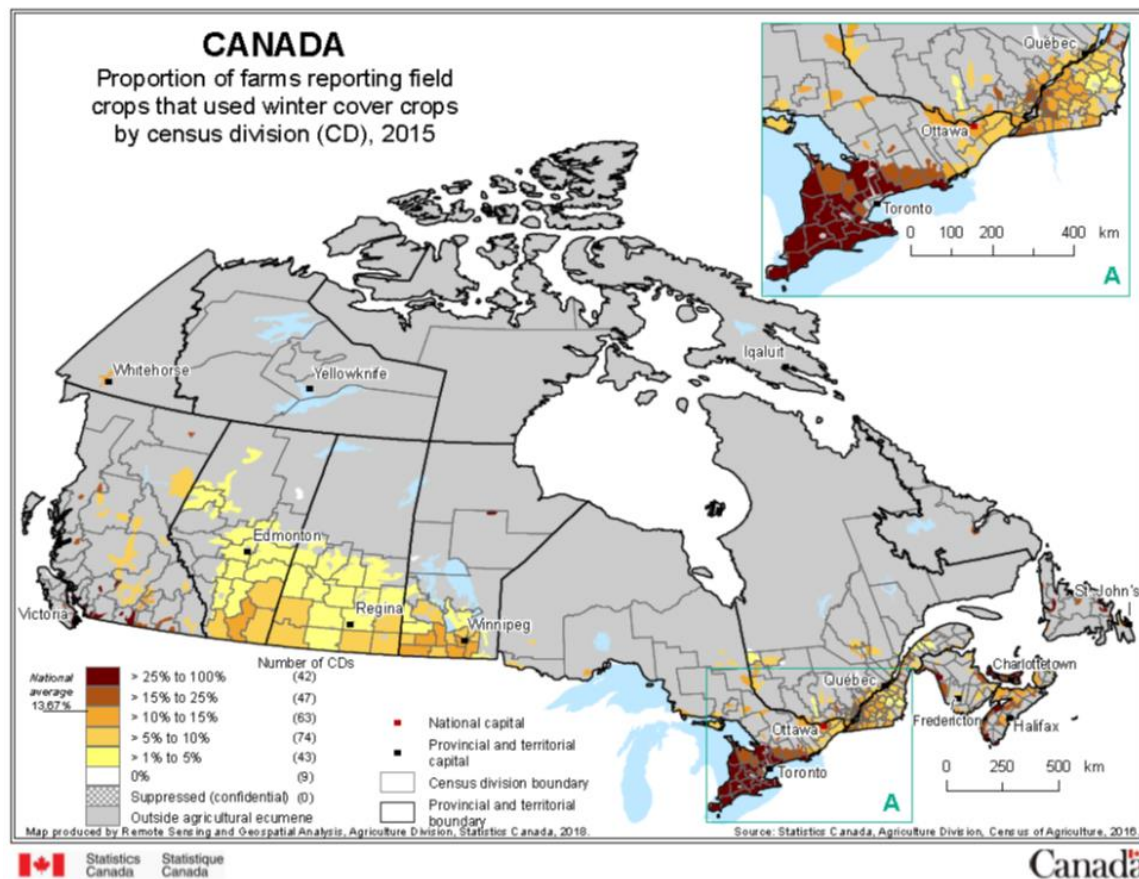


Figure 2. 1 Proportion of farms that use winter cover crops by census division. Statistics Canada-Agriculture Division, Census of Agriculture, (2016).

2.2 Cover crops in agricultural production systems

Cover crops are defined as any vegetation established outside of the cash crop growing season or as an intercrop that are not grown directly for profit, but managed to protect and improve soil fertility, crop yields, water quality (Dabney 1998), and enhance ecosystem sustainability (Liebman et al. 2018; Kaye et al. 2019). Cover cropping has been in existence for centuries, and it is a widely adopted farming practice (Blackshaw et al. 2010; Blanco-Canqui et al. 2015) in certain parts of the world. Subsequent to the invention and production of synthetic fertilizer, farmers transitioned away from a historic use of cover crops towards the reliance on synthetic N fertilizers because these products improved crop yields and reduced labor requirements, rather than cover cropping which gradually build up soil N reserves (Snapp et al. 2005a). However, the relevance of N management is not only to maintain an adequate supply of N for crop production but also to reduce N losses to the environment. Evidence suggests that, cover crops benefits are diverse, such as: reducing soil erosion, minimizing soil compaction, improving root penetration, improving soil structural and hydraulic properties, regulating soil moisture and temperature, improving microbial diversity and activities, recycling nutrients, suppressing pests and weeds, improving crop yields, a potential for mitigating climate change (Blackshaw et al. 2010; Blanco-Canqui et al. 2015) and a probable source for creating income. The cumulative effect of these individual benefits can go a long way to improve the overall soil health status.

Notwithstanding the benefits of cover cropping (Dean and Weil 2009; Blackshaw et al. 2010; Blanco-Canqui et al. 2015; Liebman et al. 2018), there are potential drawbacks which to a large extent have influenced whether or not growers will implement cover cropping. Factors such as high production cost, potential for reduced profit as a result of main crop yield penalties, (Snapp et al. 2005) can have significant impact on cover cropping, making less desirable to growers. Cover crops can be a nuisance if not adequately managed, especially with regard to cover crop termination. Problems with herbicide selection, potential for cover crops to re-grow and interfere with the main crop and compete for resources, may adversely impact crop production. Studies however show that different cover crops impact N cycling differently. For example, grass cover crops which are good scavengers and often have a high C:N ratio can temporarily immobilize available soil N during decomposition process by microbes thus, reducing the availability of N to plants (Kaye et al. 2019). Conversely, leguminous cover crops which have a low C:N ratio can also increase the availability of soil N during process of mineralization. Depending on the timing of N

immobilization or mineralization, an increase or decrease in soil N availability may increase or reduce N losses. Careful cover crop selection and management must be considered for successful cover cropping.

2.2.1 Selection of cover crops

Selecting the best cover crop depends on the farmer's objective, if it is to serve as a green manure crop, a catch crop or as a soil cover. The differences lie in the intention behind its establishment and the purpose it is to serve. In addition to identifying the objective, a crucial component that requires attention is the defining niches (spatial and temporal) where the cover crops are to be established (Snapp et al. 2005a; Thiessen Martens et al. 2015). For example, cover crops can be classified as summer and winter annuals, biennials, or perennials depending on their life cycle (SARE 2010). Winter hardy cover crops such as hairy vetch, red clover, and rye are suitable for winter zones or short season where cash crops are harvested in fall whereas heat tolerant species such as sudan-grass, berseem clover, and buckwheat are more adapted to warmer environment. Certain species are suited to the short growing windows and occasionally used to replace fallow in dryland production systems (Blackshaw et al. 2010) whilst others are suited to long season.

Cover crops which serve as green manure crops are typically incorporated into the soil in the form of mulch while the plant is still green to improve soil fertility and soil structure through soil organic matter additions. This generally corresponds to increased biological activity of soil microbes, leading to improved soil structure and soil aggregation, water infiltration, and root penetration. Catch crops and soil covers are usually fast growing and established between successive planting of main crops to make use of growing space and provide some form of vegetation during the non-growing season or fallow period. The rationale behind the catch crop is to make use of an otherwise fallow period after the growing season. After harvest of the main crops, the catch crop will immobilize available N in the soil by taking up the N and storing it in tissues. The immobilized N is recycled back into the soil after cover crop termination—and depending on the timing and rate of mineralization, the N is made available again for the subsequent main crop. The effectiveness of a catch crop depends on the fast growth and the termination dates, thus selecting a crop that establishes quickly with deep rooting system enhances the efficiency and effectiveness of catch crops. Integrating cover crops as soil covers, on the other hand, serve as

ground cover and prevent the land from lying bare and reduce its exposure to various forms of erosion. In protecting the soil against erosion, cover crops reduce nutrient losses that may result through surface run-off by water and/or wind erosion and improve infiltration rates and root penetration.

Legumes, grasses, oilseeds, and brassicas constitute a wide array of crops utilized as cover crops. Leguminous cover crops are known to be beneficial due to their ability to fix atmospheric N, add organic matter to the soil, suppress weed growth, control erosion and are adapted to different niches examples are the different types of clovers, hairy vetch, peas, alfalfa, soyabeans, etc. Legumes generally have low C:N ratio compared to grasses. The lower C:N ratio <20:1 ensures a faster breakdown of residues and results in a temporary N surplus (Oliveira et al. 2020). Legume cover crops are, however, poor N scavengers and usually it is expected that N demanding crops are established after its termination to make use of any excess N in soil that has the potential of escaping into the atmosphere or leaching below the root zone. Non-legume species such as oat, rye grass, sudan grass, barley (to name a few) do not fix N but affect soil N availability through recycling of existing soil N. Grass cover crops are mainly non-legume species, which develop rapidly and produce a considerable amount of biomass, contributing to weed suppression, erosion control and adding organic matter to the soil. Grasses generally have high C:N ratio >35 and microbial decomposition of residues with C:N ratio >30 lead to temporary N deficit (Oliveira et al. 2020). Immobilization of N is higher after grass biomass is returned to soil, and this may influence N availability and reduce the potential for N losses (Kaye et al. 2019). The extensive rooting system in grasses aids in scavenging N better (Fu et al. 2019) than legumes which tend to have fibrous roots. There is an increasing interest in the use of Brassicas as cover crops. Brassicas broadly categorized as non-legumes are fast growing and provide considerable soil coverage (SARE 2007), which aids in preventing erosion. The taproot system of brassicas such as tillage radish, helps to break down hard soil layers thus reducing compaction. Some brassica species are known to release certain chemicals that are harmful to soil borne pathogens, hence useful in suppressing pests and pathogens as well as weeds (Haramoto and Gallandt 2004).

No single cover crop can provide all the desired benefits associated with cover cropping. However, several lines of evidence point that cover crops established in mixed stands provide diversified benefits compared to pure stands due to complementarity effect (Finney et al. 2016). A

typical example is a legume-grass mix, which may be effective at managing N due to the combined effect of their distinct traits and characteristics (SARE, 2007). Mixtures of legumes and grasses leads to N functional complementarity where legumes fix N in soils while grasses exploit the soil N reserves by taking up residual N that may be subject to losses (Finney et al. 2016). Cover crop selection and management therefore remains a critical component of a successful cover cropping.

Redesigning cropping systems to ensure continuous sustainability of the agricultural systems are imperative. Research has shown that diversifying rotations influences soil N storage, mineralization, and availability of N, all of which can impact the N balance of the agroecosystem (Fu et al. 2019). This was reported in a 30-year crop rotation trial that tested the effect of diversified rotation and continuous cropping on soil N fractions. Their results showed that there was greater correlation of diversified rotation with key soil N fractions (soil total N, particulate organic N, microbial biomass N and potential mineral N) than the continuous rotations (Fu et al. 2019). Simple systems such as monocropping are regarded as nutrient exporters and can ultimately alter nutrient cycles (Thiessen Martens et al. 2015). In this regard, incorporating cover crops in rotations impacts N availability, which in turn can affect the regulation of soil ecosystem services such as nutrient recycling and greenhouse gas sequestration (Blanco-Canqui et al. 2015). Employing cover crop mixtures in crop rotations may therefore provide advantages for crop production if they improve the availability of N in soils in time for crop N use, and if N losses are reduced.

To better understand N management in soils it is essential to understand the basic principles that regulate it. Nitrogen availability in agricultural soil is tied to the N cycle, which consists of a series of transformational processes that converts N into forms for microbial and plant use. The management of N is therefore a complex action as it is regulated by processes linked to N cycling and loss. Therefore, a comprehensive understanding of the factors and processes that regulate N dynamics is necessary to manage N effectively and minimize risks associated with N losses.

2.3 The N-cycle: processes and impact of human activities

Nitrogen is a nutrient required by plants in substantial amounts and its limited supply thereof hinders plant growth and development, productivity, and primary production at global scales (Gutiérrez 2012). Yet plant available forms, nitrate (NO_3^-) and ammonium (NH_4^+) are in limited supply in both agricultural and natural systems making it a scarce resource (Erisman et al. 2018). Nitrogen exists as dinitrogen gas (N_2) in its simplest stable form and makes up about 78%

of the air in the atmosphere, but this form is predominantly inaccessible to most organisms including plants, due to its triple bond which needs to be broken. Nitrogen enters living organisms through bacteria and other unicellular organisms e.g., prokaryotes which mediate the conversion of atmospheric N into plant usable forms through a process of nitrogen fixation. The conversion of N₂ into biologically available nitrogen is energy dependent hence only specific groups of prokaryotes can perform this energy demanding process which requires eight electrons and about sixteen Adenosine Triphosphate (ATP) molecules ($\text{N}_2 + 8\text{H}^+ + 8\text{e}^- \rightarrow 2\text{NH}_3 + \text{H}_2$).

The N-cycle typically “begins” with N in its stable form, dinitrogen (N₂) gas and its conversion into accessible forms. The major transformation pathways of N in the N-cycle include N fixation, nitrification, denitrification, ammonification and anammox and its alteration into the many oxidation states, mediated by activities of diverse microorganisms such as bacteria, fungi, and archaea. Biological Nitrogen fixation (BNF) converts N gas into ammonia, which is subsequently metabolized by microorganisms. Nitrogen fixation is carried out naturally in the environment by a wide range of microorganisms termed diazotrophs, which include bacteria and archaea. These N-fixing bacteria can be symbiotic or non-symbiotic (free-living) such as *Cyanobacteria*, *Azotobacter*, *Clostridium* etc. The symbiotic groups require close association with a host to perform this process e.g., is the *Rhizobium*, a N-fixing bacteria that forms symbiotic association with leguminous plants in root nodules of legumes. The bacteria invade the root hairs of leguminous plants where root nodule formation is stimulated. Within the nodules, bacteria convert free N into ammonia with the aid of the enzyme nitrogenase and this is absorbed by plants from the soil through their root hairs by a process called assimilation. Majority of N fixation is carried out by microbes, but some amounts are abiotically fixed when energy from lightning breaks the triple bond in N atoms converting them into plant available forms. Certain industrial processes such as production of fertilizers can also fix N.

Inorganic N in the N-cycle is derived from fertilizer N, and it is in this form (NO₃⁻ and NH₄⁺) that plant can directly take up N (Bartholomew et al. 2013). Inorganic N is obtained by the manufacturing of synthetic N fertilizers through the Haber-Bosch process which synthesizes ammonia from hydrogen and N. Nitrogen supplied to plants in this form is usually through fertilizer applications in soluble forms. Addition of fertilizers aids in boosting the fertility of soils to improve productivity.

Another important source of N for plant development can be obtained from soil organic matter pool. A higher percentage of the potentially available N in the soil exists in organic forms (Bartholomew et al. 2013), in plant and animal residues, in stable soil organic matter, and microbes such as bacteria. The organic form of N is considered the reservoir of N for plant nutrition. When plants and animals die or secrete waste, the initial form of N is in the organic state (Bernhard 2010). Diverse group of bacteria and fungi are responsible for decomposing these substances with the aid of enzymes. The release of N from soil organic matter is dependent on the rate of mineralization, where organic N is converted to ammonium. The first step of mineralization is called aminization, in which microorganisms (mainly heterotrophs) break down complex proteins to simpler amino acids, amides, and amines. Formation of organic matter and its stability correlates with long term moisture and temperature trends. Higher temperatures favor soil organic matter decomposition: conversely, waterlogged conditions inhibit soil organic matter breakdown. The soil organic matter improves soil fertility through the provision of cation exchange sites that acts as a reservoir of nutrients such as N which is gradually released during mineralization.

The N cycle in the agroecosystem is not a closed one, but consists of pathways that allows N to escape from the system (Janzen et al. 2003). These pathways allow N loss through processes such as, nitrification, denitrification, ammonification, ammonia volatilization etc. Nitrification is an aerobic process involving a two-step process initiated by the conversion of ammonia to nitrite by specialized group of microbes which belong to the nitrifying bacteria genera, e.g. *Nitrosomonas*, *Nitrosospira* and *Nitrosococcus*. The nitrite formed undergoes a second reaction where it is oxidized to NO_3^- by another nitrite oxidizing-bacteria species such as *Nitrobacter*, *Nitrospira* and *Nitrococcus*. The resultant NO_3^- formed is utilized by plant for development. Below optimal oxygen (O_2) concentration levels, the process of oxidation is incomplete, resulting in the production of N_2O and NO (Skiba and Smith 2000). Additionally, nitrification process can be carried out by both autotrophs and heterotrophs but contributions by heterotrophs are relatively lesser compared to autotrophs (Skiba and Smith 2000). Nitrification is controlled by substrate availability i.e., NH_3 and NH_4^+ , microbial populations and environmental conditions such as moisture and temperature. Denitrification on the other hand is the microbial reduction of NO_3^- to dinitrogen gas (N_2) under limiting oxygen concentration. This process produces other intermediate forms of N (N_2O) which escape from microbial cells into the soil and the environment. The process is favored by anaerobic conditions, usually in waterlogged soils. Bacteria utilize the available NO_3^-

as a source of energy when oxygen levels are low to carry out respiration and consequently produce N_2 gas which plants cannot assimilate. According to Smith (2017), process of nitrification and denitrification can occur in close proximity in soils, but their dominance is determined by conditions that influence aeration status of the soil. Several other N losses occur along the N-cycle pathway. Leaching is a natural phenomenon which occurs in the soil when soil water exceeds the water-holding capacity of soils and moves soil nutrient below the root zone. Nitrate leaching occurs in soils with high percolation rates, washing residual and mineralized NO_3^- below the root zone due to its soluble nature causing eutrophication of water bodies leading to algal blooms and further contamination of drinking water, and potentially reducing biodiversity of native ecosystems (EU Nitrogen Expert Panel 2015). In respect of this, World Health Organization (WHO) has set the maximum acceptable concentration (MAC) of NO_3^- in drinking water to 50 mg L^{-1} (WHO, 2011). In Canada for instance, the MAC is 45 mg L^{-1} which is equivalent to 10 mg L^{-1} as $NO_3\text{-N}$ (Health Canada 2013) and exceeding these levels can lead to NO_3^- toxicity levels. Estimates from Canada, shows nitrate leaching varies across its regions, ranging from 2 kg N ha^{-1} in the semi-arid prairies to about 30 kg N ha^{-1} in humid regions annually (AAFC 2008). In coarse-textured soils, N loss is high due to increased percolation rates. In contrast, the amount of N lost in fine-textured soils is low due to low permeability. Ammonia volatilization another mechanism of N loss occurs when ammonium is converted to ammonia and lost to the environment. A high soil pH and temperature favors the conversion of ammonium to ammonia. Losses are high when conversion takes place at the soil surface. Several lines of evidence shows that human activities have played important roles with immense impact on N cycle. Excessive N additions in soil beyond plant requirement leaves excess N in the soil after harvest (Fu et al. 2019) which becomes subject to losses into the atmosphere as greenhouse gases or leaching to groundwater and/or adjacent fields.

2.4 Nitrous oxide (N_2O) production and emissions

Nitrous oxide (N_2O) is a potent greenhouse gas with a warming potential 265-times that of carbon dioxide on a 100-year timescale (IPCC 2014). This gas is of global concern due to its contribution to global warming and depletion of stratospheric ozone. Agriculture is reported to be the leading source of anthropogenic N_2O emissions (IPCC 2019). Naturally, nitrous oxide is produced by microbes as they transform N in soils for plant use hence the addition of large quantities of N fertilizers, manure and other amendments to farmlands has exacerbated N_2O emissions. This accelerated increase is associated with the expansion of agriculture with the aim

of meeting the increasing human demand for food. This high demand of N is due to the vital role N plays in agricultural systems. Nitrogen is an essential nutrient, and agroecosystems depends on substantial inputs to provide high crop yields; yet it is very reactive and mobile in the terrestrial system (Basche et al. 2014). Apart from these direct emissions, indirect emissions also result from the deposition and movement of N from farmlands to adjacent environments giving rise to N₂O emissions (IPCC 2006). Studies have shown that crops have low soil N recovery (40-60%) which subjects residual soil N that remains after harvest to gaseous emissions and other losses through biological processes that release N₂O to the environment (Basche et al. 2014; Fu et al. 2019). The general consensus has been that the availability of mineral N stimulates N₂O emissions through processes of nitrification and denitrification, when the soil exceeds its N supply capacity (Skiba and Smith 2000; Machado et al. 2021b). However, N fertilization for crop production cannot always be eliminated from agricultural systems. As such, management practices with potential to reduce emissions must be considered (AAFC 2008).

2.4.1 Nitrous oxide controlling factors

Nitrification and denitrification are biological processes that contribute about two-thirds of all N₂O emissions (Skiba and Smith 2000) and their relative contributions are determined by oxygen availability and the presence and activities of nitrifying and/or denitrifying bacteria (Krauss et al. 2017). In a recent study that used isotopomers to track N₂O production mechanisms, Congreves et al. (2019) reported that the ideal WFPS for nitrification under fine and coarse textured soils was approximately 55 and 40% respectively. Dong et al. (2018) observed a positive correlation between N₂O flux and WFPS when it was found that N₂O fluxes were activated by heavy rainfall post fertilization and concluded that soil water-filled pore space (WFPS) was the dominant factor driving emissions. They also reported that nitrification was the cause of the N₂O emissions produced when soil WFPS was less than 60%. Nitrification process oxidizes ammonium (NH₄⁺) to nitrate (NO₃⁻) under aerobic conditions, but at low oxygen (O₂) levels, oxidation is incomplete, resulting in the production of N₂O and NO (Skiba and Smith 2000). Additionally, nitrification process can be carried out by both autotrophs and heterotrophs but contributions by heterotrophs are relatively lesser compared to autotrophs. Denitrification on the other hand is the microbial reduction of NO₃⁻ to dinitrogen gas (N₂) under anaerobic conditions. This process produces other intermediate forms of N (N₂O) which escape from microbial cells into the soil and the environment. According to Smith (2017), process of nitrification and denitrification can occur

in close proximity in soils, but their dominance is determined by conditions that influence aeration status of the soil. The balance between N_2O production from nitrification and denitrification is reported to vary by climate, soil conditions, topography and soil management with high moisture availability, poor drainage, fine texture, and the presence of high organic carbon content associated with increased denitrification with nitrification dominating under the opposite conditions (Skiba and Smith 2000; Janzen et al. 2003). Nitrous oxide produced in terrestrial ecosystem is controlled by the availability of mineral N, soil water content, labile carbon, and soil physical properties (Basche et al. 2014). Nitrogen availability in soils comes from two sources: mineral fertilizers and organic sources such as decomposition of organic matter and addition of organic amendments like crop or cover crop residues and manure. Machado et al. (2021) point out that the release of mineral N from organic amendments is controlled by the variability in the C:N ratio of residues which affect the process of decomposition. Immobilisation dominates mineralisation when cover crop and crop residues have higher C:N ratio >25 . Additionally, residue quantity and management influence N_2O emissions (Hao et al. 2001), and Basche et al. (2019) outlined two ways cover crops can influence N_2O emissions e.g., soil mineral N can be reduced by cover crops through N storage in biomass tissues leading to reduced N_2O production, conversely, mineral N can be increased by N fixing species leading to increased N_2O production. Significant amount of N are released from residual and mineralizable N which supply N over growing season to main crops, hence there is the need to adjust N fertilizer inputs to optimize crop productivity as well as reduce losses (Taveira et al. 2020).

