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Nitrogen transfer from cover crops to the subsequent grain crop and the influence of variability in winter conditions

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Abstract

Cover crops, which are used to provide ground cover after the harvest of the grain crop, can potentially improve the sustainability of agroecosystems by reducing nutrient losses. However, few data are available to document the extent to which cover crops improve both the retention of soil nitrogen (N) and the transfer of this N to the grain crop. The efficiency of this N transfer may be further influenced by variation in winter soil temperature; for example, reduced snow cover can increase the frequency and intensity of soil freezing, which can affect the survival of cover crops and the timing of the decomposition of their residues. I quantified N transfer from cover crops (legumes, non-legumes and mixtures) to the subsequent corn crop using ^{15}N tracer. Residue swapping was used to isolate the individual contributions of the aboveground and belowground N components of the cover crops. N transfer responses to soil temperature variability over the winter were examined via snow removal and pulsed warming from overhead heaters. My results revealed that the belowground N pool contributed substantially more to N retention and N transfer than the aboveground N pool. However, less than 10% of the ^{15}N added to the soil was transferred to the corn, while the majority remained in the soil. In addition, increased soil freezing reduced the effectiveness of the cover crops in transferring N to the corn, with legumes being more susceptible to N losses than non-legumes. Year-to-year variability in cover crop establishment and productivity also had strong effects on the effectiveness of the cover crops in retaining and transferring N. Overall, my results reveal that while the scavenging of N by cover crops after harvest of the main crop may not provide a substantial contribution of N to the grain crop the following year, this scavenging may be important for reducing N losses to the surrounding environment.

Keywords

Cover crops, nitrogen, climate variability, freeze-thaw, agriculture, soil

Summary for Lay Audience

To meet the demand of increased food production required to feed the growing human population, fertilizers are used to increase nutrient inputs to agricultural fields. Nitrogen based fertilizers are often used to increase crop yield; however, the excess nitrogen not taken up by crops can become an environmental pollutant. In particular, farmers often leave their soils bare after grain crops are harvested, and bare soil is at risk of eroding and losing nutrients. Farmers can reduce these losses by using cover crops, which are planted after the harvest of the grain crop in the fall. Cover crops are not harvested but are planted to provide benefits to the soil in the absence of the grain crop. The cover crops are then terminated (killed), either by frost or from using chemical herbicide in the spring. When cover crops die, their decomposition releases any nutrients they have taken up back to the soil. Ideally, these nutrients will become available to the grain crop planted in spring, allowing farmers to use less fertilizer. However, winter conditions may affect the timing of when cover crops decompose and release nutrients. If there is reduced snow cover, soil and overwintering plants can be exposed to severe freezing, thereby reducing the positive benefits they could provide. My goals were to quantify where nitrogen taken up by cover crops ends up (e.g., in the soil or the main crop), and how different winter conditions affect the loss and transfer of nitrogen. I simulated soil freezing by reducing snow cover either by shoveling the snow off the plots or by using heaters to melt the snow. My data showed that cover crops are important at increasing the retention of nitrogen in the soil, and I also found that the effectiveness of cover crops is reduced by increased soil freezing. Overall, my results reveal that while nitrogen uptake by cover crops may not provide a substantial contribution of N to the grain crop the following year, this uptake may be important for reducing N losses to the surrounding environment.

Co-Authorship Statement

The research described in this thesis is a result of contributions from the author as well as co-authors from University of Guelph, University of Saskatchewan, and supervisors, Dr. Hugh Henry and Dr. Claudia Wagner-Riddle. The detailed contributions for each chapter are as follows:

Chapter 1 was written by the author and edited by both supervisors, Dr. Hugh Henry and Dr. Claudia Wagner-Riddle

Chapter 2 describes a project proposed by the author, Dr. Hugh Henry and Dr. Claudia Wagner-Riddle, with collaboration from Dr. Khagendra Baral (University of Guelph), and from Dr. Rich Farrell, and Dr. Kate Congreves at the University of Saskatchewan for developing the residue-swapping protocol

Chapter 3 describes a project proposed by the author and edited by both supervisors, Dr. Hugh Henry and Dr. Claudia Wagner-Riddle

Chapter 4 describes a project proposed by the author, Dr. Hugh Henry and Dr. Claudia Wagner-Riddle, in collaboration with lysimeter facility at the University of Guelph

Chapter 5 was written by the author and edited by both supervisors, Dr. Hugh Henry and Dr. Claudia Wagner-Riddle

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List of Abbreviations

AG	Aboveground pool
ANOVA	Analysis of variance
BG	Belowground pool
NH_4^+	Ammonium
C	Carbon
C:N	Ratio of mass of carbon to mass of nitrogen
CO_2	Carbon Dioxide
CC	Cover crop
GHG	Greenhouse gas
H	Hydrogen
HSD	Honestly significant difference
N	Nitrogen
N_2	Dinitrogen (nitrogen gas)
NO_2^-	Nitrite
NO_3^-	Nitrate
N_2O	Nitrous oxide
^{15}N	Heavier isotope of nitrogen
NUE	Nutrient use efficiency
NREdfF	Nitrogen recovery efficiency derived from tracer
NREdfCC	Nitrogen recovery efficiency derived from cover crop
O	Oxygen
P	Phosphorus
rpm	Rotations per minute
S	Sulfur
SOM	Soil organic matter
SR	Snow removal
TNdfCC	Total nitrogen derived from cover crop pools
10-way	Cover crop mixture with 10 species
3-way	Cover crop mixture with 3 species

Chapter 1

1 Background

1.1 Biogeochemical cycles of nitrogen

Nitrogen (N), carbon (C), oxygen (O), hydrogen (H), phosphorus (P), and sulfur (S) are the key chemical elements that contribute to the molecular building blocks of organisms (Galloway et al., 2003). These elements are constantly transformed, turned over, and moved within and between environments via both abiotic and biotic processes (Gruber & Galloway, 2008). The biogeochemical cycling of these elements provides a means for energy to flow through ecosystems, the recycling of nutrients, and exchanges of inorganic matter to living systems as organic forms and return to the environment (Moore et al., 2005).Nutrient cycling and other biogeochemical processes have been both directly and indirectly modified by humans on a global scale over the past 100 years through activities such as the burning of fossil fuels and large-scale land use changes (Vitousek et al., 1997). The N cycle (Figure 1-1) in particular has been altered dramatically, with human-driven inputs of inorganic forms of N increasing substantially with the invention of the Haber-Bosch process, which has allowed the production of synthetic N based fertilizers, and with the expansion and intensification of agricultural activity (Galloway et al., 2004).

N demand by plants is high relative to that of most elements, given that N is an essential component of amino acids, nucleic acids, and other important organic molecules such as chlorophyll; most living plant tissues are made up of 1-4% total N by dry weight (Jan Dirk Van et al., 2019). However, N is only available for plant uptake in inorganic forms and many of these forms of N are readily lost from ecosystems as they are very mobile, which can limit plant N supply (Galloway et al., 2008). Inorganic nitrogen is present in soil as ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and in various organic compounds (urea, amino acids) (Cameron et al., 2013). Although N is the most abundant element in the atmosphere (which is approximately 78% N_2), it is not accessible to most organisms in this gaseous state because of the strength of the triple bond between the nitrogen atoms (Galloway et al., 2003). Dinitrogen gas can be fixed into biologically reactive compounds via two natural processes: 1) biological N fixation, which is performed by specialized groups of bacteria and archaea, and 2) the intense heat of lightning strikes (Fowler et al., 2013).Organisms that fix N produce nitrogenase enzymes, which reduces the triple-bonded N_2 to NH_4^+ (Dixon & Kahn, 2004). There are free-living groups of organisms that fix N in both terrestrial and aquatic environments while others form symbiotic relationships There are groups of nitrogen fixing bacteria such as *Aztobacter* and *Azospirillum* form mutualistic symbiotic relationships with plants, whereby they receive photosynthates in exchange for N (Dixon & Kahn, 2004; Jan Dirk Van et al., 2019). There are two major groups of ymbionts that induce plants to form root nodules, one being Rhizobia which, nodulate leguminous plants, while the other is *Frankia*, which forms nodules on trees and shrubs (Dixon & Kahn, 2004). Biological fixation of N occurs in deoxygenated environments more readily because oxygen inhibits nitrogenase enzymes; nodules provide a protective microhabitat for these groups, reducing competition with other microorganisms, while also providing an environment with less reactive O_2 (Dixon & Kahn, 2004; Jan Dirk Van et al., 2019).

Nitrification is another specialized transformation that occurs for N, whereby NH_4^+ becomes oxidized in a two-step aerobic process that first converts it to NO_2^-, then to NO_3^-, by groups of bacteria and archaea known as nitrifiers (Mendum & Hirsch, 2002). Nitrifying bacteria such as *Nitrobact*er oxidize either NO_2^- or NH_4^+ using ammonia monooxygenase and hydroxylamine dehydrogenase (Prosser & Nicol, 2012; Jan Dirk Van et al., 2019). Nitrification is in part responsible for N mineralization, whereby organic N is converted to an inorganic state by microorganisms. Fungi are also important for N mineralization, as they break down organic N to produce NO_3^- instead of NH_4^+ using heterotrophic nitrification (Crossman et al., 1997). The rate of N mineralization often depends on the C:N ratio of decomposing organic matter, the N requirement of soil microbes and their overall biomass (Melkonian et al., 2017). N immobilization is the opposite process, in which inorganic N is converted to organic forms.

Denitrification is a process whereby reactive N in soil is converted to its gaseous state, N_2, and returned to the atmosphere (Jan Dirk Van et al., 2019). Bacteria such as *Paracoccus denitrificans* reduce $NO_2^{-+}$to NO_3^- then reduce it to N_2, although if the reaction is incomplete N_2O is produced, which is a very potent greenhouse gas (GHG) (Crossman et al., 1997). Denitrification is not a specialized function, and it occurs often across many groups of bacteria; approximately 5% of soil bacteria can denitrify using either NO_2^-, NO_3, or N_2O as terminal electron acceptors in anaerobic respiration under oxygen limited conditions, leading to the production of N_2 (Jones et al., 2008; Jan Dirk Van et al., 2019). Bacteria that reduce inorganic forms of N contain genes for nitrite reductase (nirK, and nirS), while other groups contain genes for nitrous oxide reductase (nosZ) (Jones et al., 2008). These processes occur when soils become waterlogged and anoxic, especially during the spring melt in temperate climates (Jan Dirk Van et al., 2019).

As described above, fixation of N via the Haber-Bosch process accounts for the largest anthropogenic source of N to soils. This process uses intense heat and hydrogen to produce NH_4^+, which can be converted to ammonia nitrate or urea-based fertilizers (Kitano et al., 2012). Common to soil bacteria is the enzyme urease, which is used readily to catalyze forms of urea to NH_4^+ salts, a reactive N form that can be easily absorbed by the roots of plants (Tilman et a., 2011; Jan Dirk Van et al., 2019). Fertilizer development has increased agricultural productivity and crop yield by substantial amounts to meet the demand of global food requirements and human population growth (Tilman et al., 2011). N inputs into the environment from fertilizers are expected to rise as it has been estimated that food production must increase by ~70% by 2050 to feed an estimated 9.1 billion people (FAO, 2009; Godfray et al., 2010; Paoletti et al., 2011). To meet this demand, it is expected that fertilizer use will continue to increase despite the negative effects of increased fertilization on the environment (Gomiero et al., 2011). In fact, most of the N fertilizer that is not assimilated by plant roots in agricultural fields will likely be lost from the soil through denitrification (leading to volatile gasses) and leaching of NO_3^- (Di & Cameron, 2002). For instance, increased NO_3^- entering bodies of water (e.g., rivers, lakes) can contribute to eutrophication, leading to greater losses of biodiversity in both aquatic and terrestrial systems (Tilman et al. 2001). Agricultural productivity contributes to around 24% of total GHG emissions, including both CO_2 and N_2O (FAO, 2014). To reduce the negative effects of fertilizer use, many governments have focused on developing more sustainable practices to improve soil health, with the goal of reducing rates of fertilizer application.

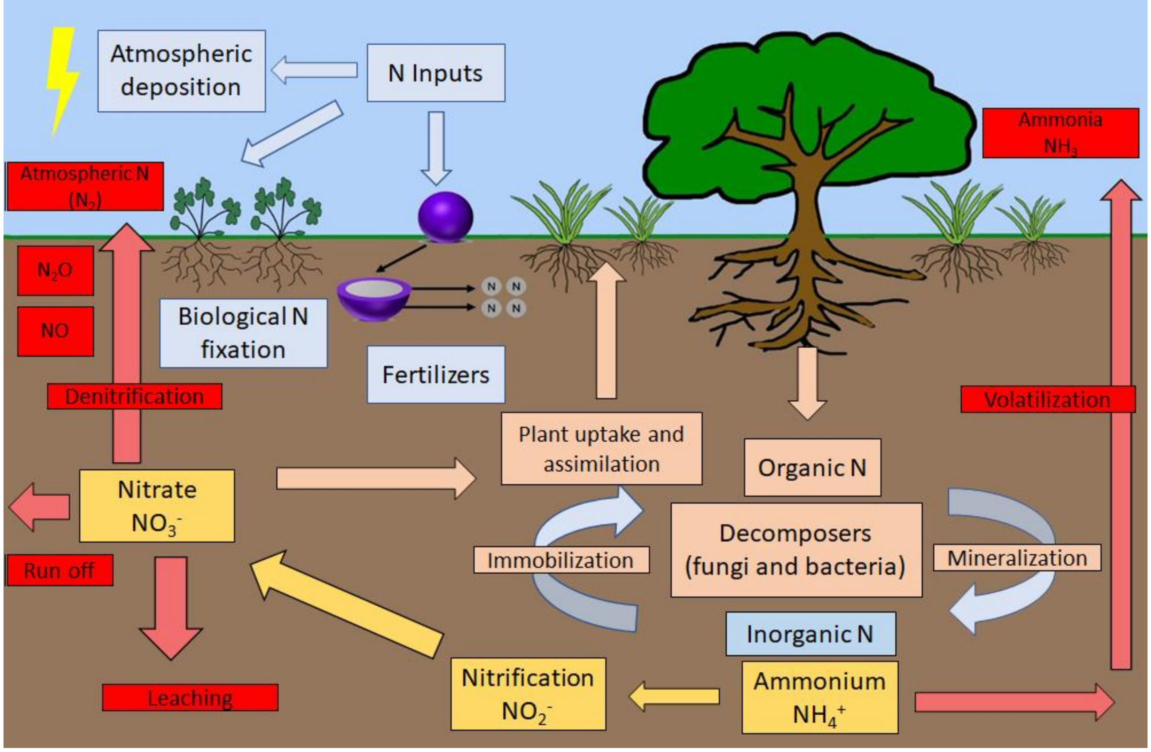

Figure 1-1 Terrestrial nitrogen cycle displaying sources, transformations, and movements of nitrogen.

1.2 Soil health within agricultural systems

The conservation and management of soil health is essential for the sustainability of crop productivity, both economically and environmentally, because soil provides the foundation for terrestrial ecosystem function (Doran & Zeiss, 2000). The capacity for healthy soils to function is defined from biological, chemical, and physical processes (Tonitto et al., 2006; Turmel et al., 2015). Maintaining healthy soils improves many essential ecosystem level services such as mitigation of GHG emissions, soil organic matter storage, disease and weed prevention, water infiltration, and climate regulation (Robertson & Vitousek, 2010; Schipanski et al., 2014).

In many agricultural systems, growers have traded long-term sustainable practices for short-term increases in agricultural production (Ponisio et al., 2015). Specifically, many current agricultural practices (e.g., heavy fertilizer usage, tillage, and non-diversified crop rotations) are disregarding future sustainability by polluting ecosystems, reducing biodiversity, and increasing GHG emissions (Tilman et al., 2001, 2002). For instance, long-term monoculture production increases the susceptibility of disease, pest infestation, and nutrient depletion (Lithourgidis et al., 2011). Monoculture crops such as corn require large amounts of N, (Vitousek et al., 1997; Tilman et al., 2001, 2011; Lithourgidis et al., 2011) and deep heavy tillage can disturb fungal networks and destabilize soil aggregates.

Current research is addressing practices to improve soil health by achieving enhanced soil fertility and maximizing nutrient retention to meet the current needs of society while also mitigating environmental degradation (Pretty, 2008). These agricultural practices promote the idea of matching the supply of soil nutrients with the nutrient demand of the harvested crop, along with optimizing the return of crop residues, promoting plant biodiversity, increasing soil organic matter, and mitigating GHG emissions (Pretty, 2008). In contrast, current agricultural practices typically focus on the maximization of economic return through the application of N based fertilizers to drive maximum plant yield rather than considering the optimization of nutrient use efficiency (NUE; defined here as the proportion of N fertilizer taken up into plant biomass) (Fowler et al., 2013; Galloway et al., 2003; Howarth et al., 2002). As a result, only a fraction of added fertilizer is typically taken up by crops, resulting in NUE as low as 30% (Galloway et al., 2003). Improving the efficiency of N delivery in agroecosystems not only has financial benefits, but it can have positive outcomes for the surrounding environment.

1.3 Cover crops

A promising strategy for the improvement of soil fertility, N delivery and nutrient use efficiency is the use of cover crops to increase the diversity of crop rotations (Gaudin et al., 2015). Cover crops are used as a nutrient management tool to maintain the soil's physical, chemical, and biological properties during the fallow period when the grain crop is absent (Tonitto et al., 2006). Cover crops can improve water filtration, prevent soil erosion, and increase soil C sequestration by improving soil structure (e.g. increasing macroporosity and decreasing soil bulk density) and soil organic material (Dabney et al., 2001, 2007). Moreover, cover crop use can increase crop yields, enhance microbial activity, reduce the requirement for fertilizer and pesticides, and reduce N loses via trace gas emissions (e.g., N_2O), runoff and leaching (Komatsuzaki & Wagger, 2015). Cover crops are typically planted after the harvest of the grain crop to promote the capture of residual soil mineral N before it can be lost (Snapp et al., 2005; Holness et al., 2008), whereas inter-seeding of low growing cover crops (e.g., clover and vetch) can be used for row crops (Norris et al., 2020; Nguyen et al., 2022). In some regions, especially for vegetable growers, cover crops are planted over the summer to restore and rehabilitate degraded soils (Snapp et al., 2005). Despite the many benefits of cover crops, cover crop adoption is still rather low in parts of North America- an estimated 2.3% of land in the Midwestern USA is cultivated by farmers who utilize cover crops (Roesch-McNally et a., 2018).

Different species of cover crops can benefit soil fertility in different ways. Non-leguminous cover crops (e.g. grasses and *Brassica* species) can immobilize residual soil mineral N before it is lost via leaching (nitrate leaching can be reduced by as much as 70%), and their N uptake can typically out-compete other nitrogen cycling processes (Ranells & Wagger, 1997; Delgado et al., 1999; Dabney et al., 2001; Zhou et al., 2020). For instance, winter rye can immobilize as much as 100 kg/ha of N (Jackson et al., 1993; Snapp et al., 2005). Moreover, *Brassica* species can control pests through the release of fumigants and can provide natural tillage of agricultural soils (Dean & Weil, 2004; Haramoto & Gallandt, 2004). Leguminous cover crops fix additional N, potentially reducing the amount of fertilizer required for the following grain crop. For example, the leguminous cover crop hairy vetch can add 50-155 kg ha^{-1} of N to a succeeding corn crop (Clark et al., 1994; Ranells & Wagger, 1997; Coombs et al., 2017). However, much like synthetic fertilizer, there is a risk that fixed N from legumes can be lost to the surrounding environment (Fageria et al., 2008; Coombs et al., 2017).

1.3.1 Cover crops and biodiversity

Cover crops are often planted as monocultures; however, cover crop mixtures may provide additional benefits and ecosystem level services (Chu et al., 2017). For instance, several studies performed within natural ecosystems have found that as biodiversity increases, so does the overall productivity of the entire ecosystem (Cardinale et al., 2012). The increased biomass production of diverse plant mixtures is a complementarity-based system, in which unrelated species utilize space and resources differently, thus enabling higher productivity

(Finney et al., 2016). Previous research has shown that grass-legume bicultures can be used in combination to both capture N and input additional N for the subsequent grain crop (Ranells & Wagger, 1997). Cover crop mixtures are determined in part by combining species that have different physiological and morphological traits. As described above, non-leguminous cover crops can obtain N from scavenging (e.g., immobilizing residual nitrate), whereas legumes provide additional N inputs through N fixation (Dabney et al., 2010; Finney et al., 2016). Patterns of N use and the life histories (phenology) of plants also can affect productivity and the capacity for nutrient-uptake. However, the success of a given cover crop can vary geographically as many plant species are constrained by hardiness zones (defined as regions were plants survive the winter – concurrent with amount of frost free days). For example, some perennial cover crops are winter-killed by frost in northern regions, whereas others are more hardy and experience regrowth in the spring (Florence et al., 2019; Freund et al., 2021; McKenzie-Gopsill et al., 2022). Although there are many potential benefits of using diverse mixtures of cover crops, several research gaps exist in terms of their demonstrated benefits to N retention as many mixes have not been characterized, especially diverse mixtures that have greater than two species (Finney et al., 2016; McKenzie-Gopsill et al., 2022). Moreover, from a management perspective, the carbon and N concentrations of cover crop residues can increase or decrease depending on species composition, along with planting and termination dates (Doran and Smith 1991, Grant et al. 2002).

1.3.2 Cover crop decomposition

By scavenging N, cover crops can increase internal cycling of N within agroecosystems (O’Reilly et al., 2012). Nevertheless, the success of cover crops in reducing N losses is contingent on the timing of N release from decomposing cover crop residues being synchronized with N uptake by the grain crop (Crews & Peoples, 2005; Nevins et al., 2020), and it can be difficult to predict how well the mineralization of decomposing cover crop residues may be synchronized with crop N demand (Vyn et al., 2000). Cover crop residues can vary widely in lignin content, cellulose, and C:N ratios, all of which can affect their decomposition and nutrient mineralization (Fageria et al., 2008; Nevins et al., 2020). Legumes typical have a low C:N ratio in comparison to grasses, which contain relatively low N concentrations and high quantities of non-labile tissues (Sievers & Cook, 2018; Perrone et al., 2022). The turnover of labile tissues from legume residues can be rapid in the early spring, given that soil microorganisms remain active at low temperatures (Henry, 2008; Campbell et al., 2014). Spring melt, in particular, is often a period of high soil N losses, which can substantially with variability in the severity of soil freezing (Campbell et al., 2014). The efficiency of N transfer from the cover crop to the main crop thus may be influenced by variation in winter soil temperatures, which can affect decomposition and residue N mineralization.

