

Assessing the Potential of Cover Crop Mixtures in a Faba Bean Cropping System Under Boreal
Climatic Conditions

By

Sharjeel Ahmad

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Abstract

Poor soil conditions and boreal climatic conditions in Newfoundland and Labrador (NL) are the major constraints in the establishment of cover crop (CC) mixtures. CC mixtures were grown as an additional forage source, which not only provided biomass but also balanced soil C:N ratio, scavenged N and improved soil health. An experiment (June 2022- September 2023) was conducted in Pasadena, NL, where fourteen CCs mixtures were introduced in a faba bean cropping system. The combinations composed of two-way and three-way CC species of legumes [red clover (RC), berseem clover (BC), hairy vetch (HV) and birdsfoot trefoil (BT)] and cereals [fall rye (CR), annual ryegrass (AR) and triticale (TR)] in a randomized complete block design. The results showed that CC mixtures were successfully established and highest DMY was observed for HVCR and RCCR. While BCAR and RCAR exhibited better fodder quality as compared to other CC mixtures. CC mixtures also improved the forage quality of the faba bean, weed suppression and benefit-cost-ratio. However, CC mixtures have a non-significant impact on faba bean yield stability. CC mixtures had a significant impact on SMBC while no significant effects on POX-C, POM-N, POM-C, MBN, and soil mineral N. PLFA analysis showed that CC mixtures have significant impact on G^- , G^+ , total bacterial population and total PLFA content while had no significant effects on fungi and protozoa population. This research concluded that CC mixtures showed good DMY and forage quality along with improvement in weed suppression, benefit-cost-ratio, SMBC, and bacterial population.

General Summary

Podzolic soil and boreal climate pose challenges in NL, and to tackle it CC mixtures were sown to improve soil health, nitrogen scavenging, and forage quality. An experiment was conducted from June 2022 to September 2023 at Pynn's Brook, Pasadena, NL, which examined the effects of fourteen CC mixtures within a faba bean cropping system. The study was a randomized complete block design with two-way and three-way combinations of legumes-cereal CC. Results indicated that the DMY of CC mixtures was significantly different among treatments and DMY of spring 2023 was lower than fall 2022. However, it significantly improved the forage quality, weed suppression, BCR and faba bean quality. Moreover, significant effects were observed on SMBC and bacterial populations. It was also observed that CC mixtures had a minor short-term impact on the microbial community and labile carbon pools of podzolic soil.

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List of Abbreviations

ANOVA - Analysis of variance

AR - Annual ryegrass

BAME - Bacterial acid methyl ester

BC - Berseem clover

BT - Bird'sfoot trefoil

C:N - Carbon/Nitrogen ratio

CC - Cover crop

CR - Cereal rye

DMY - Dry matter yield

FAME - Fatty acid methyl ester

FAO - Food and Agriculture organization

G⁻ - Gram negative bacteria

G⁺ - Gram positive bacteria

GC-FID - Gas chromatography – Flame ionization detection

HV - Hairy vetch

MBC - Microbial biomass carbon

MBN - Microbial biomass nitrogen

NL - Newfoundland and Labrador

PLFAs - Phospholipids fatty acids

POM-C - Particulate organic matter carbon

POM-N - Particulate organic matter nitrogen

RC - Red clover

SOC - Soil organic carbon

SOM - Soil organic matter

SPE - Solid phase extraction

TMSH - Trimethyl sulfonium hydroxide

TN - Total nitrogen

TR - Triticale

Σ B-PLFAs - Total bacterial phospholipid fatty acids

Σ PLFAs - Total phospholipid fatty acids

Chapter 1: General Introduction and Literature Review

1.1 Introduction

Newfoundland and Labrador (NL) soils are very acidic and the dominant factors that limit crop growth are low fertility and short growing season. The soils require regular applications of lime and fertilizers to raise soil pH and supplement elements necessary for plant growth (Kedir et al., 2021). The major challenge of cover crops (CC) establishment in boreal regions is the early frost and short growing season of primary/cash crops which do not allow enough time for CCs to establish after harvesting primary/cash crops. For example, after harvesting silage corn, the temperature drops quickly and there is not enough time for CCs to establish before the start of the winter season. In this situation, frost damage could be a significant constraint for growing CCs in the region, and it is a constant risk due to prolonged cold and frost spells, snow cover, and frequent and erratic freeze-thaw cycles (Hutchinson et al., 2007; Schlesinger & Jasechko, 2014; Yang et al., 2019). The main challenges with CC adoption in cropping systems are: (a) deciding between underseeding, interseeding, or post-harvest seeding of CCs; and (b) selecting the most appropriate and effective termination method of CCs to minimize impacts on seeding and establishment of the next cash crop (Aronsson et al., 2016; Chapagain et al., 2020b). Herbicide application (e.g., glyphosate) or plowing and the use of roller crimping machines are some possibilities for terminating CCs (Kornecki et al., 2009). Although the latter method has gained favor, it does not necessarily eliminate all CCs (Halde & Entz, 2014). An issue particular to legume CCs is that significant biomass production is required for them to boost soil N availability, therefore early establishment, such as underseeding of CCs is necessary. Although the time of underseeding is crucial for maximizing biomass and plant N, relatively few studies have been conducted to determine the suitable underseeding time (Notaris et al., 2019). It has been observed by Notaris et

al. (2020), that the early planting of legume CCs achieves N fertilizer replacement values of 40 kg ha⁻¹ and 85% of N assimilated in CC biomass which was biologically fixed by legumes. N availability and long-term soil fertility increased in this scenario, which may result in a "yield stabilization effect" over time. However, adding legume CC in rotation requires careful planning in inter-row spacing to limit crop competition and minimize the influence of CCs on the yield stability of primary crops (Notaris et al., 2019). CCs can also offer additional forages to fulfill the forage requirements of the livestock and dairy sector and improve soil health (Abdalla et al., 2019).

CCs cover the soil to reduce erosion such as catch crops, living mulches, green manure, legume, and non-legume crops (Kaspar et al., 2011). A few CCs are short-duration, winter hardy, and resistant to harsh climatic conditions, fix N, improve production of primary crops, suppress weeds, and benefit the ecosystem (Sharma et al., 2018b). Legumes CCs fix atmospheric nitrogen and enhance the soil organic matter while non-legumes are CCs that uptake nutrients from the soil and improve crop productivity. CCs improve the soil's physical, chemical, and biological properties (Abdalla et al., 2019; Ghimire et al., 2019a). The ideal CCs grow fast and can fix atmospheric nitrogen, have a deep root system to absorb moisture and nutrients, enhance biomass production in a short time duration, are tolerant to insect pests and diseases, suppress weeds, and are cost-effective (Myers & Watts, 2015). CCs with fibrous root systems show a high potential to restrict soil erosion compared to tap root system crops (De Baets et al., 2011; Gómez et al., 2018). Therefore, it increases the soil water retention capacity (Qi & Helmers, 2010; Yang et al., 2020). CCs compete with weeds on essential resources and inhibit the photo chrome-mediated emergence and growth of weeds (Mennan et al., 2020; Tursun et al., 2018). By improving the soil's physio-chemical and biological properties, CCs improve cash crop production by reducing weeds,

enhancing soil organic content, and improving soil structure, etc. (Fageria et al., 2005). CC's ability to fight weeds depends upon its species and taxon-specific phenotypic traits like canopy development and growth rate. Forbs (buckwheat) as well as grasses (cereal rye, oats) have rapid canopy development and growth which aids in suppressing weeds (Baraibar et al., 2018). Lawley et al. (2012) observed weed suppression by CCs like *Brassica* spp. is primarily due to dense canopy which reduces light penetration and availability to weeds, which inhibits weed's growth. Previous studies also observed CC biomass plays a key role in suppressing weeds (Beach et al., 2018b; Liu et al., 2022). CC mixtures are meant to enhance agroecosystem services and productivity through increased resource partitioning (Finney & Kaye, 2017). Therefore, CC mixtures may have better potential to suppress weeds than sole CC/monocultures. Increasing crop diversity acts as a driving tool in enhancing agroecosystem services (enhancing soil microbial activities and controlling soil-borne diseases) and productivity (Loreau and Hector (2001). Chapagain et al. (2020a) observed that growing a single CC species has merits and demerits. For example, oats and ryegrass can produce high biomass and are economical; however, cannot fix N and have a high C: N ratio. Red clover (RC) fixes nitrogen but has non-uniform stand establishment, low biomass, and high seed cost compared to oat and ryegrass (Chu et al., 2017). Forage radish (*Raphanus sativus* L.), rye (*Secale cereale* L.), and canola (*Brassica rapa* L.) have a better capacity to scavenge residual soil N than legume CCs (Florence & McGuire, 2020a; Smith et al., 2020). A study conducted in Michigan, USA, used three CCs (red clover, hybrid sorghum, and sudan grass), and a multi-mix species (oat, soybean, lentil, forage pea, sunflower, sweet clover, oil-seed radish, turnip, and pearl millet). The result showed that highest dry matter yield (DMY) was achieved by multi-mix species, followed by sorghum sudan grass, red clover, and control (Isleib, 2012). Snapp et al. (2005b)

observed that the mixture of legume-cereal CC species produced high-quality biomass over different growing environments.

In addition to benefits to soil physical properties, soil nutrients, and weed suppression, CCs used for forage contribute to the system's profitability. Holman et al. (2018) observed that inclusion of CCs in a wheat-fallow system enhances net profit by about 26–240%. Similarly, Plastina et al. (2018) observed an increase in net return for farmers who grazed CCs. The size of the profit from a dual-purpose CC, however, depends on factors such as the forage species, productivity, nutritive value, and cost of production (Holman et al., 2021). For example, forage multispecies CC may provide the most benefits from a CC than a single species CC (Chapagain et al., 2020a). Other studies, on the other hand, observed no advantage of CC mixtures compared with single species (DeLaune et al., 2020; Holman et al., 2018; Nielsen et al., 2015). Holman et al. (2018) concluded that CC species with low seed cost and greater forage production are more profitable. Different crop management practices are known to improve soil physiochemical properties, including manure application, crop residue management, biochar, forestry waste material or sewage sludge, and crop rotation (Snapp et al., 2005a). In recent years, there has been a much focus on CC systems to cover fallow land throughout the winter season to reduce erosion, restore soil fertility, and improve soil physical properties (Nouri et al., 2019; Tobin et al., 2020), chemical properties, and biological properties (Hallama et al., 2019; Kim et al., 2020). Roley et al. (2016) observed that a CC mixture might be used to increase soil fertility and decrease the requirement of fertilizers to enhance water quality in the Upper Mississippi River Basin, USA. Legumes and grasses are frequently combined to decrease the C: N ratio of CC residues, allowing for quicker decomposition and less risk of excessive nutrient tie-up (Nielsen; et al., 2015). Cultivation of oats and vetch in subtropical Eastern Cape, South Africa, and a blend of rye and vetch in subtropical Georgia, USA

were observed as successful CC mixtures (Koudahe et al., 2022). Furthermore, Finney et al. (2016) observed significant N scavenging with various CC mixes (hairy vetch + red clover + oat + forage radish and sun hemp + soybean + red clover + hairy vetch + forage radish + oat + canola + cereal rye) in continental Pennsylvania, USA. However, CC mixtures produce less biomass and scavenge less N compared to the most productive monoculture. CC species, on the other hand, should be chosen based on the CC functions, to meet higher productivity and N scavenging goals (DeLaune et al., 2019). Multispecies CC mixes can benefit from the strengths of each species, for example including legumes and non-legumes as CCs mixture, as most ecosystem services are connected to species' functional features (Tribouillois et al., 2015; Wendling et al., 2019). The ability to create multifunctional mixes that are more suited to satisfy the demands of producers is made possible by leveraging the link between functional features and agroecosystem services (Chapagain et al., 2020b; Storkey et al., 2015). A few studies have observed that the monocultural species could be less functional compared to the species in mixtures (Cadotte, 2011; Flynn et al., 2011; Lavergne et al., 2021; Mouillot et al., 2011).

The faba bean (*Vicia faba* L.), a short-duration pulse crop capable of growing in cool, wet environments and is used for both human and animal consumption. There are two types of faba bean varieties - tannin and low tannin (zero tannin). Tannins are anti-nutritive compounds that affect palatability and digestion in monogastric. Tannin beans are desired for human consumption in both whole form and fractionated for use as a food ingredient. Low-tannin beans, often referred to as zero tannins, can be used for both human and animal consumption (Köpke & Nemecek, 2010; Mínguez & Rubiales, 2021). Faba bean is a rich source of protein, dietary fibers, carbohydrates, and minerals. It can fix nitrogen 100-200 kg ha⁻¹ (Jensen et al., 2010). Faba bean can be employed as break crop in a crop rotation system (Abera et al., 2015; Landry et al., 2015), feed source of

pollinators and beneficial insects (Etemadi et al., 2015), enhanced soil microbial activity (van der Putten et al., 2013; Wahbi et al., 2016), medicinal crop (Etemadi et al., 2019) and control of soil-borne diseases (Jensen et al., 2010).

Currently, very little is known about the establishment of CCs mixture in faba bean cropping system in western NL. We hypothesized that CC mixtures will successfully establish in boreal climate after harvesting faba bean, suppress weeds, offer additional quality forage, improve soil health and benefit-cost ratio. Additionally, CC mixtures will keep yield stability and forage quality of faba bean. To test the hypotheses, the study was conducted with the following specific objectives:

1. To assess the establishment of CC mixtures after faba bean harvesting and yield stability and forage quality of faba bean following CC mixtures.
2. To determine the effects of CC mixtures on weed suppression in a faba bean cropping system in a boreal climate.
3. To assess the benefit-cost ratio of CC mixtures in a faba bean cropping system in a boreal climate.
4. To evaluate the effects of CC mixtures on soil mineral N, labile carbon pools, and soil active microbial population in faba bean cropping systems in a boreal climatic condition.

1.2 Literature Review

1.2.1 Effect of Cover Crop Mixtures on Subsequent Crop Yield and Quality

CCs are a vital component of sustainable cropping systems which are used to promote soil health; manage weeds, pests, and diseases; and minimize erosion. Planting two or more types of CCs simultaneously has gained popularity in recent years. Several studies have been undertaken

to assess the impact of CC mixtures on the production and quality of primary crops. Chu et al. (2017) conducted a long-term experiment assessed single-, double- and multispecies CCs in a corn-soybean rotation. Results indicated that in comparison to the less diversified treatments and a no-cover control, a multispecies combination of legumes, grasses, and brassica spp. considerably boosted soybean production, gravimetric soil water content, and soil inorganic N.

Kc et al. (2023) investigated the effects of CC mixes on maize production and quality. Annual ryegrass, crimson clover, and forage radish mixture (256 kg ha⁻¹) was the next most productive crops, followed by the annual ryegrass (174 kg ha⁻¹) and the annual ryegrass and crimson clover mixture (165 kg ha⁻¹). In addition to agricultural productivity, the impact of CC combinations on crop quality has been investigated. Uchino et al. (2012) investigated the influence of CC mixtures on potato quality and found that employing a CC mixture prior to potato cultivation, together with fertilization using manure or a combination of straw and slurry, is advisable for achieving optimal yields and quality. Singh et al. (2021) observed that CC mixes improved the quality of soybean crops. CCs improve soil health, prevent erosion, and enhance sustainability. For example, combining cowpea and rye boosted soybean seed protein content by 10% compared to control with no CC. Overall, the findings show that CCs have a considerable influence on crop yield and quality.

A vineyard study in Eastern Canada conducted by Messiga et al. (2016) examined the impacts of CC mixtures with organic and industrial wastes on grapes production and quality. Four CC mixtures: (i)-oats + peas + hairy vetch (OPV), (ii)- oats + red clover (ORCI), (iii)-timothy + + red clover (TM), and (iv)-control with no CC (CONT)-were fertilized with synthetic fertilizer or organic treatments. The CC and amendment combinations (oats + red clover (ORCI) with mussel

sediment (MS) (9.52 Mg ha⁻¹) application produced the highest grape production. Thus, the CC in combination with organic fertilizers performs well as compared to no CC with inorganic fertilizers.

Valkama et al. (2015) conducted a meta-analysis of 35 Nordic studies to examine the influence of both legume and non-legume CCs on forage yields when seeded in spring. According to the results, the best forage crop was Italian ryegrass, which depleted up to 60% of soil N and was more successful than perennial ryegrass. When CCs are grown, grain yields of the primary cash crop are often reduced in the first year (Breland, 1996; Cicek et al., 2015; Yang et al., 2019). This yield penalty is frequently equated to the density of the cash crop, which means that a denser CC frequently results in a larger yield penalty of the main cash crop; thus, selecting the appropriate CC should go hand in hand with customizing the interrow spacing (Breland, 1996; Notaris et al., 2019).

In Atlantic Canada, Gopsill et al. (2022) evaluated the biomass yield and weed control of 19 full-season CCs that were seeded as monocultures and 19 mixes with variable species makeup and functional richness (two- and three-species mixtures). The species type, their functional traits, and their abundance all had an impact on CC biomass output and weed suppression. Regardless of CC species/variety, weed biomass decreased as CC biomass increased. They found that buckwheat (*Fagopyrum esculentum Moench*), a species known for its high grain productivity, and Sorghum-Sudan grass (*Sorghum drummondii*), a species known for its high biomass, when combined, improved stand evenness, yield, weed suppression, and spatiotemporal stability compared to all CCs. Table 1.1 illustrates how different crop species have varied effects on following crop yields in various locations and time periods. Generally, legumes increase yields, especially for corn and small grain cereals, with steady gains seen in China, Europe, and North America. Crop mixtures have positive benefits on yields as well, however it's unclear how they affect soybeans. Grasses

often have a neutral or adverse effect, particularly on small grain grains like maize. Regional variations, like the beneficial effects of winter legumes in China, imply that climate and management strategies are important factors in determining these results.

Table 1.1 Summary of research conducted on the effects of cover crops on subsequent crop yields across the globe.

Region	Year	Crop species	Impact on subsequent crop yield	References
North America and EU	1910–2015	Legume (454 [†])	Corn (+31 %), small grain cereal (+33 %), soybean (ns)	Bourgeois et al. (2022)
		Brassica dicot (97)	Corn (ns), small grain cereal (ns), soybean (ns)	
		Mixture (211)	Corn (+29 %), small grain cereal (+26 %), soybean (ns)	
		Grass (357)	Corn (–2 %), small grain cereal (ns), soybean (ns)	
China	Until 2020	Winter legume (223)	Rice (+16 %), maize (+27.2 %)	

		Winter grass dicot (133)	Rice (+10.6 %), maize (ns)	Fan et al. (2021)
		Summer legume (83)	Rice (ns), maize (ns)	
Global	1980–2020	Legume (212)	Apple orchid (ns)	Wang et al. (2021)
		Grass dicot (172)	Corn (-)	
		Mixture (39)	Corn (+)	
Global	1995–2018	Mixtures (342)	Corn (ns)	Florence and McGuire (2020a)
Global	Until 2014	Legume (274)	Seed cotton (+11 %), lint (+4 %)	Toler et al. (2019)
		Grass dicot (18)	Seed cotton (-10 %), lint (ns)	

		Legume + monocot (48)	Cotton (+8 %), lint (ns)	
		Monocot (199)	Seed cotton (+3 %), lint (+4 %)	
Global	1985–2017	Legume (1005)	Corn (+27 %)	Daryanto et al. (2018)
		Grass dicot (1282)	Corn (+6 %)	
USA, Europe	1996–2017	Grass CC (32)	Vegetables (ns)	Norris and Congreves (2018)
Global	1931–2017	Winter legume (5)	Corn (ns)	Thapa (2018b)
		Winter grasses dicot (88)	Corn (ns)	

Argentine Pampa	2006–2016	Legume (68)	Corn (+7 %), soybean (ns)	Alvarez et al. (2017)
		Grasses dicot (48)	Corn (–8 %), soybean (ns)	
		CC mixture (14)	Corn (ns), soybean (ns)	
USA and Canada	1965–2015	Legumes (101)	Corn (+30 to 33 %) with low n fertilizer and no-tillage	Marcillo and Miguez (2017)
		Grasses (140)	Corn (ns)	
		Mixture (28)	Corn (+30 %) under late CC termination	
Global	1910–2012	Legumes (10)	Crop yield (+25 %) in irrigated cropping systems	Quemada et al. (2013)
		Grasses dicot (9)	Crop yield (–) in 50 % of observations	

Temperate	1970–2002	Legumes (415)	Crop yield (–7 %) relative to conventional systems	Tonitto et al. (2006)
		Legumes (115)	Crop yield (+5 %) relative to conventional systems with low N fertilizer application	
USA	1967–2003	Winter legumes (82)	Corn (+22 %)	Chapagain et al. (2020b); Gopsill et al. (2022); Miguez and Bollero (2005)
		Winter grasses (68)	Corn (ns)	
		Winter CC mixtures (10)	Corn (+21.5 %)	

‡: number of observations, +: positive, -: negative, and ns: no significant effect

1.2.2 Effect of Cover Crop Mixtures on Soil Properties

1.2.2.1 Effect of Cover Crops on Soil Physical Properties

Soil physical properties include soil porosity, structure, consistency, aggregate stability, bulk density, and water retention, which determines the soil water and nutrients holding capacity(Salazar et al., 2022). These properties have a great influence on soil chemical and biological properties through water and nutrient movement in the rhizosphere and soil microbial activities(Qi et al., 2022). Crop growth is affected by soil moisture retention due to soil particle size, pore size distribution, and bulk density(Soares et al., 2021). Good soil physical properties are elemental features of resilient soils (the soil environment's ability to retain its pivotal functions during internal and external tensions)(Blanco & Francis, 2016).

Soil bulk density (BD) is a key factor that correlates with soil compaction, physio-chemical and biological properties (Han et al., 2016). It is the ratio of soil-dried mass to its total volume (Maurya et al., 2020; Walter et al., 2016). High BD shows low soil porosity and high compaction. Soil compaction is the reduction of pore spaces because of the soil pressing due to anthropogenic activity (different tillage practices and farm machinery etc.) (Mondal et al., 2020). It causes shallow plant rooting, erosion, and poor crop productivity (Sivarajan et al., 2018). Blanco et al. (2011) observed the impacts of CCs on soil physical properties for 15 years. The findings revealed that sun hemp was reduced by 4% BD, however, no effect on penetration resistance (areas that allow identifying areas with restrictions due to compaction) compared to no CC (NCC). According to Villamil et al. (2006), hairy vetch and winter rye CCs lowered soil BD and penetration resistance (silt loam). This considerably enhanced total soil porosity at the soil surface compared to NCCs. Chen and Weil (2011) and Weil (2004) found that the forage radish roots penetrate easily in sandy loam soils and alleviate compaction at depths ranging from 15 to 50 cm in compacted soils. Haruna

et al. (2020) observed that CCs (annual medic or a clover) did not alleviate soil BD on a sandy loam. Some researchers also observed no significant differences in soil bulk density between CC and NCC plots (Cercioglu et al., 2018; Nouri et al., 2019). In boreal climates, where freeze-thaw cycles already influence soil structure, CCs can further stabilize soil aggregates and prevent compaction from machinery operation. Reduced bulk density is significant for root penetration and microbial activity. CC residues form stable soil aggregates, preventing re-compaction and enhancing soil aeration(Chirinda et al., 2019). This can benefit the establishment of faba bean after CCs by creating a more favorable seedbed and root development environment.

Soil porosity (pore spaces or voids) is the portion of soil volume not occupied with solids minerals or organics (Mondal & Chakraborty, 2022). It usually contains water and air which influences the water movement, infiltration, growth, and development of plants (De Oliveira et al., 2021). According to the Soil Science Society of America (2008), there are two different types of soil pores, i.e. macropores and micropores. Macropores (sand) easily allow air and water movement while micropores (silt and clay) restrict air and water movement(Holthusen et al., 2018). All plants need water and air for nutrient uptake and respiration. So, well-aerated soils with high water-holding capacity are good for plant growth and development (Gliński & Stepniowski, 2018). Porous soils have low water retention ability and are less saturated. These soils are good for water infiltration and hold less nutrients (Munkholm et al., 2016; Reis et al., 2021). Soil compaction, along with organic matter loss, crusting, and anaerobiosis, is an indicator of land degradation that can contribute to soil erosion and nutrient depletion. CC seeding reduces soil compaction by creating root channels in the subsoils, thus, simultaneously improving air permeability and water infiltration (Blanco et al., 2015). Root exudation, deposition, and turnover of CC roots stabilize dispersed and friable soil particles, enhancing soil aggregate stability and

structure (Hanrahan et al., 2021; Vannoppen et al., 2017). Previous research has shown that sun hemp and soybean reduced soil compaction by 5% in silt loam soil when grown over a long-term period (Adetunji et al., 2020). Villamil et al. (2006) observed that the winter rye and hairy vetch mixture with soybean and corn reduced BD and soil penetration resistance, hence, increase soil porosity compared to no CCs.

Soil hydraulic characteristics influence soil functioning in ecosystems and have a significant impact on soil management (Rawls et al., 2004). Water transport, especially water-soluble nutrients, is an important soil hydraulic activity that directly influences plant production. Increased water infiltration often enhances plant water storage, recharges groundwater, and minimizes erosion. Soil hydraulic properties include saturated hydraulic conductivity (K_{sat}), water content (WC), available water content (AWC), mean infiltration rate (MIR), and permanent wilting point (PWP) (Hao et al., 2023). Living CC roots can alter soil hydraulic characteristics by transpiring excess water out of the soil. Chalise et al. (2019) found that the mixture of winter rye and hairy vetch lowered previous soil water content, resulting in higher soil water penetration when compared to NCC management. CCs influence soil water dynamics through improved infiltration, reduced evaporation, and enhanced water retention. Studies have shown that CCs increase soil water availability by reducing runoff and enhancing soil structure (Blanco et al., 2015). The root systems of CCs create macropores, facilitating water infiltration and percolation (Abdollahi & Munkholm, 2014). Additionally, mulch from terminated CCs helps reduce surface evaporation, maintaining soil moisture levels for subsequent crops (Basche, Archontoulis, et al., 2016). In boreal climates, where precipitation patterns and snowmelt play critical roles in soil water content, CCs can aid in moisture conservation, benefiting the following faba bean crop.

