Agronomic Evaluation of Novel Green Manures for Organic Grain Production in Eastern Canada

by

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Abstract

Green manures (GrM) are used to meet organic crop-N needs. Productivity and N-accumulation of three spring-planted full-season GrM in Nova Scotia and two in Québec were compared. Hairy vetch-oats accumulated 123-292 kg biomass-N ha^{-1} and this was significantly correlated to growing degree days (GDD). In two site years out of four however, red clover was able to fix from the atmosphere a statistically equivalent amount of N as hairy vetch. Green manures were incorporated either in the fall or the spring the following year. Over the course of a 1225 GDD soil incubation, hairy vetch-oats and red clover or red clover-oats mineralized statistically similar rates and quantities of mineral-N (93.7-111.4 kg N ha^{-1} and 30.0-43.7 kg N ha^{-1} in soils from Nova Scotia and Québec, respectively). In Nova Scotia, spring incorporation of GrM resulted in greater spring wheat N-uptake (48.6 kg N ha^{-1}) than fall incorporation (40.6 kg N ha^{-1}).

List of Abbreviations and Symbols Used

Abbreviation	Definition
C	Carbon
C:N	Carbon to nitrogen ratio
cm	Centimeters
CV	Common vetch (*Vicia sativa* L.)
CVO	Green manure treatment of common vetch-oats (*Avena sativa* L.)
DM	Dry matter
DM_{legume}	Legume dry matter
GDD	Growing degree days
$GDD_{0°C}$	Growing degree days (base 0°C)
$GDD_{4°C}$	Growing degree days (base 4°C)
$GDD_{5°C}$	Growing degree days (base 5°C)
GDD_{RC}	$GDD_{4°C}$ that have accumulated since red clover planting in NS
$GDD_{vetches}$	$GDD_{4°C}$ that have accumulated since vetch planting in NS
GrM	Green manure
GrM-N	Green manure nitrogen
GWC	Gravimetric water content
ha	Hectares
HRSW	Hard red spring wheat (*Triticum aestivum* L.)
HV	Hairy vetch (*Vicia villosa* Roth)
HVO	Green manure treatment of hairy vetch-oats (*Avena sativa* L.)
k	Nonlinear regression parameter denoting the rate constant
kg	Kilograms
mg	Milligrams
MSE	Mean squared error
N	Nitrogen
N_0	Nonlinear regression parameter denoting the size of the N pool
N_{dfa}	N derived from the atmosphere
$NDFA_{15N}$	N derived from the atmosphere calculated using the isotopic method
$NDFA_{diff}$	N derived from the atmosphere calculated using the difference method
NH_4^+	Ammonium
N_{min}	Mineral nitrogen (NO_3^- and NH_4^+)
NO_3^-	Nitrate
pNDFA	Percent N derived from the atmosphere
RC	Red clover (*Trifolium pratense* L.)
RCO	Green manure treatment of red clover-oats
Sb	Soybean (*Glycine max* L. Merr.)
SOM	Soil organic matter
STE	Standard error
WFPS	Water-filled pore space
$\delta^{15}N$	^{15}N natural abundance

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Chapter 1: Introduction

In order to achieve high yields and quality, a crop needs sufficient plant available nitrogen (N) during key periods of crop development, yet caution must be practiced to avoid an excessive accumulation of N to the point that it becomes an environmental contaminant (Willson et al., 2001). With the logistical and economic constraints associated with split fertilizer applications and limited mineral N fertilizer sources available to organic farmers, great benefits could be realized by developing cropping systems that synchronize soil mineral N (N_{min}) availably with crop demand (Willson et al., 2001). Nitrogen-fixing green manures (GrM) are a tactic that organic farmers have been using for years to enhance the fertility of their soils (Badaruddin and Meyer, 1990; Nelson and King, 1996). Research from the United States and Western Canada has shown that hairy vetch (*Vicia villosa* Roth) (HV) as a fall-planted GrM system can contribute large amounts of N and provide sufficient nutrients to a subsequent crop (Badaruddin and Meyer, 1990; Clark et al., 1994; Jannink et al., 1997; Griffin et al., 2000; Mueller and Thorup-Kristensen, 2001; Cook et al., 2010; Brainard et al., 2012; Halde et al., 2014), but as of yet, this system has not been extensively tested in Eastern Canada, or when established as a spring-seeded crop. This project will determine whether a GrM of hairy vetch/oat (*Avena sativa* L.) (HVO) can provide sufficient amounts of N to subsequent hard red spring wheat (*Triticum aestivum* L.) (HRSW) in comparison to other GrM systems such as red clover (*Trifolium pretense* L.)/oat (RCO), common vetch (*Vicia sativa* L.)/oat (CVO), or a red clover monocrop (RC) at two locations in Eastern Canada (Bible Hill, Nova Scotia and Saint-Mathieu-de-Beloeil, Québec). This multi-pronged project aims to evaluate peak legume biological N_2 fixation and N accumulation in aboveground biomass of four GrM systems (HVO, CVO, RCO, RC) and also to determine optimal management (GrM type and timing of tillage) for subsequent N mineralization to the cash crop of HRSW.

1. Literature Review

1.1. Nitrogen in Agricultural Systems

Plant available N in soil takes the form of N_{min} (nitrate, NO_3^- and ammonium, NH_4^+, ions), and small organic-N molecules (Schimel and Bennett, 2004). These ions can be supplied through conventional fertilizer derived from the Haber-Bosch process, mined Chilean nitrate, through atmospheric deposition, or by mineralization of soil organic matter, crop residue, or organic amendments by microflora and fauna found in the soil (Jansson and Persson, 1982; Bohlool et al., 1992; Berry et al., 2002; Mikha et al., 2006; Sharifi et al., 2008). The Haber-Bosch process, from which the vast majority of N fertilizer is derived, requires large amounts of hydrogen, which is usually obtained from natural gas, along with significant energy investments (Bohlool et al., 1992). According to Agriculture and Agri-Food Canada (2012), natural gas accounts for 70-90% of ammonia production costs. It has been calculated that for every kilogram of fertilizer-N processed, distributed, and applied, 22,000 kilocalories are expended in production, transportation, storage, and application (Bohlool et al., 1992). Sixteen percent of total Canadian farm expenses can be attributed to fuel and fertilizer costs, and for every $0.01/kg increase in fertilizer price, approximately $61M is added to Canadian farmers' annual fertilizer bill (Agriculture and Agri-Food Canada, 2012). In a world nearing peak oil, N fertilizer prices can only be expected to increase over time, and historically, N fertilizer prices have tracked natural gas prices, with an estimated correlation of 0.74, based on monthly data from 1991-2010 (Agriculture and Agri-Food Canada, 2012). In 2011 alone, fertilizer prices in Canada rose 29%, which translates to a $969M increase in the fertilizer bill of Canadian farmers (Agriculture and Agri-Food Canada, 2012). Relying heavily on natural gas for N fertilizer is increasingly economically and environmentally unsound (Bohlool et al., 1992). In the face of these constraints, interest is being expressed in alternatives to Haber-Bosch N sources (Cook et al., 2010). As organic agriculture has never been able to rely on synthetically produced N fertilizers, organic research into fertility management will not only be beneficial to organic farmers, but can be used by conventional farmers as well.

Legumes as an N source for a subsequent crop have been utilized for thousands of years (Nelson and King, 1996). Under the Organic Production Systems, General Principles and Management Standards 5.4.2, organic agriculture systems must rely on composts and manures derived from organic sources, legumes, and crop rotations to meet the N needs of their crops (Canadian General Standards Board, 2008). Stockless organic farms have relied especially on biologically derived N sources to meet their N needs (Mueller and Thorup-Kristensen, 2001; Woodley et al., 2014). In Ontario, 15 different Ontario organic dairy farms were evaluated, and it was determined that on average, 59% of N inputs were from N derived from the atmosphere (N_{dfa}), with the rest coming from wet and dry atmosphere N deposition (21%) and feed imports (15.5%) (Roberts et al., 2008).

Nitrogen applied in organic systems is primarily in the form of organic-N, and N mineralization needs to occur for the nutrient to be made into a plant available form. The transformation of N into plant available forms in the soil is predominantly microbially driven and largely depends on soil water content, soil temperature, oxygen availability, substrate quantity and quality (Brady and Weil, 2002; Dessureault-Rompré et al., 2010, 2011; Gillis and Price, 2011; Cooper et al., 2011; Georgallas et al., 2012). The depolymerization of complex substrates is a rate-limiting step in N mineralization (Gillis and Price, 2011, 2015), yet significant amounts of N can be lost either through leaching of NO_3^- or through denitrification and/or volatilization, resulting in global warming, adverse impacts on human and animal health, impaired terrestrial and aquatic ecosystems, and stratospheric ozone depletion (Mikha et al., 2006; Zebarth et al., 2009). Nitrogen is often considered as one of the main limiting ingredients in organic production, and is responsible for lower productivity, but N balances are often positive for organic farms (Berry et al., 2002). While sufficient N is being applied in organic systems, it is either being applied in non-plant available forms; it is escaping the system, most likely as an environmental contaminate; or that it is being mineralized outside of the 3-6 months where crops take up most of their N requirements (Berry et al., 2002). The degree and timing of mineralization are influenced by many factors, including, but not limited to, soil porosity, soil moisture, soil type, temperature, the nature of the organic matter, previous crop, previous fertilization, pesticide use, intensity and timing of cultivation, cropping patterns, and biological activity

of the soils, timing mineralization to coincide with crop demand is difficult. Synchronizing N supply in both space and time with crop need is one of the major challenges facing farmers and agricultural researchers (Willson et al., 2001; Zebarth et al., 2009; Georgallas et al., 2012). An in-depth understanding of the effects of timing of tillage on N mineralization can help in designing management systems that synchronize N mineralization with crop demand, thereby increasing N use efficiency and making it possible to reduce N fertilizer applications (Thomsen and Sørensen, 2006; Zebarth et al., 2009; Georgallas et al., 2012).

The size of the pool of mineralizable N in the soil, and the rate at which it turns over, vary depending on environmental factors such as soil moisture and temperature, as well as on factors such as crop management and abiotic and biotic changes in the soil (Holland and Coleman, 1987; Sharifi et al., 2008; Van Den Bossche et al., 2009; Zebarth et al., 2009; Gillis and Price, 2011; Mazzoncini et al., 2011; Georgallas et al., 2012). The accumulation of soil organic carbon and soil total N, and thereby the N mineralization potential of a soil, are site-specific; there are important considerations that must be taken into account such as soil properties, climatic conditions, and cropping practices such as the presence of a bare fallow and tillage as a method of weed control (Drinkwater et al., 2000; Mikha et al., 2006; Tonitto et al., 2006; Thomsen and Sørensen, 2006; Van Den Bossche et al., 2009; Hubbard et al., 2013). Both soil organic C and soil total N change slowly, and long-term studies are needed to properly catalogue the change (Lynch, 2014). It is especially difficult to monitor changes in soil organic C and soil total N due to high spatial variability of a very large pool (Sharifi et al., 2008; Mazzoncini et al., 2011). Sharifi et al. (2008) found an average of 7.5% of soil organic N was potentially mineralizable.

One of the ways that organic farms, and increasingly conventional farms, have been meeting the N needs of their crops has been through GrM. Cover crops and GrM are effective ways to preserve and increase both soil organic C and soil total N (Kuo et al., 1996; Sainju and Singh, 2001; Mazzoncini et al., 2011). Green manures can be made up of multiple species, depending on the needs of the farm. They can take the form of a trap crop, whereby a non-leguminous crop is planted to take up leftover nutrients at the end of the growing season to prevent those nutrients from becoming an environmental contaminate. Legumes are also

frequently used as GrM, as they can fix N out of the atmosphere through microbial processes. Incorporating a leguminous GrM into a crop rotation can also be an attractive option from an ecological and economical perspective as it can help to reduce external inputs and improve the use-efficiency of internal resources (Bohlool et al., 1992; Lynch et al., 2012). This study focuses primarily on the N dynamics of GrM in Eastern Canada, but it should be noted that the advantages that can be harnessed through the use of GrM are many, including improved soil organic matter, increased microbial biomass, improved nutrient retention, soil moisture management, reduced nutrient losses, and weed and pest suppression (Folorunso et al., 1992; Biederbeck et al., 1998; Cherr et al., 2006; Mischler et al., 2010; Brainard et al., 2011). However, the inclusion of GrM in a crop rotation usually incurs the use of conventional tillage regimes, oftentimes increasing the frequency of tillage, which can be detrimental to soil health (Drinkwater et al., 2000; Nelson et al., 2009).

1.2. Tillage and Nitrogen Mineralization

Tillage has multiple purposes: to incorporate manure, GrM, cover crops, or crop residue; to prepare a seedbed for planting; to control weeds; or to maintain and improve soil mechanical properties (Balesdent et al., 2000; Thomsen and Sørensen, 2006; Lal et al., 2007). Plowing in and of itself, without the added benefit of fertilizers, can enhance soil nutrient availability and boost yield simply due to the mineralization of previously occluded SOM (Lal et al., 2007): tillage exposes SOM that was previously physically protected in aggregates to microbial turnover (Six et al., 2000; Balesdent et al., 2000). Tillage also affects SOM turnover by changing particle sizes and the spatial distribution of the crop residues within the soil profile, increasing soil-to-residue contact, and increasing soil aeration (Franzluebbers et al., 1999; Licht and Al-Kaisi, 2005; Thomsen and Sørensen, 2006). Depending on the nature of the organic matter incorporated, tillage can also reduce N_{min} (Franzluebbers et al., 1999; Thomsen and Sørensen, 2006), for example if a residue with a C:N ratio greater than 25-30 is incorporated (Shaffer and Ma, 2001). A carbon-rich residue will cause the decomposer community to immobilize the N_{min}-rich soil, causing net N immobilization (Holland and Coleman, 1987; Shaffer and Ma, 2001; Van Den Bossche et al., 2009).

1.2.1. Tillage, Residue Quality and Nitrogen Mineralization

Nitrogen content and concentration of the residue also have significant effects on N mineralization, as do other plant characteristics such as sulfur concentration, cellulose, hemi-cellulose, and water-soluble plant matter, although these latter are inextricably linked with the concentration of N (Janzen and Kucey, 1988). Incorporating a residue with a higher C:N ratio in the fall may result in N immobilization and as a result, a reduction in N leaching over the winter (Powlson et al., 1985).

Incorporating residue into the soil increases the residue surface area in contact with soil microbes, speeding decomposition. However, incorporating a residue with low C:N will result in a rapid net release of N_{min} shortly after incorporation. This can pose a problem as this is usually prior to when the subsequent crop is ready to take up the N_{min} (Van Den Bossche et al., 2009). In the silt loam belt of Belgium, Van Den Bossche et al. (2009) used a spade to incorporate a manually applied residue to a depth of 0-30 cm to simulate plow tillage (conventional tillage) and a two-pronged fork was used to incorporate residue to 0-10 cm (without turning the soil) to mimic soil tillage with a cultivator (reduced tillage). They found that there is slower N mineralization of an N-rich substrate in a system that mimicked reduced tillage than in a system that mimicked conventional tillage (Van Den Bossche et al., 2009). They suggest that with a low C:N residue, reduced tillage will postpone the N_{min} flush that is the result of residue decomposition to a time when there is crop demand for N (Van Den Bossche et al., 2009). Van Den Bossche et al. (2009) found that a high C:N residue will immobilize more N (expressed as a percentage of total N added) under a system that mimics conventional tillage compared to a reduced tillage system. Understanding the effects that the physical quality of an incorporated residue can have on N mineralization provides a tool whereby which the quality of the substrate can be manipulated in order to adjust mineralization patterns to best suit crop needs (Bending and Turner, 1999).

1.2.2. Tillage, Litter Particle Size and Nitrogen Mineralization

Whether a GrM or cover crop is chopped, flail-mowed, or sickle bar-mowed affects the particle size of the incorporated GrM or cover crop, a detail that is not always properly delineated in the literature. A smaller particle size of incorporated substrate yields a greater surface area that can be colonized by soil microbes, influencing the exchange of nutrients, oxygen, and water between the soil and the residue (Bending and Turner, 1999). Particle size and quality of residue were found to have an influence on microbial respiration and net mineralization (Bending and Turner, 1999). Bending and Turner (1999) found that for a substrate with a lower C:N (10:1), particle size did not have any effect on microbial processes. However, as the C:N increased to 15:1, reducing particle size resulted in increased net N mineralization during the early stages of decomposition, but reduced net N mineralization during the later stages. With rye grass roots and straw (C:N of 38:1 and 91:1 respectively), reducing particle size increased net N immobilization and caused microbial respiration to peak later (Bending and Turner, 1999). While over the long term, it was found that particle size of plant residue has no significant effect on N dynamics, Ambus and Jensen (1997) found that larger particle size (cut barley, 25 mm) net immobilized less N (42 mg N kg^{-1}) than barley ground to $\leq$ 3mm (63 mg N kg^{-1}) or cellulose+glucose (122 mg N kg^{-1}) over 60 days. However, more N was net mineralized from the ground barley than from the cut barley (3.3 mg N kg^{-1} soil vs. 2.7 mg N kg^{-1} soil respectively) (Ambus and Jensen, 1997).

1.2.3. Tillage, Residue Distribution and Nitrogen Mineralization

Tillage impacts the vertical distribution of the incorporated residue (Thomsen and Sørensen, 2006), however this aspect is not well characterized in the literature. A moldboard with its inversion of the soil, leaves the residue mostly intact, and less than 10% of it on the soil surface (Lal et al., 2007). Disk tillage with its slicing coulters, depending on the number of passes, can significantly reduce particle size, leaving anywhere between 25% and 75% of the soil surface covered in residue, with equally varying vertical distribution. With similar incorporation rates as a disk, the chisel plow does not break up residue particles to quite the

extent that the disk coulters do (Lal et al., 2007; National Crop Residue Management Survey, 2014).

It is well known that agricultural management practices influence the distribution of microbial biomass (Moore et al., 2005). Residue left on the soil surface favors a fungi-dominant microbial biomass compared to incorporated residue for numerous reasons. In incorporating residue into the soil, the moisture level of the residue is subject to change. In Fort Collins, Colorado, on a clay loam[1] , wheat straw at the soil surface maintained a moisture of 6-7%, whereas straw that was incorporated into the soil profile had a moisture of 7-16% (Holland and Coleman, 1987). Fungi are better able to withstand the drier conditions on the soil surface than bacteria (Holland and Coleman, 1987). A surface managed residue high in C will decompose slowly and immobilize N (Van Den Bossche et al., 2009). This is because the C-rich residue (~80:1) is physically separated from the N_{min}-rich soil (Holland and Coleman, 1987; Van Den Bossche et al., 2009). When residue is not incorporated, fungi predominate the soil microbial biomass as fungi can form hyphal bridges between the carbon in the surface residue and soil N (Holland and Coleman, 1987). Fungal decomposition of litter results in a more recalcitrant SOM fraction compared to a bacterial decomposition as the C that is assimilated into the fungal biomass predominantly forms cell walls, which decompose more slowly than cytoplasm-C (Holland and Coleman, 1987). Intensive tillage systems on the other hand favor bacterial pathways relative to fungal pathways. Bacteria have a faster turnover rate compared to fungi, meaning soil communities that are fungal dominant such as those in no-till systems, will have slower nutrient turnover rates (Moore et al., 2005).

[1] Nunn soil, classified as a fine, montmorillonitic, mesic Aridic Argiustoll by the United States Soil Conservation Service 1975

1.2.4. Tillage, Soil Moisture and Nitrogen Mineralization

Nitrogen mineralization is closely linked to soil moisture (Dessureault-Rompré et al., 2011; Georgallas et al., 2012). With low soil moisture, microbial activity declines as soluble substrates have decreased diffusion to microbial cells (Zak et al., 1999). Microbes also have decreased access to the substrates due to a reduction in mobility (Zak et al., 1999). Additionally, a reduction in intracellular water potential inhibits activity and alters enzyme conformation (Zak et al., 1999). Soil water-holding capacity and the spatial distribution of soil water is one of the major factors affecting crop response to fertilizer N applications (Schmidt et al., 2007b). When soil water content hovers close to field capacity, N mineralization is at its highest, and as the soil becomes progressively drier, N mineralization declines. When soils become saturated, denitrification starts to occur (Stanford and Epstein, 1974; Zak et al., 1999). In saturated soils, cellular aerobic respiration is limited, and when oxygen is limited, NO_3^- becomes an alternative electron accepter causing denitrification in the conversion of NO_3^- to N_2O or N_2 (Franzluebbers et al., 1999; Brady and Weil, 2002). However, the effects of tillage on soil hydraulic properties in space and time can be inconsistent as the specific management effects are often drowned out by spatial and temporal variability (Strudley et al., 2008).

Different residue management/tillage systems have varying effects on soil moisture. For example, the gravimetric soil moisture in the top 5 cm of a no-tillage system that had been maintained for 12 years was significantly greater (32.4%) than for a chisel plow tillage system (25.5%) and moldboard plow system (23.1%) (Karlen et al., 1994). Sometimes surface residue near the seedbed can have deleterious effects as wet soils warm more slowly in the spring. Tillage can alter the air to soil ratio and can help promote the drying out of soil as it increases the air pockets in which evaporation occurs (Licht and Al-Kaisi, 2005). It has been suggested that the implementation of no-till, while producing higher N mineralization potential, does not actually increase actual soil N_{min} due to the cooler, wetter soils found in no-till systems compared to conventional tillage systems (Sharifi et al., 2008).

In Finland, different tillage timings were examined at four different sites to study the effect of incorporating timing of GrM on soil N_{min} (Kankanen et al., 1998). Green manures of HV, RC, westerwold ryegrass (*Lolium multiflorum* Lam. *Var. westerworldicum*) and straw residue of N-fertilized spring barley (*Hordeum vulgare*) were plowed into the soil in early September, late October, and the following spring in May. They found that with higher GrM-N and preliminary soil NO_3^--N content, there was greater risk of leaching over the winter. The amount of N in the incorporated material had a greater effect on soil NO_3^- content than incorporation timing due to the rapid mineralization of the N-rich substrate. However, delaying incorporation to late October or the spring did reduce leaching risks compared to early September incorporation (Kankanen et al., 1998).

1.2.5. Tillage, Soil Temperature and Nitrogen Mineralization

The first-order rate constant (k) of net N mineralization and SOM mineralization increases with temperature (Zak et al., 1999), although Gillis and Price (unpublished) suggest a first order plus logistic model for N mineralization (a model which comprises an exponential and a logistic function representing the two potentially mineralizable pools). As temperatures increase, the microbial community shifts, and the higher temperature community has the ability to metabolize substrates which the lower temperature community could not take advantage of (Zak et al., 1999).

Residue left on the soil surface results in more stable soil temperatures and more moist soil environment when compared with soils where the residue had been incorporated (Holland and Coleman, 1987). This is because the surface residue insulates the soil surface and reflects solar radiation (Licht and Al-Kaisi, 2005). In Iowa, strip tillage[2] and chisel plowing[3]

[2] Strip tillage: mole knives had shanks 43 cm in length and 1.6 cm in width and moles 4.5 cm wide by 9 cm long with 13 cm rotor tiller blades that curved out at the end to 5 cm: this resulted in a 20 cm wide and 10-15 cm deep soil disturbance, and left a 7-10 cm mound
[3] Chisel plow: primary tillage fall chisel, followed by spring field cultivation as secondary tillage

were found to increase the soil temperature by 1.4-1.9°C over no-till[4] in the spring (Licht and Al-Kaisi, 2005).

1.3. Hairy Vetch as a Green Manure

There are over 150 species in the *Vicia* genus (Undersander et al., 1990). Hairy vetch is native to Europe and Western Asia, and is known to survive moderate to harsh winter conditions (Undersander et al., 1990). Over the last two decades, particularly in studies conducted in the United States and more recently in western Canada, HV has come to the forefront as a cover crop/GrM that can contribute substantial amounts of N to a system (see Section 1.4.). It must be noted that HV is known to contain approximately 20% hard seed (Clark, 2007). For this reason, many experts caution against letting HV go to seed as a GrM, particularly if the subsequent crop is a small grain like wheat or barley (Aarssen et al., 1986; Clark, 2007). Hairy vetch tendrils have been known to twine around cereals, causing lodging and making combining difficult, as well as increasing disease prevalence (Aarssen et al., 1986). Hairy vetch in small grains can be particularly troublesome as the seeds are approximately the same size, making it difficult and costly to clean (Clark, 2007). Just a few vetch seeds per ton of grain can markedly reduce price.

If harvesting HV for seed, it is counselled to combine HV at mid-bloom to minimize shattering (Clark, 2007), as shattered pods will contribute to the weed seed bank. In a fall-planted crop, the timing of HV flowering the following spring was found to be more controlled by HV cultivar and by temperature rather than by photoperiod, with 1095-1398 growing degree days (GDD, base 4°C) accumulating between planting and 50% flowering, with local environmental conditions (most likely soil moisture being a main contributor) around the time of flowering acting as a factor in the process (Teasdale et al., 2004). Little, if any, research has been done evaluating the effect of GDD on spring-planted HV.

[4] No-till: no soil disturbance other than planting and N fertilizer application

1.4. Nitrogen Contributions of Green Manures

Hairy vetch has been shown to accumulate a significant quantity of N, enough to meet most crop needs; however, there is great year to year and regional variation. In two Maryland locations, and a location in upstate New York, a biomass of 400 g dry matter (DM) m^{-2} (4000 kg DM ha^{-1}) was achieved at 926 GDD (base 4°C) with a fall-seeded HV monocrop planted at two dates. With a DM yield of 4000 kg ha^{-1}, and with 3.0-3.6% N content, the system can produce 120-144 kg N ha^{-1} in aboveground GrM biomass(Teasdale et al., 2004). Another Maryland study showed that HV seeded in late summer with a cereal companion crop and then terminated in mid-May could yield 9200 kg DM ha^{-1}, and at 2.2% N, yielded 219 kg N ha^{-1} (Clark et al., 1994). In Maine, which has a climate more similar to Eastern Canada, it was found that HV plus winter rye cover crop planted after a barley harvest could yield 209 kg of biomass N ha^{-1}, with a fertilizer replacement value equivalent to 156 kg N ha^{-1} in sweet corn (*Zea mays* L.) in a short-season environment (Griffin et al., 2000). On the other hand, in upstate New York, it was found one year out of two, that late summer planted HV did not stimulate a significant yield increase in corn compared to a 0-N control plot (Sarrantonio and Scott, 1988).

Similar to HV, CV is not commonly used in Eastern Canada as a GrM. However it too CV has been shown to accumulate significant amounts of aboveground biomass-N. In Australia, for example, a monocrop of CV resulted in 91-102 kg of aboveground biomass-N ha^{-1} (Rochester and Peoples, 2005). In Denmark, a monocrop of CV resulted in 80-110 kg aboveground biomass-N ha^{-1} (Mueller and Thorup-Kristensen, 2001). Red clover is a staple of many crop rotations in Eastern Canada, although often grown as a two year crop rather than a single year.

1.4.1. Biologically Fixed Nitrogen

Late-summer-seeded HV in Denmark was found to contain up to 149 kg of N_{dfa} ha^{-1}, from the atmosphere while common vetch (CV) contained 75 kg N_{dfa} ha^{-1}, as determined by the ^{15}N isotope dilution method (Mueller and Thorup-Kristensen, 2001). When different vetch

varieties were planted in late-summer in both a monocrop and in biculture with cereal rye (*Secale cereale* L.), it was found that in one year, HV grown in a mixture derived a greater percentage of N (70%) from the atmosphere compared to grown in a monoculture (58%) (Brainard et al., 2012). However, in the second year of the study, there was no detectable difference between the monoculture and the biculture, and HV averaged 73% N_{dfa} as determined through the ^{15}N natural abundance technique.

Red clover has also been found to be an efficient nitrogen fixer. In southern France, RC was found to derive 61-96% total plant N from atmospheric sources using ^{15}N enrichment methods (Warembourg et al., 1997). In an evaluation of many others, the amount of N_{dfa} was found to be in the range of 35-87% (Peoples et al., 1995).

1.4.2. Variability of Vetches

There is great variation among the amount of N_{dfa}, total kg N ha^{-1}, soil N_{min} concentrations, and fertilizer replacement values for HV systems depending on location of study, planting density, planting date, companion crop, climate, termination timing, and winter hardiness, with many studies also displaying significant differences from year to year (Sarrantonio and Scott, 1988; Clark et al., 1994; Jannink et al., 1997; Griffin et al., 2000; Teasdale et al., 2004; Brainard et al., 2012). While previous research in this crop can act as an excellent guide, the variability between results indicates that regional studies must be performed to evaluate the success of this GrM.

1.4.3. Growing Vetches in Eastern Canada

Teasdale et al. (2004) indicates an optimal fall planting date for HV in late August in New York. Due to the shorter growing season in Eastern Canada, it may not always be feasible to plant a fall-seeded GrM or cover crop after the main crop has been harvested and get sufficient establishment to achieve any benefit. Inclement weather also acts as a confounding factor in fall-seeding as farmers primarily devote good-weather days to harvesting the cash crop, with seeding a cover crop or a GrM given a lower priority.

Additionally, in Maine it was found that average daily low temperatures of -14 to -16°C in January were enough to significantly reduce over-winter survival of HV, especially with minimal snow cover (Jannink et al., 1997), common conditions in Eastern Canada. Most of the literature has focused on seeding CV and HV by either interseeding, undersowing, overseeding, or after the main crop has been removed. This research therefore focuses on GrMs that do most of their growing in the autumn in conditions of decreasing temperature and day length, and increasing soil moisture (Mueller and Thorup-Kristensen, 2001).

Research from Manitoba has found that HV planted in the spring will continue to grow until the end of the fall. By this time the HV will have accumulated between 7.9 and 10.8 Mg ha^{-1}, but is killed over the winter with no re-growth in the spring (Halde and Entz, 2014).

Little research has been conducted on spring-planted CVO and HVO as a full-season GrM in Eastern Canada. As a result, not much is known about how the N content of these crops changes over the course of the season as a function of GDD, but research from fall-seeded HV sheds some light on how N and biomass will accumulate. It has been found that late summer/fall planted HV will continue to increase in both biomass and N content until maturity (Clark, 2007), while Teasdale et al. (2004) found that a fall-seeded HV monocrop had a higher N concentration at the vegetative stage (3.6% N) than at the flowering stage (3.0% N), but total biomass was found to have a greater influence on total N of the GrM. A comparison of N_{dfa} of each GrM has also not been thoroughly researched and quantified.

1.5. Crop Nitrogen Supply from Green Manures

A single year of legume GrM tilled in the fall resulted in strong yield benefits to spring wheat compared to a canola control treatment in Winnipeg, Manitoba (Bullied et al., 2002). However, while they found that even though wheat grain yield was 73% greater after chickling vetch (*Lathyrus sativus* L.) and lentil, yield was not found to be significantly different ($p>0.05$) from the canola GrM control.

In the summer prior to planting spring wheat, GrM (Tangier flatpea (*Lathyrus tingitanus* L.) and lentil (*Lens culinaris* Medik.)) were labeled with ^{15}N and applied to the top 15 cm of soil. On average, a spring wheat crop would recover 9-27% of applied GrM-N, compared to a recovery rate of 34-37% from spring applied ammonium sulfate (Janzen et al., 1990). The large variability in the amount of GrM-N recovery is attributed to varying soil and climatic conditions affecting mineralization rates. Though GrM-N recovered by the wheat crop was lower than the fertilizer-N, it was found that the GrM contributed to the soil organic N pool at approximately twice that of the fertilizer. The proportion of GrM-N that remained in the soil profile after the spring wheat was harvested ranged from 37 to 72% of the applied GrM-N. However, this N was determined to be relatively unavailable to the following crop, which could only recover 1-2% of the initial GrM-N. This means that only approximately 4% of the N remaining in the soil after the first wheat harvest would be available to a subsequent crop (Janzen et al., 1990).

Various ^{15}N labeled GrM were buried in mesh bags in Finland in late October (Müller and Sundman, 1988). It was found that most of the N contained in the white clover (*Trifolium repens* L.) and subterranean clover (*T. subterraneum* L.) residues was released during the winter and spring prior to the start of the growing season, which was unexpected as the soil is generally frozen during that time. The highest N release from the GrM occurred prior to the main nutrient demand of the subsequent barley crop, which took up 17-25% of GrM-N applied to the soil. Only 9% and 10% of the N derived from the subterranean clover and white clover respectively was released during barley growth. The N that was released prior to barley growth was not completely lost to the system, since all but approximately 10% of N from incorporated GrM was accounted for by the end of the experiment, with most of the N found in the soil outside of the mesh bags (Müller and Sundman, 1988), presumably now a part of the soil organic N pool.

Subterranean clover material of different maturities was also buried in mesh bags in early June, just prior to planting barley (Müller and Sundman, 1988). It was found that for two and three month-old subterranean clover residue buried in the spring, the barley recovery of GrM-N was similar to subterranean clover buried in late October, 15% compared to the 18%

of total N recovered from fall-buried residue. Such similarities between the amounts of plant derived N in a cash crop from a fall incorporated and a spring incorporated GrM are surprising.

In Aurora, NY, on a Lima loam[5], in a dry year, the moister soil in the 0-7.5 cm under no-till management/HV mulch resulted in a higher N uptake (113.6 kg N ha^{-1}) compared to conventionally tilled treatments (100.9 kg N ha^{-1}) during periods of low rainfall, although corn yields were statistically similar (Sarrantonio and Scott, 1988). On a Kendaia silt loam[6] in a normal moisture year they found that corn grown after a GrM was conventionally tilled accumulated approximately 120% more N (119.4 kg N ha^{-1}) than corn that was grown under a GrM that was chemically burned down and no-tilled (55.2 kg N ha^{-1}) (Sarrantonio and Scott, 1988).

Similarly, in Drinkwater et al. (2000), the amount of N in the cash crop aboveground biomass was greater in chisel-disk and moldboard treatments than the amount of N in the incorporated GrM; this was not the case in the no-till treatments where plant N uptake was lower than the amount of N in the vetch at the time of mowing. It is proposed that the lower plant N uptake in the no-till treatments is due to decreased tillage-induced mineralization and increased immobilization, which would lead to an increase in soil organic N pools in the long-term (Drinkwater et al., 2000).

1.5.1. Green Manure Management and Incorporation: How It Affects Nitrogen Mineralization

Soil N_{min} concentrations were found to be higher after conventional tillage[7] compared to no-till[8] after a HV GrM was terminated in the spring, and more evenly distributed throughout

[5] fine-loamy, mixed mesic Glossoboric Hapludalf
[6] fine-loamy, mixed non-acid, mesic Aeric Hapludalf
[7] moldboarded to 22 cm followed by a single pass with a disk and harrow
[8] GrM terminated with paraquat (l,l'-dimethyl-4,4'-bipyridinium ion), 2.6 L ha^{-1}

the soil profile on a Lima loam[9] and a Kendaia silt loam[10] in Aurora, NY (Sarrantonio and Scott, 1988). A flush of N_{min} was found within two weeks after the HV was sprayed with herbicide and the plots plowed in the conventional treatment at both 0-7.5 cm (20 mg N kg^{-1}) and 7.5-22 cm (68 mg N kg^{-1}). After the HV was sprayed with herbicide in the no-till treatment, the N_{min} flush was highest between 0-7.5 cm (50 mg N kg^{-1}, also within two weeks of termination), and most of the N_{min} stayed concentrated near the soil surface increasing the risk of loss to volatilization or denitrification (Sarrantonio and Scott, 1988). This flush is the response of microorganisms to an addition of easily decomposable organic matter. At one to two weeks after incorporation, this first flush would be outside the window of crop need. After that first flush, N concentrations dropped by about 60% by the next sampling date, one week after. It was suggested that rapid immobilization occurred as the N that was in excess of cellular demand by the primary decomposers was released and then quickly immobilized by another population of decomposers, this second population operating on a second substrate with a higher C:N (Sarrantonio and Scott, 1988).

A second flush of N_{min} occurred in both the GrM no-till (a peak of 60 mg N kg^{-1} at 0-7.5 cm) and GrM conventional till systems (30 mg N kg^{-1} at 0-7.5 cm). Levels were highest in 7.5-22 cm under conventional tillage, remaining between two and four times higher than the no-till treatment (Sarrantonio and Scott, 1988). After May 31, N_{min} levels were the highest in the conventional till treatment at 22-45 cm throughout the growing season, and a significant amount remained after harvest (10 mg N/kg), with less than half of that in the no-till vetch treatment. While conventional till of a GrM did result in a more uniform distribution of N throughout the soil profile, early in the season this would usually be outside of the range of the crop's root system, leaving this N_{min} vulnerable to leaching (Sarrantonio and Scott, 1988).

If, as Sarrantonio and Scott (1988) suggest, the rate of denitrification under a no-till green manure system is up to 77 times greater than under conventional till, then there are serious inefficiencies occurring that need to be addressed. However, the authors also suggest that the

[9] fine-loamy, mixed mesic Glossoboric Hapludalf
[10] fine loamy, mixed nonacid, mesic Aerie Hapludalf

lower N_{min} amounts found in a no-till system could also be due to lower mineralization rates or increased immobilization rates, suggesting conservation of N over the long term (Sarrantonio and Scott, 1988).

Freeze-thaw cycles over the winter promote mineralization of incorporated substrate (Kankanen et al., 1998; Wallace, 2015). Delaying the incorporation of GrM from early September until later in the fall can reduce the amount of N_{min} hat is leached over the course of the winter (Kankanen et al., 1998). In fact, delaying incorporation until the onset of the freezing period resulted in no net N mineralization in a laboratory setting (Lahti and Kuikman, 2003). Greater understanding of the complexities of N mineralization of GrM based on management can only lead to more efficient cropping practices.

1.6. Wheat Response to Nitrogen

Hard red spring wheat responds to N by increasing in grain yield and grain protein content (McNeal et al., 1971; Ayoub et al., 1994; Ma et al., 2004). Wheat has a high N-uptake early in its development, with the maximum rate of uptake measured at tillering (Jones et al., 2011). While both grain yield and grain protein are affected by early season N applications, N applied near or after anthesis impacts only protein concentration (Ma et al., 2004). In Eastern Canada, with increased spring N fertility, increased grain yield, tillers and spikes m^{-2}, kernels per spike, and plant height were found, but also lodging (Ayoub et al., 1994). Split applications (60% at seeding and 40% at heading) showed little effect on yield, but increased test-weight across three site-years (Ayoub et al., 1994), an analysis of the effects of split applications on grain protein were not evaluated in this experiment. By grain fill, 70% of the total aboveground N has been accumulated, with more than 55% of grain N coming from redistribution of the nutrient from the plant's leaves, stems, and head, and the rest coming from the soil (Jones et al., 2011).

