

**Establishment, Productivity, and Nitrogen Dynamics in a Legume Cover Crop System with
Annual Crops in Manitoba**

by

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ABSTRACT

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In the Canadian Prairies, where growing seasons are short and falls are often dry, using legumes as a cover crop necessitates that the cover crops overlap or even coexist in the same field at the same time as cash crops. Producer confidence in seeding cover crops depends on answering questions about plant competition, cash crop productivity, profitability, and simplicity of practice. Moreover, nitrogen (N) from biological N fixation (BNF) has not been quantified accurately enough to consider legume cover crops a reliable source of N or a partial displacement of fertilizer N. This study tested the performance of a single-pass seeding system with four legume cover crops and one non-legume together with wheat at six site-years in Manitoba in 2023 and 2024, maintaining the established cover crops until the following year as a living mulch. The following spring, canola was seeded into the chemically suppressed living mulch at three site-years. All variables tested differed significantly among site-years. In no case did cover crop presence affect wheat biomass, grain yield, or protein. At all site-years, cover crop biomass was low and easy to control during the wheat phase (ranging from 11.8 to 146 kg ha⁻¹ at wheat soft dough stage), but was large and difficult to control during the canola phase at two sites (up to 10,164 kg ha⁻¹). Canola

was grown successfully in the living mulch at one of three sites, with yields similar to the canola-only control and the regional 10-year average in treatments with low mulch biomass. These results indicate that a wheat cash crop with legume cover crops succeeded at most site-years, but the canola crop seeded into the living mulch succeeded at only one out of three site-years tested and strongly depended on effective control of living mulch biomass. Measurement of N inputs was limited due to the failure of the canola crop, but soil nitrate, soil N flux, and cash crop plant N concentration did not reveal any N sharing or N benefit to either the wheat or canola when growing together with the legume cover crops.

FOREWORD

There are two research chapters of this thesis, and both have been organized in anticipation of publication in peer reviewed journals, which have not yet been identified. Jessica Frey approached Dr. Joanne Thiessen Martens with the research concept to be carried out through her employer, the Parkland Crop Diversification Foundation (PCDF) in Roblin, and at the three other sister Manitoba Diversification Center research sites in Arborg (Prairies East Sustainable Agriculture Initiative), Carberry (Manitoba Crop Diversification Center) and Melita (Westman Agricultural Diversification Organization). Dr. Thiessen Martens guided Jessica on treatment structure, methodology, data collection schedule and statistical analysis, with input from Dr. Yvonne Lawley and Dr. Xiaopeng Gao. Funding was granted through the Manitoba Crop Alliance, and in-kind labor and materials were provided at all four diversification centers. PCDF provided Jessica Frey with a vehicle for mobility between research stations. Dr. Thiessen Martens and Jessica Frey worked with the specialists at the four diversification centers to adapt plot size dimensions and timing of data collection to the available equipment. PRS® probes were provided through Western Ag Professional Agronomy and advice on their use and analysis were provided by the head of Research and Development, Dr. Eric Bremer.

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1. INTRODUCTION

1.1 Historicity of Cover Cropping

Annual cropping on the Canadian Prairies is characterized by high-input, high-output, low-diversity systems. Prior to the large-scale adoption of nitrogen (N) fertilizers, a diverse array of soil and nutrient management practices were common on the North American Great Plains and the Canadian Prairies (Brown, 1964; Harwood, 2024; Penner, 2024; Pieters, 1927). Legumes and grasses have historically been used to improve soil physical properties, conserve soil from wind and water erosion, scavenge nutrients between crops, and add N into the soil (Brown, 1964; Pieters, 1927). As early as 1917, A. J. Pieters, an agronomist at the United States Department of Agriculture, published a bulletin on using legumes for green manures. In 1927, he published a review of the practice, covering most of the mainland United States, Ontario, and southern regions of Western Canada, reporting decreased practicability in dry regions, such as the Great Plains and approaching the Rocky Mountains (Pieters, 1927). He wrote about the same cover cropping topics that are common in the literature today: above and belowground biomass, nodulation, ideal legume varieties, nutrient scavenging, N fertilizer replacement value (NFRV) for following crops, and building good soil tilth, to name a few topics (Pieters, 1927). However, since good results could be achieved with reduced labor, reduced inputs, and increased profits from using synthetic N fertilizer (Brown, 1964), a larger-scale shift to synthetic forms of N fertilizer in place of legume green manures occurred rapidly, especially after the Second World War (Blackshaw et al., 2010).

Relying heavily on inorganic fertilizers exposes producers to risk caused by volatile fertilizer prices, which are caused by disruptions to supply and fluctuating energy costs (Abay et al., 2025). This vulnerability was heightened by large-scale economic disruptions in 2001 and 2008, and more recently, by the global COVID-19 pandemic that started in 2020 and the Russia-Ukraine war that started in 2022 (Quitow et al., 2025). Additionally, there is increasing concern about the impacts that losses of N fertilizer have on water via leaching and on the atmosphere via denitrification and volatilization. These losses to the natural world equate to financial losses that have a direct impact on farm profitability. Personal economic loss comes not only from lost monetary investment in fertilizer, but also from a decrease in yield and in grain quality such as protein in crops like wheat (Holzapfel et al., 2007). With these factors in view, the prospect of achieving only a 33% N use efficiency (NUE) for cereal grains and oilseeds, as was estimated by a global review paper (Raun & Johnson, 1999) represents serious economic loss for producers. The review calculated that the 67% loss of N fertilizer globally amounted to \$15.9 billion annually. A later study by the same author (Raun et al., 2002) noted that fertilizer prices had doubled since the political instability of 2001, further increasing economic loss to producers. On a less global scale, Malhi et al. (2001) conducted a review specific to the Canadian Great Plains and estimated NUE at <70%.

Aside from the direct impact on producer profitability, losses of N to the natural environment have prompted the development of policies promoting the adoption of cover crops on an international scale, often through the use of payment incentives (De Notaris et al., 2025; Penner, 2024). Optimizing policy requires balancing the goal of maximizing production alongside the prioritization of preserving soil health and protecting water bodies and the atmosphere through judicious fertilizer use (Abay et al., 2025). With a six-fold increase in N use in the six decades since the mainstream adoption of inorganic fertilizers in agriculture (Quitow et al., 2025), the

emerging question for both cropping and animal systems is how to enhance farming efficiency and reduce harmful leakages of N from synthetic N fertilizer (Böldt et al., 2021).

However, it is not enough to identify cover cropping as part of the solution. For effective management and planning it is essential for policy makers, researchers, agronomists, and farmers to understand the logistics of implementing cover cropping on a large scale. Tools such as the 2020 Cover Cropping Survey (Morrison & Lawley, 2021) and the National Cover Crop Survey by Sustainable Agriculture Research and Education (SARE) (McGovern, 2026) help policy makers to contextualize the risks that farmers face when introducing cover crops. It also encourages the involvement of researchers and agronomists for the task of developing best management practices (BMPs) targeted to strategically address the current knowledge gaps about who is cover cropping, how they are implementing the practice, what challenges they encounter, and also what benefits that they have witnessed (Morrison & Lawley, 2021).

Low-diversity agriculture and reliance on synthetic fertilizers have revealed other weaknesses over time, such as acidification of soils, and pest and weed resistance. The result has been a renewed interest in diverse approaches to managing soil fertility (Blackshaw et al., 2010a; Carof et al., 2007). The broader concept of soil health has also risen in importance. Soil health goals, laid out by Augarten et al. (2023), are to: (1) minimize soil disturbance; (2) increase plant diversity; (3) maximize soil cover; (4) maintain year-round living roots; and (5) integrate livestock. These soil health goals align closely to the benefits of cover cropping. Research has shown that cover cropping is a management practice that can reach these goals, and that legumes, specifically, improve the soils they grow in. The benefits include, but are not limited to: N fixation, increased soil organic matter, improved water infiltration and retention, soil protection, improved soil structure leading to decreased erosion, weed suppression, increased cropping options in temperate

regions, and higher grain protein content (Blackshaw et al., 2010; Brown, 1964; Cicek et al., 2014; Thiessen Martens et al., 2001). Some producers use cover cropping only as ground cover between cash crops, seeding them in the fall after harvest of the main cash crop, in a practice known as double cropping, but another practice is to grow the legume cover crops together with cash crops. Relay cropping involves staggered seeding of the cash crop and the legume cover crop so that the two systems overlap in the same field at the same time so that they are not harvested at the same time (Thiessen Martens et al., 2005a).

Although the options for systems such as relay cropping or double cropping are more extensive in warm climates, multiple studies have shown that it is possible to manipulate these systems in the short growing seasons of the Canadian Prairies (Thiessen Martens, 2001; Blackshaw, 2010; Cicek, 2014). In the case of a short growing season, a further option is to seed the cash crop and the legume cover crop at the same time in a practice known as intercropping (Morrison & Lawley, 2021).

To design effective and practical legume–non-legume intercropped systems, it is necessary to understand: 1) climate constraints; 2) management strategies that can shift the intercrop balance in favor of the cash crop; and 3) how much N can realistically be expected to be gained from maintaining legumes in the field together with cash crops.

Identifying a practice that is suitable for the Canadian Prairies that has the most potential to improve soil health by intercropping legume cover crops without harming the cash crops necessitates identifying and eliminating the practices that do not work.

1.2 Thesis Objectives

Within the parameters of a short growing season and limited fall moisture the range of possible cover crop opportunities on the Canadian prairies is narrow. The overarching objective of this

research is to explore options for cover cropping with legumes that make use of heat and moisture when it is available, making the assumption that these constraints necessitate that cash crops and cover crops coexist in the same field at the same time, and require close management. Co-seeding cash crops and cover crops is one application (for establishment), and seeding into a pre-existing cover crop living mulch is another, lesser known, application (continuing to make use of the living cover once it is established). The specific objectives are to: 1) compare the establishment and biomass of four legume cover crops and one non-legume cover crop in four regions of Manitoba by seeding them in the same row at the same depth as spring wheat; 2) compare the establishment, biomass, yield, and protein of a wheat cash crop grown together with the different cover crop species; 3) compare establishment, biomass, and yield of canola seeded into the overwintered living mulch; and 4) quantify how much N is added by the BNF of four legumes to this two-phase, wheat-canola system of legume cover cropping.

The literature review is divided into three sections. The first section will give an overview of cover cropping practices, and focus particularly on the Canadian Prairie context. The second section will focus on interspecies interactions during establishment and subsequent growth and on management practices that can be used by growers to balance the competition between cash crop and cover crop (Objectives, 1, 2, and 3). The third section will focus on legume–non-legume N interactions — how much N is put into a legume intercropped system, and how available that N is to other non-legumes in the intercrop (Objective 4).

1.3 Overview of intercrop cover cropping

Cover cropping is the practice of cultivating a crop, not for the purpose of harvesting a cash crop, but rather to improve an agricultural system through the short-term and long-term benefits provided by a crop, such as sequestering nutrients, protecting the soil from erosion, increasing soil

organic matter and water holding capacity, and promoting healthy soil microbe and earthworm populations (Bremer et al., 2024; Morrison & Lawley, 2021; Penner, 2024). When cover cropping with legumes in particular, whether as an intercrop or as ground cover between cash crops, agricultural researchers are interested in understanding the addition of N to the soil as a result of biological N fixation (BNF). Crucially, it is important to understand not only the fixation of N by legumes, but also the movement of the fixed N between plants and the soil, as well as the accessibility of that N to co-seeded or subsequent crops (Blackshaw et al., 2010; Thiessen Martens et al., 2001; Penner, 2024).

Beyond the scientific community, farmers and other practitioners have explored numerous strategies to implement cover cropping in diverse settings, and have shared their successes and challenges online through social media and YouTube. Importantly, these individuals have shared how they implement cover cropping practices in their cropping systems, the financial implications, and their perceived long-term benefits.

Short-term economic benefits associated with the use of cover crops may be achieved through the reduction of inputs such as synthetic fertilizers and herbicides (Schipanski & Drinkwater, 2012). Longer-term benefits (listed above) may emerge over time. Although these may be less readily measured in financial terms, cover crops can protect and enhance the soil and provide fertility when they are terminated and the plant material decomposes (Blackshaw et al., 2010a; Chiriac et al., 2025).

Intercropping can also be practiced with two harvestable cash crops that are produced from the same field in the same season (Chalmers, 2015). By this definition, legumes can function as either cover crops grown for the purposes of increased soil health or potential animal integration, or as

an intercropped grain legume such as peas, beans, or lentils. In either application, legumes impart benefits to the soil.

The timing of seeding and harvest for both cover cropping and intercropping leads to further distinction between cropping systems in the form of either double cropping, relay cropping, or live mulching. Double cropping is the quick succession of seeding one crop right after the other is harvested within the same growing season, whereas relay cropping staggers seeding with a resulting overlap of the two crops in the same field at the same time (Thiessen Martens et al., 2005). A living mulch system may be established in the same way, but the use of a perennial variety allows the understory to keep up its performance throughout successive cash crops and to maintain living roots in the ground between cash crops (Cougnon et al., 2022). Maintaining a living mulch is a distinct practice that requires even more careful management than a one-season cover crop.

1.3.1 Cover Cropping on the Canadian Prairies

Although the options for systems such as relay cropping or double cropping are greater in warm climates, multiple studies have shown that it is possible to adapt these systems to the short growing seasons of the Canadian Prairies (Blackshaw et al., 2010b; Cicek et al., 2014; Thiessen Martens et al., 2001b). Even without the pressures of intercropping, plants must respond uniquely to the short growing season, the semi-arid conditions and the frequent strong winds on the Canadian Prairies (Liu, 2009).

The three most commonly seeded cover crops cited by respondents to the 2020 Prairie Cover Crop Survey were clovers (57%), oats (52%), and peas (41%). Seventy one percent of farms were mixed farms. Forty seven percent of farmers who establish cover crops in the Canadian Prairies seed them in the fall after harvesting the main crop, but with such a short growing season, an open shoulder

season with sufficient precipitation to establish a fall cover crop is not guaranteed (Morrison & Lawley, 2021). According to the 2020 Prairie Cover Crop Survey, 30% of respondents cited the short growing season as the main impediment to seeding in the shoulder season and 27% said it was lack of fall moisture. As such, 37% of survey respondents seeded cover crops contemporaneously with the main crop to make use of available heat and moisture in the early growing season. Seeding in one pass also reduces labor and equipment. Termination was generally accomplished by grazing (46%), winter kill (37%), tillage (30%), or herbicides (21%) (Morrison & Lawley, 2021).

Evaluating the success of intercropped systems requires a clear understanding of the Canadian producer goals. In the case of relatively simple intercropping systems with two harvestable products, such as pea and canola, it is common to talk about land equivalency ratios—that is, how much land would have been required to produce the same amount of the two intercrops in separate pure stands (Bremer et al., 2024; Deb et al., 2022). For example, in a pea-canola intercrop, if both the pea and canola yields are 60% of what the crops would have yielded individually in a pure stand, then the total crop yield from the same amount of land is 120%. In this example, the overall yield exceeds what would be expected in a single pure stand of each crop. In contrast, the immediate goal for an intercropped cover cropped cash crop such as a legume that is not harvested for grain is simply the successful establishment of that crop.

Once a cover crop has been established, a farmer has a range of options for what to do with it. Unlike cash crops that are typically produced and removed from the field in the same growing season, legume cover crops are more versatile. Straightforward termination and incorporation, either in spring or fall, can be useful for boosting soil organic matter and for adding N and other nutrients to the soil. For mixed farmers, grazing or baling the cover crop as a forage is also an

option. Harvesting seed from the cover crop can provide an income stream. For each option, success is measured in relation to the individual's goals, which are shaped by their farm's unique circumstance and capacity. Addressing success in terms of quantifying BNF is comparatively more ambiguous and is the subject of much research.

1.4 Interspecies Interactions at Emergence and Critical Growth Stages

There are three generally agreed upon outcomes for intercropping multiple plant species in the same system. These include complementarity, facilitation, or competition.

1.4.1 Complementarity

Complementarity occurs when plants of different species make use of the same above and below ground space and its attendant resources at different times, varying depths, and even different chemical forms. Nature itself is a complementary system, with each plant in its own niche, resulting in a diverse, resilient, and multipurpose system. Farmers who look to nature as their model are motivated to build the same resilience in their cropping systems (Thiessen Martens et al., 2015). The potential to complement each other comes about because of differences in root structure, and timing and balance of nutrient demand (Dowling et al., 2021). Root structure alone is broken down into subcategories of length, density, tissue composition, and adaptability, all lending a unique capacity to access and/or deposit nutrients at different depths of the rhizosphere (Schipanski & Drinkwater, 2012). In the case of soil N, each plant retains or releases inorganic N at distinct times and locations, thereby protecting it from pathways of loss, such as denitrification, leaching, and immobilization (Holzapfel et al., 2007).

Couëdel et al. (2023) identified complementarity between crucifers and legumes as cover crop mixtures grown in the fall shoulder season in France, where mixtures had greater uptake of C, N, potassium (K), sulfur (S), iron (Fe), copper (Cu), manganese (Mn), calcium (Ca), magnesium (Mg)

and boron (B) over their solo-seeded controls. Additionally, legumes and non-legumes grown in mixtures accumulated more biomass and C than the solo-seeded treatments, indicating improved access to light and photosynthesis. Results like these highlight that monocultures are also subject to competition and that intraspecific competition still exists within fields that are seeded to only one species (Couëdel et al., 2023).

The basic concept of a legume-cereal or legume-oilseed intercrop is very simple: legumes, which can fix their own N from the air but are poor competitors for inorganic soil pools of N, will leave those pools for the more competitive and demanding cereal. In the process of fixing its own N, the legumes ideally share their own N resources with the cereal (Xiao et al., 2004).

The differences in root morphology and density among legume crops determines where N will accumulate in the rhizosphere. Nitrogen deposits include nitrate (NO_3^-) and ammonium (NH_4^+) (which is quickly converted to NO_3^-), amino acids, cell lysates, or cells that have been sloughed off (Xiao et al., 2004). Given that white clover's root system is shallower and denser than alfalfa, these depositions occur closer to the top of the root zone. Nitrogen released by the long tap-rooted alfalfa, by contrast, impacts a deeper layer of the soil. The interactions between companion crop roots with those of legume crop roots will be a function of the depth and density of the roots of both crops (Liu, 2009).

In an ideal complementary situation, the differences in root morphology result in soil resources being used more efficiently (Liu, 2009), but there are many factors that influence how a complementary dynamic unfolds.

1.4.2 Facilitation

Facilitation is defined by Schipanski & Drinkwater (2012) as a mutually beneficial relationship between two or more species of plants that takes time to develop and mature. It is a type of

complementary relationship that results directly from plant interactions, but that takes longer to develop and generally involves one species making a limiting resource available for the other species or improving the conditions for accessing that resource. For example, Schipanski & Drinkwater (2012) discuss that rooting patterns may slightly change in length, reach, or density when responding to the presence of other roots, thereby giving the plant access to N pools that it might not have accessed as a monoculture. Given time to develop, this facilitative interaction may become stronger. The decomposition of aboveground material, such as leaves or stolons that have been shed, or death of whole plants from winter kill, is also a facilitative dynamic that develops over time.

Along the spectrum of complementary and facilitative systems is an intermediate zone, described in the literature as a *commensal* system, in which one crop is advanced by the exchange, while the other crop functions as it normally would, without any statistically significant benefit or detriment. For example, a pea-canola intercrop demonstrated in southwestern Manitoba was described as commensal because canola yield increased relative to a monocrop but pea yield was similar to the pea monocrop (Chalmers, 2015).

1.4.3 Competition

In a 2021 review of legume-oilseed intercropped systems, Dowling et al. (2021) describe a competitive system as one in which the interactions between two plant species result in negative consequences for at least one of the two species. Competition for water, N, and light can interfere with or stunt at least one of the species' growth (Mbanyele et al., 2024), resulting in decreased yields and financial loss.

Although competition resulting in economic loss is a strong disincentive to intercrop, a competitive system cannot be simply categorized as negative. Interspecific plant relationships are very

intricate. Even systems identified as complementary have some competitive aspect to them. Competition may prevail temporarily, but as the plants adapt, elements of a complementary or facilitative relationship may emerge. For example, in a trial in Northern Europe looking at white clover undersown with barley in year one, and then maintained as a living mulch for two subsequent winter wheat crops, Bergkvist (2003a) found that the presence of the clover reduced wheat yield in the first crop of winter wheat compared to a control treatment of winter wheat alone by 8%, but then increased yields in the second winter wheat crop grown with the white clover by 9% when compared to winter wheat alone. White clover was managed through herbicide application but was not killed. A facilitative relationship developed over time, potentially even because of the competition. Both inorganic soil N and N content in wheat grain increased. Soil nitrate increased 10 kg ha⁻¹ in the winter wheat treatment with a white clover living mulch compared to winter wheat alone despite that first year of competition, and N concentration in winter wheat grown with white clover was 4-34% higher than winter wheat grown alone. Various means of physical (mowing, spring harrowing) and chemical control have been proposed to contain growth, particularly of white clover (Adami et al., 2017; Carof et al., 2007).

Accordingly, a large body of literature is devoted to manipulating competitive systems to determine what types of interventions show the most potential to shift the interspecies interactions into a complementary or facilitative relationship (Carof, et al., 2007; Hiltbrunner et al., 2007; Mbanyele et al., 2024).

1.4.4 Interspecies Interaction during Critical Growth Periods

Competition between plants becomes increasingly important during key phases of development. Competition during establishment and at flowering is particularly detrimental. At flowering plants require large quantities of N, water, and light (Bergkvist, 2003a). Plant counts tracking emergence

at 2, 4, and 8 weeks after seeding provide good insight into the vigor of the crop, how it will compete to gain sufficient access to light, and how much biomass it will produce (Nelson et al., 2022). Substantial quantities of biomass in the legume, while desirable for fixing N and making it available for subsequent wheat crop uptake (Cicek et al., 2014), has also been linked to yield decreases of 19-81% for wheat when sown into a living mulch if the legume biomass leaf area is allowed to exceed that of the wheat during the critical times of stem elongation, anthesis and fertilization (Carof et al., 2007).

Additions of inorganic N fertilizer to give the non-legume an advantage is a good example of the methods that are available to manipulate access to light, space, and moisture (Bremer et al., 2024). Fertilizer addition can shift the competitive edge away from the leguminous cover crops to the companion cash crop during establishment of intercrops. Land Equivalent Ratios (LER – the area of land required for a sole crop to yield the same as a combined intercrop) of pulse-oilseed intercrops decreased when inorganic N fertilizer was added because the non-legume is quick to access the added N (Bremer, et al., 2024). Increasing leaf area of the non-legume subsequently blocks light for the less competitive legume and draws up more water (Bremer et al., 2024; Mbanyele et al., 2024). If legume grain harvest is not the goal, but rather perennial legume establishment, the decrease in LER is not problematic.

When perennial legumes for forage establishment is the goal, up to 80% of Manitoba producers choose to establish them with a cereal nurse crop. The nurse crop, such as oats, is usually cut for greenfeed in midsummer, removing it as a competitive force during establishment and stem elongation, but some producer choose to bring it to grain harvest (Leybourn, 2024). When using a nurse crop to establish a forage, researchers at Ducks Unlimited Canada have observed that hay yields in subsequent years are low compared to hay established without nurse crops (Leybourn,

2024), indicating that the establishment disadvantage continues to be seen in subsequent years. It is important therefore, for the producer to either try to advantage one or the other crop based on their goals, or to be flexible with regard to where the income is generated: a nurse crop grown for grain harvest, or a better forage in subsequent years.

Establishing a cash crop in a pre-existing living mulch presents a new type of challenge. Cougnon et al. (2022) review the current literature regarding the use of perennial plants as living mulch cover for winter cereals, documenting the methods used by growers to reduce living mulch competition with the cereal crops. These methods include sowing time and density, spatial arrangement, mechanical manipulation, herbicides, and varietal selection. The researchers point out that the current varieties for legume intercrops are best suited for the specific purpose of producing quality forages, but that quality forage production requires more water and nutrients than a companion crop would ideally take. They therefore call for breeding programs dedicated to the development of legume varieties to develop selection criteria that will provide the benefits of a legume living mulch without the intense requirements of high-yielding forage crops. Such varietal development could open the doors to the commensal relationships discussed in section 1.4.2.

Managing competition can be challenging. Bergkvist (2003a) found that applying herbicide to a white clover living mulch was effective in increasing wheat yields in the second year of living mulch, whereas mowing and spring harrowing were not effective for controlling mulch biomass. The effect was not consistent across sites and years, however. In a subsequent study, when seeding rapeseed and winter wheat into a white clover living mulch, the same researcher (Bergkvist, 2003b) used two white clover varieties with different winter hardiness. The less winter hardy white clover variety had delayed spring regrowth and reached peak biomass after the flowering of the rapeseed

and the winter wheat had already occurred, thereby reducing competition with the winter wheat and producing grain yields comparable to sole-cropped wheat in the current year compared to the treatments intercropped with the more winter hardy white clover (Bergkvist, 2003b). Carof et al. (2007) also note that in an established, multi-year living mulch with white clover and alfalfa, this type of peak N competition during flowering, measured as grains per spike, had decreased by the third year of the mulch compared to the no mulch control. A facilitative relationship developed over time between the mulch and grain crop.

Finding the “right” level of competition is sometimes the entire goal of a study. Ideal competition causes more exploitation of available resources by both plants, which can develop into complementary or facilitative systems. The “right” level of competition will be discussed further with reference to N-sharing pathways.

1.5 Interspecies N Interactions

The development of an “ideal” competition which leads to complementary resource usage applies in a very specific way with respect to N access and N usage. In an intercropping system with both legumes and non-legumes, one species can biologically fix atmospheric N and the other cannot. Additionally when oilseeds are the non-legume, there is greater competition for both soil and fertilizer N than in a monocrop (Bremer et al., 2024). However, in the presence of abundant soil N, the BNF slows and less N is fixed (Osterholz et al., 2023). Since legumes are not strong competitors for soil N compared to their non-legume companion crops, competition becomes the stimulus for the legume to fix N for itself (Szumigalski & Van Acker, 2006). Aboveground competition also influences N interactions: legumes with insufficient access to sunlight due to shading from the cash crop will not fix enough N to meet their needs (Schulze et al., 1994; Szumigalski & Van Acker, 2006). Biological nitrogen fixation is an energy intensive process,

requiring 16 ATP (adenosine triphosphate) and at least 1.5 mg C for each milligram of N fixed from the atmosphere (Schulze et al., 1994). Suppression of legume leaf area by a competing plant, decreases the legume photosynthetic potential and makes it more difficult for the legume to keep both the plant and the N fixing nodules supplied with C and ATP (Schulze, 1994).