Studies have reported that the magnitude of N_2O emissions is regulated by site-specific conditions i.e., primarily climate and soil conditions and management factors (Rochette et al. 2008). Research has shown that various soil physical properties have variable impact on the aeration status of the soil: porosity and pore size distribution, oxygen availability, soil structure, soil water determinants such as WFPS, matric potential and volumetric water content (VWC) and temperature (Smith 2017).

It is reported that precipitation amount will increase in warmer regions, hence wet areas will become wetter based on climate models and these warmer and wetter conditions will be accompanied by increased N_2O production via nitrification or denitrification (Griffis et al. 2017). They re-iterate that rising surge in anthropogenic N coupled with warmer and humid conditions

observed in agricultural zones will no doubt magnify emissions via positive radiative forcing. In regions where irrigation is used to supplement precipitation to enhance yields, N₂O emissions can be intense due to the combined effect of high moisture and increased N inputs (Hao et al. 2001).

The level of complexity that exists between N₂O controlling factors at both spatial and temporal scales makes it somewhat difficult to predict the magnitude of N₂O emissions. Rochette et al. (2008) mentioned that N₂O emissions are variable across different geographical and local conditions, influenced by climatic factors and/or management practices such as soil type, texture, land use type, tillage, irrigation, N inputs etc. In their work to develop country specific measurements to estimate the N₂O coming from agricultural soils in Canada, Rochette et al. (2008) reported a wide range of variability observed in emissions even across similar regions. Comparing emissions from Eastern Canada to the Prairies, it was realized that emissions can be high and variable across seasons; significant emissions were produced during winter and spring period in Eastern Canada due to frequent cycles of wetting and drying compared to the Prairies where the magnitude of emissions were lower during winter period due to the reduced frequency in freeze thaw events. Wagner-Riddle et al. (2017) suggested that the freeze-thaw cycles are critical factors driving emissions through three mechanisms; (i) elevated anaerobic conditions and availability of substrates leading to increased denitrification (ii) alterations in denitrifying enzyme structure and activity and (iii) release of previously trapped N₂O as snowmelt occurs. The latter factor being a significant contributor to N₂O produced in regions with prolonged periods of cold winters especially when fertilizer application occurs in fall. A study conducted in Alberta showed greatest emission occurring in Spring (March and April) from fall applied fertilizers during freeze-thaw events (Hao et al. 2001). Considering that 30-90% of annual N₂O emissions are associated with freeze-thaw cycling, neglecting to account for these emissions in agricultural systems can lead to underestimation of global N₂O emissions by up to 28% Wagner-Riddle et al. (2017). Reports show that thaw related fluxes can account for more than half of annual N₂O emissions (Wagner-Riddle et al. 2017). Snowmelt that occurs in spring creates moist conditions that stimulate N₂O emissions, and the magnitude of these emissions is controlled by the intensity of freezing (Rochette et al. 2008).

Several lines of evidence point to the fact that the high levels of reactive N in soils are due to increased mineral fertilization which contribute significantly to N₂O emissions in the presence

of key controlling factors and the network of activities that exist between (Basche et al. 2014). Studies show that cover crops can reduce the soil NO_3^- pool which is the key substrate for increasing N_2O fluxes (Basche et al. 2014; Thomas et al. 2017) by either minimizing N fertilizations or through improved crop N use efficiency.

2.4.2 Cover crop contribution to N_2O emissions

Application of crop residues in itself as amendments to soil can improve ecosystem services such as nutrient cycling, erosion control and soil carbon storage (Chen et al. 2013) with cover crop inclusion contributing additional benefits (Muhammad et al. 2019). Estimates show that crop residues produce about 0.4 million metric tons of N_2O -N yr^{-1} (Chen et al. 2013). Cover crop contribution to N_2O emissions varies considerably depending on the species, biomass, residue quality (C:N ratio and lignin content) and its management (Basche et al. 2014; Muhammad et al. 2019). For example, legume cover crops can increase N_2O emissions by inducing N availability compared with no cover crops or even with non-legumes whilst non-legumes can reduce available N by increasing N uptake (Muhammad et al. 2019). However, this theory is not consistent in literature and even among legumes differences can occur as reported by Liu et al. (2021). Crop residues contribute N to organic N pool, which subsequently increases N mineralisation (Machado et al. 2021a). During microbial mineralization process, considerable amount of the N is nitrified, stimulating N_2O production a by-product of nitrification process (Basche et al. 2014). Furthermore, the activities of microbial populations can result in low oxygen levels creating anaerobic conditions that promote denitrification (Machado et al. 2021a). A negative correlation is said to exist between N_2O emissions and crop residue C:N ratios, implying greater emissions with low C:N ratios compared with high C:N ratio residues (Toma and Hatano 2007). A study that compared C:N ratios of different residues on N_2O emissions showed an increase in emissions as C:N ratio decreased (Toma and Hatano 2007). Residue quality of cover crops impact the process of decomposition. Residues having lower C:N ratios decompose more rapidly than higher C:N ratio residues (Muhammad et al. 2019). This signifies relatively higher N_2O emissions with low than high C:N ratio residues under favorable conditions. Residue management plays a significant role on emissions. The C:N ratio of same cover crop can vary depending on kill date, e.g., a grass cover crop may have C:N ratio > 30:1 at anthesis compared to the vegetative stage with a relatively lower C:N ratio whereas a legume cover crop may have a ratio of 13:1 at an early stage compared to 25:1 C:N ratio at maturity (SARE, 2007). Furthermore, the method of incorporating cover crops into the

soil can have an influence on decomposition rates. Residues left on the soil surface decompose slowly compared to when incorporated into the soil (Oliveira et al. 2020). This is as a result of optimized contact with soil microbes when residues are incorporated increasing N₂O emissions (Muhammad et al. 2019; Oliveira et al. 2020). Biomass produced by cover crops influence C and N inputs to the soil. The greater the biomass returned to the soil, the higher the soil organic carbon and the lower the emissions (Sainju 2017). In a study conducted in Illinois, it was found that hairy vetch and cereal rye cover crop increased carbon gains between 0.1 to 0.88 Mg ha⁻¹ to a depth of 75 cm under different tillage practices following 12 years of management (Blanco-Canqui et al. 2015). Agricultural soils represent important sinks of carbon and can offset increases in N₂O emissions by increasing soil C gains; however, there is a synchronous relationship between the two as soil C increases with an increasing N (Janzen et al. 2003). Nonetheless, the magnitude of soil C increase from cover crops is site specific and contingent on several factors such as biomass input from cover crops, number of years of cover cropping, cover crop and soil type, tillage management, climate and also the history of previous level of soil C (Blanco-Canqui et al. 2015). For cover crops to influence N₂O emission, an impact on N availability and crop N use is required.

2.5 Crop nitrogen use efficiency (NUE)

Nitrogen based fertilizers have supported agricultural productivity since the mid-1900s (Dobermann 2005). Fertilizer use, though its essence is to boost yields can pose severe risk to the environment if not adequately managed. In 2015 the world consumption of N fertilizer was approximately 112.5 million tonnes and it was projected to rise to 118.2 million tonnes by 2019 as global human populations were forecast to reach about 10.5 billion between 2025 to 2050 (Baligar et al. 2015; Naeem et al. 2017; Sharma and Bali 2017). Food and Agricultural Organization (2012) projected that agricultural productivity would have to increase by 60% in order to feed the world population and to achieve this goal means substantial N inputs to agricultural lands. Regardless of vital role of N in crop growth and development, studies show that the recovery efficiency of N by crops is low, varying from 40-60% (Fu et al. 2019), 25-50% (Sharma and Bali 2017) depending on crop type. Maintaining the balance between safe environment while increasing crop yields is a big challenge in modern agriculture, as productivity is a function of N availability in soils and reducing N additions may potentially hamper crop yields (Martinez-Feria et al. 2018). Nitrogen fertilizers constitute 63% of the anthropogenic sources of all reactive N (Nr) (Dobermann 2005) and according to UNEP (2019), Nr remains one of leading risks challenging humanity due to its

contribution to climate change, environment and human health. The need to improve NUE requires immediate attention as population is increasing and producers are pressured to use the limited land resources more efficiently while also adopting environment-safe practices. However, there is a complex level of interaction between plant-soil systems and the interplay of activities that exist between climate, soil and plant factors which influence N availability (Fageria and Baligar 2005).

Nitrogen sources from inorganic N, biological N fixation, atmospheric deposition, animal manure and crop residues, collectively contribute a total of 169 Tg N yr⁻¹ to arable lands of which 46%, 20%, 12%, 11% and 7% comes from the aforementioned sources, respectively (Fageria and Baligar 2005). Nitrogen derived from biologically and atmospherically fixed processes as well as from crop and animal inputs are regulated by external factors and release of N can be sporadic hence, a greater reliance on synthetic fertilizers (Janzen et al. 2003). Soil N availability is tied to the N cycle, a leaky system which constitute several pathways for N loss. Nitrogen losses occur in agricultural system through process such as nitrification and denitrification, leaching, volatilization, and surface run-off to mention a few. These losses coupled with the low recovery of N by crops have severe consequences on the environment—terrestrial, aquatic, air, and soil quality. Plants' ability to utilize N efficiently forms a vital component in enhancing environmental sustainability. According to Baligar et al. (2007), NUE must encompass the overall capacity of soil to provide sufficient nutrient, and the plants ability to access, transport and partition to other plant parts. Greater N uptake reduces post-harvest N (Fageria and Baligar 2005) which may potentially be subjected to losses in the atmosphere and/or ground water pollution.

Studies show that there are potential environmental benefits to integrating cover crops into rotations. Cover cropping may enhance N storage by exploiting residual N in deeper soil layers by their root systems, making N available for subsequent crop to improve NUE (Basche et al. 2014). Nitrogen use efficiency has received significant attention in diverse areas all geared towards improving crop NUE. Some of the many metrics and indicators designed are suitable for tracking N and its losses from production to consumption and can provide useful inference for managing N. The use, applicability, and interpretation of the various NUE indices or indicators, however, may vary across different countries and regions due to the heterogeneity of soils, climate, and also to an extent by the type of technology and/or information accessible by farmer (Heffer and Prud'homme

2016). Overall a robust NUE estimate could be used as a tool for maintaining agronomic and environmental quality (Omara et al. 2019).

2.5.1 NUE definitions

There is no single coherent NUE metric and the different metrics available are operational under specific systems or management. On a broad scope NUE definitions can be classified as fertilizer based, plant-based, soil based, system and isotope-based (Congreves et al. 2021). In simple terms NUE indicates a system's ability to convert inputs to outputs i.e., the proportion of available N in soil (fertilizer and residual N) that is used by plant for optimum economic yields (Fageria and Baligar 2005; Hirel et al. 2007; Grahmann et al. 2013; Sharma and Bali 2017). Furthermore, every metric has its limitations and strengths that allow its use at different spatial and temporal scales (Congreves et al. 2021). For purpose of this study, plant and soil based NUE indices will be the focus.

2.5.2 Plant-based NUE

Plant-based indices determines plant N partitioning towards crop yields and can provide useful information for breeding studies. Examples of plant-based indices include (i) nitrogen harvest index—expresses the amount of accumulated plant N that is partitioned yield portions, (ii) physiological efficiency—yield increase in relation to crop uptake of N and (iii) utilization efficiency—yield per total plant N uptake (Fageria 2014; Naeem et al. 2017; Congreves et al. 2021) just to mention a few. In this study NHI index will be explored. As stated above, NHI expresses N recovered in yield to the total aboveground shoot N and is useful indicator for determining N translocation from the vegetative portions of the plant to the yield (Fageria 2014). Therefore, high NHI is an indication of increased N partitioning, however this index does not account for soil N which may potentially mask the crops actual efficiency (Naeem et al. 2017; Congreves et al. 2021). Nitrogen harvest index estimates also provides an indication of how much N could be made available to the soil after harvest based on crop residue N (Congreves et al. 2021).

2.5.3 Soil-based NUE

Soil-based indices takes into account all N sources potentially available (organic and inorganic N) for plant growth during growing season. This index serves as a tool to recognize systems with declining soil N pool which may jeopardize long-term soil fertility goals (Martinez-Feria et al. 2018). Examples of soil based NUE's (being focused on this study) includes (i) nitrogen

uptake efficiency (NUpE)—estimates the amount of available soil N taken up by the plant, (ii) apparent nitrogen recovery (ANR)—an indication of the amount of N removed from the field as yield based on available N, (iii) NUE yield—an indication of yield relative to applied N and takes into consideration uptake and utilization efficiency (Perchlik and Tegeder 2017; Congreves et al. 2021). Plant uptake of N is influenced by the availability of soil N and the synchrony between N supply and crop demand. The supply of plant available N is driven by the net rate of N release which is controlled by the balance between N mineralization and immobilisation (Ortiz-Zayas et al. 2006). However, plants take up less than half of applied N depending on the crop specie and soil conditions (Perchlik and Tegeder 2017) and the residual N that is not used by crop or immobilized into organic N pools is susceptible to losses (Martinez-Feria et al. 2018). Achieving greater uptake efficiency from applied N inputs increases overall NUE which is beneficial to enhance crop yields, reduce production cost and maintain environmental quality.

3.0 COVER CROPPING AND ITS EFFECT ON NITROGEN CYCLING, CROP YIELDS, AND NITROGEN USE EFFICIENCIES

3.1 Abstract

Cover crops are perceived as N regulators due to their ability to simultaneously influence N availability and reduce losses. However, for one of Canada's most important cropping regions, the Prairies, there is a major research gap on integrating cover crops into crop rotations. To address this gap, we implemented a four-year fully phased crop rotation trial consisting of wheat-canola-potato-pea grown with vs. without shoulder-season cover crops (red clover, berseem/oat mix, fall rye and mustard/tillage radish mix, respectively), a short rotation (wheat-canola) and a perennial alfalfa (as treatment checks) to test the viability of establishing cover crops in the Prairies, and to determine its influence on soil N availability and crop NUE. It was hypothesized that rotations with cover crop will increase soil N availability during crucial periods for crop use and therefore enhance crop N use efficiency. Cover crops were seeded after the main crop harvest in late summer or early fall (except for red clover, under-sown into wheat mid-summer). Yield and aboveground biomass of the main and cover crops were collected each year, and analyzed to determine N contents, crop NUE, and cover crop performance. Soil N availability was also monitored over study period. Cover crops were successfully established and differed significantly in terms of biomass, N content and C:N ratios. Of all cover crop types, red clover accumulated the most biomass and highest N content in the years studied. The cover crop effect on soil N dynamics was restricted to the non-growing season where cover crops reduced SIN supply and contents compared to the conventional practice without cover crop, an indication that cover crops might reduce non-growing season N losses. Yet rotations with vs. without cover crop did not differ in crop NUEs or yields or in-season N dynamics. By evaluating SIN metrics over a range of soil health management indices, we found some evidence that diversifying rotations with cover crops may help the system to function more like perennial systems in terms of regulating N metrics. To improve N availability from cover crops, future studies should focus on different ways of incorporating cover crops into rotations.

3.2 Introduction

Agricultural productivity relies heavily on N fertilization to increase crop yields, as N is considered a dominant nutrient that limits crop productivity. Most soil available N is accessible to plants only in soluble forms; thus in dryland production systems, plants may be exposed to periods of limited N supply when conditions are dry, whereas excess soil N becomes liable for losses under periods of high moisture, if not accessed by plants (Fu et al. 2019). In turn, N losses such as leaching or gaseous emissions risk yield losses as well as environmental quality. Generally, plants take up less than half of the N applied depending on the crop species and soil conditions (Perchlik and Tegeder 2017) leaving a considerable portion at risk for N loss. Therefore, regulating N fertilization to simultaneously minimize N losses *and* enhance crop productivity is necessary for developing more sustainable agriculture.

Cover cropping is a promising management practice that can influence both of these processes (Basche et al. 2016). Cover crops can be efficient in improving agroecosystem functions by enhancing crop, nutrient and water use efficiencies (Dabney et al. 2001; Finney et al. 2016), soil C sequestration and greenhouse gas mitigation (Blanco-Canqui et al. 2015). Cover crops can help to supply N for cash crops as well as reduce residual N levels during periods prone to N losses by efficiently utilizing and cycling excess N (Basche et al. 2014). This is achieved through the capacity of cover crops to increase the absorption and utilization of N during periods that would otherwise be fallow, and subsequently releasing N (ideally in time for the next crop) by mineralisation. In theory, cover crops can help to reduce the need for superfluous N fertilization to cash crops by contributing to the potentially mineralizable N pool during the subsequent growing season (Fu et al. 2019; Taveira et al. 2020). Better understanding N dynamics with and without cover crops will improve our ability to manage N additions to croplands and to balance crop yields and quality whilst conserving the environment. The challenges (short growing window and dry periods) associated with cover cropping here in the Prairies might be mitigated by understanding climate characteristics that regulate production in these regions and developing strategies to mitigate this threat. Selection of right cover crops for specific niches coupled with proper management can serve as a promising tool for overcoming the challenges of cover cropping under semi-arid conditions. This study therefore seeks to determine the viability of establishing cover crops in rotations here in the Prairies and its effect on soil N dynamics, productivity, and N use efficiencies.

3.3 Experimental design and methods

3.3.1. Study site and climate

A four-year crop rotation trial was initiated in 2018 at the University of Saskatchewan's North Management Area (52°09'22.7"N 106°36'28.8"W) in Saskatoon. The soil type is a Dark Brown Chernozem according to the Canadian system of soil classification. These chernozemic soils have dark coloured surface horizons as a result of organic matter accumulation from decomposition of mesophytic grasses and forbes which are representative of the grassland communities and are well to imperfectly drained soils (Canadian System of Soil Classification). At this site, the soil texture is a sandy loam, has organic matter of 3.7%, a pH of 6.9, cation exchange capacity of 14.5 meq 100g⁻¹, and soil bulk density of 1.35 g cm⁻³. The climate in Saskatoon is semi-arid with seasonal climate fluctuating between long cold winters and short warm summers. The mean annual temperatures range from 1-5°C and mean annual precipitation between 300-500 mm.

3.4 Experimental design and field management

The experiment comprises a four-year fully phased crop rotation of wheat-canola-potato-pea *with* and *without* cover crops (denoted CC, and LR) respectively. Also included is a four-year perennial alfalfa treatment (PR) and a short rotation of wheat-canola (SR), serving as treatment checks. All treatments (Table 3.1) are established on 6 m x 6 m plots using a randomized complete block design with four replicates. This thesis Chapter focuses on the period from spring 2019 to spring 2021.

In spring (May) of each year, the plots were seeded with the main crops (wheat, *Triticum aestivum*; canola, *Brassica napus*; potato, *Solanum tuberosum*; pea, *Pisum sativum*; alfalfa, *Medicago sativa*). Seeding rates for all main crops are shown in Table 3.2. Grain/oilseed crops were seeded using a small plot drill, with 30 cm between rows. When seeding pea, a nodulator was applied at rate of 3 kg ha⁻¹. For potato, a single row planter was used, seed pieces were planted at a depth of 20 cm, with a row spacing of 1 m x 0.3 m. For all plots, N fertilizer (urea) was broadcast on the soil surface, and rates were determined by conducting a pre-plant soil test (0-60 cm depth) and averaging the N fertilizer recommendation for each species from treatments without cover crops (determined by AgVise Laboratories). In 2019 and 2020, respectively: 142 and 186 kg N ha⁻¹ was applied to wheat, 118 and 92 kg N ha⁻¹ was applied to canola, 146 and 131 kg N ha⁻¹ was applied to potato, and 25 and 0 kg N ha⁻¹ was applied to pea. Alfalfa plots did not receive any

fertilizer. The crops receive other nutrients such as potassium, phosphorus and sulphur as needed according to the soil-test recommendations.

The entire field received irrigation after planting to enhance germination, and subsequent irrigation applied as needed. Approximately 10 to 18 cm of irrigation water was applied during the growing season. Potato plots were hilled after germination to enhance tuber formation alongside other management practices; weed and insect pest control. Potato and pea plots were sprayed with Decis insecticide at 150 mL ha⁻¹ to control Colorado beetles, and vertisan at 600 mL ha⁻¹ to control black spot, respectively in 2020. In 2019, pea plots were sprayed with other fungicides, matador at rate of 740 mL ha⁻¹ and priaxor at 490 mL ha⁻¹.

Cover crop (red clover, *Trifolium pratense*; oat + berseem clover, *Avena sativa* + *Trifolium alexandrinum*; fall rye, *Secale cereale*; tillage radish + mustard, *Raphanus sativus* + *Brassica juncea*) seed was drilled following main crop harvest in August—except for red clover, which is broadcast under-sown into wheat during the growing season on June 25 and July 2 in 2019 and 2020 respectively, to allow enough time for establishment (Table 3.2). During 2020, herbicide resistant mustard was seeded with tillage radish as a strategy to overcome potential residual injury from the herbicide applied earlier that year. Cover crops received supplemental irrigation until end of September. In spring, before seeding of main crops, overwinter cover crops biomass was collected, after which, cover crops were terminated with CleanStart at 2471 mL ha⁻¹ in 2019 and roundup glyphosate at 3311 mL ha⁻¹ in 2020. Weather data was collected from an on-site climate station for the duration of the study.

Table 3.1 The cropping sequence and cover crop treatments established at the Saskatoon site, 2018. Cover crop species are shown in parentheses.

Treatment	Phase	Crop sequence
Four-year cover crop rotation (LR with CC)	1 st Phase	Wheat (red clover) – Canola (berseem/oat) – Potato (rye) – Pea (tillage radish)
	2 nd Phase	Canola (berseem/oat) – Potato (rye) – Pea (tillage radish) – Wheat (red clover)
	3 rd Phase	Potato (rye) – Pea (tillage radish) – wheat (red clover) – Canola (berseem/oat)
	4 th Phase	Pea (tillage radish) – Wheat (red clover) – Canola (berseem/oat) – Potato (rye)
Four-year rotation without cover crops (LR without CC)	1 st Phase	Wheat – Canola – Potato - Pea
	2 nd Phase	Canola – Potato – Pea -Wheat
	3 rd Phase	Potato – Pea – Wheat – Canola
	4 th Phase	Pea – Wheat – Canola – Potato
Short rotation (SR without CC)	1 st Phase	Wheat – Canola – Wheat – Canola
	2 nd Phase	Canola – Wheat – Canola – Wheat
Perennial (PR)	No Phase	Alfalfa – Alfalfa – Alfalfa – Alfalfa

Table 3.2 Main crop and cover crop species, varieties, and respective seeding rate

Main Crop	Variety	Seeding rate		Seeding rate
		(kg ha ⁻¹)	Cover crop	(kg ha ⁻¹)
Wheat	CDC abound	83	Red clover	12
Canola	LL Canola	13	Oat/berseem clover	67/11
Potato	Norland red	1520	Fall rye	80
Pea	CDC meadow	110	Radish/mustard	11/11
Alfalfa	Equinox	12	–	–

* Main crops were seeded in May and cover crops seeded after harvest except for red clover which was under-sown into wheat during the growing season

3.5 Plant sampling and measurements

3.5.1 Main crop sampling

At grain/oilseed crop harvest, crop biomass samples were collected from near the centre of each plot, in two 0.25 m² areas. The aboveground shoots were clipped at ground level and grain separated from the crop residue. Ahead of potato harvest (~ a week), plant tops were mechanically mowed to allow for tuber skin-set. For potato yield, samples were collected by digging up tubers from a representative central area (0.25 m²) twice per plot. For all plant samples, fresh weights were recorded, and dry weights measured after oven drying the plant tissue at 60°C until constant dry weights were obtained. Grain samples were threshed, and yield and residue samples were separately ground to pass through a 1 mm sieve using Wiley grinder and stored in 8.5-dram polypropylene clear snap cap vials for %C and %N analysis.