1.4 Climate variability and soil freezing

Climate models project global surface temperatures will increase by approximately 3 °C over the next century (IPCC 2022), which is expected to alter plant litter decomposition and soil nutrient cycling over winter in temperate ecosystems (Kreyling, 2010). Warmer temperatures have the potential to reduce soil health as they increase nutrient mineralization and the decomposition of soil organic material directly effecting C and N pools (Kirschbaum, 1995). Climate variability will also increase the frequency and magnitude of extreme weather events such as drought, and heavy rainfall (Kreyling et al., 2008). The effects climate variability will directly influence vegetation and many ecological processes such as winter decomposition (early spring melts, freezing damage) and nutrient cycling within temperate climates (Kreyling, 2010). For instance, global climate models predict moisture variability to increase within temperate regions, which may impact agriculture with challenges ranging from increased soil erosion, to waterlogged soils increasing the amount of denitrification, to delaying spring plant dates and ultimately reducing crop productivity (Lal 2004). Climate models also predict an increase in the frequency of soil freeze-thaw cycles, in part due to increase variability in air temperature (e.g., mid-winter melts), but also due to reductions in snow cover (Campbell et al., 2009). Snow is an effective insulator, and when there is decreased snowpack, the soil becomes increasingly exposed to cold air at night or during cold spells (Liu et al., 2019). This relationship explains the apparent paradox that soils tend to be coldest during the warmest winters in northern temperate regions (Zhou et al., 2017). Extreme soil temperatures fluctuations from freeze-thaw events can disrupt soil microbial activity, disturb soil aggregates, and alter soil C and N transformations (Feng et al., 2007; Bai et al., 2013).

Freezing damage in plants occurs when temperatures below 0 °C cause ice crystal formation within plant cells and extracellular tissue (Gu et al., 2008). Frost damage from ice crystals usually occurs between -2 °C, and -5 °C in frost sensitive plant species (Pearce, 2001). Cellular membranes are prone to freezing damage because they contain ice nucleation sites. Soil freezing can therefore lyse plant cells, leading to the release of soluble nutrients (Cober et al., 2018).

Plant root uptake of N is reduced by intense soil freezing because fine roots are sensitive to cellular damage caused by freezing (Campbell et al., 2014; Cober et al., 2018). Alternate freezing and thawing, in addition to continuous soil freezing, also can change the physical structures of litter, increase litter fragmentation rate and decrease the stability of soil aggregates (Oztas & Fayetorbay, 2003). N mineralization of labile soil organic material (SOM) from microorganisms also can increase during periods of thaw as microorganisms are also sensitive to freeze-thaw events, increasing inorganic N losses (Yang et al., 2014). Increased nutrient leaching losses with increased freezing also have been observed in cover crop systems, potentially reducing the agronomic benefits they provide (Øgaard, 2015). Winter-hardy cover crops may also become susceptible to increased frost exposure with less snow cover, as some of these species have shallow roots (Malyshev & Henry, 2012).

1.5 The use of ^{15}N to track fate of N within cover crop systems

^{15}N, a stable isotope of N, can be used to track the fate of nitrogen fertilizer, and can be used to differentiate naturally occurring N from synthesized sources (Bedard-Haughn et al., 2003; Zhao et al., 2016). Nitrogen is composed of two stable isotopic forms, ^{14}N and ^{15}N, with the majority being present in the atmosphere as ^{14}N (99.63%), while the remaining 0.367% is ^{15}N (Bedard-Haughn et al., 2003). In ^{15}N tracer studies, an N pool of interest is enriched with artificially high levels of ^{15}N (Bedard-Haughn et al., 2003), with the quantity of added tracer sufficiently elevated so that it overwhelms any variation in the natural abundance of ^{15}N in plant tissues and soil that naturally result from physical fractionation processes (Dawson et al., 2002).

The labelling of cover crops with ^{15}N tracer can be effective for quantifying the efficiency of N transfer from cover crops to the subsequent grain crop (Collins et al., 2007; Dabney et al., 2010). ^{15}N-labelling approaches have typically only been used to track the potential contribution of aboveground cover crop biomass to the grain crop (Ranell s& Wagger, 1997; Holness et al., 2008; Gardner & Drinkwater, 2019), rather than accounting for contributions from belowground cover crop residues. This is likely due to the challenges associated with collecting below ground root residue, as well as to differences in root turnover rates among plant species (Gabriel et al., 2016; Taveira et al., 2020). Nevertheless, the importance of N from cover crop roots may be substantial to the total N contribution that they provide(Arcand et al., 2014). For example, in crimson clover, below ground residues contain 16 to 19% of the total N (Reeves, 1994), in wheat and lentil, below ground root biomass can contain between 31-50 % of the total N (Arcand et al., 2013). The belowground N contribution from cover crops is important for understanding their role in soil fertility.

1.6 Thesis Statement

Quantifying the transfer of N from cover crops to the grain crop is important from a management perspective to determine the extent to which N fertilizer application may be reduced. Although cover crops immobilize N, there is no guarantee of a successful transfer of this N to the grain crop (Crews & Peoples, 2005; Nevins et al., 2020). The success of cover crops in reducing N losses and benefitting the yield of the subsequent grain crop is contingent on the N release from the decomposition of cover crop residues being well-synchronized with the N demand of the grain crop (Hadas et al., 2002). As described above, an important distinction may exist between winter-killed cover crops, which decompose over winter, and winter-hardy cover crops which go through senescence over the winter. Winter-killed cover crops decompose and experience N mineralization earlier, which could potentially increase N leaching and trace gas losses (Schipanski et al., 2014). In contrast, winter-hardy species can continue to take up N over winter and spring, with N release from their residues occurring at a later date. This process may potentially increase synchronization with the nutrient demand of the grain crop, although the success of the latter can depend on the termination date (White et al., 2017). Nevertheless, few data are available to address how cover crops alter the dynamics of N losses vs. N transfer to the grain crop the following season . This lack of data is particularly evident for belowground cover crop residues (primarily roots), which can contain up to half of the total plant N (Arcand et al., 2014; Reeves, 1994). Winter climate variability adds another layer of complexity when attempting to assess cover crop effects on N use efficiency, which is already difficult to assess due to the increased N losses that occur over the winter. Plant survival and litter decomposition are influenced by winter conditions (Kreyling, 2010) and variation in other factors such as air temperature and snow cover (which decouples soil temperature from air temperature) may thus affect N transfer and optimal management practices (Henry, 2007; Zhou et al., 2017).

1.7 Objectives

The overall goal of my thesis was to assess how variability in winter conditions can alter the transfer of N from cover crops to the subsequent grain crops over the winter.

My three main objectives were:
Objective 1 (Chapter 2): To assess the extent to which cover crops increase the retention of soluble N present in the soil following the harvest of the grain crop, and to assess the relative contribution of cover crop N to the subsequent corn crop using ^{15}N

I compared both different cover crop monocultures and mixtures, and I assessed the relative contributions of N from aboveground versus belowground cover crop residues.
I predicted that cover crops would increase N retention and that cover crop mixtures would result in greater retention than single species cover crops. I also predicted that the belowground cover crop pool (soil and roots) would contribute more N than the aboveground residues to the subsequent grain crop.

Objective 2 (Chapter 3): To examine how variability in soil freezing affects N transfer from cover crops to the grain crop, with a focus on both winter-killed and winter-hardy cover crops.

I predicted that snow removal will increase soil freezing, leading to reductions in the transfer efficiency of N from cover crops to the subsequent grain crop. I also predicted that the N transfer from winter-hardy cover crops would be affected more by soil freezing than winter-killed cover crops.

Objective 3 (Chapter 4): To examine nitrogen losses from cover crops in response to increased soil freezing induced by pulse winter warming.

I predicted that legumes would experience the greatest losses of N as their tissues contain low carbon content and that these losses would be exacerbated by increased soil freezing.

1.8 Thesis organization

My PhD thesis is written in an integrated article format, with the three middle chapters (data chapters) written as independent manuscripts. Chapter 1: General introduction into topics, and overview of thesis. Chapter 2: The first data chapter, which addresses N retention, and N transfer from cover crops to the subsequent grain crop. Chapter 3: The second data chapter, which examines how soil freezing affects the transfer efficiency of N from cover crops to the subsequent grain crop. Chapter 4: The third data chapter, which focuses on decomposition, and N losses of various cover crop species in response to pulsed winter warming. Chapter 5: A general discussion, which synthesizes the results of the three data chapters.

1.9 References

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Chapter 2

2 Introduction

Effective nitrogen (N) fertilizer management is crucial for the reduction of environmental pollution associated with agricultural systems (Pretty, 2008; Tilman et al., 2011). Cover crops, which provide ground cover after the harvest of annual grain crops, can potentially enhance agroecosystems by reducing nutrient losses and increasing nitrogen storage in soil organic matter (Ketterings et al., 2015; Snapp et al., 2005). For example, both grasses and *Brassica* cover crops can immobilize up to 100 kg N ha^{-1} of residual soil mineral N per year, reducing denitrification and N leaching losses (Dean & Weil, 2004; Farsad et al., 2011). Leguminous cover crops also can add additional fixed N to the soil, reducing the amount of fertilization required for the following grain crop by up to 30 – 150 kg N ha^{-1} (Askegaard & Eriksen, 2007; Dabney et al., 2010; Perrone et al., 2022). While cover crops are often planted in monoculture, cover crop mixtures may provide further benefits for nutrient retention via complementarity, because unrelated species may access the soil at different depths and times, thus enabling increased total plant productivity and higher overall nutrient capture (Finney et al., 2016; de Notaris et al., 2018).

Despite the benefits of cover crop N immobilization for reducing N losses in the short term, following decomposition of their residues, only a fraction of the released N is transferred to the subsequent grain crop (Holness et al., 2008); quantifying this transfer of N from cover crops to the subsequent grain crop is needed to calibrate potential reductions in fertilizer application. Moreover, the remaining portion of N released from cover crop decomposition can be lost to the atmosphere or to the surrounding environment (Fageria et al., 2007, 2008; Coombs et al., 2017). The potential therefore exists for cover crops to be a liability from an environmental and economic standpoint if not managed correctly (Vyn et al., 2000).

Overall, the success of a cover crop in reducing N losses and potentially benefitting the yield of the subsequent grain crop is contingent on N release from cover crop decomposition being well-synchronized with the N demand of the grain crop (Crews & Peoples, 2005; Nevins et al., 2020). For example, despite the high N content of legumes, their residues contain more labile materials than the residues of non-leguminous cover crops, and thus can decompose rapidly following cover crop termination (Sievers & Cook, 2018). In addition, life history and phenology affect both cover crop nutrient uptake and the timing of residue decomposition. For example, cover crops that are killed by frost cease nutrient uptake and begin to decompose in late fall, whereas winter-hardy species can (if not terminated by herbicide in the fall) continue to take up N through spring, at which point they are terminated and the residues from their spring regrowth begin to decompose and release N (White et al., 2017). The early decomposition of winter-killed or fall terminated cover crops can potentially increase leaching and N trace gas losses (Schipanski et al., 2014; Olofsson & Ernfors, 2022). In contrast, the decomposition and release of N from spring terminated, winter-hardy species may be better synchronized with the timing of N uptake by the grain crop (White et al., 2016).

When assessing cover crop N retention and N transfer between cover crops and grain crops, it is difficult to differentiate among different sources of nitrogen (e.g., soil organic matter, cover crop residue, or inorganic fertilizer) available to the crop (Taveira et al., 2020). While cover crop shoots labelled with ^{15}N tracer can be clipped and transferred to new plots to assess their unique N contribution, this approach ignores their belowground contribution, which can be a substantial reservoir of N (Reeves, 1994; Arcand et al., 2013). However, by swapping ^{15}N-labelled and unlabeled cover crop shoots reciprocally between paired plots, ^{15}N from cover crop shoots and the belowground pool (soil and cover crop roots) can be tracked separately. This novel approach has been used to separately track the aboveground and belowground contributions of annual crop residues (corn, soy, and wheat) to the subsequent crop (Arcand et al., 2014; Taveira et al., 2020), but it has not been used for cover crop residues. For cover crop mixtures that contain both winter-killed and winter-hardy species with different termination dates, there would be a need for multiple swapping events.

I applied ^{15}N tracer to the plots of a multi-year field experiment with the objectives of 1) assessing the extent to which cover crops increase the retention of soluble N present in the soil following the harvest of the grain crop, and 2) assessing the extent to which cover crops increase nitrogen transfer to the subsequent corn crop. Both cover crop monocultures (oats or red clover) and mixtures (3-way or 10-way mixtures) were compared, and for winter-hardy species I compared the effects of both fall and spring termination. Overall, I predicted cover crops would increase retention of the added ^{15}N and increase transfer of the added ^{15}N to the subsequent corn crop compared to the no cover crop control for both the aboveground and belowground ^{15}N tracer plots. Furthermore, based on the hypothesis that species complementarity increases total N immobilization (i.e., via variation in the timing of cover crop N uptake and residue decomposition), I predicted that cover crop mixtures would increase ^{15}N immobilization and the transfer of ^{15}N to corn more than cover crop monocultures. In addition, based on the hypothesis that spring-terminated and winter-hardy cover crops increase the synchrony of N release and crop N demand, I predicted treatments with winter-hardy cover crops terminated in the spring would increase ^{15}N immobilization and ^{15}N transfer more than the treatments with winter-hardy cover crops terminated in the fall.

2.1 Methods

2.1.1 Site Description and Agronomic Details

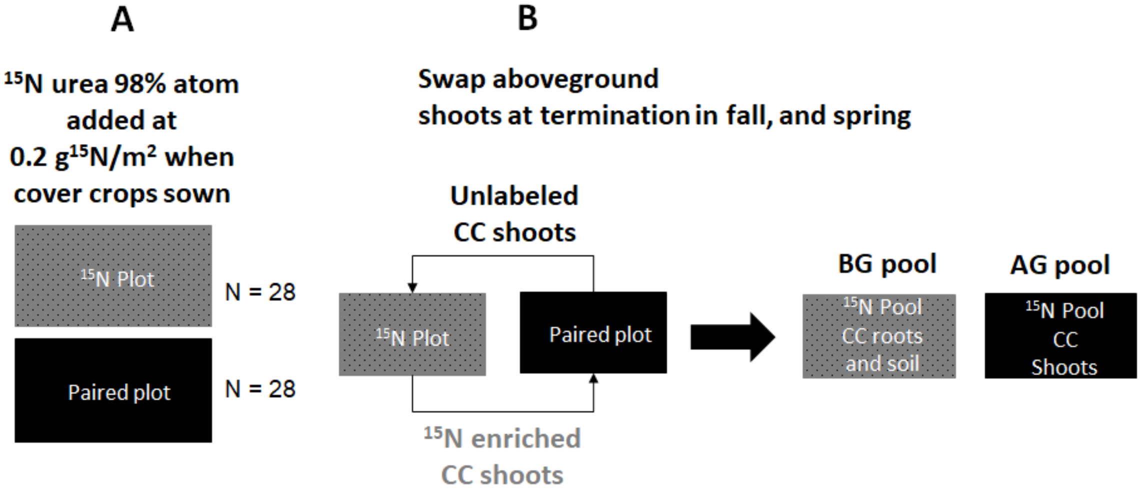
I conducted a ^{15}N tracer study in 2019 and repeated it again in 2020 by establishing subplots within the main plots of a long-term cover crop experiment (LTCC) established in 2017 at the University of Guelph's Elora Research station (43°38'25.6"N; 80°24'36.4"W) in Ontario, Canada. The goals of the LTCC are to address how different fertilization and tillage methods affect cover crop biomass production and grain yield, while my experiment was conducted within a sub-set of the plots of this larger experiment. The soil at the site is a Woolwich silt loam, which is classified as a Grey Brown Luvisol (CSSC 1998) and Albic Luvisol (FAO 2014). The soil was comprised of 16% clay, 44% silt, and 40% sand, with 0.213 g kg^{-1} of organic carbon (Munkholm et al., 2016). The main plots of the experiment (3.1 m × 7.6 m) were arranged in a multi-split plot randomized block design, with four replications per cover crop treatment embedded within the grain crop rotation (simultaneously corn-soybean-wheat) (Table 2-1). A corn-soybean-wheat rotation with each phase of the rotation present each year was used. Cover crops were sown and were seeded (see seeding rates in Table 2-1) in rows using a seed driller in the rotations after the harvest of the winter wheat crop (30 August 2019 and 12 August 2020), except red clover, which was under-seeded to winter-wheat in the spring (11 March 2019 and 21 May 2020). Five cover crop treatments were studied, and they comprised of either 1) a no cover crop (control) treatment, 2) an oat monoculture, 3) a red clover monoculture, 4) a 3-way species mixture or 5) a 10-way species mixture; the mixtures included both N scavenger species and N contributors (i.e., legumes), and they included both winter-killed and winter-hardy species (Table 2-1). Mean annual precipitation for the closest weather station (approximately 10 km distance) was 1012 mm and mean annual air temperature was 7.9 °C (based on 1981 – 2010 climate normals; Environment and Climate Change Canada, 2022).

Table 2-1. Cover crop species, seeding rate, N usage, phenology, and associated treatments (M = monoculture, 3W = 3 Way, 10W = 10 Way)

Cover crop	**Seeding Rate (Kg ha^{-1})**	**N usage**	**Phenology**	**Treatment**
Red clover (*Trifolium pratense*)	20	N_2 fixing	Winter-hardy	M
Oat (*Avena sativa*)	M50-3W16-10W5	N scavenging	Winter-killed	M, 3W,10W
Crimson clover (*Trifolium incarnatum)*	3W6-10W2	N_2 fixing	Winter-killed	3W,10W
Oilseed radish (*Raphanus sativus*)	3	N scavenging	Winter-killed	3W
Winter pea (*Pisum sativum*)	5	N_2 fixing	Winter-hardy	10W
Hairy vetch (*Vicia villosa*)	3	N_2 fixing	Winter-hardy	10W
Cereal rye *(Secale cereale)*	5	N scavenging	Winter-hardy	10W
Sudan grass (*Sorghum bicolor x var. sudanese*)	3	N scavenging	Winter-killed	10W
Buckwheat (*Fagopyrum esculentum*)	5	N scavenging	Winter-killed	10W
Daikon (*Raphanus sativus var. longipinnatus*)	1	N scavenging	Winter-killed	10W
Turnip (*Brassica rapa*)	0.5	N scavenging	Winter-killed	10W
Sunflower (*Helianthus annuus*)	0.5	N scavenging	Winter-killed	10W

2.1.2 ^{15}N tracer addition and residue exchanges

To quantify the transfer of N from the aboveground (AG) cover crop residues and the belowground (BG) pool (i.e., cover crop roots and soil) into the subsequent corn crop, I added ^{15}N tracer (98% atom ^{15}N urea at 2 kg ^{15}N ha^{-1}: <2% of the annual N fertilizer application) to 1 m^2 (150 cm × 67 cm) plots (sub-plots of the main long-term experimental plots that contained winter-wheat residues). The ^{15}N tracer was added following sowing of the cover crops in late summer 2019 (6 September), and into a new set of sub-plot for the second year study in 2020 (21 August), in the respective years preceding the 2020 and 2021 corn rotations. I used a watering can to add the ^{15}N urea dissolved in 2 L distilled water to each labelled sub-plot, and each of the latter was paired with an un labelled sub-plot that received an equivalent amount of unlabeled urea and water. By swapping the aboveground residues between these plots, the ^{15}N contributions of the AG and BG pools could be tracked separately (Figure 2-1). For the treatments with winter-killed cover crops only (oats and the 3-way mixture) and the no cover crop control, I established one pair of subplots within each main plot, while for cover crop treatments that included at least one winter-hardy species (red clover and the 10-way mixture), I established two pairs of subplots within each main plot, thus allowing for the exchange of shoots in both the fall and spring (Figure 2-1).



Winter-killed treatments 1 paired microplots (swapped in fall)
Winter-hardy treatments 2 paired microplots (swapped in fall + spring)

Figure 2-1: A) ^{15}N-Enriched urea was added to 28 microplots (1 m^2) when cover crops (CC) were sown in late August, and the remaining unlabeled paired plots were fertilized with unlabeled urea. B) Reciprocal transfer of aboveground cover crop residues: ^{15}N enriched aboveground (AG) tissues were transferred to the unlabeled paired plots at termination (fall or spring), while the plots with ^{15}N tracer remaining in the belowground (BG) pool (soil and cover crop roots) received unlabeled aboveground residues. Cover crops winter-killed (1 paired plot) by frost were sub-sampled and aboveground (AG) tissue swapped with paired sub-plots in early November 2019 and 2020 prior to the first major frost. Winter-hardy species (2 paired plots) were sub-sampled and swapped in fall at the same time as the winter-killed treatments and again in separate paired plots in late April 2020 and 2021.

I exchanged the shoots between the ^{15}N-labelled and unlabeled plots at the time of cover crop termination, with the cover crop shoots clipped using hedge trimmers. For all cover crop treatments, I exchanged cover crop shoot material for one pair of plots in early November (cover crops terminated in fall displayed reduced regrowth visually in the spring), immediately prior to the first major frost event. For the treatments that contained winter-hardy cover crops (i.e., the red clover monoculture and the 10-way mixture), I also exchanged shoot material in April for the second pair of plots, immediately before the cover crop was terminated using a broad-spectrum herbicide (glyphosate) and approximately two weeks prior to corn planting. Before the shoot material was transferred between plots, the bulk wet plant material was weighed, with subsamples (~10 – 30 g fresh weight) collected for drying and conversion of wet mass to dry mass for the subsequent ^{15}N retention calculations. Plastic netting was placed on top of each plot to hold the transferred shoot material in place. Soil cores (2 cm diameter, 0-15 and 0-30 cm depth) also were collected within the cover crops rows (including soil, and roots) at this time, and soil and plant tissue samples were collected from areas that did not receive ^{15}N to establish baselines for natural ^{15}N abundance. All plant material and soil sub-samples were dried at 60 °C.