1.5.1 Pathways of N Transfer

Transfer of inorganic plant-available N happens between plant species by many pathways. As a very first step, however, N must first be mineralized from organic N to inorganic N via aminization and ammonification. Substrates for mineralization include plant or animal residues, soil microbes, root exudates, dead roots, or shed nodules (Osterholz et al., 2023; Schipanski & Drinkwater, 2012). Root exudates may take the form of any ions, gases, enzymes, or easily broken-down organic compounds (Hupe et al., 2021). If the quantity of biologically fixed N exceeds the legume's own needs, it can be released into the soil for uptake by companion crops (Dowling et al., 2021).

The speed at which the organic N is mineralized is a function of the soil's acidity, temperature, and moisture, in addition to the C/N ratio of the material involved (Chiriac et al., 2025). The higher the C/N ratio, the longer it will take for soil microorganisms to mineralize the N. If all the available soil inorganic N is consumed by the microorganisms in the metabolic process of breaking down C, then the N is immobilized and made unavailable to the plants. A lower C/N means that there is less C to break down and N will become more readily available (Heard, 2012). Soil microorganisms themselves have a C/N ratio of 8:1, and stable soil organic matter has a ratio of 10:1. Net mineralization of N decreases as C/N ratio increases (Chiriac et al., 2025). Wheat straw, with an average C/N ratio of 60:1 (Heard, 2012), will therefore decompose much more slowly than young sweet clover, which generally has a C/N ratio of 12:1. The fine and densely rooted systems of white clover have different C/N ratios than the tap-rooted alfalfa and red clover (Louarn, et al.,

2015; Schipanski & Drinkwater, 2012). Wivstad (1999) found that yellow sweet clover resulted in greater net mineralization (38% of added N) than red clover (26% of added N) over time because of the more frequent senescence of young sweet clover leaves that are rich in N. Louarn et al. (2015) showed that white clover, at a C/N ratio of 13.9 and relatively low lignin content (8.3% in the fine roots and nodules), was well below the threshold between net mineralization and immobilization and that it therefore contributed N to the soil five times faster than alfalfa. Older alfalfa roots have a higher C/N ratio (19.3) than younger alfalfa roots (15.5), and the tap root has a higher C/N ratio (27) than the fine roots and nodules that branch off of the tap root (15.5) (Louarn, et al., 2014). Legume species more likely to provide N through turnover in the roots and leaves will be those which mineralize faster.

Mycorrhizal connections among plants also provide potential exchange sites (Dowling et al., 2021). The majority of common mycorrhizal networks are formed by arbuscular mycorrhiza and ecto mycorrhiza, with arbuscular mycorrhiza forming associations with 300,000 plant species across the spectrum of gymnosperms, angiosperms, bryophytes, and pteridophytes (He et al., 2009a). Fungal hyphae, which penetrate the roots of plants, introduce sites of nutrient exchange between the plant and the fungi: energy to feed the fungi, and N and other nutrients to feed the plant (He et al., 2009). When these networks join more than one species of plant, a complex system of nutrient exchange is made possible because of the formation of a concentration gradient which moves N from plants that have high concentrations to plants with lower concentrations (Xiao et al., 2004). Most brassicas do not form with these networks. Town et al. (2023) reports that when canola was continuously cropped over 12 years, the lack of a host for the arbuscular mycorrhizal fungi leads to a significant decline in number and diversity of fungi. They also note that canola's root tissue is known to have allelopathic qualities, and that much is unknown about the impact this

has on the soil fungal community. Allelopathy would eliminate arbuscular mycorrhizal fungi as a source to transfer N if canola was the cash crop.

Another N dynamic that arises is referred to as the “sparing effect”, whereby the poorly competing legume not only biologically fixes N, but also inhibits its uptake of existing soil N pools. Sparing of N is a different process than mineralization of N. To spare N is to leave available soil N for the ready use of the non-legume, whereas mineralization adds to the current N pool. Szumigalski & Van Acker (2006) credited the higher soil nitrate pools observed at the end of the season in wheat and canola plots intercropped with peas (compared to monocropped wheat and canola plots) to the sparing effect, as opposed to mineralization.

It should not be assumed that legumes will maintain their ability to fix N in all circumstances. A modest supply of N is required by the legume during establishment; in the absence of sufficient N, the legume will not compete with the companion crop, resulting in reduced foliage, nodulation, and BNF (Xiao et al., 2004). Establishing an intercrop during a time of N starvation can create a competitive relationship between the legume and the main crop, instead of a complementary one. For example, in a trial with corn and alfalfa at three different fertility rates (0, 112, and 221 kg ha⁻¹), Osterholz et al. (2023) found that among the three N fertility rates, adding no additional N resulted in the corn inheriting more N derived from the atmosphere as a result of higher legume fixation; however, the intercropped corn yielded 33% less than monocropped corn with no added N. Soil inorganic N was also depleted in the intercropped corn, indicating that the legume had competed for available soil N in early growth stages.

Root contact within the soil zone influences the relationship between the intercrops. For example, a pot study using three degrees of root contact or exclusion with wheat and fababean and comparing both petiole injection of ¹⁵N and natural abundance methods, demonstrated that in pots

with no barrier at all, the amount of N transferred to the wheat was 2.1 times greater than those with a mesh barrier (Xiao et al., 2004). No barrier allowed for extensive root intermingling and increased the potential for movement of N by diffusion and mass flow across the concentration gradient created by plant roots instead of relying solely upon the mycorrhizal interactions allowed by the mesh. Both the natural abundance and petiole injection of labelled ^{15}N tracked higher rates of N crossing into the wheat with increased root contact.

1.5.2 Timing of N availability

The objective of predicting and altering the timing of N availability to a cash crop is as relevant to N sourced from legumes as it is to N sourced from inorganic fertilizers. Availability of N in either case is predicated on movement and transformation of N, the presence or absence of water, C/N ratios, and microbial activity, all of which makes predicting availability a challenge. Soil N physically moves out of the rooting zone and is also transformed into different chemical forms, often resulting in net N losses. For example, a review conducted by Malhi et al. (2001) on fertilizer management in the Canadian Great Plains reported that because of immobilization, volatilization, denitrification, and leaching, fertilizer recovery efficiency in temperate regions was less than 70%. This is a startling figure when the costs of fertilizer and environmental damages are taken into consideration. These losses are partially due to environmental factors or soil management, but also to the poorly synchronized timing of fertilizer application and periods of plant demand.

Timed release of N is the topic of much research. In legume–non-legume intercrops, flushes of N into the soil have been reported in connection with setbacks in the aboveground growth of the legume, such as systematic defoliation or the use of herbicides. In a study focused on management options for biomass of white clover established with barley and then maintained as a living mulch

for two subsequent crops of wheat, Bergkvist (2003b) demonstrated that using a chemical control during wheat stem elongation provided a timely release of N from mineralization of fine roots and nodules during a critical stage of growth. While the wheat yields decreased in the first year, they increased in the second year. Grain N concentrations and inorganic soil N also increased as quantified in section 1.4.3. Louarn et al. (2015), intercropping white clover and alfalfa in a pot study with perennial ryegrass and tall fescue, also concluded that white clover, with its low C/N ratio and fine dense roots, released readily available N into the soil when the roots were mechanically severed. Nevertheless, in a pot and field study testing defoliation regimes, Sturite et al. (2006) noted that biomass management needs to be done carefully, as white clover that has taken too much damage will struggle to recover. If the goal is to maintain a living mulch, the clover may not survive the winter, particularly in northern climates.

1.5.3 Measuring N Increase

There are many methods of tracking net N increase. For the purposes of this study, net N increase is focused on fixed N that has been transferred to the non-legume in-season (total N accumulation as a product of plant N concentration x biomass) or made available in soil pools in season, as opposed to after termination.

Available soil N is typically measured as NO_3^- in the soil but another measure of N availability is N flux. This is a measure of N release to soluble inorganic forms (nitrate and ammonium) over a period of time using plant root simulator probes (PRS). It measures plant available N over time by exchanging anions and cations with the soil, similar to the action of soil roots (Western Ag Group of Companies, n.d.). Sturite et al. (2007) demonstrated the use of PRS® probes in a field study by subjecting white clover to two defoliation regimes; “frequent” (cut whenever it reached a certain height), and “infrequent” (with longer canopy recovery intervals). Anion and cation probes were

maintained over successive burial periods with the cation probe adsorbing NH_4^+ and the anion probe adsorbing NO_3^- . They found that more frequent cutting increased N mineralization but that N that had been released was rapidly taken up again by the plant and that the N that was released was from decomposition of young tissues as opposed to a release from the plant roots.

Sturite et al. (2021), comparing cut vs. uncut clover leys to cut vs. uncut mixed grass and clover leys, used PRS® probes (in addition to other above- and belowground N measurements) to demonstrate that denitrification still occurred in the soil through the winter months, especially during freeze-thaw cycles. Damaged plant tissues changes released NH_4^+ and NO_3^- into the soil (measured by the PRS® probes) and simultaneous gas chamber measurements confirmed that N_2O emissions peaked under the cool and wet freeze-thaw conditions.

Similarly, a ten-year study at three sites across Alberta and Saskatchewan and covering soil zones of Brown, Dark Brown and Black, also used PRS® probes to investigate soil nutrient fluctuations in plots seeded to crop rotations of continuous canola, canola-wheat, and canola-pea-barley (Town et al., 2023). The burial periods covered two weeks before flowering, during flowering, and two weeks after flowering. Soil nutrient fluxes in the monoculture were found to be highly variable by field location and microenvironment factors and no clear trends emerged, suggesting that characterizing nutrient movements is challenging in field environments.

1.5.4 Choice of main crops and legume varieties

Wheat and canola form a large part of the agricultural economy in the Canadian Prairies; research into compatibility of these crops with cover cropping is important if it is going to be adopted. In a four-year pilot project from 2020 to 2023 in Roblin, Manitoba, cover crops were established in the same row and at the same depth with both wheat and canola, with no impact on the main crop yield

(Frey, 2022). Most of the literature for seeding into a living mulch is from Europe. Winter wheat is a common choice for seeding a cash crop into a living mulch (Bergkvist, 2003b; Hiltbrunner et al., 2007) but spring wheat is often used as well (Carof et al., 2007). Winter oilseed rape was used as the cash crop in a living mulch trial in France (Lorin et al., 2015) and in Sweden (Bergkvist, 2003b). A number of research sites on the Canadian prairies have experimented with intercropping canola with larger-seeded legumes such as peas or fababeans (Khakbazan et al., 2025), but these are annual crops that will not provide a living mulch in subsequent years.

Canola deserves consideration as the cash crop to be seeded into the living mulch because of its dominance of seeded area on the Canadian Prairies. However, canola is known for its high N uptake (Nelson et al., 2022), and therefore the effective control of the legume biomass, both to reduce competition above and below ground and to ideally time the release of N into the soil, is especially important. The questions of how to manage the legume growth with the use of herbicide and how to effectively “time” the release of N from the clover by controlling legume biomass often arise in the literature (Bergkvist, 2003b; Holzapfel et al., 2007; Louarn et al., 2015; Osterholz et al., 2023).

White clover and alfalfa are often chosen as living mulch species for research (Adami et al., 2017; Bergkvist, 2003a; Carof et al., 2007; Leoni et al., 2024). Other common choices for cover cropping, though not necessarily for living mulch, include red clover and perennial ryegrass (De Notaris et al., 2025; Gaudin et al., 2013). Yellow sweet clover has many similarities to alfalfa, for the soils that it thrives on (loam soils and near neutral pH) and for its forage feed value (SARE, 2007); it is a lower-cost seed choice for organic growers seeding a green manure crop, and it is easier to terminate than alfalfa due to its a biennial habit (Blackshaw et al., 2010a).

1.6 Summary

Economic and environmental factors have reinvigorated interest in cover cropping and the creative use of legumes as a source of N in farming. Incorporating legumes in a systematic way to offset exogenous inorganic fertilizer N requires more knowledge than is currently available about how to predictably manage the environment so that plants compete or cooperate. Further, it is not clear how the N that is introduced to the system by legumes is, or is not, made available to the cash crops grown with or after them. Producers who grow cover crops or living mulches may need access to multiple management tools, such as special equipment for chemical or mechanical control of legume biomass, termination and incorporation of plant material into the soil, or infrastructure for animal grazing or forage harvesting. Successfully incorporating legumes into Canadian Prairie annual crop rotations is contingent upon a more thorough understanding of these issues in practice on a field scale.

1.7 References

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2. AGRONOMICS OF ESTABLISHING COVER CROPS AND THEIR PRODUCTIVITY

2.1 Abstract

In the Canadian prairie growing season of 90-110 frost-free days, cover cropping with nitrogen (N)-fixing legumes requires innovative strategies to allow sufficient time for cover crop establishment and biomass accumulation. Seeding the cover crop and cash crop at the same time in Year 1, maintaining the cover crop after harvest, and then seeding a second cash crop into the overwintered cover crop (now a “living mulch”) in Year 2 is one strategy to allow time for cover crop growth and N fixation between cash crops and throughout the subsequent growing season. Spring wheat and canola are two main cash crops of the Canadian Prairies. This study sought, in two phases, to compare 1) the establishment of five cover crop treatments (four legumes and one grass), each seeded in a single pass with wheat, 2) the impact of the cover crop treatments on the biomass, grain yield, and protein of wheat in Phase 1, and 3) the biomass and yield of canola seeded into the overwintered mulch in Phase 2. Field trials investigating cover crops in spring wheat–canola crop sequences were established in May 2023 and 2024 in six site–years at four locations. Cover crop treatments, including white clover (*Trifolium repens* L.), red clover (*Trifolium praetense* L.), sweet clover (*Melilotus officinalis* L.), alfalfa (*Medicago sativa* L.), and perennial ryegrass (*Lolium perenne* L.) as a non-legume cover crop control, were seeded in the

same row and at the same depth as wheat. Cover crop establishment varied among site–years, but generally, alfalfa had the greatest plant population and produced the most biomass. Wheat yield and grain protein were not affected by the presence of the cover crops in the establishment year. Cover crop biomass was low during the wheat growth period but was difficult to control during the canola growth period. Canola did not survive against living mulch growth in the second phase at two of the three site–years where it was seeded and could not be harvested, but it produced a harvestable yield at the third. Successfully seeding wheat and legume cover crops together without a penalty to the wheat is the most promising outcome of this research.

Keywords: cover crop, living mulch, red clover, white clover, yellow sweet clover, alfalfa, perennial ryegrass, spring wheat

2.2 Introduction

Effective cover cropping in the Canadian prairie context continues to be poorly understood due to the short growing season, the unpredictability of fall precipitation, and a lack of research (Penner, 2024). Further, much of the extension information, whether from social media or from presenters participating in speaking circuits, comes from regions with warmer climates, more precipitation, and longer growing seasons. The Farm Management Survey conducted by Statistics Canada in 2017 showed that the percent of farmers that used fall or winter cover crops as a land management practice was 19% in Quebec and 33% in Ontario, while Manitoba was 4% and Alberta was 2% (Government of Canada, 2023). Reliable data was not available for Saskatchewan. The lower figures for the prairie provinces likely reflect the generally cooler, drier, and shorter growing season. In 2021, the same survey reported an increase of farmers implementing cover crops in Quebec and Ontario (to 27% and 36%, respectively) and a proportionally greater increase in Manitoba (6%), Alberta (4%), and Saskatchewan (3%) (Government of Canada, 2023).

Successful cover cropping rests on the ability to establish a cover crop and allow sufficient time for growth with available growing degree days and moisture. In the fall, the latest date a farmer is advised to seed a cover crop on the Canadian Prairies is September 15; seeding after this date will not allow enough time for the crop to establish or provide benefits (Manitoba Agriculture, n.d. a). This early seeding deadline places additional constraints on farmers during an already busy harvest season.

According to the 2020 Prairie Cover Crop Survey (Morrison & Lawley, 2021), covering 281 farms practicing cover cropping across Manitoba, Saskatchewan, and Alberta, 30% of respondents identified the short growing season as the main impediment to cover cropping. Lack of fall moisture was cited by 27% of respondents. However, of these 281 practitioners, 71% reported seeing beneficial outcomes within three years and 35% reported benefits within one year (Morrison & Lawley, 2021). Rated from highest to lowest, these benefits included increased biodiversity (48%), increased soil organic matter (46%), decreased erosion (41%), and increased water infiltration (38%). Canadian producers have much to gain from targeted research in the prairie context, where strong winds in late fall, winter, and early spring can wick soil moisture and erode soil from bare ground (Government of Alberta, 2018). Maintaining growing plants late into the fall and early spring also imparts benefits in the form of depositing carbon in the soil, keeping active roots in the ground, and supporting thriving microbial populations (Penner, 2024).

Establishing a cover crop during the shoulder season, in late summer or early fall after the harvest of the main crop, is the approach often pursued by Canadian prairie farmers: 47% according to the 2020 Survey (Morrison & Lawley, 2021). However, with limited fall moisture, this establishment method carries a risk. Therefore, in an attempt to access moisture earlier in the season, 37% of cover cropping farmers establish their cover crops as an intercrop (Morrison & Lawley, 2021),

either by relay or double cropping using broadcasted seed, or seeding alternate rows in multiple passes on the field (Mbanyele et al., 2024). Both cover crop establishment methods have disadvantages: broadcasting causes uneven distribution of seed, and multiple seeding passes can cause soil compaction. Another option for seeding intercrops is to seed the two crops together at the same time, in the same row, and at the same depth. This method of establishing cover crops was tested from 2020-2022 at the Parkland Crop Diversification Foundation in Roblin, Manitoba, where red clover, white clover, and yellow sweet clover were seeded together with spring wheat, oats, barley, and canola as cash crops in separate trials (Frey, 2022). Even under drought conditions, Frey reported no significant effect on cash crop yields, when compared to cereal-only controls and cover crops continued to produce biomass in the subsequent year (5–14 Mg ha⁻¹ over three cuts).

Inter-seeding a cover crop and a cash crop introduces competition for nutrients and spatial variability of resource acquisition. Above ground, crop height and leaf area affect access to light. Below ground, access to water and nutrients at different soil depths is determined by the root zone and morphology of each crop type (Mbanyele et al., 2024). Establishing conditions for complementarity, rather than competition between the intercropped cover crop and cash crop, is accomplished either by selecting intercrop species that differ in their resource needs or staggering timing of development relative to the cash crop (Dzvene et al., 2023). In the cereal-legume intercrop described by Frey (2022), spatial and temporal variation could be manipulated by establishing conditions for the development of different canopy structures and root systems. as described by Mbanyele et al. (2024). This spatial and temporal advantage gives the cash crop preferential access to the critical resources of sunlight, soil nutrients, and water during the main growing season (Dzvene et al., 2023). Although the slower-growing intercropped cover crop

legume is initially suppressed by the more vigorous cash crop, after harvest it has open access to above- and belowground resources (Gaudin et al., 2013).

Identifying the ideal seeding rate and seeding depth for seeds of different sizes, as well as compatible herbicide options for weed control when combining grasses and broadleaves, present complex decisions to be made by the grower when intercropping cover crops with cash crops (Mbanyele et al., 2024).

Legumes are not strong competitors for soil N when compared to their non-legume companion crops. Consequently, in a legume–non-legume intercrop, where one plant can biologically fix atmospheric N and the other cannot, beneficial competition can stimulate the legume to increase N fixation (Mbanyele et al., 2024; Szumigalski & Van Acker, 2006). In interviews with early adopters of pea-canola grain intercropping in Manitoba, Chalmers (2015) found that a modest application of fertilizer N at seeding (34 kg ha⁻¹) was effective for boosting canola yields, while not providing enough N to reduce biological N fixation by the peas.

Seeding a second cash crop into an established cover crop is not a well-known practice on the Canadian Prairies, but it has been investigated in other northern growing regions such as Sweden (Bergkvist, 2003a) and throughout Europe and Africa (Adami et al., 2017; Carof et al., 2007; Cougnon et al., 2022; Hiltbrunner et al., 2007). It is used more frequently in fruit orchards than field crops as a means of weed management (Sosna et al., 2023). Using perennial species of cover crops to maintain living roots in the ground between cash crops is desirable for soil health, nutrient storage, and increased organic matter (Cougnon et al., 2022; Dzvene et al., 2023; Gaudin et al., 2013; Sosna et al., 2023). A living mulch that is both maintained and strategically contained throughout successive cash crops is difficult to achieve in practice, but if it could be done right, it would be a useful option on the Canadian Prairies.

Cougnon et al. (2022) present a review of the current literature regarding the use of perennial plants as living mulch cover for winter cereals and document the methods used by growers to constrain mulch growth and reduce competition with successive cash crops. These methods include manipulating sowing time, density, and spatial arrangement of the main crop and living mulch crop; suppressing living mulch biomass mechanically or chemically; and selecting appropriate crop varieties.

Chapter 3 expands upon potential N contributions from a legume living mulch to the intercropped cash crop. In a legume intercrop, flushes of N into the soil have been reported in connection with setbacks in the legume top growth, such as systematic defoliation from mowing or the use of herbicides (Bergkvist, 2003a; Louarn, et al., 2015; Osterholz et al., 2023). Timing defoliation events with critical periods of growth in the main crop can provide N supply exactly when it is needed by the cash crop and also decrease interspecies competition (Carof et al., 2007). Root contact has been shown to play a role in nutrient transfer (Xiao et al., 2004a) as well as arbuscular mycorrhizal fungi and rhizobium bacteria (Meng et al., 2015).

White clover and alfalfa are recurring choices for living mulches (Adami et al., 2017; Bergkvist, 2003a; Carof et al., 2007, Hiltbrunner et al., 2007; Louarn et al., 2015) whether in Europe, North America, or Africa. Each of these species has very different rooting patterns and plant structures. White clover has an initial transient tap root which transitions to shallow fibrous roots, while alfalfa has a tap root that penetrates deep into the soil (SARE, 2007). Above ground, white clover has a prostrate growth habit and spreads by sending out stolons, while alfalfa grows tall and bushy. On the Canadian Prairies, other legumes that are grown for forage or green manures include yellow blossom sweet clover or red clover. Yellow sweet clover is a biennial with a deep, branching taproot that begins to contribute fixed N into its environment in the year after establishment

(SARE, 2007). If terminated before blossom, 80% of plant N in sweet clover can be released into the soil because of the high concentrations of N present in the young plant parts during vegetative development (SARE, 2007). Sweet clover has been identified as a low-cost green manure crop that produces abundant biomass in a semi-arid climate (Blackshaw et al., 2010a). In Ontario and parts of the Canadian Prairies, red clover, a short-lived perennial, has historically been inter-seeded with winter wheat. This practice has fallen out of use with the evolution of industrial agriculture (Gaudin et al., 2013), perhaps because red clover stands are often patchy, leading to unpredictable fertility contributions (Loucks, 2017). All these legumes have the potential to produce abundant biomass, but require strategic management as an intercropped cover crop or living mulch.

The objectives of this study were a) to compare establishment and biomass of four intercropped legume cover crops and one intercropped non-legume cover crop in four regions of Manitoba by seeding them in the same row at the same depth as spring wheat; b) to compare the establishment, biomass, grain yield, and grain protein of the wheat cash crop grown together with the different legume cover crop species; and c) to compare the establishment, biomass, and yield of canola sown into the overwintered living mulch. It was hypothesized that a) as long as the cover crops establish and persist to the end of the season, they will grow abundantly after the wheat harvest, but performance will differ across sites due to differences in soil and climate; b) the wheat cash crop will be unaffected by the presence of the cover crop, due to the competitive advantage of wheat's quicker establishment; and c) canola can be established in living mulch but biomass and yield will be reduced in mulch treatments with more biomass, due to increased competition.

2.3 Materials and Methods

2.3.1 Experimental Design

The experimental design at each site was a randomized complete block design, with four replications. The experimental treatments included four legumes (white clover, red clover, sweet clover, and alfalfa) and one non-legume control (perennial ryegrass) plus three no-cover-crop control treatments, at six site–years for a total of 192 experimental units. The inclusion of a grass species allows for the assessment of N contributions by the legumes. The three no-cover-crop controls were established with identical management during Phase 1 to allow for manipulation of added N fertility in Phase 2. The plot areas at each site were: Arborg, 32.9 m² (8.0 × 4.11 m), Carberry, 36 m² (10.0 × 1.2 m), Melita, 41 m² (9.5 × 1.44 m), Roblin, 32.4 m² (9.0 × 1.2 m). Field trials investigating a spring wheat – cover crop establishment system were seeded in May 2023 at Arborg, Carberry (Carberry-1), Melita, and Roblin (Roblin-1), and in May of 2024 in Carberry (Carberry-2) and Roblin (Roblin-2).

The trial included two phases at three site–years (Arborg, Carberry-1 and Roblin-1), and only one phase at the other three site–years (Carberry-2, Melita, and Roblin-2). Phase 1 of the experiment was cover crops established with spring wheat and then overwintered. Phase 2 was canola seeded into the overwintered cover crop (termed living mulch in Phase 2). Phase 1 lasted from wheat seeding until the following spring soil sample. Phase 2 began with canola seeding and ended at canola harvest. The experiments at the six site–years were established in 2023 and 2024.

2.3.2 Experiment Set up and Management

2.3.2.1 Pre-seeding Operations

Spring weed growth was controlled with a pre-emergence application of glyphosate (StartUp, Bayer AG, Leverkusen Germany) in Phase 1 in all site–years. Plots were not tilled prior to seeding and the seed was disk-drilled into standing stubble. The previous crop was canola at Arborg, Melita, Carberry-1, and Carberry-2; millet at Roblin-1; and an oat-barley mix at Roblin-2.