However, CCs can also compete for soil water, especially in dry years, potentially impacting the establishment of the succeeding crop. This is particularly relevant in boreal regions, where short growing seasons and variable precipitation patterns may influence the efficiency of CCs in moisture retention (Cicek et al., 2015). Therefore, species selection and termination timing are crucial factors in optimizing the water benefits of CCs.

Haruna et al. (2022) found that the pore spaces left by CC (multi-species mixture of CCs used included hairy vetch, crimson clover, winter wheat, winter peas, oats, triticale, barley, and flax) roots enhanced cumulative water penetration by 68% two months after termination compared to NCC management. Haruna et al. (2017) found that CCs (cereal rye, oat, annual ryegrass, and Sudan grass) enhanced 18 and 11% volumetric water content (θ) at saturation and -33 kPa pressures, respectively, compared to NCC management. Furthermore, Villamil et al. (2006) found that the cereal rye and hairy vetch enhanced soil porosity, and these changes in pore size distribution resulted in large increases in transmission pores. Furthermore, Rankoth et al. (2019) investigated the effects of CCs (crimson clover, hairy vetch, cereal rye, Austrian winter pea) on soil moisture and sap flow in maize with and without CC and observed 14 %, 12 %, and 4 % higher soil water content at 10, 20 and 30 cm depths, compared to NCC plots. These authors concluded that CC-managed plots sustained higher soil moisture levels for longer than NCC plots. As a result, CCs have been shown to enhance water retention and conservation while also increasing agricultural yield when compared to NCC management (Delgado et al., 2021).

In Missouri, USA, CCs, such as barley, hairy vetch, red clover, barley, cowpeas, triticale, turnips, cereal rye, buckwheat, and winter peas improved hydraulic properties (saturated hydraulic conductivity and water retention) of claypan soils in corn-soybean rotation after 5 years (Adetunji et al., 2020). It was observed by Demir and Işık (2019) that the plantation of white clover and hairy

vetch improves the persimmon yield, soil retention capacity, and saturated hydraulic conductivity. Basche, Kaspar, et al. (2016) observed that rye on loamy soil significantly increased the rate of plant available water as compared to no CCs at the 0-15cm and 15-30 cm soil depth by 21 and 22 %, respectively. It was also observed that increased water retention capacity increases SOM and soil aggregation stability (Bilek, 2007). Liesch et al. (2011) observed increased water permeability when rye CC was integrated into the corn system. Blanco et al. (2011) observed three times increase in water infiltration rate under sun hemp in silt loam soil as compared to no CC.

1.2.2.2 Effect of Cover Crops on Soil Chemical Properties

SOM refers to the organic component of soil, which includes plant and animal residues at various stages of decomposition, cells, and tissues of soil organisms, as well as substances synthesized by soil organisms. The several soil labile C components have encouraged microbial population and activity. As a result, SOC is an important indicator of soil health (Bronick & Lal, 2005). Both inorganic C and organic C molecules make up soil C. The term "organic C compounds" refers to a group of non-living organic elements present in soil, including humus, animal, plant, and microbe leftovers (Lefèvre et al., 2017). CCs may provide a low-cost on-site management option for increasing SOM and soil health (Dabney et al., 2010). Residues from rye, oats, grazing vetch, faba bean, and clover boosted the organic matter composition of vineyard topsoil in the Western Cape, South Africa, regardless of management technique (Fourie et al., 2007). Across a wide range of environmental conditions, most studies have observed that CCs like hairy vetch, crimson clover, pea, turnip brassica, oats, and rye greatly enhanced SOC concentrations compared to control (NCCs) (Olson et al., 2014). Increased soil biomass from CCs leads to higher levels of organic C (Poeplau & Don, 2015). Additionally, by decreasing soil erosion, which may function as a pathway for such loss, CC practices may lessen SOC loss

(Blanco, 2015). SOC is thus one of the most crucial features used to investigate how CCs affect soil productivity (Fig 1.1).

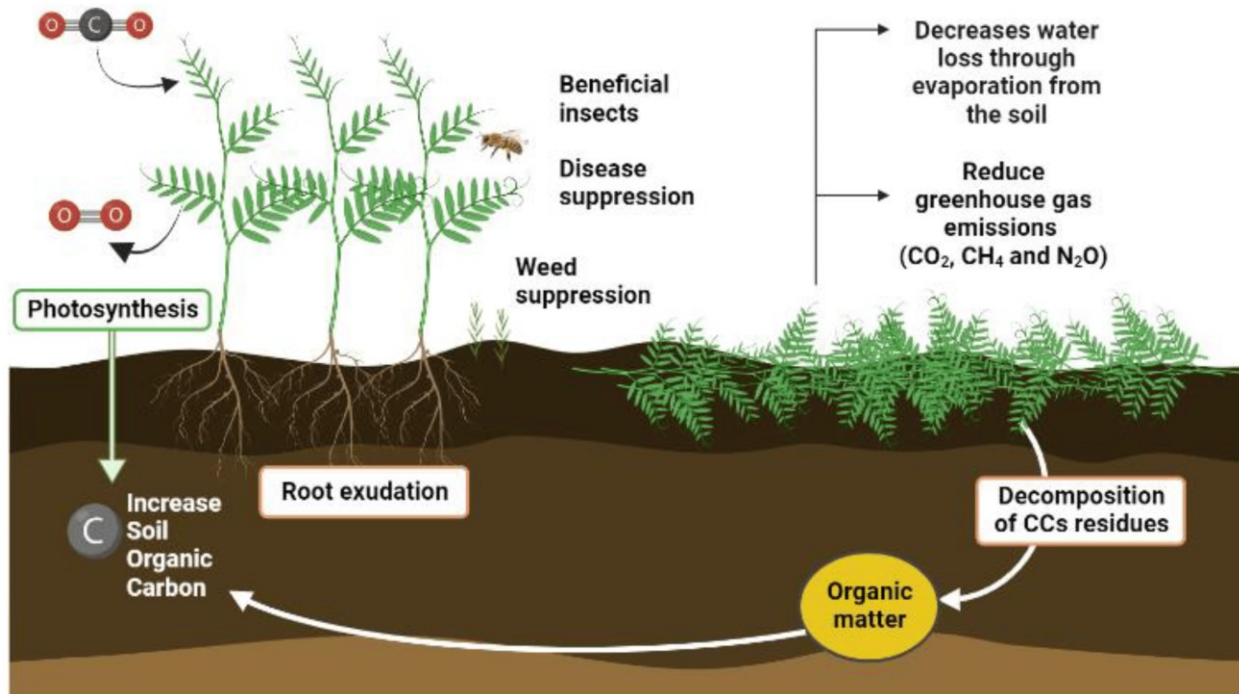


Figure 1.1 Ecological services associated with cover crops. Adapted from Quintarelli et al. (2022).

According to Blanco et al. (2015), the amount of soil C build-up is site-specific and depends on factors like CC biomass quantity, how long materials have been in existence before being terminated, the original amount of soil C, the type of soil and CC, tillage practices, and soil temperature. Because of the elevated initial soil C content, the impact of CCs on SOC is typically unnoticed until much later during the establishment process (Acuña & Villamil, 2014). When corn was rotated with oats and grazing vetch within 5 years, total SOC and water-soluble C were increased (Dube et al., 2012). In boreal climates, the decomposition rate is low due to cold temperature, CCs can aid in maintaining SOM and mitigating carbon losses (Paulsen et al., 2022).

CC cultivation can increase N levels by scavenging N and reducing leaching of N (O'Reilly et al., 2012). Compared to fallow, hairy vetch and crimson clover crops can substantially increase N concentration in soil (Yang, 2020). Soil N levels increased by 258 kg ha⁻¹ and 279 kg ha⁻¹ under soybean and sun hemp rotations, respectively, compared to no CC treatments. As a result, legume CCs provided large amounts of N to the following crops, improving soil fertility, and lowering N fertilizer needs to the following crops (Blanco et al., 2011). However, it is observed that annual winter legume CCs did not boost soil N, particularly if planted for less than 5 years (Sarrantonio & Gallandt, 2003; Villamil et al., 2006). Although grasses cannot fix N, they can rapidly uptake soil N as they develop. When the growth season is over, leftover soil N may remain in the soil and seep below the rooting zone (Jani et al., 2016). However, grasses CCs can scavenge and recycle this soil N. In comparison to legume CCs, rye, canola, and forage radish showed a greater ability to extract residual soil N (Kuo & Sainju, 1998). Jani et al. (2016) summarized the effects of CC management from 16 investigations, hairy vetch, rye, ryegrass, oats, winter wheat, purple vetch, and mustard decreased nitrate leaching by 6–94 %. In contrast, a meta-analysis in an irrigated farming system revealed that non-legume CCs significantly decreased N-leaching compared to legume by 15% and barren soil by 50 % (Quemada et al., 2013). The capacity of non-legume CCs to remove extra nutrients from the soil using inorganic fertilizers or livestock manure is crucial for reducing the likelihood of water pollution (Blanco, 2015). The amount of N fertilizer required for the highest possible yield of succeeding crops like cotton, maize, and sorghum has been shown to increase when plants with high C: N ratios slowly decompose and immobilize soil N (Hunter et al., 2019). Higher SOC (19.4 g kg⁻¹) and total N (2.8 g kg⁻¹) were found under CC mixes (radish and Australian winter pea) compared to the sole CCs (radish and Australian winter pea) (SOC: 15.9 and 17.6 g kg⁻¹ and total N: 1.5 and 1.7 g kg⁻¹) in heterogeneous soils (silt-loam, fine, illitic,

mesic Aeris Epiaqualfs) (Stavi et al., 2012). According to research conducted in the Eastern Cape of South Africa, the combination of oats and vetch produced more N in the alluvial soils during the late maize growth season compared to sole CCs (Mukumbareza et al., 2016). Whittaker (2017) conducted research trials on the effects of red clover, triticale, and legume-grass (triticale + red clover) mixture on soil N supply and soil solution N in a following potato harvest. The findings indicate that triticale and a legume-grass combination lowered NO_3^- levels than red clover only by about 66% and 86%, respectively. Therefore, red clover+ triticale reduces the NO_3^- levels in the soil. However, studies have shown that in boreal climate, the effects on soil mineral N can be minimal due to slow mineralization rate and high C/N ratio (Vankoughnett & Henry, 2014). Understanding these interactions is crucial for optimizing CC mixtures to balance N supply and retention.

1.2.2.3 Effect of Cover Crops on Soil Biological Properties

Soil biological properties include soil micro- and macro-floral activities. It represents the direct and indirect effects of living organisms habituating in a particular soil. These are living organisms that contribute to agricultural productivity and water quality. These organisms are single-celled bacteria, algae, protozoa, nematodes, fungi, earthworms, insects, arthropods, and small vertebrates (Koudahe et al., 2022).

Concern over how CC might affect soil microbial communities, and their functions have been rekindled by a revived interest in soil health and, so, biological indicators of soil health. Although the exact process by which CCs affect soil biology is complicated, it is generally thought to be connected to an increase in plant C inputs and habitat (i.e., rhizosphere) during the fallow period of the year (Budak et al., 2013; Vukicevich et al., 2016; Wang, 2020) (Fig 1.2). By acting as a

source of fuel and energy for soil microbes, the inclusion of CCs (derived from root exudates, decomposition of litter/residues) encourages the development and activity of soil microbial communities (Finney; et al., 2017; Kong & Six, 2012). According to a meta-analysis by Kim et al. (2020), CCs (hairy vetch, and red clover) increase soil microbial activity and abundance by 22 % and 27 %, respectively. Similarly, long-term cultivation of CCs (oat (*Avena sativa* L.), rye (*Secale cereale* L.), radish (*Raphanus sativus* L.), and a mixture of rye and radish) affected the structure of the soil's microbial population and increased soil microbial abundance while decreasing fungal abundance (Tosi et al., 2022). While enzyme activity did not rise with CCs, microbial functional diversity and activity raised doubts about the function of CCs in ecosystems (Nivelle et al., 2016). CCs can increase soil biological health and change the makeup of the soil microbial communities.

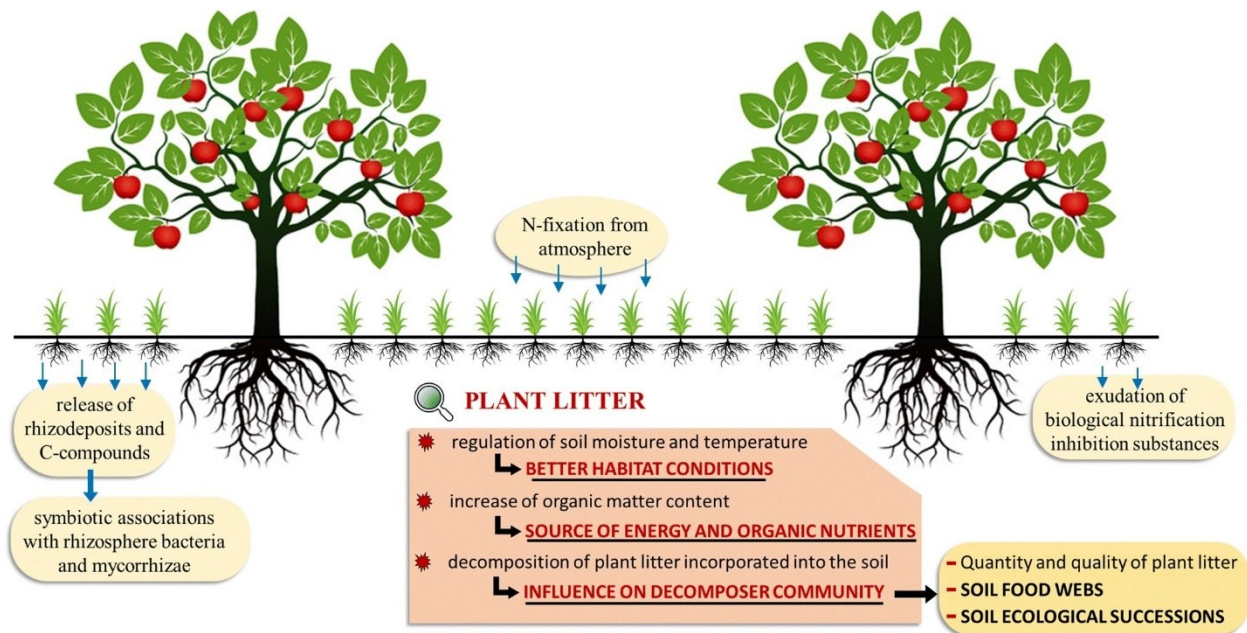


Figure 1. 2 Influence of cover crops as living mulches or dead mulches on soil microbial and faunal communities [(Scavo et al. (2022)].

The identification of the CC is vital in defining the bacterial community (Gao et al., 2022). Cover-cropped plots have greater total bacterial counts at all depths (Schmidt et al., 2018). CCs enhance total phospholipid fatty acid (PLFA) content relative to the arable weed community present in control plots (Finney, 2017). In general, Bacterial functional diversity and metabolic capability toward six carbon source categories increased in response to CC (Shen & Lin, 2021). CC treatments affected the bacterial community structure, causing alterations in dominant genera that had plant-growth-promoting and/or pathogen-antagonistic properties. For example, CCs raised the abundances of *Streptomyces*, *Arthrobacter*, and *Bacillus* spp. in wheat and wheat + legumes treatments, as well as *Gaiella* spp. in wheat + faba bean treatment (Gao et al., 2022). CCs have shown beneficial effects on relative bacterial abundance (Li et al., 2012; Mathew et al., 2012), soil microbial biomass (Chahal & Van Eerd, 2020; King & Hofmockel, 2017), and phospholipid fatty acid (PLFA) makeup, which is a hallmark of bacterial and fungal communities (Kim et al., 2020; Schmidt et al., 2018; Schmidt et al., 2019). In agricultural production systems, certain CC species, including oat and cereal rye, are also known to encourage the development of bacterial populations (Bowles et al., 2017). In comparison, Singh and Kumar (2021) claimed that neither the PLFA results, nor the overall bacterial abundance were impacted by CCs. The overall bacterial community and abundance were more affected by CC mixtures than by sole CCs (Romdhane et al., 2019a). Wheat (*Triticum aestivum* L.) treatment altered the bacterial community structure with shifts in the dominant genera, which have plant-growth-promoting and/or pathogen-antagonistic potentials, e.g., increased the abundances of *Streptomyces*, *Arthrobacter*, and *Bacillus* spp. in the wheat and wheat + wild rocket + faba bean, and *Gaiella* spp. in the wheat + faba bean (Gao et al., 2021). Research has demonstrated that fungal communities respond positively to plant-derived carbon inputs, implying that including CCs in a rotation may increase fungal community

development. CCs can improve nutrient retention and recycling for main crops, but they may also encourage soilborne diseases or inhibit beneficial root symbionts like arbuscular mycorrhizal fungi (AMF). AM-host CCs increase the quantity and variety of AMF in main crops, while non-AM-host CCs reduce them. The selection of the CCs is critical in defining the root fungal population and AMF colonization in the following major crops. For example, using ryegrass as a CC improved AMF colonization and richness in both major crops (García et al., 2023). Some CCs, such as brassicas, can reduce fungal infections while promoting disease-fighting bacteria. CCs can improve crop yield and soil microbial diversity, reducing the spread of soil-borne pathogens (Vukicevich et al., 2016). CCs were found to be a major predictor of fungal community compositions, as well as having a considerable influence on individual fungal and bacterial taxa. Strickland et al. (2019) found that CCs (alfalfa (*Medicago sativa* L.) and soybean (*Glycine max* (L.) Merr.)) increased bioavailable C and active microbial biomass by 64% and 37%, respectively. Increases in CC biomass is associated with a decline in the microbial biomass, C: N ratio, which indicates a decrease in the utilization of recalcitrant C substrates and an increase in the compounds derived from roots (Mooshammer et al., 2022; Strickland et al., 2019). AMF communities grew in semiarid Madrid, Spain, under winter barley (1.92 ± 0.25) as compared to fallow (1.72 ± 0.23) (Hontoria et al., 2019). Lehman et al. (2014) observed similar findings that the mycorrhizal fungi population increased under the inclusion of winter legumes than no CC in subtropical wet South Carolina, continental Minnesota, Nebraska, and South Dakota. Additionally, CCs (mixture of *Fagopyrum esculentum* Moench (buckwheat), *Lupinus albus* L. (white lupin), *Phacelia tanacetifolia* Benth. (lacy phacelia), *Pisum sativum* L. (common pea), *Trifolium alexandrinum* L. (berseem clover), *Trifolium incarnatum* L. (crimson clover), and *V. villosa*) in Mediterranean Tuscany, Italy, boosted the number of AMF communities in the roots of succeeding maize harvests

(Njeru et al., 2014). Numerous researchers have observed that CCs (faba bean + rye, pea + rye mustard + rye, etc.) enhanced AMF growth which accelerates P availability and abiotic stress tolerance, suppresses the infection of pathogens, and promotes crop yields compared to no CC (Brennan & Acosta, 2017; Njeru et al., 2015; White & Weil, 2010). Eight CCs, three mixed crops, and a fallow control were examined over two years by Aiyer et al. (2022), to determine the effects on soil microbiota. During the growth season of the CC and the following year, internal transcribed spacer (ITS) and 16S rRNA amplicon sequencing were conducted to characterize the bacterial and fungus populations in the soil. The alpha diversity (alpha diversity refers to the variety of species within a specific area or ecosystem) of bacteria and fungi grew significantly over time and was affected by the CCs in the following growing season. Like, oilseed radish, alfalfa, and phacelia had favorable associations with fungal pathogen abundance, while sorghum-Sudan grass had negative associations. Sorghum-Sudan grass and buckwheat were also linked to helpful symbiotic fungus and nitrification-related bacterial families. However, in boreal climates, microbial activity is constrained by low temperature, which can influence CC decomposition rates and nutrient mineralization (Hannula et al., 2021). A few studies showed non-significant effects of CCs on fungal and protozoa populations due to the temperature limitations (Liu et al., 2023). Thus, CCs offer potential benefits for microbial enhancement, their impact in boreal conditions requires further exploration to optimize their role in sustainable cropping systems.

1.2.3 Cost-Benefit Analyses of Cover Crop Cultivation

Considering the challenges of CC establishment in boreal climate, the adoption of CCs seeding might have some financial implications and both direct and indirect costs. CC incurs costs for seeding and establishment and N fertilization. In addition, no-till drills or planters with row cleaners, additional down-pressure springs, and disc openers may be required to move and

penetrate high-residue cover without dragging or hair-pinning it in the seed trench (Randall, 2002). A 2012–2013 survey conducted by the Conservation Technology Information Centre and the North Central Sustainable Agriculture Research and Education observed that 33% of respondents saw seed cost as one of the most significant obstacles in adopting CC practices. According to the survey, the median seed cost that producers were willing to pay was US\$61 ha⁻¹ (CTIC, 2013). CC planting requires the same fundamental apparatus as no-till agriculture, with a few minor additions. If a farmer already possesses a no-till planter, it is not necessary to purchase additional equipment (Bergtold et al., 2005). However, heavy residue planting environments may need row cleansers, additional down-pressure springs, and spoke-closing wheels. These additives penetrate dense detritus, guarantee appropriate seed-soil contact, and reduce hair anchoring (Bergtold & Goodman, 2007; Watson, 1999). Based on Southeast United States equipment costs, add-on investment costs can range from US \$35 to US \$70 ha⁻¹ (Bergtold & Goodman, 2007). The application of fertilizer is a potential expenditure on cultivating CCs. Although it is not necessary for all CCs, administering N to maximize grain and small-grain yield may provide additional benefits to succeeding cash crops

Sun hemp has demonstrated the capacity to fix more than 112 kg N ha⁻¹ in Kansas climates, with up to 50 % of this N readily available to the subsequent cash crop (Mansoer et al., 1997). The N fixation of hairy vetch has been measured between 101- 224 kg ha⁻¹, whereas soybean is assumed to fix N about 100.1 kg ha⁻¹ (NRCS, 2002; SARE, 2013). A study conducted by, Roland et al. (1998) in West Tennessee observed that no-till corn yields following hairy vetch could be maintained with up to 20% (40 kg ha⁻¹) less applied N than following a wheat CC and 12% (24 kg ha⁻¹) less than following a no-cover chemical-fallow system. Incorporating residue into the soil can increase the N availability, but tillage can push the N too deep and out of the root zone of the

cash crop (SARE, 2013). Certain groups of CCs release naturally occurring compounds (allelopathic) that suppress weed germination/vegetation. Cereal rye and other high-biomass-producing legumes can be used to create an environment that suppresses weeds (Lu et al., 2000). Depending on the level of weed control, a producer may be able to skip one to two herbicide applications on the subsequent cash crop, but the effects are unlikely to last the entire cash-crop growth season (Morton et al., 2006). Nevertheless, weed management through allelochemicals has the potential to reduce the cost of commercial crop production. CCs can aid in reducing or eliminating the need for deep tilling by reducing soil compaction through deep rooting (Pratt et al., 2014). Brassicas can also enhance soil structure, which may prevent compaction from ever occurring. In addition to agronomic and environmental benefits, the sale of CC residues as biofuel feedstocks can generate revenue (Anand, 2010). Increases in SOM, a product of decomposed CC dry matter, can serve as proxies for soil health, soil carbon, and nutrient content (Pratt et al., 2014). Improved SOM can benefit both the farmer and society, via mechanisms such as carbon sequestration (Schipanski et al., 2014). Pratt et al. (2014) estimated CC SOM benefits ranging from \$50.59 ha⁻¹ for oilseed radish (*Raphanus sativus* L.) to \$108.42 ha⁻¹ for red clover. These values do not include the benefits of carbon storage. The potential for profitable CC implementation in Kansas and other regions is encouraging. Opportunity costs may include the cost of a forgone revenue crop, the fixed costs of cover crop production, and the potential for annual winter grazing. The return on investment for CCs in irrigated maize is estimated to be \$17.40 ha⁻¹ while the dryland does not generate a profit, only 10% of the available N from the CC is attributed to the revenue crop. This is a conservative estimate based on the findings of Johnson and Raun (2003), who observed that 33% of applied N is typically used for assimilation, while organic nitrogen has a higher percentage. The net return increases from US \$69.21 ha⁻¹ to US

\$49.50 ha⁻¹, if 20% of the available fixed N is used by the dryland cash crop. This should be considered a base cost, subject to change based on the circumstances and preferences of each cultivator. In later years, planting and seeding expenses could be reduced if a farmer chooses a no-till cover-crop system. Costs associated with termination are predicated on either a roller-crimper or burn-down scenario. Under a winter-kill scenario, it may be possible to reduce these costs. CCs may provide smothering, allelopathic, and light-interception effects, which may reduce herbicide costs (Bergtold; et al., 2019). In a seven-year study conducted by Moonen and Bàrberi (2004), rye and clover CCs reduced weed seed bank density by 25% and 22%, respectively, compared to control sites. Utilizing CCs consistently and empirically yields additional benefits beyond the fixed N (McNally et al., 2018).

1.3 Thesis Organization

The thesis is organized in a manuscript style and divided into four chapters. The thesis has a general introduction chapter, two stand-alone chapters (manuscript format) and the last chapter (chapter four) as general discussion and conclusion.

Chapter 1: This is the general introduction chapter of the thesis. It provides the overview with background information, rationale, relevant literature, and objective of the thesis.