Ultimately, one of the main goals of using a leguminous GrM is to off-set fertilizer N inputs. While total N added to the system by the legume is of marginal interest, *if the N fixed into the GrM biomass is mineralized outside of the window of crop need, then using a GrM to*

offset fertilizer costs is a faulty practice and further research is needed to improve the efficiency of the system. The purpose of this work is 1.) to determine how various GrM accumulate N into their biomass, and 2.) to determine the effects of spring vs. fall incorporation on N mineralization of these various GrM, particularly during the period of maximum crop uptake.

2. Objectives

1.) To determine at two locations in Eastern Canada, through use of ^{15}N natural abundance (commonly denoted as $\delta^{15}N$) and through use of the N difference methods, the GrM (HV-oats) (HVO), CV-oats (CVO), RC, RC-oats (RCO)) type that will result in the greatest amount of N_{dfa} in aboveground biomass.

2.) To determine at two locations in Eastern Canada, the GrM (HVO, CVO, RCO, RC) type and timing that will result in the greatest amount of aboveground biomass and biomass-N.

3.) To determine whether aboveground biomass and/or biomass-N and N_{dfa} accumulation of different spring-planted GrM (HVO, CVO, RCO, and RC) is correlated with GDD.

4.) To determine if the type of GrM (HVO, CVO, RCO, and RC) incorporated at different timings (spring versus fall) affects N mineralization rate and total N_{min} in a controlled environment setting.

5.) To determine if the type of GrM (HVO, CVO, RCO, and RC) incorporated at different timings (spring versus fall) affects the quantity of N_{min} in situ.

6.) To determine if the type of GrM (HVO, CVO, RCO, and RC) incorporated at different timings (spring versus fall) affects yield and quality parameters of the subsequent wheat crop.

3. Hypothesis

1.) There are significant differences in the quantity and timing of peak aboveground biomass-N (kg biomass-N ha^{-1}) between HVO, CVO, RCO, and RC GrM systems.

2.) There are significant differences in total N_{dfa} (kg N_{dfa} ha^{-1}) between HVO, CVO, RCO, and RC GrM systems.

3.) The total amount of aboveground biomass, aboveground biomass-N, and N_{dfa} of HV is significantly correlated to $GDD_{4°C}$.

4.) The rate of N mineralization (kg N_{min} ha^{-1} $GDD_{0°C}^{-1}$) and quantity of N_{min} (kg N_{min} ha^{-1}) will be significantly different between GrM types (HVO, CVO, RCO, and RC) by incorporation timings (spring vs. fall) in a controlled environment setting.

5.) The amount of N_{min} available during periods of crop demand will be significantly different between GrM type (HVO, CVO, RCO, and RC) and incorporation timings (spring vs. fall) as measured by hard red spring wheat (HRSW) biomass-N (kg wheat biomass-N ha^{-1}).

6.) The quantity of soil N_{min} (kg N_{min} ha^{-1}) measured in situ at HRSW planting will be significantly different between GrM type (HVO, CVO, RCO, and RC) and incorporation timing (spring vs. fall).

7.) The peak amount of soil N_{min} in excess of crop demand (kg N_{min} ha^{-1}) will be significantly different between GrM type (HVO, CVO, RCO, and RC) and incorporation timing (spring vs. fall).

8.) Type of GrM (HVO, CVO, RCO, and RC) and incorporation timing (spring vs. fall) will have a significant effect on wheat yield (kg wheat ha^{-1}) and wheat quality parameters (protein, 1000 kernel weight).

Chapter 2: Productivity and Biological Nitrogen Fixation of Novel Green Manures in Eastern Canada

1. Introduction

Research has been conducted on the East Coast of the United States and in Europe, and to a lesser extent in Western Canada, on using hairy vetch (HV) (*Vicia villosa* Roth) as a green manure (GrM). It has been thought that perhaps a GrM of HV can be used to replace more well-known GrM in Eastern Canada such as red clover (*Trifolium pretense* L.) (RC) or the lesser known common vetch (*Vicia sativa* L.) (CV). A GrM of HV can accumulate several tons of dry matter (DM) biomass and usually well over 100 kg N ha^{-1}, and sometimes as much as 300 kg N ha^{-1}, making it an appealing choice to off-set fertilizer costs (Table 1). There is great regional and temporal variation amongst these studies, however, begetting the need for localized research into using HV as a GrM in Eastern Canada. Additionally, the majority of these studies examine the use of HV as a fall-seeded crop that is terminated the following spring. Hairy vetch is normally planted in the late-summer to early-fall as the optimum germination temperature for HV is from 15 to 25°C (Butler et al., 2014). Where HV does not winterkill, the following spring, the soil temperature for optimum growth, water use efficiency and N fixation is 10°C when the air temperature is 20°C (Zachariassen and Power, 1991; Power and Zachariassen, 1993). To achieve 50% soil cover before winter with a fall-planted HV crop, it was estimated that a minimum of 655 growing degree days (GDD) (base 4°C) would be needed (Teasdale et al., 2004). Teasdale et al. (2004) suggest a late-August planting date in New York as the optimal time for planting HV to achieve reliable performance. As New York is further south and has a longer growing season than much of Eastern Canada, an earlier planting date of mid-August could be extrapolated. However, mid-August is usually when winter wheat is being harvested in Nova Scotia (Jack van Roestel, personal communication), and is often dry, which could affect germination. In the Maritimes, farmers usually start corn silage harvest in mid-September and usually do not finish until early October (Robyn McCallum, personal communication). Late fall is usually a time of high precipitation with limited harvesting and planting dates, and as such, following

Table 1. Variations in hairy vetch biomass and nitrogen accumulation in aboveground biomass by study, place, and planting date.

Note: studies represented are from areas with similar precipitation to Québec and Nova Scotia. Also note that all studies are for late-summer to mid-fall planting dates.

Study	Place	Planting Date	Sampling Date	Kg DM ha^{-1}	Kg N ha^{-1}
Sarrantonio and Scott, 1998	Aurora, NY	Late Aug 1984	3rd week of May 1985	6256 ± 1585	246
		Late Aug 1985	3rd week of May 1986	2517 ± 284	92
Drinkwater et al., 2000	Southeastern, PA	Aug 24, 1993	5 May 1994	2860-3340	140-160
			31 May 1994	4620-5310	150-220
Teasdale et al., 2004[1]	Beltsville, MD	21 Sept 1998	4 May – 1 June 1999	5620-6360	167-191
		13 Oct 1998		3450-4410	100-140
		28 Sept 1999	2 – 24 May 2000	3750-5690	112-193
		27 Oct 1999		1700-2970	58-107
		2 Oct 2000	22 May – 11 June 2001	1900-3020	59-79
		24 Oct 2000		950-1280	18-27
	Freeville, NY	25 Aug 1998	10 May – 4 June 1999	1580-4300	55-151
		14 Sept 1998		2510-3070	85-118
Brainard et al., 2012[2]	East Lansing, MI	2 Sept 2008	29 May 2009	4500-7000	115-150
		4 Sept 2009	27 May 2010	4000-6000	80-110
Jannink et al., 1997[3]	Stillwater, ME	14 Aug 1990	22 May 1991	0 – 3977	0 – 78
		4 Sept 1990		0 – 4360	12 – 92
		14 Aug 1991	26 May 1992	Did not overwinter	
		3 Sept 1991		Did not overwinter	
Mueller and Thorup-Kristensen, 2001(Mueller and Thorup-Kristensen, 2001)	Funen, Denmark	31 July 1996	11 Nov 1996	2000	114
		5 Aug 1997	12 Nov 1997	3000	149

[1] Teasdale et al, 2004 – Results presented are ranges across cultivars and harvest dates.

[2] Brainard et al, 2012 – Results presented are ranges across monoculture and bi-culture with rye.

[3] Jannink et al, 1997 – Results presented are ranges across soil type and mono/bi-culture.

a full-season field crop with a fall-seeded HV GrM does not work with most cropping systems in Eastern Canada. This research was undertaken to determine the performance in Eastern Canada of a spring-planted, full-season HV GrM, in comparison with CV and RC.

1.1. Nitrogen Accumulation in Green Manures

Leguminous green manures (GrM) are often an N source on organic farms and can be implemented in a myriad of ways (Nelson and King, 1996; Mueller and Thorup-Kristensen, 2001; Roberts et al., 2008; Woodley et al., 2014). Examining how N accumulates in a GrM can allow for appropriate management decisions to be made: perhaps an early-season cash crop could be planted and harvested prior to the GrM, or the GrM can be interseeded into a cash-crop and still accumulate enough N to be of use the following season. It is difficult for many farming operations to justify "losing" an entire growing season to the establishment of a non-cash crop GrM, and assurances need to be made that sufficient N is accumulated in order to meet subsequent cash crop needs. Seasonal and regional variability in GrM productivity may add additional challenges with respect to their management.

In upstate New York, a fall-seeded HV GrM crop sampled in May was found to have 6256 $\pm$ 1585 kg/ha of above-ground DM, with an N concentration of 39.4 $\pm$ 5.1 g kg^{-1}, for a total of 246 kg N ha^{-1} in above-ground biomass tissue (Sarrantonio and Scott, 1988). Another 16 kg N ha^{-1} was contributed from the HV root mass (Sarrantonio and Scott, 1988). The following year had poorer establishment, and only resulted in 2517 $\pm$ 284 kg ha^{-1} above-ground DM, and 36.6 $\pm$ 3.0 g kg^{-1} N, so only an average of 92.1 kg N ha^{-1}, and 11 kg N ha^{-1} in the HV roots (Sarrantonio and Scott, 1988). Brainard et al (2012) estimate HV root contribution to N derived from atmosphere (N_{dfa}) as an additional 20% above that accumulated by shoot biomass (Brainard et al., 2012). Teasdale et al. (2004) found significant variation in the amount of biomass and N accumulated in a GrM of HV based on experiment year, location, cultivar, and early or late planting dates (summary of data found in Table 1), ranging from 5858 kg DM ha^{-1} to 1063 kg DM ha^{-1}, and 182 kg N ha^{-1} to 21 kg N ha^{-1}. In climates similar to Québec and Nova Scotia, others have found similar variations in HV overwinter survival,

biomass accumulation, and N contributions (Jannink et al., 1997; Mueller and Thorup-Kristensen, 2001; Brainard et al., 2012) (see Table 1 for further HV details).

By comparison, a monocrop of CV has been documented to accumulate 91-103 kg N ha^{-1} in an irrigated system in Australia (Rochester and Peoples, 2005) and 80-110 kg N ha^{-1} in Denmark (Mueller and Thorup-Kristensen, 2001). Peoples et al. (1995) documented that CV fixed 75% of its N from the atmosphere, for a total of 106 kg N_{dfa} ha^{-1}. In North Carolina, an overwintered cover crop of HV accumulated statistically more biomass in comparison to CV at two different sites, and had greater total N than CV in one site year out of two (Parr et al., 2011).

In a potted study using acetylene (C_2H_2) reduction assays to measure nitrogenase activity, it was found that RC does not fix N at a constant rate. Instead there are two peaks of nitrogenase activity, one in spring and one in late summer, separated by a decrease in nitrogenase activity that coincides with flowering (Warembourg et al., 1997). Red clover can derive 61-96% of total plant N from the atmosphere over the growing period, calculated using ^{15}N enrichment (Warembourg et al., 1997), although others have found a wider range of 35-87% (Peoples et al., 1995). Depending on management practices, RC can provide 69-373 kg N ha^{-1} (Peoples et al., 1995)

Teasdale et al (2004) found that, consistent across cultivars, HV productivity is positively correlated with growing degree days (GDD, base 4°C), increasing 41 g m^{-2} for every 100 GDD. With this regression model, it was estimated that 926 GDD are needed to accumulate to achieve 4,000 kg DM ha^{-1}, producing 120 to 144 kg N ha^{-1}. However, this was for HV that was planted in late-summer to early-fall in New York and at several sites in Maryland (Teasdale et al., 2004). Little is known about HV biomass or N accumulation for a spring-planted crop.

1.2. Biologically Fixed Nitrogen

A symbiotic relationship between *Rhizobium* bacteria and legumes enable legumes to derive N from the atmosphere (N_{dfa}). This legume-N can be an important source of nutrients to a subsequent crop. In legumes, fixing N has a higher energy cost than simply accessing the plant available N (PAN) in the soil (Vitousek et al., 2002), so legumes source N both from the atmosphere and from the soil. The amount of N_{dfa} is dependent on legume genetics, rhizobia species and strains, competition for soil N resources, the soil N environment, fertilization, legume growth stage and stressors such as moisture, temperature, acidity, pests, and disease (Ledgard and Steele, 1992; Power and Zachariassen, 1993; Unkovich et al., 1994). There are different methods for trying to quantify the amount of N that is biologically fixed. The two methods utilized in this study are the natural abundance of the isotope ^{15}N ($\delta^{15}N$) technique and the N difference method, explained below.

1.2.1. Natural Abundance of ^{15}N

The natural abundance ($\delta^{15}N$) technique for measuring N_{dfa} ($NDFA_{15N}$) is based on the fact that soils have a different ^{15}N:^{14}N ratio than that found in the atmosphere. Soils are enriched with ^{15}N in comparison with the atmosphere because the oxygen-nitrogen bonds of ^{14}N-NO_3^- are slightly weaker than the bonds found in ^{15}N-NO_3^- (Stevenson and Cole, 1999). As a result, ^{14}N-NO_3^- is lost more easily during denitrification in comparison with ^{15}N-NO_3^-. This results in ^{15}N enriched soils, in the range of 4-17‰ (per-thousand) (Unkovich et al., 1994; Stevenson and Cole, 1999), although cropped soils have been found to be ^{15}N enriched in the realm of 8.34 $\pm$ 1.55‰ compared to atmospheric N_2 (0.3663 atom % ^{15}N) (Shearer et al., 1978). As N_2-fixing plants typically get most of their N from the air, it is assumed that they will have a ratio more similar to the atmosphere compared to plants that do not fix N_2.

Non-legume reference plants are used to provide a measure of the $\delta^{15}N$ in the plant available soil N pool as they rely solely on soil-N for their nutritive needs, and thus should have a ^{15}N:^{14}N ratio similar to the soil (Kohl and Shearer, 1980; Rochester and Peoples, 2005). It is noted that there are several assumptions made with $\delta^{15}N$ techniques (Dakora et al., 2008;

Schipanski and Drinkwater, 2011). Reference plants are chosen in N_{dfa} calculations with the assumption that in comparison with the test legume, they have similar rooting patterns, root depth, and levels of N-isotope fractionation during N uptake (Dakora et al., 2008; Schipanski and Drinkwater, 2011). However, some argue that since most inorganic N is found in the surface 20 cm of the soil, rooting similarities between legumes and reference plants are of lesser import in comparison to the challenges of selecting an appropriate reference plant to correct for the often high spatial variability of soil $\delta^{15}N$ signatures (Høgh-Jensen and Schjoerring, 1994). Research has been conducted to determine the optimum sample size to reduce the effects of spatial variability (Holdensen et al., 2007).

It should also be noted that N_{dfa} can be transferred from the legume to the nearby reference plant, resulting in an underestimation of N_2 fixation when using $\delta^{15}N$ methods (Dakora et al., 2008; Main et al., 2013). This would presumably be a similarly flaw with the difference method ($NDFA_{diff}$, explained in the subsequent section). In both legumes and other plants, the readily decomposable organic substances contained in root exudates, as well as small sloughed off root fragments, are attacked by heterotrophic rhizosphere organisms. The subsequent mineralization of these organisms results in the transfer of ^{15}N from one species to its neighbor (Jansson and Persson, 1982). It has been found that in an RC-white clover-ryegrass system near Copenhagen, that there was between 0 and 17% transfer of N_{dfa} from clover to the grass (Høgh-Jensen and Schjoerring, 1994). However, with a young clover/grass system, this is presumed to be on the lower end of the spectrum, as the transfer is done through microbial biomass, which is less well developed in a younger system (Høgh-Jensen and Schjoerring, 1994). Mycorrhizae have an influence in the isotopic fractionation during N uptake, discriminating against the heavier ^{15}N isotopes during the transfer to the plant from the fungus (Unkovich et al., 1994; Spriggs et al., 2003; Dakora et al., 2008). It was determined that in the natural heathland vegetation of South Africa, if the mycorrhizal relationships of the legume and reference plant are different, or cannot be ascertained, then the $\delta^{15}N$ technique should not be used to estimate N_{dfa} (Spriggs et al., 2003).

Over the growing season, variations in reference crop ^{15}N enrichment levels have been documented, with levels declining as the season progresses (Høgh-Jensen and Schjoerring,

1994; Unkovich et al., 1994). This has been attributed to different levels of ^{15}N enrichment of the organic compounds being mineralized (Høgh-Jensen and Schjoerring, 1994). Despite the assumptions inherent in the $\delta^{15}N$ methodology for determining N_{dfa}, it is still a widely used practice (Kohl and Shearer, 1980; Janzen et al., 1990; Høgh-Jensen and Schjoerring, 1994; Pate et al., 1994; Mueller and Thorup-Kristensen, 2001; Huss-Danell and Chaia, 2005; Rochester and Peoples, 2005; Schipanski and Drinkwater, 2011; Brainard et al., 2012; Main et al., 2013), including for use in determining N_{dfa} in HV systems (Mueller and Thorup-Kristensen, 2001; Rochester and Peoples, 2005; Brainard et al., 2012). The popularity of $\delta^{15}N$ methodology may be because studies have found no difference in N_{dfa} when using natural abundance or applied enriched ^{15}N techniques (Huss-Danell and Chaia, 2005), or $NDFA_{diff}$ (Mueller and Thorup-Kristensen, 2001). Enriched ^{15}N techniques are also costly and unsuitable for multi-site or on-farm assessments of legume N_{dfa} (Main et al., 2013).

Previous studies have determined that a quadrat at least 0.5 m^2 in size results in a reduction of the short-range variability of $\delta^{15}N$ (Holdensen et al., 2007). Using regression analysis, Huss-Danell and Chaia (2005) found in their study of RC in Sweden that there were strong correlations between percent N_{dfa} (pNDFA) of leaves and shoots, between leaves and whole plants (including roots), and between shoots and whole plants (including roots).

1.2.2. Difference Method

There is a lower energy cost to a legume to acquire N from the soil compared to fixing the N using biological means (Vitousek et al., 2002). By assuming that the legumes in these agricultural systems were accessing the same soil-N resources and that they are equally competitive for soil-N as non-legumes, the difference in N-yield between the legume and non-legumes can be assumed to have come from non-soil (and therefore atmospheric) sources (Shearer and Kohl, 1986; Mueller and Thorup-Kristensen, 2001). Another key assumption of the difference method of estimating N-fixation is that there are similarities between species with regards to timing, rate, and total quantity of soil N uptake. Similar to

the natural abundance isotopic method of estimating N-fixation, spatial variability can be a serious source of error (Shearer and Kohl, 1986).

2. Objectives

1.) To determine at two locations in Eastern Canada, through use of ^{15}N natural abundance (commonly denoted as $\delta^{15}N$) and through use of the N difference methods, the GrM (HV-oats (*Avena sativa* L.) (HVO), CV-oats (CVO), RC, RC-oats (RCO)) type that will result in the greatest amount of N_{dfa} in aboveground biomass.

2.) To determine at two locations in Eastern Canada, the GrM (HVO, CVO, RCO, RC) type and timing that will result in the greatest amount of aboveground biomass and biomass-N.

3.) To determine whether aboveground biomass and/or biomass-N and N_{dfa} accumulation of different spring-planted GrM (HVO, CVO, RCO, and RC) is correlated with GDD.

3. Hypotheses

1.) There are significant differences in the quantity and timing of peak aboveground biomass-N (kg biomass-N ha^{-1}) between HVO, CVO, RCO, and RC GrM systems.

2.) There are significant differences in total N_{dfa} (kg N_{dfa} ha^{-1}) between HVO, CVO, RCO, and RC GrM systems.

3.) The total amount of aboveground biomass, aboveground biomass-N, and N_{dfa} of HV is significantly correlated to $GDD_{4°C}$.

4. Materials and Methods

Experimental sites were in two locations, one at the Centre De Recherche sur Les Grains (CÉROM) in Saint-Mathieu-de-Beloeil, Québec, and one at Field 206 at the Dalhousie Agricultural Campus in Bible Hill, Nova Scotia. All data are presented by location and were

analyzed separately. Statistical analysis was not performed across years. Experimental design is outlined below.

4.1. Site Characteristics and Experimental Design

At both locations, weather data was collected from weather stations in close proximity to the research plots: on-site in Saint-Mathieu-de-Beloeil, Québec, and <25 km from Bible Hill, Nova Scotia. Growing degree days were calculated by subtracting the base temperature (4°C) (Teasdale et al., 2004) from the average daily temperature starting from day of planting. In Nova Scotia in both years, as the RC-monocrop was planted on an earlier date than the HVO and the CVO, this resulted in different $GDD_{4°C}$ (growing degree days, base 4°C) having accumulated, termed $GDD_{vetches}$ for $GDD_{4°C}$ accumulated since the vetch planting date, and GDD_{RC} having accumulated since the RC planting date.

At both locations, soil samples were collected prior to GrM planting in 2013 and 2014 (Table 2) to a depth of 20 cm using a soil probe with a 2 cm diameter. A composite of 10-15 subsamples was collected for each block and sent to the Nova Scotia Department of Agriculture analytical lab for Mehlich III extractable nutrients, OM and pH. These tests revealed an acceptable pH and non-limiting macro and micronutrients at both locations in all years (Table 2).

Table 2. Pre-plant soil test results from Nova Scotia and Québec experimental sites in 2013 and 2014.

Parameter	Unit	Nova Scotia				Québec			
		2013		2014		2013		2014	
		Value	STE	Value	STE	Value	STE	Value	STE
pH	pH	6.08	0.12	6.27	0.01	7.45	0.04	7.66	0.04
Organic matter	g kg^{-1}	33.3	1.2	30.3	2.1	36.3	2.2	37.5	3.1
P_2O_5	mg kg^{-1}	498	81.9	460	67.4	106	13.1	84.8	12.7
K_2O	mg kg^{-1}	191	16.4	187	10.9	489	22.5	455	10.9
Calcium	mg kg^{-1}	1290	58.5	1310	60.8	3470	118	3320	154
Magnesium	mg kg^{-1}	217	11.2	229	2.75	955	43.4	871	13.2
Aluminum	mg kg^{-1}	1590	38.4	1590	46.5	1110	3.11	1070	9.22
Boron	mg kg^{-1}	<0.5	-	<0.5	-	1.26	0.02	1.25	0.05

Determination of Mehlich III Extractable Major and Trace Metal Ions in Soil by ICP-OES (Mehlich, 1984).

4.1.1. Bible Hill, Nova Scotia

The trial at this location was a randomized complete block design with three blocks on a Pugwash sandy loam (classified as a Orthic Humo-Ferric Podzol in Canadian soil classification (Webb et al., 1991; Lynch et al., 2012)). Green manure type was the treatment (HVO, CVO, RC).

4.1.2. Saint-Mathieu-de-Beloeil, Québec

Green manures at this site in Québec were established on a Saint-Urbain clay loam (classified as a Dark-Grey Gleysolic soil in Canadian soil classification (Lajoie and Baril, 1954)) in a randomized complete block design with eight blocks with GrM type (HVO, RCO) as the treatment.

4.2. Green Manure Establishment

Vetches were inoculated prior to planting, by *Rhizobium leguminosarum* bv. viceae. Due to the unavailability of organic RC inoculum, RC was not inoculated in 2014; however both sites had recent history RC (at least within the previous three years). The vetches, CV and HV (variety not stated), were mixed with oats (var. Triple Crown) and planted at a rate of 30 kg of vetch live seed ha^{-1} and 70 kg of oats live seed ha^{-1} (Table 3). Teasdale et al. (2004) recommend common HV over a named variety for more northern climes, although this was due to its superior overwintering capabilities. A seeding rate where the HV proportion of the GrM is ~30% has been determined to maximize cover crop biomass (Mirsky et al., 2012). In Nova Scotia, RC (var. Endure) was monocropped and seeded at 11.5 kg ha^{-1}. In Québec, RC (also var. Endure) was mixed with oats and seeded similarly to HVO. All seeding rates were adjusted after confirming germination rates. In Québec, the experimental unit was 6 m x 12 m. In Nova Scotia, the experimental unit was 14 m x 10 m.

Table 3. Dates of main field operations in Nova Scotia and Québec in 2013 and 2014.

	Seeding rate (kg ha^{-1})		Nova Scotia		Québec	
	legume	oats	2013	2014	2013	2014
Seeding RC	11.5	-	May 6	May 14	-	
Seeding RCO	11.5	70	-	-	May 8	
Seeding HVO	30	70	May 29	May 20	May 8	
Seeding CVO	30	70	May 29	May 20	-	
Primary tillage			*April 23* Disk *May 2* Disk	*May 8* Disk	*May 8* Disk	
Secondary tillage			*May 6* S-tined with rolling baskets (2x) *May 28* (vetch plots only) S-tined with rolling baskets (2x)	*May 14* S-tined with rolling baskets (2x)	*May 8* Vibroculteur spring tine cultivator (2x)	

RC = a green manure of red clover (*Trifolium pretense* L.) monocrop (Nova Scotia)
RCO = a green manure of red clover/oats (*Avena sativa* L.) (Québec)
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats

4.2.1. Bible Hill, Nova Scotia

In Nova Scotia, the RC was seeded with a Brillion seeder, and vetches were inoculated and mixed with oats prior to planting at a 17.75 cm row spacing with a Massey Fergusson 33 seed drill. Target seed depth was between 2 and 4 cm, with moderate variations based on soil conditions. Plots were not rolled after planting. The previous crop in all years was a soybean cash crop.

Red clover population was recorded on May 28, 2013, 22 days after planting, using 3-0.25 m^2 quadrats per plot. Populations of CVO and HVO were taken on June 17, 2013, 19 days after planting, when the oats were in the early leaf stage, using 3-0.25 m^2 quadrats per plot.

Similarly, in 2014, GrM populations were recorded on June 17, 34 days after RC planting and 28 days after vetch planting, prior to oat tillering.

4.2.2. Saint-Mathieu-de-Beloeil, Québec

In 2013, the previous crops at this site were barley and wheat with some mixtures under-seeded with red clover and rye grass, albeit a good catch with the red clover was not achieved and management was undertaken to terminate what remained.

The oats were planted using a Khun seed drill with 12.75 cm row spacing, and the legumes broadcasted in 2013. In 2014, GrM were planted using the same varieties and rates as in 2013, with the exception that the legumes were drilled instead of broadcast.

Population counts were performed by measuring 3-one linear meter rows for the oats on May 31, 2013, and by using 3-0.25 m^2 quadrats per plot to count the legumes on June 19, 2013. Population counts were performed on June 11, 2014 on 3-one linear meter rows in each plot.

4.3. Quantifying Green Manure Biomass Production and Nitrogen Accumulation

Biomass samples were taken throughout the course of the 2013 and 2014 season at both locations to determine biomass and N accumulation (Table 4). Green manures were sampled over three dates. Sampling dates at both the Nova Scotia and Québec sites in 2013 were originally designed to be associated with legume physiology with the first GrM sampling date to occur at 50% flowering. As a result, the first sampling dates in 2013 were staggered (Table 4). As CV is a determinate and flowers before HV or RC, the first CVO sampling date in Nova Scotia was on July 29, 2013. The first sampling date for HVO in Nova Scotia was August 22, 2013, and in Québec on August 9, 2013. These two HVO sampling dates targeted estimates of 50% flowering, but, as HVO is an indeterminate plant, 50% flower was

not achieved, despite observing the protocol outlined by Mischler et al. (Mischler et al., 2010). Once this was realized, the second and third sampling dates for CVO, HVO, RCO and RC, were set for uniform increments of time (Table 4). This however resulted in the first sampling date of RCO in Québec coinciding with the second sampling date of HVO. For the sake of simplicity of analysis, the first sampling date of RCO on September 23, 2013 in Québec is termed "Sample Date 2" for the remainder of the work, and missing values were used for RCO-Sample date 1.

Sample date 3 was in mid- to late October, just prior to when GrM would normally be fall-incorporated (Table 4). At each biomass sampling date, two 0.5 m^2 quadrats per plot were harvested to 5 cm above the soil surface and the biomass was separated into legume, non-legume, non-legume weeds, and legume-weeds. After sorting, GrM were dried at 60°C for 48 hours and weighed separately. Tissue samples were passed through a 2 mm screen on a Wiley mill (standard model number 3, Arthur H. Thomas Co., Philadelphia, USA). Total C and N concentration were analyzed through gas chromatography (GC) after dry combustion, using a Vario MAX CN analyzer (Elementar, Hanau, Germany).

The total amount of N produced in each system was quantified at each sample date by multiplying the GrM percent N by the DM aboveground biomass for legume, non-legume and weed components.

Table 4. Green manure sampling dates and growing degree day accumulation in Nova Scotia and Québec in 2013 and 2014.

GrM		Nova Scotia				Québec			
		2013		2014		2013		2014	
	Sample date	Sample date	GDD	Sample date	GDD	Sample date	GDD	Sample date	GDD
CVO	1	July 29	787	Aug. 13	996	-	-	-	-
	2	Sep. 19	1385	Sep. 16	1354	-	-	-	-
	3	Oct. 29	1571	Oct. 21	1569	-	-	-	-
HVO	1	Aug. 22	1091	Aug. 13	996	Aug. 9	1367	Aug. 12	1394
	2	Sep. 19	1385	Sep. 16	1354	Sep. 23	1964	Sep. 10	1831
	3	Oct. 29	1571	Oct. 21	1569	Oct. 28	2227	Oct. 14	2126
RC or RCO	1	Sep. 4	1393	Aug. 13	1047	-	-	Aug. 12	1394
	2	Sep. 19	1526	Sep. 16	1404	Sep. 23	1964	Sep. 10	1831
	3	Oct. 29	1713	Oct. 21	1620	Oct. 28	2227	Oct. 14	2126

GDD = Growing degree days (base 4°C)
GrM = Green manure
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.) (Nova Scotia only)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pretense* L.) monocrop (Nova Scotia)
RCO = a green manure of red clover/oats (Québec)

Growing degree days were calculated from the date of planting using a base temperature of 4°C. If the daily mean temperature was less than the base temperature, the daily mean temperature was set to the base temperature, resulting in 0 $GDD_{4°C}$ accumulated on that day (McMaster and Wilhelm, 1997).

4.4. Quantifying Green Manure Biological Nitrogen Fixation

4.4.1. Natural Abundance Technique

Biological nitrogen fixation was determined on the first GrM sampling date using the $\delta^{15}N$ method ($NDFA_{15N}$) outlined by Huss-Danell and Chaia (2005) and Main et al. (2013). In 2013, the first GrM sampling date was targeted for 50% flowering of the legume. In Nova Scotia in 2013, July 29th was the sample date used for $NDFA_{15N}$ for CVO, August 22nd was

used for HVO, and September 4th was used to calculate $NDFA_{15N}$ for RC. In Québec in 2013, the August 9th sample date was used to calculate $NDFA_{15N}$ for HVO and September 23rd for RCO. It was determined that spring-planted HVO is an indeterminate and therefore targeting sampling for 50% flowering was impractical. Therefore, in 2014, uniform sampling dates were selected for all GrM, August 13, 2014 in Nova Scotia and August 12, 2014 in Québec.

As recommended by Holdensen et al (2007), 0.5 m^2 quadrats were used for collecting biomass samples, which were harvested to 5 cm above the soil surface and species were separated into legume, non-legume, non-legume weeds, and legume-weeds. In order to achieve a very fine grind, dried and ground (<2mm), biomass samples from the first sample date were oven-dried at 50°C for 24 hours and then approximately 2 g of ground tissue were placed in square glass jars with a small round rod, a medium round rod, and a large square rod. Jars were placed on a roller-grinder for 48 hours at 70 bottle revolutions per minute (Ward, 2010). Samples were weighed out to 1.4-3.4 µg ($\pm$ 0.3) depending on species and original biomass percent N (Table 5), encapsulated in foil, and sent to the Stable Isotope Facility, Department of Soil Science, University of Saskatchewan. There they were combusted at 1800°C in a Robo Prep elemental analyzer interfaced with a continuous-flow Europa 20:20 isotope-ratio mass spectrometer (CFIRMS; Europa Scientific Ltd., Crewe, UK) for ^{14}N and ^{15}N (Main et al., 2013).

Table 5. Isotope sample weight and precision.

Sample type	Sample weight (µg)	Precision (µg)
Red clover	2.0	± 0.3
Weeds (red clover reference species in Nova Scotia)	3.1	± 0.3
Hairy vetch	1.4	± 0.2
Common vetch	1.5	± 0.2
Oats	3.4	± 0.2

Green manure $\delta^{15}N$ (measured in parts per thousand (‰) and pNDFA (%) were calculated as follows, based on Huss-Dannell and Chaia (2005):

$$\delta^{15}N = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R denotes the ratio $^{15}N/^{14}N$

and $R_{standard}$ is the international standard for atmospheric $N_2 = 0.0036765$

$$pNDFA = (\delta^{15}N_{non\text{-}legume} - \delta^{15}N_{clover\ or\ vetch}) / (\delta^{15}N_{non\text{-}legume} - \beta) \times 100$$

Oats harvested from within the same 0.5 m^2 quadrat were used as the non-legume reference plant in the HVO, RCO and CVO GrM plots. Weeds harvested from within the same 0.5 m^2 quadrat were used as the reference in the RC plots in Nova Scotia. β is the natural abundance of ^{15}N in a legume which is wholly dependent on N_2 fixation for its N nutrition, a value arrived at by growing the legume in complete absence of soil available N. However, it was decided that growing legumes in an N-free media in a greenhouse would not accurately represent field conditions. For example, in the greenhouse there would not be any transfer of N_{dfa} from the legume to the reference oats/weeds, a source of fractionation that would make the greenhouse legume values non-representative of field legume values. Additionally, it was decided that the mycorrhizal environment in the greenhouse would also not an accurate representation of field-like conditions, especially since it has been found that differences in rhizobial strain can cause unacceptable variation in the β value between host species at different sites (Unkovich et al., 1994). While some researchers just set β to zero (Høgh-Jensen and Schjoerring, 1994), it was decided that was also not an accurate representation of β. Therefore, similarly to other studies (Huss-Danell and Chaia, 2005; Ward, 2010; Brainard et al., 2012; Main et al., 2013), the minimum field $\delta^{15}N$ value was used as the β-value. This method assumes the lowest $\delta^{15}N$ value is an indicator of pNDFA closest to 100%, resulting in a conservative estimate of pNDFA.

The total amount of N produced in each system was quantified at each sample date by multiplying the GrM percent N by the DM yield. The amount of N_{dfa} in the aboveground biomass at the first sampling date was quantified by multiplying pNDFA by the total N

content in legume GrM aboveground biomass. By the time of the second sampling date (early to mid-September), plants, with the exception of HV and RC, had started to senesce, most likely resulting in changes to isotope ratios (Melillo et al., 1989), and therefore natural abundance methods were not used for the second and third GrM sampling dates.

4.4.2. Difference Method

Weeds, oats, and legumes, harvested, processed, and analyzed as outlined above, were used to calculate $NDFA_{diff}$. The amount of N accumulated (kg N ha^{-1}) was calculated by multiplying the aboveground biomass dry matter yield by the percent N content for each oat, weed, and legume. The amount of N accumulated in oat and weed biomass combined was subtracted from legume GrM biomass N (kg N ha^{-1}), giving an estimate of $NDFA_{diff}$.

$$NDFA_{diff} = N_{legume} - (N_{oat} + N_{weeds})$$

4.5. Statistical Analysis

Statistical analyses were conducted using Statistical Analysis System 9.3 software (SAS Institute, 2011, Cary, NC). The experimental design was a randomized complete block design with three blocks (Nova Scotia) or eight blocks (Québec). The factor of interest was GrM type (HVO, RC, and CVO in Nova Scotia, HVO and RCO in Québec). The factor was a fixed effect and blocks were random effects. Responses were analyzed separately by site and by year because of missing data, changing experimental units, and differences in sampling schedule between sites and years. Therefore neither the effects of site nor year were tested using statistical analysis.

All data were verified for normality and constant variance. Independence of the residuals was assumed through randomization. Assumptions of normality were met or data were transformed to bring the error distribution closer to normal. Outliers were identified using the Proc Univariate statement. All means reported in the tables and figures were back-

transformed to the original scale. The significance level is set at $\alpha = 0.05$ where α is the probability of making a Type I error and wrongly rejecting H_0. Marginal significance is occasionally noted in the text where $\alpha < 0.1$.

4.5.1. Repeated Measures

Repeated measures analysis was performed when the same response variable (for example dry matter yield) was measured on the same experimental unit on two or more occasions (for example sample dates 1, 2, 3) using Proc Mixed in SAS which computes LSMEANS that are averaged across the repeated measures, with standard errors that are reflective of the selected covariance structure. The covariance structure embodies the type of relationship of values over time (Kincaid, 2005). Covariance structures used for this analysis were compound symmetry, heterogeneous compound symmetry, and unstructured.

An F-statistic was calculated for each main effect and interaction, and was used to produce a p-value. If $p < \alpha$, then the H_0 was rejected and the H_a was accepted. In using a p-value to determine if there was a treatment effect, the highest order of interaction was considered first, and if significant, a multiple means comparison was performed. If the highest order interaction was not considered significant, then the next highest interaction was considered.

Proc Mixed and the LSMEANS statement were used in SAS to obtain paired comparisons on the least-square means. To obtain pairwise t-tests for all pairs of least-square means, the PDIFF option was used, which enabled pairwise comparisons on two-way and higher order treatment combinations of means. The variance estimate used pooled mean square error when computing the standard error of the difference between the least-square means (Kendall, 1993). The mean separation output in Proc Mixed was converted to letter groupings using a macro. The macro (pdmix800) took the data set generated from PDIFF, and converted them into groups, and then they were merged onto the LSMEANS data set (Saxton, 1998).