2.3.2.2 Seeding

Seeding rates were adjusted from thousand kernel weight and percent germination. The spring wheat seeding rate target plant stand was 250 pl m⁻², which is the low range recommended by Manitoba Agriculture (n.d. b). Cover crop seeding rates are listed in Table 2-1 and are on the high range for cover crops recommended by Imperial Seeds (Winnipeg, Canada). Clearfield® canola was seeded at 108 pl m⁻² which is a higher target plant stand than is recommended (Manitoba Agriculture, n.d. c). Clearfield® canola is resistant to AresTM SN with active ingredients imazamox and imazapyr.

In Phase 1, cover crops were seeded by placing the seed in the same row and at the same depth as the wheat seed. Seeding depth was approximately 2 cm and legumes were inoculated with Rhizobium bacteria prior to seeding. Each plot consisted of three passes of the seeder (five or six rows per pass, depending on the site), leaving the two outer passes for destructive sampling and the middle pass for determining yield.

In Phase 2, Clearfield Canola (Corteva Agriscience, Indianapolis, USA) was seeded into the cover crops (living mulch) on the opposite side of the crop row as the previous year's sideband of N. Clearfield® canola was chosen on the basis of previous research from the University of Manitoba comparing cover crop persistence under the herbicides used for LibertyLink® canola (BASF, Ludwigshafen, Germany), Roundup Ready® canola (Bayer Crop Science, Monheim am Rhein, Germany), and Clearfield® canola (Gawiak, 2026).

Table 2-1 Variety names and seeding rates for cover crops and cash crops in a two-phase cover crop system

Phase	Plant Species	Variety	Seeding rate	
			kg ha ⁻¹	seeds m ⁻²
1	Alfalfa	Stellar	14	219
1	Red clover	Double cut, variety not stated	11	139
1	Sweet clover	Yellow Blossom	11	211
1	White clover	Bombus	6	263
1	Perennial ryegrass	Soraya	14	203
1	Wheat	Landmark	105	277
2	Canola	Clearfield var. P508MCL	7	120

Seed density per m² was calculated based on the mass of the seed applied and the thousand kernel weight.

2.3.2.3 Fertility

In Phase 1, granular urea was applied to all plots to meet a target wheat yield of 4700 kg ha⁻¹ (70 bu ac⁻¹), based on a recommended total N supply of 2 lb of N per bushel of expected yield (33.4 kg of N per 1000 kg of expected yield). Urea was mid-row banded at Arborg and side-banded at the other five site-years. Table 2-2 lists background and added N fertility for each site. Starter phosphorus (P) was applied as seed-placed monoammonium phosphate: 17 kg ha⁻¹ in Arborg; 39 kg ha⁻¹ in Carberry-1; 17 kg ha⁻¹ in Carberry-2; 28 kg ha⁻¹ in Melita; 28 kg ha⁻¹ in Roblin-1, and; 17 kg ha⁻¹ in Roblin-2.

In Phase 2, the three no-cover-crop control treatments received different rates of N fertilizer, as urea, in the canola year to allow comparison of N supply from the legume cover crops. The 100% N treatment received N fertilizer to bring total N supply (soil nitrate-N to 60 cm plus fertilizer N) to 196 kg N ha⁻¹, based on a target canola yield of 3370 kg ha⁻¹ (50 bu ac⁻¹). The N application rate in the 50% N treatment and the cover crop treatments brought total N supply to 98 kg ha⁻¹ (50% of the full N supply). The 0% N rate received no N fertilizer but received a small quantity of N in the monoammonium phosphate starter fertilizer (approximately 2 kg N ha⁻¹). This small amount of N was also taken into consideration when calculating N rates for the other treatments. The N

application rates in Phase 2 are shown in Table 2-2. Due to imprecise equipment calibration, applied N was $\pm 3 \text{ kg ha}^{-1}$ of the target.

Table 2-2 Phase 1 and 2 background and added N fertility at each site–year

Site-year	Phase 1			Phase 2				
	Soil Nitrate, 0-60 cm	N fertilizer application rate, all treatments	Total starting N	Soil Nitrate, 0-60 cm	N fertilizer application rate			
					Cover Crops	100% N Control	50% N Control**	0 N control
	kg ha ⁻¹			kg ha ⁻¹				
Arborg	112	44	156	56	42	140	42	0
Carberry 1	50	112	162	74	24	122	24	0
Carberry 2	73	87	160	-	-	-	-	-
Melita	34	123	157	-	-	-	-	-
Roblin 1	43	112	155	25	70	168	70	0
Roblin 2	25	132	157	-	-	-	-	-

2.3.2.4 In-crop Herbicide

In-crop herbicide was applied three to four weeks after seeding. At Arborg and Melita, the in-crop herbicides both had Bromoxynil as the active ingredient. At Arborg, the product was Pardner (Bayer Crop Science, Leverkusen, Germany) with an active ingredient concentration of 280 g L⁻¹ and it was applied at a rate of 1 L ha⁻¹. At Melita the product was Koril with an active ingredient concentration of 235 g L⁻¹ (Nufarm Agriculture Inc, Calgary, Canada) and it was applied at 1.2 L ha⁻¹. At Melita, herbicide application resulted in the termination of the cover crops and dropping the site from the study, but at Arborg, where emergence was delayed because of the dry seed bed, it had no visible effect on the cover crops. Roblin-1 and 2 and Carberry-1 and 2 were sprayed with Basagran (BASF, Ludwigshafen, Germany) with an active ingredient concentration of 480 g L⁻¹ at a rate of 2.0 L ha⁻¹.

The herbicide used with Clearfield® canola is Imazamox and Imazapyr (Ares SN, Corteva Agriscience). Application followed the product label recommendations: two true-leaf stage at a rate of 0.4 L ha⁻¹ with Merge added as a surfactant at a rate of 0.5 L 100⁻¹. The previous research at the University of Manitoba found that while Ares appeared to pause the cover crop growth, it eventually recovered (Gawiak, 2026) making it compatible with maintaining a living mulch.

2.3.2.5 Weather

The 2023 growing season was officially classified by the province of Manitoba as a drought year (Government of Manitoba, 2023); precipitation at all sites was slightly more than 60% of the 30-year average. In 2024 all site-years received above-average precipitation. Table 2-3 outlines environmental conditions for each phase. Weather data were taken from the Manitoba Agriculture (n.d. d) Weather Program. Weather stations for Carberry and Roblin are within 5 km of the research sites, Arborg is within 10 km, and Melita is within 52 km. In the specific context of the short growing season, using growing degree days (GDD) provides information on heat accumulation rather than just using average temperature. It is calculated by averaging daily maximum and minimum temperatures and subtracting the base temperature needed for seed germination. Base temperature is 5° for wheat, canola and forage crops in Manitoba. The Manitoba Ag Weather program provides GDD as a cumulative number made up of daily values between selected dates and long-term averages from 30 years of historical data.

2.3.2.6 Irrigation

The site with the lowest precipitation was Arborg and six weeks after seeding the experiment was irrigated. It received 13 mm of sprinkler irrigation on June 12 as well as subsurface irrigation through drainage tile throughout the growing season.

Table 2-3 Growing-season Precipitation and Growing Degree Days (GDD) during Phases 1 and 2 at each site–year, compared to Manitoba Ag Weather Program long-term averages

Precipitation (mm)													
Phase	Period**	Arborg*		Carberry 1		Carberry 2		Melita		Roblin 1		Roblin 2	
		Study year	Long-term Average	Study year	Long-term Average	Study year	Long-term Average	Study year	Long-term Average	Study year	Long-term Average	Study Year	Long-term Average
1	April	24	29	23	36	65	36	28	29	15	24	20	24
1	May	18	53	18	48	121	48	43	53	6	45	78	45
1	Jun	28	78	75	70	163	70	48	101	58	73	116	73
1	July	50	60	13	68	21	68	18	69	26	71	45	71
1	Aug	53	79	39	70	30	70	29	78	59	56	75	56
1	Sep	50	49	28	48	48	48	43	35	15	53	32	53
1	Oct	51	29	25	21	8	21	53	31	40	26	1	26
1	Apr–Oct total	274	377	221	361	456	319	262	396	219	348	367	348
1	Annual total	299	460	261	455	576	455	441	510	253	441	480	441
2	Apr	33	29	65	36	-	-	-	-	20	24	-	-
2	May	74	53	121	48	-	-	-	-	78	45	-	-
2	Jun	120	78	163	70	-	-	-	-	116	73	-	-
2	Jul	62	60	21	68	-	-	-	-	45	71	-	-
2	Apr–Jul total	289	220	370	222	-	-	-	-	259	213	-	-
2	Annual total	455	46	576	455	-	-	-	-	480	441	-	-
GDD													
Phase		Arborg		Carberry 1		Carberry 2		Melita		Roblin 1		Roblin 2	
		Study year	Long-term Average	Study year	Long-term Average	Study year	Long-term Average	Study Year	Long-term Average	Study year	Long-term Average	Study year	Long-term Average
1	Apr	7	18	7	12	57	12	10	24	2	7	32	7
1	May	299	205	309	184	181	184	315	205	279	172	152	172
1	Jun	443	337	454	335	318	335	445	351	413	314	242	314
1	Jul	373	434	365	425	461	425	413	453	331	392	427	392
1	Aug	396	387	398	387	384	387	422	415	371	354	347	354
1	Sep	299	190	303	190	369	190	266	211	287	163	319	163
1	Oct	80	22	84	25	102	25	95	40	70	11	59	11
1	Apr–Oct total	1897	1593	1920	1558	1872	1558	1966	1699	2124	1413	1578	1413
1	Annual total	1898	1595	1922	1562	1880	1562	1970	1702	1757	1415	2782	2552
2	Ph.2 Apr	36	18	57	12	-	-	-	-	32	7	-	-
2	Ph.2 May	177	205	181	184	-	-	-	-	152	172	-	-
2	Ph.2 Jun	320	337	318	335	-	-	-	-	426	493	-	-
2	Ph.2 Jul	750	885	461	425	-	-	-	-	427	392	-	-
2	Apr–Jul total	533	56	556	531	-	-	-	-	426	493	-	-
2	Annual total	1861	1595	1880	1562	-	-	-	-	1582	1415	-	-

* In addition to rainfall, the Arborg site received 13 mm of sprinkler irrigation on June 12 as well as subsurface irrigation through drainage tile throughout the growing season, resulting in optimal moisture conditions after June 12.

**Data for Arborg, Carberry-1, Melita and Roblin-1 is 2023 and 2024 for Phases 1 and 2, respectively. Carberry-2 and Roblin-2 Phase 1 data is from 2024.

2.3.3 Site Properties

2.3.3.1 Site Selection and Soil Properties

Plot-scale field studies were conducted at four Manitoba research stations. Sites were chosen for their unique soil characteristics and for the wide variety of prairie topography, weather patterns, and agricultural economies that they represent. All sites are located on the lakebed of ancient Lake Agassiz, and landforms in the province were largely shaped by the recession of glaciers and soil deposition through water action. The Prairies are considered the “breadbasket” of Canada, but the agricultural productivity of each region differs based on which part of the lakebed they were on, what types of deposits the glaciation left behind, and local weather patterns. Arborg today sits between several lakes that are the low-lying remnants of Lake Agassiz, with deep lacustrine clays and silt that hold high levels of water, only half of which is plant available due to the high affinity of the clay particles to adhere water (Samuel & Dines, 2023). Carberry lies at the site where the Assiniboine River formed a temporary delta with Lake Agassiz. Melita is the southernmost site in the study and lies along the escarpment of the former lake. Roblin is in the Prairie Pothole Region, which can be characterized as hydrologically isolated. Surface water in this region often accumulates in the scattered depressions left behind by glaciation that are colloquially known as “sloughs” (Brunet & Westbrook, 2012). Table 2-4 summarizes the differences in site and soil properties at the four sites.

Table 2-4 Province of Manitoba land survey data for the four experimental sites.

Location	Latitude and Longitude	Soil Series Name	Drainage	Surface Texture	Erosion risk	Topography	Salinity
Arborg	50.9 -97.2	Tarno and Fyala	drained	Clayey Lacustrine 60% clay	none or minimal	level to nearly level 0-0.5%	non saline 0-4 dS cm ⁻¹
Carberry	49.8 -99.3	Gregg	well drained	Loamy Lacustrine 40% clay	none or minimal	level to nearly level 0-0.5%	non saline 0-4 dS cm ⁻¹
Roblin	51.1 -101.3	Erickson and Petlura	80% well drained	Clay Loam 30% clay	none or minimal	gently rolling 2-5%	non saline 0-4 dS cm ⁻¹
Melita	49.2 -101.0	Two Creeks	Well to imperfectly drained	Loamy Lacustrine 40% clay	none or minimal	level to nearly level 0-0.5%	non saline 0-4 dS cm ⁻¹

Data for the land survey were drawn from the Canadian Soil Information Service (Agriculture and Agri-Food Canada, 1967, 1978, 1982, 1983)

Sites were chosen based on low soil nitrate-N in preliminary soil tests conducted the previous fall. One site (Arborg) had higher background nitrate-N in spring than expected based on the fall soil tests. Pre-seeding soil fertility tests were analysed by AGVISE Laboratories and included a full profile of soil nutrients and chemical properties such as pH, cation exchange capacity (CEC), electrical conductivity (EC), and organic matter (OM). Soil samples were collected as six soil cores per replicate block from two depths (0-15 cm and 15-60 cm) two or three weeks before seeding, composited as one sample per depth per block. Key soil properties are summarized in Table 2-5.

Table 2-5 Soil descriptions and chemical properties in spring of Phase 1 at the six experimental site-years

Soil Property	Unit	Arborg	Carberry-1	Carberry-2	Melita	Roblin-1	Roblin-2
Sand	%	17	41	41	67	41	41
Silt	%	17	32	32	18	28	28
Clay	%	63	27	27	15	31	31
N, 0-15 cm	kg ha ⁻¹	32	14	14	8	15	6
N, 15-60 cm	kg ha ⁻¹	79	34	48	25	30	16
Olsen P, 0-15 cm	ppm	25	10	33	13	15	29
K, 0-15 cm	ppm	480	187	384	223	218	180
S, 0-60 cm	kg ha ⁻¹	228	71	74	108	75	37
CEC	meq/100g	48	23	22	16	25	14
pH, 0-15	cm	8.0	6.6	6.3	6.8	7.0	6.3
EC, 0-15 cm	dS cm ⁻¹	0.77	0.28	0.20	0.17	0.25	0.13
OM	%	6.3	4.4	5.6	3.2	4.5	3.6

N – Nitrate; P – Olsen Phosphorus; K – Potassium cations; S – Sulfate; CEC – cation exchange capacity (summation of cations); pH – H⁺ activity in solution (1:1 soil:water); EC – electrical conductivity; OM – organic matter (loss on ignition)

2.3.4 Sample and Data Collection

Dates of data collection at each site are summarized in Table 2-6. Phase 1 was conducted at all six site-years, but only Roblin-1, Carberry-1, and Arborg proceeded to Phase 2. Not all measurements took place at Arborg because of distance between sites and time allocation of researchers. Data collection stopped at Melita after the fall biomass and soil sample date because of treatment failure.

Table 2-6 Summary of field operations and plant performance variables measured for cover crops, wheat and canola at each site–year

Data	Arborg	Carberry 1	Carberry 2	Melita	Roblin 1	Roblin 2
PHASE 1						
Stubble	Canola	Canola	Canola	Canola	Millet	Oat-Barley
Spring soil sample	8-May	28-Apr	25-Apr	28-Apr	27-Apr	24-Apr
Seeding date	23-May	12-May	14-May	10-May	12-May	14-May
Spring wheat count	30-May	30-May	24-Jun	23-May	29-May	06-Jun
Spring legume count	07-Jun	07-Jun	24-Jun	31-May	09-Jun	12-Jun
Herbicide application	14-Jul	19-Jun	20-Jun	01-Jun	21-Jun	17-Jun
Summer biomass date	09-Aug	24-Jul	30-Jul	24-Jul	03-Aug	29-Jul
Fall biomass date	18-Oct	20-Oct	15-Oct	20-Oct	17-Oct	10-Oct
Spring 2 soil nitrate-N	16-May	25-Apr	8-May	-	24-Apr	5-May
PHASE 2						
Canola seeding	23-May	30-May	-	-	24-May	-
Canola plant count	21-Jun	24-Jun	-	-	17-Jun	-
1 st biomass (spring)	27-May	24-Jun	-	-	17-Jun	-
Herbicide application	10-Jun	19-Jun	-	-	21-Jun	-
2nd plant biomass	-	15-Jul	-	-	12-Jul	-
3rd biomass	24-Jul	07-Aug	-	-	31-Jul	-
Canola harvest	11-Sep	-	-	-	-	-

During Phase 1, wheat and cover crop plants were counted from flagged areas, either 0.5 or 1 m (depending on crop variability) of two adjacent rows at two locations in each plot, for a total of 4 or 8 m of row per plot. Above-ground plant biomass for cover crops, wheat, and weeds was collected when the wheat reached soft dough stage, after wheat harvest, and for weeds and cover crops the following spring. Cover crops broke dormancy in mid-April of the following spring and had about five weeks of growth at Arborg before taking a spring biomass sample on May 27, and two months of growth before spring biomass sampling at Roblin-1 and Carberry-1 (mid-June). All biomass samples were collected from 1 m of two adjacent rows in two locations per plot at an approximate height of 10 cm, for a total of 4 m of row per plot (Table 2-6). Biomass samples were sorted by species after collection. All biomass material was oven dried at ~60°C until reaching constant weight (about 48-72 hours) and weighed to determine biomass dry weights.

Wheat grain harvest took place at the end of August. Spring wheat yield was assessed from small plot combine HarvestMaster weigh systems and Mirus software (Juniper Systems, Inc, Logan, UT). Grain moisture was adjusted to 13.5% moisture. Grain protein was measured using a Perten Inframatic 9500 NIR grain analyzer (PerkinElmer, Shelton, CN), which runs 10 independent cycles on 250 g of material and takes an average of those cycles with final protein adjusted for temperature and moisture (corrected to 13.5%).

Soil nitrate-N was sampled after wheat harvest in the late fall and the following spring. The sampling pattern was six soil cores per plot that were composited for each plot. Fall samples were for one depth (0-15 cm) and spring samples were taken from two depths (0-15 and 15-60 cm).

During Phase 2, canola emergence counts were conducted on 1 m of two adjacent rows in two locations per plot. Biomass samples were collected July 24 in Arborg, July 31 in Roblin-1, and August 7 in Carberry-1 (Table 2-6) following the same biomass patterns as Phase 1 (1 m of two adjacent rows in two locations per plot at an approximate height of 10 cm) for a total of 4 m of row per plot (Table 2-6). Samples were divided into mulch, canola, and weeds and processed in the same way as described for Phase 1 samples. On the same date, soil nitrate-N was sampled, using the same sampling pattern as the fall and spring. Canola harvest only occurred at Arborg. Yield data was collected electronically with the HarvestMaster weigh system using Mirus software.

2.3.5 Statistical Analyses

Data were analyzed in SAS ODA using the generalized linear mixed model procedure PROC GLIMMIX (SAS Studio, 2023) to determine treatment (and in some cases, site–year) effect on wheat and cover crop plant populations; wheat, cover crop, and weed (where applicable) biomass; wheat yield and protein; and canola biomass and yield (where applicable). Statistical analysis was

conducted by site–year for most tests because different management became necessary at each site–year due to differences in treatment success and because not all measurements were taken at all site–years. In Arborg, the fourth block suffered from spray drift from an adjacent field, and only data from the first three blocks were used in the statistical analyses. At Carberry-1, the cover crops in three treatments (red clover, white clover and perennial ryegrass) did not persist past emergence and were removed from the data set after emergence counts. For the purposes of Phase 1, only the 100% N control treatment was used in the statistical analysis.

In tests that compared all six site–years, site–year was treated as a fixed effect with block nested within site–year as a random effect. In tests not comparing site–years to each other, block was a random effect. Plant biomass, taken at three points in Phase 1 (late July, late October, and the following spring) and Phase 2 (late July), added time to the model as a fixed factor, using repeated measures analysis and was analyzed using spatial power structure for the unevenly spaced time points.

Plant populations for wheat and cover crops were compared among all six site–years. Wheat biomass, and wheat yield and protein tests were compared at five site–years (after the failure at Melita). Canola establishment was compared at the three site–years where the experiment was continued to Phase 2.

Repeated biomass data were analyzed by individual site. Spring biomass was analyzed once for the repeated nature of the biomass samples over time, and it was also analyzed as one of the three individual sample timepoints that defined the sampling schedule of Phase 2.

Tests for common variance were conducted in PROC GLIMMIX for all tests and the denominator degrees of freedom were adjusted using the Satterthwaite method when the outcome was significant. The Kenward-Roger denominator degrees of freedom method was used for all repeated

measures analyses. Plant population followed a Poisson distribution. Biomass data followed either a normal or log-normal distribution. Yield and protein followed a normal distribution. Soil N data followed a lognormal distribution. Transformed data from non-normal distributions were back-transformed for presentation. For all data following a normal or log-normal distribution, Proc Univariate procedures were conducted to assess normality of the residuals. A probability < 0.05 was considered significant and treatment means were compared using a post-hoc Tukey-Kramer multiple comparisons test.

2.4 Results

2.4.1 Wheat establishment, biomass, yield, and protein (Phase 1)

2.4.1.1 Establishment

Wheat plant population was affected by an interaction between site–year and treatment. Dry conditions in 2023 and wet conditions in 2024 (Table 2-3) likely contributed to the significant main and interactive effects of site–year on wheat and cover crop plant population (Table 2-7).

Table 2-7 Effect of site–year and cover crop on Phase 1 wheat and cover crop establishment

Factor	Treatment	Wheat	Cover crop
		plants m ⁻²	
Site–year	Arborg	189 (5)*	29 (2)
	Carberry1	260 (6)	70 (3)
	Carberry2	257 (6)	202 (7)
	Melita	214 (5)	134 (5)
	Roblin1	79.1 (2)	150 (5)
	Roblin2	209 (5)	262 (9)
Cover Crop	Alfalfa	178 (5)	183 (6)
	Red Clover	186 (5)	83 (4)
	Sweet Clover	192 (5)	92 (4)
	White Clover	189 (5)	101 (4)
	Perennial Ryegrass	185 (5)	134 (5)
	Control	201 (5)	-
Source of Variation		p-values	
Site-year		<0.0001	<0.0001
Cover Crop		<0.0001	<0.0001
**Site x Cover Crop		<0.0001	<0.0001

*Values are least-squares means with the standard error of the mean in parentheses.

**Interactions are plotted in Figures 2-2 and 2-3.

Roblin-1 was the only site–year at which the wheat population was lower in all cover crop treatments than in the wheat-only control. Overall, wheat plant population was near the target at Carberry-1, Carberry-2, Melita, and Roblin-2, but was lower than the target at Roblin-1 and Arborg (Table 2-7), likely due to dry conditions during establishment. Wheat population was increased in Roblin-2 as compared to Roblin-1 with greater precipitation (Table 2-3). The interactions between site and cover crop are shown in Figure 2-1.

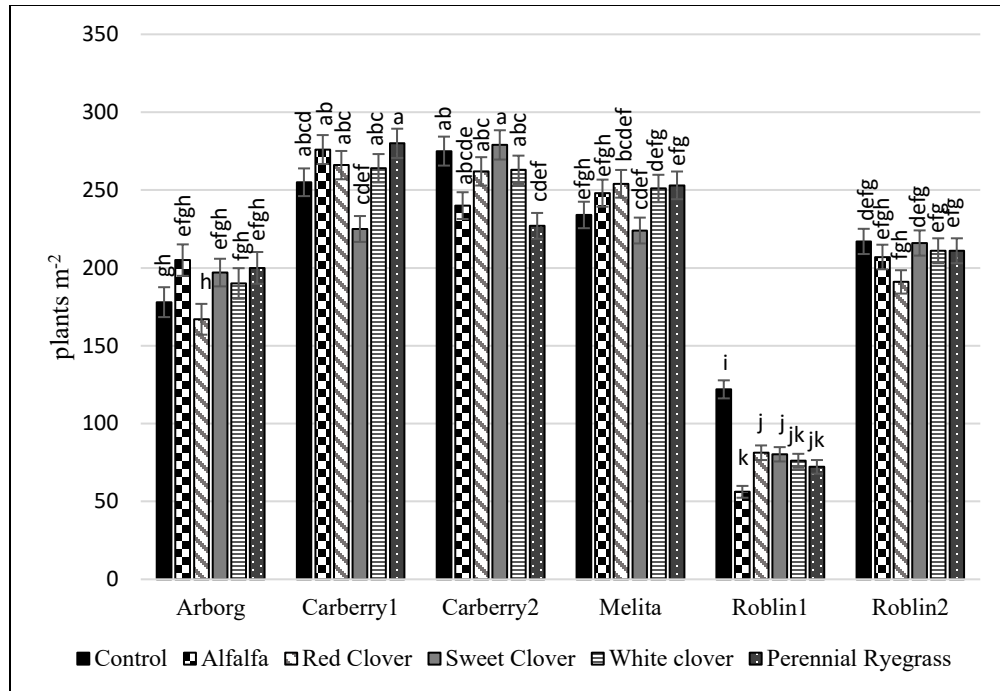


Figure 2-1: Effect of site–year and cover crop on wheat plant population at emergence. Letter groupings are based on the Tukey-Kramer multiple comparisons test and indicate significant differences between treatments for wheat plant population at emergence ($p < 0.05$). Error bars are standard error of the least squares mean. p-values: site < 0.0001 ; cover crop < 0.0001 ; site $\times$ cover crop < 0.0001

2.4.1.2 Wheat biomass, yield, and protein

At all site–years, wheat biomass, grain yield, and grain protein were similar among treatments with cover crops and the wheat-only control (Table 2-8). With no statistically significant penalty to the wheat from the cover crops, establishing cover crops by seeding at the same time and at the same depth as wheat appears to be a viable approach under the range of soil and environmental conditions tested. The initial wheat population advantage to the Roblin-1 wheat-only control treatment at emergence did not affect later performance metrics compared to the cover crop treatments.