Chapter 2: The title for this chapter is “Assessing establishment of cover crop mixtures, weed suppression and yield stability of faba bean in boreal climate”.

Chapter 3: The title of the chapter is “Impact of cover crop mixtures on soil health indicators in podzolic soils under boreal climatic conditions”.

Chapter 4: This chapter presents an overall discussion about study findings and conclusions.

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3

Chapter 2: Assessing Establishment of Cover Crop Mixtures and Their Effects on Weed Suppression, Profitability, and Faba Bean Yield in a Boreal Climate.

2.1 Abstract

The boreal climate of western Newfoundland and Labrador (NL) encounters challenges in crop growth and productivity due to short growing seasons and poor soil fertility. Insufficient crop heating units notably impact the growing season, causing a substantial delay in crop maturity and harvest. Cover crop (CC) mixtures have been recognized for providing soil cover, reducing soil erosion, improving forage quality and health, suppressing weeds, and supplying additional forages to dairy and livestock farming communities. The present study examines the feasibility of establishing CC mixtures and their effects on weed suppression, benefit-cost ratio, yield stability, and forage quality of primary crop (faba bean), in a boreal climate. A field experiment was carried out at the Western Agriculture Centre and Research Station, Pynn's Brook, NL, during 2022 and 2023 growing seasons. The experimental treatments were fourteen CCs mixtures: 1) red clover + cereal rye (RCCR), 2) RC + annual rye (AR), 3) RC + triticale (TR), 4) Berseem clover (BC) + CR, 5) BCAR, 6) BCTR. 7) Bird's foot trefoil (BT) + CR, 8) BTAR, 9) BTTR, 10) BTCRTR, 11) Hairy vetch (HV)+CR, 12) HVAR, 13) HVTR, 14) HVCRTTR, and a control (no CC or NCC). The faba bean was seeded in June 2022 and 2023 and harvested in August 2022, and October 2023, respectively. The CC mixtures were seeded after harvesting faba bean and harvested in November 2022 and June 2023. The results showed that the HVCR mixture produced higher dry matter yield (DMY) of 3.4 Mg ha⁻¹, whereas the lowest DMY of 1.07 Mg ha⁻¹ was recorded in the BTAR mixture. CC mixtures DMY was higher in spring 2023 compared to fall 2022. BCAR and RCAR exhibited superior fodder quality as compared to other CC mixtures. CC mixtures have a non-significant impact on faba bean yield stability however there was 30% reduction in faba bean

forage yield in 2023 compared to 2022 growing season. Nevertheless, the CC mixtures improved the forage quality of the faba bean. The HVCR mixture exhibited 99.9% weed suppression compared to BTAR mixture which showed 67% weed suppression. Additionally, RCCR and HVCR mixtures showed a higher benefit-cost ratio compared to other CC mixtures. Long-term studies are required to explore the production potential of CC mixtures and faba bean yield and forage quality in boreal climates.

Keywords: legume-grass CC mixtures, agronomic performance, faba bean, forage biomass, and quality.

2.2 Introduction

Cover crops (CCs) are plants grown primarily for the benefit of the soil and ecosystem rather than for direct harvest. They play a significant role in sustainable agriculture by improving soil health, reducing erosion, enhancing soil fertility, and managing pests and diseases (Rouge et al., 2023). There are pros and cons of growing CCs in boreal regions due to the region's characteristics such as low soil fertility, short growing seasons, and freezing temperatures. While CCs offer numerous benefits, there are also some potential drawbacks to consider: Implementing cover crops can incur extra costs for seeds, planting, and management. These expenses can be a burden for some farmers, especially those with limited financial resources. Selecting the appropriate CC species for specific soil and climate conditions requires substantial expertise and research (Quintarelli et al., 2022).

Inappropriate CC species selection can lead to suboptimal results or even negative impacts on the main crops. CCs can compete with the main crops for water, nutrients, and light resources, if not managed properly, hence reduce the yield of the primary crops. Certain CCs can harbor pests

and diseases that might affect subsequent crops. For example, some CCs can attract nematodes or other pests that could harm the main crops. Managing CCs requires additional labor and time for planting, maintenance, and termination which can be a challenging situation for farmers (Scavo et al., 2022).

Despite these obstacles, research into the most efficient ways to use CCs, especially mixtures of different CC species, to accomplish agronomic goals in boreal agriculture is gaining momentum (Gopsill et al., 2022). To keep the soil from becoming barren, CCs are often sown after harvesting or while growing other crops. The soil is better able to retain water, weeds are suppressed, and fertility is increased. These advantages are of utmost importance in boreal climates due to the short agricultural window and substantial soil degradation concerns (Mann et al., 2021; Nyiraneza et al., 2021). CCs can be useful in these areas, but only if you choose varieties and mixtures that do well in cold climates and short growing seasons. Selecting CC species that can rapidly establish and thrive in winter is of utmost importance in boreal areas (Finney & Kaye, 2017).

Planting different CC species/varieties can complement each other is the essence of CC combinations, in contrast to monoculture (Noland et al., 2018). CC diversity is based on ecological principles, which state that agroecosystems can be made more resilient and functional through the interaction of different species. The CC mixtures become even more important in boreal regions due to the extreme and unpredictable nature of the climatic circumstances (Coombs et al., 2017; Noland et al., 2018). Diverse CC species have varying rooting depths, development patterns, and attributes for acquiring nutrients, hence CC mixtures can create a protective barrier against these problems. CC mixtures promote biodiversity above and below the soil surface, which enhances soil health more efficiently than monocultures, according to research done by Wyngaarden et al. (2015). Legumes fix N and make it available to primary crops, improving soil structure and

reducing erosion. CCs in northern climates often consist of rye, clover, and radish. These plants are chosen for their cold tolerance and rapid establishment capabilities.

The capacity of CCs to inhibit weed growth is a major advantage (Gaudin et al., 2013). The competition between weeds and crops for water, nutrients, and light is a big problem in agricultural systems (Bowman et al., 2000; Tosti et al., 2014). Due to the relatively short growing season in boreal climates, weeds may wreak havoc on agricultural harvests. Physical smothering, allelopathy (the production of chemicals that limit weed development), and resource (water, light, and nutrients) competition are three ways CCs reduce weeds. There is substantial evidence from studies comparing various CC species and mixtures that may greatly decrease weed biomass and density (Baraibar et al., 2018; Florence et al., 2019; Florence & McGuire, 2020b; Smith et al., 2014; Smith et al., 2020).

In boreal climate, establishment of CC mixtures in faba bean cropping system is challenging but possible. As faba beans belong to the leguminous family, are a short-duration crop, and can grow in low-temperature zones. After the harvesting of faba bean, there is enough time for the establishment of CC mixtures. The high protein content and soil nitrogen-fixing capabilities of faba beans (*Vicia faba*) make it a valuable forage crop for boreal agriculture. There is a strong relationship between the CC that came before faba beans and their performance, both in terms of production and quality (Jensen et al., 2010). Soil organic matter, soil structure, and nutrient availability are all improved by CCs, which in turn improves faba bean development (Brasier et al., 2023). Soil residual nitrogen (as initial N application) is available to succeeding faba bean harvests by using legumes in CC mixtures in a rotation. This has the potential to enhance forage quality and increase yields. In addition to improving water holding capacity and reducing soil compaction, CCs may also increase soil microbial activity, which is great for growing faba beans.

CCs are a great way to increase the productivity and selling ability of faba bean since they improve their quality in many ways, including the amount of protein they contain and the size of their seeds (Karkanis et al., 2018). Most of the faba bean production in Canada is concentrated in provinces like Saskatchewan and Alberta and isn't a common forage crop in NL. However, the faba bean is gaining attention due to its ability to thrive in cool, wet conditions and high N fixation capacity.

There are some challenges of CC establishment in boreal climate due to cool summer, late harvest of primary crops, short growing season, low crop heating units or GDDs, early fall frost and low soil temperature can reduce seed germination, seedling growth, and establishment (Mann et al., 2021; Nyiraneza et al., 2021). We hypothesized that CC mixtures will be established in boreal climate after faba bean harvest. Additionally, CC mixtures will improve the yield and forage quality metrics of faba bean, weed suppression, and benefit-cost ratio in a boreal climate. To test the hypotheses, we aim to achieve the following specific objectives:

- 1- To assess the establishment, forage biomass, and nutritional quality of CC mixtures after harvesting faba bean in a boreal climate.
- 2- To evaluate the effect of CC mixtures on yield and forage quality of faba bean in a boreal climate.
- 3- To assess the effect of CC mixtures on weed suppression and benefit-cost ratio in a boreal climate.

2.3 Materials and Methods

2.3.1 Experimental Site and Treatment

A field research trial was conducted at Western Agriculture Center and Research Station (49.087°N, 57.541°W), Pasadena, NL during the 2022 and 2023 growing seasons. The

experimental treatments were comprised of a combination of four legumes (red clover (*Trifolium pratense*), berseem clover (*Trifolium alexandrinum*), hairy vetch (*Vicia villosa*), bird’s foot trefoil (*Lotus corniculatus*)) and three grasss (cereal rye (*Secale grasse*), annual ryegrass (*Lolium multiflorum*) and triticale (*Triticosecale Wittmack*)). The experiment was laid out in a randomized complete block design (RCBD) with four replications. The plot area of each experimental unit was $3.5 \times 3 \text{ m}^2$. Weather data (maximum temperature, minimum temperature, average temperature, and precipitation) was obtained from a weather station located adjacent to the experimental site at Western Agriculture Center and Research Station, Pasadena, NL. The mean average temperature during the 2022 and 2023 growing seasons was between 11.8 and 12.5 °C, respectively; the first frost date was ~October 1-10, annual rainfall was 1251 mm and 370 GDDs for CC mixtures.

Table 2.1 Cover crop species, their acronyms, and seeding rates planted at Western Agriculture Center and Research Station, Pasadena.

Treatments	Acronyms	Legume (kg ha ⁻¹)	Grass1 (kg ha ⁻¹)	Grass2 (kg ha ⁻¹)
Red clover + annual ryegrass	RCAR	4.5	6.8	
Red clover + cereal rye	RCCR	4.5	30	
Red clover + triticale	RCTR	4.5	55	
Berseem clover + annual ryegrass	BCAR	7	6.8	
Berseem clover + cereal rye	BCCR	7	30	
Berseem clover + triticale	BCTR	7	55	

Birds foot trefoil + annual ryegrass	BTAR	6.2	6.8	
Birds foot trefoil + cereal rye	BTCR	6.2	30	
Birds foot trefoil + triticale	BTTR	6.2	55	
Hairy vetch+ annual ryegrass	HVAR	9	6.8	
Hairy vetch + cereal rye	HVCR	9	30	
Hairy vetch + triticale	HVTR	9	55	
Birds foot trefoil + cereal rye + triticale [†]	BTCRTR	6.2	15	27.5
Hairy vetch + cereal rye + triticale	HVCRTR	9	15	27.5

[†]In three CC mixtures, the seeding rates of legume and grasses were 50% legume + 25% of each grass species.

2.3.2 Crop Husbandry and Quality Analyses

The faba bean cultivar CDC 219-16 was seeded with Great Plains seed drill (3P606NT, USA) on June 07, 2022, and June 29, 2023, respectively. The seed rate was adjusted to 125 kg ha⁻¹. The recommended rate of urea and murate of potash (30 kg ha⁻¹ and 60 kg ha⁻¹) were applied with Kubota VS-series spreaders on June 07, 2022, and June 30, 2023. Basagran (active ingredient: bentazon) was applied at a rate of 2.24 kg a.i./ha with a concentration of 480 g/L. Poast Ultra (active ingredient: sethoxydim) was applied at a rate of 0.19 kg a.i./ha with a concentration of 450 g/L. The herbicides were sprayed to control broadleaf and narrowleaf weeds. Faba bean was harvested on August 25, 2022, and October 18, 2023, at the pod-setting stage (Lithourgidis & Dordas, 2010). A 0.5 m x 0.5 m quadrat was placed randomly at 2 distinct sites within each plot, plants were swathed, cut at ~5 cm and fresh biomass was recorded. Thereafter, plant samples were oven-dry (Shell Labs, USA) at 65 °C for 72 h. Dried plant samples were weighed and ground with

a Hammer mill (CF198, China) and were sent to Actlabs (Ancaster, Ontario) for forage quality analyses.

CC mixtures were seeded on August 31, 2022, with Wintersteiger drill (600NT, USA). To determine the seed rate of grass-legume CC mixtures, the following formula modified by Gopsill et al. (2022) was used:

$$\text{Seeding rate (kg ha}^{-1}\text{)} = \frac{\text{Thousand kernel weight (g)} \times \text{Target plant population (m}^2\text{)}}{\text{Expected emergence (\%)}}$$

The CCs mixture in each treatment were seeded using 50% legumes and 50% grasses. The CC mixtures were first plant sampled on November 07, 2022, and then harvested manually on June 27, 2023, using 0.5m x 0.5m quadrat, and fresh biomass was measured with field weighing balance. The different CC species were separated, weighed on a scale, and put in jute bags for oven drying. Samples were oven-dried (Shell labs, USA) at 65 °C for 72 h. Dry weight of these samples were measured for DMY calculation and for specie evenness. Dry plant samples were ground with a Hammer mill (CF198, China) and were sent to Actlabs (Ancaster, Ontario) for feed quality analyses. Near-infrared reflectance analysis (NIR) technique (Foss NIR System Model 6500 Win ISI II v1.5) was employed to determine the forage protein (crude protein: CP, available protein: AP), fibers (acid detergent fiber: ADF, neutral detergent fiber: NDF), fat, net energy for gain, maintenance and lactation, total digestible nutrients (TDN), and predicted milk production.

2.3.3 Weed Suppression

Weeds were collected by clipping shoots ~2cm above the soil surface randomly from the same quadrat used for CCs harvesting from each plot. After harvesting CCs, fresh weed biomass was weighed to measure the suppression level of weeds (Strydhorst et al., 2008). The weed suppression percentage was calculated by the formula modified by Ramalingam et al. (2013).

$$\text{Weed suppression} = \left(\frac{\text{Weed biomass in control} - \text{Weed biomass in treatment}}{\text{Weed biomass in control}} \right) * 100$$

2.3.4 Benefit-Cost Ratio

The benefit-cost ratio (BCR) is the ratio of the present value of benefits (PV(B)) and the present value of costs (PV(C)) and was calculated following the method developed by Baranchuluun et al. (2014). Total revenue was calculated as forage DMY multiplied by the price of forage. The cost of field operations was used according to the estimated prices of Western Agriculture Center and Research Station, Pynn's Brook, NL. The cost of CCs as forages were taken from NL classified website (<https://www.nlclassifieds.com/Agriculture/Newfoundland-Labrador/hay-for-sale/c100010>). Total variable costs were calculated as the sum of the expenses for CC seed cost. The gross expenses and revenue were calculated on a per-hectare basis in CAD dollars. The following formula was used to calculate BCR of CC mixtures:

$$\text{BCR} = \frac{\text{PV(B)}}{\text{PV(C)}}$$

2.3.5 Statistical Analysis

One-way analysis of variance (ANOVA) was performed using XLSTAT (XLSTAT Premium 2017, Version 19.5) to evaluate the DMY of CC mixtures, forage quality, and impact of CCs on weed suppression, benefit-cost ratio, and faba bean yield and quality. A two-way ANOVA was performed using XLSTAT (XLSTAT Premium 2017, Version 19.5) to evaluate the impact of CC mixtures on the yield and forage quality of faba bean. Where treatment effects were significant, the treatment means were compared with Tukey's honest significant difference (HSD) test at $\alpha = 0.05$. PCA was employed to determine the associations between CC mixtures, nutritional quality, weed control, faba bean yield, and benefit-cost ratio. This multivariate analysis reduced the data's

dimensionality, allowing for clearer interpretation of the relationships among the variables. Figures were prepared using OriginPro® 2024b (Northampton, Massachusetts, USA) software packages.

2.4 Results

2.4.1 Dry Matter Yield of Cover Crop Mixtures

Among fourteen CC mixtures, HVCR mixture produced significantly ($p < 0.05$) higher dry matter yield (3.391 Mg ha^{-1}), compared to the lowest dry matter yield of 1.074 Mg ha^{-1} observed in the BTAR mixture (Fig. 2.1a). In the HVCR mixture, CR and HV contributed 74% and 26% of total DMY production. In BTAR and BCCR mixtures, BT and BC contribution in total DMY was 31% and 5%, respectively. In general, grass produced higher DMY compared to legumes in all CC mixtures. Among grasses, CR and TR showed superior agronomic performance and produced higher DMY compared to AR (Fig 2.1a). CR contributed 95%, 92%, and 83% in total DMY production in BCCR, BCTR and RCCR combinations. Whereas TR contributed 83%, 88% and 70% in total DMY of HVTR, RCTR, and BTTR mixtures. Among legumes, BC, RC, and HV contributed 32%, 44%, and 26% in the total DMY of BCTR, RCAR and HVCR mixtures (Fig. 2.1a). In the fall, there were no significant differences among the DMY of CC mixtures, which ranged from $0.54 - 0.62 \text{ Mg ha}^{-1}$ whereas, in spring, CC mixtures had a significant ($p < 0.05$) effect on DMY, ranging from $0.71 - 2.34 \text{ Mg ha}^{-1}$ (Fig 2.1b). ANOVA shows that there was a significant ($p < 0.05$) interaction between CC mixtures and harvesting seasons (fall vs. spring). DMY of all CC mixtures was higher in spring 2023 compared to fall 2022. During spring 2023, HVCR mixture produced higher DMY compared to the lowest was observed in BTAR mixture (Fig. 2.1b).

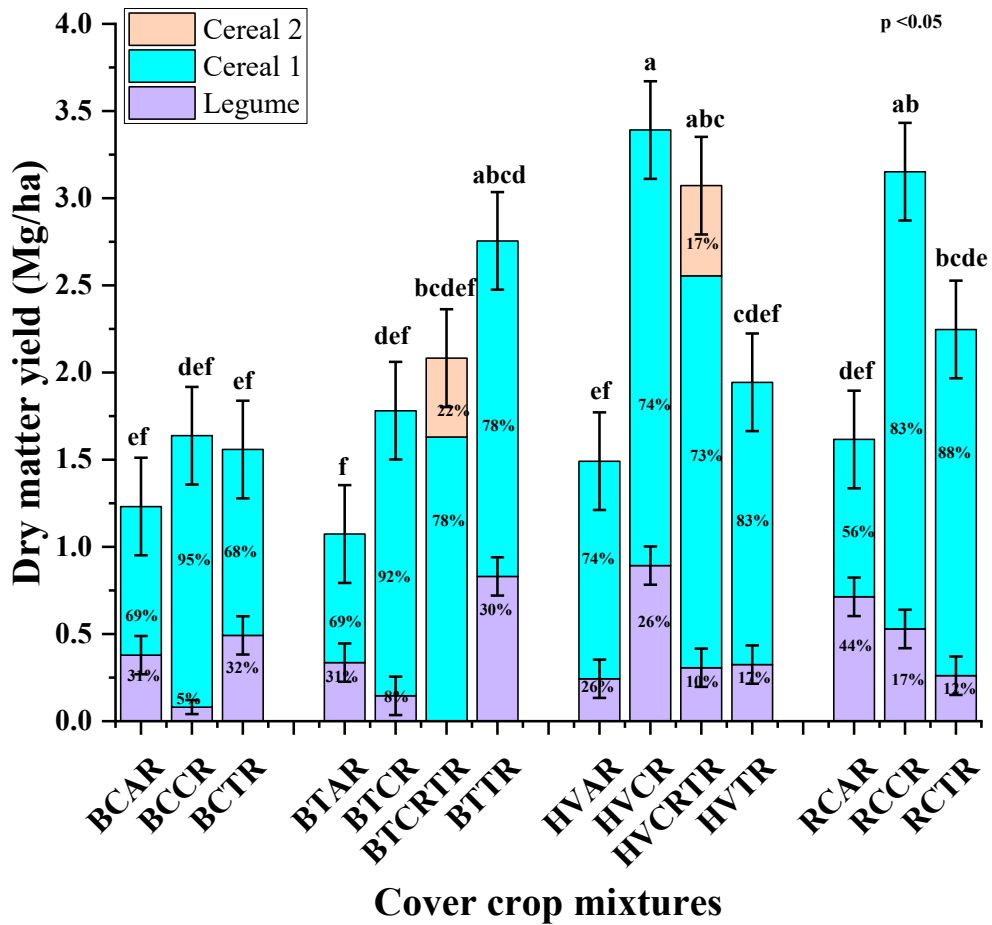


Figure 2.1 Total dry matter yield of cover crop mixtures seeded after harvesting faba bean in boreal climate, Vertical bars show the treatment means of total dry matter yield of four replications with standard errors. Lower case letters on bars indicate significant differences among treatments ($p < 0.05$, Tukey's honest significant (HSD) test). BC: berseem clover, RC: red clover, HV: hairy vetch, BT: Bird's foot trefoil, AR: annual ryegrass, CR: cereal rye, TR: triticale.

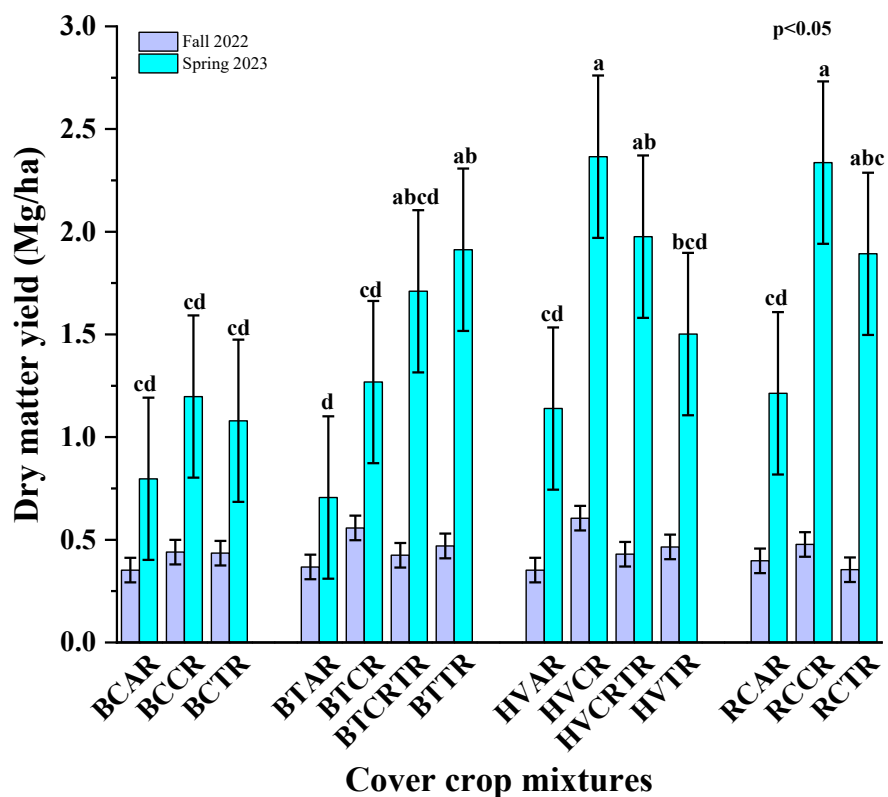


Figure 2. 2 DMY of cover crop mixtures harvested in the fall 2022 and spring 2023. Vertical bars show the treatment means of four replications with standard errors. Lower case letters on bars indicate significant differences among treatments ($p < 0.05$, Tukey's honest significant (HSD) test). BC: berseem clover, RC: red clover, HV: hairy vetch, BT: Bird's foot trefoil, AR: annual ryegrass, CR: cereal rye, TR: triticale.

2.4.2 Forage Quality Parameters of Cover Crop Mixtures

2.4.2.1 Fiber Content

The CC mixtures had significant ($p < 0.05$) effects on ADF and NDF. ADF values vary among the CC mixtures, with the BCCR mixture showed the highest (376 g kg^{-1}) ADF and the lowest (261 g kg^{-1}) were observed in the BCAR mixture (Fig 2.2a). Likewise, the highest NDF

content (624 g kg^{-1}) were observed in BCCR mixture, while the lowest NDF (388 g kg^{-1}) was observed in RCAR mixtures which was statistically at par with BCAR combination (Fig 2.2b).