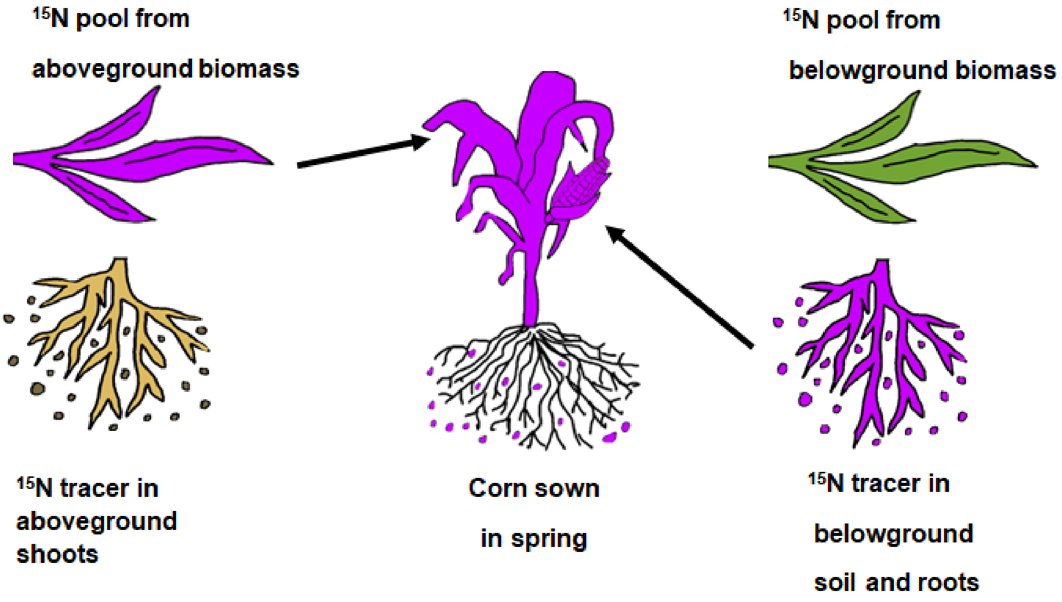


Figure 2-2: A) ^{15}N pool from aboveground (AG) cover crop shoots B) ^{15}N pool from belowground (BG) soil and cover crop roots. Arrows indicate the pool of ^{15}N in corn tissue and soil at harvest.

2.1.3 Corn establishment and harvest

Site management consisted of a strip-till/no-till rotation with strip- tilling occurring in years when corn was harvested after cover crops were terminated in the spring. The plots were strip tilled (April 7 in 2020 and April 11 in 2021) to a depth of 10-12 cm using a Remlinger, 4 rows strip-tiller to prepare the seedbed for the subsequent corn crop. Corn (var. DKC38-55RIB) was seeded using a 4-row planter (30”) (MaxEmergePlus VacuMeter) a week after strip tillage, maintaining 76 cm row spacing and targeting 76000 stalks ha^{-1}. The fertilization rate was 150 kg N ha^{-1}, which was applied as a starter at the time of seeding (30 kg N ha^{-1}) and as urea ammonium nitrate (UAN) (120 kg N ha^{-1}) a month later.

2.1.4 Corn harvest

In late September of both years, corn residue (i.e., stover – including the stalks, leaves, and cobs) and grains were harvested from the subplots, dried at 60 °C for 48 h and weighed (kernels were removed from the cobs after drying). The residues were chopped and mixed, and subsamples of 30 – 50 g each of corn residue and grains were collected. Soil cores (2 cm diameter) were collected from 0-15 cm soil depth during harvest and dried at 60 °C. Natural abundance soils sores were taken from outside the plots at a depth of 0-15 cm.

2.1.5 Plant tissue and soil ^{15}N analyses

Plant tissue samples labelled with ^{15}N, which included cover crop aboveground shoots taken at termination (in fall, and spring), and corn grain at harvest, were chopped into fragments < 2 mm using a Wiley mill (Model 3383-L10, Thomas Scientific, New Jersey, USA). I then ground subsamples of the milled plant material (~ 5 g) to a powder using a ball mill (Sample Prep Model 2000 Geno/Grinder, SPEX, New Jersey, US), with the samples shaken at 800 rpm for 2 minutes. I then weighed subsamples of the powdered plant tissue (1–4 mg) into tin capsules for isotopic analysis. I sieved the soil samples (< 2 mm), ground them using the ball mill, then weighed 18-23 mg into tin capsules for isotopic analysis. Isotopic analyses were conducted by the Saskatchewan Isotope Laboratory at the University of Saskatchewan using a gas chromatography-isotope ratio mass spectrometer (Thermo Scientific Delta V) coupled with an elemental analyzer (Costech ECS4010).

2.1.6 Calculations for determining ^{15}N recovery derived from the added tracer, and recovery efficiency of ^{15}N derived from cover crop tissues

All calculations were performed for plots that received ^{15}N-labelled aboveground shoot biomass (AG) only and for the plots that received unlabeled shoot material, and thus only contributed added ^{15}N from the belowground pool (BG) (Figure 2-2). Excess ^{15}N (i.e., ^{15}N derived from the tracer addition) was calculated by subtracting naturally occurring ^{15}N (determined for samples from unlabeled plots) from the total ^{15}N of the ^{15}N-labelled samples.

First, I calculated the nitrogen recovery efficiency derived from the tracer fertilizer (NREdfF) for the AG and BG pools at cover crop termination, and the corn grain, corn stover and soil (0-15 cm depth) at harvest, in a similar manner as Taveira et al., 2020. Then, I calculated the nitrogen recovery efficiency derived from the AG and BG cover crop pools (NREdfCC) for corn grain. For NREdfF, I expressed the efficiency of ^{15}N tracer recovered in the AG pool from cover crop shoots, and the BG pool from cover crop roots and soil at termination relative to the proportion of added tracer to each plot.

To calculate, the proportion of ^{15}N derived from the tracer relative to that applied in urea ($NdfF_{x\_i}$, %), the following equation was used:

$$NdfF_{x\_i}\,(\%) = \frac{^{15}N\ atom\%\ excess\ in\ sample\_x}{^{15}N\ atom\%\ excess\ in\ tracer} \times 100 \qquad (1)$$

where x is either cover crop (CC), corn or soil, and i refers to AG or BG. For example, $NdfF_{CC\_AG}$ represents the proportion of ^{15}N derived from tracer in AG plots recovered in the AG cover crop biomass at time of termination, while $NdfF_{Soil\_BG}$ represents the proportion of ^{15}N derived from tracer in the BG plots recovered in the soil at the time of corn harvest.

Then the total amount of tracer-^{15}N ($TNdfF_{x\_i}$, g N m^{-2}) in samples was calculated based on NdfFx_i and the total N content of the sample (g N m^{-2}):

$$TNdfF_{x\_i} = \frac{NdfFx\_i}{100} \times total\ N\ in\ sample \tag{2}$$

This information was then used to calculate the ^{15}N recovery efficiency of tracer ^{15}N in samples (*NREdfFx_i* , %) by scaling the total amount of tracer-^{15}N in the sample (g N m^{-2}) by the amount of ^{15}N tracer applied (g N m^{-2}):

$$NREdfF_{x\_i} = \frac{TNdfFx\_i\ in\ sample}{^{15}N\ tracer\ applied} \times 100 \tag{3}$$

To calculate NREdfCC, I used a similar approach as in eqn. 1-3, but expressed the ^{15}N in the corn grain at harvest as a percent of the excess ^{15}N in the AG and BG pools (i.e., the transfer efficiency from these pools to the corn):

$$NdfCC_{x} = \frac{^{15}N\ atom\%\ excess\ in\ corn\ grain}{^{15}N\ atom\%\ excess\ in\ pool\_x} \times 100 \tag{4}$$

$$TNdfCC_{x}) = \frac{NdfrCCx}{100} \times total\ grain\ N \tag{5}$$

$$NREdfCC_{x} = \frac{TNdfCCx}{TN\ in\ pool\_x} \times 100 \tag{6}$$

Where x is the AG or BG pool, $NdfCC_x$ (%) is the proportion of ^{15}N derived in corn grain derived from the AG (cover crop shoots) or BG (cover crop roots + soil), total grain N is the amount of N in corn grain (g N m^{-2}), $TNdfCC_x$ is the total amount of ^{15}N derived from the AG or BG pools (g N m^{-2}), and $NREdfCC_x$ (%) is the ^{15}N recovery or transfer efficiency of the ^{15}N for the AG or BG pools into the corn grain, i.e. ^{15}N amount in grain expressed as proportion of total N in each pool.

2.1.7 Statistical analyses

I assessed treatments effects on ^{15}N recovery, total N, , and bulk biomass in aboveground tissue (cover crop shoots, corn stover and grain) and the belowground pool (soil and cover crop roots) for each year independently using one-way ANOVA with block added as a random factor, followed by paired t-tests. All statistical analyses were conducted using JMP version 14.1 (SAS Institute, Cary, North Carolina, USA).

2.2 Results

2.2.1 Climate and environmental conditions

Each year of the experiment started when the cover crops were sown in Aug. and finished the following Oct. when corn was harvested (Figure 2-3). Mean air temperature in the fall (Sept. - Nov.) was 1.1 °C cooler in year 1 than in year 2 (10.2 °C vs. 11.3 °C), whereas mean monthly precipitation was similar between years (76.3 mm vs 67.1 mm) (Figure 2-3). Mean air temperatures over winter (Dec. – Mar.) were similar between years (-2.8 °C vs. -3.6 °C) but mean monthly precipitation in year 1 (74.7 mm) was double that of year 2 (35.8 mm) during this period (Figure 2-3). Mean spring temperature (Mar. - May) was 1.5 °C cooler in year 1 than in year 2 (7.4 °C vs. 8.9 °C), but mean monthly precipitation was similar between years during this time (46.0 mm vs. 42.9 mm) (Figure 3). Mean air temperatures over the corn growing season (May to Oct.) were 1 °C cooler in year 1 than in year 2 (15.1 °C vs. 16.1 °C) this could explain the increased cover crop growth in year 2 during the fall, and mean monthly precipitation was slightly lower in year 1 than in year 2 (62.5 mm and 76.4 mm) (Figure 2-3).

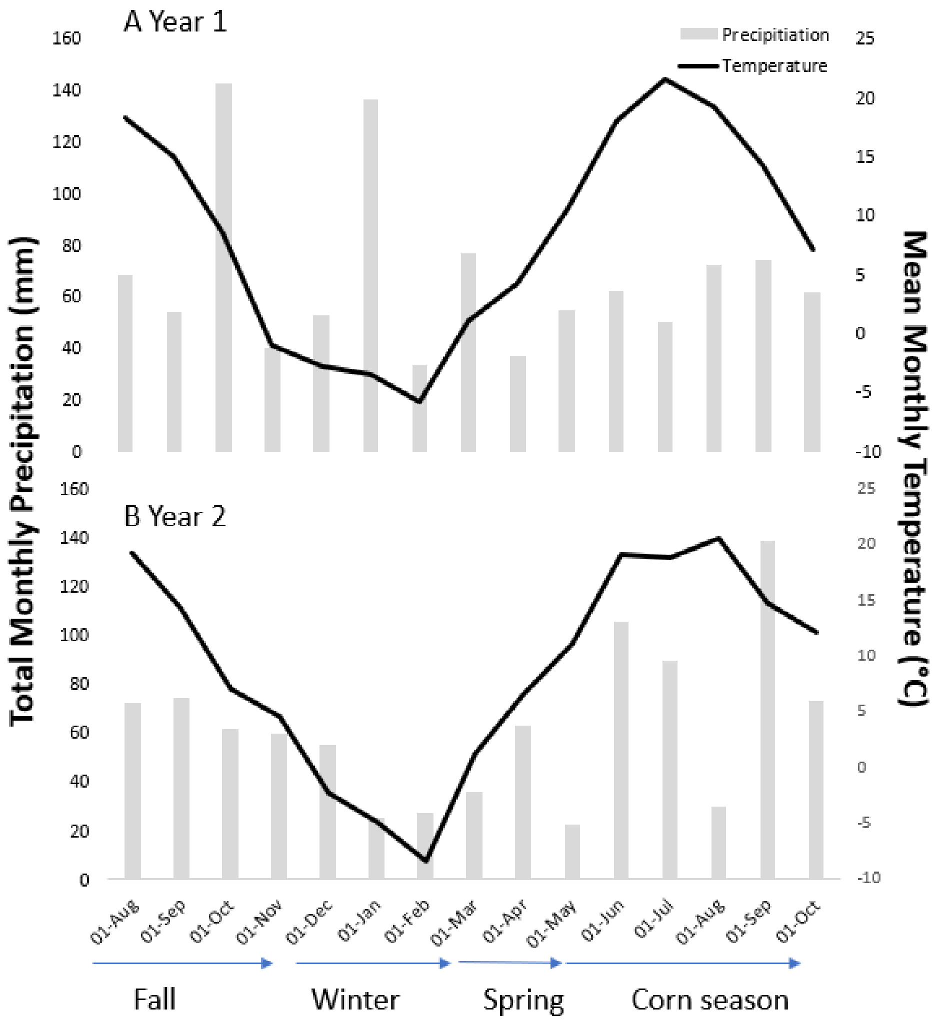
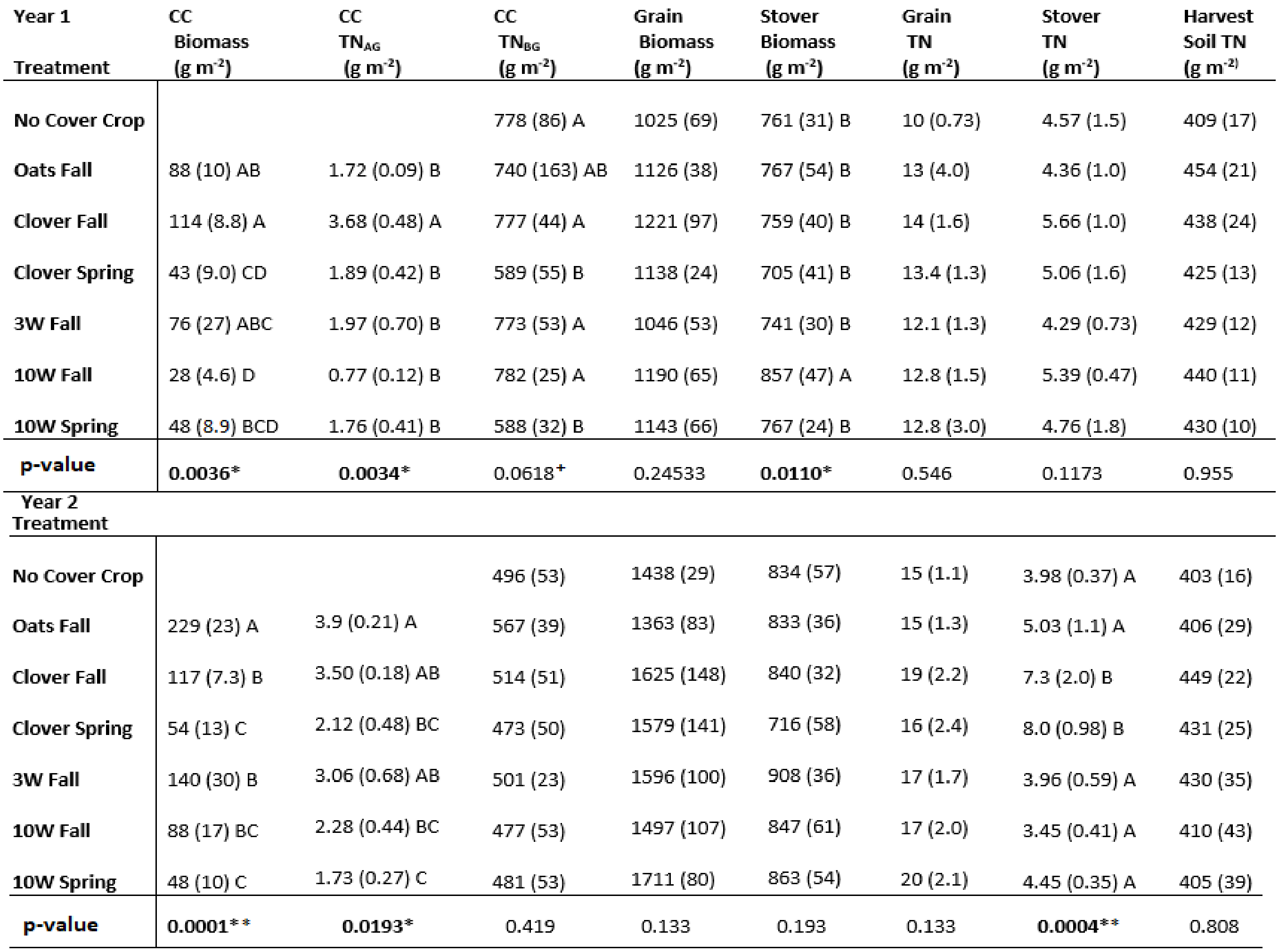


Figure 2-3. Total monthly precipitation (left y-axis: bars) and mean monthly temperature (right y-axis: line) from Aug. to Oct., showing fall, winter, and spring, and the corn growing season for A) year 1 (2019-2020) and b) year 2 (2020-2021).

2.2.2 Cover crop shoot biomass

Shoot biomass differed significantly between cover crop treatments in both years of study. In the fall of year 1, the shoot biomasses (AG) of red clover (114 g m^{-2}), the oat monoculture (88 g m^{-2}), and the 3-way mixture (76 g m^{-2}) were the highest, followed by the fall 10-way mixture (28 g m^{-2}) (Table 2-2). In the fall of year 2, oats (229 g m^{-2}) were highest, followed by the 3-way mixture (140 g m^{-2}), red clover (117 g m^{-2}) and the 10-way fall mixture (87 g m^{-2}) (Table 2-2); with the exception of red clover, AG shoot biomass was 2-3 times higher in year 2 than in year 1 in the fall terminated cover crops (it was noted that temperatures were warmer in year 2 during cover crop growth time). In both years, winter-hardy cover crops terminated in the spring had less than half the shoot mass as those terminated in the fall (48 g m^{-2} vs. 110 g m^{-2}, on average) (Table 2-2).

Table 2-2. Means (standard errors in parentheses) of total cover crop (CC) shoot biomass and CC total nitrogen in aboveground (CC TN$_{ag}$) and belowground (CC TN$_{Bg}$) (soil +roots) at the time of CC termination in fall or spring; grain yield and stover biomass, grain yield and stover total nitrogen (TN), and TN in soil at harvest for year 1 and year 2. P values from a one-way ANOVA are listed below. *, ** indicates significance $p < 0.05$, and $p < 0.001$ respectively. + indicates marginal significance. Different letters denote significant differences between treatment groups according topaired t-tests. Fall or Spring = termination date.



Year 1 Treatment	**CC Biomass (g m^{-2})**	**CC TN$_{AG}$ (g m^{-2})**	**CC TN$_{BG}$ (g m^{-2})**	**Grain Biomass (g m^{-2})**	**Stover Biomass (g m^{-2})**	**Grain TN (g m^{-2})**	**Stover TN (g m^{-2})**	**Harvest Soil TN (g m^{-2})**
No Cover Crop			778 (86) A	1025 (69)	761 (31) B	10 (0.73)	4.57 (1.5)	409 (17)
Oats Fall	88 (10) AB	1.72 (0.09) B	740 (163) AB	1126 (38)	767 (54) B	13 (4.0)	4.36 (1.0)	454 (21)
Clover Fall	114 (8.8) A	3.68 (0.48) A	777 (44) A	1221 (97)	759 (40) B	14 (1.6)	5.66 (1.0)	438 (24)
Clover Spring	43 (9.0) CD	1.89 (0.42) B	589 (55) B	1138 (24)	705 (41) B	13.4 (1.3)	5.06 (1.6)	425 (13)
3W Fall	76 (27) ABC	1.97 (0.70) B	773 (53) A	1046 (53)	741 (30) B	12.1 (1.3)	4.29 (0.73)	429 (12)
10W Fall	28 (4.6) D	0.77 (0.12) B	782 (25) A	1190 (65)	857 (47) A	12.8 (1.5)	5.39 (0.47)	440 (11)
10W Spring	48 (8.9) BCD	1.76 (0.41) B	588 (32) B	1143 (66)	767 (24) B	12.8 (3.0)	4.76 (1.8)	430 (10)
p-value	**0.0036***	**0.0034***	0.0618^{+}	0.24533	**0.0110***	0.546	0.1173	0.955
Year 2 Treatment								
No Cover Crop			496 (53)	1438 (29)	834 (57)	15 (1.1)	3.98 (0.37) A	403 (16)
Oats Fall	229 (23) A	3.9 (0.21) A	567 (39)	1363 (83)	833 (36)	15 (1.3)	5.03 (1.1) A	406 (29)
Clover Fall	117 (7.3) B	3.50 (0.18) AB	514 (51)	1625 (148)	840 (32)	19 (2.2)	7.3 (2.0) B	449 (22)
Clover Spring	54 (13) C	2.12 (0.48) BC	473 (50)	1579 (141)	716 (58)	16 (2.4)	8.0 (0.98) B	431 (25)
3W Fall	140 (30) B	3.06 (0.68) AB	501 (23)	1596 (100)	908 (36)	17 (1.7)	3.96 (0.59) A	430 (35)
10W Fall	88 (17) BC	2.28 (0.44) BC	477 (53)	1497 (107)	847 (61)	17 (2.0)	3.45 (0.41) A	410 (43)
10W Spring	48 (10) C	1.73 (0.27) C	481 (53)	1711 (80)	863 (54)	20 (2.1)	4.45 (0.35) A	405 (39)
p-value	**0.0001****	**0.0193***	0.419	0.133	0.193	0.133	**0.0004****	0.808

2.2.3 Total nitrogen in cover crop shoots and the belowground pool

Cover crop nitrogen in AG and the BG pool differed between treatments in both years of study. In the fall of year 1, total N in the shoots of red clover (3.7 N g m^{-2}) was approximately double that of the other treatments (average of 1.6 N g m^{-2}) (Table 2-2). In year 2, the total N of oats (3.9 N g m^{-2}) and red clover (3.5 N g m^{-2}) were highest, and 10-way spring had the lowest N (1.84 N g m^{-2}), while average total N was higher in year 2 (2.8 N g m^{-2}) than in year 1 (2.0 N g m^{-2}) for all treatments related to the increased productivity of all the treatments that had oats (Table 2-2). In both year 1 and 2, fall terminated cover crops (2.6 N g m^{-2}) contained more total N than spring terminated cover crops (1.9 N g m^{-2}). On average, total BG N in year 1 was higher in the fall (502 N g m^{-2}) than in spring (393 N g m^{-2}), and there were no significant differences in N among treatments in year 2 (Table 2-2).

2.2.4 Corn grain yield, stover biomass and total N

Average corn grain yields were 1127 g m^{-2} and 1544 g m^{-2} in years 1 and 2, respectively, and there were no significant differences among treatments in either year (Table 2-2). Similarly, total N was lower in year 1 (12.6 N g m^{-2}) than in year 2 (17.2 N g m^{-2}), and there were no significant treatment effects (Table 2-2). Stover biomass and total N followed similar trends, although stover biomass was significantly higher for corn following the 10-way fall mixture treatment than for the other treatments in year 1, and clover increased stover N more than the other treatments in year 2 (Table 2-2).