4.5.2. Linear Regression

Linear regression was used to determine the relationship between $GDD_{4°C}$ and legume DM yield, legume-N, and N_{DFA}. As both the independent variable ($GDD_{4°C}$, or $\boldsymbol{x}$) and the dependent variable ($\boldsymbol{y}$, for example, legume yield) were quantitative, a scatter plot (x vs. y) was made, and a preliminary evaluation of the relationship between the two was possible. By examining the pattern, it was possible to roughly determine the form, direction, and strength of the association, and to identify outliers (deviations from the pattern). The form was identified as approximately positively linear for HV. Cook's distance was calculated for each value to assist in the identification of influential values. The coefficient of determination (R^2) was used to evaluate the model for adequacy, giving the percentage of variability in the dependent variable that was explained by the model.

5. Results

5.1. Weather

Weather data was collected from weather stations in close proximity to the plots. In 2013, the seasonal (May – October) cumulative rainfall and GDD in Nova Scotia was 578 mm and 1734 $GDD_{4°C}$ respectively, and the average daily air temperature was 13.5°C. For 2014, the seasonal (May-October) cumulative rainfall and GDD in Nova Scotia was 478 mm and 1683 $GDD_{4°C}$ respectively, and the average daily air temperature was 13.1°C (Table 6). Both years were slightly cooler than the 30 year average of 14°C.

Table 6. Weather in Bible Hill, Nova Scotia, 2013, 2014.

Month	2013			2014			1981-2010 Canadian Climate Average	
	Average temp. (°C)	Precip. (mm)	$GDD_{4°C}$	Average temp. (°C)	Precip. (mm)	$GDD_{4°C}$	Average temp. (°C)	Precip. (mm)
May	10.1	72	193	8.5	54	141	10.2	106
June	14.7	103	322	13.8	125	293	15.1	96
July	19.0	99	467	19.2	40	471	18.6	91
August	17.1	33	379	16.4	54	383	18.2	90
September	13.2	148	275	12.2	93	245	13.7	109
October	6.9	123	99	8.7	112	151	8	108
Total		578	1734		478	1683		599

$GDD_{4°C}$ = Growing degree days (base 4°C)
30 year average is from Debert, NS Environment Canada weather station climate data.

In 2013, the seasonal (May – October) cumulative rainfall and GDD in Québec was 482 mm and 2325 $GDD_{4°C}$ respectively, and the average daily air temperature was 16.6°C. In 2014, the seasonal (May – October) cumulative rainfall and GDD in Québec was 519 mm and 2348 $GDD_{4°C}$ respectively, and the average daily air temperature was 16.8°C (Table 7). Both years were much warmer than the 30 year average of 15.6°C.

Table 7. Weather in Saint-Mathieu-de-Beloeil, Québec, 2013, 2014.

Month	2013			2014			1981-2010 Canadian Climate Average	
	Average temp. (°C)	Precip. (mm)	$GDD_{4°C}$	Average temp. (°C)	Precip. (mm)	$GDD_{4°C}$	Average temp. (°C)	Precip. (mm)
May	15.2	101	347	14.4	93	322	12.9	82
June	17.9	102	418	19.7	172	471	17.9	87
July	21.8	42	553	20.8	82	521	20.6	97
August	19.6	91	485	19.7	63	487	19.5	88
September	14.8	71	324	15.4	36	341	14.7	85
October	10.0	74	198	10.6	73	206	7.9	85
Total		482	2325		519	2348		524

$GDD_{4°C}$ = Growing degree days (base 4°C)
30 year average is from St-Hubert Airport Environment Canada weather station climate data.

5.2. Green Manure Establishment and Biomass Production

The interaction between sampling date and GrM was almost always significant for total biomass at both locations, with the exception of Québec in 2013.

5.2.1. Bible Hill, Nova Scotia

Despite HV and CV being seeded at the same rate (30 kg live seed ha^{-1}), in both years CV had lower establishment than the HV. The CV seed used in both years was from 2011, and even with adjustments made for germination, there was slightly lower establishment in 2014 compared to 2013 (Table 8).

Table 8. Green manure establishment in Nova Scotia in 2013 and 2014.

GrM	2013		2014	
	Legume (plants m^{-2})	Oats (plants m^{-2})	Legume (plants m^{-2})	Oats (plants m^{-2})
CVO	38	143	29	153
HVO	94	200	67	203
RC	159	-	201	-

GrM = green manure
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pretense* L.)

By the second sampling date on September 19th, 2013 after 1385 $GDD_{vetches}$ had accumulated, CVO had started to senesce, but had significantly more total biomass than the first sample date. By the third CVO sampling date (October 29, 2013), just prior to when the GrM would be incorporated in the fall, both the CV and the oats had completely senesced (Figure 1).

The HVO continued to accumulate biomass right up until the last sampling date in late October 2013, when it had a total biomass of 7034 kg DM ha^{-1}. In 2013, by the third sampling date 1571 $GDD_{vetches}$ had accumulated, HVO had accumulated the most total

biomass out of all the GrM treatments in NS, and of that biomass, 5860 kg DM ha^{-1} were HV and the oats were 1174 kg DM ha^{-1}.

The GrM of RC had statistically similar quantities of biomass between the first (September 4, 2013), second (September 19, 2013) and third sampling dates (4269, 4538 and 3339 kg DM ha^{-1} respectively) (Figure 1).

The first sampling date on August 13, 2014 was two weeks later than the first CVO sampling date in 2013 on July 29. At this point, 996 $GDD_{vetches}$ had accumulated in 2014, compared to 787 GDD_{CVO} in 2013, and approximately 70% of the CV had started to senesce, and the oats were starting to dry down. It is possible that the peak biomass accumulation of CVO occurred prior to the first sampling date in 2014. In 2014, CVO did not accumulate as much biomass as it did in 2013, with peak biomass (5567 kg DM ha^{-1}) (Figure 1) at the first sampling date on August 13, 2014, compared to peak biomass of 6430 kg DM ha^{-1} on September 19, 2013 (Figure 1). This could be in part to poorer CV establishment in 2014 (Table 8). This would also account for oats making up the majority of the CVO biomass in 2014, between 60 and 72% of the total biomass across all sampling dates.

In 2014, HVO accumulated the most biomass by the second sampling date, September 19, 2014 (1354 $GDD_{vetches}$) for a total of 8403 kg DM ha^{-1} (Figure 1). While the third sampling date in late October (1569 $GDD_{vetches}$) did not have significantly less biomass (7502 kg DM ha^{-1}) than the previous two sampling dates, it is possible that further biomass did not accumulate in 2014 after September due to deer predation.

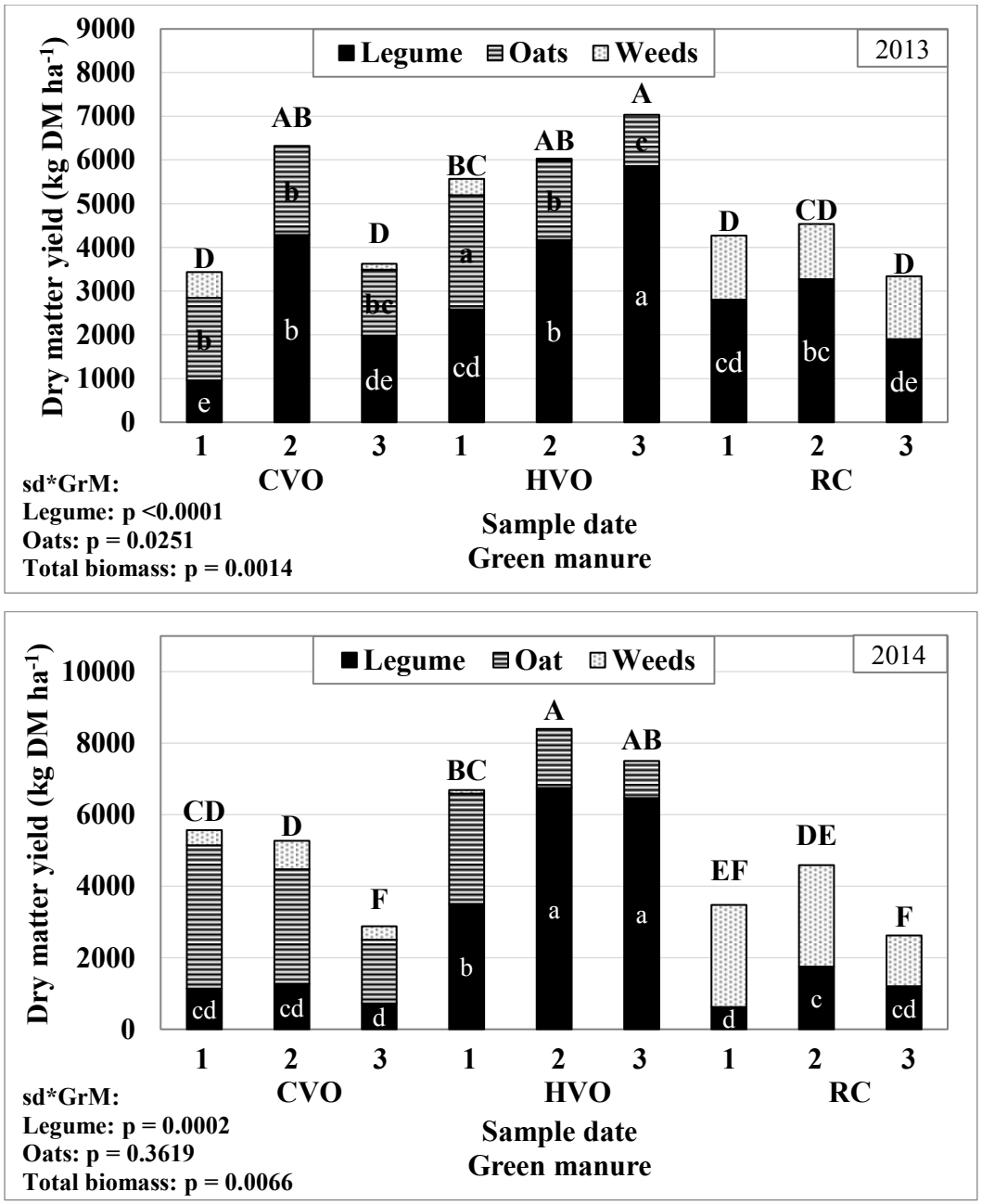

Figure 1. Biomass accumulation of green manures in Nova Scotia in 2013 (top) and 2014 (bottom).

Within each year, uppercase letters indicate significant differences between total green manure biomass accumulated (including weeds). Within each component, data with the same lowercase letter are not significantly different in biomass accumulated.

sd*GrM = sample date by green manure interaction

DM = dry matter

CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)

HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats

RC = a green manure of red clover (*Trifolium pretense* L.)

A GrM of RC hit peak biomass accumulation at the September 16, 2014 sampling date (1404 GDD_{RC}), with a total of 4586 kg DM ha^{-1} of biomass. A GrM of RC had significant differences between RC biomass between sample date 1 and 2, although there were no significant differences between total RC biomass (including weeds). Similar to HVO in 2014, deer predation could have also adversely affected the RC biomass by the third sampling date (2622 kg DM ha^{-1}). The deer were apparently disinterested in the senesced CVO plots. In comparison to 2013, while the GrM of RC had similar total biomass in 2014, much less of the biomass was legume biomass. Weeds made up a large portion of the biomass in the 2014 RC plots at each sampling date, comprising 82%, 62%, and 54% of total biomass at the first, second, and third sampling dates respectively.

While not compared statistically, there were noticeable differences between years. The second and third RC sampling dates occurred in mid-September and mid-October in 2013 (1526 GDD_{RC} and 1713 GDD_{RC}) and 2014 (1404 GDD_{RC} and 1620 GDD_{RC}) (Table 4). In 2014, at the second sampling date, 1750 kg DM_{legume} ha^{-1} had accumulated, compared to 3276 kg DM_{legume} ha^{-1} in 2013. At the third sampling date, in 2013, the RC had accumulated 1898 kg DM_{legume} ha^{-1} but in 2014, the RC had only accumulated 1206 kg DM_{legume} ha^{-1}. This reduced legume biomass could be because the RC was not inoculated in 2014 due to the inability to obtain organic RC inoculum or could be due to weed pressure.

5.2.2. Saint-Mathieu-de-Beloeil, Québec

Green manures were seeded at the same rate in 2013 as in 2014. However in 2013, the legumes were broadcast over the drilled oats, and in 2014, the legumes were drilled over the drilled oats resulting in improved legume establishment for the HV. Establishment of RC in the RCO plots may have been impacted due to the lack of inoculum in 2014 (Table 9).

Table 9. Green manure establishment in Québec in 2013 and 2014.

GrM	2013		2014	
	Legume (plants m^{-2})	Oats (plants m^{-2})	Legume (plants m^{-2})	Oats (plants m^{-2})
HVO	106	254	210	241
RCO	626	249	435	223

GrM = green manure
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)
RCO = a green manure of red clover (*Trifolium pretense* L.)/oats

In Québec in 2013, HV accumulated the most legume biomass by late October (4611 kg DM_{legume} ha^{-1}), and a GrM of HVO accumulated the most total biomass (7696 kg DM ha^{-1}) compared to RCO across all sampling dates (Figure 2). Oats had a greater amount of biomass at the second sampling date in mid-September (2525 kg DM ha^{-1}), and oat biomass decreased significantly by the third sampling date in late October, by which time the oats were starting to decompose.

In 2014, at the time of the first sampling date for all GrM in mid-August, 1394 $GDD_{4°C}$ had accumulated, and HVO had more biomass (3655 kg DM ha^{-1}) than the RCO (2710 kg DM ha^{-1}) (Figure 2). By the second sampling date, 1831 $GDD_{4°C}$ had accumulated, and the GrM of HVO and RCO had statistically similar amounts of biomass, 3751 kg DM ha^{-1} and 3282 kg DM ha^{-1} respectively, but the biomass of HV was significantly greater than the biomass of RC. By the third sampling date mid-October in 2014, RCO biomass had declined slightly but not significantly compared to the second sampling date. A GrM of HVO accumulated the most biomass of all sampling dates by the third sampling date on October 14, 2014 when 2126 $GDD_{4°C}$ had accumulated, resulting in 4769 kg DM ha^{-1}. At the third sampling date, the HVO also had the greatest legume biomass, 3727 kg DM_{legume} ha^{-1}.

In 2014, the RCO had statistically more oats (1785 kg DM_{oats} ha^{-1}) than HVO (1146 kg DM_{oats} ha^{-1}). There was less oat dry matter in the GrM at the latter two sampling dates as the oats started to senesce and decompose.

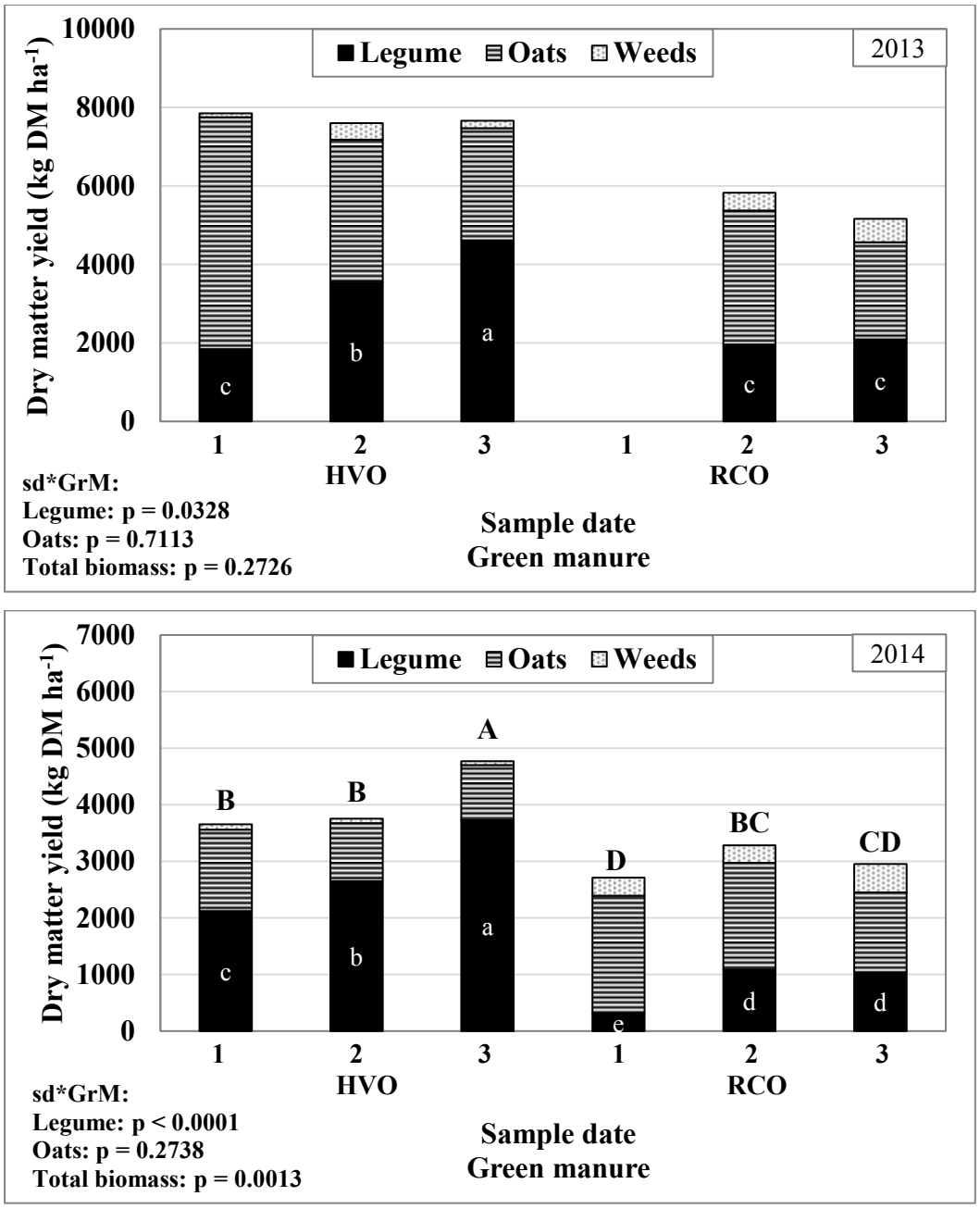

Figure 2. Biomass accumulation of green manures in Québec in 2013 (top) and 2014 (bottom).

Within each year, uppercase letters indicate significant differences between total green manure biomass accumulated (including weeds). Within each component, data with the same lowercase letter are not significantly different in biomass accumulated.

Data not available for RCO, sample date 1 in 2013.

sd*GrM = sample date by green manure interaction

DM = dry matter

HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)

RCO = a green manure of red clover (*Trifolium pretense* L.)/oats

While not compared statistically, biomass yields were less in 2014 than they were in 2013, despite similar $GDD_{4°C}$ accumulations (Table 7). Across all sampling dates, in 2013 HVO had a total biomass of 7696 kg DM ha^{-1} (Figure 2) but in 2014 it was only 4058 kg DM ha^{-1} (Figure 2).

Across all sampling dates, RCO had a total biomass of 5493 kg DM ha^{-1} in 2013, compared to 2955 kg DM ha^{-1} in 2014. This might be due to lower RC populations in Québec in 2014 (Table 9) or because the RC was not inoculated in 2014 as organic RC inoculum was unavailable. Peak biomass accumulation in 2014 occurred on the third sampling date in mid-October in HVO, a total of 4769 kg DM ha^{-1} (Figure 2). The HVO sampled at the same date in 2013 had approximately 60% more biomass (Figure 2).

5.3. Nitrogen Accumulation in Green Manures

The interaction between sampling date and GrM was always at least marginally significant for total biomass-N at both locations.

5.3.1. Bible Hill, Nova Scotia

On July 29, 2013, CVO was at 50% flower. The total CVO GrM had accumulated 68 kg N ha^{-1}, 33.8 kg N ha^{-1} was in the legume, 25.4 kg N ha^{-1} was in the oat, and the rest was in the weed biomass (Figure 3). By September 19, 2013 at 1385 $GDD_{4°C}$, the CVO reached its peak N accumulation in the aboveground biomass, with a total of 146 kg N ha^{-1}, 118 kg N ha^{-1} was in the legume, the amount of N in the oat biomass had not changed from the first biomass sampling (25.6 kg N ha^{-1}), and the rest of the N (2.4 kg N ha^{-1}) was contained in weed biomass. On the third sampling date on October 29, 2013, 1571 $GDD_{4°C}$ had accumulated, and the amount of N accumulated in the CVO biomass had declined to a total of 72 kg N ha^{-1}. Of this, statistically the same amount was contained in the oat biomass (22.6 kg N ha^{-1}), but the amount of N in the aboveground legume biomass had decreased by more than 60% to 46 kg N ha^{-1} (Figure 3).

In 2013, RC had statistically similar amounts of N in the aboveground biomass between the first and second sample dates (100 and 110 kg N ha^{-1}). By the third sample date in late October of 2013, the amount of N in the aboveground RC biomass had decreased to 70 kg N ha^{-1}. Of that 70 kg N, almost 35% of it was from weeds (Figure 3).

In the HVO, at the first sampling date (August 22, 2013), the GrM had accumulated 139 kg N $^{ha-1}$, and of that 99 kg N ha^{-1} were in the legume aboveground biomass and 33.8 kg N ha^{-1} were in the oats, with 6.2 kg N ha^{-1} in the weeds. Four weeks and 294 $GDD_{4°C}$ later, at the second sampling date, the HVO had accumulated a statistically similar amount of N (177 kg N ha^{-1}) as the first sampling date. The amount of N in the aboveground HV biomass had increased by approximately 50% from the first sampling date to 151 kg N ha^{-1}. Slightly less N was found in the oats on the second sampling date in mid-September compared to the first sampling date, but they were statistically similar (25.3 kg N ha^{-1} at the second sampling date). By the third sampling date in late-October, the amount of aboveground HVO biomass-N was 221 kg N ha^{-1}, and the amount of HV-N had more than doubled since the first sampling date to 203 kg N ha^{-1}. The amount of N in the oats in the HVO treatment by the third sampling date (18.1 kg N ha^{-1}) was significantly less than the amount of N in the oats at the first sampling date. There were no discernable weeds by the third sampling date in the HVO treatment. Peak biomass N accumulation across all of the GrM treatments trialed was in the third sampling date of HVO on October 29th, 2013 just prior to when it would be fall incorporated (Figure 3).

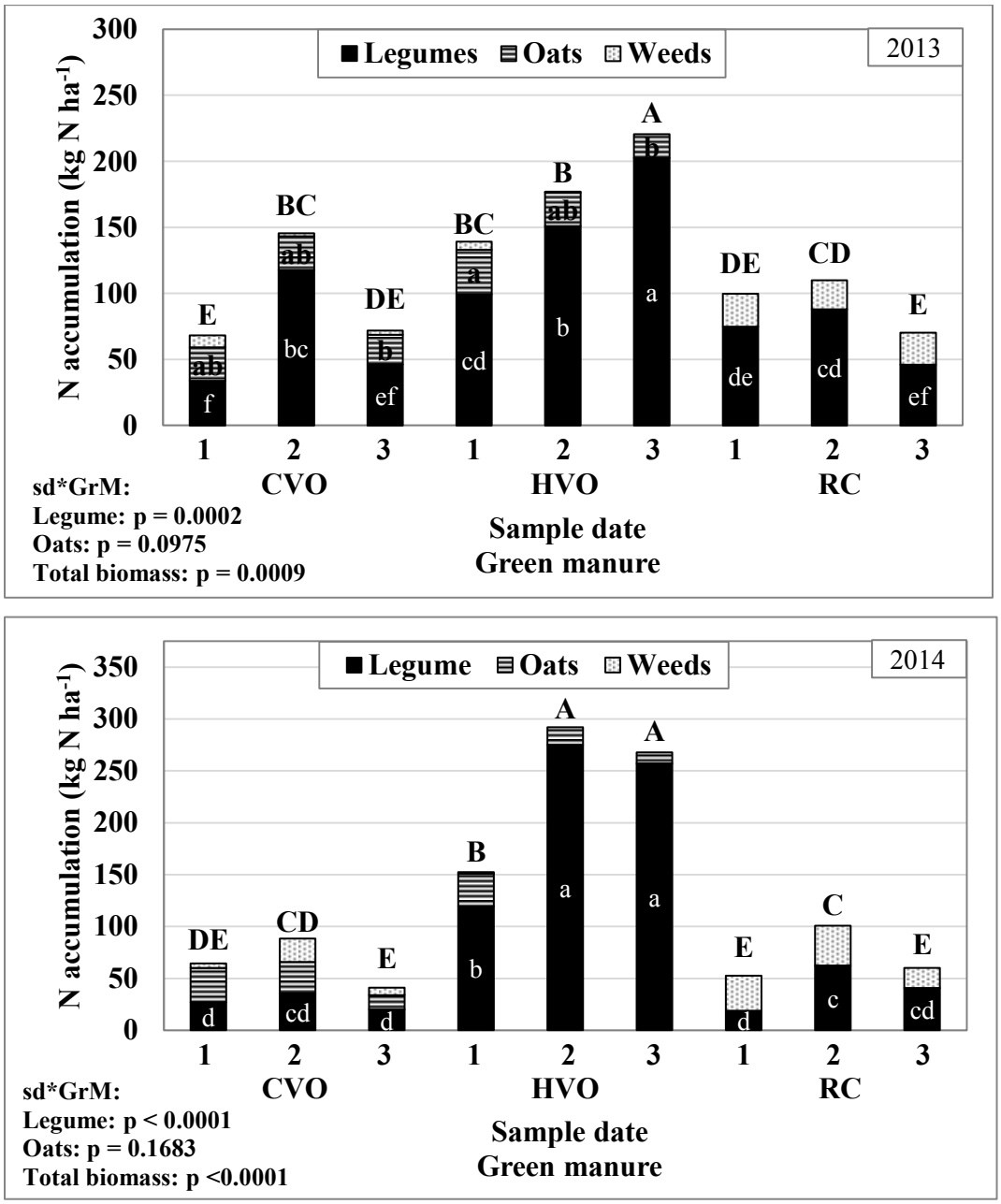

Figure 3. Biomass nitrogen accumulation of green manures in Nova Scotia in 2013 (top) and 2014 (bottom).

Within each year, uppercase letters indicate significant differences between total green manure biomass nitrogen accumulated (including weeds). Within each component, data with the same lowercase letter are not significantly different in biomass accumulated.

CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)

HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats

RC = a green manure of red clover (*Trifolium pretense* L.)

While not compared statistically, there were noticeable similarities and differences between years. In 2014, CV showed a similar pattern to 2013, whereby GrM-N accumulation peaked (88 kg N ha^{-1}) at the second sampling date, on September 16, 2014, although it was not significantly different from the amount of biomass-N on the first sample date (64 kg N ha^{-1}). However, at this stage, the CV and oats had both started to senesce already, and it is possible that peak N accumulation occurred between the first and second sampling dates. The CVO GrM in 2013 accumulated more N than the 2014 crop, and this could be either due to missed peak biomass-N accumulation in 2014 sampling, and/or due to poor CV establishment in 2014 (Table 8). By late fall 2014, after 1569 $GDD_{4°C}$, CVO above-ground biomass contained 41 kg N ha^{-1} (Figure 3).

The GrM of HVO accumulated 152 kg N ha^{-1} after 996 $GDD_{4°C}$ on August 13th. By the second sampling date on September 16, 2014, HVO had accumulated another 140 kg N ha^{-1} after 1354 $GDD_{4°C}$. However, by the third sampling date, the amount of GrM-N accumulated by the HVO had decreased, although not significantly, to 268 kg N ha^{-1}. This slight decrease could be due to deer predation as mentioned in Section 5.2.1. Similar to 2013, there were no discernable weeds by late October in the HVO treatment.

In mid-September 2014, compared to the other RC sampling dates, the GrM of RC had the most GrM-N (including weeds), 101 kg N ha^{-1}. By mid-October, there was only 60 kg N ha^{-1}, and of that, almost 33% of it was from the weeds. At the first sampling date in mid-August 2014, 64% of the RC GrM biomass-N was weeds.

While not compared statistically, there were noticeable differences between years. The RC in 2014 had less legume-N than in 2013. This reduced legume-N could be because the RC was not inoculated in 2014 due to the inability to obtain organic RC inoculum as mentioned in Section 4.2.1.

5.3.2. Saint-Mathieu-de-Beloeil, Québec

In 2013, a GrM of HVO had the greatest GrM-N accumulation by the third sampling date on October 28, 2013 (2227 $GDD_{4°C}$), a total of 200 kg N ha^{-1} (Figure 4). Of that 200 kg of N, 41.5 kg were from the oats, and 154.1 kg of N were from the HV. In 2013, RCO had statistically similar amounts of N in the aboveground biomass on the second and third sampling dates. Oats grown with HV had greater biomass-N than oats grown with RC by the third sampling date.

While not compared statistically, there were noticeable differences between years. At the Québec site in 2014, HVO continued to accumulate significantly more GrM-N at each sampling date whereas, similar to 2013, the RCO did not have significantly different legume-N nor GrM-N between the second and third sampling dates in September and October (51.4 and 51.9 kg GrM-N ha^{-1}) (Figure 4). Of the RCO biomass at the second and third sampling dates in 2014, approximately 45% of the GrM-N was from oats and weeds.

In 2014, the greatest accumulation of GrM-N was on the third sampling date in mid-October in HVO (123.0 kg N ha^{-1}) (Figure 4) and 2126 $GDD_{4°C}$ had accumulated. Of this HVO GrM-N, 112 kg N ha^{-1} was from the HV and 10.2 kg N ha^{-1} was from the oats. While not compared statistically, in total, the HVO in 2014 accumulated much less GrM-N than in 2013 (200 kg N ha^{-1}, Figure 4).

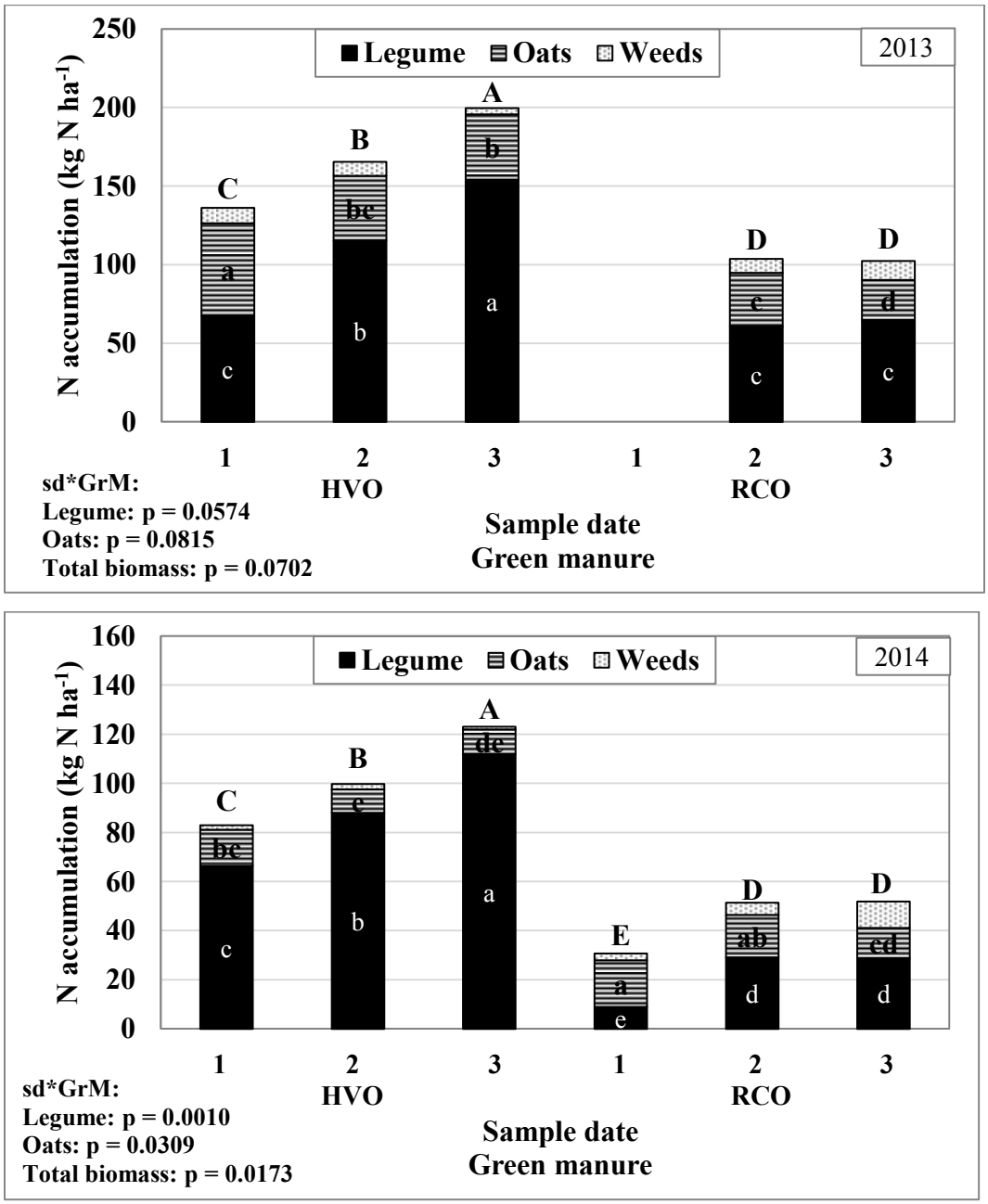

Figure 4. Biomass nitrogen accumulation of green manures in Québec in 2013 (top) and 2014 (bottom).

Within each year, uppercase letters indicate significant differences between total green manure biomass accumulated (including weeds). Within each component, data with the same lowercase letter are not significantly different in biomass accumulated.

Data not available for RCO, sample date 1 in 2013.

sd*GrM = sample date by green manure interaction

DM = dry matter

HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)

RCO = a green manure of red clover (*Trifolium pretense* L.)/oats

5.4. Estimates of Nitrogen Fixation

5.4.1. Bible Hill, Nova Scotia

5.4.1.1. Natural Abundance of ^{15}N

The interplanted oats were used as a reference species for CV and HV. Weeds were used as a reference species for RC. The first GrM sampling date for each treatment (Table 4) was used to determine δ15N. Values of δ15N for each species in each plot were used to calculate pNDFA.

In Nova Scotia in 2013, it was estimated that between 92.7 and 94.3% of legume N was atmospherically derived, with no significant differences between legume species. This suggests that the legume species were able to access and compete for soil N reserves with similar adeptness, and relied mainly on atmospheric sources of N (Figure 5). In 2013, HV fixed 91.4 kg $NDFA_{15N}$ ha^{-1}; RC had statistically similar amounts of 68.5 kg $NDFA_{15N}$ ha^{-1}, and CV was the bottom performer, only accumulating 31.7 kg $NDFA_{15N}$ ha^{-1}.

In 2014, at the first sampling date in August, GrM of RC and CVO derived the greatest percentage of N from the atmosphere, with pNDFA 95.3% and 94.0% respectively. The HVO GrM had a pNDFA of 89.0%, which was significantly less than the RC GrM (95.3%). The CVO had a statistically similar pNDFA as both the RC and the HVO in 2014.

However, in 2014, RC only fixed 17.9 kg $NDFA_{15N}$ ha^{-1}, statistically similar to CVO (25.1 kg $NDFA_{15N}$ ha^{-1}), while HVO derived 106.2 kg $NDFA_{15N}$ ha^{-1}.

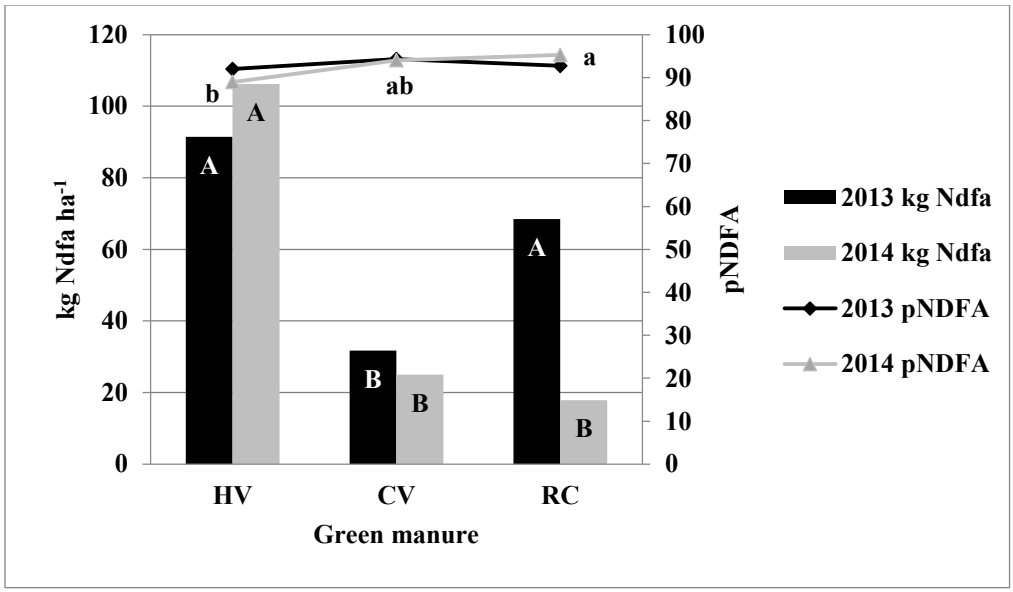

Figure 5. Nitrogen derived from the atmosphere estimates using isotopic methods in Nova Scotia in 2013 and 2014.

Within each year, values followed by the same capital/lowercase letter are not significantly different.
CV = common vetch (*Vicia sativa* L.)
HV = hairy vetch (*Vicia villosa* Roth)
RC = red clover (*Trifolium pretense* L.)
Ndfa = nitrogen derived from the atmosphere
pNDFA = percent nitrogen derived from the atmosphere

5.4.1.2. Difference Method

Using the $NDFA_{diff}$ method, in 2013, at the time of the first sampling date, HV had fixed more N than either CV or RC, but was not significantly different than RC (Figure 6). By the second sampling date, CV and HV had fixed statistically similar amounts of N (95.9 kg $NDFA_{diff}$ ha^{-1} and 116.5 kg $NDFA_{diff}$ ha^{-1} respectively). By the second sampling date, RC had only fixed an additional 15.8 kg $NDFA_{diff}$ ha^{-1}. Across the entire season, HV fixed the most N (184.5 kg $NDFA_{diff}$ ha^{-1}) in the late fall (Figure 6).