3.5.2 Cover crop sampling

After cover crop emergence (1-3 weeks after planting), plant counts were recorded in two 0.25 m² areas per plot; plant heights are recorded twice during cover crop growth, once after emergence and again before biomass sampling in early October. Biomass samples were collected (early October) by clipping all vegetation at the soil surface within three 0.25 m² areas per plot; fresh weights were recorded before tissues are oven dried at a temperature of 60°C until constant dry weights are obtained. The dry samples were ground to pass through 1 mm sieve using blender and stored in 8.5-dram polypropylene clear snap cap vials for %C and %N analysis.

3.5.3 Plant tissue %C and %N analysis

Plant tissue samples (from the main crops and cover crops) were analysed for C and N concentrations using a LECO CN628. Briefly, about 0.10- 0.15 g of plant tissues were weighed into tin foil capsules, sealed, and placed in the sample carousel for % C and %N determination using the combustion method.

3.5.4 Estimation of crop nitrogen use efficiency (NUE)

The crop NUE indices were estimated using plant dry weights in kg ha⁻¹ and pre-plant available soil nitrogen (kg ha⁻¹). Soil available N was calculated by summing pre-plant available soil N, fertilizer N, and the potentially mineralizable N estimates derived from incubated soil samples. The various NUE indices explored were calculated using a similar approach as Van Eerd (2007) (Eq. 1-4):

- i. N harvest index (NHI), estimates portioning of plant N between yield and vegetative parts

$$NHI = (Yn/Pn) * 100 \quad (3.1)$$

- ii. N uptake efficiency (NUpE), measure how much N is taken up by the plant relative to soil N

$$NUpE = (Pn/Sn) * 100 \quad (3.2)$$

- iii. Apparent N recovery (ANR), measures crop N removed from the field as yield N based on available N

$$ANR = (Yn/Sn) * 100 \quad (3.3)$$

- iv. Soil yield N use efficiency (NUE_{yield}), estimates yield potential based on available N

$$NUE_{yield} = (Yw/Sn) \quad (3.4)$$

where Yn is yield N, Pn is aboveground plant N, Yw is yield weight, and Sn is soil N. The N contents were expressed in kg N ha⁻¹; weight was expressed as kg ha⁻¹ (dry weight basis).

3.6 Soil sampling and analysis

3.6.1 Soil inorganic N release rates

For key periods throughout the year, soil N availability was monitored using ion exchange resin strips to quantify soil inorganic N (NH₄⁺ and NO₃⁻) (Schoenau et al. 1993; Qian and Schoenau 2002). The ion exchange resin strips imitate the supply of nutrients to plant roots and function by exchanging ions between the soil medium and the exchange site of the membrane. The resin strips come in two forms; i) a cation resin strip, which has a negatively charged membrane to attract and adsorb positively charged nutrients, and ii) an anion resin strip, with a positively charged membrane to attract and adsorb the negatively charged nutrients. The exchange site of the resin strips acts as a sink for ions when buried in a soil (Schoenau et al. 1993). The resin sheets (AMB-SS and CMB-SS membranes from ResinTech Inc., West Berlin NJ) were cut into 2.5 x 12 cm strips. For each strip, the top 1.5 cm was taped off with marine duct tape, leaving an exposed area of 2.5 cm x 10.5 cm for burial into the soil. Prior to burial, the cation and anion strips were pre-conditioned by soaking in 0.5M HCl for 1 hour and in 0.5M NaHCO₃⁻ solution for 3 hours, with solution changed

every hour. The strips were buried by making a slit in the soil and placing two pairs of strips (positive and negative) about 10 cm apart in each plot. To ensure good contact, the soil was pressed firmly around the strips and area flagged for easy identification. Strips were left in soil for a period of 14 days during key periods (spring, summer, fall). To capture overwinter N dynamics, resin strips were placed in the soil just prior to freeze-up, left overwinter, and removed in spring at snowmelt (capturing the ~Oct to April period). Strips due for removal were retrieved from the field by placing all four strips from each plot together in a Ziploc bag and brought to the lab. In the lab, the strips were cleaned from all soil debris by rinsing in de-ionized water.

3.6.2 Soil inorganic N extraction

Soil inorganic N was extracted from the resin strips using 2.0 M KCl solution. The cleaned strips (2 pairs) from each plot were placed in containers containing 140 mL of the KCl solution (35 mL per strip) and placed on a shaker at 55 rpm for 1 hour to desorb nutrient ions (similar to the method described by Qian and Schoenau (2007)). The extract was filtered through Whatman No. 42 paper into 16-dram polypropylene clear snap cap vials and stored in a freezer (approx. -15°C) prior to analysis. The N supply rate was determined using air-segmented (continuous) flow analysis with a SEAL AA3 HR chemistry analyser (SEAL Analytical, Kitchener, ON). The concentration obtained was transformed into rate of absorption per area of 4 strips (0.0105 m²) per number of days buried (mg N m⁻² duration of burial in days⁻¹).

3.6.3 Potentially mineralizable nitrogen

In spring 2020, soils were sampled from each plot at depths of 0-15 and 15-60 cm to determine Potentially Mineralizable Nitrogen (PMN). The PMN pool was determined by incubating soil samples anaerobically for 7 days followed by KCl extraction to determine NH₄⁺-N concentration. Sub-samples of field moist soil (5 g) were used to determine pre-incubated NH₄⁺-N using 2 M KCl solution. This was followed by submerging 5 g soil samples in 10 mL de-ionised water and incubating for 7 days at a temperature of 37°C (Curtin and Campbell 2008). Incubated samples were diluted with 3.33 M KCl solution, shaken at 120 rpm for 30 minutes and supernatant filtered in vials using Whatman No. 42 filter paper and stored for analysis. The NH₄⁺-N concentration for the pre-incubated and incubated samples was determined using air-segmented (continuous) flow analysis with a SEAL AA3 HR chemistry analyser (SEAL Analytical, Kitchener, ON). Mineralized N was estimated by deducting pre-incubated NH₄⁺-N from incubated NH₄⁺-N.

Potential mineral N values obtained were used to estimate the total N potentially mineralized over a growing season.

3.7 Statistical analysis

SAS (SAS Institute, Inc., University edition, Cary, NC) was used to perform the analyses of variance (ANOVA). PROC MEANS was used for descriptive statistics, PROC UNIVARIATE for normality check, LEVENE for testing homogeneity of variances, PROC MIXED for the ANOVA procedure, TUKEY for means comparison. A significance level of $\alpha=0.05$ was used. Each year was analyzed separately. To determine if cover crops in the rotations significantly influenced all SIN metrics, analysis was conducted to compare the LR to the CC rotation for each crop phase and year of the study. For the mixed model, fixed effects were “rotation” for crop yield, crop NUE, SIN dynamic and PMN. For all response variables, the replicates were considered as random effects. CoPlot (version 6.45) was used for all graphical presentations.

To test if cover crops—as *part of a soil health management strategy*—influenced soil N dynamics, regression analyses were conducted. The crop rotation treatments (Table 3.1) were assigned an index value from 1 to 4 based on near continuous soil cover, where in theory: 1) indicates a relatively poor management practice for soil health, a short rotation with only two crops in a 2-yr rotation; 2) indicates an incrementally better management strategy for soil health, a longer rotation with four species in a 4-yr rotation; 3) indicates an incrementally better management strategy for soil health, with four species in a 4-yr rotation *plus* four shoulder-season cover crops; 4) indicates the best theoretical management strategy for building soil health, a perennial cropping system. PROC GLM was used to test for two biologically meaningful relationships between the soil health management index and the soil N response—that being either a linear or quadratic response (cubic models were not considered as these were not deemed biologically meaningful). A significance level of $\alpha=0.05$ was used.

3.7.1 Transformations

All response variables which did not meet normality test ($Pr < SW$ greater than 0.05) were log or square root transformed. Where normality test was not met by transformations, the dataset was double checked for outliers and when points determined were erroneous (i.e., by confirming with field logbooks for issues) they were removed. All data transformed were back-transformed for presentation.

3.8 Results

3.8.1 Weather conditions

The average monthly weather during the duration of study was monitored (Table 3.3, *note that here I am reporting the weather for the study periods applicable to this Chapter 3 as well as Chapter 4*). The monthly precipitation and temperature fluctuated over the study period with differences in the amount and distribution of rainfall. Certain periods had higher or relatively similar precipitation as the 30-year normal (i.e., March 2020 and May 2021). During the growing season (May-October) precipitation amounted to 246 mm, 209 mm, and 120 mm, respectively in 2019, 2020 and 2021. Precipitation in May-June 2020 was higher than the same period in 2019 and 2021, whilst an increase was observed from July-October 2019 compared to the same period in 2020 and 2021. Some of the driest growing-season months included May and Aug in 2019; July and Aug 2020; June and July 2021. Temperatures over the study period followed similar distribution, cold winters, and hot summers. The average temperature was 4.2 °C, 3.4°C and 4.4 °C in 2019, 2020, and 2021 respectively indicating warmer temperatures in 2021 compared to 2020 and 2019.

Table 3.3 Weather data from 2019 to 2021 and 30-year normal (1991-2020) in Saskatoon, SK.

Month	Mean Temperature (°C)				Total Precipitation (mm)			
	2019	2020	2021	30-yr mean	2019	2020	2021	30-yr mean
January	-13.0	-13.2	-9.8	-14.7	8.4	9.7	10.8	15.5
February	-22.4	-10.4	-17.7	-12.2	14.2	3.5	1.7	9.1
March	-4.5	-6.3	-0.8	-5.1	3.9	12.5	3.7	11.2
April	5.9	0.5	5.4	4.4	2.9	14.8	5.4	23.3
May	10.5	11.9	11.1	11.6	11.7	33.8	35.6	37.6
June	16.8	16.0	19.6	16.3	78.0	106.4	22.5	73.9
July	18.7	19.9	22.6	19.0	82.1	26.9	5.0	60.1
August	17.0	19.8	19.0	18.3	19.9	15.9	41.8	46.4
September	13.1	12.9	15.2	12.9	44.8	20.6	8.5	33.4
October	2.6	2.6	6.7	4.7	9.6	5.3	6.1	20.4
November	-4.5	-4.9	-2.4	-4.6	14.3	32.3	13.1	13.8
December	-11.1	-8.4	-15.6	-11.9	6.1	2.8	13.4	9.9

* Data obtained from the Climate Reference station located at the experimental site operated by the Saskatchewan Research Council.

3.8.2 Cover crop biomass production, residue N content and carbon to nitrogen ratio

Cover crop biomass, N content and C:N ratios differed by species in fall of 2019 and 2020 and spring of 2021 (Table 3.4). Cover crop biomass production ranged from 99-437 kg dry matter ha⁻¹ in fall of 2019, and 90-428 kg ha⁻¹ in fall 2020; contained between 4-16 kg N ha⁻¹ each year. In fall 2019, red clover produced the most biomass and highest N content compared to the other cover crops but was not significantly different from fall rye and tillage radish—and similarly, their N content did not differ among all three. However, red clover significantly differed from the oat/berseem clover mix in terms of biomass and the N content but their C:N ratio was same. At cover crop termination in spring 2020, there was no significant difference between overwintering species (red clover and rye) for biomass and N content. Prior to termination in spring, red clover and fall rye accumulated 296 and 287 kg dry matter ha⁻¹, respectively. Even though red clover is an overwintering species, its biomass declined by 32% from fall 2019 to the subsequent spring in 2020, whereas no change in fall rye dry matter was observed. The amount of biomass that cover crops produced tended to be higher in 2019 than 2020 for all crops, except for red clover. Red clover again, accumulated highest biomass and N content in fall 2020 and was significantly different from the other cover crops. The order of decline in biomass of the cover crop specie was red clover > fall rye > tillage radish > oat/berseem clover mix in fall of 2019 vs. red clover > tillage radish/mustard > oat/berseem clover mix > fall rye. A decrease of about 68% and 71% in biomass

and N content, respectively was observed with fall rye from fall 2019 to fall 2020. Yet fall rye biomass and N content did not differ significantly from oat + berseem clover and tillage radish + mustard mix in 2020. By spring 2021, both red clover and rye had lower biomass than the previous spring, with rye performing better in terms of biomass and N content than red clover.

Table 3.4 Mean cover crop biomass, nitrogen content, and C:N ratio \pm standard error at fall before freeze-up and at spring before termination. Within each column, means followed by different letters are significantly different at $\alpha < 0.05$. Fall 2018 and spring 2019 samples were not analysed due to incomplete data and are represented by n/a when data was not available.

Year	Cover crop	Biomass (kg ha ⁻¹)	N content (kg ha ⁻¹)	C:N ratio
Fall 2018	Red clover	n/a	n/a	n/a
	Oat/berseem	8	n/a	n/a
	Rye	59	3	9
	Tillage radish	n/a	n/a	n/a
Spring 2019	Red clover	37	1.4	11
	Rye	215	10	9
	Oat/berseem	n/a	n/a	n/a
	Tillage radish	n/a	n/a	n/a
Fall 2019	Red clover	437 (121.7) ^a	16 (4.6) ^a	11 (0.1) ^a
	Oat/berseem	99 (15.2) ^b	4 (0.7) ^b	10 (0.6) ^a
	Rye	288 (15.4) ^{ab}	14 (0.3) ^{ab}	7 (0.1) ^b
	Tillage radish	258 (27.6) ^{ab}	12 (1.1) ^{ab}	7 (0.1) ^b
		<i>P</i> = 0.0419	<i>P</i> = 0.0410	<i>P</i> = 0.0001
Spring 2020	Red clover	296 (64)	12 (2.5)	10 (0.1) ^a
	Rye	287 (64)	13 (2.5)	8 (0.) ^b
	Oat/berseem	n/a	n/a	n/a
	Tillage radish	n/a	n/a	n/a
		<i>P</i> = 0.9231	<i>P</i> = 0.6821	<i>P</i> = 0.0066
Fall 2020	Red clover	428 (116.5) ^a	16 (4.5) ^a	11 (0.3) ^a
	Oat/berseem	133 (16.8) ^b	5 (0.7) ^b	11 (0.3) ^a
	Rye	90 (11.2) ^b	4 (0.6) ^b	9 (0.3) ^b
	Tillage radish	164 (12.0) ^b	7 (0.7) ^b	8 (0.1) ^b
		<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001
Spring 2021	Red clover	145 (18.6) ^b	6 (0.7) ^b	9 (0.2)
	Rye	229 (18.6) ^a	10 (0.7) ^a	9 (0.2)
	Oat/berseem	n/a	n/a	n/a
	Tillage radish	n/a	n/a	n/a
		<i>P</i> = 0.0408	<i>P</i> = 0.0158	<i>P</i> = 0.7943

* Red clover was seeded earlier than the other cover crops.

3.8.3 Cover crop influence on soil N dynamics

3.8.3.1 Pre-plant soil nitrate content in rotations with vs. without cover crops

Over the entire study period, rotation significantly influenced pre-plant soil NO_3^- content only in 2021 prior pea in the rotation W-C-Po-P ($P=0.040$), and prior wheat in the rotation C-Po-P-W ($P=0.029$) with a similar tendency prior canola in the rotation Po-P-W-C ($P=0.073$) (Fig. 3.1). By spring 2021, three years with vs. without cover cropping had accrued. For most of these cases, the rotations with cover crops had lower NO_3^- content compared to without cover crops. These SIN results show that when *canola and potato occurred early in the rotation*, NO_3^- levels were most influenced by cover cropping (Fig. 3.1). Before 2021, there were some indications—albeit weak—that cover cropping reduced NO_3^- content compared to without cover cropping at pre-plant, i.e., in 2019 before pea (and after potato) the CC rotation had less SIN than the LR although the difference was not significant ($P=0.189$). Likewise, after canola production in 2018 (prior potato) and prior pea and wheat in 2020, the CC rotation had numerically lower SIN than the LR, but again, not significantly different (Fig. 3.1). Generally, the highest pre-plant NO_3^- contents were observed prior to the production of peas in 2019; the lowest SIN content occurred prior canola in 2021 (Fig. 3.1).

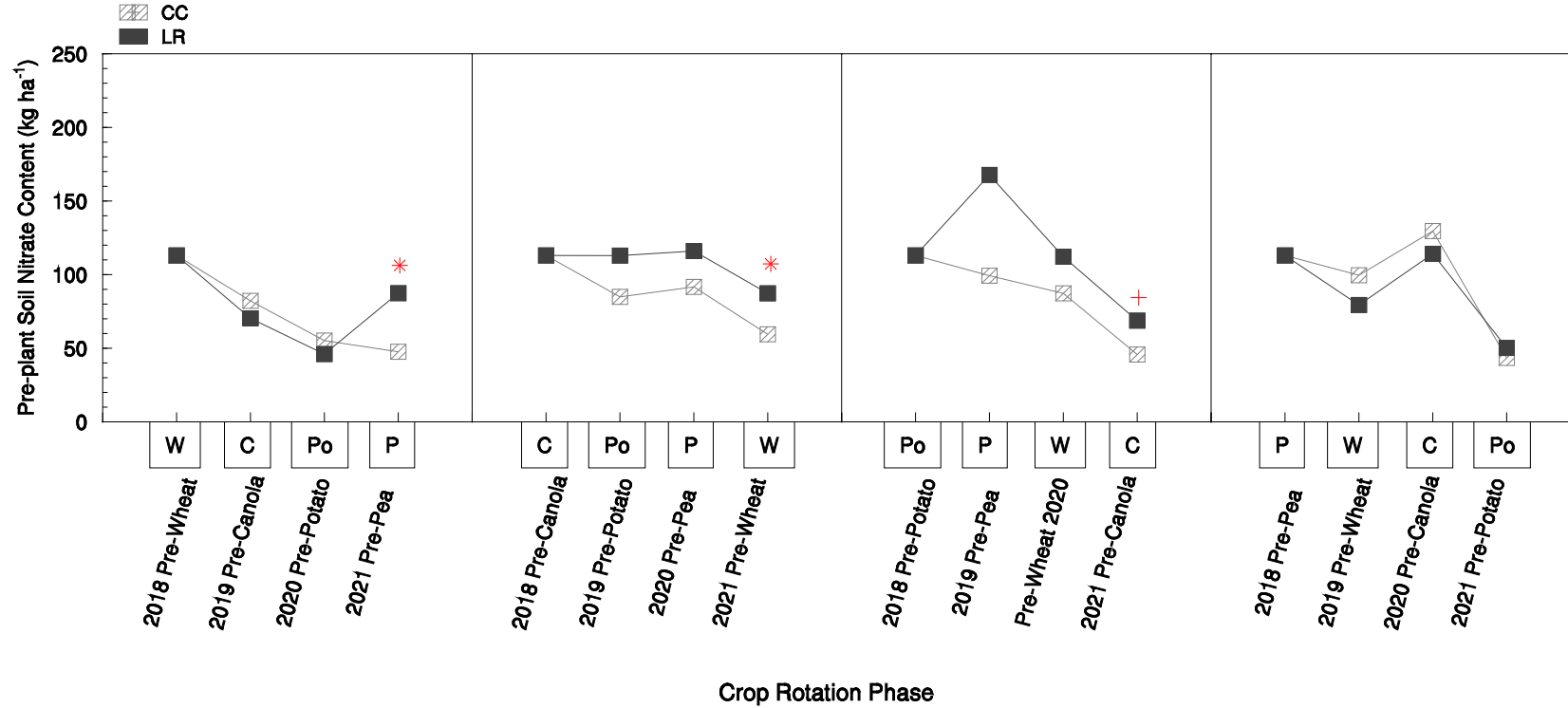


Figure 3.1 Pre-plant soil nitrate content (NO₃⁻) levels in the 0-60 cm depth as influenced by cover crops over study period (2019-2021). The SIN content beginning in each rotations represent the N level at the start of the experiment in 2018. Afterwards results are based on the period prior to seeding the main crop in any year and only the 4-year long rotations with and without cover crops are compared. CC indicates the cover cropped long rotation; LR indicates the long rotation without cover crops. Markers with red asterisks at each sampling point are significantly different (P < 0.05), while red plus (+) sign shows a tendency for significance (P > 0.05 but < 0.1). Pre-Pea in 2019 was log transformed whilst pre-canola in spring 2020 was square root transformed for analysis. The crop sequence is W (Wheat) – C (Canola) – Po (Potato) – P (Pea).

3.8.3.2 Pre-plant soil Nitrate content as influenced by soil health management

There was a significant quadratic relationship between the soil health management index and pre-plant SIN only in 2021 ($P=0.014$), but no apparent relationship in 2019 and 2020 (Fig. 3.2). As the soil health management index increased from 1 to 4 (poor to best) in 2021, there was an increase in pre-plant SIN *up to a certain point* and thereafter the SIN levels decreased. This result indicates that adopting better soil health management strategies regulates pre-plant SIN levels over time.

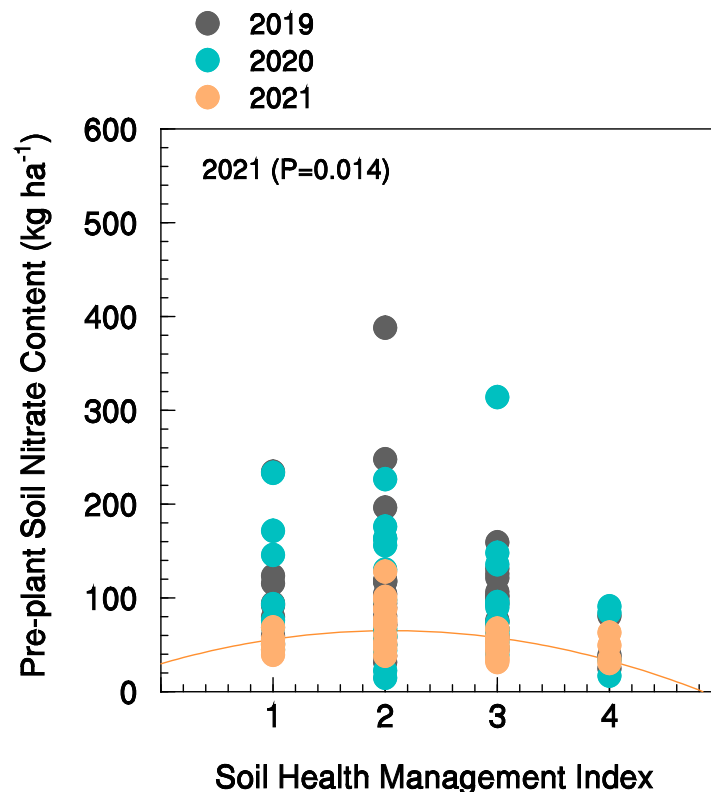


Figure 3.2 Relationship between pre-plant soil NO₃⁻ content in the 0-60 cm depth as influenced by soil health management index from 2019-2021. Numbers 1-4 on the x-axis represent the soil health management index. The lowest number is assigned to rotation with the poor soil health management and the highest number is assigned to the rotation with a better soil health management strategy. 1 = short rotation, 2 = long rotation without cover crop, 3 = long rotation with cover crop, 4 = perennial rotation with alfalfa. The regression lines represent significant quadratic relationships.