2.2.5 Recovery efficiency of nitrogen tracer added to the cover crops

In year 1, the recovery efficiency of added ^{15}N tracer in aboveground cover crop biomass ($NREdfF_{CC\_AG}$) was significantly lower in the 10-way mixtures for both fall and spring (1.3%) than the other treatments (average of 3.8%) (Figure 2-4a). BG recovery efficiency of the added ^{15}N ($NREdfF_{CC\_BG}$) was highest in the 3-way mixture treatment (53%), which was approximately two and a half times higher than in the no cover crop control (21%) (Figure 2-4a). In year 2, recovery efficiency of the added ^{15}N in AG was highest for oats

(14%) and the 3-way mixture treatment terminated in the fall (9%), and lowest for the red clover and 10-way mixture treatments terminated in the spring (2.5% and 1.9%, respectively) (Figure 2-4b). Belowground recovery efficiency of the added ^{15}N was highly variable within treatments, and thus did not differ significantly among treatments, and was 40% on average (Figure 2-4b).

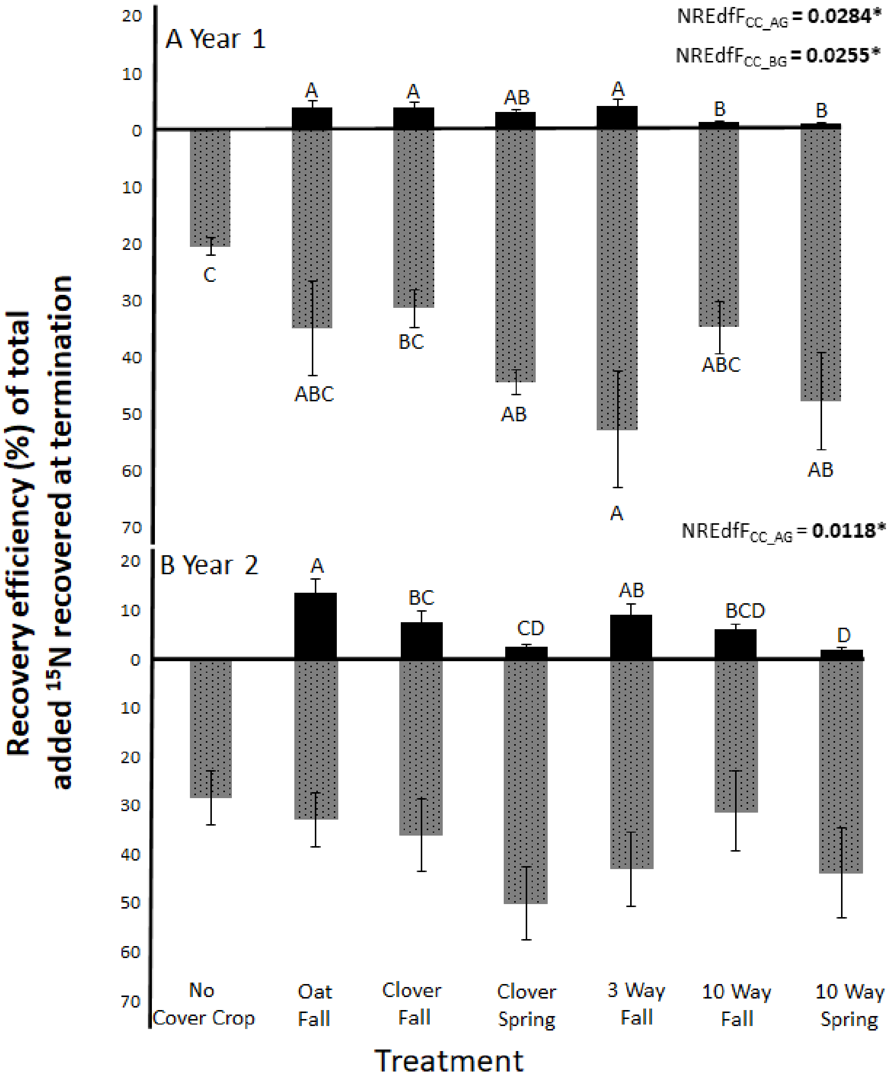


Figure 2-4. Average percent of total added ^{15}N recovered in aboveground (dark bars) and belowground pools (grey bars) in year 1 (A) and year 2 (B) at cover crop (CC) termination (lines above the bars show standard error). Aboveground (AG) refers to plots that had ^{15}N-labelled shoots exchanged onto them, while belowground

(BG) refers to plots that had ^{15}N-labelled shoots exchanged off them. Different letters denote significant differences between treatment groups $P < 0.05$, n=4.

2.2.6 Total recovery of ^{15}N tracer in corn and soil at harvest

In both years, the total recovery of the added ^{15}N was less than 10% in corn ($NREdfF_{corn\_AG}$+ $NREdfF_{corn\_BG}$) (Figure 2-5), with 75% of the latter recovered in the grain and 25% recovered in the stover, on average. The recovery of ^{15}N from AG plots treated with ^{15}N-labelled cover crop shoots was less than 1% ($NREdfF_{corn\_AG}$), whereas the recovery of ^{15}N from the plots that retained the BG ^{15}N label was less than 5% ($NREdfF_{corn\_BG}$), on average (Figure 2-5). In year 1, the total recovery of the added ^{15}N in corn ($NREdfF_{corn\_AG}$ + $NREdfF_{corn\_BG}$) was significantly higher in both the red clover treatment terminated in fall (6.1%) and the treatment terminated in spring (7.3%) than the other treatments, with more than double the recovery in the plots containing no cover crops (3.6%) (Figure 2-5a). Similarly, in year 2, both the fall (7.2%) and spring-terminated red clover treatments (6.5%) had the highest recovery of the added ^{15}N (i.e., $NREdfF_{AG+BG}$ in corn tissues). The no cover crop control (4.6%), oats (4.7%), 3-way (5.7%), and the 10-way terminated in fall treatments (5.0%) had the lowest total recoveries of the added ^{15}N in the corn (Figure 2-5b).

The recovery of added ^{15}N in soil at harvest ($NREdfF_{Soil}$) was higher in year 1 (ranging between 25% to 50%) than in year 2 (ranging between 18% to 42%) (Figure 2-5). In year 1, $NREdfF_{Soil}$ was highest (52%) for the red clover spring termination treatment, which was double that of the control plots that contained no cover crops (25%) (Figure 2-5a). In year 2, both the fall (42%) and spring termination (39%) red clover treatments had double the $NREdfF_{Soil}$ than the other cover crop treatments (19% on average) (Figure 2-5b).

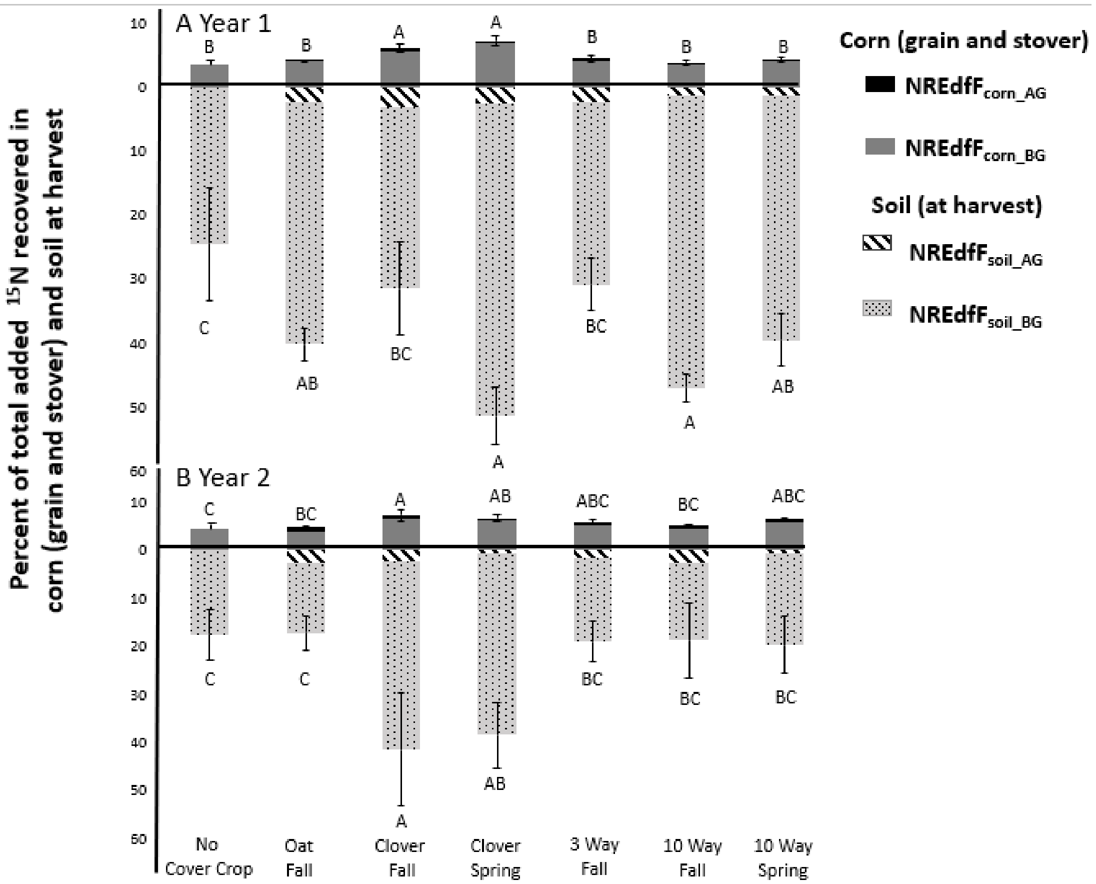


Figure 2-5. Average percent of total ^{15}N tracer recovered in corn (grain and stover) above zero, and soil below zero at harvest for AG (^{15}N pool from cover crop shoots) and BG (^{15}N pool from cover crop roots and soil at termination) in year 1 (A) and year 2 (B) (lines show standard error). For corn, the dark bars represent nitrogen recovery efficiency from the AG, whereas the grey bars represent the recovery from the BG pool. For soil at harvest, the dashed bar represents nitrogen recovery efficiency from the AG pool, whereas the dotted bar is the recovery from the BG pool. Different letters denote significant differences between treatment groups ($P < 0.05$, $n = 4$) for corn (total of bars > the zero line) and the soil (total of bars < The zero line).

2.2.7 Recovery efficiency of ^{15}N from cover crop to corn grain

In year 1, the percent of ^{15}N derived from cover crop shoots recovered in the corn grain ($NREdfCC_{AG}$) was highest for the 10-way mixture terminated in the spring (19.3%), followed by the 10-way mixture terminated in the fall (Figure. 2-6a). For the percent of ^{15}N derived from the BG pool recovered in the corn grain ($NREdfCC_{BG}$), the values ranged from 10 to 20%, but there were no significant differences among treatments (Figure 2-6a). In year 2, similar to year 1, the percent of ^{15}N derived from cover crop shoots recovered in the corn grain ($NREdfCC_{AG}$) was highest for the 10-way mixture terminated in the spring (17%) (Figure 2-6b). For the percent of ^{15}N derived from the BG pool recovered in the corn grain ($NREdfCC_{BG}$), the values ranged from 12% to 20%, but there were no significant differences among treatments (Figure 2-6b).

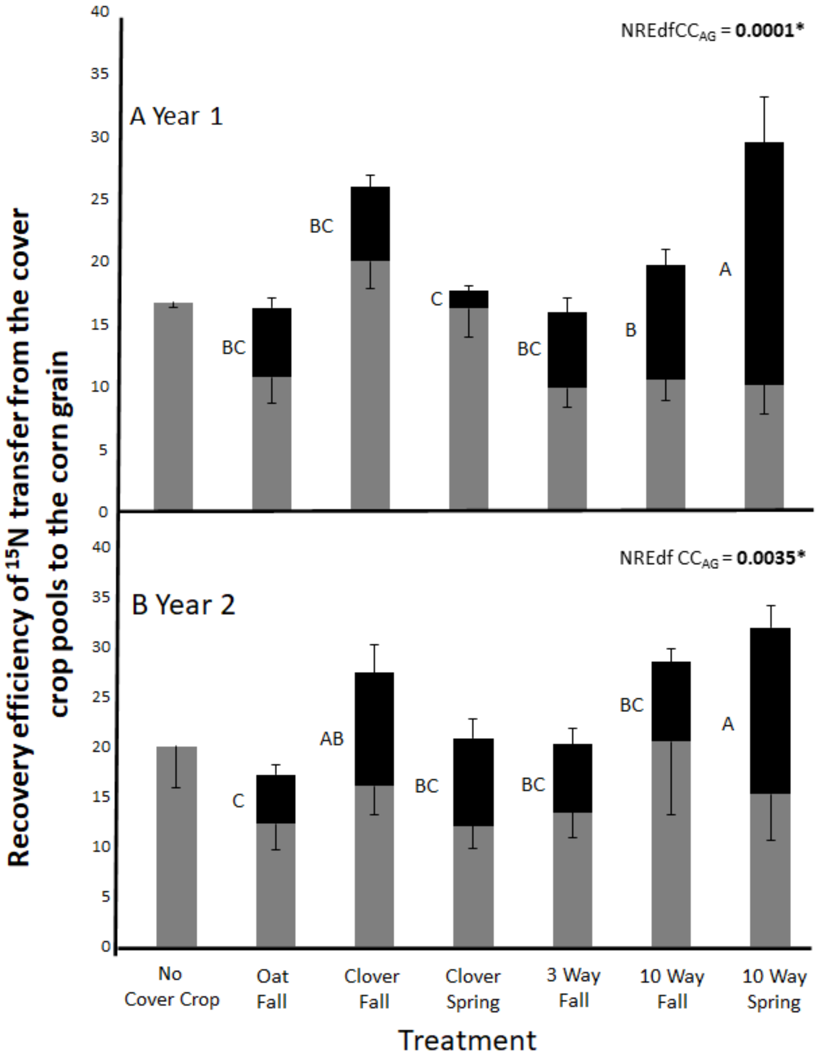


Figure 2-6. Recovery efficiencies of ^{15}N in the corn material at harvest derived from the respective AG and BG pools, expressed as a percent of the excess ^{15}N in the AG (black bars) and BG (grey bars) pools (i.e., the transfer efficiency from these pools to the corn) in (A) year 1 and (B) year 2. Different letters denote significant differences between treatment groups $P < 0.05$, n = 4 (lines show standard error).

2.3 Discussion

2.3.1 Recovery of the added ^{15}N at cover crop termination

Cover crops can improve N retention by scavenging and immobilizing residual soluble N from the soil following harvest of the grain crop (Holness et al., 2008; Tribouillois et al., 2018). Consistent with my prediction, the cover crop treatments increased retention of the added ^{15}N relative to the no cover crop control in most cases by 2-3 times, resulting in a total retention of 20-60% of the added ^{15}N. With the exception of oats in Year 2, the majority of this added retention was present in the belowground N pool, rather than in the cover crop shoots, which highlights the importance of accounting for the belowground contribution of cover crops to total N retention. While the excess shoot ^{15}N could be attributed to ^{15}N that was immobilized directly by the cover crops, the mechanism whereby the presence of cover crops increased retention of the belowground ^{15}N was less certain. Specifically, while N could have been immobilized in cover crop roots, alternatively, the presence of cover crops could have increased the retention of ^{15}N in the bulk soil through mechanisms such as altered soil microclimate, decreased soil drainage (resulting from reduced leaching of N), or increased microbial N immobilization (Steenwerth & Belina, 2008; Lapierre et al., 2022).

Cover crop establishment and growth can vary substantially in response to weather variability (Marcillo & Miguez, 2017), and in my study, variation between years in ^{15}N retention was associated with variation in cover crop biomass, which was generally higher in Year 2. ^{15}N retention in the no cover crop control also increased in Year 2, which could be explained by the control plots being partially colonized by volunteer wheat, which functioned as a cover crop. Variation in cover crop biomass also appeared to have an important influence on the relative strengths of monocultures versus cover crop mixtures in improving ^{15}N retention. Although the highest ^{15}N retention occurred for a cover crop mixture in each year, across all treatments, cover crop mixtures did not consistently exhibit higher ^{15}N retention than the monocultures, which was explained by the low total shoot biomass of some of the cover crop mixture treatments. In particular, the 10-way mixtures terminated in the fall had low total shoot biomass, which occurred because the successful species in the mixture did not adequately compensate (through increased growth) for the species that established poorly. Therefore, while it is anticipated that cover crop mixtures can improve the density and diversity of rooting systems (Amsili & Kaye, 2021), thus improving N retention, with a high number of cover crop species sown there may be an increased risk that some will not establish successfully.

As described above, winter-hardy cover crop species benefit from a spring regrowth phase to scavenge for soluble N released after spring thaw (Murrell et al., 2017; Kaye et al., 2019). Moreover, the decomposition of the spring regrowth can increase N mineralization in spring and summer (Melkonian et al., 2017), increasing the synchronization of N release with the N demand of the subsequent grain crop (White et al., 2016). In contrast, the N immobilized by fall-terminated cover crop crops is more prone to the effects of freeze-thaw cycles, which can increase leaching and trace gas losses in spring (Hadas et al., 2002). Consistent with these mechanisms, I observed a trend of higher ^{15}N retention in the spring-terminated treatments than in the fall-terminated treatments. However, given the relatively high variability in ^{15}N retention among treatment replicates, the differences between spring and fall terminated plots was not statistically significant. In addition, growers may avoid the practice of spring cover crop termination, given the potential for plants that escape termination to interfere with the growth of the subsequent grain crop (Alonso-Ayuso et al., 2014, 2020; Keene et al., 2017).

In addition to scavenging N, legumes can increase crop N availability by increasing N fixation (Tribouillois et al., 2016), and legumes are therefore often added to cover crop mixtures to reduce the C:N ratio of the cover crop residues (de Notaris et al., 2021). Although the analysis of ^{15}N tracer recovery in my study did not address this added source of N, the total N data revealed that the fall-terminated clover monoculture treatment resulted in a substantial additional N input in Year 1. However, with the poorer establishment and growth of the clover in Year 2, the residue N from clover did not exceed that of the other cover crop treatments in the second year.

2.3.2 Cover crop effect on ^{15}N recovery at corn harvest

The clover treatments significantly increased the amount of added ^{15}N recovered in the corn, with the majority of the recovery coming from the belowground pool. This result was consistent with those of both Arcand et al., 2014 and Taveira et al., 2020, who observed that the belowground N pool of crop residue contributed 2-18 times more N to the grain than the aboveground residue under field and glasshouse conditions, respectively. Such results may arise in part from asynchronies between N mineralization of cover crop shoots and N uptake by the grain crop (Crews & Peoples, 2005) caused by rapid decomposition of cover crop shoots compared to the longer belowground root turn-over rates (Holness et al., 2008). However, in my study, the total N transfer from the cover crop shoots to the corn grain was also fundamentally limited by the fact that the corn grain yield (> 1000 g m^{-2}) was far greater than the cover crop shoot biomass (typically 100 g m^{-2} or less).

Overall, the amount of added ^{15}N recovered in the soil at corn harvest (from 25 to 50%) was much higher than the recovery in the corn tissues (less than 10%), with the recovery for the clover treatment being as much as 25% higher than that of the no cover crop control. This value is consistent with the improvement in soil N retention reported for other cover crop systems (Garba et al., 2022), and with estimates of red clover contributing ~30-80 N kg ha^{-1} per year to soil (Coombs et al., 2017). A key question that remains is to what extent there may be legacy effects of these increases in soil N on crop nitrogen availability in future years (Arcand et al., 2013; Gabriel et al., 2016), and to what extent such increases could justify decreases in the amount of N fertilizer application from these potential N credits (Gaudin et al., 2015).

Among cover crop treatments, there were no significant differences among monocultures and cover crop mixtures regarding their effects on ^{15}N recovery in the corn. In the case of the 10-way mixture, the transfer of ^{15}N to the corn was equivalent to the other cover crop treatments, despite the low biomass of the 10-way mixture. This occurred as a result of 30% of the ^{15}N derived from the 10-way mixture being recovered in the corn, whereas this value was as low as 15% for other treatments. This result may be explained by the 10-way mixture containing winter rye, a highly productive winter-hardy species that is effective at scavenging residual N (Murrell et al., 2017). Surprisingly, the fall vs. spring termination dates for clover did not significantly affect the recovery of added ^{15}N in the corn, although the spring terminated clover did promote greater retention of ^{15}N in the belowground N pool at corn harvest in Year 1 compared to Year 2-.

2.4 Conclusions

Overall, while cover crops were effective at increasing the retention of soluble ^{15}N tracer added in the fall, only a small fraction of this tracer was ultimately transferred to the corn grain. Nevertheless, the high retention of the ^{15}N tracer in the belowground pool, even by corn harvest a year after the tracer addition, suggests that additional study is needed to track the cumulative effects of increased N retention by cover crops on soil fertility over the longer term. As for cover crop mixtures, although they may have a higher capacity for increased N retention in comparison with cover crop monocultures, they also may be prone to higher variability in establishment success if the successful species are not capable of filling in for those that establish poorly. Finally, regarding spring cover crop termination, while there may be concerns that plants escaping spring termination could interfere with grain crop production, my results indicated there may be benefits for increased N retention as they performed as well as fall terminated species.

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Chapter 3

3 Introduction

Cover crops can improve the sustainability of agroecosystems by scavenging and immobilizing soluble N from the soil when the grain crop is not present, thus increasing nitrogen (N) retention (Schipanski et al., 2014; Norberg & Aronsson, 2019). Leguminous cover crops also increase N inputs through fixation by as much as 30-150 kg ha^{-1}, potentially reducing the need for fertilizer application (Snapp et al., 2005). However, the success of cover crops in transferring N to the subsequent grain crop is contingent on the timing of N release from decomposing cover crop residues being adequately synchronized with N uptake by the grain crop (Crews & Peoples, 2005; Nevins et al., 2020). Cover crop phenology may influence this degree of synchronization with the timing of N uptake and decomposition of winter-killed species, which are terminated by freezing temperatures, potentially differing from those of winter-hardy species, which are terminated by herbicide or mechanically in the spring (Snapp et al., 2005; Fageria et al., 2007; Duiker, 2014; Balkcom et al., 2016). Specifically, winter-hardy cover crops can continue to take up N over winter and spring, potentially reducing nitrate leaching at these times, whereas winter-killed cover crops not only cease to take up N in early winter, but the N they take up in the fall may be mineralized earlier than that of winter-hardy cover crops and subsequently lost from the system (Rosecrance et al., 2000; Duiker, 2014).