By the third sampling date in the late fall of 2013, the oats had all senesced, as had the CV. At the third sampling date, CV had $NDFA_{diff}$ values (20.6 kg $NDFA_{diff}$ ha^{-1}) statistically similar to the first sampling date (-0.7 kg $NDFA_{diff}$ ha^{-1}).

In 2014, CV establishment was very poor, and it did not fix any atmospheric N, accumulating less biomass N than the combination of oats and weeds. Similarly, RC did not

fix any N at the first sampling date in mid-August, accumulating less N in its above-ground biomass than the surrounding weeds. By the second and third sampling date, RC had fixed 23.7 and 20.9 kg $NDFA_{diff}$ ha^{-1} respectively, although not statistically different from zero. This could be due to the RC not being inoculated in 2014 as outlined in Section 4. Hairy vetch derived well over 200 kg N ha^{-1} in the second and third sampling dates (258.1 and 246.3 kg $NDFA_{diff}$ ha^{-1} respectively).

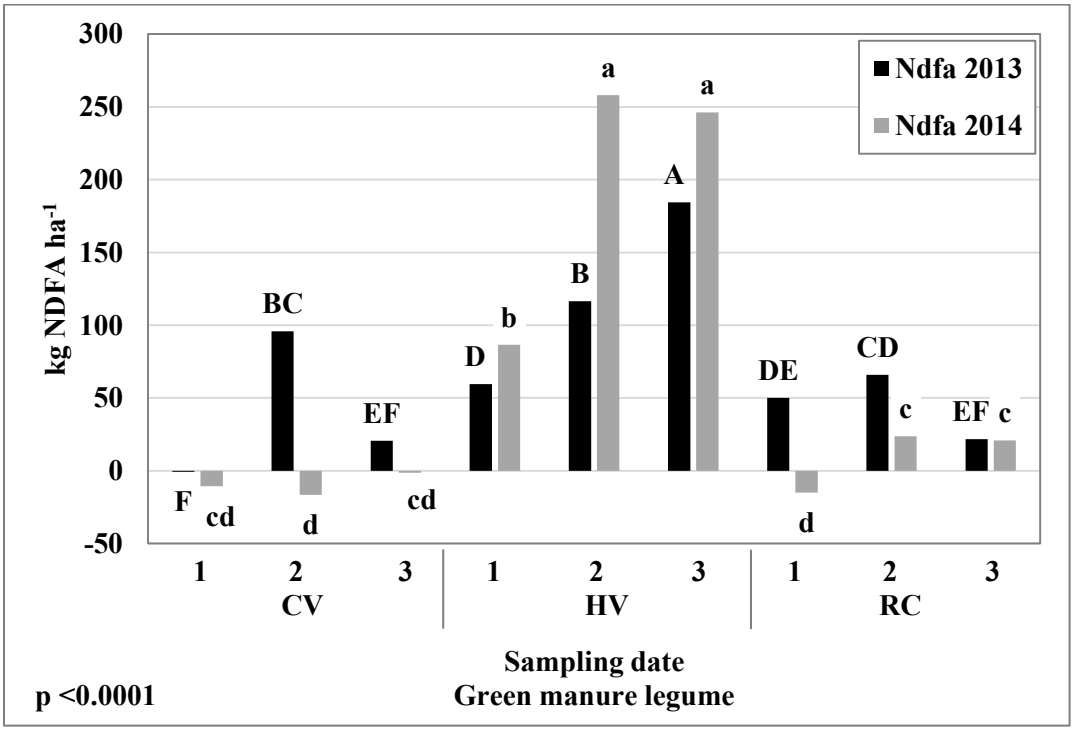

Figure 6. Estimates of nitrogen fixation in Nova Scotia using the difference method in 2013 and 2014.

Within each year, columns followed by the same capital/lowercase letter are not significantly different.
CV = common vetch (*Vicia sativa* L.)
HV = hairy vetch (*Vicia villosa* Roth)
RC = red clover (*Trifolium pretense* L.)
Ndfa = nitrogen derived from the atmosphere

5.4.2. Saint-Mathieu-de-Beloeil, Québec

5.4.2.1. Natural Abundance of 15N

In Québec, the range of δ15N for HV in 2013 was from 0.29 to -0.28‰ and between 2.37 and -0.36‰ in 2014. The range of δ15N for RC in 2013 was between -0.58 and -0.85‰ and between -048 and -1.00‰ in 2014. The oats in mixture used as a reference species ranged from -0.29 and 3.76‰ in 2013 and between 1.01 and 2.78‰ in 2014. To induce normality, two blocks containing extreme outliers were removed from both the 2013 and 2014 data sets.

In both 2013 and in 2014, the RC derived a significantly greater percentage of its N from the atmosphere (pNDFA 82.2% in 2013 and pNDFA 92.5% in 2014) compared to HV, which had 54.2% pNDFA in 2013 and 73.8% pNDFA in 2014 (Figure 7).

In 2014, the HVO and RCO were both sampled on August 12, 2014, and the HVO had significantly more $NDFA_{15N}$ (54.4 kg $NDFA_{15N}$ ha^{-1}) than the RCO, which had 10.2 kg $NDFA_{15N}$ ha^{-1}.

In 2013, RC was sampled on September 23, when 1964 $GDD_{4°C}$ had accumulated and in 2014, RC was sampled on August 12, when 1394 $GDD_{4°C}$ had accumulated, a difference of more than a month and 570 $GDD_{4°C}$. This difference could explain the difference of 47.6 kg $NDFA_{15N}$ ha^{-1} between 2013 and 2014 in the RC.

5.4.2.1. Difference Method

By the second and third sampling date in 2013, the HV contained more N then the oats+weeds. This suggests that, assuming the HV had equal access and was equally competitive as oats+weeds, the HV derived an additional 65.7 kg N ha^{-1} from the atmosphere at September 23, 2013, and 108.6 kg N ha^{-1} by October 28, 2013 (Figure 8).

Comparably, at peak $NDFA_{diff}$ on the third sampling date in 2014, HV had derived a 100.7 kg $NDFA_{diff}$ ha^{-1}; this was significantly greater than all other treatments.

The RC had only derived 13.7 kg $NDFA_{diff}$ ha^{-1} by October 14, 2014, the first time that the RC legume-N exceeded the combination of oat+weed-N. By comparison, the RC at the second sampling date in 2013 in mid-September, the RC had 17.6 kg $NDFA_{diff}$ ha^{-1}.

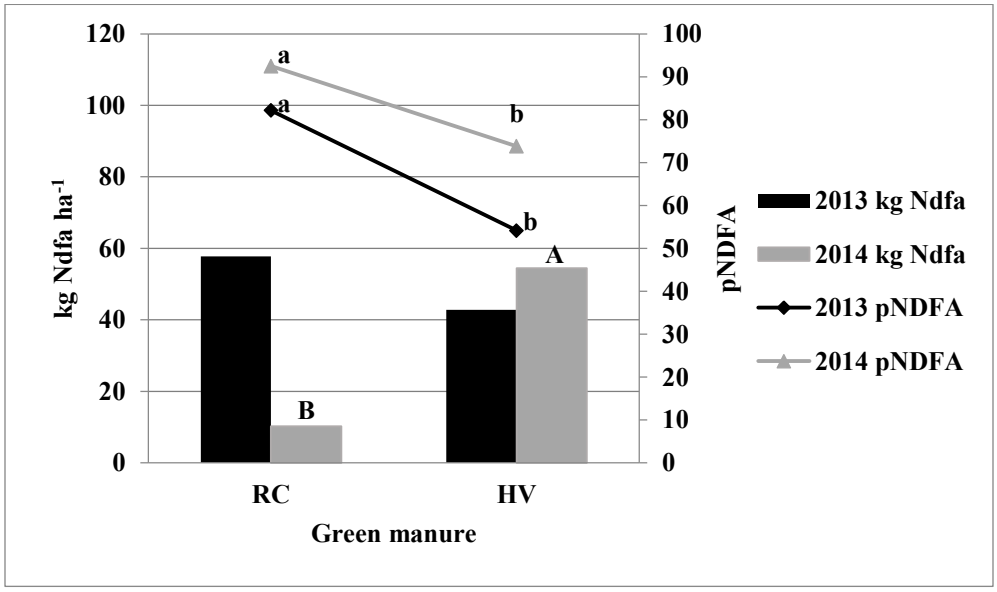

Figure 7. Nitrogen derived from the atmosphere estimates using isotopic methods in Québec in 2013 and 2014.

Within each year, values followed by the same capital/lowercase letter are not significantly different.
HV = hairy vetch (*Vicia villosa* Roth)
RC = red clover (*Trifolium pretense* L.)
Ndfa = nitrogen derived from the atmosphere
pNDFA = percent nitrogen derived from the atmosphere

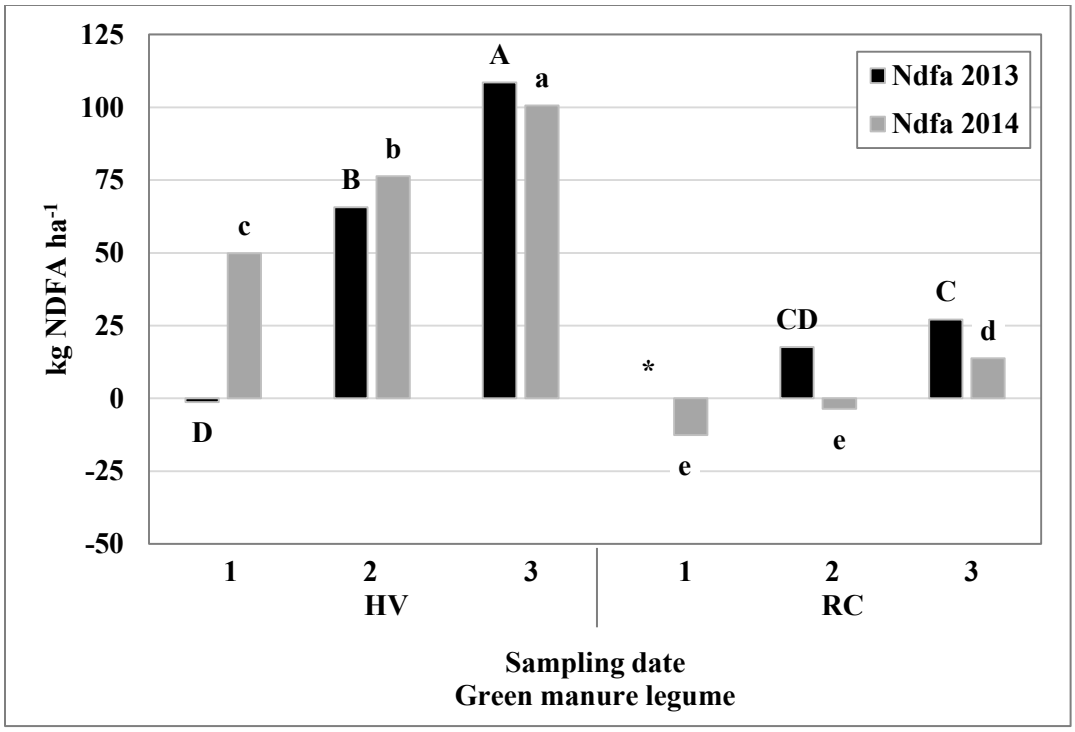

Figure 8. Estimates of nitrogen fixation in Québec using the difference method in 2013 and 2014.

Within each year values followed by the same capital/lower case letter are not significantly different.
Values replaced by an * denote a missing value.
HV = hairy vetch (*Vicia villosa* Roth)
RC = red clover (*Trifolium pretense* L.)
Ndfa = nitrogen derived from the atmosphere

5.5. Relationship between Growing Degree Days and Hairy Vetch Biomass, Nitrogen Accumulation, and Biologically Derived Nitrogen

Hairy vetch growth had a positive linear relationship to $GDD_{4°C}$ (Figure 9, Figure 10, and Figure 11). In general, HV growth parameters in Québec showed a closer linear relationship to $GDD_{4°C}$ compared to Nova Scotia.

The HV biomass of a HVO GrM in Québec in particular showed a close correlation with $GDD_{4°C}$, using sampling dates from both years (R^2 = 72.9). While Nova Scotia was significantly correlated with GDD4, it did not have as close a relationship (R^2 = 54.0) (Figure 9).

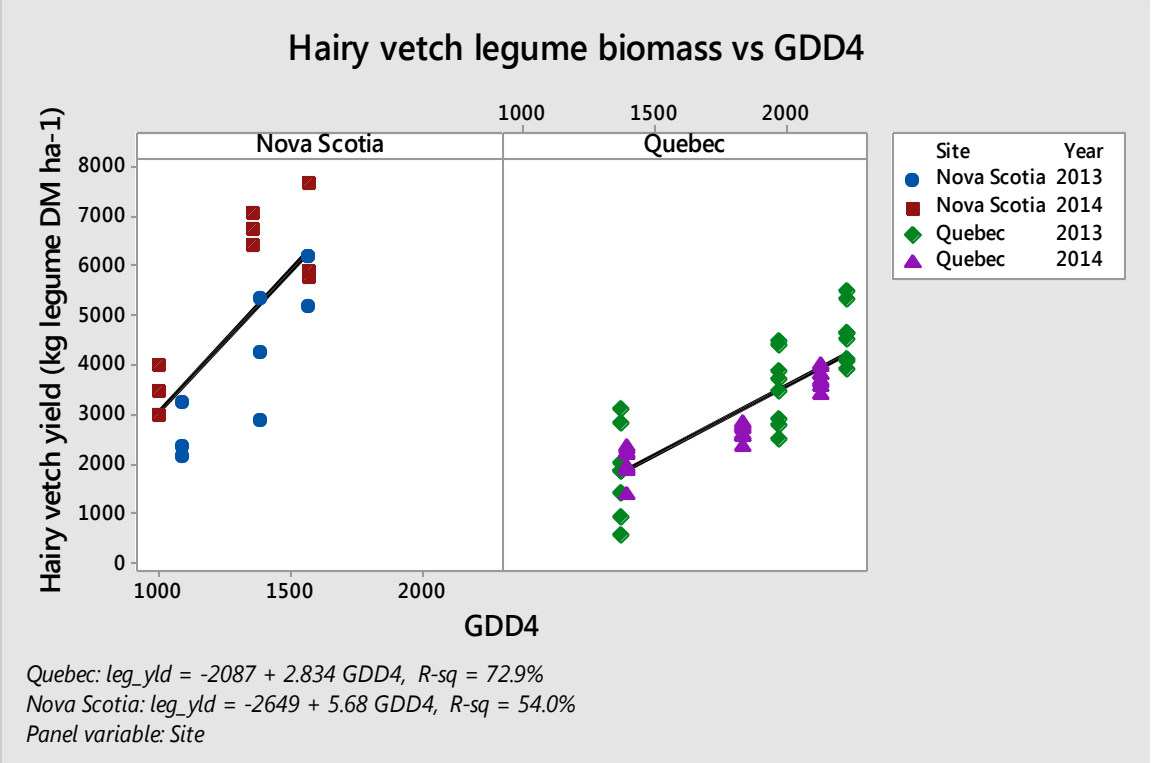

Figure 9. Hairy vetch legume biomass vs growing degree days (base 4°C) across six sampling dates over two years at two different locations.
DM = dry matter
GDD4 = growing degree days (base temperature 4°C)

Similarly, HV N accumulation was correlated to $GDD_{4°C}$, with Québec showing a closer correlation (R^2 = 57.3) than Nova Scotia (R^2 = 48.3) (Figure 10). The poorer correlation of both biomass and N accumulation in Nova Scotia is probably at least partially due to deer predation in 2014 on the third sampling date.

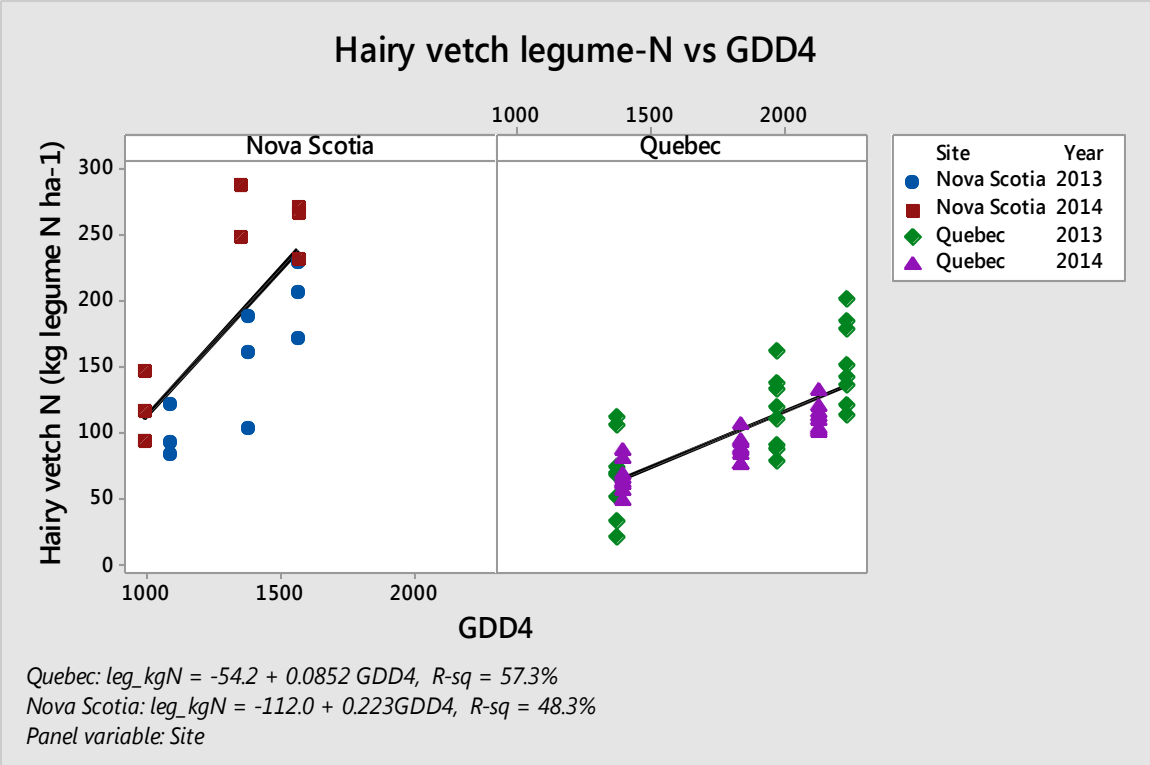

Figure 10. Hairy vetch legume nitrogen vs growing degree days (base 4°C) across six sampling dates over two years at two different locations.
GDD4 = growing degree days (base temperature 4°C)

Hairy vetch $NDFA_{diff}$ showed a positive linear relationship with GDD; R^2 in Québec was 57.3 and R^2 in Nova Scotia was 51.32. The rate of legume N and $NDFA_{diff}$ accumulation in HV in Québec was very similar, where legume N had a slope of 0.0852 and $NDFA_{diff}$ had a slope of 0.0892. In Nova Scotia legume N had a slope of 0.223 and $NDFA_{diff}$ had a slope of 0.261.

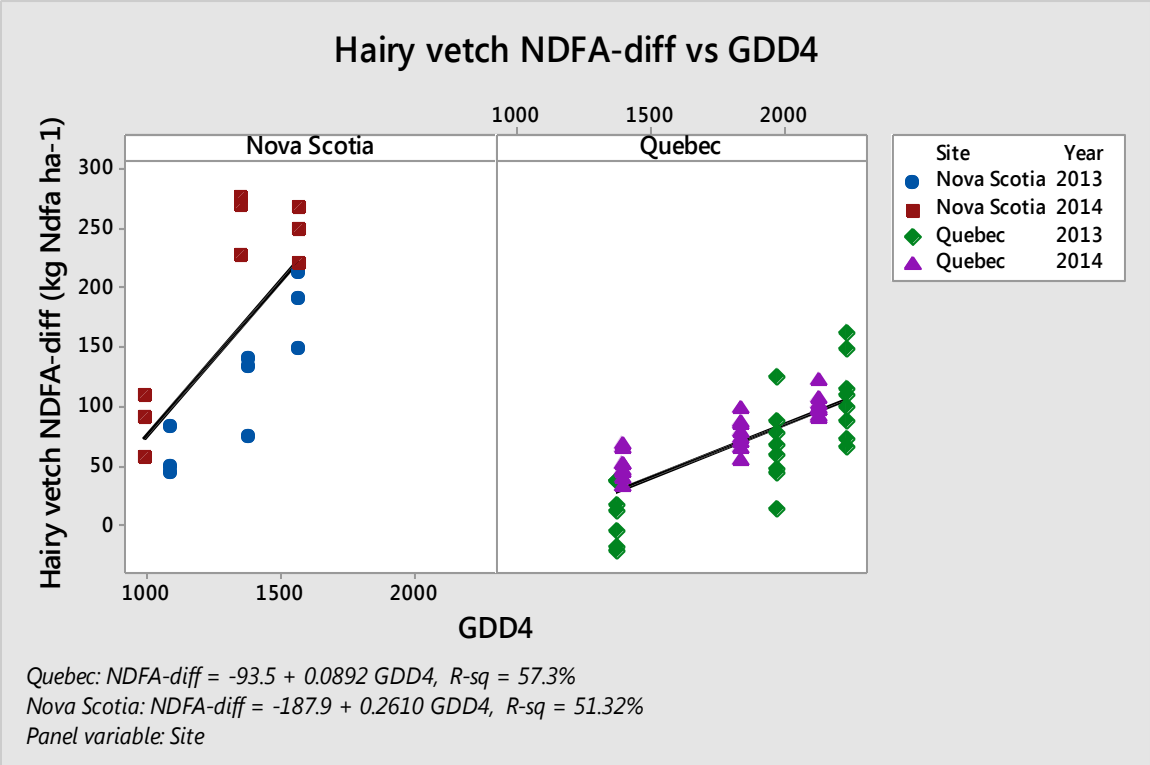

Figure 11. Hairy vetch nitrogen derived from the atmosphere (difference method) vs growing degree days (base 4°C) across six sampling dates over two years at two different locations.

GDD4 = growing degree days (base temperature 4°C)

NDFA-diff = nitrogen derived from the atmosphere calculated using the difference method

6. Discussion

The objectives of this experiment were to compare growth parameters of spring-planted GrM at two sites. Within each location by year, significant differences were found between GrM with respect to N_{dfa} as determined using both $NDFA_{15N}$ and $NDFA_{diff}$ methods (Objective 1). Within each location by year, significant differences were found between GrM in the amount of aboveground biomass and biomass-N accumulated, as well as significant differences in the timing of the greatest amounts of biomass and biomass-N (Objective 2). Aboveground biomass, biomass-N, and $NDFA_{diff}$ was found to be significantly correlated with $GDD_{4°C}$ at each location for HV, but not for RC or CV (Objective 3).

6.1. Green Manure Establishment and Biomass Production

In Nova Scotia, May of 2014 was cooler and drier than 2013, which could have impacted GrM establishment (Table 6). While not compared statistically, establishment in 2014 was poorer than in 2013. Further consideration is that the CV seed used in both years was from 2011, and even with adjustments made for germination, there was slightly lower establishment in 2014 (29 plants m^{-2} compared to 38 plants m^{-2}) (Table 8). While statistical analysis was not performed across years, the CV had observationally much less biomass in 2014 (1268 kg legume DM ha^{-1} at peak CV biomass in mid-September, Figure 1) compared to peak CV biomass in mid-September in 2013 (4281 kg legume DM ha^{-1}, Figure 1). In the CVO, oat biomass was much higher in 2014 (peak oat biomass on the first sampling date in mid-August, 4009 kg oat biomass ha^{-1}) than in 2013 (peak oat biomass on the second sampling date in mid-September, 2039 kg oat biomass ha^{-1}), most likely at least partially due to reduced competition for light and moisture from CV. A study performed on irrigated land in New South Wales Australia found that a monoculture of CV (*V. sativa,* called Blanchfleur vetch in the study) would amount to 3410-3570 kg of shoot DM ha^{-1} (Rochester and Peoples, 2005), within the range of what was observed in the biculture in Nova Scotia in 2013, but not in 2014. Mueller and Thorup-Kristensen (2001) in Denmark found that a monoculture of CV had between 1800-2800 kg DM ha^{-1} between planting in mid-July and harvesting in mid-November, which was closer to that obtained in the biculture in Nova Scotia in 2014.

While not compared statistically, observationally the HV in Nova Scotia established slightly better in 2013 (94 plants m^{-2}) than in 2014 (67 plants m^{-2}) (Table 8), again, possible due to a drier, cooler spring in 2014 (Table 6). However the HV had more biomass in 2014 (6746 kg legume DM ha^{-1} at peak biomass on the second sampling date, Figure 1) than in 2013 (5860 kg legume DM ha^{-1} at peak biomass on the third sampling date, Figure 1), despite 2014 being approximately 50 GDD cooler than 2013 and a drier year compared to the 30 year average. This suggests that HVO is able to compensate for a poor preliminary establishment. Indeed, in both years, HVO accumulated over 7,000 kg biomass ha^{-1}, and had little to no weeds present in the GrM, suggesting effective weed control through suppression.

The HVO biomass (and biomass-N) decreased by the third sampling date in 2014 in NS, contrary to the pattern observed at the same site in 2013 and to trends observed in Québec in both years. All GrM plots in Nova Scotia were surrounded by an electric deer fence soon after planting. However, in 2014, the deer fence was taken down just after the second sampling date, and the resulting deer predation could be the reason why HVO biomass and biomass-N decreased. In spite of this, the HVO outperformed the other two GrM in Nova Scotia in 2014.

The RC in Nova Scotia had better establishment in 2014 (201 plants m^{-2}) compared to 2013 (159 plants m^{-2}) (Table 8). However, the RC had much greater biomass in 2013 (3276 kg legume DM ha^{-1}, at peak biomass in mid-September at sample date 2, Figure 1) than in 2014 (1750 kg legume DM ha^{-1} at peak biomass at sample date 2 in mid-September, Figure 1). The lower biomass in 2014 is possibly due to the lack of RC inoculum in 2014 stunting RC growth, however, due to a site history of RC, it could also be assumed that there was sufficient *Rhizobium* in the soil. Another explanation for the reduced RC growth in 2014 could be because May 2014 was cool and dry, and June 2014 was very wet (Table 6).

Especially in an organic system, it is important to note that by October 29, 2013, 43% of the total biomass in the RC monocrop was weeds, 0% of the HVO GrM and less than 4% of the CVO GrM. This would most likely have significant bearings on the weed seed bank and in the subsequent crop, although that was not measured in this study.

In Nova Scotia in 2013, GrM of HVO had statistically similar amounts of biomass (7034 kg biomass ha^{-1} in late October) as the CVO (6430 kg biomass ha^{-1}) at peak biomass in mid-September (Figure 1). A GrM of RC at peak biomass in mid-September (4538 kg biomass ha^{-1}) accumulated significantly less aboveground biomass than the HVO and CVO at their respective peak biomasses.

In Nova Scotia in 2014, the HVO at peak biomass (8403 kg biomass ha^{-1} in mid-September) outperformed both CVO (peak total biomass of 5567 kg biomass ha^{-1}, including weeds, in

mid-August) and RC (peak total biomass of 4586 kg biomass ha^{-1}, including approximately 62% of that biomass as weeds, in mid-September) (Figure 1). At peak total biomass, CVO and RC were statistically similar in 2014. As May 2014 was cool and dry, and June 2014 was wet (Table 6), it is possible that the HV fared better than the CV in these conditions. The HV had better establishment than the CV in 2014 (Table 8). When looking at the performance of HV and CV within each year, the CV had much more legume biomass at peak legume biomass in September 2013 (4281 kg legume DM ha^{-1}, comparable to the HV at that same time with 4171 kg legume DM ha^{-1}) than in September 2014 (peak CV biomass 1268 kg legume DM ha^{-1} which was significantly less than the HV at the same time point with 6446 kg legume DM ha^{-1}) (Appendix A. Table 1, A. Table 2).

In Québec in 2013, oats were seeded with a Khun seed drill as outlined in Section 4.2.2, and the legumes were broadcast over. In 2014, legumes were placed in the Khun seed drill along with the oats. In 2013, there were 106 HV plants m^{-2}, and in 2014 there were 210 HV plants m^{-2}. It is likely that the differing seeding method was the cause of the improved establishment of the HV in 2014. This change in establishment, despite the same expected seeding rate, could suggest that the round-seeded HV settled in the seederbox of the drill, resulting in higher HV seeding rates. Interestingly, despite the improved establishment in 2014, by the last sampling date, the HVO treatment in Québec accumulated 7659 kg DM ha^{-1} in 2013 and 4769 kg DM ha^{-1} in 2014 (Figure 2). There could be many factors that caused this large reduction in biomass between the two years, not the least of which are climatic factors and soil moisture conditions during establishment and intraspecies competition. Most likely would be changes in oat establishment. Oat biomass was much less in 2014 (1439 kg ha^{-1} at peak oat biomass) compared to 2013 (5905 kg ha^{-1} at peak oat biomass). Hairy vetch is a twining plant and uses the oats as a support structure to climb up thereby improving photosynthetic access. It is possible that the reduced oat stand reduced the photosynthetic capacity of the HV in 2014, despite the HV having a greater density than in 2013.

Similarly, the change in seeding method most likely affected the RC population. In 2013, there were 626 RC plants m^{-2} and in 2014, there were only 435 RC plants m^{-2}. Mixing the RC into same seederbox as the oats would have resulted in the RC being drilled to the same

depth as the oats. This would result in lower RC establishment as, due to its smaller seed size, RC would not have the energy reserves to emerge if buried too deep. The RCO reached maximum biomass accumulation in both 2013 and 2014 at the second sampling date in mid- to late-September, accumulating 5822 kg DM ha^{-1} in 2013, and 3282 kg DM ha^{-1} in 2014. The RC in Québec accumulated 1091 kg legume DM ha^{-1} in 2014 at peak biomass in mid-September (sample date 2) and 2091 kg legume DM ha^{-1} at peak biomass in late October 2013 (sample date 3). The reduced biomass in 2014 could be due to the lack of inoculum used or due to the weather, as June 2014 was very wet with 172 mm of rain vs. the 30 year average of 87 mm of precipitation for June (Table 7).

In Québec in 2013, the interaction between GrM and sampling date was not statistically significant. A GrM of HVO had statistically more biomass than RCO across all sample dates (Figure 2). This could be because of the growth habit of the two legumes: HV with its twining growth habit is better able to maximize on photosynthetic resources, whereas the RC in biculture with oats is shaded for the majority of the growing season. Peak legume biomass of the HV was significantly greater (4611 kg legume DM ha^{-1}) than the peak legume biomass of RC (2091 kg legume DM ha^{-1}).

In Québec in 2014, a GrM of HVO accumulated significantly more biomass at peak biomass (4769 kg biomass ha^{-1} in mid-October) than RCO at peak biomass (2954 kg biomass ha^{-1}) in mid-September (Figure 2). At peak legume biomass, HV had significantly more legume biomass (3727 kg legume biomass ha^{-1} in mid-October) than RC (1091 kg legume biomass ha^{-1}) at peak legume biomass in mid-September. The reduction in legume biomass from 2013 to 2014 was likely due to the wet June in 2014 (Table 7).

The HV biomass attained at both sites and across both years in this study had comparable variability and productivity (3392-7697 kg DM ha^{-1}, average 4712 $\pm$ 1123 kg DM ha^{-1}) to that found by others, ranging from 0 – 6256 ($\pm$ 1585) kg DM ha^{-1} (Table 1) (Sarrantonio and Scott, 1988; Jannink et al., 1997; Drinkwater et al., 2000; Mueller and Thorup-Kristensen, 2001; Teasdale et al., 2004; Brainard et al., 2012). This variability could be a barrier to the adoption of HV as a GrM in Eastern Canada.

As both Québec and Nova Scotia sites had more HV biomass when there was a lower HV population, further studies evaluating whether a lower seeding rate results in greater biomass production are warranted. As HV seed is very expensive, this would make HV as a GrM more appealing to producers.

6.2. Nitrogen Accumulation in Green Manures

In both years in both locations, at peak biomass-N, HVO accumulated significantly more aboveground biomass-N than the other treatments, ranging from 123 to 292 kg N ha^{-1} (Figure 3, Figure 4). A GrM of HVO accumulated a total of 221 kg N ha^{-1} by late October in 2013 and 292 kg N ha^{-1} in mid-September in 2014 in Nova Scotia (Figure 3). In Québec, the HVO accumulated a total of 200 kg N ha^{-1} in 2013 and 123 kg N ha^{-1} in 2014, and peak N accumulation of the system occurred on the third sampling date in mid- to late-October (Figure 4). The majority of N in both cases was in the HV biomass, and very little to none was in the weed biomass by the third sample date, suggesting that HV would be a good GrM for supressing weeds. These values are comparable, if not superior, to that found by others with fall-seeded HV, for example: 92.1 kg N ha^{-1} in one year and 246 kg N ha^{-1} in a second year (Sarrantonio and Scott, 1988), 140-220 kg N ha^{-1} with between site and year variations within that range (Drinkwater et al., 2000), between 18 and 191 kg N ha^{-1} depending on planting date, location, and cultivar (Teasdale et al., 2004), 80-150 kg N ha^{-1} (Brainard et al., 2012), ranging from 0-92 kg N ha^{-1} depending on year, soil type and mono/bi-culture (Jannink et al., 1997), and 114-149 kg N ha^{-1} (Mueller and Thorup-Kristensen, 2001) (Table 1). In the drier climate of Manitoba, spring-planted HV-barley that was then rolled in late summer accumulated over 300 kg N ha^{-1} by early fall at two sites (Halde et al., 2014).

At peak N accumulation in mid-September, CVO in Nova Scotia accumulated 146 kg N ha^{-1} in 2013 and 88 kg N ha^{-1} in 2014 (Figure 3). The CV alone had 118 kg N ha^{-1} in the legume biomass in 2013, but only 36 kg N ha^{-1} in the legume biomass in 2014, most likely due to poor CV establishment as discussed in the previous section. The GrM of CVO in both years of this study were comparable to the findings of Rochester and Peoples (2005) in Australia,

where a monocrop of CV resulted in 91-102 kg shoot N ha^{-1}, and to Mueller and Thorup-Kristensen (2001) in Denmark, where a monocrop of CV resulted in 80-110 kg shoot N ha^{-1}.

At peak N accumulation in mid-September in NS, a monocrop of RC had accumulated 88 kg N ha^{-1} in 2013, and 62 kg N ha^{-1} in 2014. However, if the N accumulation of the weeds were included in the system, the GrM of RC in 2013 accumulated 110 kg N ha^{-1} (which includes 22 kg N ha^{-1} from the weeds, data not shown), and 101 kg N ha^{-1} in 2014 (which includes 39 kg N ha^{-1} from the weeds, data not shown) (Figure 3). For comparison, a two year old timothy (*Phleum pratense* L.)-RC biculture in Nova Scotia accumulated a total of 140.2 kg N ha^{-1} in a two cut system (June and September) and a three year old timothy-clover biculture accumulated a total of 110.2 kg N ha^{-1} in a two cut system (June and September) (Lynch et al., 2004).

Red clover-oats in Québec, in 2013, at peak N accumulation in late October, performed similarly to the Nova Scotia RC monocrop, accumulating 64.8 kg N ha^{-1} in the RC biomass, with an additional 25.6 kg in the oat biomass, and, including weeds, totaled 102 kg N ha^{-1}. The quantity of N accumulated in Eastern Canada was superior to that found by Rochester and Peoples (2005), where only 46 kg N ha^{-1} were found in the RC aboveground biomass, although this was comparable to what was found in Québec in 2014, where only a total of 51.4 kg N ha^{-1} accumulated in a RCO GrM. Total GrM-N in Nova Scotia and Québec fell on the lower edge of the range of 69-373 kg N_{dfa} ha^{-1}, which can be found in a review of the literature (Peoples et al., 1995).

6.3. Estimates of Nitrogen Fixation Using Natural Abundance of ^{15}N

The reference plants in Nova Scotia ranged in isotope signature from 3.11 to 12.48‰ and had an average of 6.57‰, which is within the range found by Stevenson and Cole (1999) and Unkovich et al. (1994) (4-17‰) for cropped soils. This suggests that the reference plants were reflective of the $\delta^{15}N$ of cropped soils. However, if the difference between the $\delta^{15}N$ of the legumes and the reference plants is less than the natural variation of the

reference plants $\delta^{15}N$, error may be introduced into the $\delta^{15}N$ method (Shearer and Kohl, 1986).

The $\delta^{15}N$ values for HV in Québec (0.18‰) and for Nova Scotia (0.03 ‰) were on par with the variability found in other sources, 0.05-0.42‰ (Rochester and Peoples, 2005), -0.62-1.71‰ (Parr et al., 2011), and 0.4‰ using isotopic dilution method (Mueller and Thorup-Kristensen, 2001). In irrigated cropping regions of eastern Australia, this resulted in 142-265 kg $NDFA_{15N}$ ha^{-1}, depending on the year and HV cultivar (Rochester and Peoples, 2005). This is much greater than the 42.8-54.4 kg $NDFA_{15N}$ ha^{-1} (Québec, Figure 7) and the 91.4-106.2 kg $NDFA_{15N}$ ha^{-1} (Nova Scotia, Figure 5) recorded in this study. However, this range was somewhat more on par with that found by Parr et al. (2011) of 59-174 kg $NDFA_{15N}$ ha^{-1}, depending on cultivar and year. In Michigan, fall-seeded HV in mixture with rye had $NDFA_{15N}$ values more closely comparable to the ones measured in this study, ranging from 22-62 kg $NDFA_{15N}$ ha^{-1} dependent upon cultivar and year (Brainard et al., 2012). Similarly, in Funen, Denmark, a mid- to late-summer seeded HV monocrop had approximately 80 kg $NDFA_{15N}$ ha^{-1} in one year, and approximately 149 kg $NDFA_{15N}$ ha^{-1} the next year (Mueller and Thorup-Kristensen, 2001).