Table 2-8 Effects of cover crop treatments and site–year on weed biomass, wheat biomass, and wheat grain yield and protein at peak wheat biomass in July at soft dough stage

Factor	Peak Wheat Biomass	July Weed Biomass	Wheat Yield	Wheat Protein
	kg ha⁻¹	Mg ha⁻¹	Mg ha⁻¹	%
Site-year				
Arborg	36.3 (8.10) c*	9.04 (0.34) b	6.11 (0.10) a	14.9 (0.20) a
Carberry1	176 (34.2) a	7.14 (0.31) c	2.58 (0.09) d	12.1 (0.20) c
Carberry2	171 (33.3) a	11.1 (0.31) a	3.19 (0.09) c	11.8 (0.20) c
Roblin1	149 (29.0) ab	7.21 (0.31) c	3.95 (0.09) b	13.1 (0.20) b
Roblin2	84.2 (16.4) b	7.84 (0.31) c	3.92 (0.09) b	11.1 (0.20) d
Cover Crop				
Control	118 (27.0) a	8.54 (0.30)	4.07 (0.09)	12.6 (0.10)
Alfalfa	128 (31.8) a	8.37 (0.28)	3.94 (0.09)	12.5 (0.20)
Red Clover	135 (18.8) a	8.17 (0.33)	3.82 (0.09)	12.7 (0.20)
Sweet Clover	77.9 (16.1) a	8.38 (0.33)	3.96 (0.10)	12.7 (0.20)
White Clover	74.4 (15.3) a	8.81 (0.25)	4.04 (0.08)	12.6 (0.20)
Perennial Ryegrass	127 (31.0) a	8.6 (0.47)	3.88 (0.09)	12.5 (0.20)
Source of Variation	p-values			
Site	<0.0001	<0.0001	<0.0001	<0.0001
Cover Crop	0.04	0.30	0.26	0.86
Site × Cover Crop	0.81	0.51	0.40	0.34

*Values are least-squares means with the standard error of the mean in parentheses. Letter groupings are based on the Tukey-Kramer multiple comparisons test and indicate significant differences between treatments biomass means at each site and sample date.

Significant differences in wheat performance were apparent between site–years (Table 2-8). Low yields in Carberry-1 are consistent with the drought conditions in this site–year. Those two sites also had significantly lower biomass at soft dough stage. Arborg wheat yields and grain protein were significantly higher than all other site–years. Carberry-2 had the highest wheat biomass at the soft dough stage but was among the lowest for wheat yield and protein. Roblin yields were statistically similar in both years, but the grain protein was higher in Roblin-1 than in Roblin-2. Weed biomass differed significantly by site–year and by treatment, although the Tukey test did not detect significant differences between individual treatment means. Both site–years at Carberry

had higher weed biomass than Arborg and Roblin-2, but neither Roblin nor Carberry were significantly different in the two years at their sites. No interaction was observed between site-year and cover-crop treatment.

2.4.2 Cover crop establishment (Phase 1) and biomass (Phases 1 and 2)

2.4.2.1 Establishment

Overall, initial cover crop plant populations were greater at Carberry and Roblin in 2024 (Carberry-2 and Roblin-2) than at those sites in 2023 (Carberry-1 and Roblin-1), and were lowest at Arborg (Table 2-7). Initial cover crop populations were affected by a treatment by site interaction caused by white clover. White clover established well in Roblin during both dry and wet years, and also in Carberry-2 and Melita, but did not establish well in either the dry conditions of Arborg or at Carberry-1 (Table 2-3, Figure 2-2). White clover plant populations at these two site-years were well below the seeded 263 seeds m^{-2} (Table 2-1) but exceeded 200 plants m^{-2} for both years in Roblin. Compared to the other sites, cover crop plant populations at Arborg were only 33-67% of the other sites for alfalfa, 15-44% for perennial ryegrass, 5-25% for red clover, 8-16% for sweet clover, and 8-16% for white clover (with exception of Carberry-1, where white clover population was similar to Arborg). Among cover crop species, alfalfa had the highest plant population among cover crop treatments across site-years (Table 2-7; Figure 2-4). The seeding rate for alfalfa was estimated to be 219 plants m^{-2} (Table 2-1) but at Carberry-2 and Roblin-2 both plant population counts reached almost 300 plants m^{-2} . This was likely a counting error in the field.

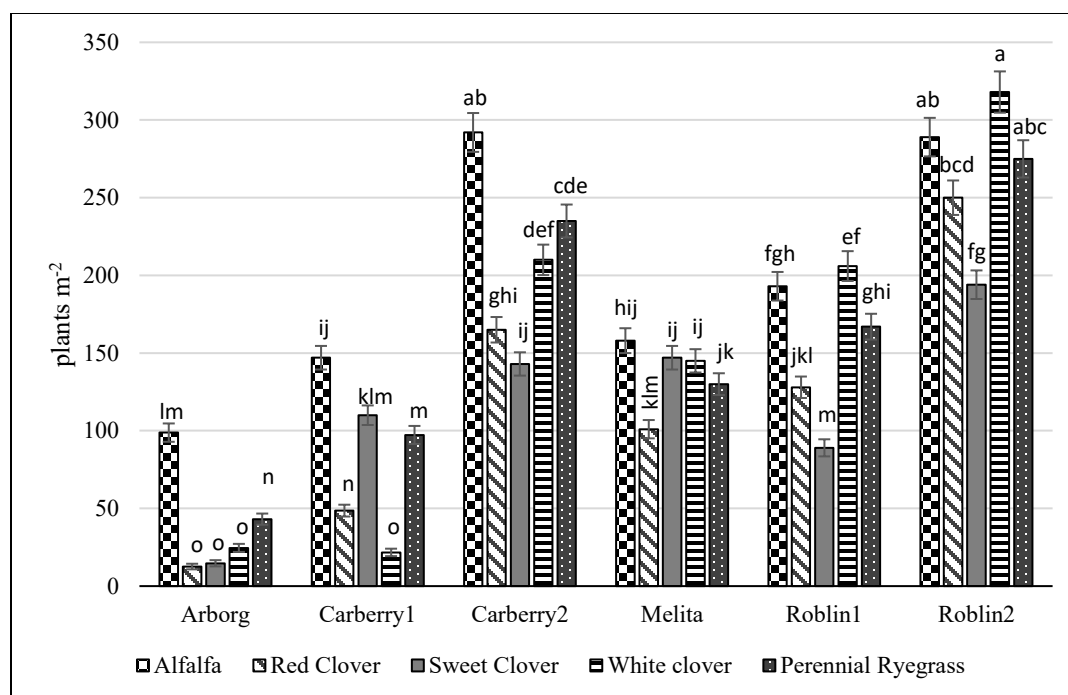


Figure 2-2 Effect of site-year and cover crop species on cover crop establishment.

Letter groupings are based on the Tukey-Kramer multiple comparisons test and indicate significant differences between treatments for cover crop plant population at emergence ($P < 0.05$). Error bars are standard error of the mean. P-values: site < 0.0001 ; cover crop < 0.0001 ; site $\times$ cover crop < 0.0001

Red clover and yellow sweet clover performed comparably to each other, but both cover crops had a lower proportion of the target plant populations at emergence (60% and 44% respectively) than alfalfa (83%). Perennial ryegrass established relatively well at all site-years except Arborg.

2.4.2.2 Cover Crop Persistence

At Melita, the cover crops did not survive the in-crop herbicide application. At Carberry-1, white clover, red clover and perennial ryegrass did not persist past emergence. For Carberry-2 and Roblin-2, no biomass data is available for the subsequent spring since these site-years did not continue to Phase 2. (Table 2-9).

Table 2-9 Effect of cover crop treatment and sampling date for Phase 1 and 2 cover crop biomass in Phase 1 July and October, and Phase 2 spring and July

Factor	Date/Treatment	Arborg	Carberry-1	Roblin-1	Carberry-2	Roblin-2
		kg ha ⁻¹				
Date	Phase 1 July	11.8 (2.92)*	68.3 (14.1) d	37.0 (6.89)	66.9 (14.0)	146 (11.9)
	Phase 1 October	185.4 (46.0)	271 (56.7) c	258 (48.0)	484 (102)	433 (35.4)
	Phase 2 Spring	58.2 (14.5)	3473 (725) b	1823 (339)	-	-
	Phase 2 July	117 (29.4)	7858 (1642) a	3841 (715)	-	-
Cover Crop	Alfalfa	425 (120)	823 (121)	1030 (201)	187 (59.0)	210 (27.1)
	Red Clover	80.0 (22.5)	-	738 (144)	266 (83.6)	430 (55.5)
	Sweet Clover	43.3 (12.2)	844 (124)	832 (162)	148 (46.5)	95.4 (12.3)
	White Clover	15.9 (4.76)	-	175 (34.2)	77.7 (24.4)	277 (35.8)
	Perennial Ryegrass	41.1 (11.6)	-	309 (60.2)	376 (118)	431 (55.6)
Source of Variation		p-values				
Date		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cover Crop		<0.0001	0.91	<0.0001	0.009	<0.0001
Date × Cover Crop		<0.0001	0.59	<0.0001	0.03	0.02

*Values are least-squares means with the standard error of the mean in parentheses. Letter groupings are based on the Tukey-Kramer multiple comparisons test and indicate significant differences between treatments and dates.

Cover crop biomass differed by sampling date in all site–years and by cover crop species at all site–years except Carberry-1 (considering only alfalfa and sweet clover at Carberry-1). In addition, a significant date by cover crop treatment interaction was present for all site–years except Carberry-1. The interactive effects of date and cover crop on biomass production over time are illustrated in Figure 2-3. At Carberry-1, no statistical date by treatment interaction was observed for the alfalfa and sweet clover cover crop treatments that persisted, but the failure of three cover crop treatments to establish could be considered an interaction, as cover crop biomass was zero for those treatments at the Phase 1 July biomass sample date.

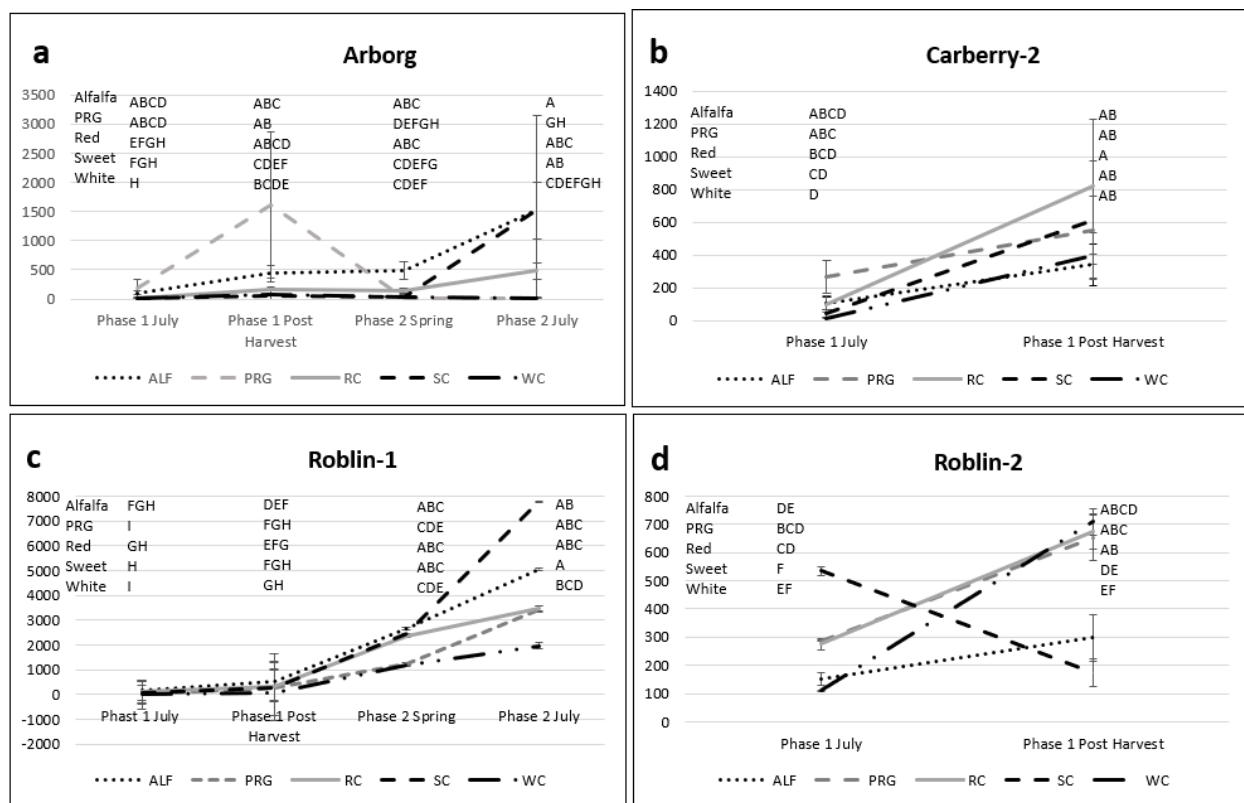


Figure 2-3: Effect of cover crop treatment and sampling date interaction on cover crop biomass at Arborg (a), Carberry 2 (b), Roblin 1 (c), and Roblin 2 (d). Cover crop biomass was taken at four time points in Arborg, Carberry-1 and Roblin-1, but at only two points in Carberry-2 and Roblin-2. P-values for each interaction: Arborg (<0.0001), Carberry-2 (0.03), Roblin-1 (<0.0001), Roblin-2 (0.02). Error bars are standard error of the mean. Letter groupings are based on the Tukey-Kramer multiple comparisons test and indicate significant differences between treatments for biomass at each sample date.

Cover crop biomass during the wheat growing period ranged from 11.8 kg ha⁻¹ (Arborg) to 146 kg ha⁻¹ (Roblin-2) but increased to a of range of 185 kg ha⁻¹ (Arborg) to 484 kg ha⁻¹ (Carberry-2) after wheat harvest (Table 2-9, Figure 2-3). Even a cover crop with very low biomass in July was able to increase biomass once the competition from wheat had been removed, as shown most dramatically in the perennial ryegrass treatment at Arborg (Figure 2-3). Fall post-harvest cover crop biomass only surpassed 1000 kg ha⁻¹ in the perennial ryegrass at Arborg. Among the legume cover crops, alfalfa showed the most consistent biomass production across site-years. Among the dry site-years (Roblin-1, Carberry-1 and during establishment at Arborg), alfalfa produced the

numerically highest aboveground biomass; however these differences were only significant at Roblin-1. Relatively low biomass was seen in red clover and sweet clover. Where red clover persisted under the wheat canopy, it also increased in biomass after wheat harvest. White clover showed good fall growth only at Roblin-2 (709 kg ha⁻¹).

Spring biomass data are available only for Arborg, Carberry-1, and Roblin-1 and were extremely variable among cover crops and site-years. At Carberry-1 and Roblin-1, spring biomass was significantly greater than the previous fall, with alfalfa and sweet clover producing the greatest quantity of growth (Table 2-9, Figure 2-3). At Arborg, spring biomass was similar to the previous fall, except for the perennial ryegrass which had the highest post-harvest growth but did not regrow in the spring due to winter kill (Figure 2-3). The lower spring biomass at Arborg as compared to Carberry-1 and Roblin-1 can be partially explained by the earlier sampling date relative to the other sites (Table 2-6). The highest-producing treatment (alfalfa) at Arborg produced only 13% and 18% of the same treatment at Roblin-1 and Carberry-1, respectively.

The large quantity of spring biomass in some cases demonstrates that even a cover crop with low productivity in the first year is capable of substantial spring biomass production the following year. At Carberry-1 the mean fall biomass in Phase 1 was 271 kg ha⁻¹, but by the next spring (June 24, just before herbicide application) the mean had increased to 3473 kg ha⁻¹.

2.4.3 Canola establishment, weed suppression, and yield (Phase 2)

The spring of 2024 was wet and cloudy, and at Carberry-1 and Roblin-1 this resulted in delayed, uneven canola emergence and it was difficult to determine when two-leaf stage had been reached across the trial because of uneven plant stages within plots. The subsequent postponing of herbicide application and plant counts (Table 2-10) allowed the mulch more time to grow and compete, further inhibiting canola emergence and growth. At Roblin-1 precipitation was 173% of

the long-term average in May and 158% in June (Table 2-3). At Carberry-1 May precipitation was 250% of the long-term average and June was 160%. Herbicide was applied at Carberry-1 on June 19 (20 days after seeding) and at Roblin-1 on June 21 (28 days after seeding). In contrast, the excess moisture was not as drastic in Arborg and canola reached the 2-leaf threshold for herbicide application 18 days after seeding on June 10, effectively gaining 9-11 days of paused mulch growth for the canola to grow into the suppressed mulch compared to Roblin-1. While Carberry-1 was closer to Arborg for timing, it also had the highest levels of precipitation of the three site-years.

Table 2-10: Effect of cover crop treatment on Phase 2 canola plant population at Arborg, Carberry-1 and Roblin-1

Treatment	Arborg	Carberry-1	Roblin-1
	plants m ⁻²		
0% N Control	84 (15)*	108 (34) bc	60 (5) a
50% N Control	85 (16)	159 (50) a	60 (6) a
100% N Control	106 (25)	115 (32) bc	53 (5) ab
Alfalfa	56 (10)	64 (20) d	45 (4) abc
Perennial Ryegrass	75 (12)	-	50 (5) abc
Red Clover	64 (15)	-	37 (4) c
Sweet Clover	66 (15)	88 (28) c	41 (4) bc
White Clover	82 (17)	-	46 (5) abc
p-value	0.15	<0.0001	0.0008

Control treatments are no-cover-crop canola at three levels of N fertilization regimens – 100% added N, 50% added N, and no added N. Values are least-squares means with the standard error of the mean in parentheses. Letter groupings are based on the Tukey-Kramer multiple comparisons test and indicate significant differences between treatment means for canola plant population

Due to the patchy and delayed emergence, the cover crops were large enough and resilient enough at the point of herbicide application that the herbicide had a brief yellowing, stunting effect, but otherwise left the living mulch unaffected (visual observation). Table 2-9 shows spring biomass accumulation before herbicide application (Table 2-9, “Phase 2 Spring”). Other than scattered individual plants, the canola that emerged in Carberry-1 and Roblin-1 did not persist.

The presence of the living mulch reduced canola plant population, relative to the controls, in some cases, but even in the control plots, the target population (108 plants m⁻²) was achieved at Carberry-1 and in the 100% N control at Arborg, but not in any of the controls at Roblin-1. At Carberry-1, the canola plant population was statistically lower in the alfalfa than in the sweet clover (Table 2-10) even though the spring biomass was comparable between those two cover crops (Table 2-11). At Roblin-1 canola plant population was only significantly lower than all three controls in the red clover and sweet clover but was very low overall (37–60 pl m²) in all treatments. At Arborg, the living mulch did not statistically reduce canola plant populations but the variability was very high, which renders the mean an imprecise representation of the individual data points (Table 2-10). All living mulch treatments reduced canola biomass relative to the 100% N control at Carberry-1 and Roblin-1 by several orders of magnitude and by mid-summer, at the late July sampling date, very little canola could be found (Table 2-11).

At Arborg, the alfalfa treatment produced the lowest canola biomass (Table 2-11), likely due to the greater living mulch biomass in this treatment, relative to the other cover crop treatments. The sweet clover treatment was the only other treatment that reduced canola biomass below the quantity produced by the 100% N control at Arborg (Table 2-11). Canola biomass in the red clover and white clover treatments was numerically lower but not significantly different from either the 100% N or 50% N canola controls.

2.4.4 Weed suppression (Phase 1 and 2)

The impact of the cover crop treatment on weed biomass at the peak wheat biomass stage in July of Phase 1 was significant by site–year with Carberry-2 having the highest weed biomass and Arborg having the lowest weed biomass (Table 2-11). During the canola phase the cover crop treatment significantly impacted weed biomass at all three sites (Table 2-11) at the 3rd biomass

date (Table 2-6). At the spring biomass date, only Carberry-1 and Roblin-1 were sampled, but there were no significant differences between treatments because of the variability. The variability in each of the treatments is an indicator that weeds grew in patches and were not evenly spread throughout the plots.

Table 2-11 Effect of cover crop treatment on Phase 2 canola, living mulch, and weed biomass

Treatment	Arborg			Carberry-1			Roblin-1		
	Canola	Mulch	Weeds	Canola	Mulch	Weeds	Canola	Mulch	Weeds
kg ha ⁻¹									
SPRING SAMPLE DATE									
0% Control	-	-	-	-	-	9.12 (3.98)	-	-	60.8 (96.1)
50% Control	-	-	-	-	-	11.7 (4.05)	-	-	7.89 (12.5)
100% Control	-	-	-	-	-	21.2 (8.93)	-	-	43.1 (68.2)
PRG	-	21.4 (45.4) b	not	-	-	-	-	1218 (261) bc	192 (305)
Alfalfa	-	475 (116) a	collected	-	3589 (909)	5.27 (1.57)	-	2660 (261) a	51.8 (106)
Red Clover	-	147 (47.5) ab	-	-	-	-	-	2364 (261) ab	75.3 (155)
Sweet Clover	-	67.3 (8.20) b	-	-	3427 (868)	6.35 (2.99)	-	2454 (261) a	32.1 (50.8)
White Clover	-	36.9 (10.1) b	-	-	-	-	-	720 (261) c	723 (1485)
p-value		0.0003			0.83	0.12		0.0006	0.32
LATE JULY SAMPLE DATE									
0% Control	914 (249) cd	-	192 (120)	5984 (2607) a	-	199 (104)	3567 (3421) a	-	28.4 (88.4) ab
50% Control	2288 (250) ab	-	42.0 (26.1)	5756 (2508) a	-	270 (141)	6672 (6399) a	-	44.0 (137) ab
100% Control	2656 (250) a	-	17.3 (10.7)	7743 (3373) a	-	138 (72.2)	4835 (4637) a	-	270 (843) a
PRG	2357 (250) ab	*	*	-	-	-	41.7 (40.0) b	3603 (930) bc	467 (1453) a
Alfalfa	517 (250) d	1473 (305) a	75.7 (58.0)	620 (270) b	6171 (1094)	177 (92.5)	22.4 (21.5) b	5178 (808) ab	7.05 (21.9) ab
Red Clover	1526 (250) abcd	467 (102) b	29.9 (23.0)	-	-	-	7.17 (6.80) b	3458 (312) bc	0.18 (0.57) b
Sweet Clover	1217 (250) bcd	1114 (388) ab	56.8 (43.8)	343 (150) b	10,164 (1535)	81.6 (42.6)	5.88 (5.64) b	7747 (672) a	22.2 (69.0) ab
White Clover	1806 (250) abc	23.5 (22.2) c	86.0 (66.3)	-	-	-	5.61 (5.38) b	1301 (452) c	685 (2133) a
p-value		0.004	0.12	<0.0003	0.12	0.53	<0.0001	<0.0001	0.017

*Arborg perennial ryegrass treatments not present in canola phase and failed treatments in Carberry were not included after emergence. **Letter groupings are based on the Tukey-Kramer multiple comparisons test. Phase 2 of the experiment included canola seeded midrow into four established legume cover crops and one non-legume cover crop. Reference treatment is solo-seeded canola at three levels N fertilization regimens – 100% added N, 50% added N and no added N. Values are least-squares means with the standard error of the mean in parentheses. Spring sampling date: Arborg (May 27); Carberry-1 (June 24); Roblin-1 (June 17). Late July sampling date: Arborg (July 24); Carberry-1 (August 7); Roblin-1 (July 31)

2.4.5 Canola yield

Canola yield, which could only be measured at Arborg, had similar Tukey groupings to canola biomass at this site (Table 2-12). The alfalfa treatment significantly reduced canola yield compared to all the controls, including the 0% N control treatment (Table 2-12). The yields of canola in perennial ryegrass, sweet clover, and white clover treatments were not significantly different than in the 50% N and 100% N controls, but the perennial ryegrass did not regrow in Phase 2 (Table 2-11).

The 50% N control had a higher quantitative yield than the 100% N control but the difference was not significant. Sweet clover, white clover, and perennial ryegrass all had a comparable canola yield to the 50% N control and only alfalfa had a significantly lower canola yield than the 100% N control.

Table 2-12 Effect of living mulch on Phase 2 canola grain yield at Arborg

Treatment	Yield
	kg ha⁻¹
0% Control	1075 (116)* c
50% N Control	2042 (102) a
100% N Control	1719 (102) ab
Perennial Ryegrass	1807 (102) ab
Alfalfa	338 (102) d
Red Clover	1391 (102) bc
Sweet Clover	1741 (102) ab
White Clover	1709 (102) ab
p-value	<0.0001

*Values are least-squares means with the standard error of the mean in parentheses. Letter groupings are based on the Tukey-Kramer multiple comparisons test.

2.5 Discussion

2.5.1 Effectiveness of cover crop establishment

Successful establishment of the cover crop can be judged by either the plant population or the biomass production. As hypothesized, the establishment and biomass of each cover crop varied by

site–year. Drought conditions in 2023 clearly impacted the establishment of cover crops at each location. The effect of drought was especially evident at Arborg, where initial poor emergence was not improved with subsequent irrigation and low cover crop plant population had the lowest biomass at all measurement dates and in all treatments, relative to the other site–years. Good seedling vigor and drought tolerance can be associated with speed of germination and seedling elongation (Loucks, 2017). In Ontario, post-emergent drought conditions have been shown to result in continued patchiness of red clover in fields intercropped with winter wheat even after the end of the drought (Loucks, 2017). Evaluating the recovery of white clover swards in New Zealand after a two-year drought, Knowles et al. (2003) found great variability during post-drought recovery related to the extent of damage caused to the white clover by the drought. Moderately damaged fields recovered within one year, but a field with 80% losses in plant population due to drought took longer to regrow and recovered only 20% of white clover biomass (Knowles et al., 2003).