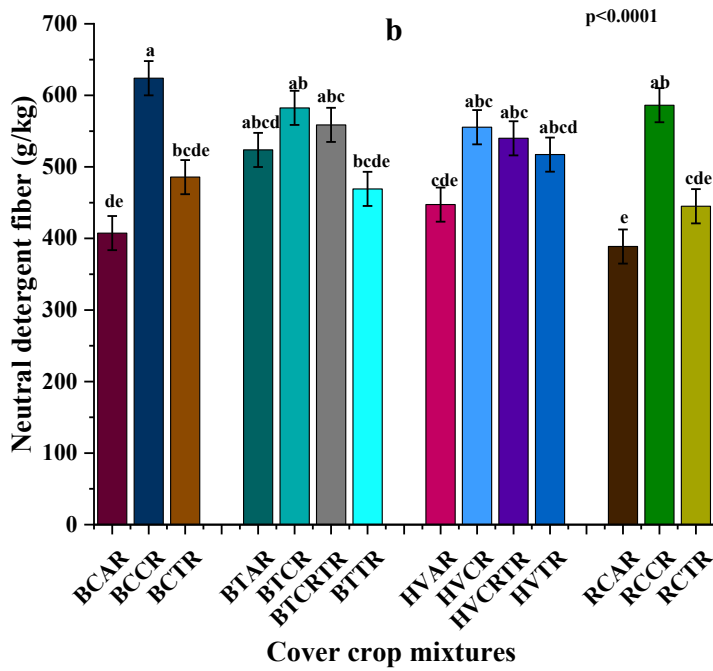
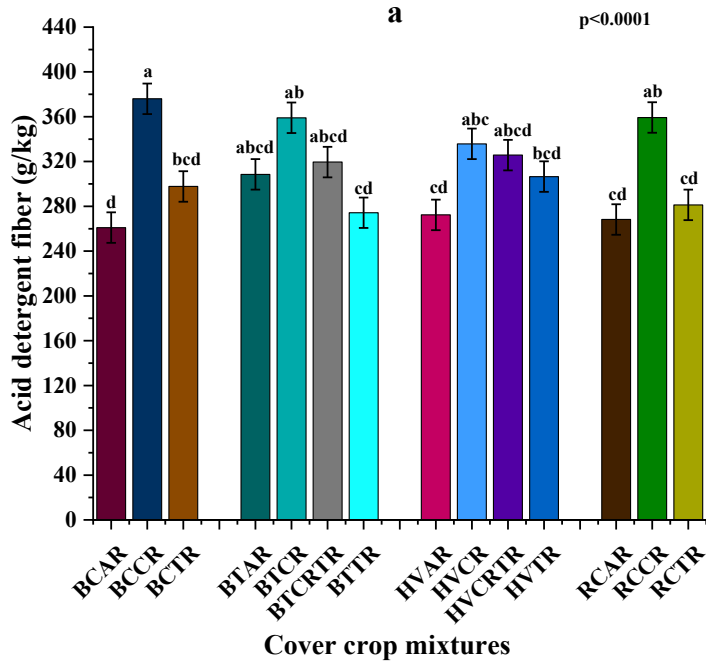
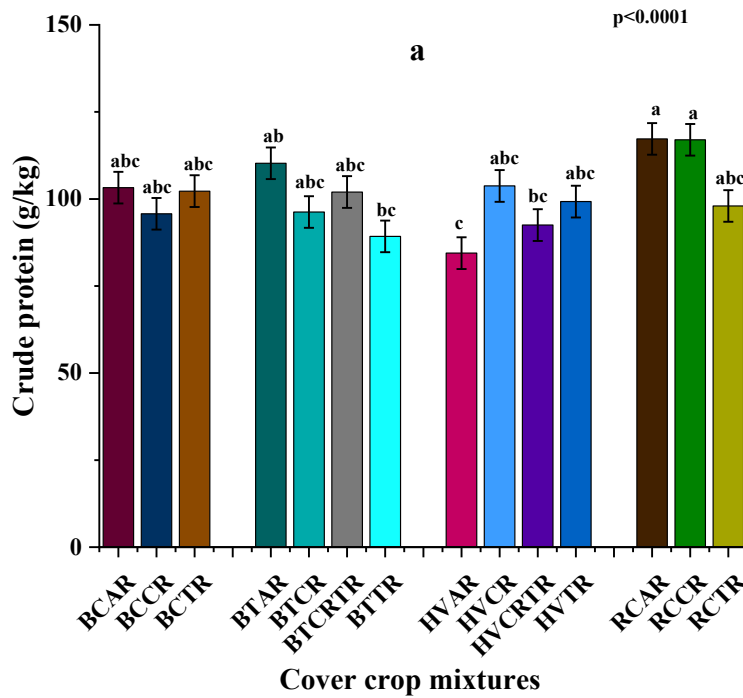


Figure 2.3 Fiber contents of cover crop mixtures: (a) Acid detergent fiber (ADF) and (b) Neutral detergent fiber (NDF). Vertical bars show the treatment means of four replications with standard errors. Different letters on bars indicate significant differences among treatments ($p < 0.05$, Tukey's honest significant (HSD) test). BC: berseem clover, RC: red clover, HV: hairy vetch, BT: Bird's foot trefoil, AR: annual ryegrass, CR: cereal rye, TR: triticale.

2.4.2.2 Crude Protein and Fat Content

Crude protein (CP) and fat content varied significantly ($p < 0.05$) among different CC mixtures. RCAR and RCCR mixtures produced the highest CP content of 117 g kg^{-1} whereas, the lowest CP (98 g kg^{-1}) was recorded in the HVAR mixture (Fig 2.3a). Fat content was significantly higher (19.7 g kg^{-1}) in BTCR mixture while the lowest (14.2 g kg^{-1}) was noted in HVTR mixture (Fig 2.3b). BTCR mixture produced 1.38% higher fat content compared to HVTR mixture.



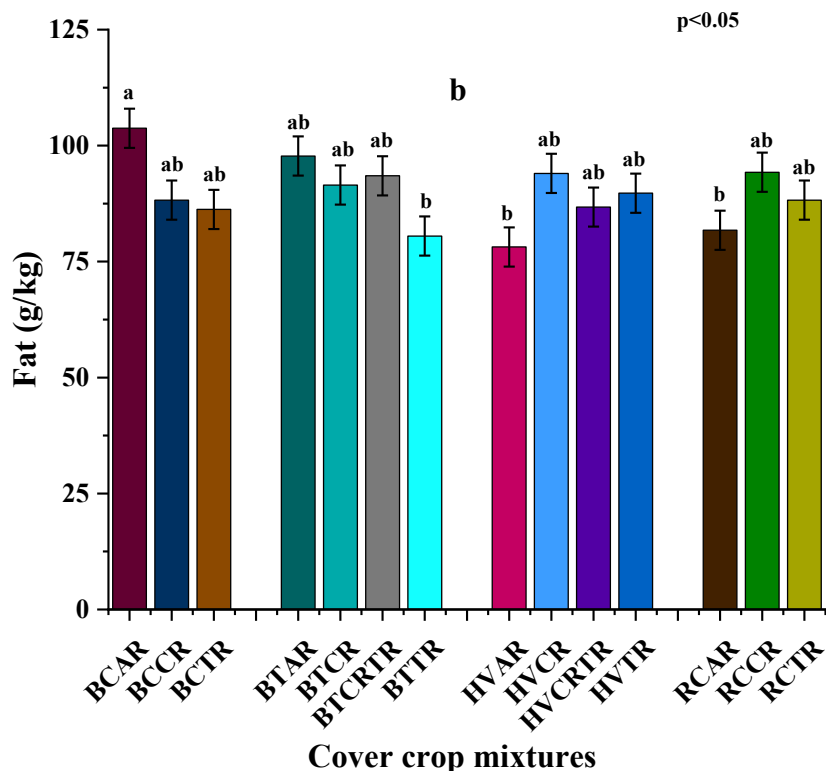
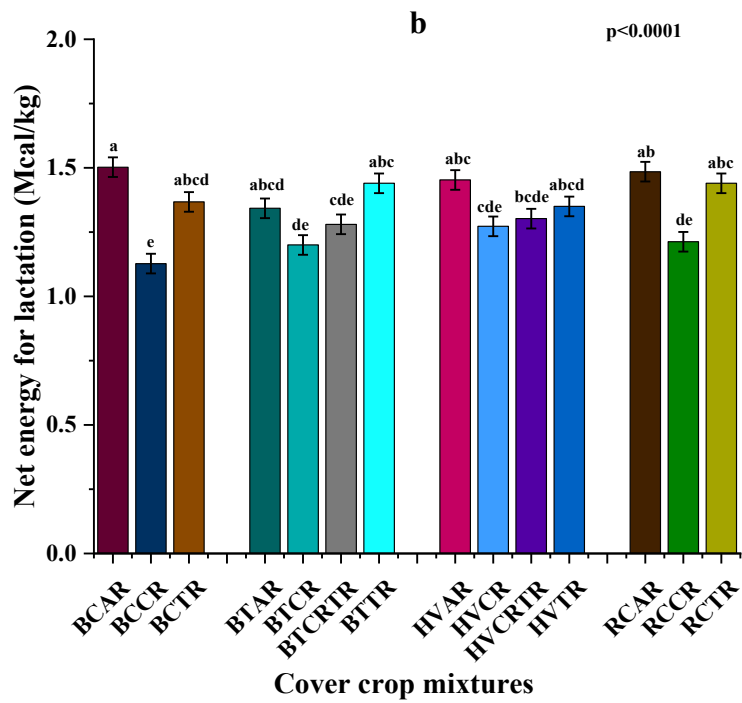
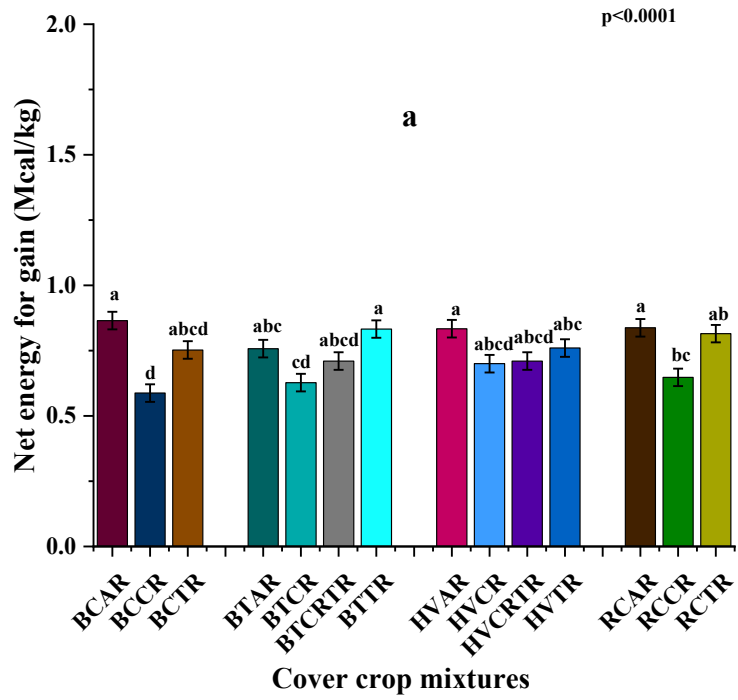


Figure 2.4 Crude protein (a) and fat (b) content of cover crop mixtures cultivated in boreal climate after harvesting faba bean. Vertical bars show the treatment means of four replications with standard errors. Different letters on bars indicate significant differences among treatments ($p < 0.05$, Tukey's honest significant (HSD) test). BC: berseem clover, RC: red clover, HV: hairy vetch, BT: Bird's foot trefoil, AR: annual ryegrass, CR: cereal rye, TR: triticale.

2.4.2.3 Forage Energies

There were significant differences ($p < 0.05$) among the CC mixtures regarding net energy for gain (NEG), net energy of lactation (NEL), and net energy of maintenance (NEM). BCAR mixture showed the highest NEG ($0.865 \text{ Mcal kg}^{-1}$), NEL (1.5 Mcal kg^{-1}) and NEM ($1.46 \text{ Mcal kg}^{-1}$) content while the lowest NEG ($0.588 \text{ Mcal kg}^{-1}$), NEL ($1.13 \text{ Mcal kg}^{-1}$) and NEM ($1.15 \text{ Mcal kg}^{-1}$) was observed in BCCR mixture (Fig 2.4 a, b & c).



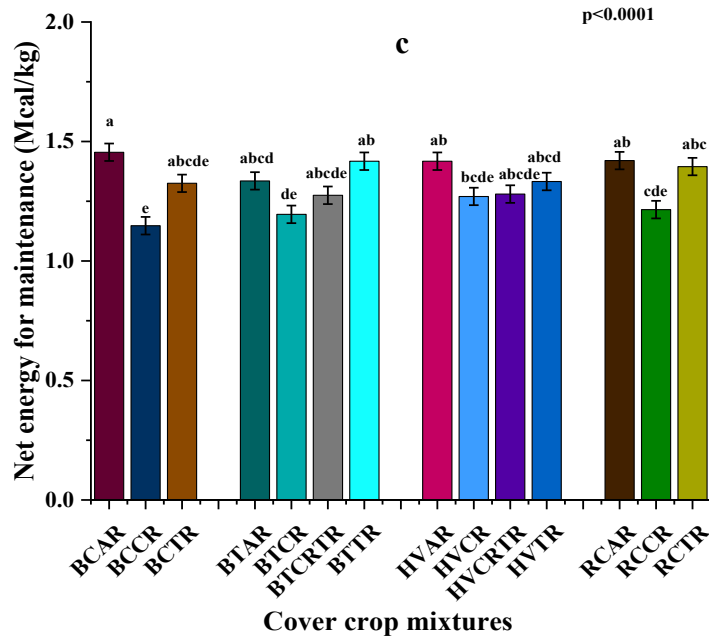


Figure 2.5 Net energy for gain (a), net energy for lactation (b), and net energy for maintenance (c) of cover crop mixtures cultivated in boreal climate after harvesting faba bean. Vertical bars show the treatment means of four replications with standard errors. Different letters on the bars indicate significant differences among treatments ($p < 0.05$, Tukey's honest significant (HSD) test). BC: berseem clover, RC: red clover, HV: hairy vetch, BT: Bird's foot trefoil, AR: annual ryegrass, CR: cereal rye, TR: triticale.

2.4.2.4 Total Digestible Nutrients and Predicted Milk Production

There were significant differences ($p < 0.05$) in TDN and predicted milk production among different CC mixtures. The highest TDN and predicted milk production was observed in BCAR (660 g kg^{-1} & 1440 L Mg^{-1} of DM) while the lowest TDN and milk production (585 g kg^{-1} & 1207 L Mg^{-1} of DM) was recorded in BCCR mixture (Fig 2.5a & b). BCAR mixture produced 1.1% higher TDN and 1.2% predicted milk production compared to BCCR mixture.

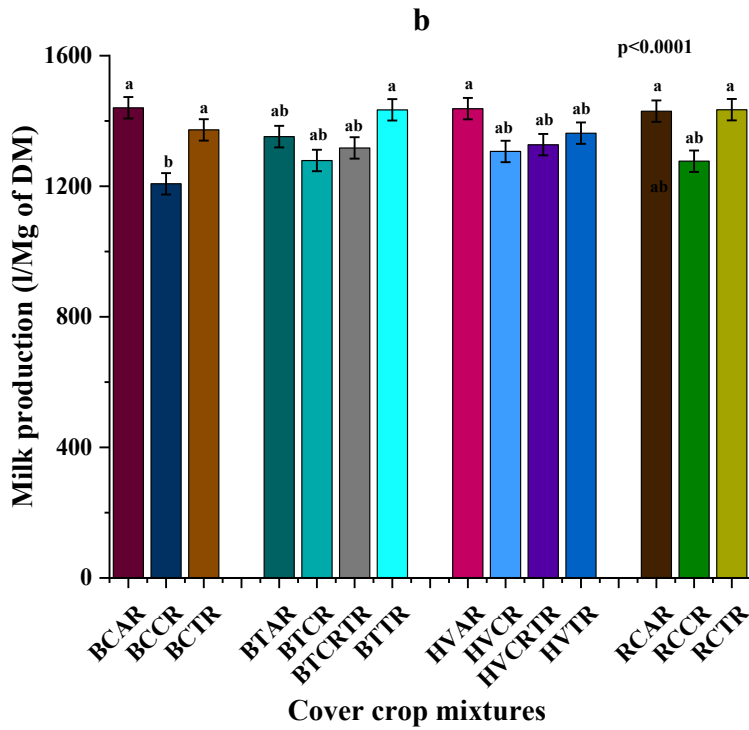
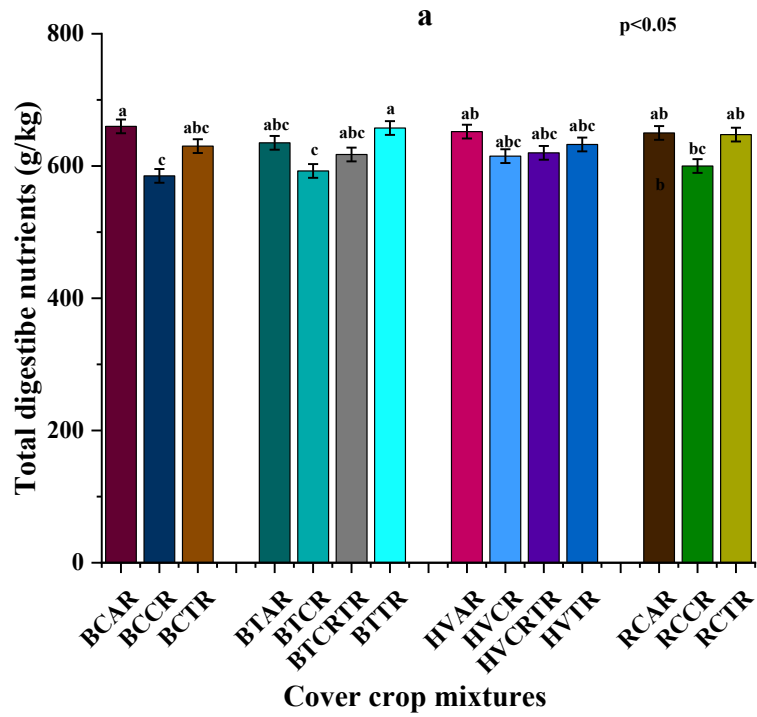


Figure 2.6 Total digestible nutrients (a) and calculated milk production (b) of cover crop mixtures cultivated in boreal climate after harvesting faba bean. Vertical bars show the treatment means of

four replications with standard errors. Different letters on bars indicate significant differences among treatments ($p < 0.05$, Tukey's honest significant (HSD) test). BC: berseem clover, RC: red clover, HV: hairy vetch, BT: Bird's foot trefoil, AR: annual ryegrass, CR: cereal rye, TR: triticale.

2.4.3 Effect of Cover Crop mixtures on Yield Stability and Forage Quality of Faba Bean

The two-way ANOVA showed a significant variability in faba bean forage yield in 2022 and 2023 growing seasons. However, there was no significant effect of CC mixtures on faba bean yield stability (Table 2.2). There seems to be a noticeable decrease in faba bean dry matter yield in 2023 compared to 2022. The maximum decline in faba bean DMY (45%) in 2023 was observed in BTCRTR mixture treatment while the lowest (18%) was observed in RCAR mixture.

Table 2.2 Two-way ANOVA showing the effect of cover crop mixtures on DMY (Mg ha⁻¹) of faba bean in 2022 & 2023.

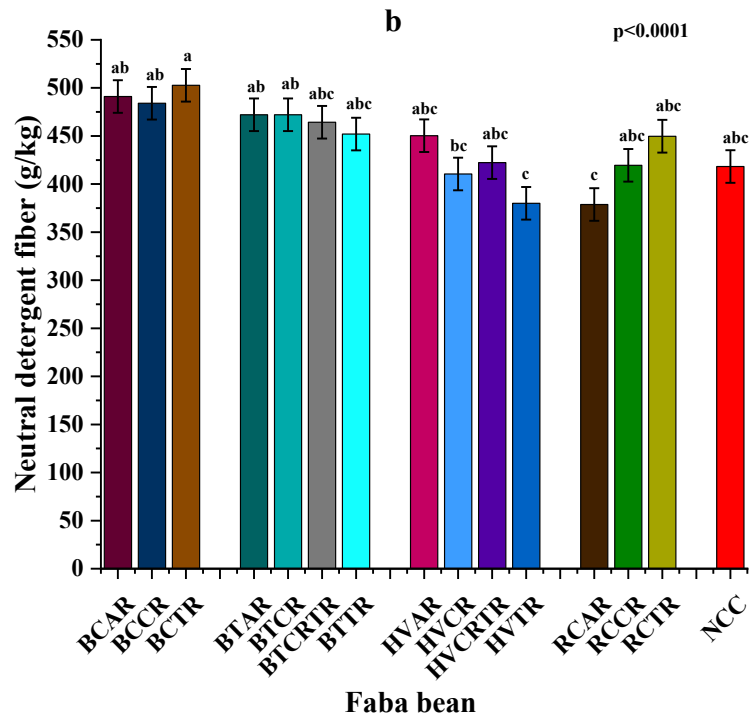
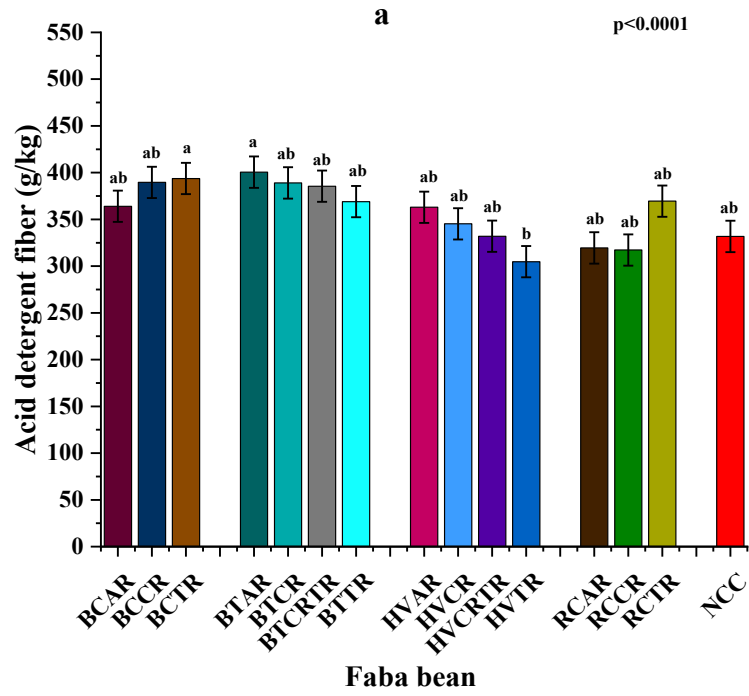
Treatments	DMY 2022	DMY 2023	Reduction in yield (%)
NCC	6.30	3.68	41.60
BCAR	5.48	3.67	33.03
BCCR	5.69	3.67	35.51
BCTR	6.28	3.47	44.74
BTAR	5.90	4.42	25.10
BTCR	4.84	3.32	31.43
BTCRTR	6.02	3.30	45.06
BTTR	4.69	3.11	33.59
HVAR	5.90	3.47	41.20
HVCR	5.21	3.22	38.14

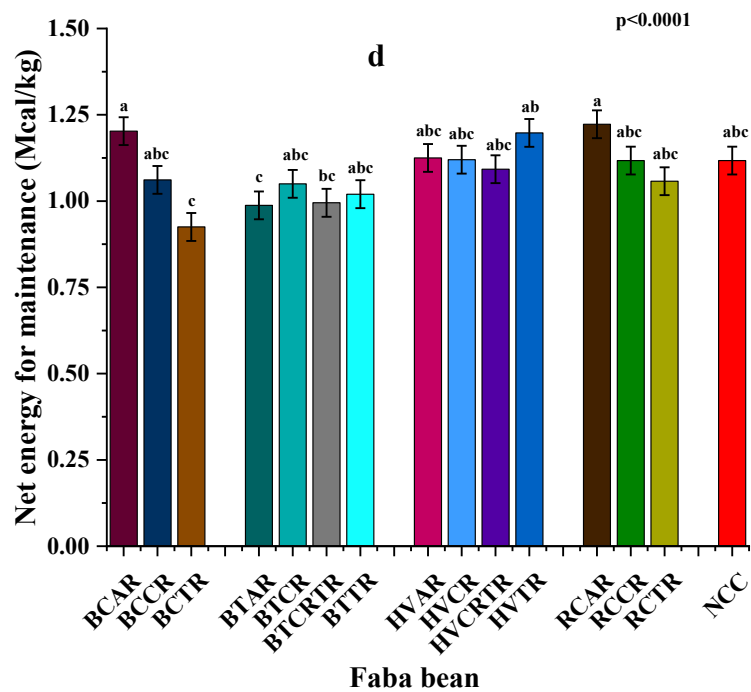
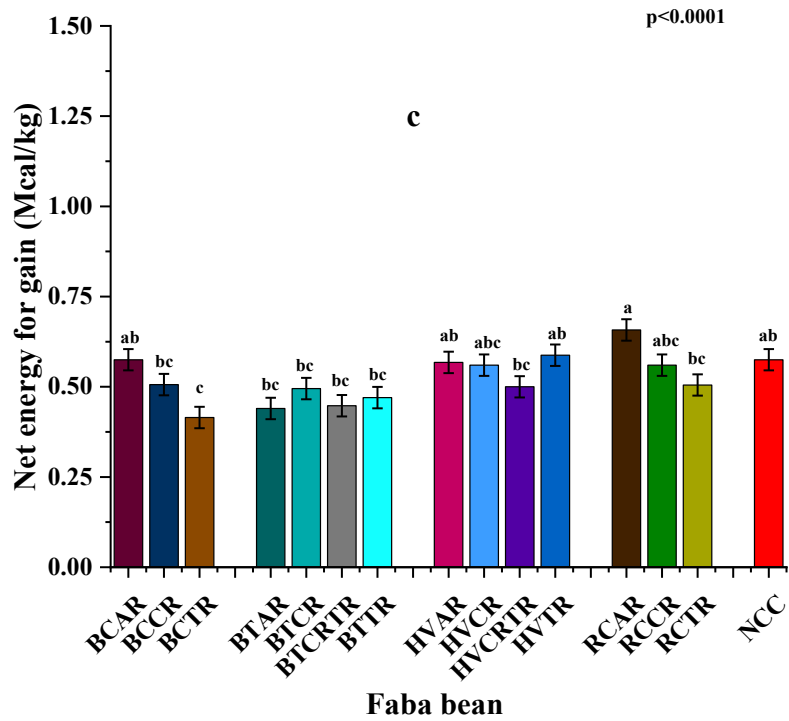
HVCRTR	6.27	3.81	39.19
HVTR	5.32	3.86	27.28
RCAR	4.67	3.83	18.10
RCCR	4.77	3.39	28.93
RCTR	5.93	3.42	42.22
P value (faba bean)	0.750	0.631	
P value (year)	<0.0001	<0.0001	
Pvalue(fababean*year)	0.877	0.877	

BC: berseem clover, RC: red clover, HV: hairy vetch, BT: Bird's foot trefoil, AR: annual ryegrass, CR: cereal rye,

TR: triticale, NCC: no CCs (control), DMY: dry matter yield

CC mixtures had significant effects on fiber content, forage energies, and TDN content of faba bean, while no significant effects on CP, fat, and predicted milk production. The highest ADF (401 g kg^{-1}) and NDF (503 g kg^{-1}) content in faba bean were observed in treatments seeded after harvesting BCTR mixture though statistically non-significant with control (NCC) while the lowest ADF (305 g kg^{-1}) and NDF (370 g kg^{-1}) contents were recorded in faba bean seeded after harvesting HVTR mixture (Fig 2.6a). The highest NEG and NEM content in faba bean forage were observed when seeded after harvesting RCAR mixture (0.658 and $1.223 \text{ Mcal kg}^{-1}$ respectively) and BCAR mixture (0.57 and 1.2 Mcal kg^{-1} respectively). Whereas the lowest NEG and NEM in faba bean were observed in plots previously seeded with BCTR mixture (0.415 and $0.915 \text{ Mcal kg}^{-1}$ respectively) (Fig 2.6 c & d). Higher NEL content ($1.268 \text{ Mcal kg}^{-1}$) in faba bean forage was noted where previous crop was HVTR mixture though statistically non-significant with RCAR, BCAR, and NCC. The lowest NEL content ($1.163 \text{ Mcal kg}^{-1}$) was recorded in faba bean where the previous CC was BTAR mixture (Fig 2.6e). The highest TDN (580 g kg^{-1}) was observed in faba bean seeded after harvesting BCAR mixture, though statistically non-significant with NCC while the lowest TDN (522 g kg^{-1}) was observed in faba bean where previous CC was BCTR mixture (Fig 2.6f).