Red clover has been found to derive 61-96% of total plant N from the atmosphere using ^{15}N enrichment (Warembourg et al., 1997), although in a review of other studies, Peoples et al. (1995) found that the proportion of N fixed was 35-87%. These numbers are similar to the ones in the study where 92.5% and 95.3% $pNDFA_{15N}$ were found in Nova Scotia (Figure 5), and in Québec, 82.2% and 92.5% of $pNDFA_{15N}$ (Figure 7). The $\delta^{15}N$ values for RC in Québec (-0.71‰) and for Nova Scotia (-0.26‰) were different from those documented by Rochester and Peoples (2005) (-0.01‰). In irrigated cropping regions of eastern Australia, this resulted in 68 kg $NDFA_{15N}$ ha^{-1} (Rochester and Peoples, 2005). This is comparable to the 2013 years in Québec and Nova Scotia when the RC was inoculated, resulting in 57.8 kg $NDFA_{15N}$ ha^{-1} and 68.5 kg $NDFA_{15N}$ ha^{-1} respectively (Figure 5, Figure 7). When the RC was not inoculated in 2014, there was only 10.2 kg $NDFA_{15N}$ ha^{-1} (Québec) and 17.9 kg $NDFA_{15N}$ ha^{-1} (Nova Scotia).

The $\delta^{15}N$ values for CV in Nova Scotia (-0.12 ‰) were different from those found by Rochester and Peoples (2005) (0.44-1.83‰) and by Mueller and Thorup-Kristensen (2001) (approximately 0.42‰ using the isotopic dilution method), but in the range found by Parr et al. (2011) (-0.74-0.83‰) in North Carolina. In Australia, this resulted in 134-142 kg $NDFA_{15N}$ ha^{-1} (Rochester and Peoples, 2005), and in North Carolina, in 57-125 $NDFA_{15N}$ ha^{-1}, between two and five times as much as recorded in Nova Scotia (25.1-31.7 kg $NDFA_{15N}$ ha^{-1}). The results found in Nova Scotia were also lower than the results found in Funen, Denmark where CV had 45 kg $NDFA_{15N}$ in one year, and 85 kg $NDFA_{15N}$ ha^{-1} in a second year, and lower than that reported by Peoples et al. (2005) of 106 kg N_{dfa} ha^{-1} (methodology not specified). This could be because the older leaves, which are richer in ^{15}N had already senesced by the time of sampling (Mueller and Thorup-Kristensen, 2001).

In Nova Scotia, the RC in 2014 had less $NDFA_{15N}$ (17.9 kg $NDFA_{15N}$ ha^{-1}) than in 2013 (68.5 kg $NDFA_{15N}$ ha^{-1}) (Figure 5), which could be due to less vigorous growth in 2014. In 2013, the RC at the first sampling date (September 4, 2013, 1393 $GDD_{4°C}$) had accumulated 2805 kg legume DM ha^{-1} (Figure 1), but in 2014, at the first sampling date of August 13, 2014 (1047 $GDD_{4°C}$), the RC had only 621 kg legume DM ha^{-1} (Figure 1). This reduced growth and therefore $NDFA_{15N}$ could be because the RC was not inoculated in 2014 due to the inability to obtain organic RC inoculum as mentioned in Section 4.

In Québec in 2013, RC had 57.8 kg $NDFA_{15N}$ ha^{-1}, statistically similar to HV, which had 42.8 kg $NDFA_{15N}$ ha^{-1} (Figure 7). However, the RCO sampling date used for $NDFA_{15N}$ was September 23, 2013 and the HVO sampling date was August 9, 2013 (Table 4). The RC had more time and 437 more $GDD_{4°C}$ to accumulate more legume-N by the September 23 sampling date (Table 4). Had the same sampling dates been used to measure $NDFA_{15N}$, it is probable that the HV would have had a greater amount of measured $NDFA_{15N}$ in 2013.

In Québec, there are many probable causes for a lower $NDFA_{15N}$ for RC in 2014 compared to 2013. As was previously mentioned, RC in 2014 had fewer GDD to accumulate legume biomass and to fix N. Additionally, the RC in 2014 was not inoculated due to the inability to find organic RC inoculum as outlined above. Red clover also had a lower population in 2014

(435 plants m^{-2}) compared to 2013 (626 plants m^{-2}) (Table 9), which could also explain why RC had much lower $NDFA_{15N}$ in the second year of the study.

Agriculture soil that is annually cropped has been documented to be 8.34 $\pm$ 1.55 $\delta^{15}N$ units with respect to $\delta^{15}N$ in the atmosphere (Shearer et al., 1978), and all soil is usually in the range of +5 to +12 $\delta^{15}N$ units (Stevenson and Cole, 1999). The oats from Québec had an average of 1.28‰, and a range from -0.89 to 3.76‰. While there is certainly variation from soil to soil, the low values observed in Québec suggest either a significant amount of transfer of N from the legumes to the oats or improper sampling and processing techniques. As a result of some of the extreme outliers observed in the reference plants in Québec, in both years two blocks had to be removed due to extreme values affecting normality.

6.4. Estimating Nitrogen Fixation Using the Difference Method

Calculating $NDFA_{diff}$ for Nova Scotia resulted in several negative values, particularly in 2014 (Figure 6). This could suggest that there was an abundance of soil-N resources in 2014 and that the legumes did not have to result to the more energetically-demanding method of biological N fixation. Negative values also suggest that the legumes are less competitive for soil-N than the oats or weeds since the oats and weeds were able to take up more soil N than the legumes. It was found that at 10°C, HV will biologically derive greater amounts of N from the atmosphere rather than utilizing soil N sources compared to temperatures of 20°C and 30°C (Power and Zachariassen, 1993).

The change in the amount of $NDFA_{diff}$ in Nova Scotia between the second and third sampling dates for CV in 2013 (95.9 and 20.6 kg $NDFA_{diff}$ ha^{-1}) suggest that perhaps this methodology should not be used on dead plant material. The CV started to lose N from its biomass much more quickly, going from 118 kg legume-N ha^{-1} on the second sampling date to 46 kg legume-N ha^{-1} on the third sampling date, a 61% reduction, than the oat-N, which went from 25.6 kg oat-N ha^{-1} to 22.6 oat-N ha^{-1} (Figure 6). This resulted in an artificially low estimate of $NDFA_{diff}$ based on the dead plant material in the third sampling date. Values in Nova Scotia of $NDFA_{diff}$ for CV ranged from -17-96 kg $NDFA_{diff}$ ha^{-1}. The higher end of

the spectrum was in order with what was found in a monocrop of CV in Denmark, which had 30-85 kg $NDFA_{diff}$ ha^{-1} (Mueller and Thorup-Kristensen, 2001).

Across both years and sample dates, values in Nova Scotia of $NDFA_{diff}$ for HV ranged from 60-258 kg $NDFA_{diff}$ ha^{-1} and from -1.2-109 kg $NDFA_{diff}$ ha^{-1} in Québec. For comparison, a HV monocrop in Denmark across years had approximately 80-150 kg $NDFA_{diff}$ ha^{-1} (Mueller and Thorup-Kristensen, 2001).

By October 28, 2013 in Québec, RC had 27.1 kg $NDFA_{diff}$ ha^{-1}, a gain in 9.5 kg $NDFA_{diff}$ ha^{-1} from the second sampling date on September 23, even though total legume-N only went up 4.8 kg legume-N ha^{-1} to 64.8 kg legume-N ha^{-1} (Figure 8). This would suggest that as the oats and weeds senesced and started to decompose, they lost N from their aboveground biomass, resulting in an inflated estimate of $NDFA_{diff}$.

The low $NDFA_{diff}$ values for RC, especially in 2014, could also suggest that the RC was unable to effectively tap into soil N sources, but based on the low RC biomass at the third sampling date (1035 kg DM_{legume} ha^{-1} in 2014, Figure 2) and the legume N content (28.8 kg legume-N ha^{-1} in 2014, Figure 4), it is most likely a result of the unharvested oats shading and limiting RC production. Poor RC $NDFA_{diff}$ in 2014 is also likely due to the RC not having been inoculated.

6.5. Comparisons and Conclusions in Using Different Methods to Determine Nitrogen Derived From the Atmosphere

Both of the methods used to calculate N_{dfa} in this study have significant flaws. As the reference plants in this study were grown in conjunction with the legumes, the bi-directional transfer of N between the legume and the reference plants most likely occurred, making the methods less sensitive than they could be otherwise be (Høgh-Jensen and Schjoerring, 2000). It has been reported that RC will transfer between 14 and 42 kg N ha^{-1}, and of that 4-25% is N_{dfa}, and 47-48% will appear as grass-N in a forage system (Peoples et al., 1995). Bi-directional transfer of N can happen through N excretion by the legume into the rhizosphere,

the decomposition of below-ground plant material during legume growth, direct interconnection via mycorrhizal fungi of the reference plant and the legume, compounds leached from the above-ground herbage during rainfall, decomposition of legume leaf litter at the soil surface, and so forth (Peoples et al., 1995). Wheat has been shown to rhizodeposit N amounting to between 18 and 33% of total N yield of the wheat plant (Janzen, 1990).

Both methods for calculating N_{dfa} in this study also use reference species which are not ideally suited for the purpose. For example, the rooting patterns of vetches are quite different from RC, which are again different from oats. This means that each species is accessing different spatial pools of N. Additionally the N uptake pattern of the legumes and the references species are quite different meaning that each species is accessing different temporal pools of N. And finally, the growth potential of the legumes and the reference species are quite different. Oats, CV, and most weeds are determinant plants, achieving maximum growth and then they senesce. Spring-planted HV appears to be an indeterminant plant that will continue to accumulate biomass and N, and RC is a perennial. In both cases, using a determinant as a reference species is based on faulty assumptions. Despite this, both methods of determining N_{dfa} do reveal interesting data, however, this data should be interpreted with full recognition of the limitations of the methodology.

Mueller and Thorup-Kristensen (2001) did not find any significant differences between estimates of N_{dfa} made using $NDFA_{15N}$ and $NDFA_{diff}$, which was not found to be true in this study. When compared to the isotopic method of determining N_{dfa}, the $NDFA_{diff}$ method was a more conservative estimate in both years (in Nova Scotia: Figure 5, Figure 7; in Québec Figure 6, Figure 8).

The variability of measurements in Nova Scotia were slightly higher with the $NDFA_{diff}$ method. The STE in 2013 for kg $NDFA_{15N}$ ha^{-1} was 10.23, and in 2014 was 8.41 (data not shown). By comparison, the STE in 2013 for kg $NDFA_{diff}$ ha^{-1} was 11.65 and in 2014 was 11.66 (data not shown).

In 2013, similar to in Nova Scotia, the variability was higher in the $NDFA_{diff}$ method in Québec (STE = 9.26) compared to the variability using the $NDFA_{15N}$ method (STE = 6.76) (data not shown). In 2014, the variability between the two measurements was much more similar, with the variability in the isotopic method (STE = 4.56) being slightly higher than that of the difference method (STE = 4.34).

In Mueller and Thorup-Kristensen (2001), only Italian ryegrass (*Lolium multiflorum* Lam.) was used as the reference species, as it was determined that it was the most suitable to calculating both $NDFA_{15N}$ and $NDFA_{diff}$ for HV, crimson clover (*Trifolium incarnatum* L.), Persian clover (*Trifolim resupinatum* L.), CV, and Egyptian clover (*Trifolium alexandrinum* L.). Italian ryegrass has a similar root growth pattern to leguminous plants in the autumn, which is when the study took place. Additionally, the rooting depth and sub-soil mineral N uptake between leguminous species and Italian ryegrass are similar. Other potential reference species were ruled out as they undergo senescence and lose their ^{15}N rich older leaves much more quickly than Italian ryegrass (Mueller and Thorup-Kristensen, 2001).

When Mueller and Thorup-Kristensen (2001) swapped out Italian ryegrass for other reference species, they found very different estimates of N_{dfa}. When using the isotope dilution method, it was found that rye resulted in 25-49% lower estimates and winter rape resulted in 13-23% lower estimates than when Italian ryegrass was used. When $NDFA_{diff}$ was calculated using rye, the estimate was 6-23% higher, and when calculated using winter rape, it was 5-13% higher compared to Italian ryegrass (Mueller and Thorup-Kristensen, 2001). In this study, it is possible that further consideration should have been given to reference species selection.

6.6. Relationship between Growing Degree Days and Hairy Vetch Biomass and Nitrogen Accumulation

When HV is seeded in the late-summer or early fall, Teasdale et al (2004) found that 1095 – 1398 $GDD_{4°C}$ accumulated between planting and 50% flowering the following spring for variety-not-stated HV seed, and 937 – 1161 $GDD_{4°C}$ for the early-flowering variety AU

Early Cover. In this study, at both locations, the spring-planted HV started to flower in mid to late July, when less than 1000 $GDD_{4°C}$ had accumulated, and 50% flowering, as determined by the protocol outlined by Teasdale et al (2004) and Mischler et al (2010), was never reached due to HV being an indeterminate plant.

Between 50% HV flowering and 50% senescence, Teasdale et al (2004) calculated a mean of 475 $GDD_{4°C}$, with little variability between cultivar and planting date. However the same study also noted that temperatures reached 30°C during that period so the stress may have also caused senescence. Varying with cultivar and planting date, the total $GDD_{4°C}$ accumulated at 50% senescence was between 1412 and 1959 (Teasdale et al., 2004). At the third sampling date in Québec between 2126 and 2227 $GDD_{4°C}$ had accumulated, and in Nova Scotia between 1569 and 1571 $GDD_{4°C}$, and the HV showed no signs of senescence, probably due to the lack of vernalization. Based on the trend observed in Nova Scotia in 2013 and in Québec in 2013 and 2014, it is possible that even more biomass could be accumulated in spring-planted HV GrM with an earlier planting, provided that the soil temperatures were warm enough to promote germination.

Teasdale et al (2004) calculated a regression model for biomass production on fall-planted HV:

$$\text{Biomass (g m}^{-2}) = 24.0 + 0.406\ (GDD_{4°C}) \qquad R^2 = 0.564$$

In Pennsylvania, a linear regression model was also calculated for fall-planted HV, however base 10°C was used to calculate GDD (Cook et al., 2010):

$$\text{Biomass (Mg ha}^{-1}) = -4.7 + 0.006\ (GDD_{10°C}) \qquad R^2 = 0.82$$

In Nova Scotia, the linear regression model calculated across both years for spring-planted HV was:

$$\text{Biomass (kg ha}^{-1}) = -2649 + 5.68\ (GDD_{4°C}) \qquad R^2 = 0.540$$

And in Québec, the linear regression model calculated across both years for spring-planted HV was:

$$\text{Biomass (kg ha}^{-1}) = -2087 + 2.834\ (GDD_{4°C}) \qquad R^2 = 0.729$$

Using the Teasdale model, it was estimated that to accumulate 4000 kg HV biomass ha^{-1}, 926 $GDD_{4°C}$ were needed. Using the regression models calculated for this study for spring-planted HV, 2148 $GDD_{4°C}$ was needed to accumulate in Québec to achieve 4000 kg biomass ha^{-1}, and 1171 $GDD_{4°C}$ in Nova Scotia. Similarly, with the Teasdale et al. (2004) model, 926 GDD resulted in 120-144 kg N ha^{-1}. By comparison, Québec would need 2045 to 2326 $GDD_{4°C}$ to accumulate similar amounts of legume N, and Nova Scotia would need 1040 to 1148 $GDD_{4°C}$.

It is interesting to note that both of the models developed for Eastern Canada in this study have a y-intercept of approximately negative 2000 kg ha^{-1}. This could be due to the Eastern Canada models being for spring-planted HV, in comparison with the models from Pennsylvania and the mid-Atlantic US which were for fall-planted HV.

While the models found in this study and others (Teasdale et al., 2004; Cook et al., 2010) are quite different and therefore suggest that one model cannot be used to predict HV growth, the positive linear trend between HV biomass and GDD does suggest that providing HV with the longest growing season will result in the greatest biomass accumulation, so long as temperatures do not exceed 30°C.

There was no linear relationship detected between $GDD_{4°C}$ and CV growth parameters, and a very weak relationship between $GDD_{4°C}$ and RC (data not shown). It is important to note that the second and third sampling dates for CV were after the CV had already started to senesce, so using GDD as a predictor for growth and N dynamics was inaccurate. A base temperature of 4°C was used to calculate GDD for CV and RC, however, this is not necessarily the appropriate base temperature for these legumes. Identifying the appropriate base temperatures for CV and RC was outside of the scope of this study. However, were the

appropriate base temperature identified, there could be a relationship between the early season CV growth and GDD and RC growth and GDD.

6.7. Management Implications of Various Green Manures

Studies have found that at 10°C, HV will have set seed by 105 days after establishment (630 GDD), and earlier at higher temperatures (Power and Zachariassen, 1993). In Nova Scotia and Québec, HV had already set seed by the second and third sampling dates. It would be expected that these seeds would contribute to the weed seed bank, although that was not examined in this study, and there was minimal volunteer HV in the following HRSW crop (data not shown). Oats had similarly set seed, and it would not be unexpected to see volunteer oats in the subsequent crop. However, it was noticed that many of the oat seeds had already germinated by the October sampling date, which would then winter kill, eliminating them as a cause for concern in the subsequent cash crop.

Although HVO consistently produced large amounts of biomass with very little weed biomass, in some GrM systems, weed management measures must be taken in order to make this a viable option for organic farmers. However, the shear amount of HVO biomass that was incorporated in the fall necessitated several passes with a disk in Nova Scotia, perhaps eliminating its appeal as a GrM in some operations with smaller farm machinery.

In both years in Québec, the RCO GrM had statistically similar amounts of N in the aboveground biomass on the September and October sampling dates (Figure 4). However, in 2014, the amount of N contributed by the weed biomass doubled from 5 kg N ha^{-1} to 10.8 kg N ha^{-1} and the weed biomass went from 310 kg weed DM ha^{-1} in September to 503 kg weed DM ha^{-1} in October (data not shown). This amount of weed growth bodes ill for organic management in the following year. Solely considering the growth and N contributions of the above-ground biomass, this could suggest that a GrM of RCO does not necessarily have to be grown for five to six months, but instead a four to four and a half month season may suffice.

A shorter growing period might also benefit a GrM of monocropped RC. According to the data collected in this trial in Nova Scotia, a GrM of a RC monocrop reached peak N accumulation by the second sampling date. This could suggest that devoting a full season to a GrM of RCO or a RC monocrop might not be within best management practices, but that there is instead a window for a short season crop prior to planting the GrM or for the seeding of a late season crop after the GrM in September. This bears further study, as photoperiod may have a greater effect on RC growth than GDD, similarly nutrient cycling and moisture availability at different times in the growing season may also have an impact. Furthermore, whether the N in the above-ground biomass is representative of the amount of total N in the system is in question. Some estimates have placed root-N contributions between 6-20% of total GrM-N (Sarrantonio and Scott, 1988; Brainard et al., 2012).

In the following chapter, the amount of N contributed to the system by these GrM will be quantified through measurements of N mineralization and plant N uptake, giving a clearer picture of the N dynamics of these GrM. Whether the amount of N contributed to the subsequent crop is enough to off-set a lost year of production with saved fertilizer costs will be determined in subsequent chapters.

7. Conclusions

Spring-planted full-season GrM crops can accumulate a significant amount of N over the course of a growing season (Objective 2). However, the accumulation of N is not linear for all GrM examined in this study (Hypothesis 1). Peak biomass and N accumulation was reached at the first or second sampling date in September for RC and CVO in Nova Scotia. A GrM of HVO followed a more positively linear pattern, with greater aboveground biomass and aboveground biomass-N accumulating as the growing season progressed.

Growing degree days using a base temperature of 4°C is an effective indicator of HV growth, N accumulation, and N_{dfa} (Objective 3, Hypothesis 3). However models developed may not be a good predictor of growth due to the variability between locations.

Using the $NDFA_{diff}$ method HV was the most successful at deriving N from the atmosphere at both locations, accumulating greater amounts of N_{DFA} ha^{-1} than the other GrM trialed. Using the $NDFA_{15N}$ method, HV accumulated greater quantities of N_{DFA} ha^{-1} than RC in years when the RC was not inoculated (2014), but when RC was inoculated (2013), HV and RC performed statistically similarly in both years. Red clover in a biculture with oats derived a greater percent of biomass-N from atmospheric sources than the HV in Québec in both years. In Nova Scotia, in one year, all legumes performed statistically similarly, and in the second year, RC derived a greater percent of its N from the atmosphere than HV (Objective 1, Hypothesis 2).

Chapter 3: Soil Nitrogen Mineralization Dynamics of Novel Green Manures at Different Incorporation Timings and the Response of a Subsequent Spring Wheat Crop

1. Introduction

As discussed in the literature review and shown in Chapter 2, the year-to-year variation in hairy vetch (*Vicia villosa* Roth) (HV) biomass and nitrogen (N) accumulation can be quite high. The fact that HV biomass production can be easily tracked through monitoring growing degree days (base 4°C) ($GDD_{4°C}$) for either a fall-planted (Teasdale et al., 2004) or spring-planted green manure (GrM) of HV allows estimation of the N content of a HV GrM. However, managing the subsequent N dynamics of the GrM after termination can be more difficult. Predicting and estimating both the amount and timing of N mineralization of GrM can be complex because there are numerous factors that can affect the process (Gaskell and Smith, 2007). Nitrogen mineralization is limited below 10°C, although not negligible, and increases with temperature (Kankanen et al., 1998). Moisture can limit mineralization if the soil is too wet (due to anaerobic conditions) or too dry. Tillage can temporarily stimulate microbial activity and therefore mineralization, and can effect soil temperature, moisture, and porosity (Brady and Weil, 2002; Gaskell and Smith, 2007; Wallace, 2015). Tillage can also affect the size of organic matter fragments and soil to substrate contact (Holland and Coleman, 1987). Further insight into how HV and other leguminous GrM-N is mineralized, will facilitate the adoption of GrM in Eastern Canada.

1.1. Mineralization of Green Manures

Nitrogen is accumulated in the above- and below-ground biomass of the GrM during the growing season. As the GrM decomposes, the N is released through the mineralization of the organic matter, turning the organic-N into plant available forms, predominantly nitrate (NO_3^-) and ammonium (NH_4^+) and small organic-N molecules (Brady and Weil, 2002). For this to happen, microbes must have easy access to the organic substrate and favorable soil

conditions, for example warm, moist, but not waterlogged soils, with available soil oxygen. Denitrification can occur in waterlogged soils as this reduces soil oxygen content, thereby limiting oxygen diffusion. For denitrification to occur, there needs to be available electrons, for example, carbon (C), for the reduction of NO_3^- to occur (Rosecrance et al., 2000). The storage of N in the soil organic matter (SOM) fraction can prove to be beneficial as warm, moist soil is also favourable to plant growth and a possible synchrony between N mineralization and plant growth can occur. However the N in the SOM can also be vulnerable to loss if mineral N (N_{min}) is in excess of crop need, resulting in environmental contamination through either gaseous or leaching losses (Rosecrance et al., 2000; Cook et al., 2010).

Incorporating residue into the soil increases the residue-soil microbial contact, which will speed decomposition. If a residue has a high C:N, then the C-rich residue will result in net immobilization of N as the N becomes bound up within the decomposer community (Holland and Coleman, 1987; Shaffer and Ma, 2001; Van Den Bossche et al., 2009). Should a residue with a low C:N be incorporated however, a rapid net release of N_{min} will occur shortly after incorporation (Van Den Bossche et al., 2009). This N_{min} is highly susceptible to leaching and denitrification. Alternatively, in many systems, after a flush of N_{min}, rapid immobilization occurs and N_{min} declines as the N_{min} that was released by the primary decomposers is immobilized by a second population of decomposers operating on this second substrate (Sarrantonio and Scott, 1988).

Residue which is left on the soil surface will result in a microbial biomass which is fungal-dominant since fungi are better able to withstand drier soil surface conditions in comparison to bacteria (Holland and Coleman, 1987). Fungal decomposition of litter results in more recalcitrant SOM fractions, slowing nutrient turnover (Holland and Coleman, 1987).

1.2. The Impact of Overwintering On Green Manure Biomass and Total Carbon and Nitrogen

Winter annual legumes such as HV that are grown in late-summer will grow vegetatively until going dormant over the winter. Vegetative growth will resume the following spring. In Michigan, winter survival of HV was improved in one year of two if the HV was intercropped with rye (Brainard et al., 2012), which is consistent with findings from Maine (Jannink et al., 1997). In both cases, the HV was seeded in late summer or early fall. Hairy vetch is expected to successfully overwinter in Nova Scotia so long as later-summer or early-fall planted HV does reach the reproductive phase prior to dormancy (Dr. Nancy McLean, personal communication). This is in agreement with others, who have found that once HV has reached full flowering/early pod set, there is minimal regrowth following mowing or roller-crimping (Mischler et al., 2010). This has been linked to HV freezing resistance decreasing with plant age (Brandsaeter et al., 2008).

Ranells and Wagger (1996) in North Carolina used nonlinear equations to estimate dry matter (DM) disappearance of winter annual cover crops and found that the range of rate coefficients of DM disappearance (-0.22 to -0.43) were similar to the rate coefficients for N release (-0.25 to -0.40) of decomposing GrM. Substrates with lower carbon to N (C:N) ratios mineralize at a faster rate (Kankanen et al., 1998). In Maryland, Rosecrance et al. (2000) observed that after the cover crop was killed, there was more N lost through denitrification and leaching from the HV treatment compared to the rye-HV, rye, and fallow treatments. The total N_{min} lost was 98 mg N per soil core (4 cm diameter x 16 cm), of which 17% was denitrified. Almost half of these losses were recorded within 30 days of cover crop termination, which is well before peak corn N demand. However, 30 days after spring GrM termination does coincide with the beginning of spring wheat (*Triticum aestivum* L.) peak N demand (Malhi et al., 2006), suggesting that perhaps a spring-tilled GrM of HV, or a GrM of hairy vetch-oats (*Avena sativa* L.) (HVO) could meet the N needs of spring wheat.

Freeze-thaw cycles can promote mineralization of incorporated GrM biomass-N over the winter (Kankanen et al., 1998; Wallace, 2015), resulting in N that is effectively "lost" as a

nutrient credit to the subsequent crop. On the other hand, in Finland it was found that a spring incorporated GrM mineralizes too late to be of use to the subsequent crop (Kankanen et al., 1998).

1.3. Impacts of Tillage Timing on the Mineralization of Green Manure Nitrogen

The presence of N_{min} in the soil over the winter poses an environmental risk. Mineral N losses from frozen soil have been attributed to denitrification and immobilization; in southern Finland, researchers experimented with manipulating incorporation timing to control mineralization. In a laboratory setting, early (40 days at 8°C followed by 40 days at 4°C) and delayed (40 days at 4°C) fall incorporations of a RC GrM resulted in significant N mineralization on a silty clay loam (Lahti and Kuikman, 2003). However, simulated late-fall incorporations (incorporation at the onset of freezing period) resulted in no net N mineralization (Lahti and Kuikman, 2003).

In a field experiment coinciding with the above laboratory experiment, manipulating GrM incorporation timing was studied using monocropped common vetch (*Vicia sativa* L.) (CV) on a silty clay soil. In comparison to early (September 1st) or spring incorporations (May 3rd), late (December 23rd at the onset of soil freezing) and delayed (October 20th at the end of the thermal growing season) incorporation timings resulted in greater N supply from the soil to a spring wheat indicator crop (Lahti and Kuikman, 2003). The late incorporated treatment also resulted in a higher grain yield. The authors theorized that spring incorporation of GrM resulted in a delay of N mineralization compared to fall incorporation. It was determined that delaying GrM incorporation to the onset of soil freezing could be used as a tool to reduce soil N losses (Lahti and Kuikman, 2003).

While laboratory incubations of soil can shed some light on the kinetics of N release from organic sources, the applicability of using that generated data in a field environment can be difficult as there is less control over variables that can have significant influence on the rate of mineralization (Woodley et al., 2014).

1.4. Green Manure Nitrogen Availability to a Subsequent Crop

Gaskell and Smith (2007) claim that only a small portion of GrM-N is used by the following crop and that N recovery rates by the cash crop are less than 50%, in part due to immobilization and asynchrony between N mineralization and cash crop need. Nitrogen from GrM can remain in the soil humus for years, slowly becoming available to subsequent crops (Gaskell and Smith, 2007). However, this makes nutrient management planning difficult for organic farmers.

Some models have used GDD to predict N availability from organic amendments (Griffin and Honeycutt, 2000). A base temperature of 0°C was successfully used to predict mineralization from livestock manures across different temperature regimes in a laboratory setting.

1.5. Nitrogen and Spring Wheat

Spring wheat yield is determined by such yield components as plants m^{-2}, spikes $plant^{-1}$, kernels $spike^{-1}$, and kernel weight (Ayoub et al., 1994; Chen et al., 2008). Amongst these yield parameters there is a compensatory relationship in response to changes in agronomic practices and environmental conditions such as seeding rate, row spacing, and moisture and nutrient availability (Longnecker et al., 1993; Malhi et al., 2006; Chen et al., 2008).

In Saskatchewan, it was found that the maximum rate of nutrient uptake occurred between tillering and stem elongation in spring wheat, which ranged from 22-36 days after emergence and from 149 to 318 GDD base 5°C ($GDD_{5°C}$). Maximum amount of nutrient uptake occurred between flowering and medium milk stages, 61-75 days after emergence and from 612 to 831 $GDD_{5°C}$. The maximum uptake rate for N in spring wheat was found to be between 3.2 and 5.7 kg N ha^{-1} day^{-1} (Malhi et al., 2006).

Spring wheat that is N deficient results in lower wheat biomass (Longnecker et al., 1993). Fewer tiller buds are found on low-N wheat as a result of fewer leaves and therefore fewer leaf axils where tiller buds form. Nitrogen deficiency can also delay or completely inhibit the growth of tiller buds (Longnecker et al., 1993). While early season N applications affect both grain yield and grain protein, N applied near or after anthesis impacts only protein concentration (Ma et al., 2004).

2. Objectives

In examining four different spring-planted, full season GrM systems [HVO, common vetch-oats (CVO), a red clover (*Trifolium pratense* L.) monocrop (RC), and a red clover biculture with oats (RCO)], the objectives of this work are:

1. To determine if the type of green manure incorporated at different timings (spring versus fall) affects N mineralization rate and total N_{min} in a controlled environment setting.
2. To determine if the type of green manure incorporated at different timings (spring versus fall) affects the quantity of N_{min} in situ.
3. To determine if the type of green manures incorporated at different timings (spring versus fall) affects yield and quality parameters of the subsequent wheat crop.

3. Hypothesis

1. The rate of N mineralization (kg N_{min} ha^{-1} $GDD_{0°C}^{-1}$) and quantity of N_{min} (kg N_{min} ha^{-1}) will be significantly different between treatments in a controlled environment setting.
2. The amount of N_{min} available during periods of crop demand will be significantly different between treatments as measured by hard red spring wheat (HRSW) biomass-N (kg wheat biomass-N ha^{-1}).
3. The quantity of soil N_{min} (kg N_{min} ha^{-1}) measured in situ at HRSW planting will be significantly different between treatments.

4. The peak amount of soil N_{min} in excess of crop demand (kg N_{min} ha^{-1}) will be significantly different between treatments.
5. Treatments will have a significant effect on wheat yield (kg wheat ha^{-1}) and wheat quality parameters (protein, 1000 kernel weight).

4. Materials and Methods

Chapter 2 outlines in detail the establishment of GrM crops in 2013. Chapter 3 is predominantly focused on the effects of these GrM treatments in 2014. Further clarification of treatments not outlined in Chapter 2 are outlined below, but for full cataloging of treatment establishment, please refer to the previous chapter.

Field experimental sites were in two locations, one at the Centre De Recherche sur Les Grains (CÉROM) in Saint-Mathieu-de-Beloeil, Québec, and one at Field 206 at the Dalhousie Agricultural Campus in Bible Hill, Nova Scotia. All data are presented by location and were analyzed separately. Experimental design is outlined below.

4.1. Site Characteristics and Experimental Design

At both locations, weather data was collected from weather stations in close proximity to the research plots: on-site in Saint-Mathieu-de-Beloeil, Québec, and <25 km from Bible Hill, Nova Scotia. Growing degree days were calculated by subtracting the base temperature (0°C) from the average daily temperature starting from day of HRSW planting (Table 11).

At both locations, soil samples were collected prior to HRSW planting in 2014 (Table 2) to a depth of 20 cm using a soil probe with a 2 cm diameter. A composite of 10-15 subsamples was collected for each block and sent to the Nova Scotia Department of Agriculture analytical lab for Mehlich III extractable nutrients, OM and pH. These tests revealed an acceptable pH and non-limiting macro and micronutrients at both locations in all years (Table 2).

4.2. Establishment of Crops Prior to Spring Wheat Year

Full details of GrM establishment can be found in Chapter 2. In brief, in the spring of 2013, GrM were seeded along with reference crops at the two sites. In the fall of 2013 or the spring of 2014, these GrM crops (plus reference crops) were incorporated (Table 10).

4.2.1. Bible Hill, Nova Scotia

In Nova Scotia, just prior to fall (2013) and spring (2014) GrM incorporation, GrM biomass was sampled using two 0.5 m^2 quadrats per plot, harvested to 5 cm above the soil surface. GrM were dried at 60°C for 48 hours and weighed separately. Tissue samples were passed through a 2 mm screen on a Wiley mill (standard model number 3, Arthur H. Thomas Co., Philadelphia, USA). Total C and N concentration were analyzed through gas chromatography (GC) after dry combustion, using the Vario MAX CN analyzer (Elementar, Hanau, Germany). The total amount of N produced in each GrM system was quantified by multiplying the GrM percent N by the DM yield.

Experimental design at this location in 2014 was a split-plot 4x2 factorial with three replicated blocks on a Pugwash sandy loam (classified as a Orthic Humo-Ferric Podzol in Canadian soil classification (Webb et al., 1991; Lynch et al., 2012)). The main plots were GrM type (planted in the spring of 2013: HVO, CVO, RC, plus Sb as a reference) and the sub-plots were incorporation timing (fall 2013, spring 2014). The main plots (HVO, CVO, RC, Sb) were 14 m x 10 m, and the sub-plots (GrM*incorporation timing) were 5 m x 3.5 m (n=24).

Chapter 2 outlines in detail the establishment of CVO, RC, and HVO in 2013. In Chapter 3, Sb, also planted in 2013, was included as a reference treatment. These GrM (plus Sb) are the main plots in the subsequent field experiments. Green manure termination timing (fall or spring incorporation) comprised the sub-plots.

On June 6, 2013, Sb (var. Prudence) were inoculated with *Bradyrhizobium japonicum* and seeded at 90 kg live seed ha^{-1} in 45.75 cm rows with an IH5100 soybean special drill (6.1 m wide drill with 15.25 cm row spacing, with 2 of every 3 rows blocked to provide a 45.75 cm actual row spacing), targeting a 2 cm seeding depth. Soybean planting was followed by a heavy rain storm, and the beans were washed out of the rows and left exposed on the soil surface. Crow predation also impacted the stand. As there was poor establishment, Sb plots were terminated using an S-tine harrow with rolling baskets (2x) on July 7, 2013. Plots were harrowed again on July 8, and then re-seeded that day with similar practices as outlined above. This second planting of Sb did not reach maturity, and were mowed and the biomass removed from the plots to mimic soybean harvest.

4.2.2. Saint-Mathieu-de-Beloeil, Québec

The experiment in 2014 at this site was on a Saint-Urbain clay loam (classified as a Dark-Grey Gleysolic soil in Canadian soil classification (Lajoie and Baril, 1954)) in a split-plot 2x3 factorial with four replicated blocks. The main plots were incorporation timing (fall, spring) and the subplots were GrM type (HVO, RCO, plus a cash-crop of oats where the straw was returned to the field as a reference). Chapter 2 outlines in detail the establishment of RCO, and HVO in 2013. In Chapter 3, oats, also planted in 2013, were included as a reference treatment. Oats were seeded at a rate of 154 kg live seed ha^{-1} with a Khun seed drill with 12.75 cm row spacing, and were harvested on August 29, 2013 and the straw returned to the plots. Experimental units (incorporation*GrM) were 6 m x 3 m (n=24).

4.3. Hard Red Spring Wheat Materials and Methods

Hard red spring wheat (cv. Helios) was seeded at a 450 live seeds m^{-2} into incorporated GrM in spring 2014 (Table 10). Wheat population was estimated prior to tillering by using three 1-m row lengths per subplot. Nitrogen uptake by the HRSW crop was monitored by a biomass subsample using two 0.25 m^2 quadrats which were taken at Zadoks 77 (Nova Scotia) and Zadoks 87 (Québec) in all GrM incorporation subplots. At this time, the number of wheat spikes were counted and weed biomass samples were also collected. Aerial wheat

and weed biomass were dried at 60°C for 48 hours and then weighed. Tissue samples were ground to pass through a 2 mm screen on a Wiley mill (standard model number 3, Arthur H. Thomas Co., Philadelphia, USA). Total C and N concentrations were analyzed through gas chromatography (GC) after dry combustion, using the Vario MAX CN analyzer (Elementar, Hanau, Germany).

Harvest protocols were different by site and are outlined below. Protein and moisture content of the wheat was determined using a SpectraStar 2500x NIR (Unity Scientific, 680-2500 nm). One thousand kernel weight was determined by weighing 100 seeds three times, taking the average and then multiplying by 10. Wheat yield, protein, and 1000 kernel weight were standardized to 12% moisture.

4.3.1. Bible Hill, Nova Scotia

On May 13, 2014, all sub-plots were soil sampled to a depth of 20 cm as an indicator of pre-plant N (Table 11). On May 15, 2014, HRSW (cv. Helios) was seeded at a 450 live seeds m^{-2} with a MF33 seed drill (4.6 m wide with 17.75 cm row spacing, dangling chains, no press wheels). All wheat plots were tineweeded on May 30, 2014. Due to impressive weed pressure, HRSW subplots were also hand-weeded on June 24-25.

On August 27, 2014, HRSW was harvested by cutting eight 1-m wheat row lengths from each sub-subplot. On September 2nd, wheat was threshed by putting each sub-subplot through a plot combine harvester, and threshed wheat was brought to <14% moisture for storage until analysis could be completed.