Variability in winter conditions also can affect the survival of cover crops, the timing of N uptake and residue decomposition, which may further influence the efficiency of N transfer to the subsequent grain crop (Alonso-Ayuso et al., 2014; Balkcom et al., 2016; Ceglar et al., 2019). In northern temperate regions, overwintering plants are anticipated to experience increased winter soil temperature variability as a result of climate warming over the next century (Cleland et al., 2007; Kreyling, 2010). This is due in part to increased variability in air temperature, which can increase the frequency of mid-winter melt or false spring events (Gu et al., 2008; Zhu et al., 2019). In addition, an increase in mean winter air temperature can decrease snow cover, which can increase the frequency and intensity of soil freeze-thaw cycles in these regions, because snow insulates the soil from air, and air temperature will continue to drop substantially in temperature at night or during cold spells, even in warm winters (Feng et al., 2007; Mellander et al., 2007; Henry, 2008). Soil freeze-thaw cycles can disturb soil microbial activity and disrupt soil aggregates, thus altering soil carbon and N transformations (Feng et al., 2007; Bai et al., 2013). Freeze-thaw cycles also can decrease plant N uptake and increase plant root mortality (Malyshev & Henry, 2012; Campbell et al., 2014). Moreover, increased soil thawing can alter litter decomposition and increase N mineralization from labile soil organic material, increasing soil inorganic N leaching losses over winter (Yang et al., 2019) and promoting the release of N_2O, a potent greenhouse gas, by up to 6-9-fold in cover crop systems (Campbell et al., 2014; Ejack & Whalen, 2021). On balance, given these potential influences of changes in soil freezing on both overwintering plants and plant residues, the effectiveness of both winter-hardy and winter-killed crops in immobilizing soil N and transferring to the subsequent grain crop may be affected by variability in winter conditions, but the extent of these effects remains unclear (Vyn et al., 2000; Abalos et al., 2014; Lombardozzi et al., 2018; Pan et al., 2022).

I used ^{15}N tracer addition to quantify the extent to which cover crops can scavenge soil N in the fall and transfer it to corn (*Zea mays*) the following growing season. In addition, I incorporated a snow removal treatment to assess how increased soil and cover crop freezing would alter the transfer of ^{15}N. I examined these effects for red clover (*Trifolium pratense*, a legume) and winter rye (*Lolium perenne*, a grass), both winter-hardy cover crops that are slow to establish in the fall, but can continue to grow in the spring, as well as for crimson clover (*Trifolium incarnatum*, a legume) and oats (*Avena sativa*, a grass), which are both winter-killed, but exhibit vigorous growth in the fall (Duiker, 2014). Based on the hypothesis that snow removal would expose the cover crops, soil, and residues to more frequent and intense freezing, I predicted snow removal would decrease the amount of ^{15}N recovered in the corn, and that this reduction would be highest for the winter-hardy cover crops. Moreover, I predicted that while the legumes would increase total corn N available to the corn, this amount would be reduced by snow removal.

3.1 Methods

3.1.1 Site Description

The cover crop experiment was established at the Environmental Sciences Western field station (43°04’29’’N, 81°20’18’’W), located near Ilderton, Ontario, Canada. The experiment began in late summer 2020, and measurements were conducted over a year until fall 2021. The soil was characterized as a Bryanston silt loam, which is a Brunisolic Gray Brown Luvisol (Hagerty and Kingston 1992), with a mean pH of 7.5 (Stover and Henry 2020). Mean annual precipitation for the closest weather station (approximately 10 km distance) was 1012 mm and mean annual air temperature was 7.9 °C (based on 1981 – 2010 climate normals; Environment and Climate Change Canada, 2022).

3.1.2 Experimental Design

In 2020, I established forty plots (150 cm × 67 cm = 1 m^2, minimum distance of 100 cm between plots) in a section of the field station that had previously been planted with a crop rotation consisting of corn, soybean, and winter wheat. Winter wheat was planted (to provide ground cover until the cover crops were planted) in April 2020 and was terminated prior to sowing the cover crops late July 2020. The experiment used a factorial block design, with eight plots assigned to each cover crop treatment; half of the latter were assigned to a snow removal treatment for each cover crop (n=4), and the other half were designated as ambient snow plots (n=4) (Figure 3-1). Cover crops were sown by hand (grasses planted in rows, and clovers were broadcast) on 20 August 2020, organized into four monoculture treatments: 1) oats (*Avena sativa*), a winter-killed grass, 2) winter rye (*Secale cereale*), a winter-hardy grass, both planted at a density of 50 kg ha^{-1}, and 3) crimson clover (*Trifolium incarnatum*), a winter-killed legume and 4) red clover (*Trifolium pratense*), a winter-hardy legume, both seeded at 15 kg ha^{-1}. An additional treatment 5) control was included, which contained no cover crops, although scattered weed establishment occurred in these plots over fall, at a sparse density consistent with the surrounding agricultural fields. Following sowing of the cover crops, I used a watering can to add 98% atom ^{15}N urea (2 kg ^{15}N ha^{-1}; <2% of the annual N fertilizer application) dissolved in 2 L distilled water to each plot.

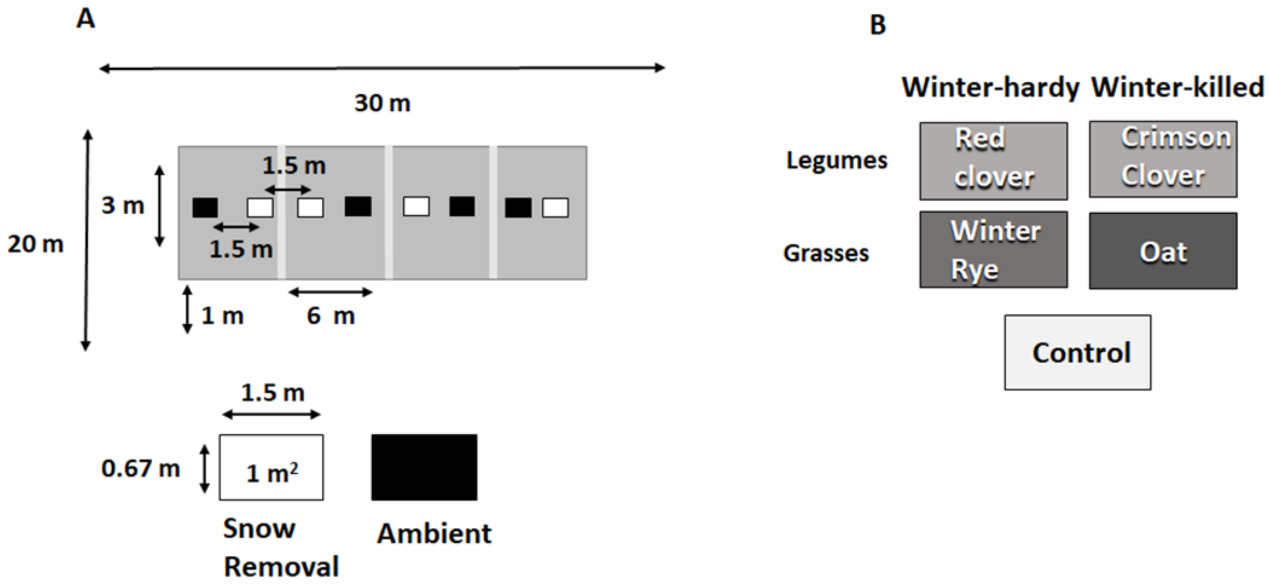

Figure 3-1. A) Depicts spacing and size of microplots and the layout of 1 of 5 possible blocks (A to E) with the large grey boxes indicating the area surrounding microplots. Each 1 m^2 plot was randomly assigned one of the 5 cover crop treatments in B, in addition to one of the 2 snow treatments ambient, or snow removal. Cover crops were placed into pairs (ambient snow or snow removal) randomly in a factorial block design. B) Cover crop treatments and phenology (winter-hardy or winter-killed) for the 40 microplots, n = 4 * 2 snow treatments (ambient/snow removal) * 5 cover crop treatments.

3.1.3 Snow removal

White plastic netting (1 cm mesh size, Protective Winter Wrap, Quest Plastics, Mississauga, ON, Canada) was placed over each plot in the late fall of 2020 (Dec 5 2020) to avoid damage to the soil and cover crops during shoveling. Following each major snow event (i.e., > 5 cm of snow accumulation), I removed snow from the snow removal plots with a shovel down to the depth of the plastic mesh. I removed snow nine times total, beginning on 8 Jan. 2021 and concluding on 18 Feb. 2021. I ceased removing snow prior to the end of winter to minimize potential snow removal effects on summer soil moisture. Snow removal effects on soil temperature were monitored for ten of the plots (5 ambient snow and 5 snow removal 1 per cover crop treatment) using LogTag, TRIX-8 (MicroDAQ, NH, U.S.A.) temperature sensors buried at 5 cm soil depth. The plastic mesh was removed 20 March 2021 to avoid interference with the regrowth of the winter-hardy cover crops. Freeze-thaw was calculated as the frequency in which temperatures went below, then above freezing between 1 January to 10 March. Average temperature was calculated based on means between 1 January to 10 March. Minimum soil temperature was calculated as the lowest recorded temperature between 1 January to 10 March.

3.1.4 Cover crop termination

The winter-killed cover crops were terminated in early winter by frost (Visually confirmed leaf dieback during the first snow removal event Jan 7, 2021). The winter-hardy cover crops (and weeds in the control plots) were terminated on 14 April 2021 using a broad-spectrum herbicide (glyphosate) that was not incorporated in the soil but sprayed onto the plant foliage. On 12 May, a second round of herbicide application was performed using a Dual 11 Magnum herbicide (S-metolachlor and R-enantiomer) (Syngenta, Arva, ON, CA), which was broadcast onto the soil to ensure the cover crop roots were sufficiently terminated.

3.1.5 Corn planting and harvest

On 12 May 2021, prior to planting, a starter fertilizer was applied to the area including and surrounding the plots at the following rates: 62.5 kg ha-^{1} N (Urea 46-0-0), 56 kg ha-^{1} phosphorus, and 55.5 kg ha-^{1} of potash (muriate K_2O). I gently disturbed the top 5 cm of soil to better incorporate the corn kernels. Corn was planted 13 May 2021 using a roundup ready field corn variety (DEKALB DKC48-56/NM5104BGLZ, ID 748W9J7JX, Bayer, Constantine, Michigan). The corn was planted over an area of 0.18 acres both inside and surrounding the microplots to prevent edge effects (1.5 m by 0.67 m) using an Almaco jab type hand planter at a depth of 6.5 cm, with a spacing of 23 cm between rows. In October 2021, the corn cobs were harvested by hand from each microplot. The cobs were dried at 60 °C for 72 h, then the grains were removed using a field sheller. The grains were weighed and mixed, then 100 g sub-samples were collected for subsequent N analyses.

3.1.6 Grain ^{15}N and total N analyses

I ground the grain samples to < 2 mm using a Wiley mill (Model 3383-L10, Thomas Scientific, New Jersey, USA), then further ground subsamples (~ 5 g) of the milled grain material to a powder using a ball mill (Sample Prep Model 2000 Geno/Grinder, SPEX, New Jersey, US), shaking at 800 rpm for 2 minutes. I then weighed subsamples of the powdered grain (1–4 mg) into tin capsules for isotopic analysis. Sub-samples of corn that were not part of the study were examined for natural abundance levels. Isotopic analyses were conducted by the Saskatchewan Isotope Laboratory at the University of Saskatchewan using a gas chromatography-isotope ratio mass spectrometer (Thermo Scientific Delta V) coupled with an elemental analyzer (Costech ECS4010).

3.1.7 Data and Statistical analyses

The efficiency of ^{15}N transfer to the corn grain was estimated by comparing the percent of ^{15}N tracer that was added at the time of cover crops being sown to the amount excess ^{15}N (i.e., in excess of naturally occurring ^{15}N) in the corn grain. The following equations were used:

$$NdfF_x\,(\%) = \frac{^{15}N\ atom\%\ excess\ in\ grain}{^{15}N\ atom\%\ excess\ in\ ^{15}N\ urea\ tracer} \times 100 \qquad (1)$$

$$TNdfF_x\,(g\ m^{-2}) = \frac{NdfFx}{100} \times total\ grain \qquad (2)$$

$$NREdfF_x\,(\%) = \frac{TNdfFx\ in\ grain}{^{15}N\ urea\ tracer\ applied} \times 100 \qquad (3)$$

where $NdfF_x$ is the proportion of N derived from tracer in corn grain, and where $TNdfF_x$ represents the total amount of tracer-^{15}N (g m^{-2}) in grain. *NREdfFx* is the ^{15}N recovery efficiency of tracer N (%) in corn grain.

I assessed treatments effects on *NREdfFx*, total grain N, and grain biomass using two-way ANOVAs with cover crop treatment and snow removal as fixed effects and block added as a random factor, followed by Tukey post hoc tests to measure differences between means.

All statistical analyses were conducted using JMP version 14.1 (SAS Institute, Cary, North Carolina, USA).

3.2 Results

3.2.1 Weather conditions

Weather conditions were reported from the London Airport station (43°01′59″N 81°09′04″W). After sowing the cover crops and through the fall (August- November 2020), mean air temperature was 12.7 °C and meanly monthly precipitation was 96 mm (Figure 3-2). Over winter (December 2020 to March 2021), mean air temperature was -1.7 °C and meanly monthly precipitation was 49 mm). During the spring (April to May 2021), mean air temperature was 10.5 °C and meanly monthly precipitation was 58 mm. For the corn growing season (May to October 2021), mean air temperature was 17.4 °C and meanly monthly precipitation was 113 mm.

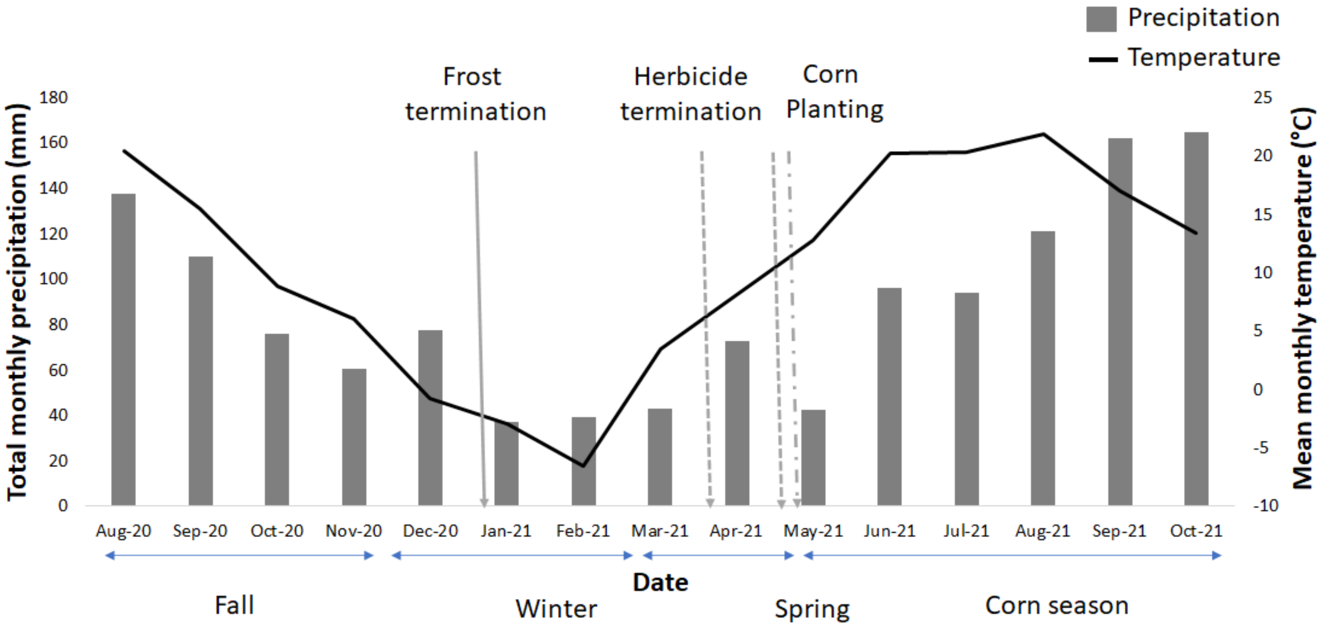

Figure 3-2. Total monthly precipitation (on left y-axis: bars) and mean monthly temperature (on right y-axis: line) between August 2020 until October 2021, showing the fall, winter, spring, and the corn growing season. Vertical lines indicate the approximate date of frost kill, the two dates of herbicide application and corn planting.

3.2.2 Snow removal effects on soil temperature

Snow removal reduced mean and minimum soil temperatures (1 January to 10 March) to 0.05 °C and -2.6 °C, respectively, compared to values of 0.4 °C and -0.1 °C for the ambient snow plots (Figure 3-3). The snow removal plots also experienced more than four times as many soil freeze-thaw cycles than the ambient snow plots (17 vs. 4). The intensity of freezing (and warming) during soil temperature cycles was typically increased by 1-2 °C in the control plots relative to cover crop plots (not shown).

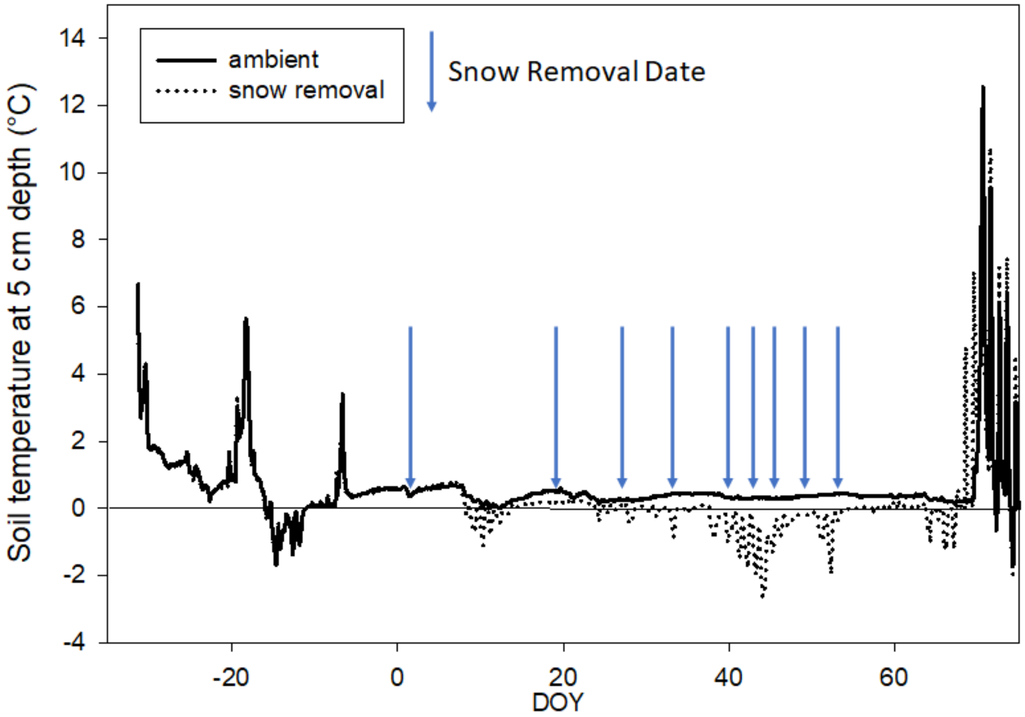

Figure 3-3. Hourly soil temperatures at a soil depth of 5 cm in ambient snow (sold black line) and snow removal (dotted black line) plots, averaged over all treatments (n=5) per snow removal treatment taken from a sub-set of plots. Arrows indicate the timing of snow removal events.

3.2.3 ^{15}N recovery in corn grain

There was a significant effect of cover crop treatment on ^{15}N recovery in corn grain (P=0.001), with *NREdfFx* almost double on average in cover crop treatments relative to the no cover crop control; however, there were no significant differences among the cover crop treatments (Figure 3-4). Snow removal also had a significant effect on ^{15}N recovery in corn grain (P=0.039), decreasing it on average relative to the ambient snow plots (Figure 3-4). There was no significant interaction between snow removal and cover crop treatment (P= 0.916).

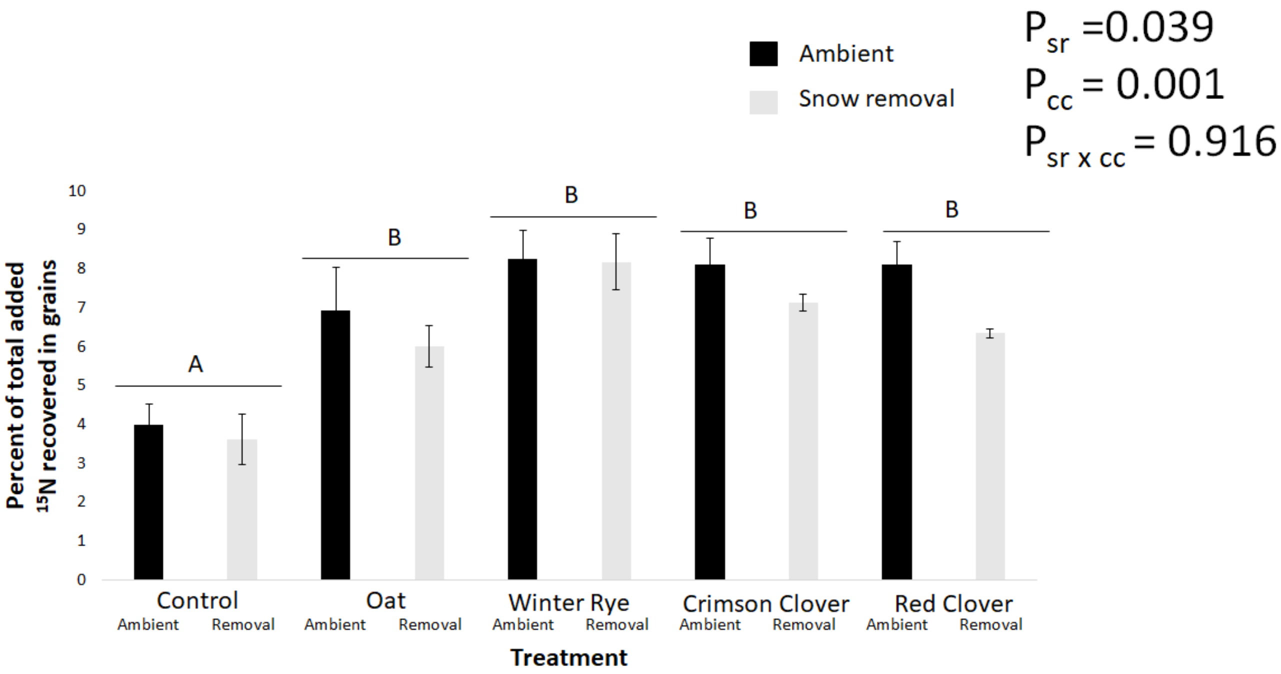

Figure 3-4. Mean percent (with standard error) of total added ^{15}N recovered in corn grain in ambient snow (black) and snow removal (grey) plots (n=4). Different letters denote significant differences between cover crop treatment groups (Tukey's HSD test, P < 0.05). P values indicate significance for the following: sr = snow removal and cc = cover crop.