At Carberry-1, three out of five cover crops failed to persist after emergence in Phase 1 because of the drought, whereas at Roblin-1 the cover crop biomass steadily increased from one sample date to the next. Qualitatively, soil in Carberry is loose to the touch and much easier to sample than Roblin, but soil tests from the spring of 2023 showed that fractions of sand, silt and clay were similar at these two sites in a 0-15 cm composite sample (Table 2-5). In the time between cover crop emergence counts and the peak biomass sample date, Carberry-1 received 12 mm less rain than Roblin-1 (88 and 100 mm, respectively) (Government of Canada, n.d.). Millet stubble at Roblin may have provided more protection from wind and temperature extremes (Cutforth & McConkey, 1997) for the legume seedlings than the canola stubble at Carberry-1, but straws were

sparse and individually scattered. In the final analysis, the reason for the difference in cover crop persistence between the two site-years is not clear (Table 2-9).

Deeply tap-rooted legumes, such as alfalfa and sweet clover, have better drought tolerance than shallow-rooted plants (SARE, 2007) and may be the reason why they survived the dry soil surface during establishment at Carberry-1. The high biomass achieved by these species in Phase 2 points to their suitability for the intercropping method of establishing cover crops even in dry conditions. Red clover is also tap rooted, but study in Minnesota found similar results to ours showing that alfalfa persistence in drought conditions was superior to red clover. The higher ratio of leaf to stem material in red clover caused greater rates of transpiration than alfalfa which was more devastating with low water supply (Peterson et al., 1992). When grown under irrigation, alfalfa consumes large amounts of water and yet it is also widely known for being drought tolerant because of the way it increases its root to shoot ratio, developing extensive and deep roots that are supplying fewer leaves (Anower et al., 2017). Some alfalfa cultivars decrease transpiration and water loss by producing less leaf area. The genetic trait that allows some alfalfa varieties to alter their physiology and thereby increase their water use efficiency is being widely investigated (Anower et al., 2017). Lack of moisture is one of the primary reasons cited by prairie producers not to grow cover crops (Blackshaw et al., 2010; Morrison & Lawley, 2021; Penner, 2024) but moisture availability is only one criterion for cover crop success. Even while producing variable biomass, because the cover crops in this trial faced drought earlier in the season, they had more time to recover from the drought and prepare for entering winter dormancy than if they had only been seeded in fall. For this same reason, relay cover cropping (rather than double cropping) is often seen as a way to increase the number of growing degree days and precipitation available to a cover crop before a killing frost (Thiessen Martens et al., 2001). Winter survival of the cover crop depends on storing

enough root carbohydrates and protein and this requires perennial forages to have five to six weeks of uninterrupted growth (Barnhart, 2012). If establishment is possible in spring as an intercrop while maintaining low cover crop biomass until wheat harvest, without hindering the wheat, then there is great potential for adoption, due to the simplicity of this approach.

2.5.2 Cover Crop Productivity

Fall (post-harvest) cover crop biomass in Phase 1 increased with the greater access to sun and moisture, but dry matter yields were usually below 1000 kg ha⁻¹. A relay cropped system in Manitoba with alfalfa and red clover seeded into winter wheat yielded between 605 and 1542 kg ha⁻¹ dry matter yield for red clover and 102 and 1193 kg ha⁻¹ for alfalfa (Thiessen Martens et al., 2001). Winter wheat is harvested earlier than spring wheat, meaning that the cover crops have more growing time after harvest, but the evidence from our trial indicates that similar cover crop biomass yields can be achieved following spring wheat despite the shorter season. Some forage crops on the Canadian Prairies are established with cereal “nurse” crops, such as oats or barley, to protect the young forages through their most vulnerable stage of development; however, the cereal is usually not brought to a seed harvest (Manitoba Agriculture, n.d. e). Although the use of a full-season nurse crop is not an officially recommended practice for establishing a forage crop (Leybourn, 2024), Phase 2 sweet clover biomass, even with the brief setback of the herbicide, reached 10,164 kg ha⁻¹ in August at Carberry-1 and 7747 kg ha⁻¹ in Roblin-1. Sweet clover in particular increases growth in the second year over the first year (SARE, 2007), as was seen in Phase 2. A review of red clover grown with winter wheat in Ontario found post-harvest biomass production that ranged from 600 to 4200 kg ha⁻¹ and 1510 to 5330 kg ha⁻¹ in spring (Gaudin et al., 2013). A fall biomass of 1700 kg ha⁻¹ was achieved for red clover sown between rows of spring barley in Denmark (De Notaris et al., 2025a). Our biomass was within this range for red clover in

fall at all site-years (except for the failure at Carberry-1). This range was also achieved in spring at Roblin-1.

This trial highlighted the challenges associated with herbicide management in cover crop and living mulch systems. Both herbicide-kill (Phase 1 at Melita) and inefficient herbicide efficacy (Phase 2 at Roblin-1, Carberry-1) occurred, underscoring the hesitancy that producers feel about herbicide management in such systems (Morrison & Lawley, 2021). As discussed in Section 2.3.3, application of herbicides with the same active ingredient (Bromoxynil) at both Arborg and Melita resulted in herbicide-kill at Melita but not at Arborg. A publication on Bromoxynil (200 g L⁻¹ active constituent) from Australia carries the warning to avoid spraying if anticipated daily maximum temperatures are greater than 20°C in the days immediately following application (Airr Apparent Pty Ltd, n.d.). Temperatures on the day of application and on the following day exceeded 20°C at Melita, so heat may have been a contributing factor to the death of the cover crops. Delayed emergence at Arborg may have been the reason for the cover crop survival, as discussed in the Materials and Methods; however, it is possible that the herbicide had more impact than was initially noted, since Arborg recorded the lowest plant populations and the lowest biomass at all sampling dates.

The issue of herbicide efficacy in Phase 2, where earlier application of Ares at Arborg showed greater efficacy than a later application in Roblin-1 and Carberry-1 (Table 2-6), underscores the importance of timing when relying on chemical control of a living mulch. Adami et al. (2017), experimenting with white clover tolerance to herbicides at three phenological stages in a living mulch system, found that younger plants with more active growth and thinner cuticle development were more susceptible to herbicide uptake. Plant age likely contributed to greater efficacy of herbicide control of living mulches in Arborg, where herbicide application took place 18 days after

seeding. At Roblin-1, there were 28 days between seeding and herbicide application. The goal for the management of the living mulch treatments was to temporarily inhibit growth of the mulch but not to kill it. Multiple combinations of mowing, brushing, or chemical control have produced mixed results across other trials (Carof et al., 2007; Dzvene et al., 2023). More research on the type of herbicide, the rate, timing of application, and combinations with mechanical control is needed to achieve the correct balance of suppression without termination.

Low mulch biomass at Arborg contributed to the conditions that allowed for the timely herbicide application in Phase 2 that did not occur Carberry-1 and Roblin-1, making Arborg the only site that produced a canola harvest in addition to the wheat harvest in Phase 1. Recommended herbicide application timing for Clearfield® canola is two true leaves (Corteva Agriscience, 2024), but blocked light and heat caused by the shade of the living mulch canopy resulted in delayed, non-uniform canola emergence and plant development at Carberry-1 and Roblin-1, and the decision to apply herbicide was delayed causing the problem to compound. The low living mulch biomass at Arborg may suggest that the cover crop seeding rates, which were already in the lower range of recommended forage monoculture seeding rates, could have been even lower. Bergkvist (2003) seeded a three-year white clover living mulch at 2.5 kg ha⁻¹ in Sweden with success. Our seeding rates were closer to what was used by White & Scott (1991) in New York for their living mulch treatments; 17 kg ha⁻¹ for alfalfa, 11 for red clover, and 5 for white clover. Hiltbrunner et al. (2007) in Germany used a seeding rate of 15 kg ha⁻¹ for a white clover living mulch. Since the low mulch biomass in Arborg played a role in more effective herbicide control (whether because of the early drought conditions, the Phase 1 application of the Bromoxynil, or winter kill) it is possible that a lower seeding rate could have established a more manageable mulch. However, previous research at the University of Manitoba in 2022 found that a targeted plant stand of 40 plants m⁻² was

insufficient to establish a cover crop as an intercrop, and that increasing the target plant stand to 100 plants m⁻² improved cover crop establishment and persistence (Gawiak, 2026)

2.5.3 Wheat performance in Phase 1 of the cover crop system

The lack of significant cover crop treatment effects on wheat performance supports the hypothesis that wheat would be unaffected by the cover crops during the establishment phase. Evidence that wheat can maintain high grain yield despite potential initial disadvantages is important since the main goal of growing wheat is profitability. At the only site–year where the wheat-only control demonstrated a significantly higher plant population than the cover cropped treatments (Roblin-1), the wheat grown with cover crops was able to compensate and no difference in wheat biomass, yield, or protein was seen between the control and the cover cropped treatments. The below-target wheat plant population at Arborg and at Roblin-1 can likely be attributed to dry soil conditions rather than to the cover crop treatments because there was no difference in wheat performance when comparing to the wheat-only controls.

The case of Arborg is unique because of the ideal moisture conditions maintained through irrigation after the first six weeks of extreme dryness and because of the higher background N that was present in the soil before establishment (Table 2-2). Arborg was second among site–years for wheat biomass production and achieved the highest wheat yields and wheat protein, both of which were significantly greater than the next best site-year. Sudden water availability, especially when plants are under drought stress, can cause a dramatic increase in the uptake of critical nutrients, such as N, when it is suddenly made available through mass flow and there is an increase mineralization activity of soil microorganisms (Peltier et al., 2023; Rakszegi et al., 2019). The starch-to-protein ratio of wheat is one reflection of moisture availability, especially during grain filling when carbohydrate is being transported and stored in the plant (Li et al., 2025). Carberry-2

and Roblin-2, having both been established in 2024 under sufficient moisture, had significantly lower protein content than in the drought years (Carberry-1 and Roblin-1). Given the lack of significant difference from the wheat-only control, the presence of cover crops is not the reason for this difference.

2.5.4 Canola performance in Phase 2 of the living mulch

Our results show that the growth performance and productivity of a second cash crop grown together with a living mulch were highly dependent upon biomass management of the mulch. This observation supports the hypothesis of the living mulch system: that effectively managing the biomass early in the season would allow the canola to access the resources it needs at the most critical times, but uncontrolled biomass will suppress canola growth. The herbicide application following canola emergence, which was intended to suppress the mulch long enough for the canola to emerge, create a canopy, and establish a competitive advantage over the mulch, was ineffective in achieving these outcomes for two out of the three site-years that included Phase 2. Canola was chosen as the second cash crop because of its dominance of seeded area on the Canadian Prairies; however, it is vulnerable to competition during early emergence and establishment because of slow early growth (Canola Council of Canada, 2022) compared to quicker growing cereals. Changing the order of the cash crop phases to take advantage of the earlier rapid growth of wheat may have made a difference. In a trial comparing winter wheat and fall rye seeded into legume living mulches, the rye demonstrated greater competition with the mulch due to early-season vigor and height (White & Scott, 1991). The complicating factors for controlling the mulch in this trial parallel real situations faced by producers, including cold and wet soils that result in uneven emergence, missed spray windows, and ineffective herbicide application (Hursh, 2025).

Arborg was the only site where canola could be harvested. The canola biomass yields in late July for treatments seeded into the mulch were not significantly lower than the no-living-mulch fertilized controls, with the exception of sweet clover and the 100% N control. The 50% N and 100% N controls had statistically similar biomass. In late July, canola biomass in mulch treatments was numerically lower than both the 50% N and 100% N controls by a substantial margin but because of high variability the white clover and sweet clover treatments both produced statistically comparable canola biomass to the 100% N control.

At harvest, canola seed yield was low in treatments with high spring mulch biomass (alfalfa) but high enough in the treatments with low spring biomass (sweet clover, white clover) to be equivalent with both the 50% N control and the 100% N control (Table 2-12) and the Riverton-Bifrost Municipality regional average canola yield (2019 to 2024) of 1816 kg ha⁻¹ (32.4 bu ac⁻¹) (MASC, n.d.). Of note is that the 100% N control yielded less canola grain than the 50% N control (1719 and 2042 kg ha⁻¹ respectively). The expected canola response to the higher N rate did not occur,

Since some of the living mulches had produced seed, it is possible that the canola yield samples collected by the plot combine contained some legume seed. This contamination issue may have inflated the canola yield in the sweet clover treatment, but not likely in the white clover treatment, given the low biomass and short stature of the plant. In each case, the lack of difference in canola biomass in the sweet clover and white clover supports the conclusion that the canola yields in these treatments were comparable with the 50% N control. Unfortunately, harvest samples of the canola were misplaced so sample contamination with legume seed could not be verified.

Biomass of the living mulch appears to have been pivotal to the performance of the canola, but it may have had more of an impact on the canola growth in the early part of the growing season than

in mid-summer. Although the spring biomass of sweet clover was significantly less than alfalfa, by late July its biomass was comparable to the alfalfa. While canola with alfalfa had lower biomass and yield at both sampling dates, sweet clover did not significantly impact canola biomass or yields (Table 2-11).

Production of enough living mulch biomass to benefit the soil, but not enough to harm the main crop, is a delicate balance. In New York, red clover and white clover living mulches showed the most potential to not decrease winter wheat and fall rye yields as compared to birdsfoot trefoil (*Lotus corniculatus* L.) and crown vetch (*Coronilla varia* L.), even while producing sufficient biomass to contribute N to the main crop (White & Scott, 1991). In France, Cougnon et al. (2022) found that a living mulch that exceeded 1 Mg ha⁻¹ of biomass negatively impacted yield. Conversely, an N fertilizer replacement (NFRV) trial in Europe found that 1 Mg ha⁻¹ was the number where residual N began to be positively impacted.

2.5.5 Producer goals for cover cropping and live mulching

Given the range of results observed in this research for cover crop type and site–year, it is important for producers to identify their objectives for cover cropping (Dzvene et al., 2023; Morrison & Lawley, 2021). This research has shown that a wheat crop may reliably be grown while establishing a cover crop. Following the wheat harvest, the producer has several potential opportunities: if the mulch is adequately managed, a canola crop may be seeded into the cover crop; if control is inadequate, the cover crop can be grown as a forage or for seed production, but these two outcomes require different markets and are not equal in terms of economic gain. In France, a threshold of living mulch biomass more than 1000 kg ha⁻¹ during wheat anthesis was identified as the point beyond which the mulch began to negatively affect yield (Cougnon et al., 2022). A cover crop that exceeds this threshold by time of main crop seeding may rather be grazed

by livestock or baled. It could also be terminated and then incorporated into the soil as a green manure. Managing the cover crop using herbicide in Phase 2 proved to be challenging, in part due to the differences at emergence in growth stage between the cover crop types. Managing a simple monoculture green manure field would likely be easier than a polyculture but would still present many of the same challenges. Both options of grazing or terminating the cover crop can increase soil fertility and organic matter. Incorporated alfalfa and red clover in Alberta produced higher yields in canola the following year than the no cover crop control treatment, demonstrating added fertility for the next crop under non-mulched management (Blackshaw et al., 2010a). Another option available to producers is to allow the cover crop to reach maturity and set seed in the year after establishment.

The more versatile a producer can be in cover crop management decisions, the more likely intercropping with legumes will be a good fit for their operation. If a producer has a flexible end goal, the simplicity of the establishment step allows them to inter-seed a legume cover crop without any wheat penalty even if they have limited knowledge of cover cropping. The flexibility allows them to decide what to do with their cover crop once it is established. The flexibility must be paired with the time and the resources to optimize their situation. For example, if a producer harvested canola with clover seed contamination, they would need to be equipped with seed separating equipment and the time to do the cleaning. They would also need access to both a canola seed buyer and a clover seed buyer.

Although best management practices for establishing forage crops do not advise bringing a cereal nurse crop to grain yield, it is still often used as a means of establishment (Leybourn, 2024; Manitoba Agriculture, n.d. e), and when fall or spring biomass of the forage is sufficient, a mixed grain and livestock producer could graze the available forage in the fall after freeze-up or in the

spring before moving animals onto summer pasture. Grazing has shown benefits to the soil, such as increasing organic matter and carbon cycling, promoting root growth and microbial activity, and returning nutrients to the soil through animal urine and feces (Augarten et al., 2023; Cardoso et al., 2025). In the event that abundant living mulch growth cannot be controlled mechanically or chemically, a mixed producer could use the mulch for forage, shifting away from the focus on growing an annual grain crop, such as canola, in the subsequent year.

If grazing is not an option, cutting the legumes as forage is also a possibility. Alfalfa yield data for Carberry-1 and Roblin-1 highlight the potential of the system to produce excellent forage yields. Average alfalfa dry matter yield in Carberry's local municipality of North Cypress-Langford from 2019 to 2024 was 3464 kg ha⁻¹ (MASC, n.d.), and the Carberry-1 dry matter yield was 6171 kg ha⁻¹. Similarly, the Roblin municipality regional average for alfalfa from 2019 to 2024 was 3497 kg ha⁻¹ and the Roblin-1 dry matter yield was 5178 kg ha⁻¹. Red clover at Roblin-1 was 3458 kg ha⁻¹ and perennial ryegrass was 3603 kg ha⁻¹. Small plot research yields tend to be higher than yields for field-scale operations, due to the intensive management maintained in small plots (White, 2019). Further, regional averages for forage crops include yields for older, less productive alfalfa stands with lower fertility, loss of physical harvest material, and unavoidable weathering while drying in the field (E. Nernberg, personal communication, December 29 2025). Therefore, while the small plot yields observed for Carberry-1 and Roblin-1 would likely be reduced when practiced on a field scale, these results nevertheless demonstrate the potential of the system.

The impact of the cover crop and living mulch on weeds during both phases of the experiment are consistent with the effect that they had on the main crops in the two phases: no impact during the wheat phase at any site-year, and suppression during the canola phase at Carberry-1 and Roblin-1, but no impact at Arborg. Using cover crops to manage weeds without suppressing the crop is

more common in orchards, but loss of yield due to competition is also a research concern in those settings and results are mixed (Sosna et al., 2023). In Iran, a crop of coriander was seeded at different densities into a living mulch of fenugreek (a legume), because of its poor competition against weeds. The researchers confirmed that while the mulch was effective at suppressing weeds, it also decreased coriander yields (Pouryousef et al., 2015).

Subterranean clover, with its prostrate growth and unique method of subsurface self re-seeding after being chemically controlled (SARE, 2007), has been recommended as a living mulch in no-till systems. In a study that tested subterranean clover as a living mulch for corn (grain and silage) over the course of three years, it was found to control weeds, provide N, require minimal maintenance, and didn't compete with the main crop (Enache & Ilnicki, 1990). White clover is popular as a living mulch for similar reasons, including low prostrate growth that is good for weed suppression but will not block light and a unique propagation method of surface creeping stolons (SARE, 2007) that allows it to quickly reestablish after chemical or mechanical control. White clover had the highest emergence population at both Roblin-1 and Roblin-2, and in Roblin-2 it also had the highest post-harvest biomass of all the treatments. However, for seeding and establishing a second successful cash crop into such a low, dense, canopy, it is even more imperative to create enough physical space so that light and heat can penetrate.

The canola performance increase that was expected from the 100% N canola control over the 50% N canola control did not occur. Canola emergence in the 50% N control exceeded the 100% N control at both Carberry-1 (159 and 115 pl m⁻² respectively) and Roblin-1 (60 and 53 pl m⁻² respectively). In the case of the Phase 2 late July canola biomass in Roblin-1, the 50% N treatment exceeded the 100% N treatment by more than 2000 kg ha⁻¹. The canola yield at Arborg was higher

in the 50% N control than the 100% N control (2042 and 1719 kg ha⁻¹, respectively) but the difference was not significant.

2.6 Conclusion

This comparison of legume species in a two-phased system of cover crop seeded with wheat followed by canola seeded into living mulch demonstrated that wheat can be grown with establishing cover crops without a yield penalty. This method of cover crop establishment uses heat and moisture when it is available earlier in the growing season, even in dry conditions, and reduces the pressure of fall seeding deadlines. Alfalfa consistently produced high levels of biomass. The effect of drought was seen in cover crop plant population and biomass but only at one site-year did wheat have a decrease in population when intercropped with cover crop treatments, an impact which the wheat was able to recover from so that there was no difference in yield. By all other metrics, wheat grown with the intercropped cover crops did not have any less biomass, yield, or protein compared to the control even under dry conditions.

Management of living mulch biomass was critical to the emerging canola plants and variability in canola emergence and development made timely herbicide application difficult. This suggests that both mechanical and chemical management would have been beneficial. All site-years out of six in Phase 1 produced a wheat harvest but only one out of three site-years in Phase 2 was brought to canola harvest. When canola failed at two of three Phase 2 site-years, alfalfa and sweet clover produced comparable forage yields to their respective regional averages at Roblin-1 and Carberry-1. At Roblin-1, red clover and perennial ryegrass also produced forage.

Finally, in keeping with the central finding of this research, this system allows producers to simultaneously grow wheat and establish a cover crop at the same time without an economic penalty. If the management capacity of the producer is high, and their goals are flexible, the system

provides a simple, cost-effective, and accessible entry point to cover cropping. More research is needed for living mulch management to make its continuous use in annual cropping feasible in the Canadian Prairies.

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3. NITROGEN DYNAMICS OF LEGUME COVER CROPS IN A WHEAT-CANOLA LIVING MULCH SYSTEM

3.1 Abstract

Sourcing plant nitrogen (N) for field crops by incorporating legumes as cover crops has long been a subject of interest in farming and research communities. This study sought to quantify in-crop N benefits from intercropped legumes to non-legumes in a two-year living mulch system at four Manitoba locations. Four legume treatments included white clover (*Trifolium repens* L.), red clover (*Trifolium praetense* L.), sweet clover (*Melilotus officinalis* L.), and alfalfa (*Medicago sativa* L.), and perennial ryegrass (*Lolium perenne* L.) was included as a non-legume control. Individual species were seeded together with wheat in a single pass during Phase 1 (six site-years), and in Phase 2 (three site-years) a canola cash crop was seeded into the resulting living mulch. Three solo cash crop controls received either no N, 50% N, or 100% N in Phase 2. Quantities of N from initial soil nitrate and in-season fluxes measured by plant root simulator probes (PRS), additions of inorganic N fertilizer, biological nitrogen fixation (BNF), and outputs from harvested grain, were measured, calculated, or estimated over two growing seasons. Wheat was grown successfully in Phase 1 at all site-years and all wheat N measurements showed that the legumes did not compete with wheat for N. The N introduced by BNF was not made available by any means

measured to either the wheat or canola, either by increasing plant N yield or by increasing soil N. Canola in Phase 2 was out-competed by the living mulch at two out of three site-years but was grown successfully at one site-year. Nitrogen budgets calculated at the end of Phase 1 showed the lowest N in the wheat-only control at two out of five site-years. A comparison of N pools in the alfalfa treatment and the 100% N control showed greater N in the control in Phase 1, but greater N accumulation in the alfalfa treatment over the course of Phase 2. More research is required on methods of biomass control of the legumes in Phase 2 to achieve both successful main crops and an agronomically important BNF contribution by legume living mulches.

3.2 Introduction

The development of large-scale, commercial farming with high-yielding varieties and hybrids on the Canadian Prairies has created a system requiring high inputs in order to continue maximizing yields. High inputs create high stakes in the form of economic investment and increases the potential for high losses. Losses come not only from the money spent on fertilizers and herbicides and lost opportunity cost, but also from leaks in the natural system resulting in lost inputs that are applied from the wrong source, or at the wrong rate, time, or place. Identifying means to reduce risky inputs by capitalizing on natural systems while maintaining, or at least balancing, productivity is an important research goal.

Cover cropping in general is of interest for building soil health. Legumes, specifically, can improve soil quality. The benefits of cover cropping with legumes include, but are not limited to: biological nitrogen (N) fixation (BNF), increased soil organic matter, improved water infiltration and retention, soil protection, improved soil structure leading to decreased erosion, weed suppression, increased cropping options in temperate regions, and higher grain protein content (Blackshaw et al., 2010; Thiessen Martens et al., 2001; Morrison & Lawley, 2021; Penner, 2024). Systems that

either rotate or intercrop with cereals, legumes, and oilseeds and are allowed to develop over many years contribute to efficient nutrient and water use, breaking disease and pest cycles, and increased productivity (Liu, 2009). Legumes in particular increase soil N supply and contribute to a thriving beneficial microbial population (Blackshaw et al., 2010; Brown, 1964; Cicek et al., 2014; Thiessen Martens et al., 2001).

The advantageous natural safeguards of cover cropping with legumes may help to lower costly inputs. Economic returns for cover crops are gained through decreased inputs such as synthetic fertilizers and reduced herbicides (Schipanski & Drinkwater, 2012a).

Given the recent increase in global conversation around the industrial-scale energy consumption of synthetic fertilizer production, N fertilizer losses through volatilization, leaching, and denitrification (Ranieri et al., 2021), and the accompanying targets of reduced N fertilizer application, the question of intercropping with legumes is receiving more attention. Offsetting inorganic fertilizer additions requires quantification of added N and a better understanding of the N dynamics that occur in legume–non-legume intercrops.

A further extension of a single-year intercrop of legumes and non-legumes is to maintain an established legume cover crop into subsequent growing seasons, thereby creating a living mulch with active roots that continues to contribute to soil organic matter, suppress weeds, fix N, and store nutrients (Cougnon et al., 2022; Dzvene et al., 2023; Gaudin et al., 2013; Sosna et al., 2023). While the practice is more common in orchards, it has also been investigated for its potential use with field crops (Adami et al., 2017; Bergkvist, 2003a; Carof et al., 2007; Hiltbrunner et al., 2007). Seeding a cash crop into living mulch is certainly the riskiest application of cover cropping for modern, high-input agricultural expectations. Nevertheless, there is historical precedent to investigate the practice. It was a very common practice in the global south prior to the 20th century

and was even acknowledged by European agronomists in the colonial era as having advantages over monocultures (Harwood, 2024).

There are three generally agreed upon outcomes for growing multiple crops together in the same field at the same time. These include: 1) Complementarity, where plants of different species make use of the same soil space and its attendant resources at different times, varying depths, and even different chemical forms (Htet et al., 2017); 2) Facilitation, where a mutually beneficial relationship between two or more species of plants that takes time to develop and mature (Schipanski & Drinkwater, 2012a); or 3) Competition, where plant interactions interfere with or stunt the other species' growth through competition for water, nutrients, and light (Mbanyele et al., 2024), resulting in decreased yields and financial loss.