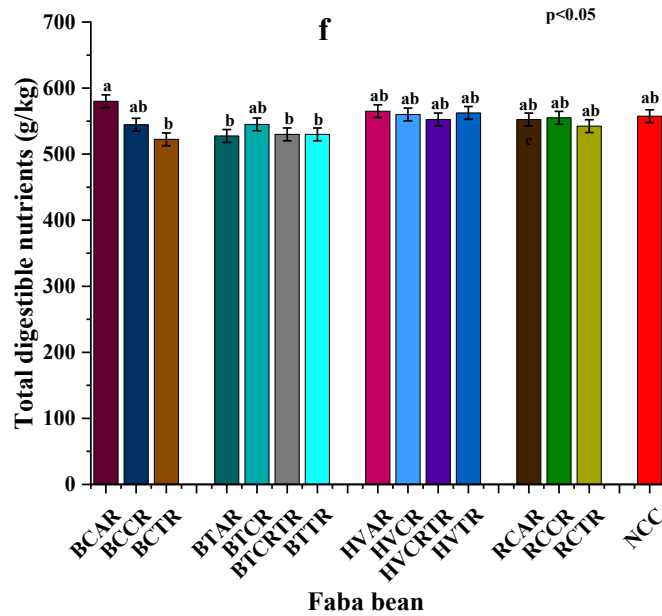
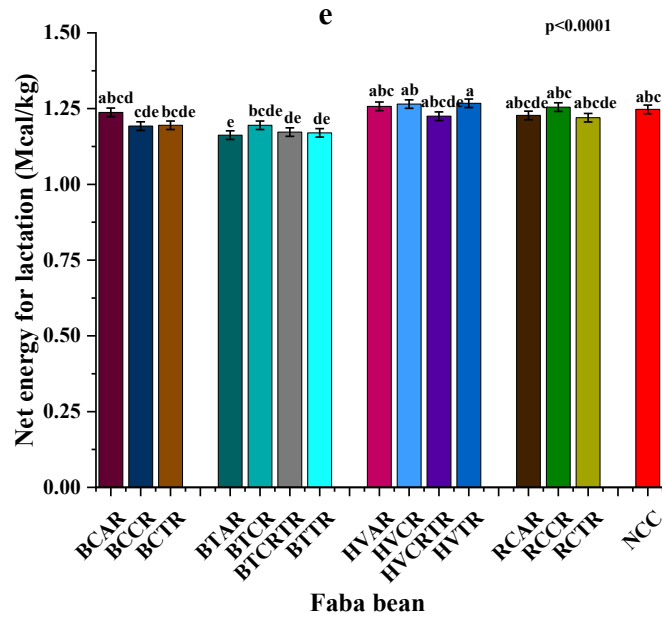


Figure 2.7 Effect of CC mixtures on faba bean forage quality parameters (a) acid detergent fiber, (b) neutral detergent fiber, (c) net energy for gain, (d) net energy for lactation, (e) net energy for maintenance, and (f) total digestible nutrients. Vertical bars show the treatment means of four replications with standard errors. Different lower-case letters on bars indicate significant differences among treatments ($p < 0.05$, Tukey's honest significant (HSD) test). BC, berseem

clover; RC, red clover; HV, hairy vetch; BT, Bird’s foot trefoil; AR, annual ryegrass; CR, cereal rye; and TR, triticale.

2.4.4 Effect of Cover Crop mixtures on Weed Suppression

The CC mixtures had significant ($p < 0.05$) effects on weed suppression. Among the CC mixtures, BCCR, BTCR, BTCRTR, HVCR, HVCRTR, and RCTR mixtures exhibited 100% weed suppression while the BTAR mixture showed 67% weed suppression which was the lowest among fourteen CC mixtures, though statistically non-significant with HVAR mixture (Fig 2.7).

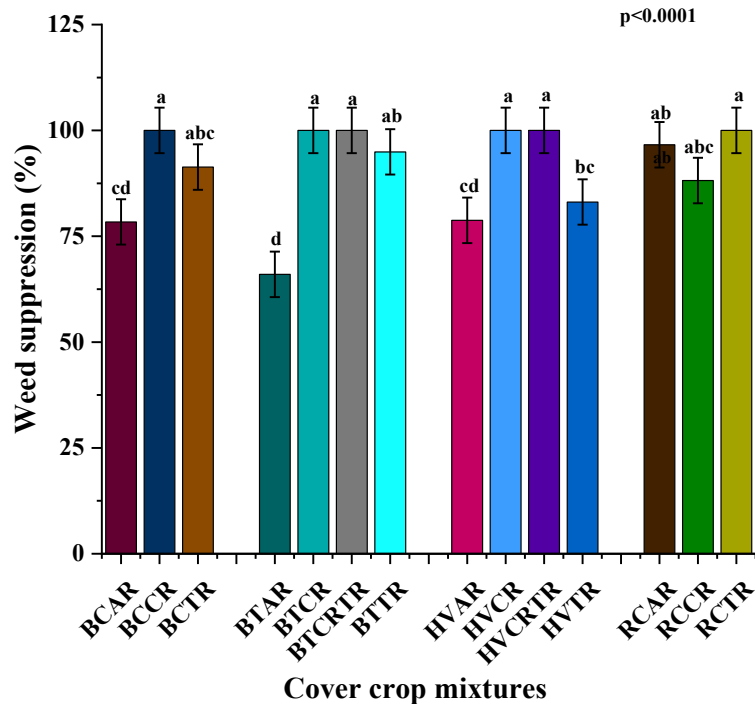


Figure 2.8 Effect of cover crop mixtures on weed suppression. Vertical bars show the treatment means of four replications with standard errors. Different letters on bars indicate significant differences among treatments ($p < 0.05$, Tukey’s honest significant (HSD) test). BC: berseem clover, RC: red clover, HV: hairy vetch, BT: Bird’s foot trefoil, AR: annual ryegrass, CR: cereal rye, TR: triticale.

2.4.5 Benefit-Cost Ratio of Cover Crop Mixtures cultivated in Boreal Climate

Gross return and benefit cost ratio (BCR) have significant ($p < 0.05$) variation among different CC mixtures seeded after harvesting faba bean. The variable cost (seed cost) varies across the CC mixtures, ranging from \$ 45.33 to \$ 197.42 ha⁻¹. Fixed costs include land lease, labor, herbicide, and farm machinery rental costs, were \$343.44. Total cost of production (COP) represents the sum of variable and fixed costs, while gross revenue reflects the overall revenue generated from selling DMY of CC mixtures. The gross revenue varies among the CC mixtures, with the RCCR mixture showing the highest gross income of \$1075 whereas, the BTAR mixture showing the lowest (\$325) gross revenue ha⁻¹. The maximum BCR of 1.76 was recorded in HVCR mixture whereas the lowest BCR (0.2.5) was observed in BCAR mixture among all CC mixtures cultivated in boreal climate. The lowest BCR (0.8) was noted when BCAR mixture was seeded in boreal climate indicating a non-feasible economic return (Table 2.3). The detailed benefit-cost ratio of CC mixtures has been shown in Table 2. 3.

Table 2.3 Benefit-cost ratio of cover crop mixtures cultivated in boreal climate.

Treatments	Variable cost (\$)	Fixed cost (\$)	Production cost (\$)	DMY (Mg ha⁻¹)	Gross income (\$)	Net return (\$)	BCR
BCAR	118.26	343.44	461.70	1.231 bc	367 bc	-95.1 c	0.79 c
BCCR	120.90	343.44	464.34	1.638 abc	551 abc	86.5 bc	1.19 bc
BCTR	197.42	343.44	540.86	1.559 abc	497 bc	-44.3 c	0.92 c
BTAR	45.33	343.44	388.77	1.074 c	325 c	-64 c	0.84 c
BTCR	47.96	343.44	391.40	1.781 abc	583 abc	191.9 abc	1.49 abc
BTCRTR	86.22	343.44	429.66	2.083 abc	787 abc	356.9 abc	1.83 abc
BTTR	124.48	343.44	467.92	2.755 abc	880 ab	411.7 abc	1.88 abc

HVAR	115.32	343.44	458.76	1.492 abc	700 abc	240.8 abc	1.53 abc
HVCR	117.96	343.44	461.40	3.391 a	1088 a	626.8 ab	2.36 ab
HVCRTR	156.22	343.44	499.66	3.065 abc	909 ab	409.3 abc	1.82 abc
HVTR	194.48	343.44	537.92	1.944 abc	691 abc	152.8 abc	1.28 bc
RCAR	84.63	343.44	428.07	1.616 abc	558 abc	130.1 abc	1.30 bc
RCCR	87.27	343.44	430.71	3.152 ab	1075 a	644.2 a	2.49 a
RCTR	163.79	343.44	507.23	2.247 abc	871 abc	363.3 abc	1.72 abc
P value				<0.001	<0.001	<0.001	<0.001

VC: variable cost, FC: fixed cost, COP: cost of production, GR: gross revenue, NP: net profit, B:C: benefit-cost ratio, BC: berseem clover; RC, red clover; HV, hairy vetch; BT, Bird's foot trefoil; AR, annual ryegrass; CR, cereal rye; TR, triticale; and NCC, no CCs control.

2.4.6 Principal Component Analysis (PCA)

PCA shows the distribution of observations on axes F1 and F2, which together explain 76.58 % of the variance in the data. PC1 and PC2 accounted for 57.16 % and 19.43 % of the total variation observed in the data. The score plot clearly segregated all CC mixtures into four quadrants based on some similarities among CC mixture groups. PCA results highlight clear variability and clustering among treatments, which may be attributed to differences in specific response variables (Fig. 2.8a). The biplot analysis revealed associations between CC mixtures, their forage quality, weed suppression capability, faba bean DMY, and benefit-cost ratio. BCCR and RCCR clustered with ADF and NDF values, represented higher fiber content while having lower CP. High DMY was observed for HVCR and RCCR, also achieved 100% weed suppression and high benefit-cost ratios (RCCR: 2.5, HVCR: 2.4), positioning them near vectors representing higher agronomic and economic performance and weed control efficacy. Treatments associated with high milk production potential and forage energy values, including HVCR, RCTR and BTTR, were clustered together. Additionally, treatments with high fat content exhibited moderate alignment with CP but were more strongly associated with milk production, forage energy values, and TDN. High faba bean DMY values were associated with treatments such as BTAR, BCAR, RCAR, BCTR and HVTR, indicating their contribution to enhanced primary crop yield while negatively associated with CC mixtures DMY. Overall, the biplot effectively differentiated CC mixture performances, linking forage quality parameters, weed suppression, economic viability and faba bean productivity (Fig 2.8b).

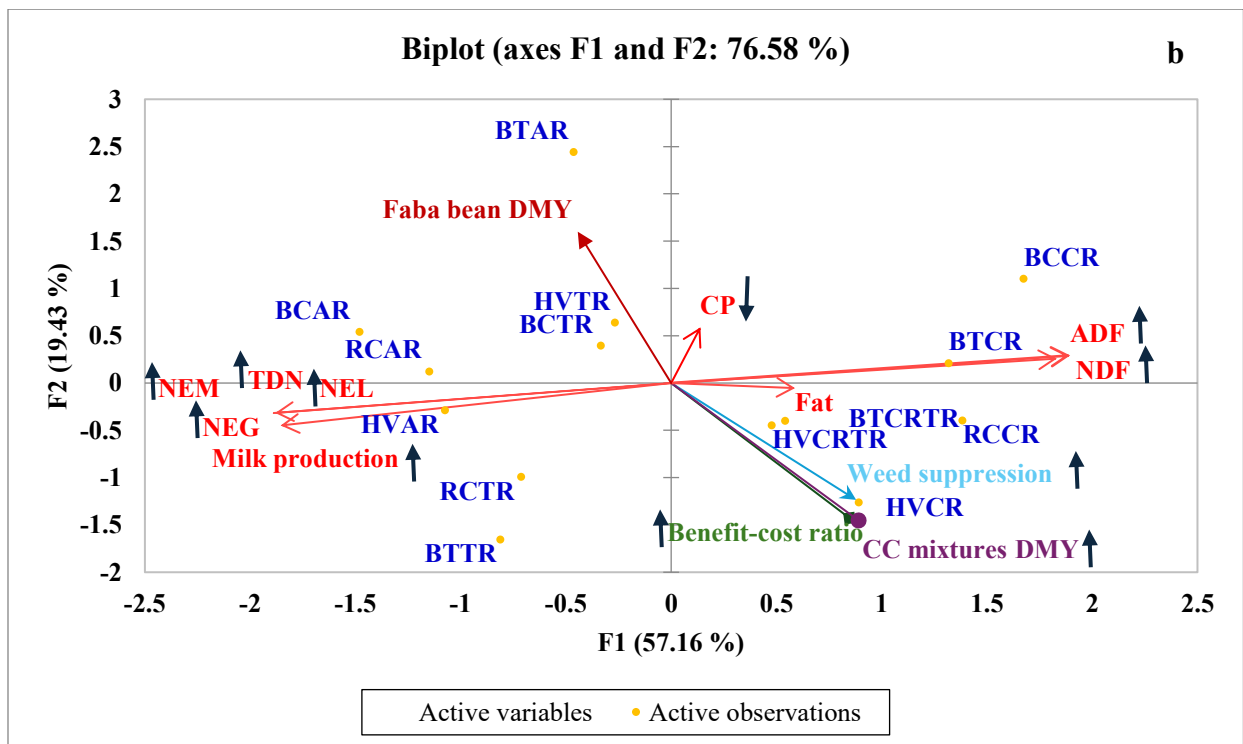
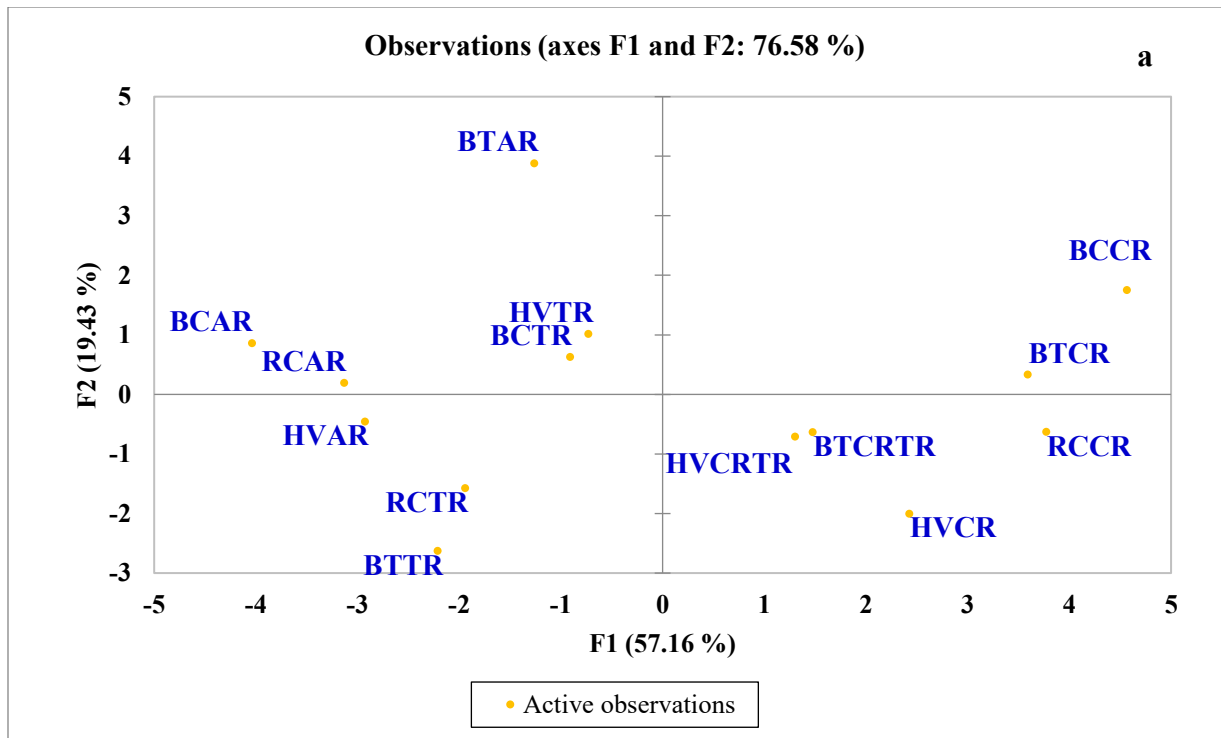


Figure 2.9 Principal component analysis showing the segregation of CC mixtures in different quadrants (a), association of CC mixtures with DMY, forage quality, faba bean yield, weed

suppression and benefit-cost ratio (b). BC: berseem clover; RC, red clover; HV, hairy vetch; BT, bird's foot trefoil; AR, annual ryegrass; CR, cereal rye; TR, triticale; ADF, acid detergent fiber; NDF, neutral detergent fiber; CP, crude protein; DMY, dry matter yield; NEM, net energy for maintenance; NEL, net energy for lactation; NEG, net energy for gain; and TDN, total digestible nutrients.

2.5 Discussion

2.5.1 Dry Matter Yield of Cover Crop Mixtures

There are various environmental and edaphic factors that can influence the germination, seedling establishment, and DMY of CC mixtures cultivated in boreal regions, hence selecting CC species with superior agronomic performance is of utmost importance. Various CC species exhibit distinct responses to growth patterns, nutritional needs, and chilling and cold temperature stresses (Blanco & Jasa, 2019). The seeding and harvesting timing of primary crops significantly impacts the growth and yield of CCs seeded after harvesting primary crops. For example, planting CCs in boreal climate after harvesting primary crops has challenges of low germination and establishment due to low temperature, early frost and short seeding window in spring and fall (Blanco, 2024; Chapagain et al., 2020b). Additionally, the growth of CCs can be influenced by nutrient availability, soil pH, soil organic matter and can substantially affect biomass production (Restovich et al., 2022).

Previous studies have reported that a few CCs species, such as HV and CR are well-adapted to cold temperatures, thriving in temperatures as low as -10 to 15 °C and can thrive in shorter growing seasons. Hence, their combined resilience and growth patterns make them a suitable CC mixture to be cultivated in boreal regions (Chintala, 2023). The higher DMY in HVCR mixture can be

attributed to their resilience to cold temperatures and robust growth pattern (Thapa et al., 2018). Additionally, HV is a leguminous crop with a high potential of biological N fixation (Ogilvie et al., 2021). HV and CR can fix between 25 and 190 kg N ha⁻¹ y⁻¹ (Ashworth et al., 2017; McKenna et al., 2018) while BC and BT can fix approximately 112 to 150 kg N ha⁻¹ y⁻¹ (Jennings, 2010). In comparison, CR has a fibrous root system which makes it a good nutrient scavenger, especially in the upper layers of the soil. Additionally, the fibrous root systems of CR enhance soil structure, build soil organic matter, and form a thick canopy that suppresses weeds. HV and CR mixture improves overall root biomass and soil health, increasing aboveground biomass, and shows complementary growth patterns (Teasdale et al., 2007). Including legumes in CC mixtures with different rooting patterns can improve soil fertility due to N fixation, nutrient availability and uptake, and enhance the growth of companion crops in the mixture resulting in higher biomass production (Thapa et al., 2018). The results of the current study are in line with the findings of Lawson et al. (2015) who observed that HVCR mixture produced higher biomass yield and N uptake compared to monocultures and other CC mixtures. The DMY of the HVCR mixture in present study was 3.39 Mg ha⁻¹ compared to studies conducted by Lawson et al. (2015) and Thapa et al. (2018) who reported 5-7.5 Mg ha⁻¹.

Conversely, due to the least germination of BT (31%) in BTAR mixture reduced the overall DMY (Fig 2.1a), only AR produced 1.074 Mg ha⁻¹ of DMY. Inadequate or suboptimal species diversity or combinations can lead to poor seedling establishment and cause a significant reduction in CCs biomass production (Finney et al., 2017).

Overall, CR showed superior agronomic performance among different grasses in CC mixtures and contributed 73% - 95% in total DMY compared to other grasses (CR > TR > AR) (Fig 2.1 a). It appears that CR suppressed the DMY of legumes in different CC mixtures except

HV. For example, BC, BT, and RC contributed only 5%, 8%, and 17% in total DMY of BCCR, BTCR, and RCCR mixtures, whereas HV contributed 26% and 10% in HVCR and HVCRTTR mixtures (2.1 a). The supportive role of CR in improving soil conditions and reducing competition from weeds allows BT to focus on lipid biosynthesis as it is slow-growing (Shoaib et al., 2016). These findings underscore the importance of selecting appropriate CC species with synergistic behavior or adequate species diversity combination among different CC mixtures to optimize biomass production in faba bean cropping system. The superior agronomic performance of HVCR and RCCR mixtures demonstrates the significance of legume - grass mixtures seeding in faba bean cropping systems in boreal climate. Conversely, the lower yields in BCAR and BTAR emphasize the selection of legumes seed with higher seed germination index, vigor, seeding depth and species diversity.

Two-way ANOVA shows a significant difference in DMY of CC mixtures. The decline of CC mixtures DMY in fall season was due to short growing season as CC mixtures seeded at the end of August and harvested in the start of November (Fig 2.1b). Spring typically offers more favorable growing conditions, such as higher temperatures and longer daylight hours, which can enhance photosynthesis and biomass production (Bélanger et al., 2020). Fall cover crops have a shorter growing season due to gradual decreasing temperatures and daylight, limiting their growth and biomass production. Temperature fluctuation and erratic rainfall in the fall may slow down root development and nutrient uptake, reducing overall DMY (Gopsill et al., 2022). Han et al. (2018) found that warmer spring temperatures significantly enhanced biomass production in cover crops compared to the cooler conditions in fall. Jones et al. (2020) reported that soil moisture levels in spring were consistently higher, resulting in increased biomass accumulation in cover crops.

2.5.2 Forage Quality Parameters of Cover Crop Mixtures

In present study, BCCR mixture produced higher ADF and NDF content compared to BCAR and RCAR mixtures (Fig. 2.2a & 2.2b) which substantiates the findings of Moore and Jung (2001). The higher ADF and NDF concentration in BCCR mixture can be attributed due to the CR containing higher amounts of structural carbohydrates (hemicellulose, cellulose, and lignin) if harvested at maturity (Khorasani et al., 1997), while BC is a legume, and generally has lower fiber content compared to grasses, the inclusion of CR in the mixture increases the overall fiber content. Additionally, the cell wall of the CR plant is more lignified, which elevates the ADF and NDF levels (Govea, 2003). In present study, BCAR, RCAR, RCTR, HVAR, BCTR, and BTTR mixtures can be considered as high-quality forages due to the optimum concentration of ADF (250-350 g kg⁻¹) and NDF (350-500 g kg⁻¹) as reported by Rivera and Parish (2010). ADF and NDF content in present study range from 261-370 g kg⁻¹ and 389-642 g kg⁻¹ among different CC mixtures which are slightly above the high-quality thresholds.

In the present study, RCAR mixtures showed higher CP content among the fourteen CC mixtures (Fig 2.3a). The legume crops such as red clover develop symbiotic relationship with rhizobia bacteria, which enables the RC plants to fix atmospheric N in the soil and make it available for RCAR mixture to uptake which might have resulted in higher CP synthesis as reported in previous studies (Frame et al., 1998). AR is known for its rapid growth, which increases N uptake, and results in relatively high N content, contributing to higher CP levels in the mixture (Dumont et al., 2015). Zupanič and Kramberger (2023) found that RC and Italian ryegrass mixtures had higher crude protein content compared to pure stands of either species. The CP in high-quality forage varies from 180-220 g kg⁻¹ (Hoffman et al., 1993) whereas, CP observed in present study was 79-117 g kg⁻¹ which was below the standard forage CP matrices.

In the present study, BTCR produced higher fat content as compared to other CC mixtures, though statistically non-significant with RCCR mixture (Fig. 2.3b). Legumes like RC and BT generally contain higher concentrations of plant lipids compared to grasses. RC has been found to contain higher levels of polyunsaturated fatty acids (PUFAs) like linolenic acid, which contributes to its overall fat content. Similarly, BT is rich in lipids due to its relatively higher oil content compared to other forage legumes (Dewhurst et al., 2003). Dewhurst et al. (2006) found that RC can have fat contents up to 38 g kg⁻¹ while BT was reported to contain lipids in the range of 30-35 g kg⁻¹. BT's inherently high lipid content, particularly its rich profile in polyunsaturated fatty acids (Wróbel & Zielewicz, 2019). The supportive role of CR in improving soil conditions and reducing competition from weeds, allows BT to focus on lipid biosynthesis (Belfry & Van Eerd, 2016). Nitrogen fixation by BT promotes lipid biosynthesis and benefits the growth of both the legume and cereal rye (Van Eerd et al., 2023). The fat of high-quality forages ranges from 20-30 g kg⁻¹ (Hatfield et al., 2007). In current study, the fat range varies from 14.2-19.7 g kg⁻¹ among CC mixtures, which is lower than the ideal range.