3.2.4 Total N in corn grain

There was a significant effect of cover crop on total N in corn grain (P= 0.00155), with winter rye increasing corn grain N relative to oats and the no cover crop control (Figure 3-5). The reduction in total grain N with snow removal was not significant (P=0.182), nor was there a significant interaction between cover crop treatment and snow removal (P=0.98).

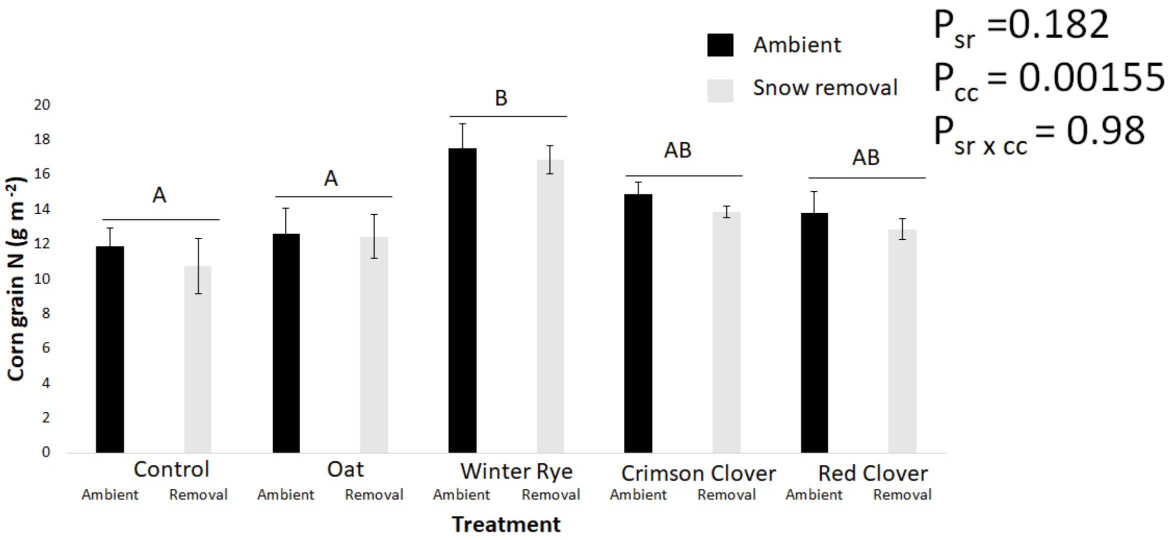

Figure 3-5. Mean corn grain nitrogen (with standard error) for ambient snow (black) and snow removal (grey) plots (n=4). Different letters denote significant differences between cover crop treatment groups (Tukey's HSD test, P < 0.05). P values indicate significance for the following: sr = snow removal and cc = cover crop.

3.2.5 Corn yield

There was a significant effect of cover crop treatment on corn yield (P=0.0023), which increased on average by 15% in cover crop plots relative to the no cover crop control (Figure 3-6). There also was a significant effect of snow removal on corn yield (P=0.0488), with ambient snow plots having 7% greater yield than snow removal plots. There was no significant interaction between the cover crop and snow removal treatments (P=0.94).

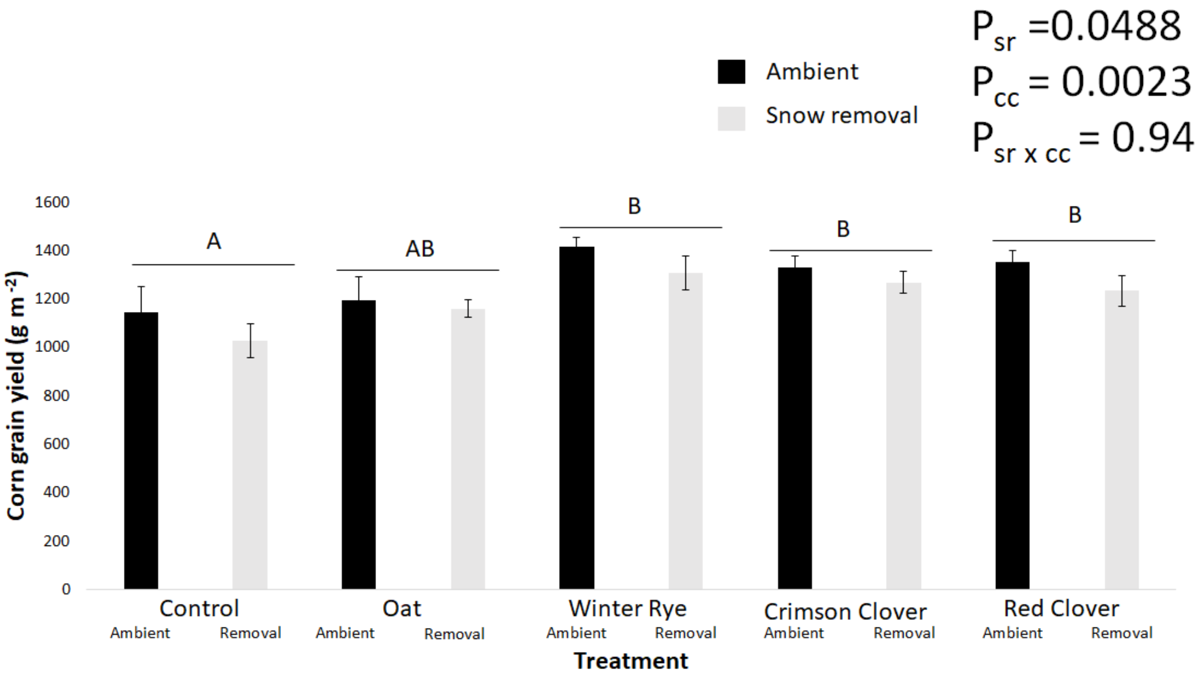

Figure 3-6. Mean corn grain yield (with standard error) for ambient (black) and snow removal (grey) plots (n=4). Different letters denote significant differences between cover crop treatment groups (Tukey's HSD test, P < 0.05). P values indicate significance for the following: sr = snow removal and cc = cover crop.

3.3 Discussion

3.3.1 Cover crop species and phenology had no effect on ^{15}N recovery in corn grain

As described above, a potential benefit of cover crops is their ability to scavenge and retain residual N in agricultural systems when the grain crop is not present, which could lead to increased N availability for the subsequent grain crop. In my study, cover crops were successful in nearly doubling the total ^{15}N recovered in the corn grain relative to the plots that lacked cover crops. The winter-killed cover crops were equally effective as the winter-hardy-cover crops in increasing recovery of the ^{15}N in the corn grain, despite the reduced window for N uptake and extended window of potential N losses, relative to winter-hardy cover crops (Duiker, 2014). This result contrasts with that of Coombs et al., 2017, who observed that red clover increased N availability and reduced the need for N fertilizer, while crimson clover had no effect on available N and did not reduce the need for N fertilizer inputs. Variation in results among cover crop studies can be explained by high annual intraspecific variation in cover crop establishment and growth (see Chapter 2), and in the study by Coombs et al., 2017 the results were explained by red clover biomass being more than double that of crimson clover. I did not quantify cover crop biomass to avoid the effects of destructive harvesting on the outcome of the experiment, but from photos of the plots it was evident that there were differences in total biomass between treatments (Appendix A1)

There also were no differences among grass and legume cover crops regarding their influence on corn grain ^{15}N recovery as there was no significant differences between the cover crop treatments The tissue quality of leguminous versus non-leguminous cover crops tends to differ substantially, with legumes containing high levels of labile compounds and N compared to non-legumes (Sievers & Cook, 2018). The low C:N ratio of legumes (10-15) versus that of grasses (20-25) has been associated with increased mineralization and N loss rates from legume residues relative to grass residues (Coombs et al., 2017; Zhou et al., 2020). In contrast, the high C:N ratio of grasses can increase N immobilization, and lead to large pools of recalcitrant material and reduced transfer of nutrients to subsequent crops (Dabney et al., 2010). For winter rye, it has even been demonstrated that N immobilization by residues can exceed N release for an extended period following termination of the cover crop (Ketterings et al., 2015). However, grasses also can be superior N scavengers, immobilizing as much as100 kg/ha of residual soil N, whereas in legumes the amount N scavenging is nearly half, ranging from 40-60 kg/ha of N (Dabney et al., 2010; Sievers & Cook, 2018). Therefore, in the context of supplying N to the grain crop, the increased recalcitrance of grass residue (increases N mineralization time) may be compensated for by increased N scavenging. In addition to N scavenging, legumes can transfer their fixed N to the subsequent grain crop. Surprisingly, the winter rye treatment resulted in the highest N content in the corn grain, in excess of the amount imparted by the legumes, which was likely explained by the high biomass production of the winter rye (visual observation).

3.3.2 Snow removal increased soil freezing and reduced corn grain ^{15}N recovery

Consistent with my predictions, snow removal increased the number of soil freeze-thaw cycles and reduced ^{15}N recovery in the corn grain. There was no significant interaction between cover crop treatment and snow removal, but as described above, there are mechanisms whereby freeze-thaw cycles can affect the N transfer from both winter-hardy crops (i.e., increased stress on overwintering plants) and winter-killed cover crops (i.e., increased over-winter N losses from cover crop residues and soil from potential tissue damage). Despite the lack of a significant interaction, the influence of snow removal on the winter rye was smallest amongst the cover crop treatments, which could be explained by this being a cold tolerant cool grass species with deep roots and extensive spring regrowth (Dabney et al., 2010). Cover crops also can alter latent heat flux and albedo relative to bare soil (Kaye & Quemada, 2017) and increase soil insulation from cold air temperature both directly (via the presence of their aboveground tissue and residues) or indirectly (via increased snow cover) (Lombardozzi et al., 2018); these may have been additional factors explaining the response of the winter rye plots, as rye could have indirectly influenced soil air temperatures resulting in a reduced freezing effect. In contrast to winter rye, red clover has relatively shallow roots (which are closer to the soil surface) that may be exposed more to soil freezing (Lubbe & Henry, 2021), and the sparser red clover cover would have provided less insultation form cold air. Surprisingly, the snow removal effect also occurred in the control plots, although as described above, there was partial weed cover in the control plots that could have allowed them to function as a cover crop, and snow removal also could have affected soil ^{15}N retention and other soil properties directly in the control plots (*sensu* Henry, 2008). Henry (2008) suggested that bare soil exposed to soil freezing and freeze-thaw events experiences greater nutrient leaching, and loss of CO_2 respiration by soil microbes, which may explain the reduced ^{15}N detected in the snow removal control plots.

3.3.3 Corn grain N

SSnow removal did significantly reduce corn yield for all cover crop treatments. Corn grain N and yield were higher in both the clover treatments and winter rye treatments compared to the no cover crop control. In comparison, in previous studies, the effects of cover crops on grain crop yield have been inconsistent, and in some cases, they had no effect or even reduced yields, this was associated with field moisture levels (O’Reilly et al., 2012; Ketterings et al., 2015). Variability among leguminous cover crop effects also have been noted, with red clover improving grain crop yield, while crimson clover had no effect (Yang et al., 2020). Nevertheless, the bulk of these studies have only addressed the potential benefits of cover crops from grain crop yield over the short term (1-2 year studies), and thus they have not accounted for the potential benefits of improved legacy effects soil quality (Marcillo & Miguez, 2017).

3.4 Conclusions

Overall, I demonstrated for a range of cover crop functional and phenological types that the benefits of cover crops for N recovery in the subsequent grain crop can be reduced by increased soil freezing. However, this effect was only monitored over a single year, and it therefore did not account for potential contributions of cover crop N that remained in the soil organic matter to grain crops in future years. Likewise, other potential benefits of cover crops for soil quality over the long term (e.g., increase soil organic matter and reduced erosion) could contribute to increased soil N retention over that time scale. Future opportunities should focus on understanding how increased soil freezing influences the belowground cover crop N contribution, the importance of which was highlighted in Chapter 2.

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Chapter 4

4 Introduction

Improving nitrogen (N) use in agroecosystems is essential for optimizing crop yield while still maintaining soil health and maximizing environmental sustainability (Galloway et al., 2008; van Meter et al., 2016). Cover crops, which can improve soil organic matter and aggregate stability (Liu et al., 2005; Snapp et al., 2005), also can potentially reduce N losses by immobilizing residual N when the cash crop is not present (Ranells & Wagger, 1997; Dabney et al., 2001). Moreover, leguminous cover crops can provide additional N inputs and improve soil fertility, potentially reducing the amount of fertilizer addition required for the following grain crop (Ranells & Wagger, 1997; Coombs et al., 2017. However, the effectiveness of cover crops in improving N efficiency is contingent on the N from their decomposing residues not being lost from the soil over winter and early spring, prior to the planting of the subsequent cash crop (Kuo & Jellum, 2002; Crews & Peoples, 2005). The decomposition of cover crop residues depends in part on plant tissue quality, and the carbon to nitrogen ratio (C:N) which typically ranges from 15-25 in non-legumes and from 9-13 in legumes (Fageria et al., 2007; Nevins et al., 2020). Given that plant residues with a low C:N ratio typically decompose faster than residues with a high C:N ratio, it is possible that much of the N fixation gain realized through the use of leguminous cover crops may be counteracted by increased N losses from their residues over winter and in early spring (Poffenbarger et al., 2015; Zhou et al., 2017). Cover crops also can be planted in multi-species mixtures that include both legumes and non-legumes, which could provide benefits via species complementary with respect to resource use and residue decomposition (Finney et al., 2016; Vogeler et al., 2019).

Over winter, variation in temperature at the soil surface, which is a function of both air temperature and snow cover, can have a strong influence on N losses from plant litter and soil, as demonstrated extensively for northern temperate forests (Campbell et al., 2014). Although warmer, shorter winters are anticipated to occur across northern temperature agricultural regions as a result of global climate warming, frost exposure at the soil surface may intensify over winter in these regions. This is in part due to reductions in snow cover; specifically, given that snow insulates the soil surface from the air, reductions in snow cover can increase the exposure of cover crop residues to cold air temperatures overnight and during cold spells, even in a warm winter (Henry, 2008; Turner & Henry, 2010). Residue frost exposure could be further intensified by increased variability in air temperature, which is also projected with global climate change (Kreyling, 2010), because an increased occurrence of mid-winter heat waves and snow melt events could increase the exposure of cover crop residues to subsequent frosts (Ruan & Robertson, 2017). Severe frost exposure can both fragment plant litter and lyse the cells of overwintering plants and soil microorganism, increasing nutrient losses from plant litter and soil (Cober et al., 2018, 2019). The increased nutrient losses resulting from more intense freezing at the soil surface could thus decrease the benefits of cover crops for nutrient retention (Øgaard, 2015). Nevertheless, the effects of increased soil temperature variability on cover crop residue N losses over winter remain unexplored.

Decomposition of cover crop residue also can be affected by soil texture, which can influence abiotic properties such as soil moisture, pH, and temperature (Sainju et al., 2021), along with soil microbial community composition (Zhang et al., 2021). For instance, sandy soils are well drained and oxygenated, which can promote increased residue decomposition (Prescott, 2010). In contrast, soils rich in clay and silt feature increased nutrient retention relative to sandy soils, along with increased protection of organic matter in soil aggregates, which can influence the soil microbial biomass (Angst et al., 2021; Mouhamad et al., 2015). Importantly, in the context of organic matter decomposition over winter, soil texture can influence microbial responses to a given level of freezing severity (Oztas & Fayetorbay, 2003).

I incubated mesh litter bags in the field over both silt-loam and sand-loam soil to examine the effects of warming pulses over winter on total mass and N losses from the residues of a four-way winter terminated cover crop mixture: oats, cereal rye, oilseed radish and crimson clover. Ceramic heaters were suspended above the plots to deliver the warming pulses and to melt the snow. After the snow was melted, heaters were turned off (this resulted in decreased soil surface temperatures). I predicted that legumes, which featured the highest N content in their tissues, would experience the highest mass loss and N losses over winter, and that these losses would be exacerbated for all cover crop residues by the pulsed winter warming. I also assessed the effects of the winter warming pulses on soil N content, along with the possible legacy effects of winter warming pulses on corn yield and corn N.

4.1 Methods

4.1.1 Site Description and Experimental Design

The litter bag experiment was conducted within an already established monolithic soil-column weighing lysimeter experiment at the University of Guelph's Elora research station in Ontario, Canada (43^{o}38'21.2" N 80^{o}22'56.2" W). The lysimeter experiment (see Brown et al., 2021; Zeitoun et al., 2021; Lapierre et al., 2022) was initiated in 2016 over an area of 0.1 ha, with each 1.5 m deep lysimeter having a surface area of 1 m^2. The site experiences cold winters and is classified as humid continental according to the Köppen–Geiger classification system (Peel et al., 2007). Specifically, the region experiences mean temperatures of 6.7 ^{o}C and an average annual precipitation of 946 mm, with 148 mm from snow (based on 1981 – 2010 climate normals; Environment and Climate Change Canada, 2022). The region experiences its coldest month in January, with a mean temperature of -7.4^{o}C, while the warmest month is July, with a mean of temperature of 20.0 ^{o}C. A total of 12 of the 18 lysimeters were used as the plots for the collection and incubation of cover crop residues (the other 6 lysimeters did not contain cover crops). Half of the lysimeters contained a soil extracted from the Elora Research Station, while the other half contained soil extracted from a farm located at Cambridge, Ontario, Canada (43^{o}27'27.6" N 80^{o}20'47.5" W), then transported to the lysimeter site. The Elora soil, an imperfectly drained, medium textured silt loam, was characterized as a gleyed gray-brown luvisol, and it was comprised of 38.0% sand, 54.5% silt and 7.5% clay; the Cambridge soil, a well-drained, coarse textured sandy loam, was characterized as a brunisolic grey-brown luvisol, and it was comprised of 79.2% sand, 17.5% silt, and clay 3.3% (Zeitoun et al., 2021). Sensors installed at a depth of 5 cm were used to assess soil temperature at 10-minute intervals (Brown et al., 2021).

The 12 lysimeter plots were managed using a diversified rotation (wheat-corn-soybean). A four species mix of cover crops 1) oats (*Avena sativa*), 2) cereal rye (*Secale cereale*), 3) oilseed radish (*Raphanus sativus*) and 4) crimson clover (*Trifolium incarnatum*) was planted after winter wheat was harvested (August 14 2020) and red clover (*Trifolium pratense*) and annual rye grass (*Lolium multiflorum*) were intercropped with corn starting in June 2021.

For 6 of the lysimeters, winter warming was administered in pulses using a single 1000 W ceramic infrared heater suspended 1 m above the center of each plot. Specifically, the heaters were turned on during major snow fall events (>5 cm) 10 times between early January through to late February and shut off when the snow in the plot was melted. Dummy heaters were suspended above the ambient temperature plots. Freezing degree days were calculated for each plot by summing the daily average soil temperatures that were below 0 °C from Jan 2021 to April 2021. Freeze-thaw was calculated as the number of times in which temperatures went below then above freezing, on average, between 1 January to April 2021. Average temperature was calculated based upon means between 1 January to April 2021. Minimum soil temperature was calculated as the lowest recorded temperature between 1 January to April 2021.

4.1.2 Litter bags

Mass loss and N losses from the cover crop residues over the winter and spring were assessed using 10 × 5 cm litter bags made of fiberglass mesh window screen (1 mm openings) and sealed with hot glue (Stover & Henry, 2020). In late fall 2020 (prior to freeze-up), 350-700 mg dry weight equivalent of shoot tissue was collected for each cover crop species to measure mass loss compared to the spring. The tissue samples were split in half, with the first half weighed then dried at 60 °C for 48 hours. The dry weight was then measured (to allow the calculation of a fresh weight to dry weight ratio for the freshly collected residue) and the samples were prepared for C and N analyses. The second half of each sample was also weighed, then air dried in the laboratory for one week and weighed again (to calculate the ratio of fresh weight to air-dried weight) and placed into the litter bags. Within each lysimeter plot, a litter bag for each cover crop was positioned on the soil surface on 27 November 2020, before snowfall. The litter bags were then collected on 23 April 2021, two weeks prior to the corn planting, and the remaining litter was rinsed in deionized water, with adhering soil particles gently removed with a paint brush. The litter was then dried at 60 °C for 48 h and weighed. The final dry weight was then subtracted from the estimated initial air dry weight to determine mass loss, and the samples were prepared for C and N analyses (see below). There was insufficient residue material remaining for radish to conduct C and N analysis.

4.1.3 Soil sampling

I collected a single 1 cm diameter by 10 cm deep soil core from each lysimeter near when the litter bags were placed in the lysimeters in November 2020, and again in spring 2021, when the litter bags were collected (larger cores were not collected to minimize disturbance to the long-term lysimeter experiment). The soil cores were dried at 60 °C for 72 hours, then crushed with a mortar and pestle to ensure heterogenous sub-sampling, then prepared for C and N analyses (see below).

4.1.4 Corn planting and harvest

The lysimeters followed no-till management. Prior to the corn being planted, the lysimeters and surrounding area were fertilized on 13 May 2021 with 16.7 kg N ha^{-1} and 87 kg phosphorus ha^{-1}. A second fertilization on 22 May occurred prior to planting of the corn, in which the lysimeters received 74 kg N ha^{-1} applied as urea. Corn was planted by hand (Pioneer variety P9188AM at rate of 23.6 kg ha^{-1}), in the lysimeters and the surrounding area, on 23 May. Crimson clover and ryegrass were under-seeded by hand into the corn at the 6th leaf stage on 26 June 2021. Under-seeding was chosen for this experiment because corn is harvested relatively late in this region as it experiences cooler fall temperatures, leaving only a short period for cover crop establishment if seeded post-harvest. Corn was harvested on 27 October 2021 by hand. Sub samples of corn grain and stover were collected for analysis of C and N.

4.1.5 Carbon and Nitrogen analyses

The pre- and post-incubation cover crop residue samples as well as the corn grain and stover samples were ground to < 2 mm using a Wiley mill (Model 3383-L10, Thomas Scientific, New Jersey, USA), then ground to a powder using a ball grinder (Sample Prep Model 2000 Geno/Grinder, SPEX, New Jersey, US) and shaken at 800 rpm for 2 minutes.

I then weighed a subsample of the powdered plant tissue (1–4 mg) into tin capsules for carbon and nitrogen analysis. Soils from the lysimeters were ground using a mortar and pestle, then further ground with the ball mill. A sub-sample that weighed 18-23 mg was placed into tin capsules for C and N analysis. C and N analyses were conducted by the Saskatchewan Isotope Laboratory at the University of Saskatchewan using a Costech ECS4010 elemental analyzer.