A good example of an overtly complementary relationship is a study conducted in France by Louarn et al. (2015) where grasses intercropped with white clover showed improved Nitrogen Nutrition Index (NNI) already 3-10 days after cutting the white clover. White clover also released the N twice as fast as alfalfa.

Although competition resulting in economic loss is a strong disincentive to intercrop, a competitive system cannot be simply categorized as negative. Interplant relationships are very nuanced. Competition during establishment and at flowering is particularly detrimental because of the large quantities of N, water, and light required by plants at those times (Hiltbrunner et al., 2007) but competition can also encourage facilitative relationships to develop, as seen in a multi-year clover living mulch trial in Northern Europe where the mulch reduced wheat yield by 8% in the first crop of wheat, but then increased it in the second wheat crop by 9% when compared to winter wheat alone (Bergkvist, 2003b). Researchers also observed a 10 kg ha⁻¹ increase in inorganic soil N and a 4-34% increase in grain N concentration in the second year.

When growing legumes, substantial quantities of biomass are desirable for fixing N. However, when growing legumes and non-legumes together, it is necessary to give the main crop an intentional advantage by manipulating sowing time, density, spatial arrangement, and mechanical management; applying herbicides; and selecting strategic varieties (Cougnon et al., 2022; Gaudin et al., 2013; Mbanyele et al., 2024). If the legume is an established living mulch that will have a crop seeded into it, manipulating the level of competition through biomass control is necessary to create the time and space needed for the new crop to establish successfully. Various means of control, both physical (mowing, spring harrowing) and chemical, have been researched to contain growth of living mulches (Adami et al., 2017). Use of chemical control on white clover living mulch was found to be effective in increasing wheat yields, whereas mowing and spring harrowing were not effective (Bergkvist, 2003b). Natural setbacks, such as the use of legume varieties that are more sensitive to winter temperatures, are also useful, as seen in Sweden where rapeseed and winter wheat were sown into a white clover living mulch. White clover varieties with delayed spring regrowth reached peak biomass after flowering of the main crop, and were less competitive at critical time periods resulting in higher grain yields than in treatments intercropped with winter-hardy varieties (Bergkvist, 2003b).

In a legume–non-legume intercrop, one plant can biologically fix atmospheric N and the other cannot. However, the N fixer is known to fix less N in the presence of abundant soil N (Osterholz et al., 2023). Since legumes are not strong competitors for soil nutrients compared to their non-legume companion crops, competition becomes the incentive that stimulates the legume to fix N for itself (Szumigalski & Van Acker, 2006). Leaving N in the soil and instead fixing more atmospheric N is the “sparing effect”, whereby the poorly competing legume potentially fixes N in excess of its own needs, and makes more N available in the soil for uptake by the non-legume

in the process (Dowling et al., 2021). Even in the absence of all other benefits, legumes act as a nutrient bank when available N might otherwise be at risk of leaching out of the system, thereby making that N available later through mineralization of the legume residues (Szumigalski & Van Acker, 2006).

Belowground transfer of inorganic plant-available N from legumes to non-legumes happens by many pathways. In addition to N mineralization of soil N for uptake, mycorrhizal connections and direct root contact and intermingling provide exchange sites for translocating N (Dowling et al., 2021). Nitrogen can follow the concentration gradient from plants that are naturally high in N (legumes) to plants that are low in N (Xiao et al., 2004b).

The timing of N availability is important not only because of critical periods of uptake, but also because of the constant movement and chemical transformation of soil N as it interacts with soil water and changes forms through microbial action. Timed release of N from fertilizers is the topic of much research but a similar timed release from growing legumes is also possible (Holzapfel et al., 2007). In a legume–non-legume intercrop, flushes of N into the soil have been reported in connection with legume defoliation events (Bergkvist, 2003a; Louarn et al., 2015; Osterholz et al., 2023). White clover, with its low C/N ratio and fine dense roots, was shown to release waves of readily available N into the soil when those roots were severed from top growth (Louarn et al., 2015). Similarly, alfalfa has been shown to respond to top growth damage by shedding its nodules, which are then made available for mineralization (Osterholz et al., 2023).

Estimating BNF from legumes is one of the largest sources of error when using indirect means to estimating N contributions from legumes (Nimmo et al., 2013). Some methods of estimating N contributions from legumes include calculations of N budgets, N fertilizer replacement values (NFRV), or statistical regressions based on aboveground biomass or plant N yield. Nitrogen

budgets are useful to visualize how inputs and outputs add up, but they can significantly vary based on how BNF has been calculated. Nimmo et al. (2013) found that using an N balance method tended to estimate higher BNF contributions than $\delta^{15}\text{N}$ assessment suggesting that quantifying total N can vary depending on the method used by the researcher.

Nitrogen fertilizer replacement value (NFRV) is another method to estimate BNF. The N benefit from legumes is usually associated with decay of the residues and subsequent mineralization and so NFRV is an estimation of how much N fertilizer can be displaced in the growing season after a legume cover crop as N is mineralized from legume residues. It is calculated in a few different ways. Oats grown as a following crop after alfalfa, red clover, chickling vetch, and lentil in Manitoba showed that NFRV was greatest for alfalfa (51-62 kg ha⁻¹), approximately double the NFRV of red clover, even though red clover produced the most biomass (Thiessen Martens et al., 2005b). A trial in Denmark with red clover and perennial ryegrass also calculated NFRV and found that the year 1 fall biomass growth was a better predictor for the NFRV for the following crop than the following spring biomass. Positive residual N was only observed when dry yields in the fall exceeded 1 Mg ha⁻¹ (De Notaris et al., 2025b).

Another method for estimating N contributions from legumes is through established regression relationships between easily measured variables such as aboveground biomass or plant N content and measured fixed N. A review paper across multiple studies in 2015 found that 0.75-0.80 kg N was fixed in the aboveground biomass for every 1 kg of aboveground shoot N across legume forage species (Anglade et al., 2015). To extend the estimate from aboveground fixed N to include belowground the calculation includes a multiplier to account for belowground N.

Little is known about the N contributions from legume cover crops grown as living mulches in the Canadian prairie region. Therefore, the objective of this study was to quantify how much N was

added by BNF to a two-phase system of four legume cover crops seeded as separate treatments together with wheat in the first phase and with canola seeded into the established legumes as a living mulch in the second phase. The hypothesis is that the treatments including legumes will have a measurable increase in wheat and canola crop N yield, and/or in plant-available soil N accumulation, when compared to treatments without legumes due to physical belowground contact and N shedding by the legume after being set back by herbicide application in Phase 2. The effect will vary by site, by legume biomass production, and by effective chemical control of the legume species.

3.3 Materials and Methods

3.3.1 Conceptual framework for N dynamics in the living mulch system

To support understanding of the N dynamics in an annual crop system with living mulches, Figure 3-1 proposes a model for understanding how N pools shift between soil and plants across eight timepoints in the above-described phases when N-fixing legumes are added. Timepoint 1 shows a typical no-till Manitoba field at seeding. Nitrogen is found in the field residue, in the two depths of soil, and in the added fertilizer. At Timepoint 2 (wheat soft dough stage), legume fixed N is starting to enter the equation in both above and belowground legume biomass. Legume N is not seen in the wheat plant N because the legumes are still very small and are using fixed N for their own growth. Both plants still access soil and fertilizer N. Timepoint 3 (roughly six weeks after wheat harvest) depicts that fertilizer N has been depleted either by plant uptake or by other unavoidable losses (leaching and denitrification) or has been immobilized as organic N. With greater access to light and moisture the legume has increased growth and increased N fixation. It will also continue to scavenge soil nitrate. Not all wheat plant N has been removed with the grain because of the residue left on the field. At Timepoint 4 (the following spring) there is some increase

to soil nitrate because of mineralization of legume residues, but that N is also subject to losses from overland movement during spring melting and denitrification. Wheat residue N remains on the field but is also subject to losses during spring melt. Mulch N is a combination of soil N and fixed N from new growth. Timepoint 5 is at the point of seeding the next cash crop (canola). Fertilizer N has increased the soil pool, and mulch growth both above and below ground (roots and legume exudates) results in greater BNF. Timepoint 6 (canola 2-leaf stage) shows a transfer of mulch N from all pools into mulch exudate N and N from root death and nodule shedding because of the application of herbicide to halt, but not kill, mulch growth. Mulch N from these sources is available to emerging canola along with soil and fertilizer N. At Timepoint 7 (3rd biomass and soil sample), renewed mulch growth has occurred with an increase in N fixation over soil N due to competition with the canola. Canola shows an increase in N transferred from mulch fixation. Soil nitrate continues to be drawn down. Timepoint 8 (after canola harvest) shows that a significant quantity of N has remained on the field, even after the canola harvest, which would not be present if there was no mulch.

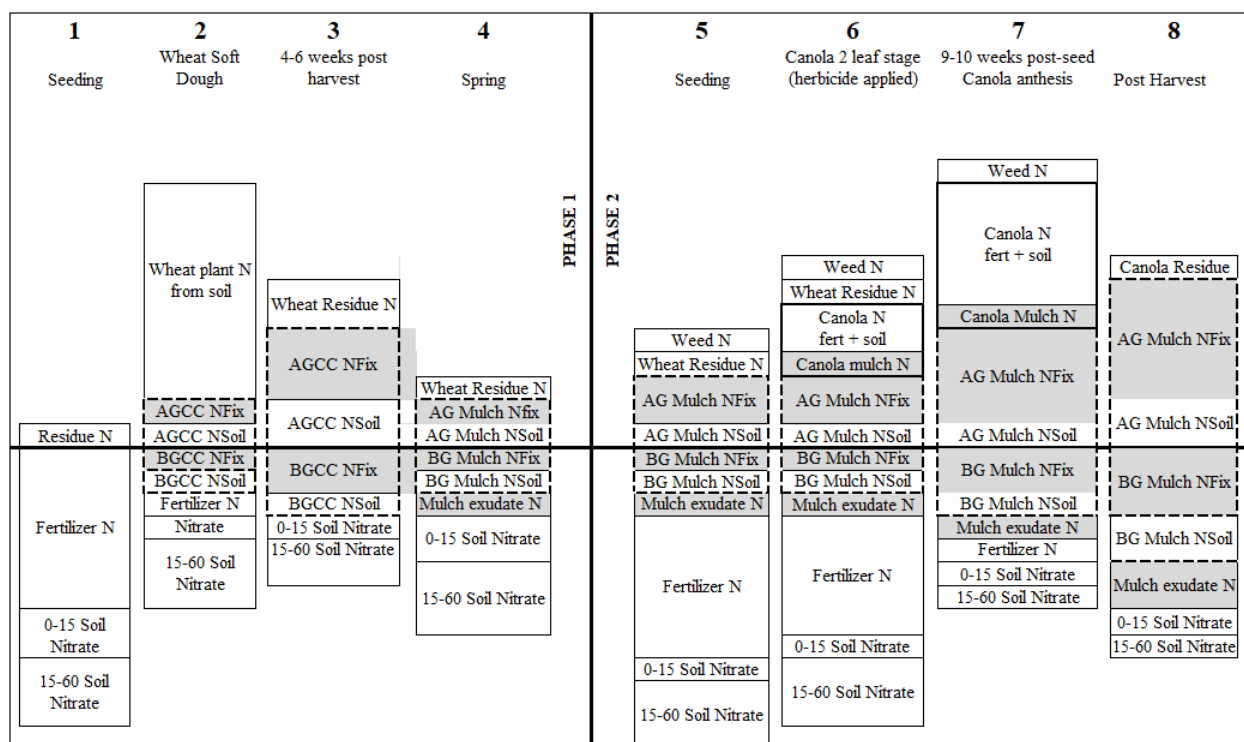


Figure 3-1 Model of N sources in a multi-year legume–cereal intercrop. N pools at eight timepoints over two years with spring wheat in Year 1, canola in Year 2, and a legume living mulch spanning both years. Solid line across the middle represents the soil surface. Boxes above and below the line represent N pools above or below ground. Dashed line represents N found in the legume cover crop. Shaded boxes specifically represent N pools from legume N. Thicker solid lines represent total N in the pool belonging to canola. Overlapped solid and dashed lines represent N transfer from legume to main crop. AGCC NFix is aboveground N sourced by cover crop fixation. AGCC NSoil is aboveground N in the cover crop that has been sourced from the soil. BGCC Nfix is belowground N sourced from cover crop fixation. BGCC NSoil is belowground N in the cover crop that has been sourced from the soil. AG Mulch NFix is N fixed by the aboveground mulch. AG Mulch NSoil is N taken up from the soil by the mulch.

3.3.2 Experimental Design

Four field trials investigating a spring wheat – cover crop establishment system were seeded in May 2023 at Arborg, Carberry (Carberry-1), Melita and Roblin (Roblin-1). Two additional trials were seeded in May 2024 at Carberry (Carberry-2) and Roblin (Roblin-2). The experimental design at each site was a randomized complete block design, with five cover crop treatments plus three no-cover-crop control treatments, and with site–year and/or time as additional factors for

some variables. The four legume treatments were white clover, red clover, sweet clover, alfalfa, and one non-legume treatment was perennial ryegrass (as a non-legume control to assess N contributions from the legumes). Three no-cover-crop control treatments receiving different fertility treatments (100% N, 50% N and no N) were also included. There were four replications at each of six site-years for a total of 192 experimental units. The no-cover-crop controls were established with identical fertility during Phase 1 but had different fertility levels in Phase 2. In Phase 1, only one control treatment was used in the statistical analysis.

The trial included two phases at three site-years (Arborg, Carberry-1 and Roblin-1), and only one phase at the other three site-years (Carberry-2, Melita, and Roblin-2). In Phase 1, cover crops were established with CDC Landmark spring wheat (FG Genetics, Regina, Canada) and then maintained over the winter. In Phase 2, Clearfield® Canola (var. P508MCL) by Corteva Agriscience (Calgary, Canada) was seeded into the overwintered cover crop (now termed living mulch). Clearfield® canola is resistant to Ares™ SN with active ingredients imazamox and imazapyr.

3.3.3 Experiment Set up and Management

3.3.3.1 Pre-seeding Operations

Pre-emergence weed control used glyphosate (StartUp, Bayer AG, Leverkusen, Germany). Plots were not tilled and the seed was disk-drilled into standing stubble. The previous crop was canola at Arborg, Melita, Carberry-1, and Carberry-2; millet at Roblin-1; and oat-barley mix at Roblin-2.

3.3.3.2 Seeding

Seeding rates were based on the low range of recommended wheat seeding rates at 250 pl m⁻² and the high range of recommended seeding rates for the cover crops (Table 2-1). The canola target plant stand of 108 plants m⁻² was recommended by personal communication with two agronomists

(personal communication, S. Wilson and A. Delaquis). All seeding rates accounted for percent germination and mortality.

In Phase 1, cover crops were seeded in the same row and at the same depth as the wheat seed at an approximate depth of 2 cm. All legumes were pre-inoculated with the appropriate strain of powdered *Rhizobium* bacteria. Each plot consisted of three passes of the seeder (5 or 6 rows per pass, depending on the site), leaving the two outer passes for destructive sampling and the middle pass for determining grain yield. The plot dimensions at each site were: Arborg (8×1.37 m), Carberry (10×1.2 m), Melita (9.5×1.44 m), Roblin (9.0×1.2 m).

In Phase 2 at Arborg, Carberry-1 and Roblin-1, Clearfield Canola (Corteva Agriscience, Indianapolis, USA) was seeded into the cover crop living mulch on the opposite side of the wheat row as the previous year's sideband of N. Clearfield® canola was chosen on the basis of previous research from the University of Manitoba comparing cover crop persistence under the herbicides used for LibertyLink® canola (BASF, Ludwigshafen, Germany), Roundup Ready® canola (Bayer Crop Science, Monheim am Rhein, Germany), and Clearfield® canola (Gawiak, 2026).

Variety names and seeding rates were listed in chapter 2 (Table 2-1).

3.3.3.3 Fertility

In Phase 1, granular urea was applied to all plots to meet a target yield of 4700 kg ha^{-1} (70 bu ac^{-1}), based on a recommended total N supply of 2 lb of N per bushel of expected yield ($33.4 \text{ kg of N per } 1000 \text{ kg of expected yield}$). Five of the six site-years side-banded N fertilizer, and only Arborg used a mid-row band. Table 2-2 lists background and added N fertility for each site. Starter phosphorus (P) was applied as seed-placed monoammonium phosphate (MAP): 17 kg ha^{-1} at Arborg; 39 kg ha^{-1} at Carberry-1; 17 kg ha^{-1} at Carberry-2; 28 kg ha^{-1} at Melita; 28 kg ha^{-1} at Roblin-1, and 17 kg ha^{-1} at Roblin-2.

In Phase 2, urea was applied at three different rates to the three no-cover-crop control treatments, to compare to the N supply from the legume cover crops. All plots were sampled individually in spring of Phase 2 and an average was used as a background rate of N supply. The 100% N treatment received enough N to bring total N supply from 0-60 cm soil depth and added fertilizer to 196 kg N ha⁻¹ (targeting a canola yield of 3370 kg ha⁻¹). Both the 50% N treatment and the cover crop treatments received enough N to bring total N supply to 98 kg ha⁻¹ (50% of the full N supply). The 0% N rate only received a small quantity of N in the MAP starter fertilizer (approximately 2 kg ha⁻¹ as a side-band). This small amount of N was also taken into consideration when calculating N rates for the other treatments. The N application rates in Phase 2 are shown in Table 3-1.

Table 3-1 Phase 1 and 2 background and added N fertility at each site-year

Site-year	Phase 1			Phase 2				
	Soil Nitrate, 0-60 cm	N fertilizer application rate, all treatments	Total starting N	Soil Nitrate, 0-60 cm	N fertilizer application rate			
					Cover Crops	100% N	50% N	0 N
						Control	Control**	control
	kg ha ⁻¹			kg ha ⁻¹				
Arborg	112	44	156	56	42	140	42	0
Carberry 1	50	112	162	74	24	122	24	0
Carberry 2	73	87	160	-	-	-	-	-
Melita	34	123	157	-	-	-	-	-
Roblin 1	43	112	155	25	70	168	70	0
Roblin 2	25	132	157	-	-	-	-	-

*Target Phase 2 total starting N for 50% control treatment and all cover crops was 98 kg ha⁻¹ and the 100% N control was 196 kg ha⁻¹ ** N fertilizer was added to reach 50% of the total N required (soil + fertilizer).

Due to imprecise equipment calibration applied N was +/- 3 kg ha⁻¹ of the target.

3.3.3.4 Herbicide

In-crop herbicide was applied three to four weeks after seeding. At Arborg and Melita, the in-crop herbicides both had Bromoxynil as the active ingredient. At Arborg, the product was Pardner (Bayer Crop Science, Leverkusen, Germany) with an active ingredient concentration of 280 g L⁻¹ and it was applied at a rate of 1 L ha⁻¹. At Melita the product was Koril with an active ingredient

concentration of 235 g L⁻¹ (Nufarm Agriculture Inc, Calgary, Canada) and it was applied at 1.2 L ha⁻¹. At Melita, herbicide application resulted in the termination of the cover crops and dropping the site from the study, but at Arborg, where emergence was delayed because of the dry seed bed, it had no visible effect on the cover crops. Roblin-1 and 2 and Carberry-1 and 2 were sprayed with Basagran (BASF, Ludwigshafen, Germany) with an active ingredient concentration of 480 g L⁻¹ at a rate of 2.0 L ha⁻¹.

The herbicide used with Clearfield® canola is Imazamox and Imazapyr (Corteva Agriscience, 2024). Application followed the product label recommendations: two true-leaf stage at a rate of 0.4 L ha⁻¹ with Merge added as a surfactant at a rate of 0.5 L 100⁻¹. The previous research at the University of Manitoba found that while Ares appeared to pause the cover crop growth, it eventually recovered (Gawiak, 2026) making it compatible with maintaining a living mulch.

Differences among sites related to equipment, dates of data collection, and necessary adjustments are summarized in Table 3-2.

3.3.3.5 *Weather*

The 2023 growing season was officially classified by the province of Manitoba as a drought year (Government of Manitoba, 2023); precipitation at all sites was slightly more than 60% of the 30-year average. In 2024 all site-years received above-average precipitation. A detailed table of weather data has been included in the Chapter 2 (Table 2-3)

3.3.3.6 *Irrigation*

The site with the lowest precipitation was Arborg and six weeks after seeding the experiment was irrigated. It received 13 mm of sprinkler irrigation on June 12 as well as subsurface irrigation through drainage tile throughout the growing season.

Table 3-2 Summary of key dates for field operations and data collection during Phase 1 and Phase 2 at all site-years.

Data	Arborg	Carberry 1	Carberry 2	Melita	Roblin 1	Roblin 2
PHASE 1						
Spring 1 soil nitrate-N	08-May	28-Apr	25-Apr	28-Apr	27-Apr	24-Apr
Seeding	23-May	12-May	14-May	10-May	12-May	14-May
Mid season herbicide	14-Jul	19-Jun	20-Jun	01-Jun	21-Jun	17-Jun
Biomass	09-Aug	24-Jul	30-Jul	24-Jul	03-Aug	29-Jul
Harvest	13-Sep	29-Aug	29-Aug	17-Aug	30-Aug	17-Sep
Fall soil nitrate-N	18-Oct	20-Oct	15-Oct	20-Oct	17-Oct	10-Oct
Fall Biomass	18-Oct	20-Oct	15-Oct	20-Oct	17-Oct	10-Oct
Spring 2 soil nitrate-N	16-May	25-Apr	03-May	-	-	-
PHASE 2						
Canola seeding	23-May	30-May	-	-	24-May	-
1 st PRS ® burial	-	03-Jun	-	-	24-May	-
Spring plant biomass	27-May	24-Jun	-	-	17-Jun	-
Ares TM application	10-Jun	19-Jun	-	-	21-Jun	-
2 nd PRS ® burial	-	24-Jun	-	-	21-Jun	-
2 nd plant biomass	-	15-Jul	-	-	12-Jul	-
2 nd soil test nitrate-N	-	15-Jul	-	-	12-Jul	-
3 rd PRS ® burial	-	15-Jul	-	-	12-Jul	-
3 rd plant biomass*	24-Jul	07-Aug	-	-	31-Jul	-
3 rd soil test nitrate-N	-	07-Aug	-	-	31-Jul	-
PRS ® removal	-	07-Aug	-	-	31-Jul	-
Canola harvest	11-Sep	-	-	-	-	-

*Technically, this was not the third biomass at Arborg, but the label has been kept to align with the timing at Carberry-1 and Roblin-1

3.3.4 Site Properties

Low soil nitrate-N in preliminary soil tests conducted the previous fall was a selection criterion. One site (Arborg) had higher background nitrate-N in spring than expected based on the fall soil tests (Table 3-1). Pre-seeding soil fertility tests were analysed by AGVISE Laboratories (Northwood, ND). The tests included a full profile of soil nutrients and chemical properties such as pH, cation exchange capacity (CEC), electrical conductivity (EC), and organic matter (OM). Soil samples were collected as six soil cores per replicate block from two depths (0-15 cm and 15-60 cm), composited as one sample per block.

Sites were chosen to represent a wide variety of soil characteristics, landforms, and weather patterns. The Province of Manitoba classifies the soil of each site in the following ways (Agriculture and Agri-Food Canada, 1967, 1978, 1982, 1983).. The Arborg site is a clayey lacustrine soil, non-saline, nearly level, and drained. Carberry and Melita are loamy lacustrine soils that are classed as level to nearly level, and non-saline. Carberry is classified as well drained and Melita is well drained to imperfectly drained. The Roblin site is a clay loam, well drained, level to nearly level and non-saline. Key soil properties have also been summarized in Chapter 2 (Table 2-5).

3.3.5 Sample and Data Collection

3.3.5.1 Phase 1 – Wheat

At wheat soft dough stage, above-ground plant biomass of all species was collected. This was repeated after wheat harvest, and also the following spring (Table 3-2). Biomass samples were collected from 1 m of two adjacent rows in two locations per plot, for a total of 4 m of row per plot. Samples were divided into cover crops, wheat (where applicable), and weeds. All biomass material was oven dried, weighed, and ground to pass a 2-mm screen using a Wiley Mill (Thomas Scientific, Swedesboro, New Jersey). Some samples were additionally ground in a ball mill to achieve the necessary fineness. All wheat phase sampling dates were analysed for plant tissue N concentration. Biomass samples were analyzed for percent N content (combustion method) at Central Testing Laboratories in Winnipeg, MB. Plant N yield was calculated by multiplying biomass yield in kg ha⁻¹ by the percent N content. If the sample was too small for N analysis, averages of percentages from other replicates of the same treatment were applied to that sample. Wheat grain was harvested at the end of August. Spring wheat yield was assessed from small plot combine HarvestMaster weigh systems and Mirus software (Juniper Systems, Inc, Logan, Utah).

Grain protein was measured using a Perten Inframatic 9500 NIR grain analyzer (PerkinElmer, Shelton, Connecticut), which runs 10 independent cycles on 250 g of material and takes an average of those cycles with final protein adjusted for temperature and moisture (corrected to 13.5%). Grain N concentration was estimated by converting grain protein to %N by dividing by a factor of 5.8. The total grain yield in kg ha⁻¹, corrected to a standardized 13.5% moisture, was multiplied by the %N to determine grain N yield.

Soil nitrate-N was sampled two months post harvest and at the end of April the following spring at all site-years, and three times in-season during Phase 2 at Carberry-1 and Roblin-1. The sampling pattern was six soil cores composited for each plot. Post-harvest samples and in-season samples were for one depth (0-15 cm) and spring samples were taken from two depths (0-15 and 15-60 cm). Soil nitrate-N was analyzed on oven-dried soil using the cadmium reduction method with 0.2 M KCl (Gelderman & Beegle, 2015.).