Table 10. Dates of main field operations in Nova Scotia and Québec in 2013 and 2014.

	Seeding rate		Nova Scotia		Québec	
			2013	2014	2013	2014
	Legume (kg ha^{-1})	Oats				
Seeding RC	11.5	-	*May 6*	-	-	-
Seeding RCO	11.5	70	-	-	*May 8*	-
Seeding HVO	30	70	*May 29*	-	*May 8*	-
Seeding CVO	30	70	*May 29*	-	-	-
Seeding Oats		154	-	-	*May 8*	-
Seeding Sb	90		*June 6* Replant *July 8*	-	-	-
GrM sampling			*Oct 29*	*May 7*	-	
GrM termination			*Oct 30* Flail mowed Rototilled (RC only, 2x) Disk (HVO, CVO, Sb)	*May 8*	*Nov 8* Moldboard	*May 20*
Secondary tillage			*May 6* S-tined with rolling baskets (2x) *May 28* (vetch plots only) S-tined with rolling baskets (2x)	*May 14* S-tined with rolling baskets (2x)	*May 8* Vibroculteur spring tine cultivator (2x)	*May 20*
Seeding HRSW	450 live seeds m^{-2}		-	*May 15*	-	*May 21*
Weed control				*May 30* Tineweed *June 24-25* Hand weed		
HRSW population count				*June 4*		*June 11*
HRSW biomass sample				*July 25*		*July 24*
HRSW harvest				*August 27*		*August 20*

4.3.2. Saint-Mathieu-de-Beloeil, Québec

On May 21, 2014, all sub-plots were soil sampled to a depth of 20 cm as an indicator of pre-plant N (Table 11). Hard red spring wheat was planted using a Khun seed drill with 12.75 cm row spacing on May 21st, 2014.

Wheat was harvested on August 20th, 2014 using a plot combine harvester. Wheat was brought to <14% moisture for storage until analysis could be completed.

4.4. In Situ Nitrogen Mineralization

An additional treatment acted as a reference for the in situ N mineralization experiment. A brown fallow was maintained by occasional disking or harrowing in both 2013 and 2014. It is important to note that the fallow treatment is not included in in situ analyses as it was not maintained as part of the factorial design due to no incorporation timing treatment imposed on the fallow. It was also decided that the fallow was not representative of "background mineralization" as the fallow was a second year brown fallow and so would have had dissimilar conditions (i.e. smaller aggregates, different drainage, etc.) compared to what would have been found in the other treatments. When present in the following in situ sections, it is left in for purely demonstrative purposes.

In order to gauge GrM type and management influences on soil N_{min} quantity (Objective 2), composite soil (0-20 cm) samples were collected from all GrM incorporation sub-plots (including Sb sub-plots) just prior to planting HRSW as well as from the fallow treatment, and then biweekly until the wheat reached physiological maturity (Table 11). At each soil sampling date (Table 11), HRSW development stage was determined using the Zadoks scale (Zadoks et al., 1974). After wheat harvest, all sub-plots were sampled for post-harvest residual soil mineral N. Soils were sampled to a depth of 20 cm, and five cores per subplot were taken. Samples were kept at 4°C until they could be frozen. Soils were thawed and then extracted with 0.5M K_2SO_4 (Voroney et al., 2008). Extracts were analyzed

colourimetrically for mineral N (NH_4-N and NO_3-N) content by a segmented flow Auto-Analyzer II (Technicon Industrial Instruments, Tarrytown, NY).

4.1. Incubation

In May 13, 2014, prior to planting wheat, all subplots in Nova Scotia, and all subplots in Québec on May 21, 2014, were soil sampled. Three 5 cm diameter cores were taken with a split-core sampler to a depth of 20 cm at each subplot, including Sb treatment in Nova Scotia, the brown fallow in Nova Scotia, and the oat treatment in Québec. Soils were kept at 4°C until the incubation experiment started. As outlined below in the Statistical Methods section (4.2.2.1), unlike in the previous sections, the brown fallow in Nova Scotia *was* included as a treatment and as part of the analysis for the incubation study on the Pugwash sandy loam. However, it was decided that the fallow was not representative of "background mineralization" as the fallow was a second year brown fallow and so would have had dissimilar conditions (i.e. smaller aggregates, different drainage, etc.) compared to what would have been found in the other treatments.

The in situ soil bulk density was determined at both sites in late summer. This was done by collecting 0-20 cm soil cores with a split-core sampler (diameter of 5 cm, n=2 cores per sample) for each plot. Samples were weighed, and a subsample was taken to determine soil gravimetric water content. Subsamples were over-dried at 105°C for 24h.

$$\text{Gravimetric water content (GWC)} = \frac{[\text{wet soil weight (g)} - \text{dry soil weight (g)}]}{\text{dry soil weight (g)}} \times 100$$

Table 11. Soil sampling date and associated wheat growth stage and growing degree day (base 0°C) accumulation in 2014.

Nova Scotia			Québec			Growth stage
Sampling date	Zadoks stage	$GDD_{0°C}$	Sampling date	Zadoks stage	$GDD_{0°C}$	
May 13, 2014	0	0	**May 21, 2014**	Z.0	0	
May 23, 2014	Z.12	109				Seedling development (Z.10-19)
June 10, 2014	Z.23	311	**June 20, 2014**	Z.21	551	Tillering (Z.20 – 29)
June 23, 2014	Z.33	476				Stem Elongation (Z.30-39)
			July 2, 2014	Z.45	814	Boot (Z.40-49)
July 7, 2014	Z.53	717				Heading (Z.50-59)
			July 15, 2014	Z.60	1083	Flowering (Z.60-69)
July 25, 2014	Z.77	1058				Milk (Z.70-77)
			July 24, 2014	Z.87	1274	Dough (Z.80-89)
Aug. 27, 2014	Z.92	1628	**Aug. 20, 2014**	Z.92	1795	Harvest
Sept. 9, 2014	Post-harvest	1822	**Aug. 29, 2014**	Post-harvest	1976	Residual soil N_{min}

The incubation protocol was based on the Nationally Coordinated Evaluation of Soil Nitrogen Mineralization Rate using a Standardized Aerobic Incubation Protocol (Griffin et al., 2008). Soils from both sites were gently mixed and packed into 100 mL graduated plastic test tubes (DigiTUBE, SCP, Baie D'Urfé, Québec) to 50 mL to equate the natural bulk density for that soil. Deionized water was added using a disposable pipette to bring the soils up to 60% water-filled pore space (WFPS) as calculated based on the soil's original GWC and bulk density. Test tubes were coved with pin-pricked parafilm to minimize water loss without rendering the samples anaerobic. All soils were kept at 4°C until placed in an

incubator set at 25°C. A small humidifier (Honeywell Top Fill 1.0 Gallon Cool Moisture Humidifier, Model number HCM-710C) was placed in the bottom of the incubator to help maintain incubator humidity and reduce the risk of soils desiccating too quickly. Growing degree days (base 0°C) have been found to be an accurate predictor of N transformations in soils amended with livestock manure (Griffin and Honeycutt, 2000).

By physiological maturity, wheat no longer takes up N from the soil (Malhi et al., 2006; Jones et al., 2011). At the time of biomass sampling, 1058 $GDD_{0°C}$ (Zadoks 77, Nova Scotia) and 1275 $GDD_{0°C}$ (Zadoks 87, Québec) had accumulated (base 0°C). Therefore samples were incubated at 25°C and 70% humidity for 7 weeks, equating 1225 $GDD_{0°C}$.

Every seven days, test tubes were brought back up to 60% WFPS, or removed from the incubator and destructively sampled and extracted with 0.5 M K_2SO_4 (Voroney et al., 2008). Extracts were analyzed colourimetrically for mineral N (NH_4-N and NO_3-N) content by a segmented flow Auto-Analyzer II (Technicon Industrial Instruments, Tarrytown, NY).

4.2. Statistical Analysis

All statistical analyses were conducted using Statistical Analysis System 9.3 software (SAS Institute, 2011, Cary, NC). All data were verified for normality and constant variance. Independence of the residuals was assumed through randomization. Assumptions of normality were met or data were transformed to bring the error distribution closer to normal. Outliers were identified using the Proc Univariate statement. All means reported in the tables and figures were back-transformed to the original scale. The significance level is set at $\alpha = 0.05$ where α is the probability of making a Type I error and wrongly rejecting H_0. Marginal significance is occasionally noted in the text where $\alpha < 0.1$.

4.2.1. In Situ Measurements

Data were analyzed using the Proc Mixed procedure in SAS.

4.2.1.1. Bible Hill, Nova Scotia

In Nova Scotia, in-field measurements of N_{min} and wheat growth parameters were in a split-plot 4x2 factorial design where GrM was the main plot and incorporation timing was the subplot with three blocks. All treatments except block were considered fixed effects. Overwintering GrM were analyzed using repeated measures.

4.2.1.2. Saint-Mathieu-de-Beloeil, Québec

In Québec, in-field measurements of N_{min} and wheat growth parameters were in a split-plot 2x3 factorial design where incorporation timing was the main plot and GrM was the subplot with four blocks. All treatments except block were considered fixed effects.

4.2.2. Incubation Measurements

The Nova Scotian soil, a Pugwash sandy loam, and the Québec soil, a St. Urbain clay loam, were analyzed separately. Shelves in the incubator were used as a blocking factor. Seven sampling time points were performed at 0 $GDD_{0°C}$, 175 $GDD_{0°C}$, 350 $GDD_{0°C}$, 525 $GDD_{0°C}$, 700 $GDD_{0°C}$, 875 $GDD_{0°C}$, and 1225 $GDD_{0°C}$.

4.2.2.1. Pugwash Sandy Loam (Nova Scotia)

An additional treatment was included in the N mineralization incubation experiment. As mentioned previously, a brown fallow was maintained by occasional disking or harrowing in both 2013 and 2014. The brown fallow *was* included in the incubation analysis. As there was no incorporation timing treatment, the Pugwash sandy loam incubation was instead analyzed as a randomized complete block design with three replications with one factor of interest: previous crop*incorporation timing, thereby including the fallow treatment (n=27). After a preliminary evaluation of the data, it was determined that a linear regression model would accurately represent the relationship between the dependent variable ($GDD_{0°C}$) and the factor of interest (N_{min}). Linear regression of total N_{min} was fitted to each treatment

(GrM*incorporation) in each block using Proc Reg in SAS, and an analysis of variance using Proc Mixed was performed on the parameters (slope and y-intercept) to detect significant differences (Er and Ögüt, 2015).

4.2.2.2. St. Urbain Clay Loam (Québec)

Québec soils were arranged in a 2x3 factorial with four replicates, where incorporation timing and previous crop were the respective treatments (n=24). A preliminary evaluation of the data revealed that a first order non-linear regression model where mineralization rate is proportional to the amount of substance remaining would accurately represent the relationship between the dependent variable ($GDD_{0°C}$) and the factor of interest (N_{min}). Seven sampling dates were collected for incubated St. Urbain clay loam samples, however, due to a flawed extraction, one sampling date needed to be removed. As a result there were insufficient degrees of freedom in the error terms when a model was created with three parameters (intercept, N_0, and k) to detect true differences between the treatments. To rectify this, the amount of mineral N at 0 $GDD_{0°C}$ was subtracted from all time points so that the model went through the origin and parameters were determined using Proc Nlin in SAS.

5. Results

5.1. Overwinter Effects on Aboveground Biomass and Nitrogen of Green Manures in Nova Scotia

In Nova Scotia, biomass was sampled on October 29, 2013, and again on May 7, 2014, the following spring. Aboveground biomass (kg DM ha^{-1}, including weeds) of all GrM was reduced by 25-35% over the winter (Table 12).

Table 12. Total aboveground green manure biomass and biomass-N of green manures sampled in fall 2013 and spring 2014 in Bible Hill, Nova Scotia.

Sampling date	Green manure	Total biomass kg DM ha^{-1}		N kg ha^{-1}		Biomass C:N	
Fall	CVO	3627	BC	71.9	C	22.8	B
Fall	HVO	7034	A	220.7	A	13.8	C
Fall	RC	3339	CD	70.4	C	20.7	B
Spring	CVO	2573	D	37.1	D	26.1	A
Spring	HVO	4533	B	105.4	B	16.2	C
Spring	RC	2480	D	48.6	CD	20.0	B
sd*GrM	p-value	0.0631		0.001		0.0347	

Values within a column followed by the same letter are not significantly different.
DM = dry matter
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pratense* L.)

A GrM of HVO had the highest total biomass-N, whether measured in the fall or the spring. Both CVO and HVO lost approximately half of the total N content of the biomass overwinter, most likely due to leaching (Table 12).

The fall sampling of HVO had significantly higher aboveground biomass, than all other treatments (Table 12). The C:N of the biomass ranged between 13.8 for HVO in the fall to 26.1 for CVO in the spring, all within the range of readily mineralizable substrate (Shaffer and Ma, 2001). The C:N of HVO and RC did not significantly change whether measured in the fall or the spring (Table 12) although the C:N of HVO was consistently significantly lower than RC in both seasons.

5.2. Nitrogen Mineralization after Green Manure Incorporation

5.2.1. Bible Hill, Nova Scotia

5.2.1.1. Nitrogen Mineralization in a Controlled Environment – Pugwash Sandy Loam

An analysis of variance was performed on linear regression parameters to detect significant differences in nitrogen mineralization dynamics, with the intercept representing the initial amount of N_{min} present and the slope characterizing the mineralization rate (Table 13, Figure 12). Spring incorporated treatments of RC and HVO had statistically similar intercepts at 72.4 kg N_{min} ha^{-1} and 61.5 kg N_{min} ha^{-1} respectively.

As can be observed in Table 13 and Figure 12, there were no significant differences in the slope between treatments. This indicates that the treatments mineralized at a similar rate (Objective 1). A high STE for intercept and slope (Table 13) made it difficult to find significant differences between the treatments and suggests that there are additional uncontrolled sources of variability in the system.

The RC and HVO treatments, regardless of termination timing, had the greatest amount of N_{min} (kg N_{min} ha^{-1}) by the end of the incubation period (Table 14). When the amount of N_{min} at the start of the incubation (0 $GDD_{0°C}$) was subtracted from the total N_{min} at the end of the incubation (1225 $GDD_{0°C}$), RC-Spring and RC-Fall treatments had the greatest amount of soil N_{min} over the course of the incubation period (Table 14), although RC-Fall was not significantly different from HVO-Spring and Fall, nor from CVO-Fall and Sb-Spring.

When biomass was measured in the fall of 2013, CVO had 72 kg of aboveground biomass-N ha^{-1} (Table 15). Contributions of Sb aboveground N were minimal, and the fallow treatment had no biomass N added other than what weeds may have grown up in between cultivations. Interestingly, CVO treatments that had had that amount of N incorporated in either the fall, or the following spring had intercepts that were statistically similar to the treatments that

previously had a cash crop of soybean and fallow treatment (Table 13). Treatments with CVO were also statistically similar to Sb treatments and the fallow for total N_{min} (Table 14).

Table 13. Nitrogen mineralization of green manures incorporated at two timings on a Pugwash sandy loam from Bible Hill, Nova Scotia in a controlled environment.

GrM	Incorporation	Intercept		Slope	Intercept STE		Slope STE	
RC	Spring	72.4	A	0.0328	3.35	BCD	0.00497	BCD
RC	Fall	51.1	BC	0.0367	2.86	CD	0.00420	CD
HVO	Spring	61.5	AB	0.0276	4.40	B	0.00653	B
HVO	Fall	54.1	B	0.0376	5.93	A	0.00880	A
CVO	Spring	37.0	CD	0.0223	4.03	BC	0.00600	BC
CVO	Fall	34.9	CD	0.0280	2.02	DE	0.00300	DE
Sb	Spring	36.1	CD	0.0270	3.03	CD	0.00450	CD
Sb	Fall	29.5	D	0.0252	1.04	E	0.00154	E
Fallow	-	29.6	D	0.0273	2.30	DE	0.00340	DE
	p-value	0.0004		0.1159	0.0002		0.0001	

Values followed by the same letter are not significantly different from values within the same column.
GrM = green manure
STE = Standard error
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pratense* L.)
Sb = a proceeding cash crop of soybeans (*Glycine max* L. Merr.)

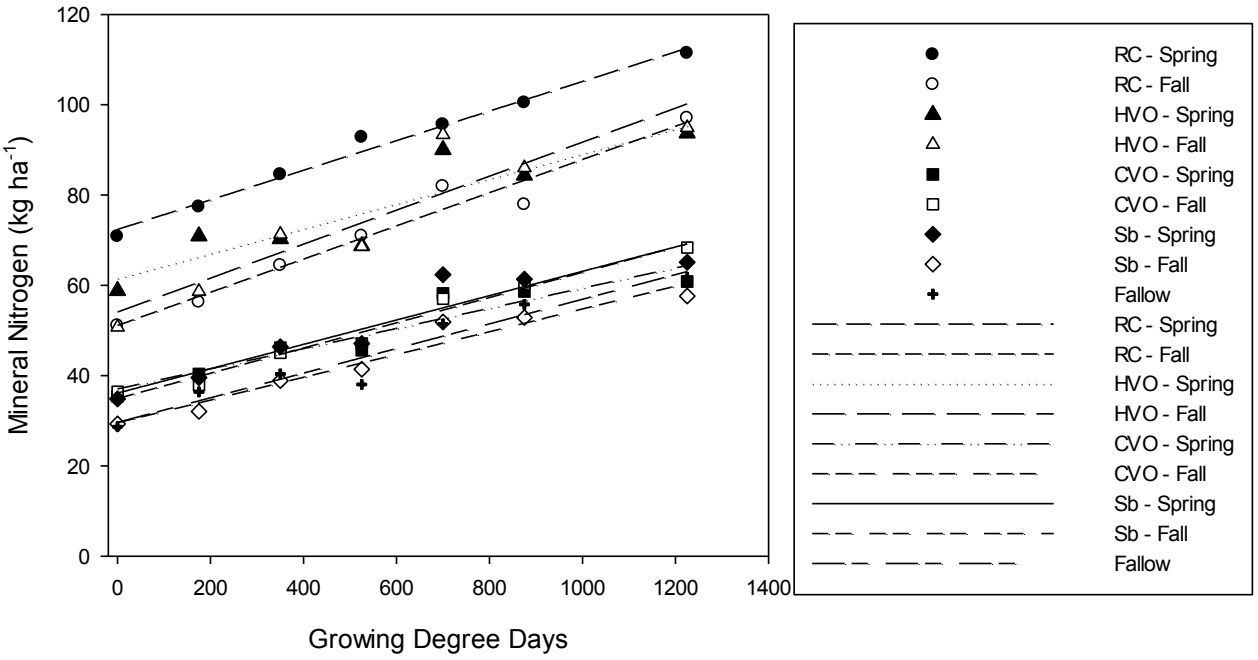

Figure 12. Cumulative soil mineral N of green manures incorporated at two timings on a Pugwash sandy loam from Bible Hill, Nova Scotia in a controlled environment.
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pratense* L.)
Sb = a proceeding cash crop of soybeans (*Glycine max* L. Merr.)

Table 14. Total mineral nitrogen and change in mineral nitrogen during incubation of a Pugwash sandy loam soil from Bible Hill, Nova Scotia containing green manures incorporated at two timings.

GrM	Incorporation	Total mineral N		Net mineral N since 0 $GDD_{0°C}$	
		---------- (kg N_{min} ha^{-1}) ----------			
RC	Spring	111.4	A	79.2	A
RC	Fall	97.0	A	54.3	AB
HVO	Spring	93.7	A	38.0	BC
HVO	Fall	94.9	A	33.4	BC
CVO	Spring	60.8	B	20.0	C
CVO	Fall	68.3	B	27.6	BC
Sb	Spring	65.1	B	31.9	BC
Sb	Fall	57.6	B	15.6	C
Fallow	-	61.8	B	14.8	C
	p-value	0.0012		0.0122	

Values followed by the same letter are not significantly different from values within the same column.
GrM = green manure
N_{min} = mineral nitrogen
GDD = growing degree days base 0°C
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pratense* L.)
Sb = a proceeding cash crop of soybeans (*Glycine max* L. Merr.)

In late October 2013, RC had 70 kg aboveground biomass-N ha^{-1}. At the beginning of the incubation, the RC treatments averaged across both fall and spring incorporation had 96 kg N_{min} ha^{-1}, meaning that the RC had in a sense already mineralized 137% of the amount of aboveground biomass-N incorporated into the system (Table 15). By the end of the incubation period, RC had more than double the amount of N_{min} than what was incorporated in the original amount of fall aboveground biomass-N. In the HVO treatment, the amount of soil N_{min} by 1225 GDD was only about half of the incorporated aboveground biomass-N, although it is unclear if this is because some was lost prior to the start of the incubation or because not all of the GrM-N was completely mineralized by the end of the incubation, or because immobilization occurred concurrently with mineralization (Table 15). All of the incorporated CVO aboveground N was recovered as soil N_{min} by the end of the incubation period.

Table 15. Percent estimated nitrogen release from green manure systems from Bible Hill, Nova Scotia based on initial aboveground biomass-nitrogen over the incubation period.

GrM	Incorporation timing	Fall aboveground biomass-N	Cumulative mineral N 0 GDD		Cumulative mineral N 1225 GDD	
		kg N ha^{-1}	Percent of aboveground biomass-N			
RC		70	137%	A	235%	A
HVO		221	25%	C	44%	C
CVO		72	54%	B	100%	B
	Spring		70%		129%	
	Fall		58%		123%	
		GrM	<.0001		<.0001	
		Incorp	0.1861		0.6809	
		GrM*Incorp	0.3207		0.5367	

For each factor, values followed by the same letter are not significantly different from values within the same column.
GrM = green manure
GDD = growing degree days base 0°C
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pratense* L.)

5.2.1.2. Nitrogen Mineralization In Situ – Nova Scotia

Substantial amounts of N_{min} were present in the field soils (0-20 cm) at 0 $GDD_{0°C}$ (Table 16) just prior to wheat planting on May 15, 2014. There was no significant effect of incorporation timing and there was no interaction between GrM and incorporation timing (Table 15). Green manures of RC and HVO had significantly more N_{min} at 34.8 and 33.1 kg N_{min} ha^{-1} respectively, compared to CVO and Sb. As in the incubation study, CVO and Sb performed statistically similarly in the field.

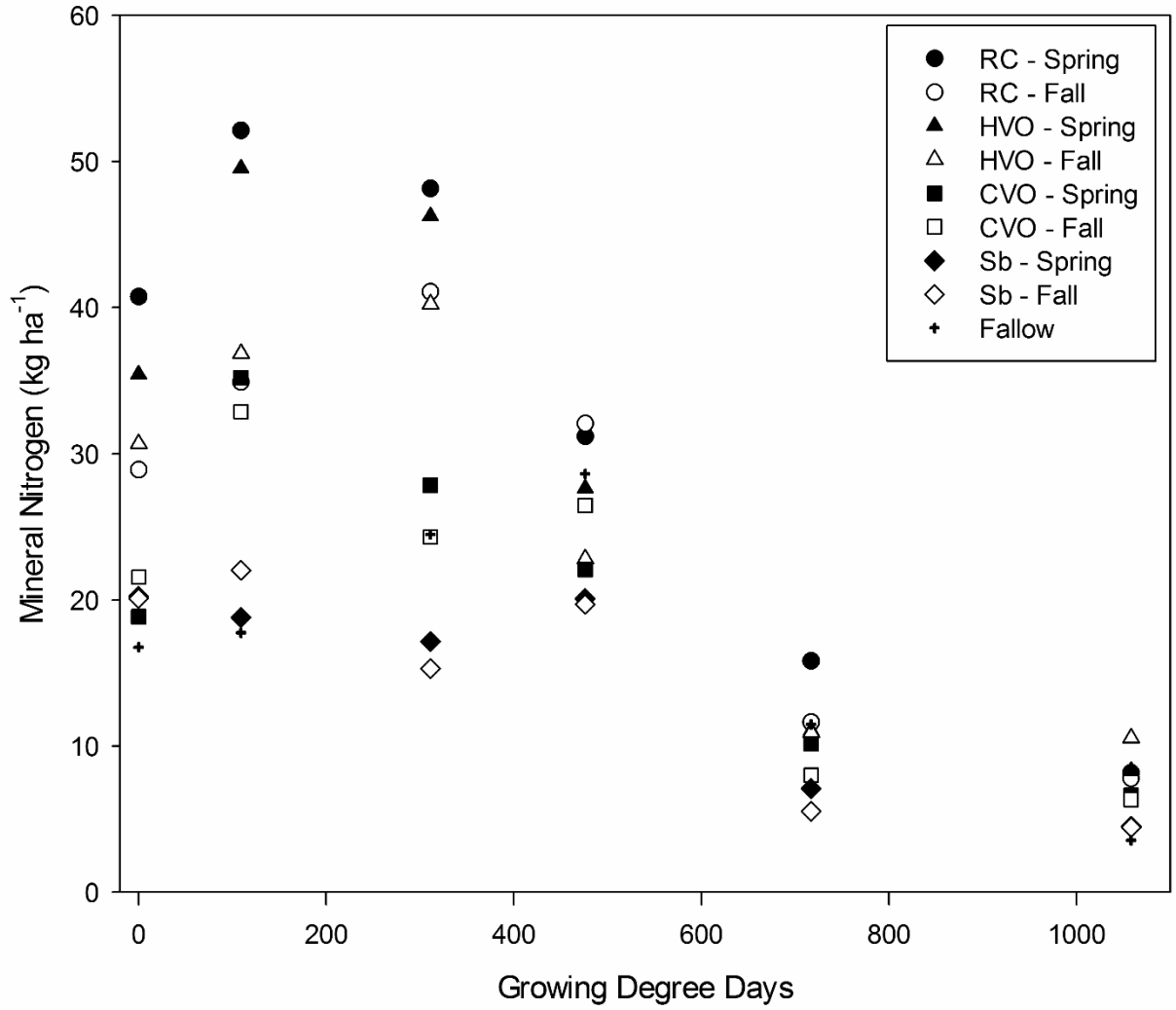

Figure 13. Net soil mineral nitrogen measured in a hard red spring wheat crop following green manure treatments incorporated at two timings on a Pugwash sandy loam in Bible Hill, Nova Scotia.
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pratense* L.)
Sb = a proceeding cash crop of soybeans (*Glycine max* L. Merr.)

By the time the HRSW crop had stopped taking up N at late milk (1058 $GDD_{0°C}$, Zadoks 77), GrM of HVO and RC had the greatest amount of net N_{min} measured in the field at 9.43 kg N_{min} ha^{-1} and 7.97 kg N_{min} ha^{-1} respectively (Table 16). There was no significant difference in net N_{min} between RC and CVO at the end of late milk. Again, as in the incubation study, CVO and Sb performed statistically similarly. The effect of GrM incorporation timing was not significant at either 0 $GDD_{0°C}$ or 1058 $GDD_{0°C}$.

Treatments where HRSW was preceded by RC or HVO had the most soil residual N_{min}, although RC was only marginally significantly greater than CVO and Sb (Table 16).

Table 16. Mineral nitrogen present in the field at spring wheat planting (0 GDD), late milk (1058 GDD), and post-harvest residual soil mineral N in Bible Hill, Nova Scotia.

GrM	Incorporation	0 GDD		1058 GDD		Post-harvest residual soil N_{min}	
		kg N_{min} ha^{-1}					
RC		34.8	A	7.97	AB	12.7	A
HVO		33.1	A	9.43	A	9.9	AB
CVO		20.2	B	6.46	BC	7.6	B
Sb		20.2	B	4.47	C	6.6	B
	Spring	28.8		6.90		9.6	
	Fall	25.3		7.27		8.8	
	GrM	0.0238		0.0149		0.0558	
	Incorp	0.1221		0.5060		0.2783	
	Incorp*GrM	0.1387		0.3246		0.4052	

For each factor, values followed by the same letter are not significantly different from values within the same column.
GrM = green manure
Incorp = incorporation timing
N_{min} = mineral nitrogen
GDD = growing degree days base 0°C
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pratense* L.)
Sb = a proceeding cash crop of soybeans (*Glycine max* L. Merr.)

The amount of N_{min} in excess of crop uptake peaked at GDD 109 and GDD 311 (Figure 13). During this time, spring incorporated treatments had a greater amount of N_{min} than their fall counterparts (Table 17). Green manures of RC and HVO had significantly more soil N_{min} (44.1 kg N_{min} ha^{-1} and 43.2 kg N_{min} ha^{-1}) than CVO or Sb at both sample dates. Treatments with a proceeding crop of CVO had greater amounts of N_{min} at 109 $GDD_{0°C}$ and 311 $GDD_{0°C}$ than Sb, differing from the controlled environment study where the two treatments acted statistically similar.

At GDD 109 and 311, the HRSW crop was at Zadoks 12 and Zadoks 23 (Table 11). Zadoks 12 is prior to significant crop demand, however at Zadoks 23, tillering has just begun and crop demand starts to increase. This is why N_{min} measured in the field started to decline after GDD 311.

Table 17. Peak soil mineral nitrogen measured in situ in spring wheat in Bible Hill, Nova Scotia.

GrM	Incorporation	GDD	kg N_{min} ha^{-1}	
RC			44.1	A
HVO			43.2	A
CVO			30.1	B
Sb			18.5	C
	Spring		37.0	a
	Fall		31.0	b
		109	35.4	
		311	32.5	
	GDD		0.1724	
	GrM		<0.0001	
	Incorp		0.0057	
	GrM*GDD		0.3590	
	Incorp*GDD		0.4980	
	Incorp*GrM		0.1369	
	Incorp*GrM*GDD		0.5626	

For each factor, values followed by the same letter are not significantly different from values within the same column.
GrM = green manure
Incorp = incorporation timing
N_{min} = mineral nitrogen
GDD = growing degree days base 0°C
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pratense* L.)
Sb = a proceeding cash crop of soybeans (*Glycine max* L. Merr.)

5.2.2. Saint-Mathieu-de-Beloeil, Québec

5.2.2.1. Nitrogen Mineralization in a Controlled Environment – St. Urbain Clay Loam

Nitrogen mineralization dynamics in the Québec soil, a St. Urbain clay loam was described best by a first order non-linear regression model where the rate of the transformation of the substrate (in this case potentially mineralizable N) is proportional to the amount of mineralizable substrate remaining (Figure 14). As previously discussed in Section 4.1. , insufficient degrees of freedom in the error terms when a model was created with three parameters (intercept, N_0, and k) led to the decision to pass the model through the origin and so the amount of mineral N at 0 $GDD_{0°C}$ was subtracted from all time points.

$$\mathbf{N_{min} = N_0 * (1 - e^{(-k * GDD)})}$$

N_0 is the size of the potentially mineralizable N pool (units kg N_{min} ha^{-1}), essentially the amount of soil organic N that is susceptible to mineralization following a first order kinetic model at *k* rate of mineralization. The mineralization rate constant (*k*, units GDD^{-1}) is a property of the soil and reflects the temperature at which mineralization is occurring. It is also affected by environmental factors such as water content, oxygen availability, etc.

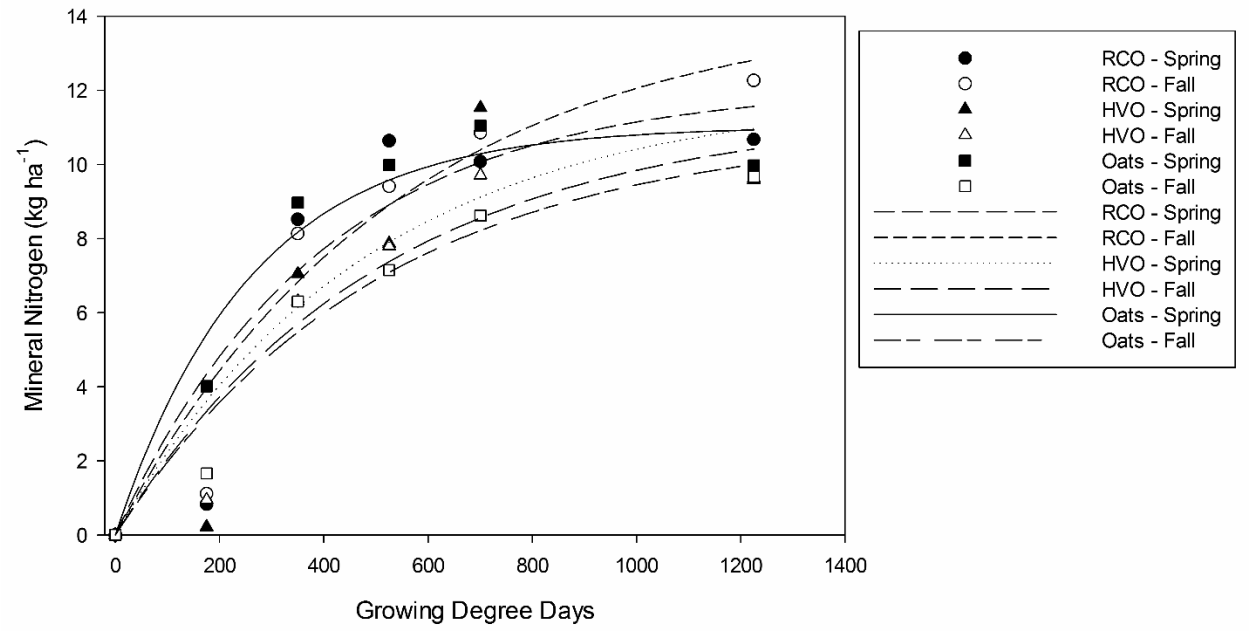

Figure 14. Cumulative soil mineral N of green manures incorporated at two timings on a St. Urbain clay loam from Saint-Mathieu-de-Beloeil, Québec in a controlled environment.

A non-linear curve was fit to the treatment means. Each treatment in each block was analyzed separately for analysis of variance in Table 18.

HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)

RCO = a green manure of red clover (*Trifolium pratense* L.)/oats

Ultimately, there were no significant differences in the size of the N pool (N_0), nor in the rate constant (k) (Table 18) (Objective 1). The standard error for the rate constant was marginally significantly different ($p = 0.0552$) between spring and fall incorporation timings, signifying that there was greater variability in spring-incorporated treatments.

Table 18. Non-linear regression parameters of the nitrogen mineralization curve for green manures incorporated at two timings on a St. Urbain clay loam from Saint-Mathieu-de-Beloeil, Québec in a controlled environment.

GrM	Incorporation	Size of N pool (N_o)	N_o STE	Rate constant (k)	k STE	
	Spring	11.92	3.47	0.00295	0.00193	A
	Fall	18.68	3.70	0.00189	0.00134	B
RCO		20.74	4.07	0.00203	0.00172	
HVO		12.88	4.66	0.00221	0.00169	
Oats		11.51	2.06	0.00306	0.00148	
	GrM	0.2428	0.2191	0.9033	0.2847	
	Incorp	0.2062	0.8144	0.3208	0.0552	
	GrM*Incorp	0.3148	0.2095	0.6491	0.2705	

For each factor, values followed by the same letter are not significantly different from values within the same column.
GrM = green manure
Incorp = incorporation timing
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)
RCO = a green manure of red clover (*Trifolium pratense* L.)/oats

The effects of GrM and incorporation timing had a marginally significant interaction ($p = 0.0614$) for total N_{min} (Table 19). The HVO-Spring treatment had the most N_{min} by the end of the incubation, statistically similar to RCO-Spring and RCO-Fall. However, RCO-Spring, RCO-Fall, and HVO-Fall were not significantly different than the fall incorporated oats. If soil N_{min} at 0 $GDD_{0°C}$ was subtracted from all values, there was no significant difference between GrM, incorporation timing, or their interaction (Table 19).

Table 19. Total mineral nitrogen and change in mineral nitrogen during incubation of a St. Urbain clay loam soil from Saint-Mathieu-de-Beloeil, Québec containing green manures incorporated at two timings.

GrM	Incorporation	Total mineral N		Net mineral N since 0 $GDD_{0°C}$
		---------- kg N_{min} ha^{-1} ----------		
RCO		36.7	ǂ	10.5
HVO		36.9	ǂ	9.7
Oats		26.0	ǂ	9.9
	Spring	35.1		10.1
	Fall	31.3		9.9
RCO	Spring	38.1	AB	10.7
RCO	Fall	35.4	AB	10.3
HVO	Spring	43.7	A	9.7
HVO	Fall	30.0	BC	9.6
Oats	Spring	23.5	C	10.0
Oats	Fall	28.5	BC	9.8
	GrM	0.0112		0.9318
	Incorp	0.1929		0.9104
	GrM*Incorp	0.0614		0.9980

For each factor or interaction among factors, values followed by the same letter are not significantly different from values within the same column.
Values followed by an ǂ have a higher order interaction and therefore do not have letter groupings representing significant differences.
GrM = green manure
Incorp = incorporation timing
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)
RCO = a green manure of red clover (*Trifolium pratense* L.)/oats

5.2.2.2. Nitrogen Mineralization In Situ – Québec

Amounts of N_{min} in field soils at 0 $GDD_{0°C}$ in Saint-Mathieu-de-Beloeil were statistically significant between GrM treatments, with treatments that included a leguminous GrM prior to the HRSW crop having significantly more soil N_{min} (Table 20, Figure 15).