4.1.6 Statistical analyses

I assessed treatments effects on mass and N losses for the cover crop residues using three-way ANOVA with cover crop species, warming, and soil type as fixed effects with block added as a random factor, followed by Tukey post hoc tests to examine differences between means. Corn tissue and soil samples were assessed using two-way ANOVA with soil type and warming as fixed effects with block added as a factor, followed by Tukey post hoc tests to examine differences between means There were 3 replicate lysimeters (3 blocks) for each treatment. All statistical analyses were conducted using JMP version 14.1 (SAS Institute, Cary, North Carolina, USA).

4.2 Results

4.2.1 Weather conditions

Average air temperature in the fall (September – November 2020 see Chapter 2, figure 2-3b page 34 from the same weather station), when the cover crops were sown, was 11.3 °C, and mean monthly precipitation was 67.1 mm. The average air temperature over winter (December – March) was -2.8 °C and mean monthly precipitation was 35.8 mm. Average spring temperature (Mar – May 2021) was 8.9 °C and mean monthly precipitation was 42.9 mm. Average air temperature over the corn growing season (May to October 2021) was 16.1 °C and mean monthly precipitation was 76.4 mm. During the time the pulse warming occurred between Jan. and Feb., the air temperature on average was -6.6 °C, and mean monthly precipitation was 26.1 mm.

4.2.2 Freeze thaw cycles and soil temperature at 5 cm depth

For the sandy soil, the ambient plots experienced 11.6 freeze-thaw cycles on average, while the warmed plots experienced 12.0 freeze-thaw cycles between 1 Jan, and 10 March 2021. Likewise, average soil temperature from Jan to March was slightly colder for the warmed plots, as expected when insulating snow is melted away, (-0.36 °C) compared to the ambient plots (0.10 °C). However, the minimum soil temperature over the course of the pulsed warming experiment (1 Jan to 10 March 2021) was colder in the warmed plots (-6.1 °C), than in the ambient plots (- 3.1 °C), and the warmed plots experienced more than double the number of freezing degree days as the ambient plots (65 versus 26) (Figure 4-1).

For the silt-loam soil, the ambient plots experienced 10.6 freeze thaw cycles on average, while the warmed plots experienced 13.3 freeze-thaw cycles between 1 Jan , and 1 March 2021. Average soil temperature (Jan to March 2021) was slightly lower in the warmed plots (-0.48 °C) than in the ambient plots (0.063 °C) over the course of the snow removal experiment. However, minimum soil temperature was colder in the warmed plots (-5.4 °C), than in the ambient plots (- 2.2 °C), and the warmer plots experienced 60 freezing degree days, which is more than double compared to 22 for the ambient plots (Figure 4-1).

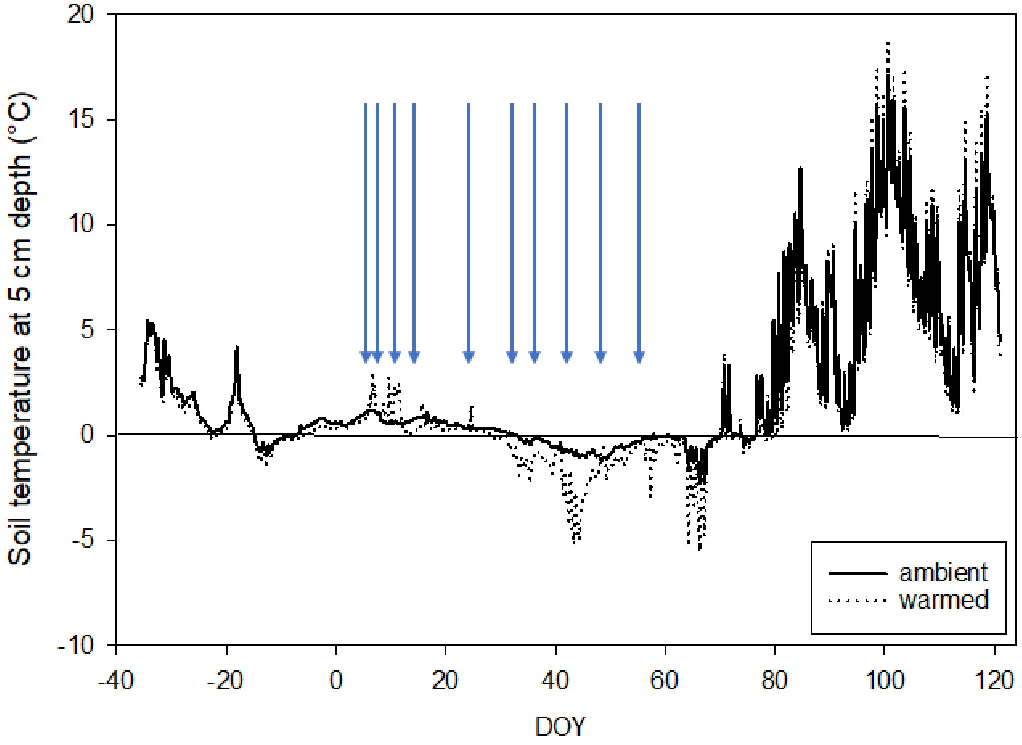

Figure 4-1. Mean hourly soil temperatures at a soil depth of 5 cm in ambient temperature (solid black line) and pulse warmed (dotted black line) lysimeter plots, averaged over all soil treatments (n=6). Arrows indicate the approximate timing of pulsed warming events.

4.2.3 Cover crop litter bag mass loss and decomposition

Oilseed radish experienced the greatest mass loss (78.0%), followed by rye (50.5%), clover (47.7%) and oats (34.5%) (P =0.0001; Figure 4-2). There was no significant effect of soil type on mass loss, but the interaction between cover crop and soil type was marginally significant (P=0.087). Residues in the warmed plots experienced greater mass loss (59.5 %) than those of the ambient temperature plots (46.7%) (P = 0.00126), but there was not a significant interaction between cover crop and warming (Figure 4-2).

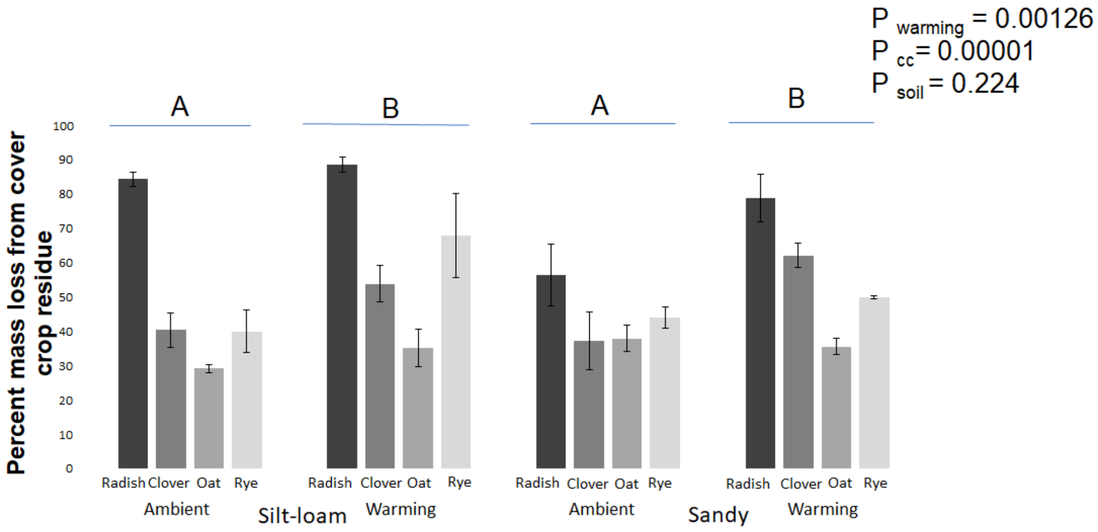

Figure 4-2. Mean percent mass loss from cover crop residues (with standard errors reported) in the pulse warmed and ambient temperature plots (n=3). Litter bags were incubated from late November 2020 to late April 2021, before the corn was sown. Different letters indicate significant differences between warming treatments for each averaged across soil type, and cover crop species (Tukey's HSD test).

4.2.4 Residue N losses

Nitrogen losses initial dry weight (dw) from clover residues were highest (0.011 g N per g dw), followed by rye (0.0098 g N per g dw) and oat (0.0051 g N per g dw) (P = 0.005) (Figure 4-3). Soil type did not significantly influence residue N losses, but there was a close trend that silt-loam (0.010 g N per g dw) lost slightly more N than sand (0.0075 g N per g dw) (P =0.059). Residue N losses from warmed plots (0.0106 g N per g dw) were higher than those from ambient temperature plots (0.0071 g N per g dw) (P = 0.015) (Figure 4-3). There was no significant interaction between cover crop and warming.

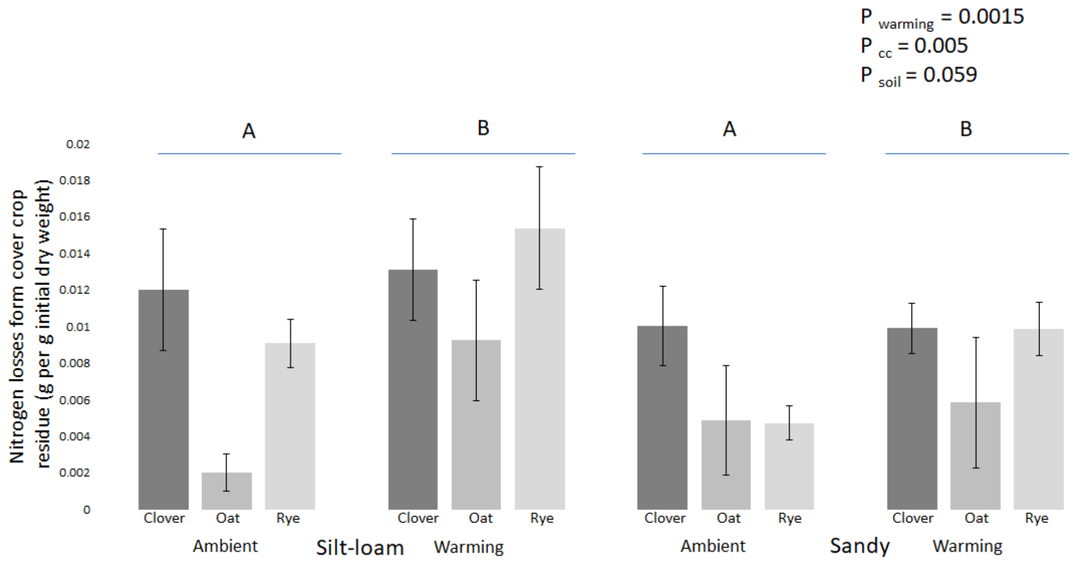

Figure 4-3. Mean nitrogen losses from cover crop residues based on initial dry weight (g N per g dw) (with standard errors reported) in the pulse warmed and ambient temperature plots (n=3). Litter bags were incubated from late November 2020 to late April 2021, before the corn was sown. Different letters indicate significant differences between warming treatments for each soil type, averaged across soil type, and cover crop species (Tukey's HSD). P values indicate significance for the following: warming = warming treatment, cc = cover crop, soil = soil type.

4.2.5 Soil N content

The silt loam and sandy soils differed in N concentration (0.260% and 0.088% N, respectively) (P = 0.0001) (Figure 4-4). There were no significant differences in soil N concentrations between ambient, and warming plots. There were also no significant differences between fall, and spring soil samples (Figure 4-4).

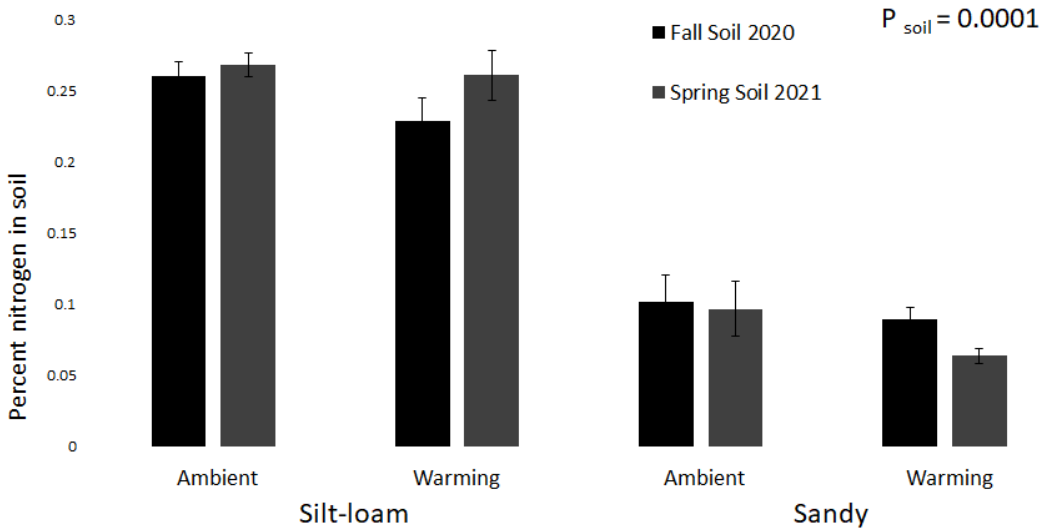

Figure 4-4. Mean soil nitrogen concentration from 0-10 cm depth (with standard errors reported) for the ambient temperature and pulse warmed plots with silt loam and sandy soil in fall (Nov. 2020) and spring (Apr. 2021) (n=3).

4.2.6 Cover crop and soil C:N

Cover crop C:N was affected by cover crop species and season with few significant interactions among factors. Soil type did not significantly effect C:N. C:N was higher in fall (19.8) than in spring (17.2) (Table 4-1) (Table 4-2); P=0.0038), and slightly interacted with cover crop species (P = 0.09). Oat had the highest C:N in the fall (25.6), followed by rye (18.3) and clover (15.5) (Table 4-1). Similar results were observed in spring, with oat (20.0) having the highest C:N, followed by rye (16.3) and clover (15.4) (Table 4-1) (P = 0.0001). There were no significant interactions between cover crop species and both treatment, and season (Table 4-2). There were no significant interactions between cover crop species, and warming treatment for soil samples taken in fall, and spring (Table 4-2).

Table 4-1. Mean cover crop and soil C:N ratios in the fall 2020, and in spring 2021 (standard errors in parentheses), for the warmed and ambient temperature plots including both soil types.

Cover Crop and Soil	Loam Ambient (C:N)	Loam Warming (C:N)	Sand Ambient (C:N)	Sand Warming (C:N)
Fall Clover	15.2 (0.5)	16.1 (0.6)	14.8 (0.6)	15.9 (1.4)
Fall Oat	24.3 (0.7)	22.2 (1.1)	27.4 (3.0)	28.7 (2.9)
Fall Rye	17.4 (2.2)	17.7 (1.2)	19.2 (0.2)	18.9 (2.0)
Spring Clover	24.0 (9.0)	13.5 (1.1)	11.4 (0.9)	13.3 (1.2)
Spring Oat	19.6 (0.92)	16.4 (0.3)	22.4 (1.9)	21.8 (1.2)
Spring Rye	17.9 (3.1)	16.7 (1.8)	14.3 (1.1)	16.3 (2.1)
Fall Soil	11.0 (0.4)	11.5 (0.6)	11.5 (0.1)	11.2 (0.6)
Spring Soil	11.4 (0.9)	11.2 (0.5)	11.3 (0.2)	10.2 (0.2)

Table 4-2 Three-way ANOVA results for the cover crop C:N data displayed in Table 4-1 (significant effects, $p < 0.05$, show in bold; species - clover, oat, rye; season - fall, spring; warming treatment – warming, no warming). Data were pooled over soil type, which did not have a significant effect on cover crop C:N).

C:N Cover Crop Tissue Interactions	**P-value**
Species	**0.0001**
Season	**0.005**
Warming Treatment	0.49
Species * Season	0.09
Species * Warming Treatment	0.81
Season * Warming Treatment	0.37
Species * Season * Warming Treatment	0.52

4.2.7 Grain yield, stover mass, and total nitrogen

Corn grain yield and N content in grain were significantly higher for the silt loam than for the sandy soil. There were no significant effects of warming on corn grain yield and N, nor were there significant effects on corn residue biomass and N content.

4.3 Discussion

4.3.1 Pulsed warming increased residue N losses

There has been a substantial focus in winter biogeochemistry literature on how freeze-thaw cycles can increase soil N losses by disrupting soil aggregates and lysing microbial and root cells (Feng et al., 2007; Kejadian et al., 2016; Ejack & Whalen, 2021). This concept has been extended to exploring whether there are increased plant litter N losses with increased freezing exposure, with increased N losses possibly occurring as a result of more litter fragmentation coupled with increased microbial activity during periods of thaw (Cober et al., 2018, 2019; Øgaard, 2015). Contrary to my expectation, the main effect of pulsed warming was one of increased soil freezing alone, rather than an increased in number of freeze-thaw cycles. This occurred because the pulsed warming was only conducted when snow was present when the heaters were turned off at the completion of snow melt, the ambient air temperatures typically remained below freezing. Nevertheless, consistent with my prediction, pulsed warming increased cover crop residue N losses. This result suggests that increased exposure of cover crop residues to freezing could increase N losses from residues at a time when the grain crop is not present to take up the nitrogen, leading to increased N trace gas or leaching losses. The latter is consistent with the results of Lapierre et al. (2022), who observed increased concentrations of nitrate in drainage water during spring melt in the warmed lysimeters where I deployed the litter bags, albeit in an earlier year. Although there was a marginally significant interaction between soil type and warming, there was no significant interaction between cover crop and warming for mass loss or N losses, despite high variability among cover crops in N losses (see below).

4.3.2 Variability in N losses among cover crops

Based on the wide range in tissue quality of the cover crops I examined (e.g., C:N ratios of 15:1 for clover, 17:1 for rye and 23:1 for oats; N data were not obtained for oilseed radish), I anticipated high variability in mass loss and N losses among residues, both of which were observed. Variation in residue quality can result in residues contributing to either net immobilization (e.g. for high C:N residues) or net mineralization (for low C:N residues) (Roth et al., 2022). Grasses like rye or oats may also exhibit slower release of N because their tissues contain higher lignin and cellulose concentrations than that of forbs such as legumes (Jahanzad et al., 2016; Coombs et al., 2017). Despite substantial total N losses from all cover crop residues, in general, residue C:N ratios declined over the course of the incubation, which was consistent with microbial colonization of the residue material. The exception was for clover residue on the silt loam soil, which was influenced by an outlier litter bag where only recalcitrant stem material remained in the spring which thereby inflated its average C:N. As expected, total N losses over winter were highest for clover, which suggests that despite their high capacity for increasing N inputs through fixation (e.g., as much as 100 kg N ha^{-1}; Sainju et al., 2021), a significant quantity of this N can be lost from the residues prior to planting of the grain crop in spring. For example, Yang et al. (2019), who conducted a litter removal experiment over winter, noted a substantial reduction in N losses in litter removal plots. In addition, previous cover crop residue decomposition experiments conducted over spring have revealed different phases of decomposition, related in part to variability in weather, but with initial decomposition usually peaking 2-3 weeks after the beginning of litter bag incubation, then slowing as time progresses (Roth et al., 2022). Although interactions between warming and cover crop type were not significant for residue mass and N losses, the variability within treatment groups was high, which suggests that greater replication may be needed to obtain a more conclusive result regarding these potential interactions.

4.3.3 There were no interactive effects of soil texture and pulsed warming on soil N

Soil N concentrations were vastly different between the two soil types, and as described above, sandy soil is expected to have less nutrient and water holding capacity than finer textured soils (Prescott, 2010; Mouhamad et al., 2015), while the latter also exhibit greater soil aggregation and higher quality SOM than sandy soils (Angst et al., 2021). Soil texture also can influence the freezing sensitivity of soil microorganisms (Oztas & Fayetorbay, 2003). However, I did not detect a significant interaction between soil type and pulsed warming for residue mass loss or N losses. Likewise, although there were trends of pulsed warming reducing soil N, and soil type interacting with season (fall versus spring), these effects were only marginally significant.

4.3.4 Soil freezing did not influence corn yield

Variability in soil freezing can alter summer production indirectly by influencing soil nutrients, moisture, or soil physical properties (Henry, 2008; Campbell et al., 2014). However, I did not observe significant effects of pulsed warming on corn yield or N content; only soil type had a strong influence on corn tissue mass and total N. While all the cover crops that, I examined were terminated in the late fall, the success of winter-hardy cover crops may be affected substantially by pulsed warming, which could have a stronger effect on corn yield and N content (see previous Chapter 3).

4.4 Conclusions

Overall, my results revealed an important role of variability in winter conditions in influencing mass loss and N losses from cover crop residues. While the latter did not ultimately influence the yield or N content of the subsequent grain crop, increased N losses with reduced snow cover and increased soil freezing could nevertheless lead to more N losses to the environment over winter and early spring. In particular, many of the potential benefits of increased N inputs from N-fixing cover crops such as clover could be offset by increased leaching and gaseous N losses during spring-melt.

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Chapter 5

5 General discussion

5.1 Summary of thesis goals

Despite emerging evidence in recent decades that cover crops provide benefits to agroecosystems, the practice has only been adopted slowly by farmers in the United States and Canada (Roesch-Mcnally et al., 2018). One area that has remained unclear has been the potential benefits (or possible negative consequences) with respect to N pollution and fertilizer management. A theme throughout my thesis has been that even though cover crops immobilize N, and some associate with microorganisms that fix N, benefits for the N nutrition of the main crop may be contingent on the decomposition of the cover crop being synchronized with the N demand of the subsequent crop to minimize potential N losses (Vyn et al., 2000). The overarching goal of my thesis was to evaluate this ability for cover crops to retain and scavenge residual N and to trace the transfer efficiency to the subsequent grain crop under variable climate conditions. This information may be used to make recommendations on the value of cover crop N credits to growers that could be used to justify reductions in fertilizer use.

In the second chapter of my thesis, I evaluated the relative N contributions from aboveground and belowground cover crop components using a novel method that involved swapping of enriched N tissues. My second and third chapters also examined the transfer efficiency of enriched N fertilizer from cover crops to corn tissue. My fourth chapter focused on the decomposition and release rate of N from various cover crop residues. Chapters 3 and 4 also addressed how interactions between climate variability and soil freezing can alter N transfer in cover crop systems. A recurring theme of these chapters involves how variation in phenology between winter-killed and winter-hardy cover crops interacts with N transfer, and I also explored differences in effectiveness between legumes and non-legumes, as well as cover crop monocultures versus mixtures.