3.3.5.2 Phase 2 – Canola

Phase 2 occurred at Roblin-1, Carberry-1, and Arborg, but distance between sites and time allocation of the researchers meant that not all N measurements were taken at Arborg for Phase 2 (Table 3-2). Soil was sampled in the spring (end of April) by individual plot at each site. Mulches broke dormancy in mid-April and biomass samples were taken at Arborg after five weeks of growth (May 27). At Carberry-1 and Roblin-1, spring biomass taken after two months of growth (mid-June) (Table 3-2).

After the point of seeding canola, N measurements differed by site-year, with measurements at Carberry-1 and Roblin-1 being marked by three major data collection dates, the timing of which were determined by the 21-day burial schedule of the PRS® probes at those sites, weather and weekend dependent.

The PRS® probes were placed on the opposite side of the row to the placement of the N sideband put in at wheat seeding, but adjacent to the cover crop, and kept in situ for 21 days for each burial period. Probes were initially buried at seeding (Table 3-2). Eight probes (four anion and cation pairs) per plot were composited for one sample per plot. When the probes were changed, they were always replaced as close as possible to the original location, but not necessarily in the same slot in the soil. On the same date as probe removal and replacement, soil cores were taken from the vicinity of the probes and sent to AGVISE Laboratories for nitrate-N analysis, and plant material biomass samples were also taken from the same area in order to measure aboveground plant N. Each soil nitrate sample was a composite made up of six cores per plot. Plant biomass samples were taken from 4 m of crop row per plot and also composited. The three-week pattern was repeated two more times for a total of three burial periods (Table 3-2). Roblin-1 kept probes in all eight treatments. Carberry-1 had probes in only five treatments because of the three failed treatments (red clover, white clover, sweet clover). PRS data from all dates were analyzed (Table 3-6).

In addition to the spring biomass date, mulch, canola, and weeds from the 3rd biomass date (Table 3-2, roughly coinciding with canola anthesis) were also analyzed for plant N at all site-years. At Carberry-1 and Roblin-1, this was also the day when the PRS® probes were removed. Biomass samples were analyzed for percent N content by Central Testing Laboratories in Winnipeg, MB, as total N by combustion (as was described in Section 3.3.4.1) and plant N yield was calculated by multiplying biomass yield in kg ha⁻¹ by the percent N content. If the sample was too small for N analysis, averages of percentages from other replicates of the same treatment were applied to that sample.

Post-wheat harvest N balance was calculated on a plot-by-plot basis. The calculation for N balance in each cover crop treatment was starting soil N + added fertilizer N – grain N + estimated legume fixed N. In the case of perennial ryegrass, no additional N was added through BNF. The calculation for control treatments was starting soil N + added fertilizer N – grain N. Losses were also assumed to have occurred through denitrification, leaching, and volatilization but were not included in the balance calculations.

Calculations for estimating fixed N used the formula presented by Anglade et al. (2015) which is a regression relationship between shoot N and fixed N ($R^2=0.92$) with slope and intercept values specific to forage species and with a multiplier to account for both aerial and belowground N. The equation below was used for BNF calculations, expressed as $\text{kg N ha}^{-1} \text{ yr}^{-1}$:

$$\text{BNF (kg N/ha/yr)} = \left[\alpha_{cult} \times Y + \beta_{cult} \right] \times \text{BGN}$$

where the slope and intercept are represented by α_{cult} (0.79) and β_{cult} (-0.49), respectively, and BGN is the multiplicative factor for belowground N (1.7). The regression coefficients are for forage legumes as a group (Anglade et al., 2015), and were used for all legume treatments in the present study.

3.3.6 Statistical Analyses

In Arborg, the fourth block suffered from spray drift from an adjacent field, and only data from the first three blocks were used in the statistical analyses. At Carberry-1, the cover crops in three treatments (red clover, white clover and perennial ryegrass) did not persist past emergence and were removed from the data set after emergence counts.

Data were analyzed in SAS ODA using the generalized linear mixed model procedure PROC GLIMMIX (SAS Studio, 2023) to determine treatment effects on main crop plant N, cover crop

plant N, estimated fixed N, soil nitrate-N, nitrate and ammonium fluxes, and fall N balance. No comparisons were made across site-years because of differences in management and sampling approaches at each site. Block was considered a random effect. Tests for common variance were conducted in PROC GLIMMIX for all tests and the denominator degrees of freedom were adjusted to Satterthwaite when the outcome was significant. Plant N and soil N data followed either a normal or log-normal distribution. Data from the PRS probes followed a lognormal distribution and was analyzed using compound symmetry structure for the evenly spaced time intervals of repeated measures analysis using the Kenward-Roger denominator degrees of freedom method. Transformed data were back-transformed for presentation. For all data following a normal or log-normal distribution, Proc Univariate procedures were conducted to assess normality of the residuals. A Shapiro Wilk test W statistic > 0.9 was the threshold for normality. A probability < 0.05 was considered significant and treatment means were compared using a post-hoc Tukey-Kramer multiple comparisons test.

3.4 Results

3.4.1 Phase 1 N Status (Figure 3-1 Timepoints 1, 2, 3)

Chapter 2 reported the results of the impact of the cover crop on wheat performance. In those results, no difference was observed between the wheat-only control and the wheat grown together with cover crops for wheat biomass at soft dough stage, wheat yield, or wheat protein.

3.4.1.1 Wheat plant N yield and grain N yield in Phase 1

For both wheat plant N yield and wheat grain N yield there was only one significant effect of cover crop treatment observed which was in the Arborg grain N yield (Table 3-3). The Tukey test failed to categorize the difference among means, but quantitatively, the highest grain N yield for that site-year was found in the white clover treatment, in which cover crop biomass was negligible at

the wheat soft dough stage. Grain N yields in the red clover, sweet clover, white clover, and wheat-only control treatments were numerically very close (ranging from 160 – 166 kg ha⁻¹). Means for alfalfa and perennial ryegrass treatments were also very close (148 and 147 kg ha⁻¹, respectively). Wheat plant N yield was similar among treatments within each site with means falling within a 25 kg ha⁻¹ range at individual sites. The highest values were at Arborg (127 kg ha⁻¹ to 148 kg ha⁻¹) and the lowest values were at Roblin-2 (73 kg ha⁻¹ to 94.8 kg ha⁻¹) (Table 3-3).

3.4.1.2 Cover crop N yields and fixed N in Phase 1 – peak wheat biomass

At peak wheat biomass, the cover crop was still very small and cover crop N yield was orders of magnitude lower than the wheat N yield at all sites. The effect of treatment in the ANOVA was significant at each site except Carberry-1 (Table 3-3). Alfalfa N yield was significantly higher than that of white clover at all site-years except Roblin-2. At both Roblin-1 and Roblin-2, alfalfa N yield was significantly higher than that of sweet clover. Arborg alfalfa had a higher N yield than red clover, and at Roblin-1 alfalfa N yield was higher than that of perennial ryegrass.

Table 3-3 Effect of cover crop treatment on aboveground wheat plant N, aboveground cover crop N, estimated cover crop fixed N, wheat grain N, and soil nitrate-N in three sampling periods in Phase 1 (peak wheat biomass, post-harvest, and subsequent spring) at five site-years.

Site-year and treatment	*Peak biomass sample			**Post-harvest sample				*** Subsequent spring pre-seed sample
	Wheat plant N yield	CC N Yield	Estimated Fixed N	Grain N yield	CC N Yield	Estimated Fixed N	Soil N (0-15)	Soil nitrate-N (0-60cm)
kg ha ⁻¹								
ARBORG								
Alfalfa	145 (9.36)	4.85 (1.76) a	5.51 (1.78)	148 (12.5) a	16.3 (3.67) ab	21.7 (10.2) a	11.9 (1.48)	41.7 (9.10)
Red Clover	129 (6.00)	0.11 (0.03) b	0	162 (10.8) a	5.17 (3.67) b	5.92 (2.77) ab	12.7 (1.48)	47.5 (8.72)
Sweet Clover	127 (24.3)	0.48 (0.79) a	1.01 (0.47)	160 (17.7) a	2.73 (3.67) b	3.60 (2.0) ab	15.3 (1.48)	48.1 (8.82)
White Clover	148 (12.4)	not present	-	166 (9.38) a	2.26 (3.67) b	2.16 (1.01) b	14.0 (1.81)	64.7 (11.9)
PRG	134 (1.19)	4.09 (1.20) a	-	147 (1.71) a	23.3 (3.67) a	-	9.70 (1.48)	41.2 (7.53)
Control	142 (4.20)	-	-	162 (9.38) a	-	-	12.3 (2.56)	61.2 (11.2)
p-value	0.37	0.001	0.19	0.03	0.002	0.02	0.26	0.29
CARBERRY 1								
Alfalfa	96.7 (19.9)	2.31 (0.59)	2.27 (0.79)	96.6 (19.5)	10.5 (1.97)	13.2 (2.37)	17.4 (1.68)	55.7 (10.5)
Red Clover	97.9 (14.5)	-	-	-	-	-	-	-
Sweet Clover	109 (4.63)	2.02 (0.80)	1.88 (1.07)	109 (5.0)	9.05 (1.54)	11.3 (2.37)	18.5 (3.78)	50.7 (9.12)
White Clover	115 (1.49)	-	-	-	-	-	-	-
PRG	97.5 (10.6)	-	-	-	-	-	-	-
Control	97.5 (5.82)	-	-	97.5 (5.91)	-	-	26.1 (4.11)	73.4 (9.13)
p-value	0.22	0.79	0.78	0.40	0.61	0.61	0.24	0.38
CARBERRY 2								
Alfalfa	111 (7.97)	2.99 (1.59) ab	3.12 (2.48)	70.1 (3.11)	12.0 (4.04)	15.2 (5.04)	8.4 (2.07)	8.40 (2.09)
Red Clover	122 (11.6)	2.61 (1.39) ab	1.99 (1.58)	60.7 (3.11)	22.2 (7.61)	28.7 (9.60)	8.96 (1.52)	8.96 (1.54)
Sweet Clover	112 (9.09)	1.11 (0.59) bc	2.19 (2.51)	61.4 (3.11)	17.2 (4.58)	22.6 (7.55)	10.1 (2.29)	10.1 (2.31)
White Clover	113 (9.97)	0.43 (0.23) c	0.16 (0.19)	66.5 (3.11)	10.3 (3.29)	13.0 (4.33)	10.4 (0.70)	10.4 (0.72)
PRG	128 (11.8)	5.19 (2.76) a	-	65.5 (3.11)	11.9 (1.63)	-	6.72 (2.29)	6.72 (2.31)
Control	103 (14.2)	-	-	65.2 (3.11)	-	-	16.8 (2.29)	16.8 (2.31)
p-value	0.67	0.001	0.09	0.19	0.34	0.34	0.12	<0.0001

ROBLIN 1

Alfalfa	96.4 (8.51)	4.09 (1.07) a	4.57 (1.07) a	85.5 (5.63)	16.5 (1.83) a	21.4 (2.58) a	16.5 (1.82)	17.7 (4.32)
Red Clover	113 (7.78)	2.23 (0.58) ab	2.54 (0.68) b	86.1 (5.63)	9.46 (1.83) b	11.9 (2.58) b	16.2 (1.82)	16.2 (3.95)
Sweet Clover	108 (8.86)	2.01 (0.53) b	1.98 (0.69) b	93.5 (5.63)	8.15 (1.83) b	10.1 (2.58) bc	16.8 (1.82)	21.2 (5.18)
White Clover	114 (8.01)	0.25 (0.08) c	-0.83 (0.75) c	88.2 (5.63)	3.68 (1.83) b	4.11 (2.58) c	15.4 (1.82)	24.4 (5.95)
PRG	105 (8.70)	0.24 (0.06) c	-	93.1 (5.63)	6.15 (1.83) b	-	11.8 (1.82)	23.3 (5.66)
Control	121 (10.5)	-	-	90.5 (5.63)	-	-	17.4 (1.82)	35.9 (8.77)
p-value	0.08	<0.0001	0.0004	0.71	0.0002	<0.0001	0.12	0.28

ROBLIN 2

Alfalfa	84.5 (4.04)	3.68 (0.89) a	4.01 (0.39) a	75.6 (5.70)	9.55 (2.45) ab	11.3 (2.68) ab	7.56 (1.64) ab	15.2 (0.89)
Red Clover	73.0 (6.60)	6.87 (1.67) a	8.71 (1.82) a	71.0 (2.54)	17.2 (1.87) a	22.0 (2.57) a	9.53 (2.84) ab	13.4 (1.11)
Sweet Clover	94.8 (7.05)	1.18 (0.29) b	0.95 (0.49) b	77.6 (4.96)	4.58 (0.88) b	5.06 (1.15) b	10.1 (1.32) ab	16.7 (1.47)
White Clover	94.0 (6.03)	2.71 (0.66) ab	3.02 (0.84) ab	71.9 (3.98)	18.4 (2.14) a	23.5 (2.95) a	6.44 (0.58) b	14.5 (0.48)
PRG	90.2 (21.0)	5.52 (1.34) a	-	69.9 (4.48)	11.8 (0.55) a	-	7.30 (1.98) ab	12.6 (0.69)
Control	94.5 (11.9)	-	-	84.2 (5.77)	-	-	15.2 (2.33) a	23.5 (4.55)
p-value	0.26	0.002	0.0002	0.36	<0.0001	0.0002	0.01	0.13

*Peak biomass sample date: Arborg (Aug 9), Carberry-1 (Jul 24), Carberry-2 (Jul 30), Roblin-1 (Jul 24), Roblin-2 (Jul 18).

**Post-harvest sample date: Arborg (Oct 18), Carberry-1 (Oct 20), Carberry-2 (Oct 15), Roblin-1 (Oct 17), Roblin-2 (Oct 10).

***Pre-seed spring sample date: Arborg (Apr 25), Carberry-1 (Apr 25), Carberry-2 (Apr 25), Roblin-1 (Apr 25), Roblin-2 (Apr 25). PRG = perennial ryegrass. CC = cover crop. Values are least-squares means with the standard error of the mean in parentheses. Letter groupings are based on the Tukey-Kramer multiple comparisons test. Fixed N values are calculated using the formula proposed by Anglade et al. (2015) which uses a linear regression based on aboveground shoot N with slope and intercept specific to forage crops, and a multiplicative factor to adjust for belowground contributions. Dashes are present in table cells where the test was not relevant.

3.4.1.3 *Post-harvest cover crop N yield and N fixation*

The ranges of cover crop N yield and estimated N fixation at the post-harvest sample date were 1.42 – 23.9 and 3.6 – 21.7 kg ha⁻¹, respectively, and differed due to cover crop treatment at three out of five site-years for both metrics (Table 3-3). Since they are associated with plant biomass, the estimated fixed N values for cover crops at the wheat soft dough sampling date are very small and only two treatments exceeded 5 kg ha⁻¹ (red clover at Roblin-1 and alfalfa at Arborg).

At Carberry-1 and Carberry-2, differences between treatments for both cover crop N yield and estimated N fixation were within the margin of error. The means for the two surviving treatments at Carberry-1 were very close to each other (10.5 and 9.05 kg ha⁻¹ respectively for alfalfa and sweet clover plant N yield and 13.2 and 11.3 kg ha⁻¹ respectively for alfalfa and sweet clover estimated fixed N).

Arborg post-harvest cover crop N yield was statistically higher for perennial ryegrass than all treatments except alfalfa. Although it does not fix N, its post-harvest N yield was five times higher than red clover and 10 times higher than sweet clover and white clover. Figure 2-5 in Chapter 2 illustrated a spike in biomass for Arborg perennial ryegrass after harvest which is the reason for the high N yield. The estimated N fixation of alfalfa at Arborg was significantly greater than white clover ($p = 0.02$) with white clover fixing only 9% of what was fixed by alfalfa (Table 3-3). Red clover and sweet clover are estimated to have fixed 27% and 17%, respectively, of the quantity of N fixed by alfalfa.

At Roblin-1, the N yield of non-fixing perennial ryegrass was higher than that of white clover but lower than that of the other three cover crops (37% of alfalfa N yield). Alfalfa had a significantly higher post-harvest N yield than any of the other cover crops and the estimated fixed N for alfalfa

is accordingly higher than the other treatments as well ($p < 0.0001$). Red clover, sweet clover, and white clover N yield was 53%, 49%, and 22%, respectively, of alfalfa N yield.

At Roblin-2, sweet clover N yield was significantly lower than all other treatments. Estimated N fixation at this site-year was greatest for white clover and red clover. The difference was not significantly higher than alfalfa, but it was higher than sweet clover.

3.4.1.4 Post-harvest soil nitrate-N

Values for fall soil nitrate-N samples from the 0-15 depth ranged from 9.7 to 26.1 kg ha⁻¹, with significant differences due to cover crop treatment occurring at only one site-year (Roblin-2) out of five (Table 3.3). At Roblin-2, fall soil N for white clover was significantly lower than the wheat-only control, but was comparable to the other cover crop treatments.

3.4.1.5 Post-harvest N balance

With the conclusion of Phase 1 after the spring biomass, a N budget was calculated (Table 3-4) to show the ending fall balance. Two site-years of the five had significant results: Carberry-2 ($p=0.0007$) and Roblin-2 ($p=0.0074$).

Table 3-4 Effect of cover crop on post-wheat-harvest N balance at each site-year in Phase 1

Treatment	Arborg	Carberry-1	Carberry-2	Roblin-1	Roblin-2
	kg ha ⁻¹				
Alfalfa	26.7 (12.6)	115 (3.07)	103 (10.3) bc	83.4 (8.30)	90.3 ± 7.33 ab
Red Clover	2.26 (12.6)	-	123 (10.3) a	80.2 (8.34)	103 ± 7.33 a
Sweet Clover	0.93 (12.6)	115 (6.78)	117 (10.3) ab	63.4 (8.30)	83.3 ± 7.33 ab
White Clover	-4.93 (12.6)	-	104 (10.3) bc	67.8 (8.30)	103 ± 7.33 a
PRG	12.1 (12.6)	-	94.4 (10.1) c	63.7 (8.30)	74.7 ± 7.33 b
Control	-3.51 (12.6)	107 (5.77)	94.7 (10.3) c	66.4 (8.30)	72.2 ± 7.33 b
p-value	0.43	0.40	0.0007	0.11	0.007

PRG = perennial ryegrass. Calculation for all cover crop treatments: starting soil N + added fertilizer N – grain N + estimated legume fixed N. Calculation for control: starting soil N + added fertilizer N – grain N. Values are least-squares means with the standard error of the mean in parentheses. Letter groupings are based on the Tukey-Kramer multiple comparisons test.

At Carberry-2, the red clover treatment had a higher fall N balance than the alfalfa, perennial ryegrass, and white clover treatments, but it was similar to sweet clover. At Roblin-2 the only treatments that had significantly more fall N than the control were the red clover and the white clover.

At Roblin-1, alfalfa and red clover fall N balances were very similar (83 and 80 kg ha⁻¹) and the other values were all in the range of 60 kg ha⁻¹, but these differences were not significant. The range of fall balances at Carberry-1 was very narrow with an 8 kg ha⁻¹ difference between the highest and lowest values (107-115 kg ha⁻¹), and here again, no difference was detected.

Arborg showed small negative N balances for the white clover treatment and the wheat-only control treatment, indicating an N source not accounted for in the equation, but no significant difference among treatments was detected. Arborg also had the highest export of N in their wheat harvest.

3.4.2 Phase 2 N Status (Figure 3-1 Timepoints 4 – 8)

Measurements of N in Phase 2 were limited because of the failure of the herbicide to effectively control the living mulch at both Roblin-1 and Carberry-1. Cool, damp conditions (see Chapter 2, Table 2-3) delayed canola emergence which also delayed herbicide application due to lack of canola uniformity. Uncontrolled mulch growth suppressed the canola, and no canola grain harvest was possible at these sites.

3.4.2.1 Soil nitrate-N

The difference in spring soil nitrate-N was significant at only one site-year out of five (<0.0001 at Carberry-2) (Table 3-3) where sweet clover (46 kg ha⁻¹) had lower spring nitrate-N than perennial

ryegrass (91.3 kg ha⁻¹) and red clover (88.7 kg ha⁻¹). Soil nitrate-N at the other sites ranged from 12.6 to 73.4 kg ha⁻¹.

Other than the spring soil nitrate-N, Arborg soil was not sampled in Phase 2.

By the spring biomass date (Table 3-5) three weeks after seeding and N fertilizer application, the 0-15 cm soil nitrate-N was significantly higher in the 100% N control treatment than all the legume cover crop treatments at Roblin-1 and Carberry-1 (Table 3-5). Perennial ryegrass (30 kg ha⁻¹) in Roblin-1 was the only treatment not significantly lower than the 100% N control (84.7 kg ha⁻¹) at the three-week sampling date but quantitatively it was three times lower. The other values ranged from 13.9 to 24.8 kg ha⁻¹.

At the 3rd soil sample date in Carberry-1, the only control sampled was the 50% N but there was no significant difference between it and the alfalfa or sweet clover. Also at the 3rd soil sample date in Roblin-1 (Table 3-5), there was no longer any significant difference between the 100% N control and the legume mulches in the top 0-15 cm of soil. Soil nitrate-N in the perennial ryegrass was still lower than in the 100% N control, the alfalfa, and the sweet clover.

3.4.2.2 Phase 2 aboveground mulch N yield and estimated fixed N

As fixed N is calculated from mulch N yield, they follow the same trends and groupings. The measurement of mulch N yield includes perennial ryegrass at the only site where it was present (Roblin-1), but the estimated fixed N does not. Carberry-1 had no significant differences in mulch N yield or estimated fixed N for either date, whereas at Arborg and Roblin-1 mulch N and fixed N were affected by cover crop treatment at both dates (Table 3-5).

At Arborg, alfalfa mulch N yield and fixed N at the spring sampling were three times greater than the nearest treatment (red clover) and at least ten times greater than the other three treatments (p=0.01). By late July, white clover had a very small presence and the perennial ryegrass was not

even visible. Sweet clover, however, which started with a spring mulch N yield comparable to that of white clover, accumulated enough N by the 3rd biomass date that it was not significantly different from alfalfa (34.1 and 45.9 kg ha⁻¹ respectively for mulch N yield; 44.4 and 60.1 kg ha⁻¹ respectively for fixed N).

At Roblin-1, there were significant differences between treatments for N yield at both the spring biomass date and the 3rd biomass date (first PRS® probe switch and three weeks after seeding and final PRS® probe removal and ten weeks after seeding, respectively – Table 3-2). Whereas red clover (69.7 kg ha⁻¹) was grouped with alfalfa (87.2 kg ha⁻¹) and sweet clover (81.5 kg ha⁻¹) at the spring sampling date (both with higher plant N yield than perennial ryegrass and white clover), by the 3rd biomass date red clover was grouped with the poor-performing white clover and perennial ryegrass. Alfalfa and sweet clover both increased biomass and N yield between those two dates (31% increase for alfalfa and 52% increase for sweet clover). Perennial ryegrass and white clover both increased by only a few kg ha⁻¹, and red clover N yield declined slightly. This pause in growth, with only slight increases or even a decrease for red clover, may be a product of partial senescence caused by the herbicide application.

Table 3-5 Phase 2 effect of cover crop treatment on soil nitrate-N, plant N yield, and estimated fixed N at Arborg, Carberry-1 and Roblin-1 at the spring biomass and soil sample date and the 3rd biomass and soil sample date

Site-year and treatment	*Spring biomass and soil sample date				**3 rd biomass and soil sample date				
	Soil N (0-15 cm)	Weed N Yield	Mulch N Yield***	Estimated Fixed N****	Soil N (0-15 cm)	Canola N Yield	Weed N Yield	Mulch N Yield	Estimated Fixed N
kg ha ⁻¹									
ARBORG									
Alfalfa	^t not sampled	1.43 (0.97)	17.0 (3.50) a	22.0 (4.72) a	not sampled	12.2 (7.08) b	1.43 (0.97)	45.9 (13.0) a	60.1 (14.7) a
Red Clover	-	0.52 (0.36)	5.72 (1.96) ab	6.80 (2.64) a	-	30.5 (7.08) b	0.52 (0.36)	14.2 (4.03) b	17.9 ± (4.39) b
Sweet Clover	-	1.14 (0.78)	1.44 (0.75) b	1.20 ± 1.87 a	-	27.3 (7.08) b	1.14 (0.78)	34.1 (9.70) ab	44.4 ± (10.9) ab
White Clover	-	1.72 (1.18)	1.54 (0.98) b	1.30 (1.80) a	-	32.1 (7.08) ab	1.72 (1.18)	0.64 (0.23) c	0.64 (0.26) c
PRG	-	^{tt}	1.91 (2.67) b	-	-	43.9 (7.08) ab	**	**	-
0 N Control	-	3.88 (2.19)	-	-	-	16.5 (7.08) b	3.88 (2.19)	-	-
50% N Control	-	0.80 (0.45)	-	-	-	43.3 (7.08) ab	0.80 (0.45)	-	-
100%N Control	-	0.42 (0.24)	-	-	-	65.6 (7.08) a	3.88 (2.19)	-	-
p-value		0.08	0.01	0.04		0.002	0.08	0.001	0.001
CARBERRY 1									
Alfalfa	17.5 (1.70) b	0.91 (0.37)	103 (21.1)	138 (31.3)	8.96 (0.46)	10.5 (13.1) b	0.91 (0.37)	154 (23.6)	206 (31.6)
Red Clover	-	-	-	-	-	-	-	-	-
Sweet Clover	18.1 (1.71) b	1.58 (0.65)	85.5 (18.4)	125 (28.5)	14.3 (2.44)	16.6 (13.1) b	1.58 (0.65)	207 (40.7)	277 (54.7)
White Clover	-	-	-	-	-	-	-	-	-
PRG	-	-	-	-	-	-	-	-	-
0 N Control	20.7 (3.59) ab	1.39 (0.57)	-	-	not sampled	85.0 (13.1) a	1.39 (0.57)	-	-
50% N Control	32.8 (4.46) ab	2.20 (0.91)	-	-	10.9 (2.52)	99.6 (13.1) a	2.20 (0.91)	-	-
100%N Control	21.4 (2.55) a	4.16 (1.72)	-	-	not sampled	137 (13.1) a	4.16 (1.71)	-	-
p-value	0.01	0.12	0.28	0.51	0.17	<0.0001	0.12	0.34	0.34
ROBLIN 1									
Alfalfa	21.5 (4.77) bc	1.00 (2.10)	87.2 (6.58) a	116 (18.1) a	16.5 (1.47) a	0.69 (0.40) b	0.37 (0.30) a	126 (14.9) a	165 (15.1) a
Red Clover	13.9 (3.07) c	1.14 (2.39)	69.7 (6.58) ab	88.0 (8.03) b	12.3 (0.79) ab	0.23 (0.17) b	0.11 (0.01) b	66.2 (4.88) b	87.6 (8.03) b
Sweet Clover	23.6 (5.23) bc	0.62 (1.0)	81.5 (7.53) a	224 (20.6) a	15.4 (1.85) a	0.30 (0.22) b	0.79 (0.80) ab	168 (14.6) a	224 (20.6) a
White Clover	24.8 (5.50) bc	11.5 (24.1)	41.2 (7.53) bc	58.0 (5.30) c	14.3 (4.27) ab	3.13 (3.73) ab	3.50 (1.26) a	44.0 (3.51) b	57.8 (5.30) c
PRG	32.0 (7.10) abc	3.28 (5.29)	36.6 (6.58) c	-	7.00 (1.15) b	1.84 (1.08) b	2.48 (0.66) a	43.4 (8.31) b	-
0 N Control	21.0 (4.65) bc	0.45 (0.20)	-	-	12.9 (3.25) ab	36.5 (18.1) a	0.74 (0.65) ab	-	-
50% N Control	45.2 (10.0) ab	0.09 (0.10)	-	-	11.7 (1.50) ab	75.1 (37.4) a	0.29 (0.21) ab	-	-
100%N Control	84.7 (18.8) a	0.53 (0.41)	-	-	17.9 (1.37) a	71.0 (35.3) a	1.82 (1.23) a	-	-
p-value	0.0004	0.31	0.0005	0.31	0.0002	<0.0001	<0.0001	<0.0001	<0.0001

*Spring biomass sample date: Arborg (May 27), Carberry-1 (Jun 24), Roblin-1 (Jun 17).