The higher forage energies content was observed in BCAR mixture (Fig 2.4a, b & c). Bargo et al. (2003) examined the digestibility of different grasses and legumes and found that AR, compared to CR, has higher digestibility and provides more energy per kilogram of dry matter due to its lower fiber content and greater non-structural carbohydrate content. Lemus (2013) found that BC consistently provides high quality forage, rich in proteins and energy, which makes it an ideal legume for mixtures aimed at high energy output. BC's low fiber content and high digestibility improve its energy yield when combined with complementary grasses.

In current study, higher TDN was observed in BCAR (Fig 2.5a) and the TDN content ranges from 585-660 g kg⁻¹ among different CC mixtures. BC typically has a TDN range of 560-710 g

kg⁻¹, while AR also contributes significantly to the energy content when used in mixtures (Stout et al., 1997). The combination of these two forages provides a balanced diet with high energy and protein content, leading to better animal performance (Enriquez et al., 2020). The TDN contents in high-quality forage ranges from 550-650 g kg⁻¹ (Mullenix & Dillard, 2018), whereas TDN in present study were 585-660 g kg⁻¹ which is within the prime quality forage range.

In the present study, the higher milk production (1440 L Mg⁻¹ of DM) was observed in BCAR mixture (Fig 2.5b). BCAR mixture is known for its high TDN and potential to enhance milk production (Enriquez et al., 2020). BCAR was also observed to have higher CP, TDN and forage energies which were aids in higher milk production. BC is a high-protein legume, while AR provides good fiber and energy. Together, they create a balanced diet that supports high milk yield which was also endorsed by Enriquez et al. (2020). Previous studies have shown that forages with balanced nutrient profiles can significantly improve milk production (Molle et al., 2017). Enriquez et al. (2020) has shown that mixtures of BC and AR can achieve predicted milk production estimates ranging from 1200-1300 L of milk Mg⁻¹ of DM. The high-quality forages can support predicted milk production of 1500-2000 L Mg⁻¹ of DM or more, depending on the specific forage composition and management practices (Lalman et al., 2019).

2.5.3 Effect of Cover Crop Mixture on Yield Stability and Forage Quality of Faba Bean

Environmental, edaphic factors and management practices influence crop growth and yield. Bridger (2017) observed that late seeding and suboptimal environmental conditions, such as cold temperature and shorter growing seasons, can limit the growth and biomass accumulation of faba bean. Late seeding can affect nutrient access and uptake, leading to lower biomass production (Andersen et al., 2020). Additionally, competition for light, space, and other sources, as well as allelopathic effects of CC mixtures may inhibit the growth and yield of faba beans

(Tallman & Kilian, 2017). In the present study during 2023, faba bean forage yield decreased when seeded after harvesting CC mixtures (Table 2.2). The reduction in faba bean forage yield during 2023 compared to 2022 can be attributed to late seeding and weed pressure. However, no disease symptoms were observed in faba bean in both years.



Figure 2.10 Faba bean at harvesting stage in 2022 (left) and 2023 (right) growing season.

Fiber refers to the indigestible portion of plant materials, primarily consisting of cellulose, hemicellulose, and lignin. It is categorized into two main types: NDF and ADF. NDF includes all cell wall components, while ADF consists of cellulose and lignin. In the present study, BCTR mixture might have led to higher ADF and NDF content in faba beans due to the residual effects of TR. TR, being a cereal, can leave behind more fibrous residues, which might influence the

subsequent crop's fiber content. Additionally, BC, while a good nitrogen fixer, might not have provided as balanced a nutrient profile as HV, leading to higher fiber content in the faba beans (Angeletti et al., 2022). Hans et al. (2022) experimented on barley and faba bean mixture indicated that the presence of cereals could increase the fiber content in the subsequent crops due to the higher cellulose and hemicellulose residues left by cereals. Faba beans typically exhibit moderate levels of fiber, with ADF often ranging from 250-350 g kg⁻¹ and NDF from 350-500 g kg⁻¹ of DM, values exceeding this range may indicate reduced digestibility and palatability (Jung & Allen, 1995).

Forage energy values, including NEG, NEL, and NEM, reflect the energy available to livestock from forage for maintenance and growth. These metrics are crucial for evaluating the energy efficiency of feed. NEG and NEM are crucial for assessing the energy potential of the forage. In the present study, higher NEG and NEM in RCAR mixtures was likely to provide a balanced nutrient profile, enhancing the energy content in faba bean forage. RC is known for its high nitrogen fixation, which improves soil fertility, while AR contributes to soil structure and organic matter (Andersen et al., 2020). Ghorbi et al. (2023) showed that RC can significantly improve soil nitrogen levels, leading to better growth and higher energy content in subsequent crops. Like RCAR, the BCAR mixture also enhances soil fertility through nitrogen fixation by berseem clover and organic matter contribution by annual ryegrass (Andersen et al., 2020). The higher NEL content in faba bean seeded in HVTR plots can be attributed to high N fixation capability of HV, which can improve soil fertility and provide a more balanced nutrient profile for the subsequent crop, leading to higher NEL content (Etemadi et al., 2018). Ghorbi et al. (2023) demonstrated that HV can enhance soil nitrogen levels more effectively than other legumes, leading to improved growth and higher energy content in subsequent crops. The observed NEG

values in this study (e.g., 0.658 Mcal kg⁻¹ in RCAR) are within a desirable range for forage legumes, which typically range from 0.55 to 0.75 Mcal kg⁻¹ (NRCS, 2002). Similarly, NEM values should ideally fall between 1.2 and 1.5 Mcal kg⁻¹ for optimal performance.

TDN is a measure of the overall digestibility of feed, encompassing the sum of digestible fiber, protein, fat, and carbohydrates. It provides an estimate of the energy content available from the feed. TDN is a key measure of forage quality, reflecting the digestibility of the feed. This indicates that faba bean forage from specific CCs can meet or exceed the nutritional requirements for high-performance livestock, corroborating research by Enriquez et al. (2020) which emphasized that higher TDN values correlate with improved animal health and productivity. Higher TDN value of faba bean in BCAR mixture is because of BC's nitrogen fixation and AR's organic matter contribution improve soil fertility and forage quality (Andersen et al., 2020). Angeletti et al. (2022) indicate that BC can significantly improve soil nitrogen levels, leading to better growth and higher digestibility in subsequent crops. TDN value of 580 g kg⁻¹ BCAR aligns well with optimal ranges for high-quality legume forages, which are typically between 550 to 650 g kg⁻¹ (Van Soest et al., 1991).

2.5.4 Effect of Cover Crop Mixtures on Weed Suppression.

High biomass-producing CCs like HVCR and RCCR create a dense canopy which prevents light penetration to the crops under canopy and suppress weed germination and growth (White et al., 2015). In the present study, BCAR, BCCR, BCTR, HVAR, HVCR, HVCTR, and RCTR mixtures exhibited the most effective weed suppression approximately over 90% (Fig 2.7), probably due to fast-growing grass species and high efficacy of CCs combinations (Baraibar et al. (2018). Conversely, the BTAR mixture showed 60% weed suppression which was the lowest among all CC mixtures. These results are in line with the study conducted by Gopsill et al. (2022) who

observed that few species combinations provided greater weed suppression due to their complementary traits. Higher biomass production from CCs was linked to better weed suppression. Mixtures that included highly productive species like cereal rye and triticale showed increased weed suppression. BTCR, HVTR, and HVAR mixtures demonstrated moderate levels of weed suppression, with rates ranging from 70% to 80% (Fig 2.7). This research suggests that CC mixtures can differ greatly in their ability to control weeds. The most effective weed control was attained by using combinations of rapidly growing and highly competitive species (Cordeau et al., 2020). These findings have significant ramifications for the development and administration of CCs intended to decrease weed infestation in agricultural areas.

2.5.5 Effect of Cover Crop Mixtures on Benefit-Cost Ratio

This study provides insight into the economic viability of different CC mixtures. The cost-benefit ratios or net profitability can help farmers and policymakers in the adoption of CC mixtures in boreal climates. Hence, it is essential to consider the variable and fixed costs, and the overall yield or revenue generated from the CC mixtures (Snapp et al., 2005a). In the present study, the variable cost ranges from \$ 45 - \$197 which is consistent with the previous studies reported by Bergtold et al. (2019) and Acharya et al. (2019), who observed that variable costs can vary significantly depending on the crop type and the farm location. Fixed costs, including land lease, fuel, labor, pesticide application, and farm machinery rental, vary from \$ 343 to \$ 538 ha⁻¹ (Table 2.3). Our results are consistent with the findings of Singh et al. (2021), who reported that fixed costs vary depending on various factors such as the size of the farm, the type of machinery used, and the labor cost. Gabriel et al. (2013) noted that the revenue generated from different CC mixtures can vary depending on the type of CC mixtures, forage quality, and selling price of hay/forages.

Both HVCR and RCCR mixtures produced high DMY (3.39 and 3.15 Mg ha⁻¹, respectively), which directly contributed to higher gross revenue (GR) and BCR. High DMY is crucial for economic viability of the farms, as it ensures sufficient forage for livestock, reducing the need for additional feed purchases. Despite having moderate VC and FC, the high DMY resulted in a lower cost of production per unit of forage produced, enhancing the BCR (Table 2.3). This is consistent with the previous findings reported by Beach et al. (2018a) who reported that high-yielding CCs can offset production costs through increased revenue.

BTAR and BCAR show lower BCR (0.84 and 0.79, respectively), which is directly linked to their lower DMY (1.07 and 1.23 Mg ha⁻¹, respectively) and COP (\$388.7 and \$467.7, respectively) (Table 2.3). Yucel et al. (2018) have shown that the efficiency of grass-legume mixtures can be affected by various factors such as seed mixture ratios, cutting frequency, and environmental conditions. For example, BC mixed with AR has been found to have higher forage quality, but lower yield compared to other CC mixtures which leads to lower net profit.

2.5.6 Principal Component Analysis

PCA of CC mixtures demonstrated that two principal components (PC1 and PC2) accounted for 57.16 % and 19.43 % of the total variation observed in the data, respectively. This indicates that these two components capture a significant portion of the variability in the dataset, allowing for meaningful interpretation of the relationships between CC mixtures and these variable responses (Fig 2.8a).

The clustering of BCCR and RCCR with ADF and NDF values suggests higher fiber content but lower CP levels. This finding aligns with previous studies indicating that cereal-dominant

mixtures, particularly those including CR, are associated with increased structural carbohydrate content, which is reflected in higher ADF and NDF values (Clark, 2008).

High DMY was recorded for HVCR and RCCR, which also achieved higher weed suppression and high BCR. These results position these mixtures as highly effective in both agronomic and economic terms, as indicated by their proximity to vectors representing productivity and profitability in the biplot. HV/RC and CR are known for their complementary growth habits; HV/RC contributes nitrogen through biological fixation, while CR suppresses weeds through rapid biomass production and allelopathic effects (Finney et al., 2016; Teasdale et al., 2007).

HVCR, RCTR, and BTTR were closely associated with parameters representing milk production, TDN, NEM, NEL, and NEG and negatively associated with fiber content. The inclusion of legumes such as RC, HV and BT contributed to the elevated energy and milk production in these mixtures. The positive association between forage energy content and milk production parameters aligns with Singh et al. (2021) who highlighted the significance of lipid profiles in enhancing forage quality for lactating dairy animals.

High faba bean DMY was observed in treatments such as BTAR, BCAR, RCAR, BCTR and HVTR). These treatments enhanced the subsequent primary crop yield while exhibiting negative associations with CC mixture DMY, reflecting a trade-off in resource allocation. Finney et al. (2016) and Blanco (2015) similarly observed that lower CC biomass enhances soil resource availability, particularly N and water, for subsequent crops, resulting in improved yield.

This PCA analysis provides a comprehensive understanding of the performance of CC mixtures across a range of agronomic and forage quality traits. The observed relationships align with findings from previous studies, such as those by Blanco (2015) and Teasdale et al. (2007)

emphasizing the multifunctionality of certain CC mixtures in enhancing biomass production, weed suppression, economical profitability and forage quality.

2.6 Conclusion and Future Prospects

In the present study, hairy vetch + cereal rye (HVCR) and red clover + cereal rye (RCCR) mixtures established successfully after harvesting faba bean and showed superior agronomic performance and produced higher biomass production in boreal climate. Conversely, berseem clover + cereal rye (BCAR) and bird's foot trefoil + annual ryegrass (BTAR) mixtures underperformed, likely due to suboptimal species combinations. These results highlight the importance of selecting appropriate species and managing their growth conditions to maximize biomass production. Mixtures such as HVCR and RCCR were highly effective in suppressing weeds by creating dense canopies that limit light availability and resource access for weeds. In contrast, low biomass-producing mixtures like BTAR are less effective in weed suppression. These results demonstrate that careful selection of cover crop species and their growth dynamics can significantly impact weed management strategies. The benefit-cost ratio (BCR) revealed that high DMY mixtures, such as HVCR and RCCR, not only increased forage availability but also improved the BCR by optimizing the cost of production. High-yielding mixtures contributed to greater gross revenue and reduced the cost of production per unit of produced forage, highlighting their financial advantages. The study provides valuable insights for farmers and policymakers, emphasizing the importance of considering both agronomic performance and economic factors in decision-making regarding cover crop adoption.

Overall, HVCR and RCCR produced higher DMY, showed higher weed suppression, superior forage quality and a high BCR compared to other CC mixtures. However, long-term research

trial at multiple locations is required to fully explore the potential effects of CC mixtures on yield and soil health in different crop rotations in boreal climate.

2.7 References

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Chapter 3: Impact of Cover Crop Mixtures on Podzolic Soil Health in Boreal Climatic Conditions

3.1 Abstract

CC mixtures may enhance soil carbon pools (CP), soil organic matter (SOM), add nitrogen (N), improve soil physicochemical properties, reduce erosion, and boost active microbial population and abundance. The current research investigates the effects of CC mixtures on soil labile C pools, mineral N, and soil active microbial population in faba bean (*Vicia faba* L.) production systems in boreal climate. A field trial was conducted at the Western Agriculture Centre and Research Station (49.087°N, 57.541°W), Pasadena, NL. Faba bean was planted on June 6, 2022, and June 30, 2023, and harvested on August 25, 2022, and October 18, 2023, respectively. After harvesting faba beans, CC mixtures were seeded on August 31, 2022, and harvested on June 27, 2023, respectively. The experimental treatments were: 1) red clover + cereal rye (RCCR); 2) RC + annual ryegrass (AR); 3) RC + triticale (TR); 4) berseem clover (BC) + CR; 5) BCAR; 6) BCTR; 7) bird's foot trefoil (BT)+CR; 8) BTAR; 9) BTTR; 10) BTCRTR; 11) hairy vetch (HV)+CR; 12) HVAR; 13) HVTR; 14) HVCRTR; and a control (no CC/NCC). After harvesting CC mixtures, soil sampling was done from 0-20 cm depth to determine SCP (soil permanganate oxidizable C (POX-C), particulate organic matter N (POM-N) and C (POM-C), soil microbial biomass carbon (MBC) and N (MBN)) and soil mineral N. Phospholipid fatty acid analysis (PLFA), was performed to determine the active microbial communities and abundance. The results showed that CC mixtures had significant effects on MBC. In contrast, there were no significant effects on POX-C, POM-N, POM-C, MBN, or soil mineral N. Higher soil MBC (26.4 mg kg⁻¹) was observed in BTAR mixture compared to the lowest recorded in the RCCR (21.5 mg kg⁻¹). The PLFA analysis showed a higher gram-negative population (93.08 nmol g⁻¹) in RCTR mixture whereas the lowest was recorded in

HVCRTR (75.9 nmol g⁻¹). Contrarily, the highest gram-positive bacterial population (54.1 nmol g⁻¹) was observed in control. The highest total bacterial population was observed in control and RCTR (143 nmol g⁻¹). However, CC mixtures had no significant effects on fungi and protozoa population. This research concluded that the CC mixtures had a minor impact on the microbial community population, and labile C pools of podzolic soil in the short term. A long-term field trails needed to assess the impact of CC mixtures on soil health indicators.

Keywords: Winter hardy CCs mixtures, soil health indicators, soil mineral N.

3.2 Introduction

CCs are grown to cover the soil and may be incorporated into the soil to enhance soil organic matter (SOM) and soil fertility (D. J. C. r. m. Reeves, 2018). CCs are generally seeded during off-seasons when soil would otherwise remain fallow. The advantages of CCs encompass: (i) to reduce soil erosion (Lamichhane & Alletto, 2022), (ii) mitigating topsoil loss through establishing CCs root system which enhance soil cohesion, while also forming channels that improve soil structure and porosity, water infiltration and diminishing surface runoff (Hoorman, 2009), (iii) add organic matter, enhance soil fertility and water retention (Scavo et al., 2022), (iv) capture and recycle nutrients, especially N, thereby reducing leaching and making them accessible to the companion crops or the following crops (Lamichhane & Alletto, 2022), (v) suppress weeds proliferation by overshadowing and creating competition for light, water, and nutrients (Scavo et al., 2022). CCs are frequently employed in agroecosystems to improve the soil physical, chemical, and biological properties (Finney, 2017). CCs are getting the attention of farmers and researchers across the globe due to their multifaceted benefits (Austin et al., 2017; Ghimire et al., 2019b). CCs enhance soil health and nutrient cycling (White et al., 2020), organic matter (Kopittke et al., 2020),

microbial biomass (Kim et al., 2020; Rankoth, 2019; Ritz, 2019), and aggregate stability (Adeli et al., 2020; Domagała et al., 2019). Additionally, different CCs under varying crop rotations have unique impacts and roles on soils and microorganisms (Balota et al., 2014; Ritz, 2019). As potent N-fixers, legume CCs transform atmospheric N into forms accessible to plants i.e., NH_4^+ and NO_3^- (Somenahally et al., 2018). These CCs yield residue with a comparatively greater N content that decomposes fast and release nutrients for the following crops (Joshi et al., 2024). The grasses or cereal CCs with well-developed root systems are excellent nutrient scavengers, particularly to lessen N and phosphorus (P) leaching (Sanchez et al., 2019). Ryegrass CC biomass can increase SOM, decrease N leaching over the winter, and shield bare soil from wind and water erosion even during intense early spring rainfall (Kaye et al., 2019). Brassica species CCs such as radish with deep root systems can help alleviate soil compaction and provide additional organic matter and nutrients for the next cash crop (Abdollahi & Munkholm, 2014). However, sole CC, either grass or legume, can't offer multiple advantages simultaneously. Consequently, multi-species CCs, such as combination of grasses and legumes may offer more advantages to improve soil health since they may increase the diversity of substrates available to the ecosystem and offer a variety of ecosystem services (Chu et al., 2017; Drost et al., 2020).

CCs are widely recognized for their ability to enhance soil health by improving soil structure, increasing organic matter, and promoting biodiversity (Seitz et al., 2024). One of the key benefits of CCs is their impact on soil microbial populations, which play a crucial role in nutrient cycling, soil fertility, and plant health (Muturi et al., 2024). Research has shown that CC mixtures can significantly influence soil microbial diversity and abundance (Wang, 2020). For instance, a study by Muturi et al. (2024) found that CC species such as cereal rye, wild pennycress, and a mix of pea, clover, radish, and oat enhanced soil fungal richness and altered fungal community

structure. The genus *Fusarium*, which includes some economically destructive pathogens, was more abundant in control plots without cover crops, while beneficial fungi like *Mortierella* were more abundant in cover crop treatments.

CC root exudates play a significant role in shaping soil microbial communities. Seitz et al. (2024) demonstrated that root exudates from CCs such as sorghum, hairy vetch, and cereal rye selectively enriched specific microbial taxa and functionalities. These exudates activated the rhizosphere microbiome, leading to changes in microbial metabolic patterns and nitrogen cycling. Despite practicing cover cropping as a regenerative agricultural strategy, there is a lack of evidence regarding the chemical diversity of CC root exudates and their effects on soil biogeochemistry (Hu et al., 2018). Plants emit a variety of metabolites as root exudates, including sugars, organic acids, amino acids, enzymes, and secondary metabolites (Blanco et al., 2022). These predominantly water-soluble, low-molecular-weight chemicals affect the rhizosphere, modifying the chemical environment surrounding plant roots and influencing the recruitment and relationships of microbes that benefit plant hosts (Hu et al., 2018; Sasse et al., 2018). Exuded sugars and amino acids serve as C and N substrates for rhizosphere microorganisms, hence boosting microbial activity around roots, which benefits the host plant through improved nutrient absorption and disease control (Otto et al., 2020). Additionally, secondary metabolites in exudates, such as indoles and their derivatives, facilitate microbially mediated plant defense mechanisms, growth, and plant-microbe communication, significantly influencing the entire ecology of the rhizosphere (Khare et al., 2017; Lopes et al., 2023; Otto et al., 2020). Nonetheless, utilizing exudates for precise biological enhancement of the rhizosphere is complicated by the variability in both the quantity and quality of root exudate compounds produced by different crop species and their cultivars, which is

influenced by growth stage, abiotic and biotic stressors, and various edaphic conditions (Seitz et al., 2023).

Living CCs have immediate impacts on soil microbial community structure and function. Finney (2017) found that CCs increased total phospholipid fatty acid (PLFA) concentration, indicating higher microbial biomass. Specific CC species favored microbial functional groups, such as arbuscular mycorrhizal (AM) fungi under oat and cereal rye, and non-AM fungi under hairy vetch. Long-term cover cropping has been shown to increase soil nutrient availability and microbial diversity. Schmidt et al. (2018) found that cover cropping increased organic C, which stimulated the abundance and diversity of microbial communities. Additionally, CC management practices, rather than the composition of CCs, were found to have a significant impact on soil microbial communities. CC mixtures have a profound impact on soil microbial populations, enhancing microbial diversity, abundance, and functionality. These benefits contribute to improved soil health, nutrient cycling, and overall crop productivity (Muturi et al., 2024). Future research should focus on optimizing CC mixtures and management practices to maximize their positive effects on soil microbial communities.

According to Sauer et al. (2007), podzolic soils usually have a coarse-sandy texture and a low pH in the topsoil layer, ranging from 4 to 4.5. These circumstances indicate a deficient crop nutrition supply. Podzolic soils are common in Atlantic Canada and are characterized by their acidic nature and high organic matter content. The addition of CC residues can significantly increase the soil organic matter content in podzolic soils, improving soil structure and fertility. This is crucial for maintaining soil health in the acidic conditions of Atlantic Canada. Studies have shown that CC mixtures can enhance microbial activity in podzolic soils, leading to improved nutrient cycling and soil fertility. This is particularly important in the boreal climate of Atlantic

Canada, where microbial activity is often limited by low temperatures (Sanborn et al., 2011). The province is experiencing rapid agricultural development, it is necessary to improve our knowledge regarding soil C pools, microbial composition and abundance under land use changes and CCs cultivation. CCs are increasingly recognized for their ability to enhance SOM and soil fertility (Reeves, 2018). Traditionally, CCs are sown during off-seasons to reduce soil erosion (Lamichhane & Alletto, 2022), improve soil structure and porosity, increase water infiltration, and diminish surface runoff (Hoorman, 2009). CCs enhance water retention, recycle nutrients (especially nitrogen), and suppress weeds by creating competition for light, water, and nutrients (Scavo et al., 2022). While these benefits are well-documented for studies conducted in temperate or tropical climates, leaving a gap in understanding how CCs interact with soil health in boreal climates. The boreal climate, characterized by cold temperature and short growing seasons, presents unique challenges and opportunities for CC management. Cold temperatures and early fall frost often limit crop growth and microbial activity, making it crucial to understand how CCs can influence microbial populations and nutrient cycling in such environments. This study hypothesized that the CC mixtures will improve the soil labile C pools, active microbial population and abundance, and mineral N in podzolic soil in boreal climate. To test the hypothesis, a field trial was set up with the following specific objectives: 1) to investigate the effects of CC mixtures on POM-C, POM-N, POXC and soil mineral N, 2) to assess the effect of CC mixtures on microbial biomass C and N (MBC and MBN), and active microbial population in podzolic soils in boreal climate.

3.3 Materials and methods

3.3.1 Experiment Design

A field research trial was conducted in Western Agriculture Center and Research Station (49.087°N, 57.541°W), Pasadena, NL during the 2022 and 2023 growing seasons. Faba bean was planted on June 04, 2022, and after the harvesting of faba beans, CC mixtures were seeded on August 31, 2022. The experimental treatments were fourteen CC mixtures of legumes and grasses. Legumes were red clover (RC), berseem clover (BC), hairy vetch (HV) and bird's foot trefoil (BT) and grasses included were cereal rye (CR), annual ryegrass (AR) and triticale (TR). The experiment was laid out in randomized complete block design (RCBD) with four replications. Each experimental treatment plot was 3.5 m x 3 m with a row-to-row distance of 0.5 m. Details about the seeding rate of CC mixtures are given in Table 2.2.