5.2 Summary of factors that influence cover crop N transfer

Reactive N can go through many different transformations when in soluble forms in the soil. This can complicate the understanding of how cover crops are involved in internal N cycling within agroecosystems, particularly because the belowground influences of cover crops are difficult to assess (Gardner & Drinkwater, 2009). However, the simplified biogeochemical pathway I explored was one of N immobilization by cover crops, followed by mineralization of residues, then immobilization by the grain crop (Vyn et al., 2000; Crews & Peoples, 2005; Snapp et al., 2005). By tracing N through this pathway, I was able to construct a model for which factors are most significant in influencing N movement in cover crop systems (Figure 5-1).

First, cover crop species selection is extremely important in influencing N transfer, and in particular, non-legumes and legumes are vastly different in the ways they access and release N (Kaye et al., 2019; McKenzie-Gopsill et al., 2022). As addressed previously, whereby all cover crops can scavenge residual N and immobilize it within their tissues, leguminous cover crops can provide additional N inputs through fixation (Dabney et al., 2010). N transfer from cover crops is further complicated by phenology, with cover crops that die from frost early in the winter potentially differing from winter-hardy cover crops, which continue to grow in the spring until they are terminated (Finney et al., 2016). Cover crop phenology also affects the timing of nutrient release and mineralization (Dabney et al., 2010; Congreves & van Eerd, 2015).

Over-winter N mineralization is also influenced by variation in environmental conditions, including soil temperature, moisture, snowpack cover and soil texture, all of which can influence microbial activity (Henry, 2007; Congreves & van Eerd, 2015). For instance, snow cover provides insulation from freezing temperatures for decomposing crop residue and for plants that overwinter at or below the soil surface (Malyshev & Henry, 2012). Climate variability is expected to increase extreme events, such as increased frequency of freeze-thaw events or prolonged soil freezing from lack of snowpack, which can increase N losses (Turner & Henry, 2010). Mechanisms for increased N losses include increased soil N_2O efflux during freeze-thaw events, because under anaerobic conditions microbes often use reactive N as a terminal electron acceptor (King et al., 2021). The N_2O can become trapped in the frozen soil and released once thawed (Ruan & Robertson, 2017; Baral et al., 2022). In addition to trace gas emissions, there is also damage that occurs to microbial and plant cells; this lysis can contribute to increased leaching of nitrate (de Notaris et al., 2018).

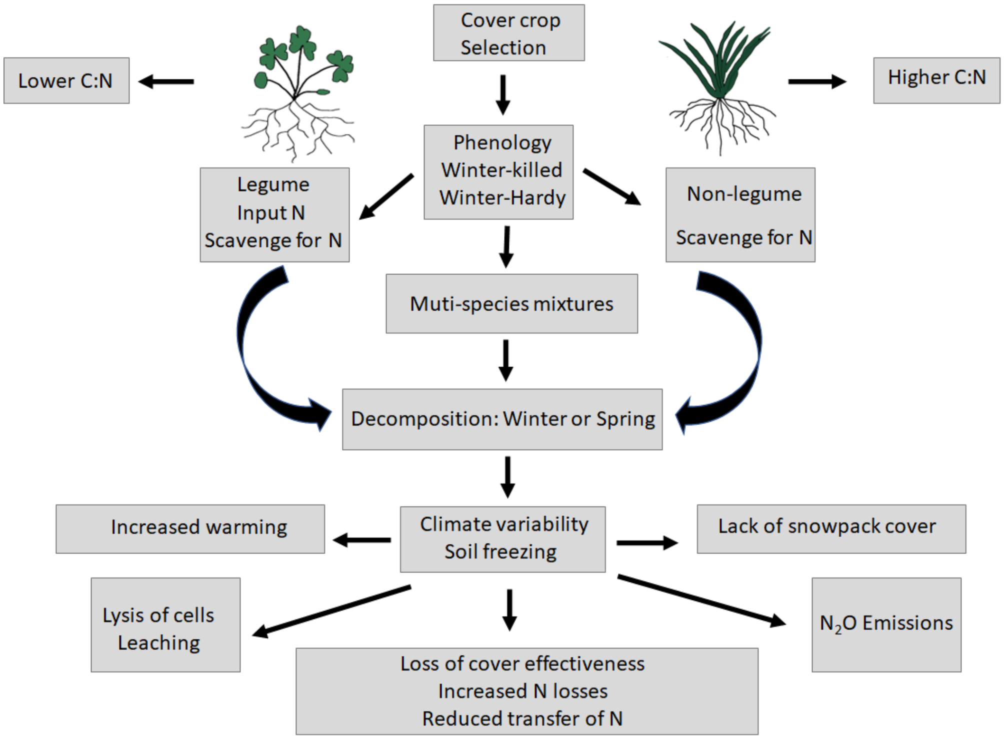

Figure 5-1 Factors influencing N transfer and losses in cover crop systems.

5.3 Major trends

5.3.1 Aboveground versus belowground N contributions

The belowground contributions of cover crops to N fertility are difficult to assess and often overlooked (Arcand et al., 2013; Taveira et al., 2020). While root to shoot ratios have been used to estimate the belowground contributions of N, this approach is best suited for cover crop monocultures and is difficult to apply to mixtures (Roth et al., 2022). The data I presented in Chapter 2 were some of the first to address differences between the aboveground and belowground N contributions from cover crops to the subsequent grain crop. The recovery efficiency of enriched N fertilizer from the aboveground pool was quite variable year-to-year, given that the retention was highly correlated with cover crop biomass. For the belowground pool, the mechanisms behind the retention are less clear (Sievers & Cook, 2018), and this applied to my experiment, where the root and soil N contributions were not separated. Moreover, the aboveground and belowground pools can interact, given that the presence of cover crops can alter the soil microclimate (Arcand et al., 2013). The presence of cover crops also can reduce drainage (leaching) and alter microbial activity, which can influence belowground N retention substantially (Lapierre et al., 2022). My results follow similar patterns to other studies that have performed experiments with crop residues, which have demonstrated that the belowground contribution can range from 2 to 18 times more than the aboveground pool with respect to N retention (Arcand et al., 2014; Taveira et al., 2020).

The transfer efficiency of enriched N from mineralized cover crops to corn was increased in both Chapters 2 and 3 by nearly double compared to the no cover crop control, although in both cases less than 10% of the added ^{15}N was recovered in the corn. The data from Chapter 2 followed a similar trend as previously reported data, in which the belowground pool (< 5% recovery) contributed much more N to the grain crop, than the aboveground pool (<1% recovery). The low N contribution from aboveground tissues resulted from cover crop productivity only being a small fraction of corn grain productivity (approximately 1000 kg/ha versus 10000 kg/ha). Typically, a corn crop requires around 0.450 kg of N per 25 kg of corn; therefore, for a cover crop to transfer the required amount of N (given that % N of the cover crop is around ~2-3%), it would have to transfer 180 kg of N, which is an order of magnitude higher than what the aboveground pool offered in my experiments (20-30 kg of N). Nevertheless, legumes can add as much as 30-150 kg N ha^{-1} to the soil, which would provide a more substantial credit towards fertilizer reduction (Clark et al., 2007; Coombs et al., 2017).

Significant portions of N remained in the soil following the grain harvest in chapter 2, suggesting that indigenous soil N can be increased by the presence of cover crops as they improve soil organic matter (Gabriel et al., 2016; Taveira et al., 2020). The transfer efficiency of enriched soil N at harvest improved N recovery in comparison to the no cover crop treatment by nearly double in some cases (20% versus 50% in the case of legumes). These results are in line with the increased reported by Arcand et al. (2013). Despite the relatively short time scale of my experiments (i.e. single years), I was still able to detect significant influences of cover crops on soil N. However, the accumulation of organic matter from crop residues may have important cumulative effects over years to decades (Garba et al., 2022). In some cases, the build of soil N is sufficient to justify further reductions in annual N fertilization (Gaudin et al., 2015).

5.3.2 Non-legumes, legumes, and mixtures N contributions and differences

As described above, species selection plays an important role in cover crop N retention, given that the quality of tissue and functional characteristics of different species contrast substantially (Freund et al., 2021). For instance, non-legumes are superior at scavenging for N, and they can serve as a large sink for residual soil N (Zhang et al., 2022). In contrast, legumes improve the fertility of soils mostly through N fixation (Tribouillois et al., 2016). In Chapter 2, I found that cover crop biomass was highly correlated with the ability of the non-legumes to retain N; for example, in year 1 there was no difference between non-legumes and legumes, but in year 2, when the biomass of non-legumes was much higher, they outperformed the legumes. This result was consistent with that of Fageria et al. 2007.

Also as described in the previous chapters was the tendency for non-legumes and legumes to have vastly different C:N and physical properties, with the less recalcitrant legume residues tending to release N more quickly than those of non-legumes (Dabney et al., 2010). Jahanzad et al., (2016) reported that grasses like rye or oats exhibit a slow release of N because their tissues contain higher lignin and cellulose content than legumes such as winter pea and clover. Depending on residue C:N, microorganisms can also become a potential sink for inorganic N (Sainju et al., 2021). The N losses demonstrated in Chapter 4 confirmed that legume residues lose more N from their tissues than the non-legumes over the winter.

Cover crop mixtures potentially offer the combined benefits of N scavenging and N fixation, along with a mix of fast and slow residue decomposition. Mixtures typically reduce the C:N ratio of aboveground residues in comparison to non-legume monocultures (de Notaris et al., 2018). It also has been speculated that cover crop mixtures should improve total root biomass compared to monocultures, and extensive rooting systems are useful for building up soil organic matter (Amsili & Kaye, 2021). Mixtures and single cover crop monocultures of legumes and non-legumes were examined in Chapter 2 of my thesis. While the multi-species mixtures performed as well as the monocultures with respect to N retention, aboveground biomass tended to decrease as diversity increased in the mixtures (10-way versus 3-way). This appeared to occur because some species established poorly, while the species in the mixtures that established successfully could not fully compensate for the reduced biomass of the other species. Therefore, the potential benefits of biodiverse cover crop mixtures appear to be contingent on high germination and establishment success across species.

5.3.3 Cover crop phenology

The synchrony of crop residue N mineralization and grain crop N is predicted to vary among winter-killed and winter-hardy cover crops (Finney et al., 2016), with the potential for additional spring N uptake by winter-hardy species; N release from the residues of these species may be better synchronized with grain crop N demand (Kaye et al., 2019; White et al., 2022). Nevertheless, variation in residue quality can modulate the degree of synchronization (Nevins et al., 2020), as demonstrated previously for winter rye, which can vary substantially in C:N ratio (Roth et al., 2022). While winter-hardy cover crop survival may be vulnerable to severe conditions over winter, freeze-thaw events, leaching and immobilization of N by microorganisms can likewise alter the fate of N mineralized from the residues of winter-killed cover crops at this time (Hadas et al., 2002; Melkonian et al., 2017). Although I did not to detect significant differences between winter-hardy and winter-killed crops in Chapters 2 and 4, high variability in N recovery may have obscured the trend of winter-hardy cover crops exhibiting higher N retention than the winter-killed cover crops. However, this effect was only observed for the cover crop mixtures, whereas legumes showed no differences in N retention when terminated in the fall versus in the spring.

5.3.4 Soil freezing may reduce cover crop effectiveness

A theme from Chapters 3 and 4 was that, in temperate regions, more variable climate conditions are expected to influence soil temperatures and snow cover (Henry, 2008). The non-growing season in agroecosystems typically featured substantial N losses, which can be exacerbated by increased soil freezing. Both Chapters 3 and 4 demonstrated that increased soil freezing could potentially reduce the effectiveness of cover crops in transferring N to the main crop. For instance, in Chapter 3, I demonstrated that enriched N recovery was reduced by snow removal in comparison to ambient conditions and while pulsed warming in Chapter 4 also caused greater N losses, both events led to increased soil freezing. In Chapter 3, the frequency of freeze-thaw cycles was four times greater than under ambient conditions. Meanwhile, the pulse warming in Chapter 4 did not increase the number of freeze-thaw cycles, but did result in a lower minimum soil temperature. Therefore, both an increased frequency and intensity of freezing may contribute to increased N losses.

Leguminous cover crops appeared to be influenced most by soil freezing and freeze-thaw events. For instance, legumes exhibited trends of having the highest decreased in N recovery in corn grain in response to soil freezing in data Chapter 3, particularly in red clover. Red clover has low lying roots, which could have been more exposed to soil freezing than other species (Malyshev & Henry, 2012; Zhou et al., 2017). Since legumes tissues contain a high percent of N and are made up of a high proportion of labile materials they are at risk of increased losses from soil freezing compared to grasses and other non-legumes (Crews & Peoples, 2005). Soil freezing also affects physical characteristics of soil. For instance, the expansion of ice as water freezes reduces soil aggregate stability and increases root cell lysis, thereby increasing nutrient leaching during snow melt (Øgaard, 2015; Cober et al., 2018;Lapierre et al., 2022). Ultimately the effects of increased soil freezing may be a reduced effectiveness of cover crops in improving N retention.

Even though increased soil freezing may reduce the effectiveness of certain cover crop species, there may be options to mitigate the negative effects of increased soil freezing. Such methods could include utilizing more hardy cover crops that have deep and robust root systems (Dabney et al., 2010; Roth et al., 2022). For instance, in Chapter 3, I observed that winter-rye was least effected by soil freezing. This was likely due to it being a cold tolerant species with deep fibrous roots, compared to other grasses (Dabney et al., 2010), which was associated with it being highly productive during spring regrowth (visual observation). However, this species also has a high C:N ratio and increased recalcitrance, which may impair N transfer to the grain crop (Congreves & van Eerd, 2015; Nevins et al., 2020).

5.4 Future directions

Future opportunities for cover crop research should focus on characterizing the long-term effects that cover crops have on soil fertility (O'Reilly et al., 2012). Likewise, given that year-to-year variability in cover crop performance appears to be high, the effects of weather variability on cover crops should also be further examined (Schipanski et al., 2014). Future work also should focus on optimizing muti-species mixtures, given they can form a complementarity-based system, in which unrelated species utilize space and resources differently, thus enabling higher productivity and nutrient retention (Dabney et al., 2010). Cover crop mixtures also may help reduce some negative effects of soil freezing and other stresses, given the broad range of species tolerances to different stresses (White et al., 2017). Mixes of legumes and non-legumes also may combine the benefits of N fixation and N scavenging, along with staggered N release (Duiker, 2014).

5.5 Anticipated Significance

The use of cover crops is a sustainable but potentially complex agricultural practice that can enhance many ecosystem level services (Schipanski et al., 2014). The goals of my thesis were to characterize internal N cycling from cover crops to inform growers the extent to which conventional N fertilizer application can be reduced when cover crops are used, and to explore how climate variability in soil freezing may impede cover crop effectiveness. My data provide insight into the effectiveness that cover crops have as a N management tool, and how climate variability may affect their use and N cycling mechanisms. Understanding the dynamics and mechanisms of litter decomposition of cover crops residues over the winter and under a variable climate is extremely important, as it gives insight into the timing of N mineralization and transfer to the subsequent crop. My data suggest there is the potential for high variability in establishment and productivity when using cover crops that could affect the overall effectiveness of cover crop systems. Therefore, to better recommend cover crops to new and current growers within a changing climate, strategies for reducing this variability should be explored.

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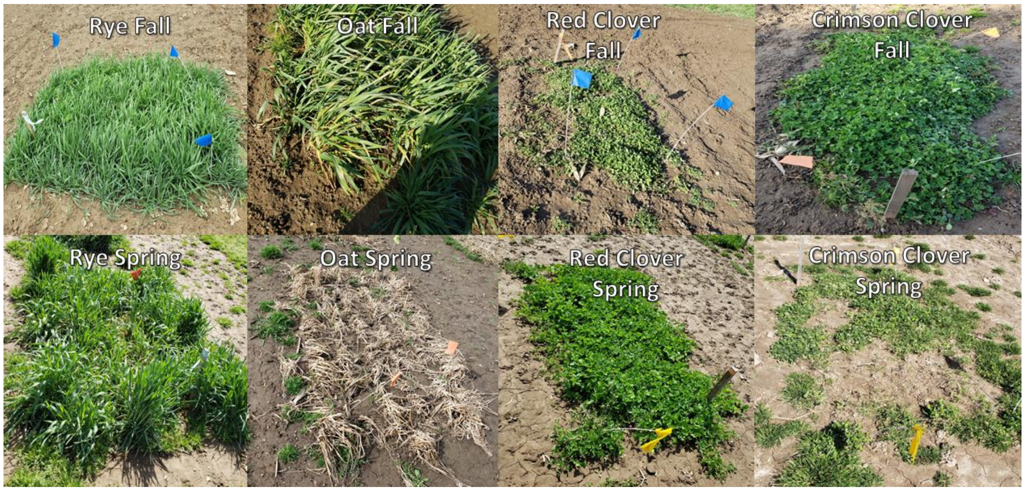
Appendix

Appendix A Supporting Information



Appendix A1: Chapter 3: Cover crops species visually; top October 2020 and bottom April 2021

Curriculum Vitae

Education

Doctor of Philosophy, Department of Biology, 2018-2022 (Fall 2022)
University of Western Ontario (UWO), London ON
Thesis Nitrogen transfer from cover crops to the subsequent grain crop: the influence of variability in winter conditions
Supervisors: Dr. Hugh Henry, and Dr Claudia Wagner-Riddle

Master of Science, Department of Biology, 2016-2018
University of Western Ontario (UWO), London ON
Thesis: Plant stimuli-responsive biodegradable polymers for the use in timed release fertilizer coatings
Supervisors: Dr. Hugh Henry, and Dr. Elizabeth Gillies

Bachelor of Science, Department of Biology, 2012-2016
University of Western Ontario (UWO), London ON

Teaching Experience (2016-2022)

Teaching Assistant, Mycology
Biology Department, University of Western Ontario (2016-2022)
- Assisted with fungal identification in lab and taught tutorials

Lead Teaching Assistant, Plant Pathology
Biology Department, University of Western Ontario (2018-2021)
- Supervised and developed labs, educated students on plant disease identification, designed assignments and final exams

Teaching Assistant, Organismal Physiology
Biology Department, University of Western Ontario (2016-2018)
- Supervised wet labs, and taught tutorials

Awards

Ontario Graduate Scholarship (OGS) - $15,000 University of Western Ontario (UWO) 2019-2020
Ontario Graduate Scholarship (OGS) - $15,000 University of Western Ontario (UWO) 2020-2021
Ontario Graduate Scholarship (OGS) - $15,000 University of Western Ontario (UWO) 2021-2022
Biology Department Travel Award - $300 University of Western Ontario (UWO) 2022
Graduate Teaching Award For Science - $500 University of Western Ontario (UWO) 2022

Deans Honour’s list 2014-2016

Contributions to Research and Development

a) Articles published or accepted in refereed journals

Heuchan, S.M., Fan, B., Kowalski, J.J., Gillies, E.R., and Henry, H.A.L. (2019) Development of fertilizer coatings from polyglyoxylate-polyester blends responsive to root-driven pH change. Journal of Agriculture and Food Chemistry. 67(46), 12720–12729 (Msc work)

Heuchan, S. M., MacDonald, J. P., Bauman, L. A., Fan, B., Henry, H. A. L., and Gillies, E. R. (2018) Photoinduced Degradation of Polymer Films Using Polyglyoxylate-Polyester Blends and Copolymers. Acs Omega 3, 18603-18612. https://doi.org/10.1021/acsomega.8b02826 (MSc work)

b) Non-peer reviewed contributions

Conference Poster Presentations

1. **Heuchan, S.M*.,** Wagner-Riddle, C., Henry., H.A.L.(2022) Can variability in snow cover affect nitrogen transfer between cover crops and the subsequent grain crop? Ecological Society of America, Montreal, Ontario, Canada (PhD work international)
2. **Heuchan, S.M*.,** Wagner-Riddle, C., Henry., H.A.L.(2021) Tracing nitrogen transfer from aboveground and belowground cover crop residues to the subsequent grain crop. Ecological Society of America, Virtual, (PhD work international)
3. **Heuchan, S.M*.,** Gillies E.R., Henry, H.A.L (2018) Plant root responsive slow-release fertilizer coatings for the improvement of nutrient delivery. Ecological Society of America, New Orleans, Louisiana, USA. (MSc work international)
4. **Heuchan, S.M*.,** Gillies E.R., Henry, H.A.L. (2018) Plant-responsive polymers for the use in controlled release fertilizer coatings. Fallona Family Interdisciplinary Showcase, University of Western Ontario, London, Ontario. (MSc work institutional)
5. **Heuchan, S.M*.,** Gillies E.R., Henry, H.A.L. (2017) Plant stimuli-responsive biodegradable polymers for the use in timed release fertilizer coatings. Ontario Ecology, Ethology, and Evolution Colloquium. Queens University, Kingston, Ontario. (MSc work provincial)

Conference Oral Presentations

1. **Heuchan, S.M.,** Gillies E.R., and Henry, H.A.L. (2019) Improving nutrient delivery with plant root responsive slow-release fertilizer coating. Ecological Society of America, Louisville, Kentucky, USA. (MSc work international)
2. **Heuchan, S.M.,** Wagner-Riddle, C., Henry, H.A.L. (2019) Reducing nitrogen fertilizer inputs and increasing nitrogen use efficiency: are cover crops the answer? Envirocon. University of Western Ontario, London Ontario. (PhD work institutional)
3. **Heuchan, S.M.,** Gillies E.R., and Henry, H.A.L. (2018) Plant stimuli triggered polymers for the use in slow release fertilizer. Ontario Ecology, Ethology, and

Evolution Colloquium. University of Western Ontario, London Ontario. (MSc work provincial)

Relevant Activities

Institutional organizations

Environmental and Ecological Planning Advisory Committee (EEPAC) for the city of London on a 2019-2022 contract.

I am asked to review and provide my expert and technical opinion on environmental assessments to the city of London on matters that are relevant to the cities official plan. I provide feedback to the city's municipal council through the Planning and Environment Committee on a monthly basis. This includes London's natural heritage systems, Environmentally Significant Areas (ESAs), woodlands, and stream corridors.

Graduate Education Council (GEC) at Western University on a 2019-2021 contract.

This is a nominated position that enables me to play an active role and as representative of the science department for school of Graduate and Postdoctoral Studies. I serve to propose and comment on academic polices that been approved by the senate. I also provide feedback and guide the development of studies relevant to graduate and postdoctoral studies at western. I also provide advice on the aspects of graduate education.

Leadership

I have provided mentorship for numerous work study and undergraduate students at Western. I have taught and provided them with lab, and field experience.

Committees

Honour Thesis Advisory Committee – providing guidance and grading honour thesis projects for senior level undergraduate students completing an honours degree.