**3rd biomass sample date: Arborg (Jul 24), Carberry-1 (Aug 7), Roblin-1 (Jul-31).

***MulchN is Aboveground Mulch N measured from biomass sample without any conversions or multipliers.

****Estimated Fixed N is the estimated BNF of the cover crop, based on regression relationship established by Anglade et al. (2015) including a multiplier to account for belowground N . Values are least-squares means with the standard error of the mean in parentheses. Letter groupings are based on the Tukey-Kramer multiple comparisons test.

[†]At Arborg biomass was sampled only in early spring and in late July, and soil was sampled only in spring.

^{††}PRG had limited spring regrowth and failed completely early in the season. PRG is perennial ryegrass.

3.4.2.3 *Canola N yield*

Canola N yield at the 3rd biomass date was significantly different among treatments at all site–years. At Arborg, canola N yields in the red clover, sweet clover, and alfalfa treatments (30.5, 27.3, and 12.2 kg ha⁻¹, respectively) were significantly lower than the 100% N control (65.6 kg ha⁻¹) but not different from the 50% N control and the 0 N control (43.3 and 16.5 kg ha⁻¹, respectively). White clover and perennial ryegrass treatments did not have a significant impact on canola N yield when compared to the 100% N control. The legume mulches that produced more biomass caused the largest reductions to canola N yield.

At Roblin-1 and Carberry-1, even the 0 N control treatment had significantly more canola N yield than the mulch treatments because of the low canola biomass produced in the mulch treatments. Canola N yield in the white clover treatment was highly variable and was not significantly different from the control treatments, even though it was 10-25 times lower than in the controls.

3.4.2.4 *Weed N yield*

Overall, weed N yield was relatively low at all site–years. Weed N yield was highly variable, but at Arborg and Carberry-1 it was quantitatively much smaller than both the canola and the mulch N yields. At Roblin-1 weed N yield was significantly lower than in the 100% N control, alfalfa, white clover, and perennial ryegrass treatments (Table 3-5).

3.4.3 In-season Soil N Flux

All cover crop treatments received the same fertility as the 50% N control so it was the only control monitored with PRS® probes. Soil N fluxes were compared among cover crop treatments and the control treatment that received the same N fertilizer rate (50% N control) to remove any confounding effects of N fertilizer rates. PRS® probes were installed at Roblin-1 and Carberry-1 and changed every three weeks over a period of nine weeks (i.e., three burial periods). No probes

were installed at Arborg. The results did not show any interactions between the mulch treatment and the burial period (Table 3-6), indicating that patterns over time were similar for all treatments. Burial periods were significantly different from each other for nitrate at both site-years, and for ammonium at Carberry-1.

Table 3-6 Effect of living mulch treatment on soil nitrate and ammonium flux measured by PRS® probes at two site-years

Factor	NO ₃ ⁻		NH ₄ ⁺	
	Roblin-1	Carberry-1	Roblin-1	Carberry-1
	μg ⁻¹ 10cm ⁻² burial period ⁻¹			
Mulch				
50% N Control	117 (25.0) a	93.0 (11.7) a	1.5 (0.5)	1.7 (0.4)
PRG	72.3 (15.4) ab	50.4 (6.30) b	1.8 (0.6)	1.3 (0.3)
Alfalfa	28.6 (6.10) c	-	1.8 (0.6)	-
Red Clover	29.0 (6.20) c	-	1.1 (0.4)	-
Sweet Clover	48.3 (10.3) bc	63.2 (7.90) b	1.1 (0.3)	1.8 (0.4)
White Clover	35.2 (7.50) bc	-	1.8 (0.6)	-
Burial period				
		372.7 (46.8)		
1 st	137.5 (20.6) a	a	1.6 (0.4)	3.5 (0.3) a
2 nd	68.0 (10.2) b	67.9 (8.50) b	1.0 (0.2)	1.2 (0.4) b
3 rd	11.3 (1.70) c	11.7 (1.50) c	2.0 (0.4)	0.9(0.3) b
Source of Variation				
	p-value			
Cover Crop	<0.0001	<0.0001	0.76	0.60
Burial period	<0.0001	<0.0001	0.09	0.0005
Burial period x Cover crop	0.42	0.49	0.11	0.93

PRG = perennial ryegrass. 1st burial period: Carberry-1 (Jun 3 – Jun 24), Roblin-1 (May 24 – Jun 17); 2nd burial period: Carberry-1 (Jun 24 – Jul 15); Roblin-1 (Jun 17 – Jul 12); 3rd burial period: Carberry-1 Jul 15 – Aug 7), Roblin-1 (Jul 12 – Jul 31). Values are least-squares means with the standard error of the mean in parentheses. Letter groupings are based on the Tukey-Kramer multiple comparisons test.

At both Roblin-1 and Carberry-1, NO₃⁻ was steadily drawn down as the season progressed. The effect of mulch treatment was also significant, with the 50% N control retaining the highest levels of NO₃⁻ supply. At Roblin-1, alfalfa NO₃⁻ supply (72.3 μg⁻¹ 10 cm⁻²) was statistically similar to the control (117 μg⁻¹ 10 cm⁻²). The other mulch treatments had lower NO₃⁻ supply than the control but

were not significantly different from each other (Table 3-6). At Carberry-1, sweet clover ($63.2 \mu\text{g}^{-1} 10 \text{ cm}^{-2}$) and alfalfa ($50.4 \mu\text{g}^{-1} 10 \text{ cm}^{-2}$) both had a significantly lower NO_3^- supply than the control ($93.0 \mu\text{g}^{-1} 10 \text{ cm}^{-2}$).

The effects of treatment and burial period were not significant for NH_4^+ at Roblin but at Carberry-1 there was a significant difference among burial periods. Ammonium flux was greater during the 1st burial period than the later periods, which did not differ from each other (Table 3-6).

3.5 Discussion

Ideally, the study would show that legume additions of N by BNF into a legume–non-legume intercrop were measurably present during the living mulch growth period and were able to offset high input of N by inorganic N sources. As such, identifying the chronology of accumulation and translocation of legume N and where it is being stored at different crop stages is as important as knowing how much N the legumes fixed.

In light of the failure to effectively control the mulch so that canola could establish, the original objective of quantifying N supply to the canola crop in Phase 2 of the living mulch system cannot be addressed. Numerous gaps in knowledge are apparent in trying to interpret the results of the research. However, since the same gaps exist across treatments, it is still meaningful to fill in the N values that are known from the data that was collected, and estimate the missing values where precedent exists to do so. Using the proposed Figure 3-1 as a guide, we identified the pools of N that we measured or calculated, and compared alfalfa and the 100% N control treatments across both phases of the trial. Alfalfa was chosen because across site–years and sampling dates, it repeatedly yielded high biomass and therefore also high amounts of fixed N. Other mulches varied in their yields, but any time there was a significant difference for N yield and N fixation, alfalfa

was usually significantly higher, or was at least in the top grouping, and showed consistent potential of injecting the most atmospheric N.

	1		2		3		4		5		6		7		8	
	Phase 1 Seeding CN AL		Wheat Soft Dough CN AL		Post Harvest CN AL		Spring CN AL		Phase 2 Seeding CN AL		Canola Seeding CN AL		Canola Anthesis CN AL		Spring 2 CN AL	
Total Aboveground			121	103	31	39					175		71	211		
Weed N											4	2		0.4		
Legume Not fixed				2		7		?		?		37		45	?	?
Legume fixed				5		21		?		?		138		165	?	?
Main crop/residue plant N			121	96	31	11	?	?			too small		71	1	?	?
0-15	15	15	?	?	17	8	20	9	20	9	91	24	18	18	15	23
15-60	30	30	?	?	?	?	19	9	19	9	?	?	?	?		
Fertilizer	112	112							168	70						
Total Belowground	157	157			17	8	39	18	207	88	91	24	18	18	15	23
Total N	157	157	121	92	47	46	39	18	207	88	91	199	89	229	15	23

Figure 3-2 Changes to N from intercrop cover cropping with alfalfa N sources and pools of stored N for 100% N Control (CN) and alfalfa (AL) during Phase 1 and 2 at Roblin-1. Values in boxes for each component are the LS means from their respective tables (Tables 3-3, 3-5). Deep soil samples (0-60 cm) were only taken in the two springs, pre-seeding. Comparisons of totals and subtotals are meaningful only at each given time point, and not across timepoints because not all of the same data is available across timepoints. However, within each timepoint, both treatments have the same gaps. The size of the boxes is not meant to represent the size of the difference. Differences may have occurred between treatments for N lost to leaching, volatilization, denitrification, or erosion are assumed to have occurred but are not accounted for in the figure. Estimates for Fixed N were calculated using the approach of Anglade et al. (2015). Fixed N is displayed above ground but it is actually physically located both above and below ground. In Phase 2, the total N supply (0-60 cm soil nitrate plus fertilizer) provided to alfalfa was 50% of that provided to the 100% N control.

At the eight timepoints that were tracked, measured N tended to be higher in the 100% N control than in the alfalfa treatment in Phase 1, even accounting for the N found in the post-harvest cover crop biomass. A shift to having more N in the alfalfa treatment than in the 100% N control treatment did not occur until Timepoint 6, later in Phase 2.

3.5.1 Wheat plant and grain N yield

At Timepoint 2, the higher mean of the wheat N yield compared to the control at the wheat soft dough stage was not significant (p-value of 0.08). The same holds true for the wheat stubble in the post-harvest period (Timepoint 3 - soft dough wheat plant N minus wheat grain N).

Despite the potential of the cover crops to contribute N to the system (represented by the amount they were calculated to have fixed) the lack of overall impact to wheat plant N yield and grain N yield as in-crop N measurements indicate that it was neither moving into the soil to be taken up by the wheat nor moving to the wheat by root contact by any of the means that we could use to measure; rather, the wheat sourced its N from what was present in the soil and the inorganic N applied as fertilizer. Additionally, the potential for fixed N to accumulate in the soil and contribute to the main crop is dictated by the growth of the cover crop. Little N was fixed by the legumes at the wheat soft dough stage so the potential to act as an N source was limited. Lack of biomass means lack of photosynthetic energy to drive the legume's nitrogenase complex for fixing N (Schulze et al., 1994). Low cover crop biomass was a result of giving the wheat an intentional advantage, so suppressed cover crop growth is the desired outcome (Gaudin et al., 2013; Mbanyele et al., 2024). Importantly, while the legumes (exemplified in Figure 3-2 in the alfalfa) did not act as a source for N, they were also not competing with the wheat for N, as indicated by the size of the difference between wheat plant N yield and cover crop plant N yield at soft dough stage, as well as the lack of difference between the wheat N yield in the alfalfa and control treatments (Table 3-3).

Potential trade-offs between B transfer and yield suppression are highlighted by Osterholz et al. (2023) where maize was seeded with alfalfa in Wisconsin under three different fertility regimes and ^{15}N was traced through the natural abundance approach. They found that although the maize

was able to source an average of 28% of its total N from the alfalfa with no added N, they also suffered a 33% reduction in yield.

No significant evidence was seen for more or less N being made available in the soil from the fall to spring soil samples (Timepoints 3 and 4) between the alfalfa and the 100% N control treatments, or in fact for any of the treatments (Table 3-3) even though the legumes were continuing to grow while the control plots remained bare. Despite the lack of difference, nitrogenous exudates are assumed to have been present in the form of ions, gases, enzymes, and low C/N organic matter which breaks down easily (Hupe et al., 2021). Quantitatively, the alfalfa spring soil nitrate-N in Figure 3-2 (Timepoint 4) was actually somewhat lower than the 100% N control, though not statistically significant. However, overall, the increase in alfalfa biomass with no significant decrease in soil N indicates that the alfalfa relied on atmospheric sources of N that were being stored in the plant and that the fixed N would become available at a later date. Both the fixing and storing of N is the quality that sets legumes apart from non-legumes (Bergkvist, 2003a; Cicek et al., 2014; Sturite et al., 2006; Thiessen Martens et al., 2005b). Non-legumes store N, but they do not fix it.

Overall, soil nitrate-N increased in all treatments from post-harvest to spring sample dates, which would be expected from mineralization, and it is expected that some of the N that was mineralized was lost to surface runoff during spring thaw, or through leaching and denitrification; however, neither the alfalfa treatments nor any of the other legumes showed greater contributions from mineralization than the control treatments. In many cases, unless the cover crop is being terminated, increased N mineralization may depend on how much shedding of dead leaves has occurred. White clover tends to contribute more N because of the low C/N ratio (Louarn et al.,

2015). Immediate increases to soil nitrate-N only represent a portion of the N that will become available over the course of years (Blackshaw et al., 2010b).

Losses to the environment through the seasonal transition must also be considered. Spring N is a function of soil moisture (leading to leaching or denitrification) and nutrient losses to spring melt (Corriveau et al., 2025). Environmentally, legumes that have been damaged by winterkill contributes to N leaching and runoff losses (Böldt et al., 2021) and unstable winters have also been identified as a source of N₂O emissions in pure white clover stands in Sweden (Sturite et al., 2006). While the study was designed with the aim of tracing influxes of N both into the soil and the canola using ¹⁵N natural abundance techniques (anticipating a release of N from the legumes after growth setback from herbicide application), the failure to suppress mulch growth meant legume N contributions to canola could not be measured directly. If successful, some of the alfalfa N seen in the aboveground boxes at the point of seeding in Figure 3-2 (165 kg ha⁻¹ fixed and 45 kg ha⁻¹ from the soil) would likely have been released from suppressed mulches into the soil N pool below ground and from there would have been available to the vegetative canola. Release of N was observed in the soil of treatments with winter wheat and white clover in Sweden, where the white clover was sprayed to reduce biomass, resulting in increases both to grain N and soil N (Bergkvist, 2003b).

Figure 3-2 also does not account for the mulch biomass present in spring between breaking dormancy and Phase 2 seeding (Timepoint 5). If the biomass sample had been taken earlier in the spring there would be a better understanding of how much N was accumulating via BNF or by scavenging soil N that might otherwise have been lost to the environment.

In Figure 3-2 (Timepoint 6) it is possible that the calculation used to estimate fixed N after seeding and adding fertility is an over-estimate of the potential fixed N additions because of the priming

effect, where adding inorganic N to cover crops has been shown to depress N fixation (Gaudin et al., 2013; Louarn, et al., 2015; Osterholz et al., 2023). However, as biomass increased to the 3rd sample date, and the shallow soil N decreased (deep N is not accounted for here), it is reasonable to conclude that BNF was occurring at the full estimated rate. This assumption increases the total measured N in the alfalfa plots to the 229 kg ha⁻¹ of total stored above and belowground N indicated in Figure 3-2 as compared to the 89 kg ha⁻¹ calculated in the 100% N control.

Figure 3-2 highlights the question of the destination of the fertilizer N, particularly in Phase 2.

From seeding (Timepoint 5) until canola anthesis (3rd biomass, Timepoint 7), total N from above and belowground pools in the 100% N control decreased from 207 to 89 kg ha⁻¹ N. During the same period, total N from above and belowground pools in the alfalfa increased from 88 to 229 kg ha⁻¹ despite the fact that the 100% N control treatment received twice the quantity of N that the alfalfa did at seeding (Timepoint 5). Of the starting N in the 100% N control, 57% cannot be accounted for. Not all soil pools could be measured, but with the same soil pools unaccounted for, the alfalfa treatment increased in total N by 62%.

The alfalfa plots took up less soil nitrate-N than the control plots did (Total belowground N, Timepoint 7), but it was estimated to have a higher N yield from the total plant biomass than the 100% N control did. This additional N in the alfalfa treatment can be attributed to BNF.

If there had been a canola harvest from the 100% N control, then soil pool N would have translocated to the aboveground canola N, but since there was no harvest, a large portion of the starting belowground N may have been lost. The cold, wet spring conditions (Table 2-3) very likely increased losses of fertilizer N to denitrification and leaching from all treatments (Manitoba Agriculture, n.d.), but more of the inorganic fertilizer N may have been captured by the actively growing alfalfa, whereas the canola was still having difficulty emerging. The greater aboveground

N in the alfalfa at canola 3rd biomass date (Timepoint 7) likely also includes N that was scavenged from the soil rather than being lost to the environment, as seen in a review of red clover and winter wheat intercrops in Ontario (Gaudin et al., 2013). The presence of living cover crops (mulch) can also decrease leaching through lowering soil moisture and increasing water holding capacity (Bergström & Kirchmann, 2004). Conversely, decreased air movement across the soil's surface caused by the presence of an alfalfa canopy in moist conditions can also increase N losses to denitrification (Gaudin et al., 2013).

Figure 3-2 illustrates that a slow change occurred in the aboveground alfalfa N pools over two seasons of growth. Whereas the control had N only in the main crop, weeds, or stubble, the alfalfa treatment also had N stored in the cover crop, an estimated 80% of which had been fixed from the atmosphere (Anglade et al., 2015). Although stored N may not console a disappointed canola producer, as they would hope to see N yield in the form of a grain harvest, a producer who is able to pivot in their overall strategy has: 1) not lost their N to leaching or volatilization; 2) the opportunity to harvest the living mulch for seed as a marketable product; and 3) the potential to harvest the mulch as a forage or graze it in the field.

3.5.2 Plant root simulator probes

There has been little research to account for soil nutrient dynamics and nutrient availability in living mulch systems (Carof et al., 2007; Dzvene et al., 2023). A review of living mulch systems states that short-term changes to nutrient concentrations and availability (within the first year) are not realistic (Dzvene et al., 2023). Adding soil organic matter or improving the microbial populations take time to manifest (Schipanski & Drinkwater, 2012b; Wivstad, 1999). Moreover, older stands of mulches have also shown that they release or share more N than younger stands (Louarn et al., 2015).

The steady drawing down of soil NO_3^- and NH_4^+ , as measured by PRS® probes, indicates that N was being taken up from the soil by the mulches rather than deposited. Fixing N is an energy intensive process for legumes, so they take up freely available N when it is available (Schulze et al., 1994). The competition from canola, which would have reduced mulch uptake of soil N by growing into the space that was created by controlling the growth of the mulch, was not present. However, the role of a living mulch to store nutrients, rather than letting them erode aboveground or leach belowground, was still at work here. A different result was found for red clover grown in pure stands or in grass mixes in Denmark, where bare fallow treatments and non-legume cover crops both decreased in soil mineral N over the course of a year while treatments with red clover increased (De Notaris et al., 2025).

The consistent lack of difference at Carberry-1 between the two remaining treatments of alfalfa and sweet clover in all Phase 2 tests may indicate that they are very similar to each other in the field, a pattern that has also been noted by the organization, Sustainable Agriculture Research and Education (SARE, 2007). Even at the other site-years, alfalfa and sweet clover were in the same groupings across metrics (Table 3-5 – Arborg “3rd” biomass, Roblin-1 “spring biomass”). Additionally, the standard errors of the mean were relatively high across tests for sweet clover and alfalfa at Carberry-1, limiting our ability to detect differences between these treatments.

3.6 Conclusion

The purpose of this research was to determine if the additions of N by the presence of legumes could be measured in the companion crops of wheat and canola (Phase 1 and 2), or in the soil nitrate-N supply as compared to solo-seeded canola plots (Phase 2) at 100% N and 50% N levels of N fertility. The results show that the N that was introduced into the two cropping system phases by the presence of legumes was not made available in the soil nitrate, or in the soil N fluxes for

the wheat or canola during the time that they were growing together with the cover crops. There was no measurable N benefit to the companion crop plant or grain N when compared to treatments without legumes. Changes to soil N were seen only in the canola phase at the 3rd soil sample date past seeding at one site–year (Roblin-1) of the three where the canola phase was carried out. At this site–year, despite receiving only 50% of the fertilizer of the canola-only control, all mulch treatments had the same 0-15 cm soil N as the 100% N fertilizer control. However, no canola harvest was possible due to uncontrolled mulch growth. There were also no increases to N soil flux detected by PRS® probes in plots with legumes. The benefits of N additions to the system by legume fixation were seen over a longer period of time, and came at the expense of the Phase 2 canola crop. This points to a more traditional understanding of legume cover cropping, as a source of organic material to be released through plant decay and subsequent mineralization.

The accumulation and translocation of N in legume cover cropping systems continues to prove difficult to anticipate and to control. While the general principles and processes of the N cycle are well understood, the N dynamics observed in this research suggest a system that balances and counterbalances N movement, based on subtle mechanisms within the micro-environment. Future research should investigate means for living mulch control, so that in-season N benefits to the canola crop can be measured by means such as natural abundance and so that canola can be grown successfully.

3.7 References

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4. APPLICATION

The ability to grow wheat and simultaneously establish a cover crop with one pass of the seeder without an economic penalty is the most impactful finding of this research. While lower seeding rates may have contributed to lower biomass in Phase 2, they were sufficient to establish the cover crop and produce a forage harvest. On the Canadian Prairies, more producers (especially on mixed farms) would likely be more interested in producing forage than in seeding canola into a living mulch. Since none of the legumes had a negative impact on the wheat, and alfalfa consistently produced the highest biomass, the seeding approach used in this study would be a good method of establishing an alfalfa stand. In Roblin white clover established well in both years.

Despite the promising results for establishing the living mulch system, seeding into a living mulch cannot yet be recommended for any of the cover crop species that were tested, and more research is needed to identify methods of biomass control.

Nitrogen losses from agricultural systems cannot be completely avoided, but the practice over the last seven decades has often been to recommend inorganic N application rates that are in excess of what is actually used by the crop — a practice that enables high-yield grain farmers to turn a profit (as long as fertilizer prices remain low enough), but also obscures the losses. Excess application has provided a measure of predictability both for farmers and for consumers at the dining room table, both of which are necessary. However, targeted research has begun to frame the loss of inorganic N fertilizers in more tangible ways, putting dollar values to the amount of N that cannot be seen volatilizing during dry spells, denitrifying during wet spells, flowing over land during spring melts, or leaching into lower soil horizons. Nitrogen that is not taken up by a harvested, marketable crop is felt by the farmers as a real financial loss; it is an investment that literally does not yield results. The N does not disappear, however, and the further consequences

of N accumulating in water bodies or as volatilizing as greenhouse gasses are environmental, economic, and societal.

If a producer is going to feel confident relying on legumes to replace any amount of external fertilizer N additions, they either need the BNF to be more predictably quantified, or they need to have flexibility built into their farming goals. This research challenges the view of the farm as a factory. A factory can optimize its outcomes and increase productivity by decreasing diversity and removing or obscuring sources of unpredictability. Re-evaluation of standard fertility programs requires changing our view of the farm.

A farm using the approach demonstrated in this research has a more holistic view than the dominant practice, recognizing that nature is highly variable, that sometimes a great success (a successful wheat harvest and a well-established cover crop) can translate into a great challenge (biomass control), and that the individual objectives and management capacity of the farmers drives the decision of what their farming model will be. This research shows that the practices of legume cover cropping can be integrated into farming systems that also make use of inorganic fertilizers, and that legume cover cropping is not relegated solely to the domain of organic producers. Integrating legume cover cropping practices introduces undeniable management challenges, but for a versatile farmer, legume cover crops also present many opportunities: soil cover outside the growing season, moisture management, forage production, livestock grazing, and seed production, to name a few. Nevertheless, challenges are not unique to systems that incorporate legume cover crops. Risk and inefficiency will never be absent from farming. The challenge this research presents for policy makers is to identify the target audience for cover crop implementation, while recognizing that N gained from legumes is largely a deferred benefit.

Ideal candidates for implementation of legume cover crops are flexible in their management goals. In the face of unpredictable natural systems, they are able to pivot and are comfortable with variable outcomes. Each growing season develops differently and farmers that are able to adapt their goals are better equipped to handle the situation that arises. Producers such as these are often found on mixed farms, or have partnerships with other farms that manage livestock. The array of tools they have at their disposal must have a wide range and include fencing, mowers, sprayers, and cultivators. Working with a complex land setup has required them to keep informed by good resources which provide them with a range of “off ramps” in the event of a failed cash crop, ineffective herbicide application, or overabundant legume biomass. Farming cannot be divorced from profitability, but profitability can take many different forms.