3.3.2 Soil Sampling and Analysis

After harvesting CC mixtures on June 26, 2023, soil samples were collected to determine the soil properties. Three soil samples were collected randomly from distinct places of each plot at 0-20 cm depth using an auger, mixed uniformly to make a composite sample for that plot, and were taken to the boreal ecosystems research facility, Grenfell Campus Memorial University. Soil samples were sieved (~2mm) and divided into two parts: (i). One part of the soil sample was sent to Soil, Plant and Feed Laboratory, Department of Fisheries, Forestry and Agriculture, Saint John's to determine particulate organic matter (POM), and (ii). The second part was preserved at 4 °C to assess soil mineral N, permanganate oxidizable C (POX-C) and soil microbial biomass C (SMBC) and N (SMBN).

To assess active soil microbial communities, 4 plants from each plot were randomly selected and uprooted with the help of a shovel. The soil from roots (rhizosphere) was gently removed and put in a Ziploc bag in the ice box and stored at -20 °C in the lab. Each time the shovel was cleaned with ethanol to avoid contamination (Benitez et al., 2016; Fernandez et al., 2016; Nivelles et al., 2016).

3.3.2.1 Soil labile Carbon Pools

Soil Microbial Biomass Carbon and Nitrogen

Soil MBC and MBN were determined by the fumigation–extraction method (Brookes et al., 1985; Vance et al., 1987). Briefly, a 10 g soil sample was fumigated with ethanol-free chloroform for 24 h at 25 °C. The remaining samples were treated as control. Fumigated and non-fumigated soils were extracted with 40 mL 0.5 mol l⁻¹ K₂SO₄ (soil: extractant = 1:4) and shaken for 1 h on a shaker. The extracts were filtered using Whatman No. 42 filter paper and were re-filtered by 0.45 µm filter paper. Then, froze and stored at -15 °C before analysis. Total OC and N in the extract were measured using a Shimadzu TOC-LCPH/TN analyzer (Shimadzu Inc., Japan). All data was expressed on an oven-dry (105°C) soil weight basis.

MBC was calculated as:

$$\text{MBC} = E_C / k_{EC}$$

where E_C = (organic C extracted from fumigated soils) (Organic C extracted from non-fumigated soils) and $k_{EC} = 0.45$ (Wu et al. 1990).

MBN was calculated as:

$$\text{MBN} = E_N / k_{EN}$$

where $E_N = (\text{total N extracted from fumigated soils}) / (\text{total N extracted from non-fumigated soils})$
and $k_{EN} = 0.54$

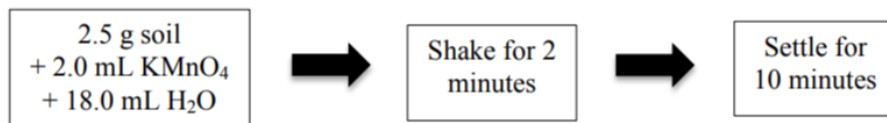
Particulate Organic Matter Carbon and Nitrogen

POM (53 – 2000 μm diam.) was determined using a procedure modified from the method of Moni et al. (2012). Briefly, 25 g of air-dried pre-sieved soil (<2 mm) and 60 glass beads (5 mm diam.) were shaken for 16 h at 130 rpm with 100 mL water. After shaking, the suspension was poured onto a < 2 mm sieve overlaying a <53 μm sieve to separate the beads and POM residues. The residue collected on the <53 μm sieve was then dried in an oven at 60 $^\circ\text{C}$ for 48 h and pulverized using a ball mill grinder. The samples were sent to the soil, plant, and feed laboratory, St. John's, NL, to determine the POM-N and POM-C by dry combustion using a LECO TruSpec CN autoanalyzer (LECO Corporation, St. Joseph, MI, USA).

Permanganate Oxidizable Carbon

Soil POX-C was measured by the protocol developed by Weil et al. (2003). Fresh soil samples were air-dried and sieved (<2mm sieve). The following procedure was employed to measure the POX-C from these soil samples, as shown in Figure 3.1.

Sample Reaction



Sample Dilution



Figure 3.1 Procedure for determination of soil permanganate oxidizable carbon

3.3.2.2 Soil Active Microbial Communities and Abundance

Phospholipid fatty acid analyses (PLFA) method was used to determine the active soil microbial population and abundance (Gómez et al., 2010). Total fatty acids were extracted from 4 g soil with 10 mL chloroform-methanol, 2:1 (v/v) in 20 mL glass vials. The samples were homogenized with a sonicator for 5 minutes (amplitude 50; pulse on time: 5 s; and pulse off time; 10 s), and the mixture was allowed to separate at room temperature for 24 h. The supernatant was filtered and collected in glass vials and then evaporated to dryness under a stream of oxygen-free N₂ gas. The total lipid extracts obtained were dissolved in 2 mL chloroform and fractionated into neutral lipids, glycolipids, and phospholipids, with chloroform (2.5 mL), acetone (4 mL) and methanol (2.5 mL), utilizing solid phase extraction (SPE) on silicic acid columns (Discovery R DSC- Si SPE tube, 50 µm, 70 Å, 100 mg mL⁻¹). The separated phospholipids evaporated to dryness under a stream of oxygen-free N₂ gas. Phospholipids extracts were dissolved in 500 µL of methyl tertiary-butyl ether. Aliquots (100 µL aliquots) were taken from the 500 µL extracts and placed in screw-cap vials with 50 µL of derivatization agent trimethyl sulfonium hydroxide (TMSH), vortex mixed for 30 s and allowed to react for 30 min. 10 µL of internal standard methyl nano-deconate (19:0 @ 160 ug mL⁻¹) was added to the extract of methylated PLFAs and the samples were analyzed with Gas Chromatography-flame ionization detection (GC-FID). PLFAs was conducted on a Thermo Scientific Trace 1300 gas chromatography coupled to a flame ionization detector (Thermo Fisher Scientific, Waltham, MA, USA). The methylated fatty acids were separated with a DB-23 column (30 m × 0.32 mm × 0.25 µm; Agilent Technologies, Canada) by supplying helium (He) as carrier gas at a continuous 1 ml per min flow rate. The GC injector will run in splitless mode and 1 µL of each sample was applied to the injection system using a Tri-plus auto-sampler. The initial oven temperature was 50 °C (1 min hold), then increased using a heating rate of 20

°C/min to 175 °C, kept at 175 °C for 1 min, and further increased at a rate of 4 °C/min to 230 °C (hold up to 5 min). To identify the methylated PLFAs, the retention times and mass spectra were compared with those obtained from commercial standards (NIST database) (Thermo Scientific, ON, Canada, Supelco 37 Component Fatty acid methyl ester (FAME) mix, and Bacterial acid methyl ester (BAME) Mix purchased from, Sigma Aldrich, ON, Canada). Methylated PLFAs were quantified using internal standards and the results were expressed in nmol g⁻¹ soil.

3.3.2.3 Soil Mineral Nitrogen

Soil mineral N was determined by extraction of 5 g sieved (<2 mm) field-moist soil with 50 mL 2M KCl. After extraction, samples were sent to the University of Alberta for the determination of soil ammonium and nitrate concentration. Dissolved target analytes, including NO₃-N, NH₄-N, were measured via well-known color reactions. Samples and method reagents were injected into reaction cuvettes, generating colored complexes after reaction. A separate analysis method was run on each cuvette. The intensity of color change in the solution was dependent on the concentration of analyte in the sample and was measured by light absorbance at a specific wavelength. The amount of light absorbed by the solution follows the Beer-Lambert law:

$$A_{\lambda} = \epsilon_{\lambda}lc$$

where: A_{λ} is the absorbance of light at the target wavelength, ϵ_{λ} was the extinction coefficient of the color complex at that wavelength, l is the path length of the cuvette in cm, and c is the concentration of the analyte in solution. The samples were analyzed using Thermo Gallery Plus Beermaster Autoanalyzer (Thermo Fisher Scientific, Vantaa, Finland, 2017).

3.3.3 Statistical Analysis

One-way ANOVA was performed to evaluate the impact of CC mixtures on soil health parameters by employing the XLSTAT (XLStat Premium 2017, Version 19.5). Where treatment effects were significant, the treatment means were compared with Tukey's honest significant difference (HSD) test at $\alpha = 0.05$. Figures were prepared using OriginPro® 2024b (Northampton, Massachusetts, USA) software packages. PCA was done using a XLSTAT (XLStat Premium 2017, Version 19.5) to determine the association between CC mixtures, soil C pools, soil mineral N and active soil microbial communities.

3.4 Results

Table 3.1 Analysis of variance shows the effect of cover crop mixtures on soil labile carbon pools, active microbial populations and mineral nitrogen.

Cover crop mixtures	POX-C	SMBN	POM-N	POM-C	Fungi	Protozoa	NH ₄ ⁺	NO ₃ ⁻
	mg kg ⁻¹		g kg ⁻¹		nmol g ⁻¹		mg	
RCAR	82.6	4.71	4.5	23.2	21.5	7.60	0.036	0.19
HVCR	83.3	4.06	8.3	24.0	21.3	7.62	0.035	0.17
NCC	77.7	5.14	6.8	20.9	17.2	7.59	0.036	0.17
BTCR	76.9	4.54	4.6	24.9	23.9	7.58	0.037	0.17
BTAR	80.4	5.05	4.3	22.2	20.5	7.60	0.036	0.17
HVCRTR	72.8	4.41	4.8	23.2	19.1	7.55	0.034	0.18
BCTR	71.2	5.07	5.2	22.4	19.0	7.61	0.035	0.19

BTCRTR	81.5	3.82	5.7	24.0	22.8	7.55	0.038	0.15
RCCR	76.0	5.11	4.4	23.8	21.5	7.56	0.031	0.15
BCAR	78.6	4.66	4.6	22.3	17.6	7.61	0.034	0.15
RCTR	73.6	4.34	8.5	22.1	22.4	7.55	0.032	0.20
HVTR	71.4	3.76	6.3	22.5	22.4	7.58	0.037	0.19
HVAR	69.6	3.82	7.0	22.6	20.9	7.55	0.035	0.15
BCCR	81.8	4.62	4.4	20.5	17.1	7.61	0.031	0.13
BTTR	72.1	3.95	3.7	21.9	19.1	7.57	0.031	0.13
Standard error	4.822	0.431	0.12	0.177	1.89	0.03	0.003	0.039
P value	0.539	0.223	0.207	0.951	0.258	0.831	0.932	0.919

SMBN: soil microbial biomass N, POX-C: permanganate oxidizable C, POM-N: particulate organic matter N, POM-C: particulate organic matter C, NH_4^+ : ammonium ion, NO_3^- : nitrate ion, BC: berseem clover, RC: red clover, HV: hairy vetch, BT: Bird's foot trefoil, AR: annual ryegrass, CR: cereal rye, TR: triticale, and NCC: no CCs (control),

3.4.1 Effect of Cover Crop Mixtures on Soil Active Carbon Pools

CC mixtures had significant ($p < 0.05$) effects on soil MBC, whereas no significant effects on soil MBN, POX-C, POM-C, and POM-N (Table 3.1). Soil MBC was significantly higher in BTAR (26 mg kg⁻¹ mixture, though statistically non-significant with all other treatments including control except RCCR mixture which showed the lowest soil MBC (21.5 mg kg⁻¹).

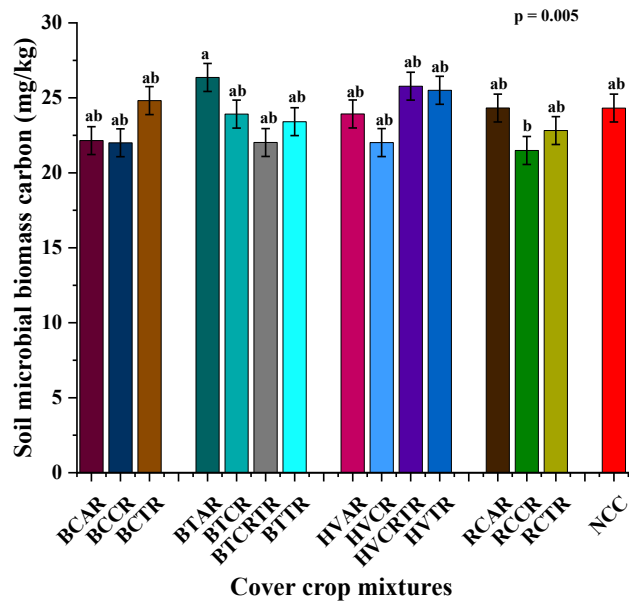
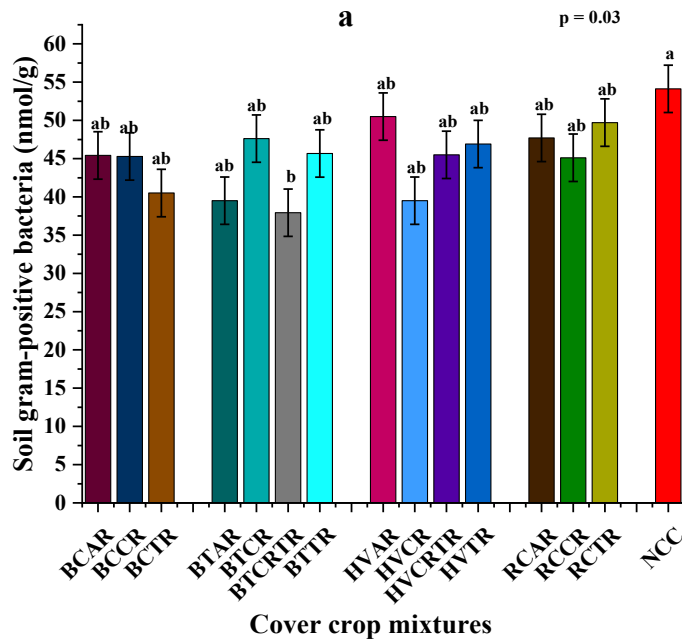


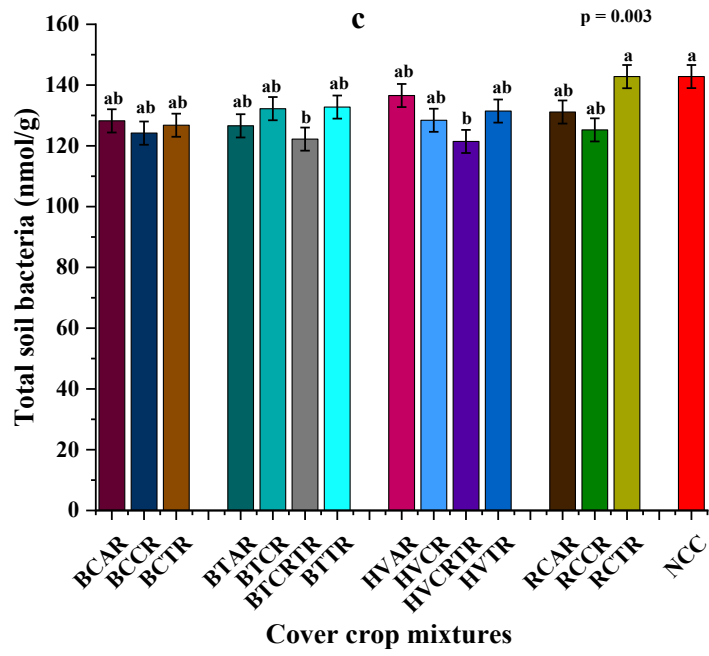
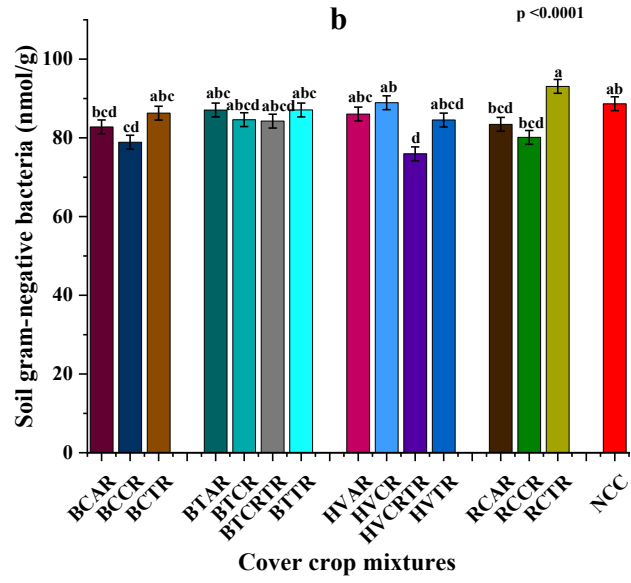
Figure 3.2 Effect of cover crop cover crop mixtures on soil microbial biomass carbon. Vertical bars show the treatment means of four replications with standard errors. Lower case letters on bars indicate significant differences among treatments ($p < 0.05$, Tukey's honest significant (HSD) test). BC: berseem clover, RC: red clover, HV: hairy vetch, BT: bird's foot trefoil, AR: annual ryegrass, CR: grass rye, TR: triticale, and NCC: no CCs (control).

3.4.2 Effect of Cover Crop Mixtures on Phospholipid Fatty Acid Analysis

Soil G⁺, G⁻, total bacterial population, and total PLFA contents were significantly ($p < 0.05$) different, meanwhile, fungi and protozoa populations (Table 3.1) show non-significant differences

among different CC mixtures. NCC showed higher (54.1 nmol g^{-1}) G^+ bacteria and lowest (37.9 nmol g^{-1}) were in BTCRTR mixture. Higher soil G^- (93.1 nmol g^{-1}) bacteria were recorded in RCTR mixture, though statistically non-significant with control whereas, the lowest (75.9 nmol g^{-1}) were observed in HVCRTR mixture. Likewise, total bacterial community was significantly higher (143 nmol g^{-1}) in RCTR mixture and were statistically non-significant with all CC mixtures including control. The lowest total bacterial community (122 nmol g^{-1}) were observed in HVCRTR and BTCRTR mixture (Fig. 3. 3d).





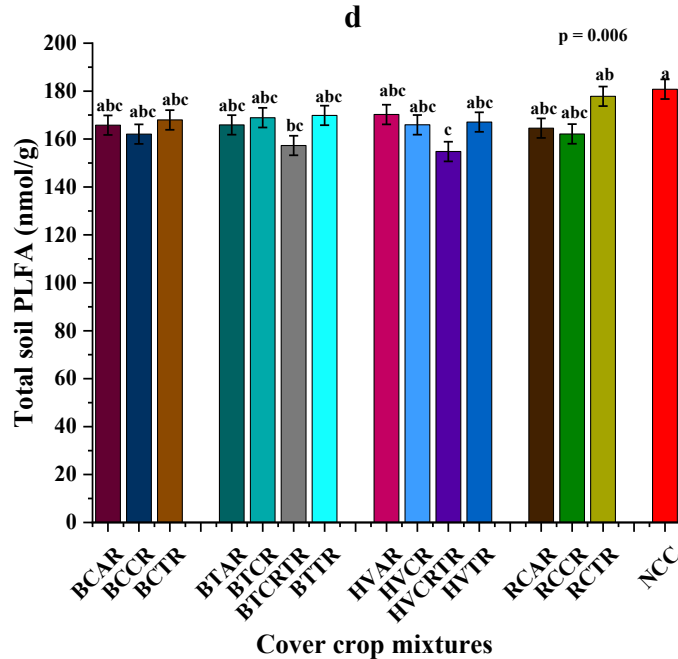


Figure 3.3 Effect of cover crop mixtures on soil gram-positive bacteria (a), gram-negative bacteria (b), total bacterial population (c), and phospholipid fatty acid analysis (d). Vertical bars show the treatment means of four replications with standard errors. Lower case letters on bars indicate significant differences among treatments ($p < 0.05$, Tukey's honest significant (HSD) test). BC: berseem clover, RC: red clover, HV: hairy vetch, BT: bird's foot trefoil, AR: annual ryegrass, CR: grass rye, TR: triticale, and NCC: no CCs control.

Total PLFA was significantly higher (181 nmol g^{-1}) in control though statistically non-significant with all CC mixture treatments except HVCRT mixture which showed the lowest (155 nmol g^{-1}) total PLFA (Fig. 3.3d).

3.4.3 Effect of Cover Crop Mixtures on Soil Mineral Nitrogen

No significant difference was observed in soil mineral N among different CC mixtures and NCC. The soil nitrate and ammonium content ranges from $1.3 - 2.1$ and $3.1 - 3.8 \text{ mg g}^{-1}$ respectively among different CC mixtures (Table 3.1).

3.4.4 Principal Component Analysis

PCA shows the distribution of observations on axes F1 and F2, which together explain 49.68 % of the variance in the data. PC1 and PC2 accounted for 28.80 % and 20.88 % of the total variation observed in the data. The observations were spread across all four quadrants, indicating diverse relationships with the principal components. (Fig 3.4a). Biplot showed the relationship between the CC mixtures with soil labile C pools, mineral N and active microbial population. HVTR, HVAR and HVCRTTR exhibited high SMBC, POM-N, NO_3^- availability, and protozoa population while negatively associated with fungi and POX-C. Protozoa values were relatively uniform across observations, suggesting this variable is not strongly discriminating against groups in the biplot. NCC and RCTR have high values for G^- and G^+ , reflected by their position. BTAR, RCCR, BCAR, BCCR and BCTR have relatively high values for fungi and POX-C. HVCR and HVCRTTR have lower POM-C. RCTR has the highest total bacteria and total PLFA, confirming its strong positive alignment with these variables along the F1 axis. While HVTR, BTCR, and BTTR have moderate to high values for these variables, supporting their proximity to these vectors in the biplot. HVTR was strongly associated with NO_3^- , however, HVCR has relatively high NH_4^+ . BCCR has a high SMBN value. BTCRTTR has the highest SMBC, aligning closely with this variable in the biplot (Fig 3.4b).

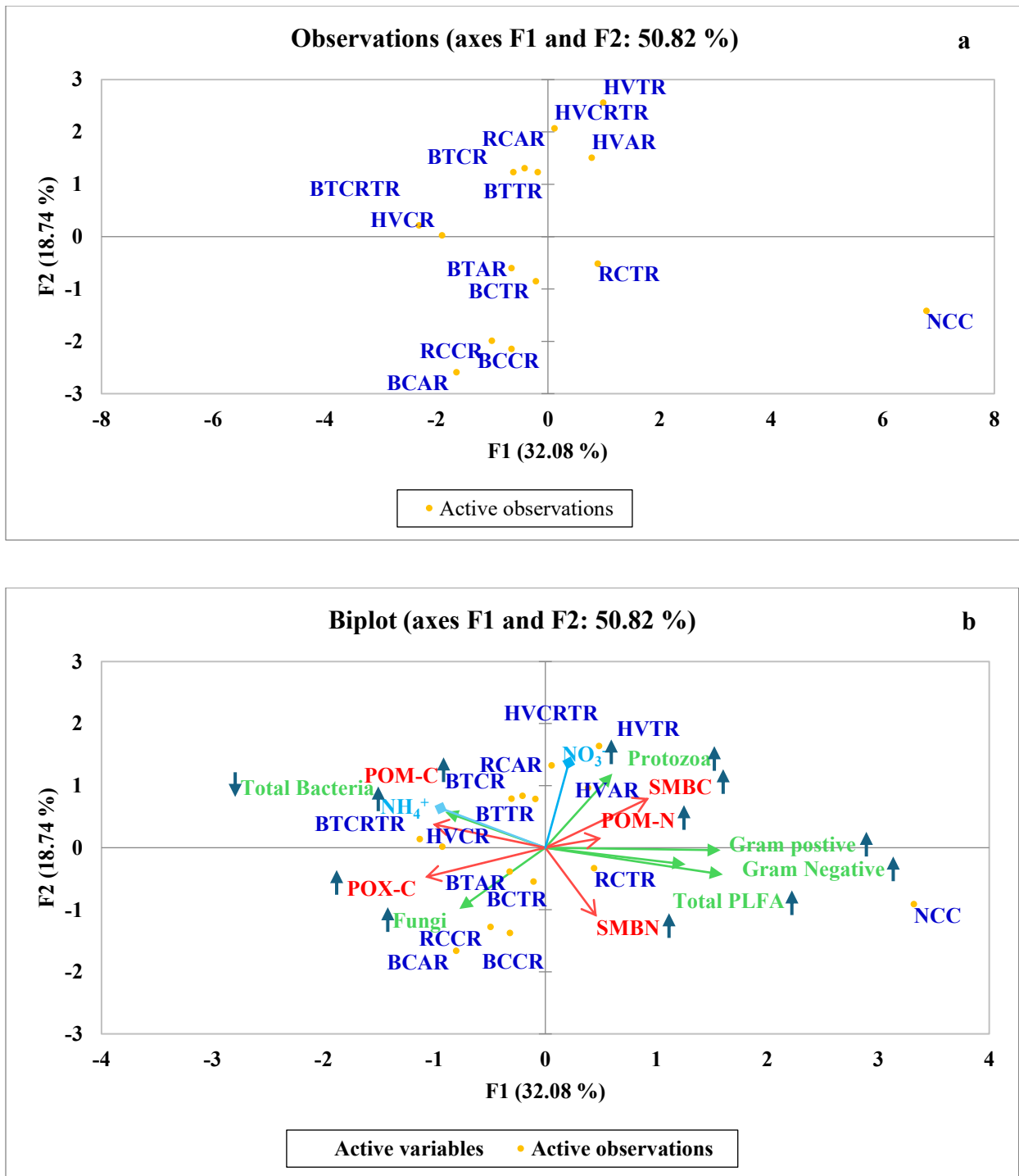


Figure 3.4 Principal component analysis segregated cover crop mixtures in different quadrants (a), Biplot showing association between cover crop mixtures and soil health indicators (labile carbon pools, soil mineral nitrogen and active microbial population) in podzolic soils in boreal

climate (b). BC: berseem clover, RC: red clover, HV: hairy vetch, BT: bird's foot trefoil, AR: annual ryegrass, CR: grass rye, TR: triticale, SMBC: soil microbial biomass C, SMBN: soil microbial biomass N, POX-C: permanganate oxidizable C, POM-N: particulate organic matter N, POM-C: particulate organic matter C, NH_4^+ : ammonium ion, NO_3^- : nitrate ion and PLFA: phospholipid fatty acid analysis.