3.8.3.3 Supply rate of soil inorganic N in rotations with vs. without cover crops

Including cover crops in the rotation significantly influenced SIN supply rates only during the fall season, and never for spring or summer (Fig. 3.3). Notably, the cover crop effect was apparent after harvest for all grain/oilseed crops (i.e., canola in 2019, pea in 2020, and wheat in 2020) but never for potato (Fig. 3.3). The rotation starting with wheat in 2018 (W-C-Po-P) had lower SIN supply rates with vs. without cover crops in fall after canola harvest in the second year of the rotation ($P=0.0137$). When potato and pea were grown later in this same rotation (during the third and fourth year), no significant effects were observed. The rotation beginning with canola in 2018 (C-Po-P-W) showed lower SIN supply rates with vs. without cover crops in the third year of the rotation, after pea harvest. Similarly, the rotations with cover crops had lower SIN supply rates compared to the long rotation without cover crop in fall of 2020 only ($P=0.0007$). No significant effect was observed for other crop phases in the rotation. The rotation beginning with potato (Po-P-W-C) in 2018 also resulted in a significant effect of rotation in fall 2020 after wheat was grown. The cover cropped rotations lowered SIN compared to the long rotations without cover crop during fall 2020 ($P=0.0010$). The rotation starting with pea in 2018 did not show any influence of rotation on SIN in any of the crop phases from fall 2019 to spring 2021.

Generally, the trend across all the crop phases was an increase in SIN supply rate during spring and a subsequent decrease from summer to fall. The cover crop rotations *numerically* increased SIN in spring (except for when pea appeared in the C-Po-P-W sequence in 2020). Subsequent decrease was observed from summer 2020 across all crop phases followed by a steady increase from fall 2020 to spring 2021. Across the whole sampling period, the highest N supply rate was observed during the wheat phase grown with red clover in spring 2020 whilst the lowest was observed in summer when wheat was grown with and without cover crop but, relatively higher with cover crop than without.

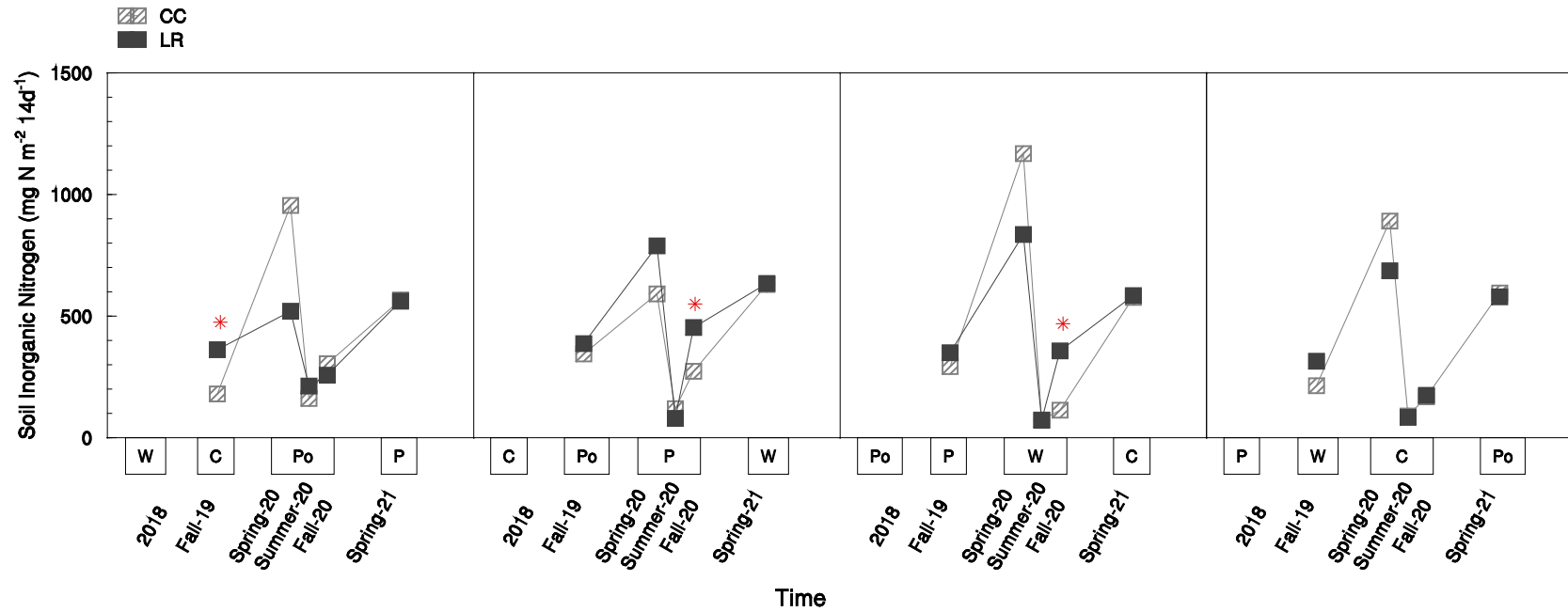


Figure 3.3 Soil inorganic N supply rate (NH_4^+ and NO_3^-) in the top 10 cm depth, as influenced by rotation from fall 2019 to spring 2021. Only the 4-year long rotations with and without cover crops are compared. CC indicates the cover cropped long rotation; LR indicates the long rotation without cover crops; Markers with red asterisks at each sampling point are significantly different, otherwise are not significantly different. Spring and summer are period during the growing season for any specific crop phase while fall periods are during the non-growing season after crop harvest. Data for wheat in Spring 21 was log-transformed for analysis. The crop sequence is W (Wheat) – C (Canola) – Po (Potato) – P (Pea).

3.8.3.4 Soil inorganic N supply rate as influenced by soil health management

The SIN supply rate as influenced by soil health management showed a quadratic relationship in summer 2020 ($P=0.061$) and spring 2021 ($P=0.0002$) (Fig 3.4). For the spring 2021 period, moving from the short rotation to the long rotations without cover crop (represented by the soil health management index 1 and 2), an increase in soil N supply rate was observed. However, this was followed by a steady decrease in the SIN supply rate for the cover crops rotations and perennial systems (indices of 3 and 4). During the summer of 2020, a similar (albeit more prominent) pattern was observed (Fig 3.4). These patterns indicate that including cover crops in annual crop rotations may behave similar to perennial systems—in terms of N cycling and dynamics. For the post-harvest fall periods, the soil health management practice was not related to SIN supply.

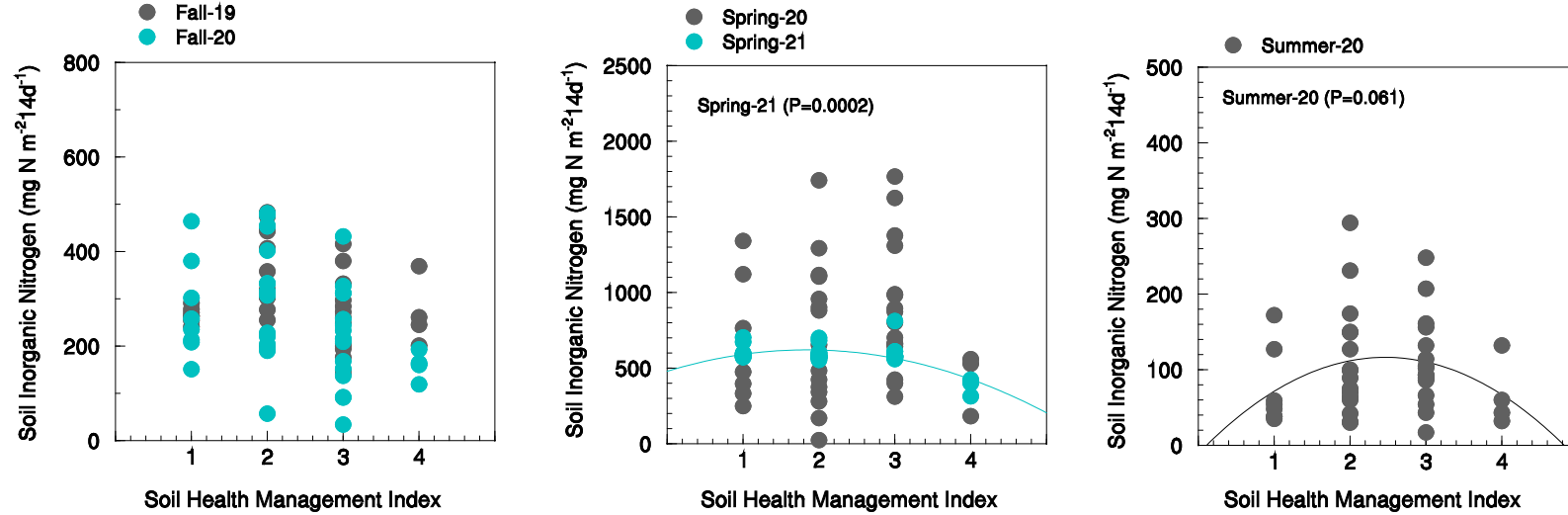


Figure 3.4 Relationship between soil inorganic N (NH_4^+ and NO_3^-) supply rate in the top 10 cm as influenced by soil health management index. Numbers 1-4 on the x-axis are the soil health management. The lowest number is assigned to rotation with the poor soil health management and the highest number is assigned to the rotation with a better soil health management strategy. 1 = short rotation, 2 = long rotation without cover crop, 3 = long rotation with cover crop, 4 = perennial rotation with alfalfa. The regression lines represent significant quadratic relationships.

3.8.3.5 Overwinter soil inorganic N in rotations with vs. without cover crops

The supply rate of SIN for the overwinter period (October-April) was not significantly influenced by rotation in either 2019/2020 or 2020/2021 (Fig. 3.5). Over the winter period in 2019/2020, the rotation starting with canola (C-Po-P-W) in 2018 gained the highest N supply rate over both years. This increase was observed during the second year when potato appeared in the long rotation without cover crops, supplying more N than the corresponding rotation with cover crop— but the difference was not significant. Subsequently a decrease of about 48% was observed in the following year after pea harvest. The rotation beginning with pea (P-W-C-Po) in 2018 and studied after wheat was grown in 2019 showed the lowest supply rates for both rotation sequences studied (with or without cover crops). Following wheat, canola was grown in 2020, doubling the overwinter N supply rate in both rotations with and without cover crops. Similarly, the rotations beginning with wheat (W-C-Po-P) and potato (Po-P-W-C) in 2018 also increased N supply rate by the third overwinter period. Across all crop phases during the second overwinter period (2019/2020), the long rotations with cover crops numerically reduced SIN supply rates compared to their respective rotations without cover crops. However, by the third year, the cover crop rotations performed similar to the long rotations without cover crops. Generally, SIN steadily increased or remained same among all crop phases from second to third over-winter period except during the pea phase of the rotation beginning with canola in 2018 (C-Po-P-W).

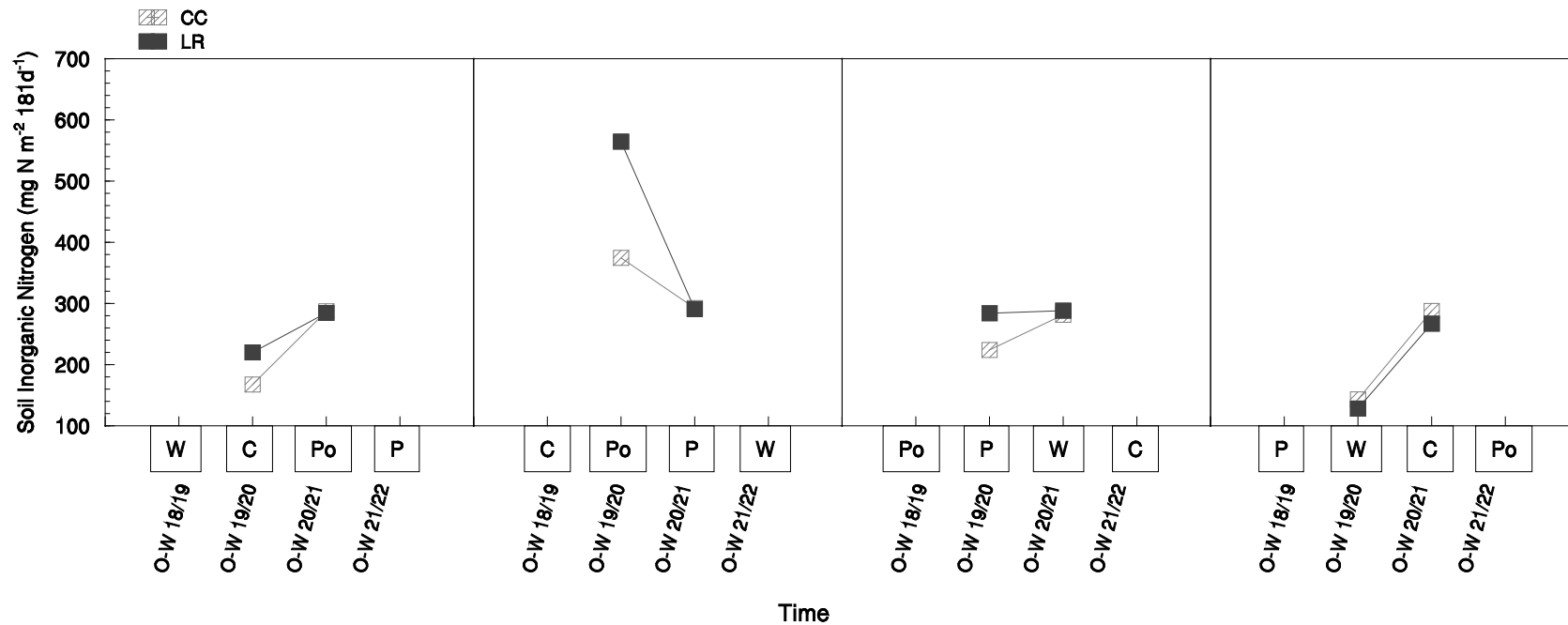


Figure 3.5 Over-winter soil inorganic N (NH_4^+ and NO_3^-) supply rate as influenced by cover crops overwinter (Fall 2019 to Spring 2020 and Fall 2020 to Spring 2021), $\text{mg N m}^{-2} 181 \text{ d}^{-1}$. Only the 4-year long rotations with and without cover crops are compared. CC indicates the cover cropped long rotation; LR indicates the long rotation without cover crops; no significance difference were detected between treatments at $P < 0.05$. Overwinter SIN data after canola in 2020/2021 was transformed for analysis. Overwinter is the period from soil freeze-up (end of October) to spring thaw (April). The crop sequence is W (Wheat) – C (Canola) – Po (Potato) – P (Pea).

3.8.3.6 Overwinter soil N supply rate as influenced by soil health management

The influence of soil health management on overwinter SIN supply did not show any significance in both 2019/2020 and 2020/2021.

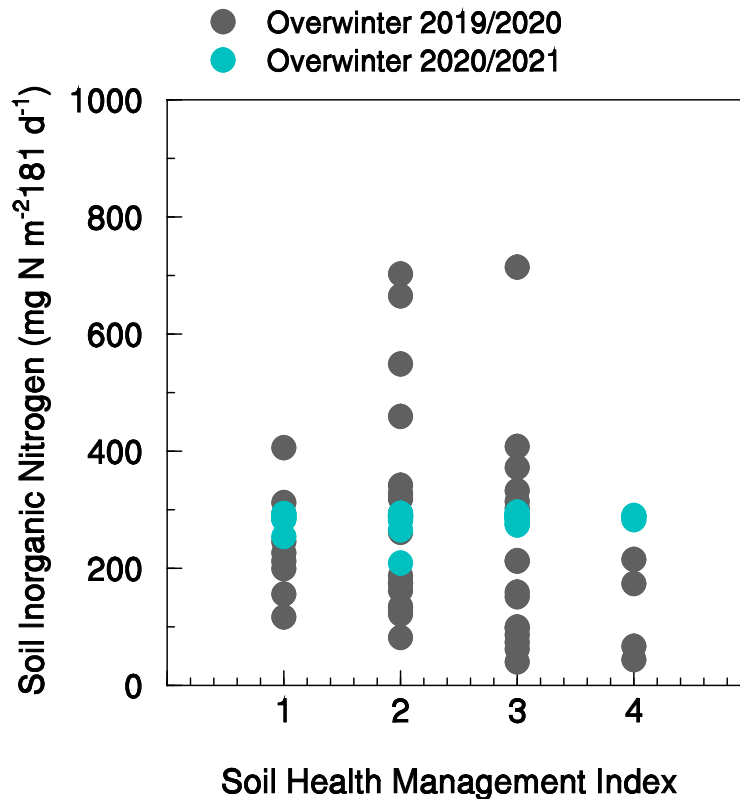


Figure 3.6 Relationship between overwinter SIN supply rate as influenced by soil health management index. Two overwinter periods are shown (Fall 2019 to Spring 2020; Fall 2020 to Spring 2021). Numbers 1-4 on the x-axis denotes the soil health management index. The lowest number is assigned to rotation with the poor soil health management and the highest number is assigned to the rotation with a better soil health management strategy. 1 = short rotation, 2 = long rotation without cover crop, 3 = long rotation with cover crop, 4 = perennial rotation with alfalfa. The absence of regression line represents no significant quadratic relationship. Overwinter is the period from soil freeze-up (end of October) to spring thaw (April).

3.8.3.7 Potentially mineralizable N in rotations with vs. without cover crops

Potentially mineralizable N was not significantly influenced by rotation in any phase (Fig. 3.7). However, prior to wheat production in 2020, PMN had a tendency to be influenced by cover crops (at $P < 0.1$ but > 0.5). Across all rotations, the cover crop rotations had numerically greater PMN than the rotations without cover crops (except for the rotation starting with wheat in 2018, W-C-Po-P). The lowest PMN was observed in the rotation without cover crop beginning with canola in 2018 (C-Po-P-W). This was detected prior to the pea phase in 2020. Comparing PMN across all cover crop treatments, the phase prior to canola showed the highest. This phase was the post-wheat period when red clover was under-sown into wheat in the growing season for P-W-C-Po sequence. The W-C-Po-P rotation showed the lowest PMN from the cover crop rotations, and this was following canola phase seeded with oat and berseem clover mix.

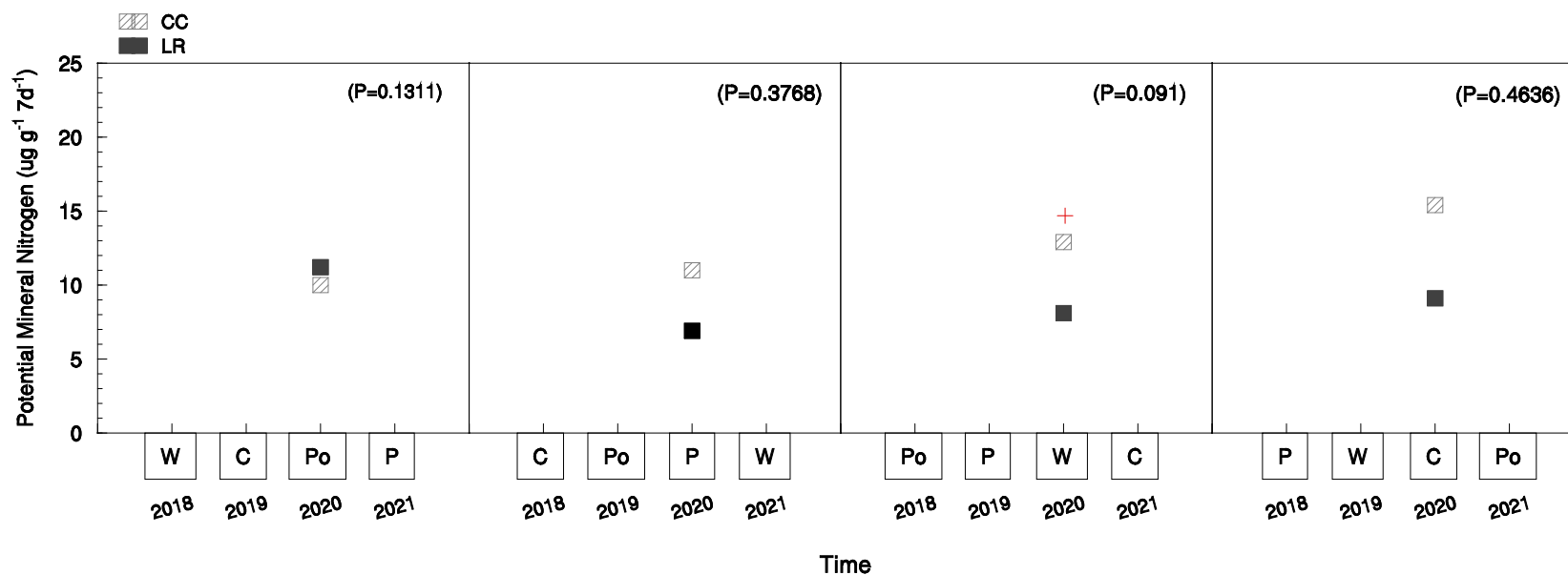


Figure 3.7 Soil potentially mineralizable N (PMN) in the 0-15 cm depth in 2020. Only the 4-year long rotations with and without cover crops are compared CC indicates the cover cropped long rotation; LR indicates the long rotation without cover crops. P values for the comparisons are shown directly on the figure. Red plus (+) sign shows a tendency for significance ($P>0.05$ but <0.1). Potential mineral N for potato and pea was log-transformed for analysis. Soil samples for incubation was collected in spring 2020 prior to seeding. The crop sequence is W (Wheat) – C (Canola) – Po (Potato) – P (Pea).

3.8.3.8 Potentially mineralizable N as influenced by soil health management

Soil health management strongly influenced potentially mineralizable N ($P=0.007$), moving across the indices of 1-4 (Fig. 3.8). There was a significant quadratic relationship as the index moved from 1 to 2 (representing the short rotation and long rotations). As index moved from 3 to 4 (representing the cover cropped and perennial rotation), soil PMN began to increase again.

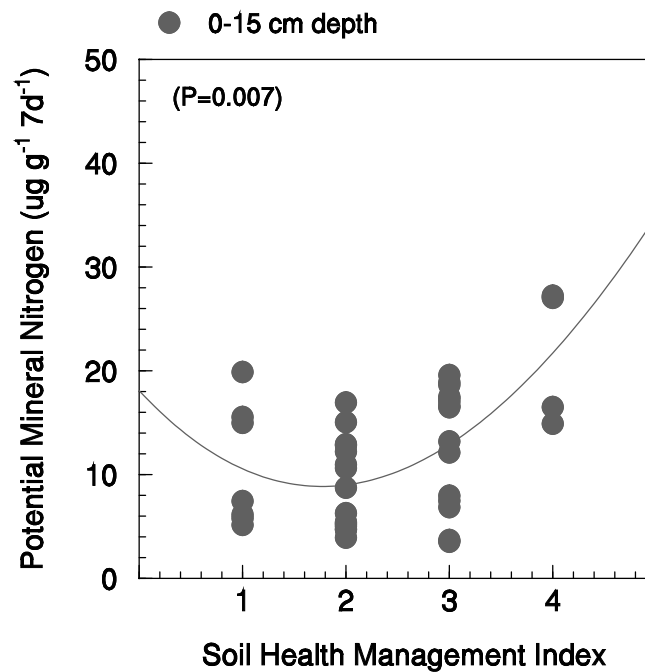


Figure 3.8 Relationship between soil potentially mineralizable N (PMN) in the top 15 cm and soil health management index. The lowest number is assigned to rotation with the poor soil health management and the highest number is assigned to the rotation with a better soil health management strategy. 1 = short rotation, 2 = long rotation without cover crop, 3 = long rotation with cover crop, 4 = perennial rotation with alfalfa. The regression line represents a significant quadratic relationship.