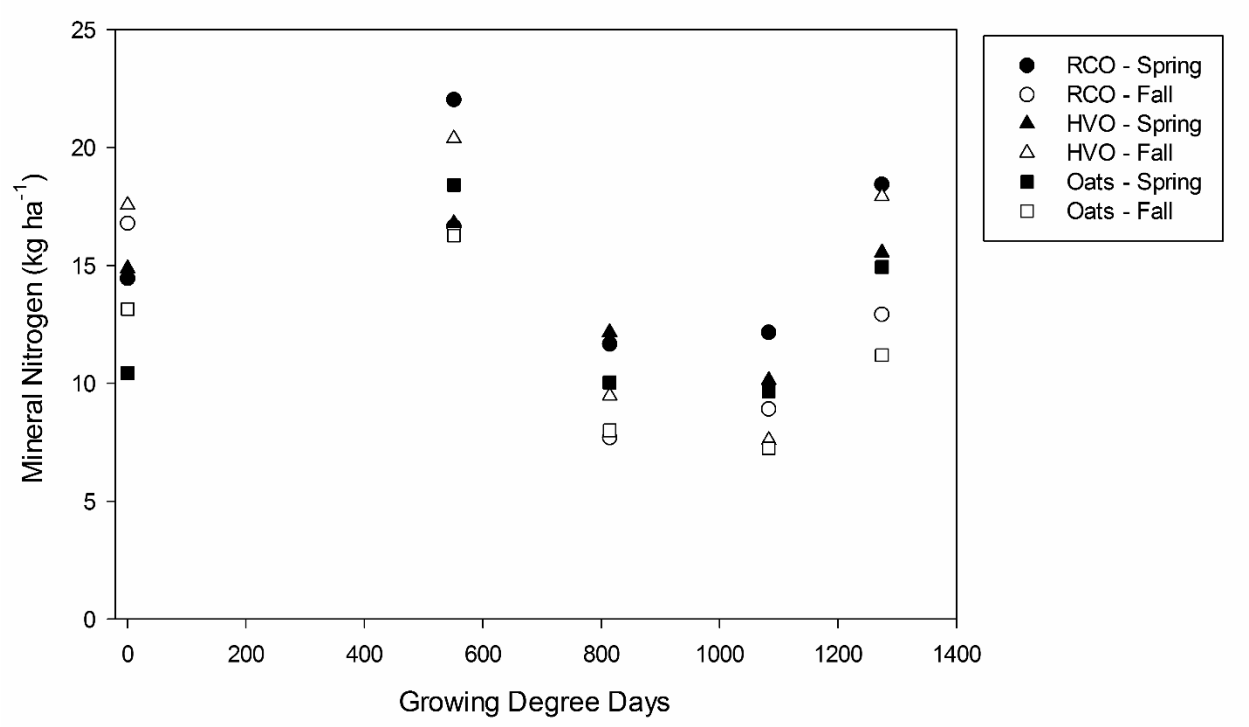

Figure 15. Net soil mineral nitrogen measured in a spring wheat following green manure treatments incorporated at two timings on a St. Urbain clay loam in Saint-Mathieu-de-Beloeil, Québec.
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)
RCO = a green manure of red clover (*Trifolium pratense* L.)/oats

By peak soil N_{min} at 511 $GDD_{0°C}$, there was no significant differences between treatments (Figure 15, Table 20). By HRSW physiological maturity, where the crop is no longer taking up soil N_{min}, there were significant differences between GrM by incorporation timing. Red clover-Spring had soil N_{min} in excess of net N_{min} statistically similar to HVO-Spring, HVO-Fall, and Oats-Spring. After the HRSW crop had been harvested, on August 29, 2014, there were significant differences in post-harvest residual N_{min} between GrM treatments, with RCO having significantly more residual soil N_{min} than HVO and Oats, and HVO having significantly more soil N_{min} than Oats.

Table 20. Mineral nitrogen present in situ at spring wheat planting (0 $GDD_{0°C}$), at peak soil mineral N (511 $GDD_{0°C}$), at dough (1274 $GDD_{0°C}$), and post-harvest residual soil mineral N in Saint-Mathieu-de-Beloeil, Québec.

GrM	Incorporation	0 $GDD_{0°C}$		511 $GDD_{0°C}$	1274 $GDD_{0°C}$		Post-harvest residual soil N_{min}	
		---------- kg N_{min} ha^{-1} ----------						
RCO		15.6	A	19.1	15.7		18.7	A
HVO		17.2	A	23.6	16.4		16.1	B
Oats		11.5	B	16.7	13.4		13.5	C
	Spring	13.9		22.4	16.1		14.9	
	Fall	15.6		17.1	14.2		17.3	
RCO	Spring	14.5		22.0	18.4	A	17.4	
RCO	Fall	16.8		16.4	12.9	B	20.1	
HVO	Spring	16.9		27.9	14.9	AB	14.4	
HVO	Fall	17.6		19.8	17.9	A	17.9	
Oats	Spring	10.4		18.4	14.9	AB	12.9	
Oats	Fall	12.5		16.3	11.9	B	14.0	
	GrM	0.0015		0.1718	0.1413		0.0025	
	Incorp	0.1647		0.1536	0.2069		0.2494	
	Incorp*GrM	0.7326		0.8454	0.0317		0.5934	

For each factor or interaction among factors, values followed by the same letter are not significantly different from values within the same column.
GrM = green manure
Incorp = incorporation timing
N_{min} = soil mineral nitrogen
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)
RCO = a green manure of red clover (*Trifolium pratense* L.)/oats

5.3. Wheat Response to Green Manure Type and Incorporation Timing

5.3.1. Bible Hill, Nova Scotia

There were no significant differences between treatments for plant population (plants m^{-2}) or the number of heads m^{-2} (data not shown). Incorporation timing had a significant effect on the number of spikes per plant, although tillering was minimal in 2014 in Nova Scotia

(Table 21). There was a marginally significant effect of GrM treatment on weed biomass in the subsequent HRSW crop (Table 21).

Wheat biomass-N was marginally significantly higher for spring incorporated GrM, and similarly, spring incorporated GrM had significantly higher total biomass-N (including weeds) (Table 21). Red clover and HVO GrM resulted in greater total-biomass-N, and all three leguminous GrM resulted in marginally significantly greater wheat biomass-N, although the CVO resulted in wheat biomass-N that was similar to a crop of HRSW following a Sb crop.

Table 21. Wheat growth response to green manure type and incorporation timing at late milk (Zadoks 77, 1058 GDD) in Bible Hill, Nova Scotia.

GrM	Incorp-oration	Plants m^{-2}	Spikes $plant^{-1}$	Wheat biomass (kg ha^{-1})	Weed biomass (kg ha^{-1})	Wheat biomass-N (kg N ha^{-1})	Total biomass-N (kg N ha^{-1})
RC		258	1.36	4652	822 A	56.8 A	70.2 A
HVO		228	1.55	4392	508 AB	55.4 A	66.6 A
CVO		208	1.34	3063	347 B	36.7 AB	41.6 B
Sb		236	1.20	2868	306 B	29.5 B	32.8 B
	Spring	225	1.47 A	3965	504	48.6 a	58.0 a
	Fall	240	1.26 B	3522	487	40.6 b	47.6 b
	GrM	0.2951	0.5708	0.1501	0.0829	0.0774	0.0148
	Incorp	0.1954	0.0783	0.1429	0.3625	0.0335	0.0070
Incorp*GrM		0.9539	0.7370	0.7440	0.2573	0.6184	0.1914

For each factor, values followed by the same letter are not significantly different from values within the same column.
GrM = green manure
Incorp = incorporation timing
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pratense* L.)
Sb = a proceeding cash crop of soybeans (*Glycine max* L. Merr.)

There was no significant effect of incorporation timing on wheat yield, percent protein, or 1000 kernel weight (Table 22). A proceeding GrM of HVO resulted in the highest wheat yield regardless of incorporation timing, most likely due to an increased number of kernels

plant^{-1}. A monocropped GrM of RC resulted in statistically similar grain yields as a GrM of CVO (Table 22). Green manure had no effect on protein content and 1000 kernel weight (Table 22).

Table 22. Wheat yield parameters in response to green manure type and incorporation timing in Bible Hill, Nova Scotia.

GrM	Incorporation	Wheat yield (kg ha^{-1})		Protein (%)	Kernels plant^{-1}		1000 kernel weight (g)
RC		2428	B	13.1	27.1	B	34.2
HVO		3480	A	13.2	45.7	A	34.2
CVO		2081	BC	12.9	30.3	AB	33.3
Sb		1147	C	12.1	14.9	B	32.8
	Spring	2236		12.7	29.8		34.0
	Fall	2332		13.0	29.2		33.2
	GrM	0.0085		0.1775	0.0215		0.3929
	Incorp	0.7559		0.2992	0.9029		0.2330
	Incorp*GrM	0.3951		0.6837	0.7639		0.7840

For each factor, values followed by the same letter are not significantly different from values within the same column.
GrM = green manure
Incorp = incorporation timing
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pratense* L.)
Sb = a proceeding cash crop of soybeans (*Glycine max* L. Merr.)

5.3.2. Saint-Mathieu-de-Beloeil, Québec

Oats had a marginally significantly higher wheat population compared to RCO, although not statistically different from HVO (Table 23). No significant differences were found between treatments for the number of spikes per plant. There were marginally significant differences in wheat biomass between GrM*incorporation timing, which the two oat treatments having the least amount of biomass, although surprisingly not significantly different from HVO-Spring and RCO-Fall. Oats incorporated in the fall had the most weeds. Hairy vetch-oats incorporated in the fall and RCO-Spring had the most wheat biomass-N, although RCO-

Spring was not significantly different from Oats-Fall. If wheat biomass-N and weed biomass-N were combined, while there were significant differences between previous crops with the leguminous GrM resulting in more total biomass-N compared to the oats, there was no effect of incorporation timing.

A crop of HRSW following a leguminous GrM had significantly greater wheat biomass, wheat biomass-N, and total aboveground N, and significantly less weeds, although a higher order interaction between GrM and incorporation was marginally significant for wheat biomass and wheat biomass-N (Table 23). Significantly higher wheat yields, higher protein content, more kernels per plant, and higher 1000 kernel weights was observed for HRSW following a leguminous GrM (Table 24).

Table 23. Wheat growth response to green manure type and incorporation timing at dough (Zadoks 87, 1274 GDD) in Saint-Mathieu-de-Beloeil, Québec.

GrM	Incorp-oration	Popu-lation m^{-2}		Heads m^{-2}		Spikes $plant^{-1}$	Wheat biomass		Weed biomass		Wheat biomass-N		Total biomass-N	
							kg ha^{-1}		kg ha^{-1}		kg N ha^{-1}		kg N ha^{-1}	
RCO		375	B	472		1.12	4415	ǂ	67.9	ǂ	49.6	ǂ	50.8	A
HVO		376	AB	461		1.11	4474	ǂ	63.3	ǂ	55.5	ǂ	56.2	A
Oat		414	A	482		1.08	3272	ǂ	229	ǂ	38.7	ǂ	42.1	B
	Spring	372		460		1.11	3857		72.9	ǂ	45.7		47.3	
	Fall	404		484		1.10	4251		168	ǂ	50.2		52.1	
RCO	Spring	364		458	AB	1.12	4622	AB	54.6	B	51.9	AB	52.4	
RCO	Fall	386		486	AB	1.12	4209	ABC	81.2	B	47.3	BC	49.3	
HVO	Spring	341		420	B	1.12	3774	BC	101	B	49.1	B	50.2	
HVO	Fall	411		502	A	1.11	5175	A	25.6	B	61.9	A	62.1	
Oat	Spring	412		501	A	1.10	3174	C	63.2	B	36.0	C	39.5	
Oat	Fall	416		463	AB	1.04	3371	C	396	A	41.4	BC	44.7	
	GrM	0.0828		0.5185		0.2707	0.0212		0.0124		0.0047		0.0137	
	Incorp	0.1604		0.4132		0.5588	0.2872		0.0876		0.2207		0.2080	
	Incorp*GrM	0.2233		0.0223		0.7030	0.0887		0.0065		0.0913		0.1485	

For each factor or interaction among factors, values followed by the same letter are not significantly different from values within the same column.

Values followed by a ǂ have a higher order interaction and therefore do not have letter groupings representing significant differences.

GrM = green manure

Incorp = incorporation timing

HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)

RCO = a green manure of red clover (*Trifolium pratense* L.)/oats

Table 24. Wheat yield and quality parameters in response to green manure type and incorporation timing in Saint-Mathieu-de-Beloeil, Québec.

GrM	Incorporation	Wheat yield (kg ha^{-1})		Protein (%)		Kernels plant^{-1}		1000 kernel weight (g)	
RCO		1907	A	12.9	A	17.7	A	29.4	A
HVO		1901	A	13.1	A	17.8	A	29.8	A
Oat		1307	B	12.4	B	11.4	B	27.7	B
	Spring	1685		12.7		16.5		28.9	
	Fall	1725		13.0		14.8		29.0	
	GrM	0.0021		0.0076		0.0032		0.0071	
	Incorp	0.7427		0.2580		0.2850		0.8270	
	Incorp*GrM	0.4428		0.5254		0.8462		0.8818	

For each factor, values followed by the same letter are not significantly different from values within the same column.
GrM = green manure
Incorp = incorporation timing
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)
RCO = a green manure of red clover (*Trifolium pratense* L.)/oats

6. Discussion

6.1. The Effect of Overwintering On Green Manure Biomass and Nitrogen Content

In both locations, HVO did not survive the winter, nor did the CVO (Nova Scotia site only). The RC in Nova Scotia and the red clover in the RCO treatment in Québec both survived the winter. In Nova Scotia, between late October and mid-May, CVO and HVO lost approximately half of their aboveground biomass and biomass-N (Table 12). In Finland, aboveground biomass N of a spring-planted HV monocrop went from 153-236 kg biomass-N ha^{-1} in the late autumn to 40-82 kg of aboveground biomass-N ha^{-1} in the spring, losing 58-83% of aboveground biomass-N (Kankanen et al., 1998), similar to the findings in this study (Table 12). Previous work done in Nova Scotia with a two-year old RC that was either

taken as forage, or clipped, found that the most substantial of annual N losses was during the winter and spring thaw (Wallace, 2015).

The N content of the spring-planted monocrop of a RC GrM was not significantly different from the fall to the spring; the still-living RC only lost 30% of the total N content, decreasing from 70.4 kg N ha^{-1} to 48.6 kg N ha^{-1} (Table 12). This is in contrast to earlier findings in Atlantic Canada where delaying plow down of RC until spring resulted in an additional 20-30 kg N ha^{-1} uptake in the following potato crop (Woodley et al., 2014). Spring-planted RC in Finland, on the other hand, went from 36-115 kg of aboveground biomass-N ha^{-1} in the late fall to 16-99 kg aboveground N ha^{-1} in the spring, ranging in a loss of over 50% of aboveground N to a gain of 10% of aboveground N (Kankanen et al., 1998).

In Manitoba it was found that overwintering spring-planted barley/HV mulches in a no-till study lost between 14 and 47% of their aboveground biomass (Halde and Entz, 2014). In another study, also in Manitoba, it was found that during the winter, GrM mulch biomass decreased between 19 and 59% after being roller-crimped (Halde et al., 2014). Overwinter, a spring-planted monocropped HV that had been fall roller-crimped decreased from approximately 9 Mg biomass ha^{-1} (290 kg N ha^{-1}) to 7.75 Mg ha^{-1} (200 kg N ha^{-1}) at one site and from 10 Mg biomass ha^{-1} (300 kg N ha^{-1}) to 6 Mg ha^{-1} (150 kg N ha^{-1}) at another, more northern site. Similarly, a spring-planted GrM of barley/HV that was roller-crimped in the fall decreased from 10.5 Mg biomass ha^{-1} (330 kg N ha^{-1}) to 7.5 Mg biomass ha^{-1} (180 kg N ha^{-1}) over the winter at one site. At another, more northern site, the spring-planted GrM of barley/HV that was roller-crimped in the fall went from approximately 11 Mg biomass ha^{-1} (305 kg N ha^{-1}) in the fall to 6.75 Mg biomass ha^{-1} (170 kg N ha^{-1}) the following spring (Halde et al., 2014). However, Manitoba is a drier climate than in Eastern Canada.

Due to errors in communication, data on overwintered GrM in Québec was not collected. Similarly, oat straw that was incorporated into the control treatment was also not sampled and analyzed for total N or C:N.

6.2. Nitrogen Mineralization of Green Manures

6.2.1. Bible Hill, Nova Scotia

6.2.1.1. Green Manure Mineralization in a Controlled Environment (Pugwash Sandy Loam)

The best fit model to predict N mineralization for the Pugwash sandy loam soils from Nova Scotia was a linear model (Figure 12). This was slightly unexpected as most (although not all) mineralization models are first order exponential functions (Griffin, 2008) such as was obtained for the St. Urbain clay loam from Québec (Figure 14). The N dynamics in soil are best understood as having two pools of soil N, one more labile, and one more latent or recalcitrant. After the first flush of N where there is rapid mineralization, there is a period of slower mineralization if not immobilization (Sarrantonio and Scott, 1988; Gillis and Price, 2015). In this study, it is likely that this first flush after incorporation was not captured due to the delay between incorporation and sampling. However the ultimate purpose of the incubation was to capture the dynamics of N mineralization in a controlled environment that would be representative of what was happening in the field after HRSW had been planted.

In the incubation of the Nova Scotia soil, spring incorporated treatments of RC and HVO had statistically similar intercepts at 72.4 kg N_{min} ha^{-1} and 61.5 kg N_{min} ha^{-1} respectively (Figure 12, Table 13). This shows large amounts of N at 0 $GDD_{0°C}$, occurring when crop uptake would be minimal and therefore stand the risk of NO_3^- leaching. It is worth noting that while the incubated samples had 61.5-72.4 kg N_{min} ha^{-1} at 0 $GDD_{0°C}$ (Figure 12), the range of N_{min} in the field at 0 $GDD_{0°C}$ was between 20.2 and 34.8 kg N_{min} ha^{-1} (Table 16). It was expected that these values would be similar. Samples that were set aside for incubation were refrigerated at 4°C until the beginning of the incubation trial. However, at one point those fridges ceased functioning over a weekend, causing the samples to warm, resulting in mineralization that would account for the doubling to tripling in the amount of N_{min} at 0 GDD found in samples used for the incubation that should have been identical to that found in situ.

Treatments that were incorporated in the fall, while generally possessing lower intercepts than their spring counterparts, were rarely significantly different within the same GrM in the incubation study (Figure 12, Table 13). When the incubation data were modeled, HVO-Spring and HVO-Fall had statistically similar intercepts, as did CVO-Spring and CVO-Fall, and Sb-Spring and Sb-Fall respectively (Figure 12, Table 13). Within the same GrM, only RC had significantly different intercepts between the Fall and Spring incorporations. A lower intercept, regardless of the treatment, does not necessarily pose a lower leaching risk, as it is likely that leaching occurred over the winter prior to measurements being taken (Schmidt et al., 2007a).

A high STE, as was found in the incubation experiment, can make it difficult to find significant differences between treatments suggesting that there were uncontrolled sources of variability in the system. As there was plot to plot variation within each treatment in the amount of GrM-N in the aboveground biomass incorporated from the previous year, it is unsurprising that the STE is large. It is interesting to note that within the same GrM, STEs for spring incorporations for RC, CVO, and Sb were higher than the fall incorporations, suggesting greater variability in spring incorporated systems. Also of note is that a GrM of HVO had some of the highest variation for both the slope and intercept. When it was sampled in the late fall, the HVO had a C:N of 13.8 (Table 12). The growth habit of the HVO was that the HV grew up and over the oats, and by late October the HV had formed a thick mat on top of the oats. The sheer quantity of biomass (greater than 7034 kg biomass ha^{-1}) made it difficult to incorporate evenly. Composite soil samples were taken from the plots, and gently mixed prior to packing into the DigiTUBEs, one tube for each sampling date for each plot. It is possible that there were "hot" spots in the DigiTUBEs with a higher concentration of HV:oats, which would have a lower C:N. This would result in faster mineralization of those "hot spots", and vice versa for higher concentrations of oats:HV, creating larger variability as captured in the incubation study.

As can be seen in Figure 12 and Table 13, there was no significant difference in the slope between treatments. This indicates that the treatments mineralized N at a similar rate, and

thus there were no detectable differences between the quality of the GrM in each treatment (Objective 1, Hypothesis 1). While the C:N ratios of the incorporated biomass of the treatments were significantly different (Table 12), the ratio ranged from 13.8 to 26.1: all well within the bounds of a C:N ratio that should foster mineralization (Shaffer and Ma, 2001), although Rosecrance et al. (2000) suggest that a C:N of as low as 21.4 can result in immobilization.

According to Malhi et al. (2006), maximum rate of spring wheat nutrient uptake (3.2 – 5.7 kg N ha^{-1} day^{-1}) is between tillering and stem elongation, which in their study ranged from 22-36 days after emergence and from 149 to 318 $GDD_{5°C}$. Green manure-N mineralization on a Pugwash sandy loam was found to be linear in a controlled environment, so the rate of mineralization was constant within each treatment. There was no significant difference between rate of mineralization of the treatments, but they ranged from 0.0223 kg N ha^{-1} GDD^{-1} (CVO-Spring) to 0.0376 kg N ha^{-1} GDD^{-1} (HVO-Fall), which in the incubator set at 25°C would be from 0.55 kg N ha^{-1} day^{-1} to 0.94 kg N ha^{-1} day^{-1}, far below the need of spring wheat during maximum crop uptake as identified by Malhi et al. (2006).

It could be postulated that overwinter N "losses" from the aboveground biomass, as described in Table 12, might not be lost to the system but captured instead by SOM as soil organic N. It was found that GrM contributes almost twice the amount of N to the organic N pool compared to a synthetic fertilizer (Janzen et al., 1990). They found that the N contributed to that pool was fairly recalcitrant thereby contributing to the long-term stable soil organic N reserves of the soil.

Table 15 makes a comparison of GrM aboveground biomass-N at the end of the growing season in Fall 2013, to the amount of net soil N_{min} measured in a controlled environment after GrM incorporation timing treatments had been implemented in the spring of 2014. Of the 70 kg of aboveground biomass N ha^{-1} in the RC at the end of the fall of 2013, 235% of that was captured as N_{min} by the end of the incubation period, a total of 112.6 kg N_{min} ha^{-1}, for RC-Spring, and a total of 96.1 kg N_{min} ha^{-1} RC-Fall (Table 15, Figure 12). In Finland, it was found that RC that was spring-planted and then incorporated in the late autumn had

between 36-115 kg of aboveground biomass-N ha^{-1}, and 24-135 kg of below-ground biomass-N ha^{-1} (Kankanen et al., 1998), showing that the below-ground biomass-N from 0-20 cm was approximately equivalent to the aboveground biomass. On the other hand, in the same study, spring-planted HV aboveground biomass-N incorporated in the late-fall ranged from 153-236 kg N ha^{-1}, while root-N in the 0-20 cm profile ranged from 4-29 kg-N ha^{-1}, or between 2 and 15% of aboveground biomass (Kankanen et al., 1998). Others in the US mid-Atlantic have found that HV root biomass comprised about 12% of the aboveground biomass (Cook et al., 2010).

In Nova Scotia, the RC in the spring incorporated treatment probably continued to grow after the October 29, 2013 sampling date, and since RC is a perennial, a little bit of regrowth had started in the spring of 2014 prior to the incorporation. However in the spring of 2014, the aboveground biomass-N was only 48.6 kg of biomass-N ha^{-1} (Table 12), suggesting that the a large amount of the substrate that was mineralized might have come from the RC root structure, or what had leached from the aboveground biomass at least partially made its way into SON. This root biomass generally has a higher lignin content and decomposes slower than the aboveground biomass (Cook et al., 2010). In Finland, while the aboveground RC biomass-N from fall to spring was reduced by over 50% in two sites, at one site, the spring RC aboveground biomass actually gained almost 10% of N in the aboveground material. Combining above- and below-ground RC biomass, at one site in the study, overwintering resulted in almost 75% loss of biomass-N, while another site only lost 14% of biomass-N (Kankanen et al., 1998).

At the end of fall 2013 in Nova Scotia, HVO had 221 kg biomass-N ha^{-1}, but by the end of the incubation period, only 44% of that biomass had been captured as N_{min} (95.4 to 100.2 kg N_{min} ha^{-1}) (Table 15). The fact that the HVO did not contribute more N_{min} to the system suggests that the HVO-N was either recalcitrant or very labile, and based on the C:N ratio of 13.8-16.2, it seems like it was most likely very labile (Table 12). This most likely resulted in N being lost over the winter. This is in comparison to CVO, which had a slightly higher C:N ratio (22.8 in the fall and 26.1 in the spring, Table 12), which resulted in net N_{min} that was 100% of the fall aboveground biomass-N (Table 15).

Alternatively, as the pattern for HVO mineralization on a Pugwash sandy loam was linear, it also possible that not all of the added biomass-N had been mineralized by the end of the incubation period, although in situ post-harvest residual soil mineral N (Table 16) showed that HVO did not have significantly different soil N_{min} compared to the other GrM treatments. A GrM in Pennsylvania of a fall-planted HV subsequently terminated in late May, and having accumulated 305 kg biomass-N ha^{-1}, resulted in 78 kg soil N_{min} ha^{-1} by late June (Cook et al., 2010). A HV GrM terminated just two weeks prior had accumulated 211 kg biomass-N ha^{-1}, resulting in 59 kg soil N_{min} ha^{-1}. This equals 26% and 28%, respectively, of biomass-N turning into soil N_{min} (Cook et al., 2010). In North Carolina, the release of N from cover crops was studied by looking at biomass-N decreases over time (Ranells and Wagger, 1996). They found that the N release from a winter-seeded HV monocrop totaled 118 kg N ha^{-1} in one year, and with an HV-rye biculture there was as much as 170 kg N released ha^{-1} in another year (Ranells and Wagger, 1996).

The GrM of CVO had 72 kg biomass-N ha^{-1} by late fall 2013 (Table 15) and the plants had long since senesced. In the incubation, the CVO did not act significantly different from the Sb reference, nor from the fallow in terms of the total N_{min}, and in the amount of N mineralized over the course of the incubation period (Table 14). At the beginning of the incubation, 37.0 kg N_{min} ha^{-1} (CVO-Spring) and 34.9 kg N_{min} ha^{-1} (CVO-Fall) were measured, roughly half of the amount of aboveground biomass that was originally incorporated. By the end of the incubation, 100% of the aboveground biomass-N that had been incorporated into the system was recovered as N_{min} (Table 15), suggesting that CVO would not contribute large amounts of N_{min} to a crop in year two after the GrM.

As the Sb crop in 2013 did not reach physiological maturity, plots were raked in the fall of 2013 to remove Sb residue. Nodulating soybeans grown in Illinois were mineralizing at a rate of 0.96 kg N ha^{-1} day^{-1} in early June (Gentry et al., 2001). Soybean residue was found to result in a cumulative net soil N mineralization amount of 112 kg N ha^{-1} in the subsequent year (Gentry et al., 2001). Based on the poor stand of Sb observed in Nova Scotia in 2013, it was not expected that Sb would contribute much N to the subsequent crop, and indeed,

visual observations of the HRSW in 2014 in the former Sb plots did suggest severe N deficiency manifested as short, yellowing HRSW plants. It is surprising however that at the end of the incubation period, the net mineralization of Sb, regardless of incorporation timing, was not significantly different from the fallow, which had no leguminous-N additions, from CVO, which had 72 kg GrM-N ha^{-1} added to the system, nor from HVO, which had 221 kg GrM-N ha^{-1} added to the system (Table 14).

One of the primary goals of a leguminous GrM is to provide plant available N at a time when it is most needed by the crop. Having a high amount of total N_{min} is immaterial if that N_{min} is available outside of the window of crop need. That window of maximum wheat nutrient uptake, as identified by Malhi et al (2006), is between tillering and stem elongation, in their study ranging between 149 to 318 $GDD_{5°C}$. In Nova Scotia, tillering occurred at 275 $GDD_{0°C}$ and stem elongation was completed by 503 $GDD_{0°C}$. In the controlled environment incubation, between the GDD timing of when tillering and stem elongation occurred in situ, the net amount of N_{min} that was released was between 5.7 kg N_{min} ha^{-1} and 8.58 kg N_{min} ha^{-1} (the minimum by Sb-Fall and the most by HVO-Fall respectively); not enough to meet crop demand.

In Saskatchewan, depending on the variety and year, maximum total amount of nutrient uptake occurred at flowering to medium milk stages, 61-75 days after emergence and from 612 to 831 $GDD_{5°C}$ (Malhi et al., 2006). In Nova Scotia in 2014, when spring wheat was at medium milk in the field, 1042 $GDD_{0°C}$ had accumulated. At this point in the controlled environment incubation, the RC-Spring treatment had resulted in net N mineralization of 106.6 kg N ha^{-1}, the most of any of the treatments, and the Sb-Fall treatment had net mineralized 55.8 kg N ha^{-1}, the least of all the treatments, even less than the fallow (58.1 kg N ha^{-1}) (Figure 12). Mineral N in the field prior to plant demand is very likely to leach, especially in Nova Scotia's wet springs. Were it to be assumed that any N_{min} in the soil would be leached away prior to tillering, then the amount of net N_{min} from the RC-Spring treatment during the period of crop uptake would actually be only 25.2 kg N ha^{-1} (the amount of net N_{min} measured between 275 and 503 $GDD_{0°C}$) and all other treatments would

be even less. In Nova Scotia, standard N recommendations are 100 kg N ha^{-1} for spring feed wheat, meaning that RC-Spring could not meet crop demand.

6.2.1.2. Green Manure Mineralization Measured In Situ

While in the incubation study, plant competition for mineral N was excluded, in the field N_{min} measurements captured what N_{min} was present in excess of what the crop had taken up. In Nova Scotia, standard N fertilizer recommendations are 100 kg N ha^{-1} for spring feed wheat.

Based on the amount of biomass-N incorporated into the systems (Table 12), it was expected that there would be greater quantities of soil N_{min} in excess of crop demand (Table 16, Table 17). However, based on the incubation study, only 17-28 kg N_{min} ha^{-1} were released between tillering and medium milk, which is far below crop demand. This would suggest that what little soil N_{min} was found in the field was pretty quickly taken up by N-starved HRSW. In Figure 13, the curve demonstrates when crop uptake begins. Tillering commenced at 311 GDD in Nova Scotia (Table 11), and any N that was mineralized before 311 $GDD_{0°C}$ was really prior to significant crop growth and therefore there were high levels of observable N_{min} in the field. As tillering commenced at 311 $GDD_{0°C}$, the amount of detectable N_{min} in the soil declined as the HRSW crop took it up and incorporated it into wheat biomass.

At planting (0 $GDD_{0°C}$), there were significant differences between GrM treatments, with the RC and the HVO having significantly more N_{min} than the CVO and the Sb (Table 16), as would be expected based on the net soil N_{min} results from the incubation study. At peak soil N_{min}, the RC and the HVO continued to have significantly more N_{min} in the field than the CVO and the Sb (Table 17). By Zadoks 77 of the HRSW (1058 $GDD_{0°C}$), RC and HVO still had the highest N_{min}, although surprisingly, given how much more net soil N_{min} was found in the incubation, RC was not significantly different than the CVO (Table 16). Timing of incorporation did not have any significant effect on soil N_{min} in excess of crop demand, nor was the interaction between GrM and incorporation timing significant.

6.2.2. Saint-Mathieu-de-Beloeil, Québec

6.2.2.1. Green Manure Mineralization in a Controlled Environment (St. Urbain Clay Loam)

There were no significant differences between GrM or incorporation timing in the size of the N pool (N_0) or the rate of mineralization as measured in a controlled environment setting on a St. Urbain clay loam from Saint-Mathieu-de-Beloeil, Québec (Figure 14). This is despite HVO and RCO having 154.1 and 64.8 kg of biomass-N ha^{-1} respectively when they were incorporated in the fall in Saint-Mathieu-de-Beloeil, Québec, and a fall C:N ratio ranging from 16.4 to 21.1 (HVO and RCO respectively, data not shown). Due to the lateness of sampling (May 20), it is to be expected that there was significant leaching and denitrification that occurred prior to sampling. For example, Saint-Mathieu-de-Beloeil had a storm on May 17, 2014, receiving 25.9 mm of rain (data not shown), which would have leached NO_3^-, and the subsequent waterlogged soils would have resulted in further losses through denitrification (Jansson and Persson, 1982). However, plowing and planting on heavy soils can be a challenge in a wet spring, and late planting is not unrepresentative of what can commonly happen on-farm.

It is interesting to note that there was higher variability in the rate constant (*k* STE) for a spring incorporated GrM compared to a fall incorporated GrM (Table 18). This could suggest that overwintering conditions lead to a greater variability in biomass that is left on the soil surface over the winter, in both/either the amount or the quality of the remaining litter. Microbial biomass has been found to peak over the winter, and as a result so does plant litter decomposition (Schmidt et al., 2007a). The overwintering process at the soil surface is dominated by fungal decomposition, the dynamics of which greatly influence the flush of N mineralization observed the following spring which is often lost to leaching (Schmidt et al., 2007a). Factors such as inconsistent snow cover and heterogeneous distribution of microbial populations could lead to higher variability in the composition of residues that overwinter on the soil surface.

There were marginally significant differences between GrM by incorporation timing in the total amount of net N_{min} (Table 19), however, the change in the amount of N_{min} since 0 $GDD_{0°C}$ was not significant between the treatments. The total amount of net N_{min} in the fall incorporated oats, which would presumably not contribute a large amount of N_{min} to a system in the first season, was not significantly different than RCO-Spring, RCO-Fall, nor HVO-Fall. As seen in Chapter 2, these latter systems contributed a large amount of N to the system in their aboveground biomass, (200 kg biomass-N ha^{-1} from the HVO and 102 kg biomass-N ha^{-1} from the RCO in late October). In the controlled environment study, none of the treatments resulted in sufficient net N_{min} to meet Québec wheat N fertilizer recommendations of 90-120 kg N ha^{-1} (70-90 kg N ha^{-1} for baking wheat).

While not compared statistically, following the findings of others, the averages for the amount of NO_3^- accumulated in the coarser textured soil (Pugwash sandy loam) was much higher than found in the lighter textured soils (St. Urbain clay loam). Soil texture will have an influence on soil water content and aeration, with greater air-filled porosity to be found in coarser textured soils, resulting in more N mineralization and reduced N immobilization (Gordillo and Cabrera, 1997; Griffin et al., 2002). A strong positive correlation was found between the sand content of a soil and the rate and extent of mineralization of broiler litter organic-N and a strong negative correlation was found with the silt + clay content (Gordillo and Cabrera, 1997). A regression equation using silt + clay as the only variable was able to explain 62% of the variation observed in potentially mineralizable N of broiler litter on nine different soil types in an 146 day incubation (Gordillo and Cabrera, 1997). With a higher soil clay content, the C and N substrates are physically protected from microbial activity, reducing the mineralization of C and N (Griffin et al., 2002).

Gordillo and Cabrera (1997) also found a negative relationship between pH and the size of N pools, suggesting that as soil pH increased, the size of the N pools decreased. They hypothesized that this could be due to the microbial biomass that was driving the N mineralization in their soils performed better in a more acidic environment. This too could have played a role in the differences between the amount of net N_{min} in Québec vs. Nova

Scotian soils, as the Québec soils averaged a pH of 7.66 and the Nova Scotian soils averaged a pH of 6.27.

The graduated plastic test tubes for this incubation were packed to the in situ bulk density measured for the soil. The natural bulk density as measured for the Pugwash sandy loam was 1.24 g cm^{-3} and the bulk density for the St. Urbain soils was 1.18 g cm^{-3}. The clayey soils from Québec were more difficult to pack to that density. When water was added with a pipette to bring the soils to 60% WFPS, sometimes the water sat on top of the soil and did not percolate down. It was feared that this would create an anaerobic environment. Anaerobic conditions hamper microbial activity, stimulating the formation and accumulation of NH_4^+ and/or NH_3, resulting in the loss of NO_3^- due to denitrification (Jansson and Persson, 1982). However, the NH_4^+ levels in the incubated samples from Québec were not unusually high, ranging from 0 – 0.5 kg NH_4^+ ha^{-1}, averaging 0.105 kg NH_4^+ ha^{-1} (data not shown), suggesting that anaerobic conditions did not occur.

6.2.2.2. Green Manure Mineralization Measured In Situ

In Québec, significant differences were found between GrM at 0 $GDD_{0°C}$ in situ, with the leguminous GrM having significantly more N_{min} than the oat control (Table 20). However, this is prior to crop uptake. Similar to what was measured in the controlled environment, these values are much lower than would be expected, ranging from 11.5 to 17.2 kg N_{min} ha^{-1}.

In Québec, at peak soil N_{min} at 511 $GDD_{0°C}$, just as tillering was starting (Zadoks 21), there were no significant differences measured in N_{min} between treatments in the field. However, there were marginally significant differences found between incorporation timing for the number of spikes per plant (Table 21). As tillering is related to N supply to the crop (Longnecker et al., 1993), this suggests that differences in the soil N_{min} did happen over the course of tillering (Zadoks 20-29) that were not captured by soil sampling.

Throughout the growing season, the change in the amount of N_{min} in the soil measured in situ stayed within the same range of between 10 and 20 kg N_{min} ha^{-1} (Figure 15), regardless

of GrM type or incorporation timing. A change of this magnitude would generally not be considered agronomically significant.

Significant differences were found in residual soil N_{min} post-harvest in Québec (Table 20). The two leguminous GrM both had significantly more residual soil N_{min} compared to the oats, and this N_{min} would not only be subject to leaching but also show that mineralization was happing outside of crop need.

6.2.3. Nitrogen Mineralization in a Controlled Environment

In an incubation study, the competition for N_{min} from plants is excluded, which can be a useful tool in observing N dynamics. One of the many disadvantages of incubation studies are that they disrupt the soil structure, allowing greater oxygen permeation throughout the sample which would not necessarily be the case in a field-like setting.

There are several factors working in conjunction with N mineralization such as denitrification and leaching which were not captured in this study. In Maryland, for example, they found in a denitrification incubation, that five days after HV termination, high denitrification rates were recorded (Rosecrance et al., 2000). It was hypothesized that this was due to high NO_3^- availability due to rapid N mineralization and also high soil organic C availability. This was demonstrated by high microbial respiration rates (Rosecrance et al., 2000). This window was not captured in Nova Scotia as there was a 5-6 day delay between spring incorporation and HRSW planting/soil sampling for N_{min} content and for the incubation study.

While there was a fallow treatment at the Nova Scotia site, it was decided that the fallow was not representative of "background mineralization" as the fallow was in its second year, and so would have had dissimilar conditions such as smaller aggregates, different drainage, different microbial populations, etc. compared to what would have been found in the other treatments. In Maryland, after correcting for N mineralization from fallow controls (0.53 mg $(NO_3^- + NH_4^+)$-N d^{-1}), decomposition of a monocropped vetch green manures in soil cores in

a controlled environment study resulted in net mineralization (C:N 10.3) at a rate of 0.98 mg $(NO_3^- + NH_4^+)$-N d^{-1} (Rosecrance et al., 2000). Monocropped rye (C:N 21.4) resulted in net N immobilization 0.34 mg $(NO_3^- + NH_4^+)$-N d^{-1}. A mixture of rye and vetch (C:N 14.8) resulted in net mineralization of 0.10 mg $(NO_3^- + NH_4^+)$-N d^{-1} after correcting for N mineralization from fallow controls (Rosecrance et al., 2000).