3.5 Discussion

3.5.1 Effect of Cover Crop Mixtures on Soil Active Carbon Pools

Soil MBC is the carbon contained within the living component of soil organic matter, primarily bacteria and fungi. This biomass C plays a crucial role in soil health and fertility by decomposing organic matter, releasing CO_2 , and nutrients available to plants (Cookson et al., 2008). Different plant species might have varying effects on the soil microbial community (Wang et al., 2023). CCs can increase soil microbial C which aids in regulating soil microbial biomass (Ning et al., 2023). However, changes in MBC may not impact MBN (Romdhane et al., 2019b). Different CCs contribute varying amounts and types of organic matter to the soil, which can influence microbial activity and biomass (Seitz et al., 2024). The root systems of different CCs can affect soil structure and microbial habitats (Eisenhauer et al., 2017). While AR and CR both have fibrous root systems, BT has a deeper, more extensive root system compared to RC, which can contribute more organic matter to deeper soil layers (Meza et al., 2022). RC typically fix high nitrogen in soil, which can favor nitrogen-mineralizing microbes rather than a diverse microbial community that increases SMBC (Bardgett et al., 2005). In contrast, AR residues may favor a broader microbial community. BT didn't emerge but the field had a history of legume (faba bean) establishment, the legacy effects (e.g., residual nitrogen from previous primary crops) might still favor microbial growth (McDaniel

et al., 2014). This could explain why the BTAR mixture has higher SMBC compared to the RCCR mixture (Eisenhauer et al., 2017). CCs can alter soil moisture, temperature, and nutrient availability improving the soil microbial biomass. The specific combinations of CCs might create more favorable conditions for microbial growth in some mixtures (Quintarelli et al., 2022).

MBN is the nitrogen content within the living microbial biomass in the soil. It plays a crucial role in nutrient cycling, as microbes decompose organic matter, releasing nitrogen and other nutrients which plants use (Schmidt et al., 2020). The non-significant differences in MBN among different CC mixtures could be due to the no N application across the treatments (Muhammad et al., 2021). The soil sampling time might not have captured significant differences in SMBN among the treatments, as microbial biomass can fluctuate seasonally (Finney, 2017). Muhammad et al. (2021) found that while CCs increased SMBC and SMBN compared to NCC, the mixture of legume and non-legume CCs decreased SMBC, SMBN, and SMBC/SMBN ratios relative to sole legume or non-legume CCs. Garba et al. (2022) assessed the effects of cover cropping in drylands on soil N content and found that CCs reduced soil mineral N by 25% compared to control fallows. The study highlighted that CCs did not significantly impact SMBN.

POM is the fraction of soil organic matter consisting of partially decomposed plant and animal residues larger than 0.053 mm but smaller than 2 mm. POM is an important source of nutrients for soil organisms and plants and contributes to soil structure (Fohrafellner et al., 2023). The non-significant differences in POM among CC mixtures might be attributed to the similar rates of organic matter decomposition and incorporation into the soil. Kim et al. (2022) have indicated that while CCs can increase POM, the specific mixture of CCs might not lead to significant differences if the overall organic matter input and decomposition rates are comparable. Restovich et al. (2022) demonstrated that while CC mixtures can increase soil organic matter, the specific mixtures did

not lead to significant differences in POM, likely due to similar organic matter inputs and decomposition rates.

POX-C is the labile (easily decomposable) fraction of soil organic matter. It is sensitive to changes in soil management practices and is involved in nutrient cycling (Svedin, 2022). The non-significant differences in POX-C among different CC mixtures could be due to the equilibrium between labile carbon inputs and microbial activity across the treatments (Decker, 2021). Studies have found that while CCs can enhance POX-C, the specific CC variety might not significantly impact POX-C if the overall carbon inputs and microbial activity are similar (Sarker et al., 2023).

3.5.2 Effect of Cover Crop Mixtures on Soil Active Microbial Communities and Abundance

Gram-positive (G^+) bacteria play a crucial role in the decomposition of organic matter, breaking down complex organic compounds into simpler substances that can be utilized by plants and other microorganisms. In the current study, the soil G^+ bacteria population was significantly different among CC mixtures and the highest was observed in NCC. In the NCC, there might be less competition for resources (e.g., nutrients, space etc.) compared to the CC mixtures, allowing G^+ bacteria to thrive (Sharma et al., 2018a). The introduction of CCs can alter the microbial community dynamics, favoring certain microbial groups over others. G^+ bacteria might be less favored in the rhizosphere of CCs compared to the control (McDaniel et al., 2014).

Contrarily, Gram-negative (G^-) bacterial population thrive in nutrient-rich environments, particularly where there is a continuous supply of easily decomposable organic matter (like root exudates and plant residues). The higher active G^- population observed in RCTR mixture in the present study may be due to presence of labile C through root exudates and residue decomposition (Fig 3.3b). Research by Bossio and Scow (1998) observed that gram-negative bacteria often flourish in more nutrient-rich environments due to their fast growth and ability to utilize fresh

organic matter. Legumes like RC typically exude more nitrogen-rich compounds, which favor gram-negative bacteria. G^- bacteria have been shown to utilize these labile C and N sources more efficiently than G^+ bacteria, which tend to dominate in environments with less available organic matter (Fierer et al., 2007). TR, being a grass, contributes substantial C to the soil through its residues (February et al., 2020). Additionally, TR tends to have a higher C: N, when paired with a legume like RC, this balance between carbon and nitrogen enhances decomposition rate (Moore et al., 2000). The faster breakdown of organic matter, facilitated by the more balanced C: N, provides readily available nutrients for microbial communities. G^- bacteria are often more competitive in such environments, where labile carbon and nitrogen are abundant, due to their ability to grow rapidly and exploit fresh organic matter (Wan et al., 2021). Schmidt et al. (2019) observed that legume-grass mixtures lead to more balanced residue decomposition, which supports higher microbial activity and favors G^- bacterial populations as observed in present study (Fig 3.3b).

RCTR both release a variety of root exudates that can serve as substrates for soil bacteria. RC, being a legume, releases exudates rich in C and N, which attract microbial communities (Neumann, 2007). TR, a hybrid between wheat and rye, has a fibrous root system that also contributes to microbial diversity and supports a wide range of bacteria. The combination of these root exudates in the RC+TR mixture likely leads to an increase in both the bacterial population and the microbial biomass, as evidenced by the higher PLFA content (Gaudin et al., 2013) (Fig 3.3d). While in NCC, it could also have a relatively high bacterial population and PLFA compared to CC mixtures due to differences in nutrient dynamics. For instance, the presence of organic matter from previous crops or soil amendments in the control might contribute to a stable and diverse microbial

community. In some cases, the lack of aggressive competition from fast-growing CCs could allow microbial populations to thrive (Romdhane et al., 2019a; Sharma et al., 2018a) (Fig 3.3c & d).

CCs may provide only limited organic input to substantially impact active microbial diversity. CCs can increase soil organic matter, but if they don't drastically alter nutrient availability or structure in the soil, changes in fungal and protozoal populations may not be significant. Studies show that short-term changes in organic inputs do not always translate to major shifts in microbial populations (Sánchez et al., 2018; Vukicevich et al., 2016). Fungal and protozoal populations are strongly influenced by more stable soil factors, such as pH and soil texture, rather than just plant cover. Research suggests that soil microbial populations are influenced by long-term conditions and stable properties, which may explain why temporary or seasonal cover crops don't lead to significant population changes (Bowles et al., 2020). The influence of CCs on soil microbial communities varies according to the species used (Taning et al., 2024). Some CCs may have little effect on specific microbial populations. Soil factors including nutrient content, pH, and structure can enhance microbial community. If the CC mixtures do not affect these qualities result in stable fungus and protozoa populations. The impact of CCs on soil microbial populations may not be immediately noticeable and may take many growing seasons to materialize (Romdhane et al., 2019b). Interactions between microbial groups can potentially have an impact on population dynamics. Bacteria and fungi, for instance, frequently fight for the same soil nutrients. If CC mixtures enhance bacterial development, they may inhibit the growth of fungi and protozoa (Cloutier et al., 2020).

3.5.3 Effect of Cover Crop Mixtures on Soil Mineral Nitrogen

The mineralization process depends on environmental factors that might limit N release.

Conditions such as temperature, moisture, and microbial activity can affect the mineralization of

CC residues. In boreal or temperate climates, these conditions can slow down mineral N release, leading to no significant increase in mineral N relative to control plots (White et al., 2017). Quemada et al. (2013) observed that short growing seasons and low temperatures in temperate and boreal climates hinder the breakdown of cover crop residues, which limit mineral N availability in the soil.

3.5.4 Principal Component Analysis

PCA results indicated that the first two principal components (PC1 and PC2) accounted for 28.80% and 20.88% of the total variance, respectively, explaining 49.68% of the total variability in the data. The relatively high percentage of explained variance suggests that these two components adequately capture the key patterns in the dataset (Fig 3.4a). The biplot revealed a diverse distribution of observations across all four quadrants, indicating distinct relationships with the principal components. HVCRTTR and RCAR have strong associations with POX-C, POM-C and SMBN. Chaudhary (2023) showed that legume-grass mixtures like RC and HV paired with cereals enhance microbial biomass and labile C pools due to balanced residue C:N ratios.

BTCRTTR, BTAR, BCCR, RCCR, HVCR, and BCAR observed to have relatively low influence on labile C pools and microbial biomass. Six et al. (2006) observed that certain CC combinations do not significantly increase labile C pools, especially those with low biomass production. HVTR, BTTR, and HVAR show strong positive associations with active microbial populations, NO_3^- , and PLFA. Vukicevich et al. (2016) demonstrated that CCs like HV paired with grasses enhance microbial diversity and NO_3^- availability due to their fast decomposition rates and high N content. RCTR highlighted its strong association with total bacteria, PLFA, and POM-N. Similar results were reported by Zong et al. (2024), who observed that RC improves microbial biomass and labile C pools when paired with TR.

NCC and RCTR were strongly associated with G⁺ and G⁻ bacteria, indicating their positive effects on bacterial biomass. Fierer et al. (2007) emphasized that bacterial dominance is often observed in systems with minimal residue complexity or in treatments with high-quality residues like RC. BTAR and BCTR showed strong alignment with fungal biomass, suggesting that these treatments favor fungal growth. Yan et al. (2023) observed that fungal populations thrive under CCs with moderate C:N ratios, like BT and BC.

3.6 Conclusion

Cover crop (CC) mixtures showed variable effects on soil C pools, active microbial populations, and soil mineral N. These attributes may be influenced by CC mixture composition, growing season length, and site-specific environmental conditions. Birds foot trefoil + annual ryegrass (BTAR) and hairy vetch + cereal rye + triticale (HVCRTTR) showed higher SMBC, while red clover + cereal rye (RCCR) exhibited the lowest. However, no significant differences were observed in other active C pools, such as SMBN, POX-C, POM-C, and POM-N. This suggests that specific CC mixtures can enhance microbial activity but might not change in labile C pools. CC mixtures have varying impacts on soil microbial communities, with significant differences observed in certain bacterial groups. NCC and RCTR showed the highest total bacterial population, compared to the lowest observed in HVCRTTR and BTCRTTR mixtures. However, there were no significant effects on fungi and protozoa. These results suggest that certain CC mixtures can stimulate bacterial growth, but their influence on other microbial groups may be limited. The study found no significant differences in soil mineral N among the CC mixtures which may be gradual release of N from legumes and the nutrient-scavenging properties of grasses in CC mixtures. This study concludes that CC mixtures, such as BTAR and RCTR, can significantly enhance certain soil health parameters, particularly microbial biomass and bacterial populations. Future research

needs to explore the long-term effects of CC mixtures on multiple sites to determine the soil C pools and microbial community structure and abundance in podzolic soils in boreal climate.

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Chapter 4: General Discussion and Conclusion

The objectives of this research study were:

1. To assess the growth, forage DMY, and nutritional quality of CC mixtures after harvesting faba bean in a boreal climate.
2. To evaluate the yield stability and forage quality of faba bean in rotation with CC mixtures in a boreal climate.
3. To assess the effect of CC mixtures on weed suppression and benefit-cost ratio in a boreal climate.
4. To measure the impact of CC mixtures on soil labile C pools, active microbial populations and mineral N

Field experiments, which are detailed in Chapters 2 and 3, were carried out to achieve these objectives. Assessing the impact of CC mixtures on weed control, benefit-cost ratio, and faba bean yield and quality in boreal climate were discussed in Chapter 2. Whereas the effect of CC mixtures on soil health indicators in podzolic soils under boreal climate were described in Chapter 3.

4.1 Forage Yield and Nutritional Quality of Cover Crop Mixtures after Harvesting Faba Bean in a Boreal Climate.

Present research investigates the establishment of CC mixtures after harvesting primary crop (faba bean) in boreal regions. It highlights the necessity of selecting CC species with superior agronomic performance, responses to growing conditions, and forage quality indices in cold weather conditions of western NL. The study further detailed that seeding and harvesting time of primary crops affects CC establishment, growth and DMY due to low temperature, early frost, and short growing season in boreal climates. Among the examined CC mixtures, the HVCR

combination emerged as the most resilient, yielding 3.4 Mg ha⁻¹, attributed to its robust growth and cold tolerance (Fig 2.1a). This performance aligns with prior research demonstrating that certain CC species are well-suited to colder climates and shorter growing seasons (Ranaldo et al., 2020). HV is recognized for its N-fixing capabilities, potentially contributing 25-190 kg N ha⁻¹ y⁻¹, which enhances soil fertility and benefits companion crops (Thapa, 2018a). In contrast, BTAR mixture demonstrated less DMY due to poor germination of BT, highlighting the importance of species diversity in optimizing biomass production (Finney et al., 2016; Smith et al., 2014). In fall 2022 CC mixtures DMY were lower as compared to Spring 2023 CC mixtures DMY (Fig 2.1b). Overall, the study concludes that combining legumes like HV with grasses like CR can improve soil health and biomass through complementary growth patterns. The findings underscore the need for careful selection of CC species to enhance agronomic performance in faba bean cropping systems in boreal climates.

ADF and NDF are crucial in forage quality, as they affect digestibility and nutrient availability. High fiber content, especially lignin, can reduce digestibility (Sindelar et al., 2019). Balancing fiber levels is key to ensuring efficient nutrient availability and absorption (Katoch, 2023). Grasses generally contains higher NDF and ADF compared to legumes (Moore & Jung, 2001). In the present study, the BCCR mixture had higher ADF and NDF content compared to BCAR and RCAR mixtures, aligning with the findings of Moore and Jung (2001) (Fig 2.2a & b). The higher fiber content in BCCR can be attributed to the structural carbohydrates in CR (Govea, 2003; Khorasani et al., 1997). CP is vital for muscle development, milk production, and overall growth of animals (Katoch, 2023). Legumes enhance CP concentrations in CC mixtures, while grasses influence carbohydrate and fiber fractions (Sanderson et al., 2018). The RCAR mixture showed higher CP content due to the higher nitrogen fixation capability of RC (Dumont et al., 2015;

Zupanič & Kramberger, 2023) (Fig 2.3a). Fat plays a critical role as a concentrated energy source, providing essential fatty acids for cell membrane integrity and hormone production (Bracey et al., 2022). The BT CR mixture produced higher fat content compared to other CC mixtures. Legumes like BT contain higher concentrations of plant lipids (Dewhurst, 2013; Dewhurst et al., 2003) (Fig 2.3b). Forage energies refer to the energy content necessary for growth, maintenance, and productivity of dairy and livestock (Lemus, 2020; Weiss & Hall, 2020). The BCAR mixture showed higher forage energy content (Fig 2.4a, b & c), TDN (Fig 2.5a) and predicted milk production (Fig 2.5b) providing a balanced diet with high energy and protein (Beck et al., 2024; Enriquez et al., 2020; Lemus, 2020).

4.2 Yield Stability and Forage Quality of Faba Bean in Rotation with Cover Crop Mixtures in a Boreal Climate.

Faba bean yield stability observed to decline from 2022 to 2023, with several plausible contributing factors such as resource competition, allelopathic effects, and adverse weather conditions (Table 2.2). This indicates the importance of selecting appropriate CCs to avoid detrimental effects on faba bean yield (Snapp et al., 2005a; Teasdale & Abdul-Baki, 1998). Additionally, late sowing of faba bean in 2023 might have reduced faba bean forage yield.

Fiber, a crucial component of plant materials, is categorized into two main types: NDF and ADF. Increase in fiber content of faba bean was because of the residual effects of the grass (TR) and the N fixer (BC) (Angeletti et al., 2022) (Fig 2.6a & b). Forage energy values, including NEG, NEL, and NEM, are essential for evaluating the energy efficiency of forages. Higher NEG and NEM in RCAR mixture likely provided a balanced nutrient profile, enhancing the energy content in faba bean forage (Fig 2.6c & d). RC fix N (upto 150 N kg h⁻¹ y⁻¹) which improves soil fertility, while AR contributes to soil structure and organic matter (Andersen, 2019). Ghorbi et al. (2023)

showed that RC can significantly improve soil N levels, leading to better growth and higher energy content in subsequent crops. The higher NEL content in HVTR is due to HV's N fixation capability ($112\text{--}224 \text{ N kg h}^{-1} \text{ y}^{-1}$), which might have improved soil fertility and provided a more balanced nutrient profile for the subsequent crop (Etemadi et al., 2018) (Fig 2.6e). The faba bean forage grown following specific CCs can meet or exceed the nutritional requirements for high-performance livestock, corroborating research by Enriquez et al. (2020), which emphasizes that higher TDN values correlate with improved animal health and productivity. The higher TDN value in BCAR mixture likely provided a balanced nutrient profile, enhancing the digestibility of faba bean forage (Fig 2.6f). BC's N fixation and AR's organic matter contribute to improve soil fertility and forage quality (Andersen et al., 2020).

4.3 Effect of Cover Crop Mixtures on Weed Suppression and Benefit-Cost Ratio

CC mixtures can significantly enhance weed suppression by competing with weeds for light, water, and nutrients, releasing allelochemicals that inhibit weed germination and growth, forming a mulch layer that blocks sunlight, and covering more ground area (Boquet et al., 2004; Mirsky et al., 2013). These benefits lead to fewer weeds, delayed weed emergence, and smaller weed sizes, ultimately reducing the weed seed bank for future growing seasons (Ackroyd et al., 2019). High biomass-producing CC mixtures such as HVCR and RCCR might have created a dense canopy that shades the soil, reducing the amount of light available for weed germination and growth (White et al., 2015). Vigorous CC mixtures compete more effectively for soil nutrients, leaving fewer resources available for weeds. Mixtures that include a variety of species with different growth habits can more effectively occupy different niches in the soil, further reducing opportunities for weeds to establish (Finley, 2021). Low biomass-producing CC mixtures like BTAR create a sparse canopy, allowing light to reach the soil and promoting weed germination

(Baraibar et al., 2018). The most effective weed suppression can be achieved by using combinations of rapidly growing and highly competitive species as observed by (Gopsill et al., 2022). These findings have significant implications for the development and administration of CC mixtures to decrease weed infestation in agricultural production systems.

This study also explores the economic feasibility of different CC mixtures in boreal climates. The high cost-benefit ratios or net profitability can aid farmers and policymakers in adopting CC mixtures. The cost of production varies across different CC mixtures. HVCR mixtures produced high DMY though statistically non-significant with RCCR mixture, contributing to higher gross revenue, net return and benefit-cost ratio. High DMY is crucial for farm economic viability, as it ensures sufficient forage for livestock and reduces the need for additional feed purchases. Despite moderate VC and FC, high DMY resulted in a lower COP per unit of forage produced, enhancing the B:C ratio. This is consistent with the previous findings reported by Beach et al. (2018a) who reported that high-yielding CCs can offset production costs through increased revenue.

4.4 Impact of Cover Crop Mixtures on Soil Labile Carbon Pools, Active Microbial Populations and Mineral Nitrogen in Podzolic Soils

CC mixtures had a significant impact on soil MBC while no significant effect on soil MBN, POX-C and POM. MBC is the C in soil organic matter's living constituents, mostly bacteria and fungi, decompose organic matter, releasing CO₂, and supplying nutrients to plants, is vital to soil health and fertility (Cookson et al., 2008). CCs help regulate soil microbial biomass by increasing microbial C (Ning et al., 2023). However, variation in MBC may not affect MBN (Romdhane et al., 2019b). CCs provide different amounts and quality of organic matter to the soil, which may affect microbial activity and biomass (Seitz et al., 2024). CCs root systems affect soil structure and

microbial communities through root exudates or release of secondary metabolites. In current study, the highest SMBC content was observed in BTAR and lowest in RCCR. BT produces high-quality root exudates rich in soluble C compounds (e.g., organic acids, amino acids) that are readily utilized by soil microbes. AR has a dense, fibrous root system, which contributes more root biomass and labile organic matter to the soil. While RC also contributes to organic C soil, its root exudates might not be as labile or abundant as those from BT. CR contributes a high amount of biomass, but its residues are typically high in C:N, decomposing slower and providing less immediate energy for microbial growth (Eisenhauer et al., 2017). Results observed in present study corroborate the findings of previous studies which have reported that certain CC combinations may promote microbial growth in specific combinations or mixtures (Quintarelli et al., 2022). Additionally, climate change alters soil moisture, temperature, and nutrient availability, increasing microbial biomass.

Legume based mixtures increased G^- bacteria and arbuscular mycorrhizal fungi and decreased saprophytic fungi (Moreno et al., 2021). CCs change soil physicochemical properties, affecting the bacterial population. For example, wheat grown with legumes, changes soil properties and bacterial populations the most (Gao et al., 2022). Legume CCs have nodulation of rhizobium bacteria in their roots, potentially leading to changes in microbial communities and soil health. (Li et al., 2023). G^+ bacteria thrive in low-C soils because they use more recalcitrant organic C (Romdhane et al., 2019a) whereas, G^- bacteria thrive in nutrient-dense environments with abundant root exudates and plant wastes. Root exudates and residue degradation might have provided labile C from the RCTR mixture, increased gram-negative bacterial populations (Fig 3.3b). G^- bacteria thrive in nutrient-dense environments due to their rapid growth and ability to exploit fresh organic materials, as observed by Bossio and Scow (1998). Legumes like RC fix N while grasses like TR

have high C:N ratios, balance the soil C: N, speeding up decomposition (Moore & Jung, 2001). By changing biosynthesis of fatty acids and phospholipid structures, bacteria can precisely regulate their membrane lipid composition. This lets them excel in various physical adaptations (Zhang et al., 2024). Soil nutrients affect bacterial proliferation and phospholipid fatty acid composition. C compounds can affect soil nutritional dynamics, including bacterial population and PLFA concentration (Fig 3.3c & d) (Sharma et al., 2018a). Environmental factors like temperature and moisture can affect bacterial growth and PLFA levels. In control or NCC plots, there was not enough competition of different microbial groups for food which might have increased total bacteria and PLFA (Tobin et al., 2020). Soil microbial communities, including fungus and protozoa, are resilient and can maintain structure and function after disturbances (Cloutier et al., 2020). CCs affect soil microbial communities differently by species (Taning et al., 2024) and might have little effect on some microbial populations. Microbial communities can benefit from soil pH, structure, and nutrient composition. Fungi and protozoa populations may be generally resilient and stable over short time periods. Introducing CC mixtures may not immediately create significant changes, particularly if the existing microbial community is well-adapted to the soil environment. CCs may take a long duration to alter soil microbial populations (Romdhane et al., 2019a). Microbial group interactions may affect population dynamics. Fungal and bacterial competition for soil nutrients is common. Specific CC mixtures may inhibit fungi and protozoa growth by promoting bacterial growth (Cloutier et al., 2020).

Environmental variables that could restrict N release affect the mineralization process. The mineralization of CC residues can be influenced by variables like temperature, soil pH, moisture content, and microbial activity. Mineral N release may be slowed down in temperate or boreal climate, preventing a discernible rise in mineral N in comparison to NCC (White et al., 2017). In

temperate and boreal areas, short growing seasons and low temperatures prevent cover crop residues from breaking down, which reduces the amount of mineral N available in the soil (Quemada et al., 2013).

4.5 Conclusion

In conclusion, this research highlights the importance of selecting appropriate CC mixtures for boreal climates to optimize agronomic performance, enhance soil health, and improve forage quality. The study demonstrates that resilient CC mixture, such as hairy vetch + cereal rye (HVCR), is compatible in these regions due to its cold tolerance and robust growth. The research confirms that legumes like HV, with their nitrogen-fixing capabilities, significantly contribute to soil fertility, while grasses, such as CR, complement these benefits by improving biomass production and soil structure. Additionally, the timing of planting and harvesting plays a crucial role in CC growth, as early frost and short growing seasons present challenges in boreal regions. CC mixtures with high biomass, such as HVCR and red clover + cereal rye (RCCR), effectively suppress weeds by competing for resources and creating dense canopies. This, in turn, reduces weed growth and minimizes future infestations. Moreover, specific mixtures, such as berseem clover + annual ryegrass (BCAR), enhance the nutritional quality of faba bean forage, providing balanced protein and energy levels beneficial for livestock. The economic feasibility of CC mixtures is also evident, as high-yielding mixtures can reduce production costs while increasing revenue. CC mixtures have a positive impact on soil microbial activity, improving microbial biomass and altering microbial communities. However, CC mixtures have no effect on soil labile C pools and soil mineral N. Short cool growing season, erratic rainfall and podzolic soils significantly influence the establishment of CC mixtures and their impact on different agronomic and soil factors, highlighting the need for careful selection of CC mixtures in boreal climates. This study's

limitation was that it was a one-site experiment and 2-year research. Overall, this research emphasizes the need for strategic selection and management of CC mixtures to enhance the sustainability and productivity of farming systems in boreal regions.

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