3.8.4 Cover crop influence on crop yields and crop nitrogen use efficiency

3.8.4.1 Crop yields in rotation with vs. without cover crops

In both 2019 and 2020, crop yields were not significantly different with vs. without cover crops (Table 3.5)—except for pea where there was a tendency for yields to be significantly reduced due to cover cropping ($P < 0.1$ but > 0.05). Numerically, all crops grown in the long or short rotations without cover crops had higher yields in 2019 than with cover crops. The 2020 yields were relatively higher than those from 2019; however, unlike 2019, yields from the cover crop rotations were numerically higher than from the conventional rotation without cover crops.

Table 3.5 Mean crop yields in 2019 and 2020 \pm standard error (SEM). CC indicates the cover cropped long rotation; LR indicates the long rotation without cover crops; SR indicates the short rotation without cover crops.

2019 Yields (Mg ha ⁻¹)					
Crop	CC	LR	SR	SEM	P-value
Wheat	4.5	4.8	4.9	0.35	0.673
Canola	1.8	2.1	2.1	0.35	0.710
Potato*	40.6	44.0		4.59	0.596
Pea	1.6	2.1		0.15	0.087
2020 Yields (Mg ha ⁻¹)					
	CC	LR	SR	SEM	P-value
Wheat	6.6	6.4	6.3	0.26	0.658
Canola	3.9	3.5	3.9	0.36	0.656
Potato*	54.5	48.3		6.46	0.270
Pea	1.9	3.0		0.43	0.089

* Potato yields reported on a fresh weight basis as per convention for this crop; all other yields are reported on a dry weight basis.

3.8.4.2 Crop nitrogen use efficiency in rotations with vs. without cover crops

Crop N use efficiency did not differ by rotation in any year (Fig. 3.4). Nitrogen harvest index ranged from 29-86%; uptake efficiency from 73-169%; recovery efficiency from 34-93%; NUE_{yield} from 9-46%.

Although no significant differences were observed, key resulting trends and patterns are described here. Generally, the NHI values were higher in 2020 than in 2019 for all main crops except pea. Among the crops in 2020, potato had the greatest NHI reaching 86% in the rotation with cover crops; but in 2019, wheat had the highest NHI of about 59%. For NUpE, the general trend was a decrease in NUpE in 2020 compared to 2019 for all crops, except for pea grown without cover crop. The NUpE was greatest in pea for both years far exceeding 100% (likely due to the N supplied by N fixers in pea, which is not accounted for in the calculation). In 2019, pea grown with cover crops had the highest NUpE, but the reverse was observed in 2020. No clear pattern for NUpE was observed for canola or wheat, other than higher values in 2019 than 2020. Interestingly, potatoes showed numerically higher NUpE with vs. without cover crops over both years. Apparent N recovery was highest in 2019 for wheat in the long rotation without cover crop and also for potato in the cover crop rotation both reaching 66%. In 2020, pea had the highest ANR, about 69% for the rotations without cover crop. The general trend for ANR was a decrease for wheat and potato from 2019 to 2020 for the rotations and an increase for canola. Yield efficiency was greatest in potato for both years and lowest in canola in 2019. In 2020 however, pea in the cover crop rotation had the lowest yield efficiency. No clear pattern for yield efficiency was observed for wheat.

Table 3.6 Mean crop N use efficiency indices of NHI (nitrogen harvest index), NUpE (nitrogen uptake efficiency), ANR (apparent nitrogen recovery, and NUpE_{yield} (yield efficiency) for wheat, canola, potato, and pea in 2019 and 2020. CC indicates the cover cropped long rotation; LR indicates the long rotation without cover crops; SR indicates the short rotation without cover crops. NUE indices for pea in 2020 were log transformed for analysis.

2019 NUEs						2020 NUEs				
	CC	LR	SR	SEM	<i>P</i> -value	CC	LR	SR	SEM	<i>P</i> -value
Wheat										
NHI (%)	59	58	59	1.9	0.825	71	70	68	1.3	0.354
NUpE (%)	93	114	97	12.9	0.524	73	77	74	5.7	0.847
ANR (%)	55	66	58	8.1	0.626	52	55	51	4.0	0.762
NUE _{yield}	18	22	18	2.5	0.516	19	19	18	1.5	0.793
Canola										
NHI (%)	32	38	29	3.6	0.243	64	60	64	3.2	0.594
NUpE (%)	111	118	145	30.0	0.538	82	92	75	14.7	0.705
ANR (%)	34	46	43	9.1	0.289	52	55	49	9.1	0.899
NUE _{yield}	9	11	11	2.4	0.348	13	14	13	2.5	0.941
Potato										
NHI (%)	48	52		3.9	0.234	86	85		1.1	0.548
NUpE (%)	135	112		13.0	0.259	98	89		10.7	0.298
ANR (%)	66	58		8.4	0.532	58	48		8.2	0.271
NUE _{yield}	44	40		6.8	0.681	46	39		5.5	0.248
Pea										
NHI (%)	38	42		2.5	0.326	32	43		4.6	0.069
NUpE (%)	146	113		34.6	0.429	130	169		52.6	0.480
ANR (%)	55	47		14.3	0.706	41	69		12.3	0.256
NUE _{yield}	14	12		3.9	0.758	10	19		8.0	0.255

3.9 Discussion

3.9.1 Cover crop production in a short season growing region: low biomass and N content, and opportunities for improvement

For the duration of this study, all cover crops were successfully established in fall with subsequent re-growth in the spring for the overwintering species. Biomass, N contents, and C:N ratios generally differed by the type of cover crop and how it was incorporated. Red clover under-sown into wheat accumulated more biomass and N content than the rest of the shoulder season cover crops. This pattern is similar to another study where 31 kg N ha⁻¹ was accumulated in cover crop tissues when under-sown compared to 25 kg N ha⁻¹ when sown after harvest (Doltra and Olesen 2013). The winter hardy species (red clover and fall rye) in our study accumulated greater biomass than non-hardy species (oat/berseem clover mix and tillage radish/mustard mix).

Generally, the aboveground biomass produced in this study was lower than that observed from other studies conducted under similar short growing windows and colder climates (Ruis et al. 2019; Farzadfar et al. 2021). Even within this study, there was interannual variation—2019 had better cover crop production than 2020, a trend that corresponds to mean precipitation over the cover crop growth period (August-October), i.e., more in 2019 (74 mm) than in 2020 (42 mm). Even with supplemental irrigation during the cover crop establishment phase (late August/early September before irrigation was shut down for the fall), the soil moisture likely had an impact on the relative amount of biomass produced. Cover crop performance varies with climatic conditions, a conclusive remark made based on a meta-analysis study under temperate conditions (Ruis et al. 2019). Their results showed that humid regions with precipitation >750 mm produced greater biomass compared to semi-arid regions with less precipitation (Ruis et al. 2019).

Among the groups of cover crops (legumes, grasses, legume-grass mix and non-legume), the legume cover crop (red clover) produced the greatest N content (i.e., an average of 16 kg N ha⁻¹ in 2020) whereas a lower average of 4.5 kg N ha⁻¹ was accumulated in the legume-grass mix (oat/berseem clover) over 2019 and 2020. This result is comparable to a study which reported that N accumulated in red clover alone was 88% higher when grown alone compared to when it was mixed with grasses (Nyiraneza et al. 2021). However, the average N content observed for red clover in this study was much lower compared to an average ranging between 34 to 102 kg N ha⁻¹ normally observed for temperate climates (Gentry et al. 2013; Coombs et al. 2017). In this study, the order of N accumulated in the cover crop tissue from a high to a low N content was red clover > fall rye

> tillage radish > oat/berseem clover mix in fall 2019 compared to red clover > tillage radish/mustard > oat/berseem clover > fall rye in fall of 2020. Among the groups of cover crops, our results suggest that legumes might be better N builders. However, this may not always be the case, as observed with the trend in biomass and N accumulation with the cover crops across the years. Fall rye out-performed tillage radish/mustard and oat/berseem clover in 2019 and vice versa in 2020. This is an indication that cover crops can perform differently under varying conditions. In this study, the order of performance for N content accumulation based on the class of cover crop was legumes > grass only > brassica > legume-grass mix in 2019. In fall 2020, the order of performance changed with legume-grass mix out-performing grass only. The similarity in N content produced in red clover and fall rye is an indication that grass cover crops can scavenge N and accumulate as much N in the tissues as legumes when considerable biomass is produced.

Comparing C:N ratio by cover crop species, the C:N ratio was similar between the legume cover crop and the legume-grass mixtures in 2019 and 2020 (i.e., red clover and the mixture of oat/berseem clover attained similar C:N ratio across the periods when sampling occurred). This can be attributed to the complementary effect of growing legume-grass mix together (Finney et al. 2016). A similar C:N ratio was observed between grass only and non-legume (brassica) cover crop. Fall rye and tillage radish/mustard acquired similar C:N ratio. This was anticipated as these species would basically make use of any residual N and store in its tissues. Cover crop establishment as reviewed is dependent on many factors. Site-specific factors such as climate, N availability, cover crop specie, cropping system, growing season, irrigation, seeding rate and time, herbicide selection and application rates are responsible for the variability in biomass production as well as uptake and amount of N utilized by cover crops (Dabney et al. 2001; Ruis et al. 2019) under any environment. This supports our hypothesis that cover crop biomass accumulation and N content will differ by species and how it is incorporated in the rotation. It was observed that red clover seeded earlier accumulated more biomass and N content than the other shoulder season cover crops. Therefore, on the Canadian prairies under-seeding cover crops during the growing season might be a useful strategy to help maximize cover crop biomass production, rather than waiting until main crop harvest to seed the cover crops.

3.9.2 Cover crops reduce soil inorganic N levels and supply rates during the non-growing season, but not during the growing season

During the fall, the SIN supply rate in the top 10 cm was generally lowered by including cover crops—the effect being most pronounced after canola, pea, and wheat when oat/berseem clover, tillage radish, and red clover were growing, respectively. This result was not surprising because as the cover crops were growing, they were taking up SIN thereby reducing the SIN supply rates as observed in other studies where cover crops reduced soil nitrate concentrations in fall (Dean and Weil 2009; Lapierre et al. 2022). It is curious that the rye cover crop after potato harvest did not have a more pronounced effect on reducing the SIN supply rate in the 0-10 cm; however, the SIN supply rate in the fall-2020 after potato harvest was already relatively low (likely due to the high NHI of potato tubers in 2020) which may have precluded a cover crop effect in this soil depth post-harvest. High amounts of SIN are often present at pea harvest (Arcand et al. 2013); thus, it is possible that a cover crop effect on SIN during the fall depends on the amount of SIN remaining at harvest. The ability for cover crops to reduce N available for loss during the post-harvest period has been observed in other studies, especially if the levels of SIN would have otherwise been high, had a cover crop not been grown (Coombs et al. 2017).

Other than the post-harvest fall period, there was limited evidence that SIN supply rates were influenced by cover crops—be that spring, summer, or overwinter. However, if cover crops influence the 0-10 cm soil SIN supply rates in the fall, then it is possible that this translates into different N dynamics in deeper depths, i.e., altering N supply and N movement of deeper depth layers over time. Although the spring SIN supply rates in the 0-10 cm depth did not significantly differ with or without cover crops, the 0-60 cm SIN contents at pre-plant were generally reduced with *vs.* without cover crops in rotation—the effect being most pronounced in 2021 after three years of cover cropping, and when heavy N feeders like canola and potato occurred early in the rotation. The reduction in 0-60 cm SIN content at this timepoint indicates cover crop N uptake and immobilization before the cover crop tissues have started to decompose/re-mobilize N. Through this process, it is possible that cover cropping will increase the PMN pool over a longer period of time. The PMN results point towards higher N supply with *vs.* without cover cropping, but after only two years of including cover crops in rotation the difference remained non-significant. Perhaps a longer period of study will reveal larger differences. It is critical to acknowledge that fertilizer N applications at planting increase the SIN supply to the main crops, and in this study the fertilizer

was applied at seeding, and that the rotations with and without cover crops received the same amount of fertilizer (depending only on crop type). As such, the application of fertilizer might have masked any cover crop effect on SIN supply during the summer period. The amount of biomass produced by cover crops generally dictates its influence on the soil N (Ruis et al. 2019). Other plausible explanations for why cover crops did not markedly influence SIN supply rate in the top 10 cm depth in spring and summer may be due to the prevailing factors responsible for the process of N movement, decomposition, and plant N uptake.

3.9.3 Characteristics of different cover crop species on soil N availability

The cover crop species evaluated in this study are known to have different key characteristics. Tillage radish is a brassica cover crop with an extensive tap root and can recycle N from the deeper soil layer and subsequently make N available for succeeding crop (Jahanzad et al. 2017b). Red clover and rye are winter hardy species that can grow during fall and spring, conditions depending. In this study, active growth was observed during both fall and spring. The earlier termination (due to the onset of winter conditions) of the tillage radish and oat/berseem clover mix may have resulted in an earlier decomposition hence earlier supply of SIN in the spring, compared to the overwintering cover crops (rye and red clover) as observed with SIN supply rates for spring 2020 (Fig 3.3). Correspondingly, it can be deduced that the overwinter species (red clover and fall rye) may mineralize N later in the growing season after termination.

If mineralization is not synchronized with crop N demand, the mineralized N can be prone to losses. Perhaps monitoring N supply over the entire growing season from seeding to harvest would have increased the likelihood of detecting the period of N mineralization from rye and red clover residues. By summer prior to harvest, the N supply rate reduced across all phases of the rotations with and without cover crop with no significance. The greatest decline (over the period from spring to summer) was observed when wheat was grown with red clover (relative to the other cover cropped rotations). Again, only the main crops appeared in the rotation at this time except when wheat was under-seeded with red clover. Therefore, the low N supply rate with wheat under-sown with red clover compared to the other crop phases may be the result of N uptake by both wheat and red clover.

Generally, there was a clear pattern in SIN availability throughout the year depending on season, and exacerbated by the presence of cover crops. In other words, rotations with cover crops

had the greatest range in SIN supply over the study duration. The SIN supply had the greatest increase during springtime when cover crops were included in the rotation, and subsequently had the greatest drop in SIN supply during fall. This pattern was also observed in the study by Coombs et al. (2017). This evidence to an extent supports our hypothesis that cover crop rotations will have lower post-harvest season soil N levels due to plant N uptake but higher in-season levels as a result of plant tissue N mineralization compared to rotations without cover crop—though the effect was ambiguous if only certain phases of the rotation were focused on.

Potentially mineralizable N was higher with cover crop rotations compared to the rotations without cover crop though no significance was detected. It is essential to note that sampling for incubation studies took place prior to seeding the main crops in 2020 hence, the crops that appeared in the rotation in 2019 predominantly influenced the rate of mineralization. Among the cover crop rotations, the post-wheat and red clover phase in 2019 mineralized more N than the other cover crop rotations prior to seeding canola. This was anticipated as in 2019, red clover accumulated the greatest biomass and N content in fall. This has been observed in other studies where red clover was associated with higher soil nitrate levels compared with other cover crops (Nyiraneza et al. 2021). Under the right conditions, decomposition of the residues will result in more N being mineralized. Mineralization from tillage radish and fall rye was similar just as observed with their biomass and N content. Oat/berseem clover mix accumulated the lowest biomass and N content hence it was expected that mineralization will be low. Coombs et al. (2017), reported lower biomass and N content with crimson clover compared to other legumes such as red clover and alfalfa.

The C:N ratio of residues serve an important indicator for predicting mineralization (Muhammad et al. 2019; Oliveira et al. 2020). Crop residues have variable C:N ratios and depending on the ratio, a temporary N deficit or surplus can result (Oliveira et al. 2020). This N deficit or surplus will lead to an initial N immobilization or mineralization during decomposition. In this study the C:N ratios among all the cover crops were low, < 12:1; hence mineralization time of the residues will be similar depending on the composition of the residues and if conditions were favourable. This means, biomass quantity and N content of the residues will be the determining factor in predicting the amount of N to be mineralized. Comparing cover crop rotations to the rotations without cover crop, mineralizable N was higher with cover crop rotations due to the

diversity of crops in the rotations, as observed in other studies (McDaniel et al. 2014; Chatterjee et al. 2016).

3.9.4 Temporal crop diversity regulated soil health management

In evaluating the relationship between soil health management index and soil N metrics—be that SIN supply rates and SIN contents all pointed towards a similar pattern: cover crop and perennial rotation helped to redirect N dynamics towards a trajectory that continually reduced SIN. This indicates that these systems have potentials to reduce N losses. The greater chance of agricultural soils becoming sources of nitrate pollution (Lapierre et al. 2022) can therefore be minimized under such systems. Furthermore, the similarity in N dynamics for the cover crop and perennial system can be linked to their characteristic of having near continuous soil cover. This raises interesting questions on if including cover crops in annual crop rotations can help cropping systems to function more like perennial systems. Although a perennial alfalfa cropping system is not diverse, it has benefits of continuous soil cover. Tighter N cycling might be achieved by “perennializing” annual cropping systems via the inclusion of cover crops. The SIN results showed strong effect during fall periods, but no significant relationships were observed for soil health management. However, the ensuing period (spring and summer) showed significant relationship between SIN and soil health management but only for certain periods (summer 2020 and spring 2021). The relationship between soil health management index and PMN was significant. Results showed a decreasing PMN for the short and long rotations without cover crop and an increasing PMN for the cover crop and perennial rotation. Potentially mineralizable N constitute an important indicator of soil health (Allen et al. 2011). In a meta-analysis study that looked at how crop rotations influence nutrient cycling, it was concluded that crop rotations diversity, particularly ones that include cover crops improves soil C and N pool (McDaniel et al. 2014). A 6.3% and 12.5% gain in SOC was observed for cover cropped and perennial rotations respectively, when SOC was evaluated in a these systems compared to a grain-only system (King and Blesh 2018). In another study that evaluated microbial biomass C and N (MBC and MBN) in different cropping systems, a higher MBC and MBN was associated to a 4 year crop rotation with corn-soybean-grain crop-alfalfa than a 2 year rotation with corn-soybean (King and Hofmockel 2017). This is an indication for improved soil health status and quality in diversified and perennialized cropping systems.

3.9.5 Crop yield was not influenced by the presence of cover crops

Crop yields over the study period were not significantly influenced by cover crop rotations. Several confounding effects of cover crops on crop productivity have been reported. In a recent survey conducted in the Prairies, 24% of farms reported an increase and a no change in farm profit when cover crops were included compared to 4% which saw a reduction in farm profit (Morrison and Lawley 2021). Studies have shown increased (Farzadfar et al. 2021), decreased (Abdalla et al. 2019), and no change (Dozier et al. 2017) in yields when cover crops were included in the rotation. In 2019, the mean precipitation over the cash crop growing season (May-August) was 48 mm compared to a mean of 46 mm in 2019 and 2020 respectively. The corresponding mean air temperatures over the same period was 16°C vs. 17°C in 2019 and 2020 respectively. The average climatic conditions over the two-years were similar hence the variation in yields though not significant may be due to factors such as temporal variations in climatic conditions over growing season, irrigation supplied to the crops and whether plant nutrient demand was synchronized with nutrient supply. Research suggests that the impact of cover crops on soil properties and productivity to a large extent can be variable in the short term (Blanco-Canqui et al. 2015; Ruis et al. 2019). Additionally, the relatively higher moisture conditions following seeding in spring 2020 compared to 2019 and the warmer temperature in 2020 than in 2019 during the growing season, may aid crop establishment. In May and June of 2020, precipitation was 56% higher than the same period in 2019. Gentry et al. (2013), observed something similar in their study and explained that the sufficient amount of rainfall produced in August after the dry periods in July led to a flush of soil N mineralization across their treatments which coincided with periods with rapid corn N uptake and remobilization into yield portions. Though the period of rainfall varies across both studies, we speculated in our study that the period of sufficient rain possibly coincided with period of rapid crop N uptake rather than grain N partitioning as reported in the aforementioned study. It is said that crop growth and productivity may be negatively impacted as soil water availability is an important determinant of crop productivity (Ihuoma and Madramootoo 2017; Holzman et al. 2018).

Furthermore, the effect of cover crop on yields can be variable, depending on whether it is a legume, non-legume, grass, or a legume-non-legume mixture. A study found out that legume and non-legume cover crops reduced grain yields whilst a mixture of legume-non-legume increased yields (Doltra and Olesen 2013; Abdalla et al. 2019). In this study, this observation was obscure as

the legume, non-legume, and legume-grass mix all reduced yields compared to their controls without cover crop in one of the 2-years. However, in 2020, the non- legume, the legume-grass mixture as well as legumes increased yields than the rotations without cover crop. A study conducted in Guelph, Ontario to determine N dynamics and corn yields in a cover crop-corn rotation reported a positive effect of red clover and alfalfa cover crop on corn yields in one of the 2 years (Coombs et al. 2017). Alfalfa and red clover are leguminous plants and can increase soil N availability through N fixation.

In this study there was a general increase in yields across all the crops in the rotations with and without cover crop in 2020 though no significance was detected. The greatest increase in yields was observed when red clover preceded canola in 2020. Canola yields more than doubled between 2019 and 2020, and this may be a result of N mineralized from red clover after its termination in spring. A synthesis study conducted across Canada and the US reported that canola yields can reach 4 Mg ha⁻¹ with potential maximum yield of 7 Mg ha⁻¹, though in North America actual yields have been averaged at 1.7 Mg ha⁻¹ (Assefa et al. 2018). The canola yields produced in this study falls within the attainable yield of canola but higher than the average for North America as yield from our study ranged between 1.8-3.9 Mg ha⁻¹. On the other hand, the lowest crop yield was detected among the cover crops when rye preceded pea in 2020. Pea yield increased only by 0.3 Mg ha⁻¹ compared to canola where yields increased by 2.1 Mg ha⁻¹ from 2019-2020 with cover crops in the rotation. Tillage radish in the rotation also increased wheat yields by 2.1 Mg ha⁻¹ while mixture of oat and berseem clover increased potato yields by approximately 14 Mg ha⁻¹ between 2019 and 2020 with cover crops in the rotation. It is reported that potatoes following leguminous cover crops such as red clover and alfalfa supplemented with low N fertilizations leads to significant increase in yields (Jahanzad et al. 2017b). In this study, potato was followed by oat/berseem clover in the cover crop rotation and yields were higher in the rotations with than without cover crop in 2020. In the long rotation without cover crop, yields increased by 1.6, 1.4, 4.3, 0.9 Mg ha⁻¹ for wheat, canola, potato, and pea respectively. In the short rotation without cover crop, the increase across wheat and canola was 1.4 and 1.8 Mg ha⁻¹. The increase in yields observed across all crops and rotation from 2019 to 2020 indicates that the presence of cover crops in the rotations may not be the only contributing factor to the higher yields observed in 2020 but, preceding crops in the rotation could be a factor. A plausible explanation to this is a cumulative mineralization effect of the cover crops and the previous year crop residues as reported by Farzadfar (2021) and fertilizer

N contributing N towards crop. The N returned to the soil from the crop/cover crop residues serves as a source of nutrient for next crop if mineralization and plant N demand are synchronized. However, Taveira et al. (2020) in his study to trace crop residue N into subsequent crops mentioned that yield benefits associated with diversified crop rotations may not always be tied to improved N supply from crop residues. In his study results showed that belowground residues N recovery in subsequent crops were about 8 to 18 times higher compared to aboveground residues (Taveira et al. 2020). This indicates a lower potential for aboveground residue derived N transfer to subsequent crops. This further illustrate that other N sources such as indigenous soil N, could supply significant amount of N towards plant growth.

3.9.6 The presence of cover crops did not impact plant- or soil-based N use efficiencies

No evidence was found to support our hypothesis that cover crops increase crop NUE and improve crop yields and quality. Nitrogen harvest index which is categorized as a plant-based NUE was not influenced by cover crop across all the crops and years, not even when yields numerically increased in 2020 vs. 2019 for some cover crop rotations. Yet in some studies, cover crops positively influenced crop NHI (Farzadfar et al. 2021). A higher NHI is an indication for improved N availability with cover crops and a subsequent synchronization of plant N uptake with N supply as mentioned by Farzadfar et al. (2021). The NHI estimates N allocation between yield and vegetative parts of the plant, and it ranged between 32-71% in the grain crops and 48-86% in the tuber crop in this study. Studies have reported that NHI varies among crop species and genotypes of the same species (Fageria 2014). A high NHI signifies increased re-allocation of N to the grain resulting in improved grain development and filling (Ali et al. 2018), thus it can be inferred from this study that potato, wheat and canola in one of the 2 years showed greater potential for N partitioning in the yields. A positive relationship has been reported to exist between grain yields and NHI for some crops (Fageria 2014) and in this study, a numerical increase in NHI was associated with a numerical gain in crop yields from 2019 to 2020 for all the rotations with and without cover crop. The similarities in the NUE values observed across the rotations suggest that cover crops did not tighten the N cycle. The highest NHI for grain crops was observed with wheat over the two years whilst the lowest was recorded in canola in 2019 and pea in 2020 irrespective of the type of rotation. A review of different studies revealed that NHI for grain crops can vary between 17-70% depending on genotype, environment and management (Sharma et al. 1987; Fageria 2014; Ali et al. 2018). By estimating NHI, an indirect amount of N returned to the soil in

the form of residue-N is determined (Congreves et al. 2021). A caveat associated with NHI is that a high value does not always signify an increased NUE as this index does not account for an important soil N pool (Congreves et al. 2021). In this study, soil inorganic N was not significantly influenced by cover crops during the growing season. Though SIN increased numerically at springtime, a decrease was observed by summer prior to harvest, implying that cover crop impact on N availability was not robust enough to influence plant N.

Similar to the plant-based index, all soil based NUEs (NUpE, ANR and NUE_{yield}) were not impacted by cover crops in the rotation. The NUpE was greater than 100% for most of the crops in the rotation in 2019. A NUE index value that exceeds 100% means that more N was taken up by the plant than was applied (Van Eerd 2007) suggesting an underestimation of soil available N. However, in the subsequent year, NUpE dropped below 100% for most of the crops except pea. In 2020, mineralizable N from the potentially mineralizable pool was estimated as a source of N made available towards plant growth. Accounting for soil mineralized N increased the N pool as estimated in the denominator and led to a potentially improved estimation of NuPE in 2020. Estimating N release from mineralization of organic matter pool represent a significant source of N for plants (Karklins and Ruza 2015). It is important to recognize that the assumptions in estimating the PMN pool could lead to an underestimation of actually available N, as it does not represent the gross N mineralized but only the net N mineralized. Secondly the mineralised N represented the amount of N present in the soil at the beginning of growing season and did not include that after the growing season. Other sources such as N from biological fixation and deposition, and perhaps the depth of soil N measured may have resulted in an underestimation of N. Correctly accounting for this soil inorganic N availability can be made when non-fertilized control plots are included in the study. But in our case the control plots also received fertilizer N. This further draws an attention to the fact that a considerable amount of N may have been supplied by indigenous soil N pool, as discussed by Taveira et al. (2020). Among the crops, pea was associated with the highest NUpE. According to Congreves et al. (2021), N-fixers can be classified as effective N utilizers by virtue of their ability to utilize energy to ensure their own N supply by nodulating or biologically fixing N especially when the soil N supply capacity is lower than the plants demand. Owing to the fact that pea plots did not receive much fertilizer N, it can be deduced that the status of the soil N supply capacity may have influenced pea crops to act in the manner explained above.

Apparent N recovery estimates the amount of N removed from the field as yield based on available N, and it did not exceed 70% in our study. In a study conducted in the UK, ANR ranged between 77-111% for winter wheat which far exceeds the recovery efficiency observed for this study (Karklins and Ruza 2015). Recovery efficiency highlights the apparent increase in yield in response to soil available N unlike fertilizer recovery efficiency where the focus is on how much of applied N is recovered. However, ANR does not specify the direct amount of N recovered in plant as ^{15}N technique does (Congreves et al. 2021). Fertilizer N and soil N contribute significantly to the soil N supply yet are influenced by temporal and spatial factors (Karklins and Ruza 2015). Apparent recovery can be said to be a function of N uptake by plants. Uptake efficiency is an indication for N capture by root of plants and the subsequent use by the plant (Congreves et al. 2021) but N uptake is dependent on the ability of plants to efficiently take up nutrient from the soil which is controlled by other factors such as the internal transport of N, storage, and remobilization of N (Perchlik and Tegeder 2017).

The $\text{NUE}_{\text{yield}}$ is also considered as a soil-based NUE as it gives an indication of the yield potential based on soil N available. In this study, the crop NUE values were expressed as a fraction of yield dry weight and available N and values ranged between 9-22 for grain crops and 39-46 for tuber crop. These values depict an overall low NUE especially for the grain crops as values did not approach 50. This is however not surprising as studies have shown that crop NUE is variable and globally differs by crop type, climate, water and N management etc. (Dobermann 2005). It has been documented that organic residues from previous crops in a rotation to a greater extent influences NUE (Hirel et al. 2011) and recent advancements have shown that plants are capable of taking up N directly in the organic form (Farzadfar et al. 2020). In this study the higher N uptake did not have any apparent effect on yield NUE. To influence NUE, cover crops must first affect N availability. Considering that cover crops did not markedly influence N availability in this study, it was anticipated that NUE metrics measured may not be impacted. Nitrogen dynamics and plant N utilization traits are important factors that can influence NUE. However, these processes are intricate as they are controlled by factors such as climate, soil, and plant factors and the relationships that exist among them and how they interact to regulate the process in plant-soil systems.

3.10 Conclusion

Cover crops are multifunctional and can provide multiple agronomic, environmental, and economic benefits under proper management. It is said that N cycling can be improved using cover crops, leading to a reduction in potential N losses and further minimizing excessive N fertilizations. Yet, studies have shown several conflicting results of cover cropping globally. This study was not an exception, as results show variations in cover crop effects on N cycling and subsequently crop productivity and quality. Findings of this study partially supported some of our hypotheses and vice versa. Cover crop biomass differed by species, N content and C:N ratio suggesting that in a short season growing region, like the prairies, selecting the most suitable shoulder season cover crops is an important choice. It was evident that cover crops influenced N cycling. During the non-growing season, it was observed that cover crops had a potential for reducing residual N accumulations available for loss via uptake. However, there was limited evidence that rotations with vs. without cover crops had different N dynamics during the growing season. Subsequently, crop NUE and yield was not enhanced by the presence of cover crops in the rotation. It is reported that for cover crops to enhance NUE, it must improve N availability and its uptake by plants. For this to occur, several factors come into play: (i) method of incorporating cover crops, (ii) management, (iii) synchrony between soil N availability and crop N uptake. The higher N uptake efficiency in this study did not reflect a higher impact on the other indices (be that NHI, ANR, NUE_{yield}). This means that though the plants took up more N, the ability of the crop to remobilize N to the yield and or vegetative part was low. Furthermore, soil health management strategy pointed to a potential increase in soil organic matter inputs from cover cropped and perennialized systems compared to the long and short rotation systems. Though not much difference was observed in SIN dynamics over the years (2019-2021), there is a greater chance for cover cropped and perennialized systems overtime to sequester soil N which can overall improve the soil health status. It is stated that the amount of biomass produced by cover crops regulates its effect on N cycling, yet in semi-arid regions like the prairies, climate remains a dominant factor that regulates the success of cover cropping. Innovative agronomic research is needed to improve cover crop biomass accumulation, future research should explore the development of fast growing, cold hardy cover crops. As a next step, cover cropping should be considered in conjunction with N fertilizer adjustments to move towards improved crop N use efficiencies.

4.0 NITROUS OXIDE EMISSIONS AS INFLUENCED BY INCLUDING COVER CROPS IN ROTATION

4.1 Abstract

Abundant soil N supply supports agricultural productivity, yet there is an environmental cost due to the emission of the potent greenhouse gas nitrous oxide (N_2O). One promising strategy towards regulating N for crop use and reducing losses is by integrating cover crops into crop rotations—a practice known to increase soil N supply for subsequent crops through biological N fixation and/or crop residue N mineralization. By providing N, cover crops may help to avoid excessive N fertilization and, in turn, reduce N_2O emissions. However, cover crop effects on N_2O emissions are uncertain and there is no consensus in literature. To understand how cover crops influence N_2O emissions, samples were collected from a fully phased crop rotation in 2020/2021. The trial, which was initiated in 2018, compared a wheat-canola-potato-pea rotation grown with cover crops (CC) vs. without cover crops (LR), a short rotation of wheat-canola (SR), and a perennial alfalfa (PR) system. Emissions were quantified during the spring thaw, growing season, and post-harvest, and both cumulative annual and yield-scaled emissions were evaluated. In general, peak fluxes were associated with spring thaw and after fertilizer applications partially supporting hypothesis. Growing season, post-harvest and spring thaw contributed 51, 7, and 42% to the cumulative annual emissions. Generally, cover crops did not significantly influence cumulative N_2O emissions—but where they did, N_2O emissions were higher for the CC than the LR and SR. There was increasing evidence that legumes can increase soil N availability and enhance N_2O emissions. The higher cumulative emission for PR translated into greater emissions over the hypothetical 4-year period, with perennial rotation emitting $2617 \text{ g N ha}^{-1} \text{ N}_2\text{O}$, which was 21, 42, and 46% higher than CC, LR and SR treatments, respectively. Yield-scaled N_2O emissions were never impacted by rotation. Low yield accompanied with higher annual emissions increased yield-scaled emissions for pea while higher potato yields and higher annual emissions, generated lowest yield-scaled emissions. Based on the results from this study, including cover crops into crop rotations without adjusting

other aspects of agronomic management (e.g., reducing fertilizer rates or adjusting timing of application) may increase the risk of greater N₂O.

4.2 Introduction

Nitrous oxide is a potent greenhouse gas with a global warming potential 265 times greater than carbon dioxide on a per mass basis and agricultural soils are the leading contributor of anthropogenic N₂O emissions globally (IPCC 2019). Better managing agricultural soils to minimize N₂O production and emission is necessary for climate change mitigation. Some have proposed that reducing N fertilizer applications seems the only feasible means of reducing N₂O emissions (Venterea et al. 2012). However, N fertilizer application cannot be entirely eliminated from crop production due to the important role that N has in supporting plant growth and yield development; therefore, it is necessary to explore a diversity of management practices that have potential to reduce N₂O emissions (AAFC 2008).

Cover cropping has been identified as a sustainable management practice that can reduce N availability during periods when N is prone to losses, alongside other benefits such as building soil organic matter. Consequently, there is growing interest in incorporating cover crops into prairie cropping systems. Collectively, cover crops influence key agroecosystem services such as regulating greenhouse gas emissions, carbon sequestration, and nutrient cycling (Blanco-Canqui et al. 2015). Cover crops can reduce N₂O production by reducing the availability of mineral N in the soil by way of storing N in plant biomass; conversely, cover crops can increase N₂O production by releasing mineral N into the soil via N fixation or N mineralization of residue biomass (Basche et al. 2014). The availability of soil mineral N stimulates N₂O emissions through microbial processes—namely nitrification and denitrification. Nitrification is an aerobic process in which microbes oxidize ammonium to nitrate whilst denitrification is anaerobic, and nitrate is reduced to nitrogen gas by microbes. During these processes N₂O is formed as a by-product of nitrification and as an intermediate during denitrification. These processes are therefore key microbial mechanisms that lead to N₂O production and emission. When cover crops increase the soil N supply, there is need to adjust N fertilizer inputs to optimize crop productivity as well as reduce losses (Fu et al. 2019; Taveira et al. 2020). Though cover cropping seems to be a promising tool for mitigating N₂O emissions, they can have both positive and negative impacts on N₂O production, thus, effective management strategies are required to ensure success. Carbon can act as a substrate

to reduce N₂O emissions (favouring the stepwise complete reduction of N₂O to N₂) but the contribution of cover crops to the labile C pool also can intensify these emissions, i.e., denitrification is a heterotrophic process that may rely on labile C as substrate for heterotrophic activity (Basche et al. 2014). At the same time, mineralization of N rich residues that potentially enhance N₂O fluxes can be repressed via the addition of C-rich residues that temporarily results in N immobilization (Venterea et al. 2012). Studies are therefore required to determine how inclusion of cover crops (legume, non-legume, grass, and legume-grass mix) into crop rotation systems can influence N cycle and N₂O emissions. Therefore, this study aimed to determine the amount of N₂O produced in rotations with and without cover crop and ascertain if cover crops have potential to reduce N₂O emissions by influencing soil N availability at different times of the year.

4.3 Experimental design and methods

The site and experimental design for this study was described in detail in Chapter 3. Briefly, a four-year fully phased crop rotation was established in 2018 (Table 3.1) to determine the impact of cover crops on N cycling. In this Chapter, I focus on soil N₂O dynamics from spring 2020 to spring 2021.

4.3.1 Nitrous oxide gas sampling

Starting from the growing season in 2020 and continuing to the next spring in 2021, gas samples were collected and analysed to determine N₂O fluxes. The gas samples were collected using a standard static chamber technique as described by Liu et al. (2021). Non-steady state vented chambers (27.9 cm x 22.4 cm dimensions, with volume of 6.8 L headspace) made by modifying stainless steel serving trays were designed to fit onto welded rectangular aluminium bases that were inserted to a depth of 3.8 cm (1 m from the top and side row of each plot,) in the soil. The chamber bases remained where they were installed for the duration of the experiment—being removed only during seeding and harvest operations. To collect gas samples, the chambers were sealed onto the bases allowing gases to accumulate in the chamber headspace. A single gas sample was collected from the chamber headspace at a pre-determined time, i.e., after 14 min (T₁₄). Intervals of 1 min were used to move from one plot to the next. Gas samples were collected using a 20 mL syringe fitted with a 22-gauge needle and injected into an evacuated 12 mL Exetainer® tube (Labco Limited, UK). In addition, 16 ambient air samples (four per replicate) were collected on each sampling day. These samples were then used to determine the N₂O concentration at time-zero (T₀).

Gas samples were collected between 10 am and 2 pm when soil temperatures were near the daily mean. Overall, gas samples were collected after seeding and fertilizer application and continued until just prior to seeding of the next crop in the rotation. Samples were collected frequently after fertilization (at least twice weekly) and thereafter collected weekly during the growing season. All gas samples were analysed for N₂O concentration using a gas chromatograph equipped with electron capture and thermal conductivity detectors (Bruker 450 GC, Bruker Bio- sciences, Billerica, MA) with actual conditions of 330 °C in the electron capture detector, 120 °C in thermal conductivity and flame ionizer detectors and 85 °C in the column, injector, and the valves.

At fertilizer application, the amount of fertilizer needed to cover the soil surface of each chamber base area was calculated and hand-applied to the soil surface within the base so that each chamber received the correct amount of fertilizer and to ensure uniform application within the small chamber base areas. (Note: these areas were covered when the whole plot received a broadcast application of fertilizer.) The bases received the equivalent of 190, 90, and 130 kg N ha⁻¹ (i.e., 19, 9, and 13 g m⁻²) of fertilizer for wheat, canola, and potato plots in 2020. Pea and alfalfa plots did not receive any N fertilization. Gas sampling resumed the day after fertilizer was applied. Due to the nature of potato production, the potato plots had two bases installed after planting to capture the spatial variability of the hilled and furrowed areas: one base was directly on top of a potato hill, and another directly in a furrow.

4.3.2 N₂O calculations

Initial daily fluxes (g N₂O-N ha⁻¹d⁻¹) were calculated by subtracting average air samples from N₂O gas concentrations from each plot. The N₂O concentrations were converted from mixing ratios (µl L⁻¹) to molar concentrations (g ha⁻¹ d⁻¹) using ideal gas law. Prior to N₂O calculations, potential outliers from averaged air samples were inspected using coefficient of variation (CV) values and adjusted where CV values were more than 1%. Daily N₂O-N fluxes were estimated as average daily flux of the 4 replicates using a similar approach as (Venterea et al. 2020; Machado et al. 2021b) (Eq. 5):

$$F_N = \left(\frac{C_{14} - C_0}{T_{14} - T_0} \right) \left(\frac{V}{A} \right) \quad (4.5)$$

where F_N is the daily N_2O flux ($g\ ha^{-1}\ d^{-1}$), C_0 is concentrations of N_2O -N taken at T_0 (ambient air) and C_{14} is the concentration taken at T_{14} (units), V is the volume of the chamber headspace ($6.82\ m^3$), and A is surface area of the chamber ($0.0625\ m^2$).

4.3.3 Cumulative N_2O emission calculations

Cumulative N_2O emissions [$C_N = \text{total } N_2O\text{-N} (g\ N\ ha^{-1})$] were estimated for (i) the growing season period (seeding to harvest); (ii) post-harvest period; (after harvest to freeze up); (iii) the spring thaw period (March to start of growing season); and (iv) annually (for the period encompassing the crop's growing season, the post-harvest period until freeze-up, and the subsequent spring thaw). Cumulative emissions were calculated using area-under-the-curve analysis involving trapezoidal integration of the daily fluxes over specified evaluation periods (Machado et al. 2021a). Emissions were assumed to be negligible during the winter as Saskatchewan soils remain frozen during the winter period (Liu et al. 2021).

4.3.4 Estimating total N_2O emissions over a full crop rotation

The total N_2O emitted over a full crop rotation was estimated based on the cumulative annual emissions data (2020/2021), similar to how others have estimated 'rotation emissions' using a shorter time frame of two years (Machado et al. 2021b). This was done by summing cumulative annual emissions for all crop phases starting from when the crop was established (growing season, 2020) to the next year when a new crop appeared in the rotation (after spring thaw, 2021). It was assumed that all crop phases appearing in a particular year represent a full crop rotation. To compare all rotations (including the SR and PR treatments), total emissions were estimated for a hypothetical 4-year period.

4.3.5 Yield-scaled N_2O emission calculations

Yield-scaled N_2O -N emissions were calculated as ratio of cumulative N_2O emissions to yield biomass ($g\ N_2O\text{-N}\ Mg^{-1}$) and yield N ($g\ N_2O\text{-N}\ kg^{-1}$) by dividing the total cumulative annual N_2O emissions (area scaled) by crop yield and yield N (Venterea et al. 2011) (Eq. 6 & 7):

$$YSE_B = (C_N/Y) \quad (4.6)$$

$$YSE_N = (C_N/Y_N) \quad (4.7)$$

where YSE_B and YSE_N are yield biomass and yield-N scaled N_2O -N, C_N is cumulative emissions ($g\ N\ ha^{-1}$) over the entire sampling period and Y is crop yields ($Mg\ ha^{-1}$) and Y_N is yield N ($kg\ ha^{-1}$).

4.4 Statistical analysis

SAS (SAS Institute, Inc., University edition, Cary, NC) was used to perform the analyses of variance (ANOVA). PROC MEANS was used for descriptive statistics, PROC MIXED for mixed procedure analysis, PROC UNIVARIATE for normality check, LEVENE for testing homogeneity of variances, TUKEY for means comparison. Due to the variable nature of agricultural soil N_2O emissions, a significance level of ($\alpha=0.1$) was used. Data which did not meet normality assumptions ($Pr < SW$ greater than 0.05) were log-transformed for analysis and then back-transformed for presentation. For the mixed model, rotation was considered as fixed effect, and replicates as random effect for all response variables and each crop type was analysed independently. CoPlot (version 6.45) was used for graphical presentations.

4.5 Results

4.5.1 Daily nitrous oxide emissions

Nitrous oxide emissions are shown by crop type (Fig. 4.1). Starting from the growing season, emissions generally peaked after fertilizer application, across all the crops and rotations. The highest daily flux during the growing season was observed for CC-Wheat ($26\ g\ N\ ha^{-1}\ d^{-1}$) and LR-Potato ($19\ g\ N\ ha^{-1}\ d^{-1}$), occurring DOY-143 and DOY-160, respectively during the growing season. Daily N_2O emissions during wheat phase were slightly higher with vs. without cover crops. However, during the spring thaw period in 2021, the SR-Wheat produced the highest flux ($33\ g\ N\ ha^{-1}\ d^{-1}$). During canola phase there was little impact of the cover crop on emissions, except for slightly higher N_2O emissions with vs. without cover crop during the spring thaw period in 2021. The highest emissions produced from canola was $9\ g\ N\ ha^{-1}\ d^{-1}$ after fertilization and $12\ g\ N\ ha^{-1}\ d^{-1}$ at spring thaw. For potato production, the LR had the highest fluxes after fertilization and during the spring thaw period in 2021. Emissions from Potato furrows were slightly higher than from potato hills with and without cover crops. During the spring thaw of 2021, the highest flux ($45\ g\ N\ ha^{-1}\ d^{-1}$) followed potato production in the LR without cover crop. During pea production N_2O fluxes peaked after fertilization and reached $13\ g\ N\ ha^{-1}\ d^{-1}$ in LR. During the spring thaw period emissions increased in the CC rotation up to $25\ g\ N\ ha^{-1}\ d^{-1}$.

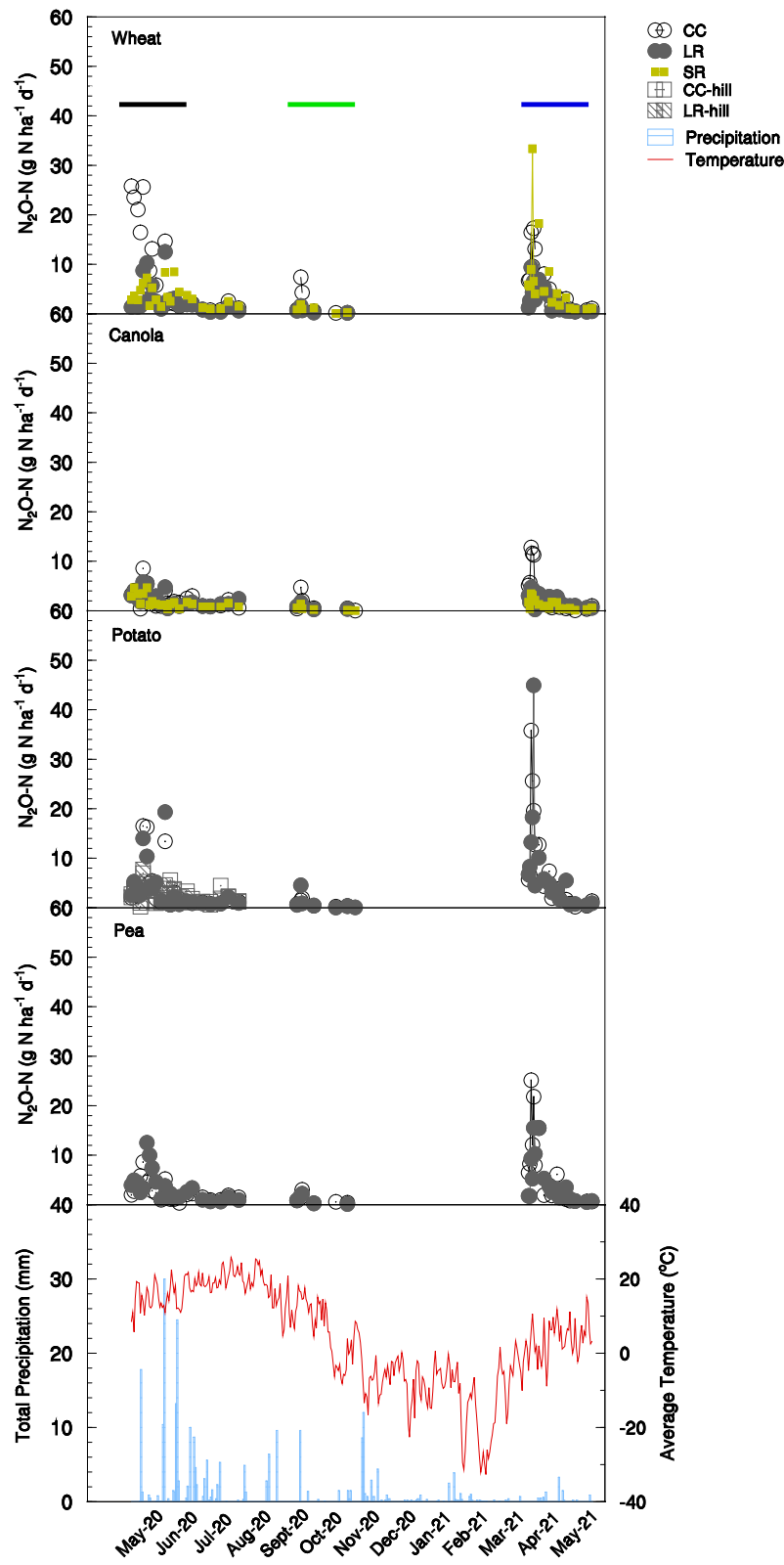


Figure 4.1 Daily $\text{N}_2\text{O-N}$ emissions for wheat, canola, potato (with hills and furrows) and pea in 2020/2021 crop rotation year. CC indicates the cover cropped long rotation and LR indicates the long rotation without cover crops (both of which followed a rotation sequence of Wheat-Canola-Potato-Pea); SR indicates the short rotation without cover crops (Wheat-Canola sequence). Black, green, and blue lines in the graph represent emissions during growing season, post-harvest, and at spring thaw, respectively. Gaps in graph represent breaks in sampling. Weather conditions are represented by precipitation (blue bars) on left y-axis and temperature (red line) on right y-axis.

Generally, it was observed that emissions during the growing season were associated with fertilization and periods of precipitation. Following harvest, emissions remained relatively low among all the crops. The highest fluxes produced during the post-harvest period ranged between 3-4.7 g N ha⁻¹ d⁻¹ among the crops. Emissions subsequently increased following spring thaw in 2021 for all crops and rotations.

4.5.2 Cumulative nitrous oxide emissions annually, at thaw, during the growing and post-harvest season

In 2020/2021, cumulative N₂O-N emissions ranged from 124 to 303 g N ha⁻¹ during the growing season, 13 to 46 g N ha⁻¹ during the post-harvest period, 43 to 306 g N ha⁻¹ during the spring thaw period, and 180 to 654 g N ha⁻¹ annually (Table 4.1).

Table 4.1 Cumulative N₂O-N emissions during 2020/2021 period for each crop grown in rotation with vs. without cover crops. Within each column for each crop type, means followed by different letters are significantly different at $p < 0.1$ (highlighted in bold). CC indicates the cover cropped long rotation; LR indicates the long rotation without cover crops; SR indicates the short rotation without cover crops. Annual emissions for wheat and canola, post-harvest and growing season emissions for wheat and canola, respectively were log transformed for analysis and back-transformed for reporting. One outlier was removed from wheat (growing season and annual emission).

Rotation	Main crop/ cover crop	Preceding crop/ cover crop	Cumulative N ₂ O-N emissions (g N ha ⁻¹)			
			Growing season	Post-harvest	Spring thaw	Annual
LR	wheat	pea	200 ^b	13 ^b	107	317 ^b
CC	wheat/red clover	pea/radish	238 ^{ab}	46 ^a	188	447 ^{ab}
SR	wheat	canola	263 ^a	33 ^a	229	495 ^a
			0.015	0.005	0.270	0.097
LR	canola	wheat	154	25 ^{ab}	78	248 ^{ab}
CC	canola/oat+clover	wheat/red clover	165	33 ^a	92	288 ^a
SR	canola	wheat	124	13 ^b	43	180 ^b
			0.134	0.056	0.274	0.065
LR	potato	canola	241	28	230	498
CC	potato/rye	canola/oat+clover	241	30	239	509
			0.991	0.826	0.917	0.912
LR	pea	potato	224	25	189	439
CC	Pea/radish	potato/rye	183	40	207	430
			0.494	0.222	0.889	0.949
PR*	alfalfa	alfalfa	303	45	306	654

*Cumulative emissions for perennial rotation are raw data (not statistically analyzed). Annual emissions are from May to the subsequent May (in other words, from fertilizer application to subsequent spring thaw).

Rotation significantly influenced N₂O emissions during the growing seasons, post-harvest period, and annually but not for the spring thaw period in any of the crop phases. During the growing season, only the wheat phase was significantly influenced by rotation ($P=0.015$). The short rotation had emissions up to 263 g N ha⁻¹ but was not significantly different from the cover crop rotation (238 g N ha⁻¹) which also did not differ from the LR (200 g N ha⁻¹) without cover crop. For the other crops, there were no consistent pattern of increased emissions with vs without cover crop during the growing season. There were some cases with numerically higher emissions from the CC rotation compared to LR and SR, and vice versa. Among the rotations, perennial alfalfa numerically produced the highest growing season emissions (303 g N ha⁻¹).

For the post-harvest period, emissions were affected by rotation for the wheat ($P=0.005$) and canola ($P=0.056$) phase only. In both cases, the cover crop rotations yielded significantly greater emissions than the rotations without cover crops. This pattern was observed for the post-harvest period of potato and pea phase where cover crops numerically increased N₂O emissions. Wheat in the cover crop rotation and perennial alfalfa produced the highest post-harvest emissions, 46 and 45 g N ha⁻¹, respectively.

The spring thaw period following the crops growing season were not impacted by rotation. Even though no significant differences for N₂O emissions at spring thaw were detected, there was a consistent trend where most CC rotations had numerically higher spring thaw emissions than LR or SR (Table 4.1). Comparing emissions numerically among the rotations, alfalfa produced the highest emissions (306 g N ha⁻¹) at spring thaw.

Cumulative annual emissions were significantly different due to rotation type with or without cover crops for the wheat ($P=0.097$) and canola phase ($P=0.065$). The wheat phase had higher emissions in the short rotation but did not differ significantly from the cover crop rotation. Canola on the other hand had higher emission in the CC rotation but did not differ from the LR. For the other crops, potato, had numerically higher emissions with vs without cover crop and vice versa for pea. On an annual basis, perennial alfalfa emitted the most N₂O (654 g N ha⁻¹) followed by wheat + red clover (447 g N ha⁻¹).

4.5.3 Estimated total N₂O emission across a full complete crop rotation

The estimated amount of N₂O emitted over a complete crop rotation was influenced by the type of rotation (Table 4.2). The complete perennial rotation produced the highest emissions (2617

g N ha⁻¹ d⁻¹) over the hypothetical 4-year period while the SR yielded the lowest total emissions. In general, total N₂O emissions for the 4-yr rotations decreased in the order: PR > CC > LR > SR, with total emissions from the perennial rotation being 21, 42, and 46% higher than those from the CC, LR, and SR, respectively. Statistically, however, there were no significant differences between the cover crop and long rotations. The complete short rotation produced significantly lower emissions than the perennial rotation, but not the complete cover crop and long rotation.

Table 4.2 Cumulative N₂O-N emission for full crop rotation grown with vs. without cover crops for 2020/2021 rotation period. CC indicates the cover cropped long rotation; LR indicates the long rotation without cover crops; SR indicates the short rotation without cover crop; PR indicates perennial rotation. Emissions were estimated from beginning of the crop year (May) to the following year when next crop appeared in the rotation (May).

Rotation	Estimated cumulative N ₂ O-N produced for a complete crop rotation (g N ha ⁻¹) over a hypothetical 4-yr period
CC (Wheat red clover-Canola berseem clover/oat -Potato rye -Pea tillage radish)	2062 ^{ab}
LR (Wheat-Canola-Potato-Pea)	1521 ^{ab}
SR (Wheat-Canola)	1418 ^b
PR (Alfalfa)	2617 ^a
<i>P</i> -value	0.077

4.5.4 Yield-scaled N₂O emissions

Yield-scaled N₂O emissions expressed on grain- or tuber-yield and N-yield basis during the individual phases of the rotations were not affected by the composition (with vs. without cover crops) of the rotation in the 2020/2021 crop year (Table 4.3). However, there was a general pattern towards higher yield-scaled N₂O emissions with vs. without cover crops. When emissions were scaled for yield, pea in the CC rotation produced the highest emissions (248 g N₂O-N Mg⁻¹) among the crops whilst potato in the CC rotation produced the lowest emissions (47 g N₂O-N Mg⁻¹). The N₂O emissions expressed per unit of grain/tuber N removed at harvest ranged from 1.8 to 6.3 g N₂O-N kg⁻¹. A similar trend of higher N-yield scaled emissions was observed for rotations with cover crop. Pea produced the highest yield-N scaled emissions (6.3 g N₂O-N kg⁻¹) while canola in the SR produced the lowest emissions (1.3 g N₂O-N kg⁻¹) when yield-scaled emissions were estimated on grain N basis.

Table 4.3 Yield-scaled N₂O emissions expressed on a yield biomass and yield N basis for 2020/2021 rotation period. Within each column for each crop type, means followed by different letters are significantly different at $p < 0.1$ (highlighted in bold). CC indicates the cover cropped long rotation; LR indicates the long rotation without cover crops; SR indicates the short rotation without cover crops. Yield-scaled N₂O for grain yield and grain N for wheat was log-transformed for analysis and back-transformed for reporting.

Year	Rotation	Main crop/ Cover crop	Grain/tuber yield (Mg ha ⁻¹)	Grain/tuber yield scaled (g N ₂ O-N Mg ⁻¹)	N-Yield scaled (g N ₂ O-N kg ⁻¹)
2020/21	LR	wheat	6.4	50	1.8
	CC	wheat/red clover	6.6	98	3.6
	SR	wheat	6.3	79	2.8
			<i>0.658</i>	<i>0.167</i>	<i>0.122</i>
	LR	Canola	3.5	74	1.9
	CC	Canola/oat+clover	3.9	76	2.0
	SR	Canola	3.9	47	1.3
			<i>0.656</i>	<i>0.100</i>	<i>0.105</i>
	LR	potato	9	54	4.4
	CC	potato/rye	11	47	3.9
			<i>0.211</i>	<i>0.546</i>	<i>0.611</i>
	LR	Pea	3.0	173	4.7
	CC	Pea/ tillage radish	1.9	248	6.3
			0.089	<i>0.135</i>	<i>0.203</i>
	PR*	Alfalfa	5.1	131	5

* Emissions for perennial rotation are raw data for inference (not statistically analysed). Yield for alfalfa is based on residue biomass. Potato yields are reported on a dry weight basis.

4.6 Discussion

4.6.1 Growing and non-growing season emissions

Seasonal flux patterns were observed for N₂O emissions across the seasons (growing season, post-harvest, and spring thaw period). Among these periods, the highest emissions were observed for the growing season followed by the spring thaw and the post-harvest period. Averaged across all rotations, growing season, post-harvest, and spring thaw contributed 51, 7 and 42%, respectively to the annual totals. Growing season emissions increased across the rotations after plots were fertilized. This was evident in the daily N₂O emissions figure (Fig. 4.1) which showed emissions peaking after fertilization. This trend was consistent to what is commonly seen following spring applications of N fertilizer (Burton et al. 2008; Venterea et al. 2011; Thilakarathna et al. 2020). Emissions began peaking 3-days post fertilization across all crops and rotations and continued to increase 25 days post fertilization for potato before significant decreases were observed. Studies have shown that N₂O emissions resulting post-fertilization can take place up to 2-3 months (Takeda et al. 2021) or longer (Wang et al. 2016). These periods can be considered

“hot moments” for N₂O intensification (Wagner-Riddle et al. 2020). Together, with an increase in the supply of readily available N, the addition of fertilizer to the soil likely stimulated microbial activities (Wagner-Riddle et al. 2020) that, in turn, resulted in an increase in emissions. Although the pea plots did not receive any N fertilization, N₂O emissions from these plots did follow similar trend with N₂O peaking after seeding though the effect was mild. This is an indication that seeding operations can potentially trigger N₂O release by disrupting soil aggregates and potentially releasing physically protected soil organic matter that can induce N₂O production (via nitrification or denitrification) after mineralization (Liu et al. 2021). Averaged across all rotations, cumulative emissions were generally higher during the growing season (212 g N ha⁻¹) than the spring thaw period (173 g N ha⁻¹) and after harvest (30 g N ha⁻¹). During the growing season (May-August) the average temperature was 17 °C with precipitation amounting to 183 mm over the same period. After harvest (September to November), temperature reduced to an average of 3.5 °C with 58 mm of precipitation. By spring thaw (March-May), temperature had increased to an average of 5.2 °C with a total of 45 mm precipitation. In this study the differences in the amount of N₂O produced across the seasons can be associated with intra-annual and seasonal variations in weather conditions as remarked by Liu et al. (2021). Spatial and temporal fluctuations in temperature and soil moisture influences N₂O emission rates (Butterbach-Bahl et al. 2013). The episodic nature of N₂O emissions have been correlated with thaw events (Wagner-Riddle et al. 2017; Thilakarathna et al. 2020), fertilization (Burton et al. 2008; Venterea et al. 2011; Thilakarathna et al. 2020), temperature and/or precipitation (Burton et al. 2008). In temperate regions, spring thaw is regarded as an important period due to its contribution to annual N₂O estimates. A significant amount of annual N₂O emissions from croplands (29-53%) can be associated with freeze-thaw related events (Griffis et al. 2017; Machado et al. 2021a). In this study, 42% of annual emissions was associated with spring thaw events. Studies have reported that regions experiencing periods of frequent freeze-thaw events usually have the spring thaw periods as critical times for high N₂O production (Wagner-Riddle et al. 2017; Machado et al. 2021a). According to Congreves et al. (2018), the mechanisms that control freeze-thaw cycles can range from biogeochemical—such as substrate (NH₄⁺, NO₃⁻) availability, oxygen concentration, denitrifier activity—to physical processes such as soil temperature, pre-existing soil water, soil texture, solute concentration, etc. Nitrification and denitrification contributions to N₂O emissions are contingent on the aeration status of the soil which is determined by the amount of WFPS occupied by water (Congreves et al. 2019). Snowmelt that

occurs in spring creates moist conditions that drive anaerobic processes that stimulate N₂O emissions (Rochette et al. 2008). Studies have shown that nitrous oxide emissions intensified when high soil N concentrations were synchronized with increasing soil moisture (i.e., WFPS > 40%) (Machado et al. 2021b). The daily N₂O emission figure (Fig. 4.1) shows higher emissions corresponding with increased precipitation amounts and higher temperatures during the growing season and higher temperatures with relatively lower precipitation in the subsequent spring thaw period. The interplay of favourable conditions (available mineral N after fertilization, higher temperature and high soil water filled pore space) resulted in enhanced denitrification which, in turn, triggered the hot moments observed during these periods. Similar results were described by Liu et al. (2021).

Under field conditions, factors such as soil moisture and oxygen concentrations, temperature, C and N availability, and microbial communities control N₂O emissions (Lemke et al. 2007). For example, temperature can directly influence N₂O emissions by influencing enzymatic processes that regulate N₂O production, and indirectly by enhancing soil respiration that results in soil anaerobiosis, a major factor that drives N₂O emissions (Butterbach-Bahl et al. 2013). The latter factor could be the cause of N₂O emissions observed during the spring thaw period as temperature markedly increased than the amount of water entering the soil during snow melt.

4.6.2 Short- and long-term crop rotation impacts on N₂O emissions

Rotation impacts on N₂O emissions were variable in this study. In general, higher cumulative emissions were observed in rotations with vs without cover crops. In terms of total annual N₂O emissions, the perennial rotation emitted the most N₂O, followed by the long rotation with cover crops, and long and short rotations without cover crops. Comparing cumulative emissions across sampling periods (i.e., growing season, post-harvest, spring thaw, and annual), wheat showed significant differences during the growing season, post-harvest period, and for annual emissions but not for the spring thaw period. On all occasions, the SR produced higher emissions during the growing season (263 g N ha⁻¹) and annually (495 g N ha⁻¹) while the CC rotation produced highest emission during the post-harvest period (46 g N ha⁻¹). For the spring thaw period emissions from the SR generally exceeded those from the long rotation ± cover crops, though the differences were not significant.

Under canola production, a significant rotation effect was noted for the post-harvest period and annual emissions—with the CC rotation producing higher emissions after harvest (33 g N ha^{-1}) and annually (288 g N ha^{-1}) compared to the conventional LR and SR systems. Although there was no significant rotation effect on cumulative N_2O emissions during the growing season and spring thaw, emissions were 24–33% greater with CC vs without CC. For potato and pea production, rotation effects on cumulative N_2O emissions were not significant for any of the sampling periods. Indeed, emissions in the potato year for the CC and LR systems were numerically similar during all sampling periods. For pea, emissions were numerically greater in the CC rotations during the post-harvest and spring thaw periods, but were greater in the LR rotations during the growing season. On an annual basis, however, emissions from the two rotations were virtually the same (i.e., LR = $439 \text{ g N}_2\text{O-N ha}^{-1}$ vs. CC = $430 \text{ g N}_2\text{O-N ha}^{-1}$). Growing season emissions during the canola phase were 23% to 53% lower than during the wheat phase, which can be linked to the amount of fertilizer N applied. In spring 2020, the canola plots received 90 kg N ha^{-1} compared to 190 kg N ha^{-1} applied to the wheat plots. Thus, given that the pool of readily available mineral N in the soil increases following fertilizer addition (Machado et al. 2021b), there was undoubtedly more N available to the microbial communities responsible for nitrification and denitrification in the wheat plots. In turn, this resulted in greater N_2O losses during the growing season for the wheat phase. A similar effect was observed when comparing canola and potato, i.e., growing season emissions from the canola plots were about 33% lower than those from the potato plots, which had received 130 kg N ha^{-1} in spring 2020.

Residue incorporation coupled with N fertilization can result in a synergy between the fertilizer N and the decomposing residue that may increase denitrification (Frimpong and Baggs 2010; Duan et al. 2018). Thus, the crop/cover crop residues incorporated in fall 2019 likely contributed to the high growing season emissions—especially those observed right after the spring 2020 application of fertilizer N. The daily emissions figure (Fig 4.1) illustrates fluxes peaking post-fertilization, though the impacts were subtle for some crops—canola in particular. Furthermore, the soil inorganic N pool in the spring (prior to fertilization) was greater in the canola plots than in the wheat plots for the CC (129 vs. 87 kg N ha^{-1}) and SR (143 vs. 77 kg N ha^{-1}) treatments, and were comparable in the LR (114 vs. 112 kg N ha^{-1}) treatment. As a result, the canola received a relatively small amount of fertilizer N (90 kg N ha^{-1}), which despite the relatively large amount of soil N resulted in lower emissions during the growing season. This stresses the usefulness of

adjusting N fertilizer rates based on soil N test to maximize productivity and minimize losses (Liu et al. 2021).

An exponential increase in N₂O emissions has been observed as fertilizer rates exceed the optimum rate for some crops (Wang et al. 2016; Song et al. 2018; Takeda et al. 2021). Additionally, canola as a high N feeder crop may have increased N uptake and reduced any excess N available for N losses throughout the canola phase. It is believed that legume cover crops can decrease N fertilizer requirements, and potentially lower N₂O emissions (Fiorini et al. 2020). However, the relatively lower pre-plant SIN for the wheat phase (which was preceded by pea ± a cover crop) warranted a higher fertilizer N application, which resulted in higher emissions during the wheat phase compared to the canola phase. Similarly, the potato phase had lower pre-plant SIN (55 kg N ha⁻¹) and received a higher N fertilizer rate (130 kg N ha⁻¹), resulting in a slightly higher emissions (241 g N ha⁻¹) by the end of the growing season. Pea and alfalfa plots which did not receive any fertilizer N also increased growing season emissions with PR alfalfa producing the largest total emission of all crops. It is also worth noting that the wheat and canola crops were preceded by a legume crop and/or legume cover crop, i.e., prior to seeding in 2020, wheat was preceded in the rotation by pea/tillage radish while the canola was preceded by wheat + red clover. The common denominator is the legume crop/cover crop (pea and red clover). For wheat production, cumulative emissions were about 10% greater for the SR than the CC, though the difference was not significant. Furthermore, emissions during the post-harvest period after wheat + red clover were 1.4- to 3.5-times greater than those following wheat alone. The early seeding of red clover into wheat increased its available window for establishment and hence early N fixation may have occurred throughout the growing season and continued post-harvest to increase N availability and emissions. A study that determined cover crop N capture and transfer to subsequent crops using ¹⁵N-labelled fertilizer found that legumes have lower N capture capacity than non-legumes (Langelier et al. 2021)—providing a plausible explanation for the higher post-harvest emissions for wheat + red clover and alfalfa.

The impact of pea + tillage radish and wheat + red clover on emissions from wheat and canola, respectively, occurred during different time periods. This is a reflection of the temporal dynamics that occur with residue decomposition. When pea + tillage radish preceded wheat, a significant impact of rotation was observed as early as the growing season. An early decomposition

of pea + tillage radish residues may have increased mineral N in the spring, hence the impact during growing season. For canola, the impact of the preceding wheat + red clover crop may have had a delayed impact, i.e., because red clover is an overwinter species, its decomposition was likely delayed until the growing season—resulting in a late impact that was not apparent until after harvest. Results from a study by Duan et al. (2018) revealed that the most recent residue input may not completely affect available N and the resultant N₂O. Considering annual emissions, the presence of a legume, regardless of when it was present in the rotation, may have influenced N₂O emissions (i.e., PR-alfalfa, CC-wheat, and CC and LR-Pea). There is increasing evidence that legumes can increase soil N availability and enhance N₂O emissions (Ruser et al. 2001; Basche et al. 2014; Fiorini et al. 2020), which is supported by the evidence presented here. Compared to the other cover crops, rotations including canola + oat/berseem clover yielded a consistent pattern of lower emissions during the growing season (165 g N ha⁻¹), at spring thaw (92 g N ha⁻¹), and annually (288 g N ha⁻¹).

Emissions estimated for a hypothetical 4-year period were significantly impacted by rotation. Emissions were greatest in the PR system and did not differ significantly from those in the CC and LR systems, but did differ significantly from those in the SR system. A recent study by Machado et al. (2021) reported that N₂O emissions during the non-growing season were greater under a diverse crop rotation system compared to a simple system. This observation did not appear to hold, however, our study showed a potential for cover crop rotations to increase emissions compared to the other rotations during the post-harvest and the subsequent thaw periods and also for complete crop rotation emission. Increased plant residue N from diversified systems can increase the soil available N pool, making N available for losses. Since alfalfa is a high N builder (Snapp et al. 2005b; Fu et al. 2019), the N₂O emissions observed were not unexpected; indeed, continuous N inputs from alfalfa increased the mineralizable N pool (as observed in Chapter 3) and may have contributed to higher emissions under conditions that favoured mineralization. Machado et al. (2021) explained that emissions were higher in diverse vs. simple rotations due to variations in soil organic C, abundance of genes that control nitrification and denitrification, and soil physical properties. Another explanation that I propose is excessive N supply, i.e., not adjusting N fertilizer rates in the more N-rich diverse systems may heighten the risk for N losses.

4.6.3 Crop and cover crop residue composition on N₂O emissions

Residue left in the field after harvest provides a form of organic matter that can influence N₂O emissions by providing substrates for denitrification and nitrification (Wagner-Riddle et al. 2020)—the microbial pathways that produce N₂O. In this study annual cumulative emissions decreased in the order: perennial alfalfa > wheat + red clover > potato/rye > pea/tillage radish > canola/oat + berseem clover. This same pattern held during the growing season, except that, emissions were higher in potato/rye than in wheat + red clover. During post-harvest period, the order was wheat + red clover > alfalfa > pea/radish > canola/oat + berseem clover > potato/rye. At spring thaw, emissions were higher in alfalfa > potato/rye > pea/radish > wheat + clover > canola/oat + berseem clover. It can be inferred from above trends that the ability of cover crops to reduce N₂O emissions is highly variable. In my study, canola with the legume-grass cover crop mixture (canola + oat/berseem clover mix) frequently lowered cumulative emissions compared to the legume and non-legume cover crops in the rotations. Legume-grass mixtures often exhibit N functional complementarity where legumes fix N in soils while grasses exploit the soil N reserves by taking up residual N (Finney et al. 2016). This may help explain the lower emissions with oat and berseem clover noted in the present study. Comparing legumes to non-legumes, it is reported that legumes can intensify N₂O emissions by way of increasing soil mineral N availability, whereas non-legumes may enhance N immobilization and reduce emissions (Duan et al. 2018; Muhammad et al. 2019). To an extent, this effect was observed in my study as alfalfa consistently—and red clover occasionally—increased cumulative emissions. Nonetheless, studies have shown that even among legume cover crops, emissions can be variable. A study conducted in Saskatoon investigated how residues of different pulse crops influence soil N₂O emissions and showed higher cumulative N₂O emissions with lentil compared to pea over the 4-year rotation period (Liu et al. 2021). Generally, the variation in emissions associated with different cover crops is controlled by several factors including cover crop species, biomass yield, residue C:N ratio, lignin content, and residue management (Basche et al. 2014; Muhammad et al. 2019) while the magnitude of N₂O emissions is known to be influenced by the chemical composition of residues incorporated into the soil (Frimpong and Baggs 2010). In the present study, the cover crop contribution to N₂O emissions varied depending on the species, biomass, residue quality (C:N ratio), and its management. In fall of 2019, the oat + berseem clover produced the lowest biomass (99 kg ha⁻¹) and N content (4 kg N ha⁻¹) but did not differ significantly from rye (288 kg ha⁻¹ with 14 kg N ha⁻¹) or tillage radish (258

kg ha⁻¹ with 12 kg N ha⁻¹). In fall of 2020 however, rye yielded the lowest biomass (90 kg ha⁻¹) and N (4 kg N ha⁻¹) content yet did not differ significantly from the oat + berseem clover (133 kg ha⁻¹ and 5 kg N ha⁻¹) or tillage radish/mustard (164 kg ha⁻¹ and 7 kg N ha⁻¹). Among the cover crops, red clover accumulated the greatest biomass (437 and 428 kg ha⁻¹) and N content (16 kg N ha⁻¹) in fall of 2019 and 2020 and differed significantly from all the other cover crops. Compared to the other crops, alfalfa biomass collected mid-summer was greater and had a higher N content (6400 kg ha⁻¹ and 192 kg N ha⁻¹) in 2019 and (5089 kg ha⁻¹ and 133 kg N ha⁻¹) in 2020. It is therefore not surprising that alfalfa emitted higher cumulative N₂O. Mineralization of organic matter drives microbial activities that can enhance microbial respiration to create hypoxic conditions for denitrification to occur (Machado et al. 2021a).

An important factor that predicts residue decomposition is the C:N ratio (Duan et al. 2018). The addition of residues with low C:N ratio can stimulate N₂O emissions (Toma and Hatano 2007) by (i) serving as an N source for nitrification and/or denitrification, (ii) providing a source of easily decomposable C as an energy source for denitrifiers, and/or (iii) increasing microbial activity and respiration, temporarily inducing oxygen-limiting microsites that promote denitrification (Lemke et al. 2007). Residues that contain higher N concentrations undergo rapid decomposition and result in net N mineralization while residues with low N concentration lead to net N immobilization (Muhammad et al. 2019; Oliveira et al. 2020). The C:N ratios of the cover crop residues were 8:1 for tillage radish and rye, 10:1 for red clover and the mixture of oat + berseem clover, and 15:1 for alfalfa. Since the C:N ratios were <20, a net N mineralization can be implied for all the cover crop species. However, it has been reported that the usefulness of the C:N ratio in predicting mineralization can be undermined as different materials may possess distinct stabilities; e.g., residues that contain different proportions of these compounds (carbohydrates, cellulose or hemicellulose) will have contrasting mineralization rates (Oliveira et al. 2020). Furthermore, the timing, or rate of mineralization plays a significant role in N release to plants. The oat + berseem clover mix and tillage radish are not winter hardy, hence early termination at freeze-up in the fall may result in early decomposition of their residues compared to red clover and rye, which are winter-hardy and were actively growing at springtime. Decomposition of residues at springtime generates N₂O (Duan et al. 2018) and the tillage radish that was winter-killed in fall numerically increased spring thaw emissions compared to red clover that was spring-killed but not the rye that was spring-killed. However, this effect was not observed with the fall-killed oat/berseem clover. A

recent study by Machado et al. (2021) found that N₂O derived emissions from freshly incorporated residues contributed minimally to N₂O totals. Rapid decomposition occurs following residue incorporation into the soil compared to surface placed residues and this is due to the minimal contact with soil microorganisms and other contributing factors such as temperature, moisture etc. that regulate the process (Oliveira et al. 2020). In this study, cover crop residues were left on the soil surface and the impact on decomposition is not known. However, PMN results point towards higher N input with vs. without cover cropping by 2020, indicating a relatively higher SOM input from residue decomposition process. The biomass accumulation and C:N ratios were low in all the cover crops, suggesting that decomposition time may be relatively rapid. Furthermore, soil N supply rates monitored in fall, spring, and summer (see Chapter 3) showed higher N supply rates in spring than all other periods. This may have contributed to the higher contribution of growing season emissions to the total annual N₂O estimated if the higher N supply at spring were not utilized by plants.

In a study to trace crop residue N into subsequent crops, Taveira et al. (2020) showed that (i) long-term rotation did not influence residue turnover to the next crop and (ii) the magnitude of recovery of belowground residue-N was 4–10 times greater than above ground residues. It is however ambiguous whether the N₂O emissions observed in my study were due to the aboveground crop and cover crop residues returned to the soil. Using a ¹⁵N-tracer technique to determine above and below-ground residue contributions to N₂O emissions, they showed that only 0.3–1.3% of residue-N was lost as N₂O (Machado et al. 2021b). Indigenous soil N has been identified as an important contributor to soil N pools and crop N use (Taveira et al. 2020). It is therefore possible that N₂O emissions observed were coming from the indigenous soil N pool considering that emissions, most of the time were not significantly different among the rotation which received similar management. In our case, the majority of comparisons showed no difference with vs without cover crops, indicating that other N sources may be contributing to N₂O production other than the crop and/or cover crop residues returned to the soil.

4.6.4 Yield-scaled emissions

Yield-scaled emissions based on grain/tuber biomass yield (YSE_B) and N-yield (YSE_N) basis did not appear significant in any of the crop phases (Table 4.3). However, emissions were generally (though not significantly) higher in rotations with vs. without cover crops. Yield-scaled

emissions are an important metric to help ensure that food production is accompanied by lower emissions (Qin et al. 2012). In the present study, YSE_B were highest for the pea crop, which was attributed to the combination of low yields and high cumulative annual emissions—especially in the CC rotation when the yield attained was only 1.9 Mg ha^{-1} compared to the LR with yield a of 3.0 Mg ha^{-1} . Among the grain crops, wheat yields were higher compared to canola and pea across all rotations. Yield scaled emissions (YSE_B) reported by Venterea et al. (2011) in the corn phase of a corn-soybean rotation ranged from 46 to 100 g N Mg^{-1} , which was similar to the range for my study ($47\text{--}131 \text{ g N}_2\text{O-N Mg}^{-1}$). The magnitude of annual cumulative emissions and yield dictate the amount of yield-scaled emissions (Liu et al. 2021). Though alfalfa produced the highest annual area-scaled emissions, the higher residue yield resulted in moderately low yield-scaled N_2O emissions compared to pea. Potato however was associated with the lowest yield scaled emissions averaging 50 g N Mg^{-1} for the CC and LR systems. The national average potato yield in Canada is estimated at 36 Mg ha^{-1} (Statistics Canada, 2021), but yields in my study were higher than the national average—ranging between 49 and 52 Mg ha^{-1} for fresh tuber weight. The higher yields for potato meant that the YSE_B for potato were low even though the area-scaled annual emissions were relatively high. In terms of N-yield scaled emissions, alfalfa and pea produced the highest YSE_N averaging $5.3 \text{ g N}_2\text{O kg}^{-1}$. The ranking for YSE_B was alfalfa > pea > wheat > canola > potato. This order changed for YSE_N , pea > alfalfa > potato > wheat > canola. The difference noticed here can clearly be attributed to the differences in crop yields and the amount of N recovered in the grain/tuber yields. Evaluating N_2O production on a yield-scaled basis provides a knowledge base and a better understanding of how intensifying agriculture can impact the environment for certain crop productions (Qin et al. 2012).

4.7 Conclusion

Cover crops have been promoted as a sustainable and suitable management practice that can offset N_2O emissions by contributing multifunctional agronomic and environmental advantages such as sequestering C and N, reducing nutrient losses, improving soil aggregation and water infiltration among many others. Results from cover crop studies and their impacts on N_2O emissions have been inconsistent with cover crops increasing or decreasing N_2O emissions. The variability observed with cover cropping effects on N_2O emissions can be attributed to (i) environmental factors that control cover crop growth and performance such as climate, temperature, and moisture conditions and (ii) cover crop factors such as type of cover crop species,

biomass accumulated, residue quality (C:N ratio, lignin content) and management factors. In this study, N₂O emissions showed a trend towards higher N₂O emissions over the growing season, after harvest, at spring thaw and annually with cover crop rotations—but in most cases, no significant difference was observed when compared with the conventional rotations without cover crops. Comparing full crop rotation emissions over a hypothetical 4-year period showed a significant effect with the largest emissions observed in the perennial rotation alfalfa—which was comparable to the cover crop and the long rotation treatments—and the lowest emissions in the short rotation. Apart from the cumulative emissions, daily N₂O emissions peaked post-fertilization and at spring thaw across all the crop rotations and crops—an important observation that partially supported my hypothesis that N₂O emissions from cover crop rotations would be similar to rotations without cover crops during spring thaw and at fertilization. There was increasing evidence that legumes can increase emissions than non-legumes though not significant for most part, with the exception of alfalfa, which consistently increased cumulative emissions. Red clover showed a similar pattern of increasing emissions though the impact was random. The rye cover crop in the potato phase and the tillage radish in the pea phase did not show any significant impacts, but both pointed towards an increasing emission with cover crops. The oat + berseem clover, however, showed a trend of lowering emissions. The consistent decrease in emissions in the canola/oat + berseem clover (area and yield-scaled emissions) demonstrates a viable mitigation option for canola production. Consequently, both yield-scaled and yield-N scaled emissions were not influenced by rotation, but low pea yields were accompanied by a higher yield-scaled emissions on a yield and yield N basis. Overall, the subtle impact of the cover crops on N₂O emissions could be due to the low biomass, N content and C:N ratio among the cover crop species. Based on the results of my study, combining cover cropping with other agronomic practices —such as adjusting the fertilizer rates — is needed to better mitigate N₂O emissions. Understanding the mechanisms that control residue effect on N₂O emissions may contribute to developing management practices that can lessen N₂O emissions from cover crops.

5.0 SYNTHESIS AND CONCLUSIONS

5.1 Summary and synthesis of key findings

Studies have demonstrated that cover crops are multifunctional and integrating them into existing cropping systems can strengthen ecosystem services such as food production, nutrient and water cycling, and soil, water and air quality (Blanco-Canqui et al. 2015). Adoption of cover crops is a dominant practice in the US and the eastern Canada (Ontario and Quebec) and farmers in the prairies are beginning to adopt this practice on the Canadian prairies (Morrison and Lawley 2021). Yet several questions remain unanswered on the viability, impact, and benefits associated with adopting cover crops in a region with short growing season like the prairies. This study sought to provide a knowledge base for the many stakeholders (farmers, researchers, environmentalists, and policy makers etc.) who are seeking answers to some of these questions. To address this research gap, different cover crop species were included in various crop rotation systems to ascertain if cover crops can grow in the semi-arid regions of Canada and additionally provide a comprehensive understanding on how the choice of a suitable cropping system is vital to the long-term sustainability of agriculture. Thiessen Martens et al. (2015) specified that the long-term sustainability of Canadian agriculture is dependent on its capacity to progress profitably and ensure the preservation of its natural resource and at the same time strengthen its capacity to withstand stress and shocks. Attaining this goal relies on redesigning cropping systems to ensure continuous sustainability of the agricultural systems. In adopting cover crops, farmers are moving away from the conventional perennial practice by redesigning their systems through diversification to imitate perennial systems. One of the many benefits of cover cropping include its potential to recycle nutrients and make them available for successive plant to maximize yield and minimize the risk associated with intensive fertilizations such as N₂O emissions. With proper management, cover crops can ensure a balance by improving the synchrony between plant N demand and soil N supply whilst reducing N losses.

Key findings of this study showed that cover crops do have a potential for increasing and decreasing N availability but at different times. There was a trend towards decreasing soil inorganic

N during the non-growing season due cover crop N uptake, and a potential increase in soil inorganic N supply during the growing season due to cover crop residue mineralization (although not always significant). However, comparing crop yields and NUEs showed that including cover crops did not differ a great deal from the conventional rotations without cover crops. This effect was likely due to the relatively low biomass and N content of the cover crops which did not markedly affect the growing season dynamics of N when compared to the other rotations. As mentioned earlier, the success of cover crops here in the Prairies is contingent very much on climate and the available window for cover crop growth. Semi-arid regions are characterised by low amount of precipitation and short period of high temperature (Floate and McGinn 2010) and these factors predominantly regulate the success of production. Coupled with the short growing window means testing different methods of incorporating cover crops is a vital means to ensuring cover crop success and its adoption.

Red clover among all the cover crops was under-sown into wheat therefore increasing its window for establishment. This method proved viable as red clover accumulated the highest biomass and N content compared to the other cover crop species. However, rye, for example, included as a shoulder season cover crop, accumulated sufficient biomass similar to red clover in 2019. But in 2020, its biomass declined (about 67%) and was not comparable to red clover. On the other hand, oat and berseem clover accumulated the lowest biomass in 2019 but an increase, about 34% was observed in its biomass in 2020. The variations in the performance of the cover crop species can be attributed to interannual variations in climate and other management factors such as irrigation, and the species ability for rapid growth. There was a challenge ensuring cover crop growth in 2020 due to the herbicide regime implemented by the weed ecology specialists, and this was an eye opener to why proper herbicide selection was critical to the success of cover cropping. The wrong herbicide choice can lead to residual effect that can linger into the next season and hinder cover crop growth and development.

With respect to N dynamics, not much difference was observed for SIN supply rate and SIN content among the rotations though cover crops had potential to influence N. A study also reported that dissolved inorganic N (DIN) over a 2-year and 4-year crop rotations of corn-soybean and corn-soybean-grain crop-alfalfa were not different (King and Hofmockel 2017). However, potentially mineralizable N numerically increased with the cover crop rotations with much greater

increase for the perennial systems, signifying increases in organic matter inputs. In evaluating the relationship between soil health management strategy and all soil N metrics measured, there was an obvious pattern where annual crop rotations that included cover crops more closely resembled the perennial system with alfalfa. Overtime, the relatively high SIN levels in the short and long rotations may represent a poor soil health management strategy as the readily available SIN may drive N losses if the available N is not utilized by plants. When viewed over the range of soil health management indices, the PMN supply from the PR and CC rotations were higher than the SR and LR—an indication that incorporating cover crops into annual crop rotations may help mimic perennial systems. Perennial agriculture is known to promote resilient and sustainable cropping systems as a result of the continuous soil cover, enhancement of microbial biodiversity, moisture retention, C storage and greenhouse gas mitigation (Thiessen Martens et al. 2015). Nonetheless, the cover crops did not have any impact on crop productivity. The crop yields in the LR and SR did not differ from the CC rotations. Subsequently, no effect of cover crop was observed on any of the NUE metrics measured. Nitrogen harvest index, uptake efficiency, recovery efficiency and yield efficiency were similar among the rotations. The high N uptake by the crops did not influence N partitioning and remobilization into other plant parts and yield components.

In evaluating the impact cover crops had on N₂O emissions, there were periods of increased emissions with cover crops and vice versa. In responding to the subject if legume cover crops increase emissions than non-legumes, the data pointed towards a pattern where legumes increased emissions though results were not significant. However, the legume alfalfa, consistently increased emissions. Overall, cover crops showed a potential for reducing as well as increasing emissions. For example, at spring thaw, (wheat + red clover and canola +oat/berseem clover) numerically reduced emissions than the other cover crops while (wheat + red clover, potato/rye, and pea/tillage radish) increased annual emissions. It was presumed that the low SIN supply in fall for the cover crop rotations would reflect lower emissions at spring thaw, yet this effect did occur. Surprisingly none of the crop phases was significantly impacted by rotation. The ample soil N availability (partly due to cover crop residue mineralization in addition to N fertilization) may explain the numerically higher N₂O emissions that were recorded during the growing season for the rotations with cover crops. But it is important to note that, N₂O emissions are regulated by other factors such that substrate availability (NH₄⁺, NO₃⁻) are not the only factors that ultimately determine the magnitude of N₂O emissions produced.

5.2 Recommendations for future research

Based on results and discussions that emerged from this study, recommendations for future research will be geared towards achieving successful cover crop establishment and enhancing the possibilities for deriving any benefit associated with cover crops. Future research should explore cold hardy and drought resistant cover crops for the prairies. It is recommended that future research work towards addressing key questions such as: (i) are cover crops a viable option across a range of crop rotations and growing conditions in the Canadian Prairies? (ii) will rotations with cover crop produce similar or different yields compared to the other rotations over time? (iii) what potential agronomic, environmental, and economic benefits or drawbacks are associated with including cover crops in the semi-arid prairies? (iv) will cover crops tighten the N cycle and reduce N₂O emissions?

Choosing the right cover crop can go a long way to ensure its success here in the Prairies, and the correct choice depends on the overall goal of the end-user. One of the most important factors to ensure successful cover crop establishment is how it is incorporated into the system. Intercropping cover crops into cash crops is regarded as an efficient and effective way to obtaining the benefits related to cover crops and has received a lot of attention in other regions. Further research can build on this study by also including cover crops in the short rotations to determine if potential differences could arise. This might be helpful for growers who may only be interested in a simple crop rotation like the short rotation (comprising only two crops over a 4-year period). In this way growers may be diversifying their systems and increasing the length of soil cover. Furthermore, since it is the overall aim of every farmer to maximize profit and the practice of cover cropping is sometimes regarded as an additional cost to be incurred by the farmer, it will be prudent for future works to focus on the cost-benefit analysis of engaging in cover cropping. This will provide insight to farmers on the costs associated with the practice. Additionally, future research can focus on long-term cover crop rotations *vs.* long-term conventional systems to ascertain if cover crops do influence N availability and have potential to reduce N losses as cover crops effects build up gradually. Nitrous oxide mitigation strategies involve increasing crop N use in agricultural systems. This may involve exploring methods of managing cover crops and/or residue in cropping systems that can lessen N₂O emissions. Obtaining a comprehensive knowledge base and establishing the optimal system under which cover crop functions best, will provide an extensive information that would be beneficial for all stakeholders in this field.

5.3 General conclusions

Many environmental issues threaten the sustainability of agricultural sector and most of these are attributed to the adoption of simplified production systems. It is reported that simple systems such as monocropping are nutrient exporters and can ultimately alter nutrient cycles. It is there crucial that systems that have potential to improve and maintain agricultural systems are developed and promoted. Including cover crops into crop rotations have become part of the process of redesigning cropping systems for agricultural development. Cover crops are known to provide numerous benefits ranging from (i) agronomic: increased yields, improved soil fertility, reduced loss of soil water, pest, and disease control etc. (ii) environmental: reduced greenhouse gas emissions that promote climate change, increased C sequestration, improved air quality, biodiversity enhancement etc. to (iii) socio-economic benefits: income, natural habitat enhancement. As mentioned earlier in previous chapter of this study, Prairie region of Canada are characterised by short growing window and growers may be interested in which cover crop species, methods of incorporation and management practice would ensure its success. Overall, this study has demonstrated that cover crops may be viable on the Canadian prairies if properly managed; but there is room for improvement. However, for cover crops to sufficiently tighten N cycling, improve crop NUE, and reduce N₂O emissions—other agronomic practices/adjustments should be made, such as reducing N fertilizer rates, applying N fertilizers in a timely manner and at the right place to meet crop demands.

Since the study involved a variety of cover crop species and different rotation systems, growers would be able to assess and choose which species and system fit their needs. This study also provides policy makers with additional information on farming practices that promote more sustainable agricultural development. This study, however, does not provide comprehensive knowledge on the topic due to the fact that the studies spanned a period of 2 years, thus, further research will be required to provide extensive information and more conclusive evidence. Collectively, longer-term research will strengthen the sustainability of agricultural systems in the prairies by providing the information needed to support improvements in crop productivity, food security, environmental quality and at the same time promote social and economic development.

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