Rannells and Wagger (1996) found that the initial N concentration and the C:N ratio of GrM biomass prior to incorporation were valid indicators of GrM decomposition rate, outstripping hemicellulose and cellulose as predictors. It would not have been unreasonable to expect that there was immobilization occurring in treatments with a higher C:N ratio, for example, while not measured, it could be presumed that the oat straw in Québec would have a high C:N. The incubation study captured net N_{min}, and did not capture to what extent immobilization occurred. Rosecrance et al. (2000) found net N immobilization in monocropped rye (C:N 21.4).

Higher GrM-N and soil NO_3-N increases the risk of leaching and denitrification (Kankanen et al., 1998), which could be why net N_{min} in HVO was only 44% of incorporated-N by the end of the incubation period on the Pugwash sandy loam from Nova Scotia (Table 15). Based on low C:N ratios and large amounts of biomass-N, it is very possible that some of the HVO N_{min} was lost to denitrification in both Nova Scotia and Québec which was not captured in this study.

It has been found that incubating soils outside of their normal temperature range can alter soil biological characteristics, which in turn will impact the estimates of mineralization parameters (Cooper et al., 2011). In retrospect a temperature of 25°C was perhaps not the best choice for the incubation temperature for this study.

6.3. Hard Red Spring Wheat Response to Green Manures As Affected By Timing of Incorporation

Yield components in wheat are plastic and interdependently compensatory (Beavers, 2005). Grain yield is affected by multiple parameters such as heads m^{-2}, kernels $plant^{-1}$, and kernel weight with one compensating for the others to stabilize yield (Beavers, 2005). Generally it is expected that with increasing N fertility there would be an increase in tillers m^{-2}, spikes m^{-2}, kernels $plant^{-1}$, grain yield, and grain protein, however there are always other environmental factors than can play a role such as inherent soil fertility, water availability, disease pressure and other physiological factors. Nitrogen available just after seeding results in increased tillering, whereas later N availability results in increased grain protein (Ayoub et al., 1994).

6.3.1. Bible Hill, Nova Scotia

There was no significant difference in plant population or spike density between GrM treatments in Nova Scotia (Table 21). The HRSW was seeded at 450 live seeds m^{-2}, however the average population across all treatments just prior to tillering was 233 plants m^{-2}. This could be due to high mortality after tineweeding, or perhaps poor seeder calibration to account for the 48% mortality. Based on the observed weed pressure, this lower HRSW stand density was ineffective in competing against weeds for both light and N_{min}, limiting the ability of HRSW to maximize N uptake.

Chen et al. (2008) found that N treatments do not affect spike density. Similarly, it was found that GrM type (including RC and HV) did not affect the number of spikes m^{-2} in a study in North Dakota (Badaruddin and Meyer, 1990). In Nova Scotia, there was a marginally significant difference ($p = 0.0783$) between incorporation timing with respect to spikes m^{-2} (Table 21). Spring incorporation had a 17% increase in the number of spikes compared to fall incorporation, consistent with in situ field sampling where treatments that

were incorporated to in the spring had greater N_{min} than the fall incorporations early in the growing season (Table 17).

While there was no significant treatment effect on total wheat biomass, there was a significant effect of incorporation timing (p=0.0.335) and a marginally significant effect (p=0.0774) of GrM on wheat biomass-N (Table 21). The amount of N_{min} measured in the field (Table 17) at peak in situ N_{min} (109 and 311 $GDD_{0°C}$) indicated that the spring treatments should have more aboveground wheat biomass-N, and indeed, spring incorporated GrM resulted in 20% greater crop N uptake (48.6 kg wheat biomass-N ha^{-1}) compared to fall incorporated GrM (40.6 kg wheat biomass-N ha^{-1}, Table 21). There were marginally significance differences between GrM treatments in wheat biomass-N, with RC having similar amounts of wheat biomass-N as HVO and CVO treatments (Table 21). This is unexpected since treatments with CVO usually had less N_{min} in the incubation and field measurements (Table 14, Table 16, Table 17, Figure 12). Treatments with CVO and Sb had statistically similar amounts of total biomass-N, which relates back to there being no significant differences in the net total amount of N_{min} and the change in N_{min} found in the controlled environment study (Table 14).

A GrM of RC resulted in a 92% improvement in wheat crop N uptake compared to the Sb control. Similarly, a GrM of HVO resulted in an 88% improvement in wheat crop N uptake compared to the control, while CVO only improved crop N uptake by 24%.

In Nova Scotia on the Pugwash sandy loam, the percent of crop N uptake (wheat biomass-N, Table 21) of the total amount of N mineralized as measured in the controlled environment study (Total N mineralized, Table 14) ranged from 45% (Sb-Fall) to 66% (HVO-Spring) (data not shown). In western Canada, a spring wheat crop recovered only 14% of GrM-N using Tangier flatpea (*Lathyrus tingitanus* 'Tinga') and lentil (*Lens culinaris* 'Indianhead') compared to 36% of fertilizer N (Janzen et al., 1990). Legume-N recovery by a subsequent crop such as corn, wheat, or barley, range from 11-28%, approximately half of the mineralized estimate (Ranells and Wagger, 1996). In Nova Scotia, there is room for further work to be done to improve on the N use efficiency (cumulative N_{min} from the controlled

environment study divided by the amount of wheat biomass-N) on a Pugwash sandy loam. It is likely that better weed control would improve this.

There was no significant effect of incorporation timing on wheat yield parameters (Table 22), despite spring incorporated GrM having marginally more spikes plant^{-1} (Table 21). Wheat following a GrM of HVO resulted in the highest yield. The monocropped RC treatment tended to have similarly high amounts of soil N_{min} as HVO when measured in a controlled environment (Figure 12, Table 14), and yet performed statistically similar to CVO which tended to have some of the lowest amounts of soil N_{min} in the incubation. Since RC and HVO have a statistically similar plant population, number of spikes plant, wheat biomass, and wheat biomass-N (Table 21), it could be surmised that most of the yield difference observed between RC and HVO is due to fewer kernels plant^{-1} in the RC treatment than the HVO treatment (Table 22). Kernel number is determined at the beginning of stem elongation (Zadoks 30) (Beavers, 2005), which, based on observed growth stages in Nova Scotia, would have been at approximately 400 $GDD_{0°C}$ (Table 11). What is perplexing is that according to the incubation study (Figure 12), RC and HVO should have had similar amounts of N_{min} at stem elongation, but they do not. Similarly, the results from the incubation would have suggested that a GrM of HVO provided significantly more N_{min} to the subsequent crop than a GrM of CVO at key points of crop development (Figure 12). However, HVO was not significantly different from CVO in the number of kernels plant^{-1}.

In North Carolina, 16 winter annual GrM cultivars (including CV and HV) were trialed and despite each treatment containing more than 100 kg N ha^{-1} in the GrM biomass, after being terminated via roller-crimping, several of the treatments resulted in grain corn yields that were statistically similar to a 0 kg N ha^{-1} control (Parr et al., 2011). It was hypothesized that this might be in part due to GrM re-seeding acting in competition with the corn crop but also due to non-synchrony of GrM mineralization with crop needs. It was proposed by the authors that this non-synchrony had much to do with the GrM characteristics such as N concentration, C:N ratios, lignin, phenolic contents, and cellulose and hemicellulose concentrations (Parr et al., 2011).

As mentioned in the previous chapter, the monocropped RC treatment experienced very high weed pressure due to the lack of a nurse crop to outcompete the weeds before the slower-establishing RC could shade out competition. However, what was unexpected was that in the HRSW, the HVO would have similar weed pressure as the RC (Table 21) considering that the HVO was virtually weed-free in 2013. The amount of weeds in each treatment as measured in (Table 21) are slightly subjective however, as the plots were hand-weeded in late June (Table 10), which could have resulted in inconsistent weed control between treatments. Hand weeding in late June however was well into HRSW crop growth and therefore the retrospective effects of competition for light, nutrients, and moisture are hard to determine.

Organic Sb plantings are traditionally very weedy, and it is surprising that there were not more weeds in the subsequent HRSW. This could be due to hand weeding, or also because the Sb were tilled in on July 8, 2013 and replanted (Table 10), terminating many weeds that had sprouted but not yet set seed, and thereby lowering the number of weeds in the weed seed bank.

Observations of the treatment effects on wheat growth habit over the course of the 2014 growing season seemed to signify significant differences between the treatments. Wheat where the preceeding crop had been RC or HVO was taller, greener, and lusher than wheat grown in plots that were formerly CVO or Sb. Similarly, within the same GrM, spring incorporated treatments were visibly less N deficient than the fall incorporated counterparts (i.e. taller, greener). However, there was within-treatment variation in GrM biomass-N grown in 2013. This within-treatment variation carried forward into the incubation and in situ soil N_{min} sampling and wheat measurements. It is highly probable that this resulted in an inflated MSE making differences between the treatment means harder to detect.

Only a small area of wheat was harvested by hand to determine yield, and then run through a stationary plot combine. It was determined halfway through the combining of the treatments that the machine was malfunctioning at which point debris was removed from the feeder and threshing cylinder. After the debris was removed, the yields of the plots improved. It is also

highly likely that there was intermingling of some treatments within the combine, resulting in a dilution of treatment effects.

6.3.2. Saint-Mathieu-de-Beloeil, Québec

No significant differences were found between treatments in the number of spikes per plant. However, there were also no significant differences in N_{min} as measured in the field at the onset of tillering (Zadoks 21, $GDD_{0°C}$ 511) (Table 20), suggesting that the HRSW plants in all of the treatments had similar amounts of available N_{min}.

While there were no significant differences in the change in N_{min} as measured in a controlled environment setting, there were marginally significant differences in the total amount of net N_{min} (Table 19). This does not quite match up with wheat growth response to N as there were marginally significant differences in wheat biomass and wheat biomass-N (Table 23). For example, the total net amount of N_{min} in the controlled environment study was greatest for HVO-Spring (43.7 kg N_{min} ha^{-1}). It would be expected that the HVO-Spring would have resulted in the highest wheat biomasses and wheat biomass-N, however, this was not the case. As HVO-Spring had some of the highest net N_{min} and yet not the highest wheat biomass nor wheat biomass-N this suggests that N_{min} availability happened outside the window of crop uptake. It is also possible that low wheat biomass in the HVO-Spring could be due to weed competition as the HVO-Spring was the second weediest plot (101 kg weed DM ha^{-1}), although not statistically different from all other treatments except Oat-Fall (Table 23).

Normally with heavy soils, fall tillage is preferred as plowing in the spring is difficult and can delay planting. What is particularly interesting is that while there were no significant differences between RCO-Spring and RCO-Fall for wheat biomass and wheat biomass-N, there were significant differences between HVO-Spring and HVO-Fall. It would have been expected that the effect would be consistent across GrM, but it is likely that the nature of the GrM impacted N mineralization. For example, the HVO left a deep mulch layer in the spring which would have prevented the soils from warming to the same extent that would have

occurred with HVO tilled in the fall. Cooler soils have slower N mineralization rates, and this was seen in the in situ soil sampling (Figure 15) where the in situ N_{min} for HVO-Fall was higher than for HVO Spring at 0 $GDD_{0°C}$ and 511 $GDD_{0°C}$, although not significantly different (Table 20).

To continue this line of reasoning of cooler soils in the early growing season underneath a layer of mulch impacting mineralization rates, it is interesting to note that the amount of N contained in the aboveground biomass in the HRSW crop was much more than was net mineralized during the incubation period, especially for HVO. The amount of total biomass-N captured in the HRSW + weeds for HVO-Fall (62.1 kg biomass-N ha^{-1}, Table 23) was 207% the total amount of N_{min} from the incubation study (30.0 kg N_{min} ha^{-1}, Table 19). The amount of total biomass-N captured by HRSW + weeds for HVO-Spring on the other hand (50.2 kg biomass-N ha^{-1}, Table 23), is only 115% of the total amount of N_{min} from the incubation study (43.7 kg N_{min} ha^{-1}, Table 19). While the total amount of N_{min} captured in the incubation study for HVO-Spring is significantly higher than the amount from HVO-Fall, the fact that this does not play out in the field suggests that the environment in situ played a large role on the amount of N_{min} during significant periods of crop growth.

In situ biomass recovery of N (HRSW + weeds, Table 23) in all treatments outstripped the total amount of N_{min} measured in the incubation study in all treatments (Table 19). Similar to above, RCO-Spring mineralized a total of 38.1 kg N ha^{-1} in the controlled environment study (Table 19), however total biomass-N in the HRSW + weeds was 52.4 kg biomass-N ha^{-1} (Table 23), almost 1.5 times the amount that would have been expected. This continues to suggest that in situ conditions had an effect on N mineralization outside of what was captured in this study, and in retrospect, field soil temperature and moisture conditions should have been monitored.

In Québec, a leguminous GrM resulted in higher yields, protein content, kernels $plant^{-1}$, and 1000 kernel weight compared to oats (Table 24). This is similar to findings in North Dakota, where it was found that spring wheat following a leguminous GrM will have a greater yield and 1000 kernel weight compared to wheat following wheat in one year at two sites

(Badaruddin and Meyer, 1990). There were no significant effects of incorporation timing on wheat yield and quality parameters (Table 24).

It is important to note that wheat grain yields in Québec were quite low for the region. This is most likely due to the late seeding on May 20. Later planting has been shown to reduce yield in organic spring wheat production (Darby et al., 2011, 2013, 2014).

7. Conclusions

In Nova Scotia there were no significant differences found in the rate of N mineralization (slope, Table 13) between treatments (Hypothesis 1). In Nova Scotia, there were significant differences found in the quantity of cumulative N_{min} between treatments, where RC and HVO had significantly more total N_{min} in comparison to CVO, Sb, and the fallow, regardless of incorporation timing (Table 14). Spring incorporation of RC resulted in the most N_{min} over the course of the incubation, although not statistically significant from RC-Fall (Table 14) (Hypothesis 1).

Similarly, in Québec, there were no significant differences found in the rate of N mineralization (*k*, Table 18) between treatments (Hypothesis 1). There were marginally significant differences among the treatments for the total amount of N_{min}, with HVO-Spring resulting in the greatest amount of N_{min} (43.7 N_{min} ha^{-1}), although not statistically significant from RCO-Spring and RCO-Fall (Table 19). There were no significant differences between treatments for the amount of net N_{min} over the course of the incubation (Table 19) (Hypothesis 1).

The quantity of soil N_{min} measured in situ at HRSW planting was significantly different between treatments in Nova Scotia (Table 16) and GrM of RC and HVO resulted in significantly more soil N_{min} compared to CVO and Sb, regardless of incorporation timing. In Québec, RCO and HVO resulted in significantly more soil N_{min} compared to oats (Table 20), regardless of incorporation timing (Hypothesis 3).

In Nova Scotia, the peak amount of soil N_{min} in excess of crop demand occurred prior to significant crop growth at 109 and 311 $GDD_{0°C}$ (Table 17). Spring incorporation of GrM resulted in significantly higher soil N_{min} than fall incorporation, and RC and HVO had significantly higher soil N_{min} than CVO and Sb (Hypothesis 4).

In Québec, peak soil N_{min} was recorded at 511 $GDD_{0°C}$, but there were no significant differences between treatments (Table 20) (Hypothesis 4). This is most likely because the HRSW was already tillering (Zadoks 21, Table 11), and had started the period of high crop demand resulting in short retention timing for soil N_{min}.

There was a marginally significant effect ($p = 0.0774$) of GrM type on wheat biomass-N in Nova Scotia (Table 21), with a GrM of RC resulting in the highest wheat biomass-N, although not significantly different from HVO, and CVO. Across all GrM, spring incorporation resulted in more wheat biomass-N than fall incorporation (Hypothesis 2).

There was a marginally significant interaction ($p = 0.0913$) of GrM and incorporation timing in Québec for wheat biomass-N (Table 23). A fall incorporated GrM of HVO resulted in the most wheat biomass-N, although not significantly different from RCO-Spring (Hypothesis 2).

Regardless of incorporation timing, in Nova Scotia, a GrM of HVO resulted in the highest wheat yield (Table 22, Hypothesis 5). A GrM of HVO also resulted in the highest number of kernels per plant, but not significantly different from CVO. Neither GrM type nor incorporation timing had any effect on protein nor on 1000 kernel weight.

In Québec, a leguminous GrM resulted in significantly greater wheat yield, grain protein, kernels $plant^{-1}$ and 1000 kernel weight in comparison to wheat following oats (Table 24, Hypothesis 5). Incorporation timing of GrM had no effect on yield or quality parameters of HRSW.

Using GrM to meet the N needs of a cash crop is a common practice in organic agriculture, and arguably one of the cheaper methods of doing so. However, great thought should be put into not only GrM selection, but also management practices. Further understanding of the dynamics of N mineralization will help organic farmers choose a GrM where the N is released at a time when the cash crop needs it. Cook et al. (2010) suggested modifying GrM termination and cash crop planting times to synchronize N release with cash crop need. It is perhaps also necessary to tailor GrM selection depending on the intended following cash crop to fully synchronize N mineralization with crop N uptake.

Chapter 4: Conclusions and Future Directions

The use of leguminous green manure (GrM) is a common method for providing nitrogen (N) to a cash crop in organic systems, however selecting the best GrM for the system and synchronizing soil mineral N availability with crop demand are ongoing challenges. If GrM are to be used effectively, further understanding is needed of how different GrM systems accumulate N and how to predict when and how much of that N is mineralized over the course of the growing season.

1. Understanding Green Manure Growth Patterns

1.1. Conclusions

Through a greater understanding of how GrM accumulate N, a farmer will be better equipped to make management decisions that best fit his or her agricultural system. For example, results from this work reveal that a spring planted GrM of hairy vetch (*Vicia villosa* Roth)-oats (*Avena sativa* L.) (HVO) continues to accumulate N in its aboveground biomass linearly with increasing growing degree days (base 4°C) in quantities ranging from 123 to 292 kg N ha^{-1} (Chapter 2, Hypothesis 3). Conversely, a spring-planted GrM of common vetch (*Vicia sativa* L.)-oats (CVO) accumulates less N in comparison to HVO and is a determinate: after senescence, the amount of N in the aboveground biomass and biomass-N start to decline (Chapter 2, Hypothesis 1). Spring-planted monocropped red clover (*Trifolium pratense* L.) (RC) reaches peak aboveground N accumulation in mid-September, accumulating approximately 100 kg N ha^{-1}, and after mid-September the aboveground biomass starts to decline. However, RC in a biculture with oats (RCO) in mid-September will have similar amounts of aboveground biomass-N as in mid-October, ranging from 51 to 104 kg N ha^{-1} (Chapter 2, Hypothesis 1).

Red clover is very effective at fixing nitrogen, deriving a greater percent of N from the atmosphere than HV in both years when measured with the natural abundance method at the

Québec site and in one year at the Nova Scotia site. (Chapter 2, Hypothesis 2). In Nova Scotia in one year, HV fixed a greater quantity of N from the atmosphere than CV and RC, although statistically similar amounts as RC in a second year as determined using natural abundance methods. Using the difference method, HV derived more N from the atmosphere than CV and RC in both years in Nova Scotia. In Québec, HV was better at fixing N than RC in one year of two using natural abundance methods. Hairy vetch derived greater amounts of N from the atmosphere than RC in both years using the difference method (Chapter 2, Hypothesis 2). While not measured directly, the lack of RC inoculum in the second year of this study appeared to have a great impact on RC productivity when RC was both monocropped or in a biculture. The lack of an organically certified RC inoculum is of rising concern in the organic industry.

1.2. Future Directions

Perhaps a farmer cannot undertake the economic losses of putting a piece of land into a full-season GrM, but must examine alternative arrangements that maximize land use such as planting a short season crop before or after a GrM. Alternatively, further examination of intercropping a GrM into a cash crop would help to offset the costs of having a field out of production for an entire year. Further understanding of how a GrM accumulates N with respect to photoperiod as well as growing degree day models will help aid in this decision making process. Future work could also examine how GrM accumulate N when interseeded into a cash crop, for example undersowing a GrM into cabbage, broadcast planting a GrM into a recently harvested small grain stubble, etc.

2. Understanding Nitrogen Mineralization of Green Manures

2.1. Conclusions

While a GrM of HVO generally accumulates significantly more aboveground biomass-N than RC or RCO, when mineralization was monitored both in situ and in controlled

environment conditions, the two GrM acted very similarly in N mineralization patterns and quantity. In a controlled environment study, there were no significant differences between a fall versus spring incorporation of HVO or RC in terms of total N mineralized in Nova Scotia, and marginal significance in Québec ($p = 0.0614$) (Chapter 3, Hypothesis 1). In the system trialed in Nova Scotia of a spring-planted, full-season GrM of CVO, this GrM did not mineralize significantly differently than what was recorded following a cash crop of soybeans (*Glycine max* L. Merr.) suggesting that the termination timing for CVO in this study was not well suited that GrM.

In Nova Scotia, regardless of incorporation timing, a GrM of RC and HVO had significantly more soil N_{min} present at hard red spring wheat (*Triticum aestivum* L.) (HRSW) planting than did a GrM of CVO or a previous crop of soybeans (Chapter 3, Hypothesis 3). In Québec, a proceeding leguminous GrM, regardless of incorporation timing, resulted in significantly more soil N_{min} at HRSW planting than did a previous crop of oats (Chapter 3, Hypothesis 3).

In Nova Scotia, at peak soil N_{min} measured in situ (109 and 311 GDD), spring incorporated GrM resulted in significantly more soil N_{min} than did fall incorporated GrM. A GrM of RC and HVO as resulted in significantly more soil N_{min} than did CVO or soybeans (Chapter 3, Hypothesis 4). At peak soil N_{min} measured in situ (511 GDD), there was no significant differences between previous crop nor incorporation timing in Québec (Chapter 3, Hypothesis 4).

The GrM in situ N mineralization was only conducted in one year at both sites, and should be repeated for a second year. Additionally, root exclusion cores in situ would give a clearer picture of field N mineralization in the absence of plant competition.

2.2. Future Directions

One of the benefits of a leguminous GrM is not only the amount of fixed-N returned to the system, but also the conserved soil N: essentially the N that was already present in the soil

that the GrM does not have to take up as it is creating its own N (Peoples et al., 1995). Soil N dynamics are driven by numerous factors such as soil temperature and water content, oxygen availability, and substrate quantity and quality (Brady and Weil, 2002; Dessureault-Rompré et al., 2010, 2011; Cooper et al., 2011; Georgallas et al., 2012; Gillis and Price, 2015). Well over 250 models have been generated to predict biogeochemical processes in soil (Manzoni and Porporato, 2009), attempting to describe and quantify a complex system. However, while these models contribute to the greater understanding of elemental cycling of soils, they are necessarily mathematically complex making them inaccessible to farmers trying to predict mineralization within their agro-ecosystems. Adapt-N is an online decision support tool that is designed to help farmers manage in-season N inputs developed through Cornell University in New York. This computer model incorporates high resolution weather information with user information on soil and crop management to provide N sidedress recommendations for grain, silage, and sweet corn (*Zea mays* L.). However, this model as yet does not include GrM, cover crops, organic systems, or crops other than corn.

Further work is needed in how on-farm management can alter GrM quality and thereby affect N mineralization. For example, if a farmer were to adjust his or her seeding rate, how would that affect the final C:N ratio and other quality parameters of a GrM and the subsequent mineralization? Suppose a GrM has been grown, and it has been found that it has a low C:N, as was found with the HVO in this study (13.8:1 in late October). Are there management practices that can be used to increase the C:N ratio (i.e. adding straw or a high carbon compost to the system prior to GrM incorporation) and thereby fend off a rapid flush of mineral N early in the growing season, prior to crop demand?

Additionally, do GrM incorporation strategies play a role in the GrM mineralization? For example, what kind of effect could be expected if the crop is terminated with an herbicide versus flail mowed or sicklebar mowed prior to incorporation? Different types of tillage affect litter particle size, litter placement within the soil profile, aeration, soil moisture, and soil temperature: how does this affect GrM mineralization?

3. Hard Red Spring Wheat Yield Responses to Green Manures

3.1. Conclusions

Based on the similarities between RC, RCO, and HVO in terms of how N_{min} was quantified in this study, it was not expected that there would be significant differences in HRSW yield and yield parameters. But what was surprising was that CVO, HVO, and RC were similar in the amount of N found in the wheat biomass ($p = 0.0774$), and even more surprising was that there were no significant differences between a GrM of CVO, cash crop of soybeans, HVO and RC with regards to total wheat biomass in Nova Scotia, protein, or 1000 kernel weight (Chapter 3, Hypothesis 2 and 5). However, ultimately, HVO did result in significantly higher wheat yield, almost 1000 kg ha^{-1} more than RC, the latter which was statistically similar to CVO (Chapter 3, Hypothesis 5).

In Québec, yields were very low, less than 2000 kg ha^{-1}, most likely due to late planting. A previous GrM of RCO and HVO performed statistically similarly with respect to wheat yield, protein, 1000 kernel weight, and total biomass-N, which was reflective of the amount of mineral N monitored in the system (Chapter 3, Hypothesis 2 and 5).

3.2. Future Directions

Future work in Eastern Canada would benefit from further analysis of planting date by seeding rate interactions of HRSW, and how N fertility at different timings affects yield parameters. Additionally, this study did not analyze the effect of GrM on HRSW quality parameters such as impact on *Fusarium graminearum* and deoxynivalenol levels which are important considerations for the end use of the grain.

4. Future Directions: Green Manure Establishment Costs

Observational data from this study suggested that a lower population of HV, as seen in Nova Scotia in 2014 and in Québec in 2013, results in a higher HVO biomass. This suggests that more work is needed to determine if 30 kg live seed ha^{-1} is indeed an appropriate seeding rate for a spring-planted GrM of HVO in Eastern Canada. This is certainly worth further research particularly as one of the primary differences between different GrM treatments is seeding cost. Hairy vetch seed, for example, is very expensive, approximately $130 for a 50 lbs bag of organic seed, and oats are relatively cheap at approximately $20 for a 55 lbs bag of organic seed. Red clover is equally expensive as HV at $177 for a 55 lbs bag of organic seed. However, using the seeding rates in this study, HVO cost $227 ha^{-1} in seeding costs, whereas RC cost $85 ha^{-1}. Additionally, while the HVO could be disked in, RC has to rototilled or moldboard plowed in order to terminate the RC, a more energy intensive incorporation strategy.

Initially looking at the amount of GrM-N in Nova Scotia, where HVO had almost twice the amount of biomass-N as RC in late October of 2013, it seemed like the HVO might be worth the added expense. However, in both in situ and in a controlled environment, the two treatments performed very similarly in the amounts of N mineralized. Additionally, when it came to cash crop N-uptake, RC and HVO had statistically similar amounts of total wheat biomass-N, protein, and 1000 kernel weight. When looking at the amount of N in the aboveground wheat biomass following the different GrM, fall-incorporated HVO ended up costing $3.99 (kg wheat biomass-N)$^{-1}$ and spring-incorporated HVO cost $2.98 (kg wheat biomass-N)$^{-1}$ in Nova Scotia. On the other hand, fall-incorporated RC cost $1.34 (kg wheat biomass-N)$^{-1}$ and spring-incorporated RC cost $1.10 (kg wheat biomass-N)$^{-1}$. However, HVO did result in almost an additional 1000 kg ha^{-1} of wheat, which might suggest that perhaps the added expense is worth it.

5. In Conclusion

There are many leguminous GrM that an organic farmer can choose from to meet the N needs of his or her crop. Ultimately, N demand and N uptake patterns of the subsequent crop should play a large role in GrM selection, along with costs of GrM seeding, management and termination. Different incorporation practices may impact N mineralization and should be tailored to fit the agricultural system with further consideration given to erosion control, weed management, and field moisture in the following season.

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Appendix

A. Table 1. Biomass accumulation in Nova Scotia in 2013.

Sample date	GrM	Legume (kg DM ha^{-1})		Oats (kg DM ha^{-1})		Total biomass$^{\Phi}$ (kg DM ha^{-1})	
1	CVO	956	E	1889	B	3436	D
1	HVO	2579	CD	2619	A	5572	BC
1	RC	2805	CD	-		4269	D
2	CVO	4281	B	2039	B	6430	AB
2	HVO	4171	B	1822	B	6028	AB
2	RC	3276	BC	-		4538	CD
3	CVO	1979	DE	1509	BC	3627	D
3	HVO	5860	A	1174	C	7034	A
3	RC	1898	DE	-		3339	D
sd*GrM	p-value	<.0001		0.0251		0.0014	

Values followed by the same letter are not significantly different.

$^{\Phi}$ Total biomass includes weed biomass.

GrM = green manure

sd = sampling date

DM = dry matter

CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)

HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats

RC = a green manure of red clover (*Trifolium pretense* L.)

A. Table 2. Biomass accumulation in Nova Scotia in 2014.

Sample date	GrM	Legume (kg DM ha^{-1})		Oats (kg DM ha^{-1})	Total biomass$^{\Phi}$ (kg DM ha^{-1})	
1	CVO	1137	CD	4009	5567	CD
1	HVO	3500	B	3089	6689	BC
1	RC	621	D	-	3474	EF
2	CVO	1268	CD	3205	5273	D
2	HVO	6746	A	1657	8403	A
2	RC	1750	C	-	4586	DE
3	CVO	719	D	1786	2881	F
3	HVO	6464	A	1038	7502	AB
3	RC	1206	CD	-	2622	F
sd	p-value	<.0001		<.0001	0.0002	
GrM	p-value	<.0001		0.0009	<.0001	
sd*GrM	p-value	0.0002		0.3619	0.0066	

Values followed by the same letter are not significantly different.

$^{\Phi}$ Total biomass includes weed biomass.

GrM = green manure

sd = sampling date

DM = dry matter

CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)

HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats

RC = a green manure of red clover (*Trifolium pretense* L.)

A. Table 3. Biomass accumulation in Québec in 2013.

Sample date	GrM	Legume (kg DM ha^{-1})		Oats (kg DM ha^{-1})		Total biomass$^{\Phi}$ (kg DM ha^{-1})	
1		1841	ǂ	5905	*	7846	
2		2751	ǂ	3525	a	6711	
3		3351	ǂ	2667	b	6411	
	HVO	3330	ǂ	4136		7696	A
	RCO	2027	ǂ	2942		5493	B
1	HVO	1841	C	5905		7846	
1	RCO	NA		NA		NA	
2	HVO	3539	B	3639		7600	
2	RCO	1962	C	3411		5822	
3	HVO	4611	A	2862		7659	
3	RCO	2091	C	2472		5163	
sd	p-value	<.0001		<.0001		0.6266	
GrM	p-value	<.0001		0.1646		<.0001	
GrM*sd	p-value	0.0328		0.7113		0.2726	

Values followed by the same letter within each column are not significantly different.

Values followed by a ǂ have a higher order interaction and therefore do not have letter groupings representing significant differences.

NA – data not available for RCO, sample date 1.

Φ Total biomass includes weed biomass.

GrM = green manure

sd = sampling date

DM = dry matter

HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)

RCO = a green manure of red clover (*Trifolium pretense* L.)/oats

A. Table 4. Biomass accumulation in Québec in 2014.

Sample date	GrM	Legume (kg DM ha^{-1})		Oats (kg DM ha^{-1})		Total biomass$^{\Phi}$ (kg DM ha^{-1})	
1		1155	ǂ	1761	a	3183	ǂ
2		1871	ǂ	1392	b	3550	ǂ
3		2381	ǂ	1195	b	3861	ǂ
	HVO	2864	ǂ	1146	b	4058	ǂ
	RCO	812	ǂ	1785	a	2955	ǂ
1	HVO	2122	C	1439		3655	B
1	RCO	309	E	2082		2710	D
2	HVO	2651	B	1025		3751	B
2	RCO	1091	D	1881		3282	BC
3	HVO	3727	A	974		4769	A
3	RCO	1035	D	1416		2954	CD
sd	p-value	<.0001		<.0001		0.0008	
GrM	p-value	<.0001		<.0001		<.0001	
GrM*sd	p-value	<.0001		0.2738		0.0013	

Values followed by the same letter are not significantly different.

Values followed by a ǂ have a higher order interaction and therefore do not have letter groupings representing significant differences.

$^{\Phi}$ Total biomass includes weed biomass.

GrM = green manure

sd = sampling date

DM = dry matter

HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats(*Avena sativa* L.)

RCO = a green manure of red clover (*Trifolium pretense* L.)/oats

A. Table 5. Green manure biomass nitrogen accumulation in Nova Scotia in 2013.

Sampling date	GrM	Legume (kg N ha^{-1})		Oat (kg N ha^{-1})		Total biomass$^{\Phi}$ (kg N ha^{-1})	
1	CVO	34	F	25.4	AB	68	E
1	HVO	99	CD	33.8	A	139	BC
1	RC	75	DE	-		100	DE
2	CVO	118	BC	25.6	AB	146	BC
2	HVO	151	B	25.4	AB	177	B
2	RC	88	CD	-		110	CD
3	CVO	46	EF	22.6	B	72	DE
3	HVO	203	A	18.1	B	221	A
3	RC	46	EF	-		70	E
sd	p-value	0.0007		0.0194		0.0039	
GrM	p-value	<.0001		0.5872		<.0001	
GrM*sd	p-value	0.0002		0.0975		0.0009	

Values followed by the same letter within each column are not significantly different.
Φ Total biomass includes weed biomass nitrogen.
GrM = green manure
sd = sampling date
CVO = a green manure of common vetch (Vicia sativa L.)/oats (Avena sativa L.)
HVO = a green manure of hairy vetch (Vicia villosa Roth)/oats
RC = a green manure of red clover (Trifolium pretense L.)

A. Table 6. Green manure biomass nitrogen accumulation in Nova Scotia in 2014.

Sampling date	GrM	Legume (kg N ha^{-1})		Oat (kg N ha^{-1})	Total biomass$^{\Phi}$ (kg N ha^{-1})	
1	CVO	27	D	33.1	64	DE
1	HVO	119	B	31.5	152	B
1	RC	19	D	-	53	E
2	CVO	36	CD	29.9	88	CD
2	HVO	275	A	16.9	292	A
2	RC	62	C	-	101	C
3	CVO	20	D	14.1	41	E
3	HVO	257	A	10.6	268	A
3	RC	41	CD	-	60	E
sd	p-value	<.0001		0.0002	<.0001	
GrM	p-value	<.0001		0.0314	<.0001	
GrM*sd	p-value	<.0001		0.1683	<.0001	

Values followed by the same letter within each column are not significantly different.
Φ Total biomass includes weed biomass nitrogen.
GrM = green manure
sd = sampling date
CVO = a green manure of common vetch (*Vicia sativa* L.)/oats (*Avena sativa* L.)
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats
RC = a green manure of red clover (*Trifolium pretense* L.)

A. Table 7. Green manure biomass nitrogen accumulation in Québec in 2013.

Sampling date	GrM	Legume (kg N ha^{-1})		Oat (kg N ha^{-1})		Total biomass $^{\Phi}$ (kg N ha^{-1})	
1		67.5	ǂ	58.8	ǂ	136	ǂ
2		88.1	ǂ	37.7	ǂ	135	ǂ
3		109.4	ǂ	33.5	ǂ	151	ǂ
	HVO	112.4	ǂ	47.2	ǂ	167	ǂ
	RCO	62.7	ǂ	29.9	ǂ	103	ǂ
1	HVO	67.5	C	58.8	A	136	C
1	RCO	NA		NA		NA	
2	HVO	115.6	B	41.1	BC	165	B
2	RCO	60.7	C	34.2	C	104	D
3	HVO	154.1	A	41.5	B	200	A
3	RCO	64.8	C	25.6	D	102	D
sd	p-value	<.0001		<.0001		0.0007	
GrM	p-value	<.0001		<.0001		<.0001	
GrM*sd	p-value	0.0574		0.0815		0.0702	

Values followed by the same letter within each column are not significantly different.
Values followed by a ǂ have a higher order interaction and therefore do not have letter groupings representing significant differences.
NA – data not available for RCO, sample date 1.
Φ Total biomass includes weed biomass nitrogen.
GrM = green manure
sd = sampling date
DM = dry matter
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)
RCO = a green manure of red clover (*Trifolium pretense* L.)/oats

A. Table 8. Green manure biomass nitrogen accumulation in Québec in 2014.

Sampling date	GrM	Legume (kg N ha^{-1})		Oat (kg N ha^{-1})		Total biomass $^{\Phi}$ (kg N ha^{-1})	
1		37.7	ǂ	16.8	ǂ	56.8	ǂ
2		58.6	ǂ	13.1	ǂ	79.0	ǂ
3		70.4	ǂ	11.3	ǂ	87.5	ǂ
	HVO	88.8	ǂ	11.6	ǂ	101.9	ǂ
	RCO	22.4	ǂ	16.0	ǂ	44.1	ǂ
1	HVO	66.4	C	14.7	BC	83.0	C
1	RCO	9.0	E	18.9	A	30.7	E
2	HVO	88.0	B	9.6	E	99.7	B
2	RCO	29.2	D	17.2	AB	51.4	D
3	HVO	111.9	A	10.2	DE	123.0	A
3	RCO	28.8	D	12.3	CD	51.9	D
sd	p-value	<.0001		<.0001		<.0001	
GrM	p-value	<.0001		<.0001		<.0001	
GrM*sd	p-value	0.001		0.0309		0.0173	

Values followed by the same letter within each column are not significantly different.
Values followed by a ǂ have a higher order interaction and therefore do not have letter groupings representing significant differences.
Φ Total biomass includes weed biomass nitrogen.
GrM = green manure
sd = sampling date
DM = dry matter
HVO = a green manure of hairy vetch (*Vicia villosa* Roth)/oats (*Avena sativa* L.)
RCO = a green manure of red clover (*Trifolium pretense* L.)/oats

A. Table 9. Range of δ15N measurements in Nova Scotia.

GrM	δ15N (‰)			
	2013		2014	
	high	low	high	low
CV	0.08	-0.27	-0.06	-0.54
HV	0.29	-0.19	0.25	-0.02
RC	0.12	-0.60	-0.28	-0.43
CV - reference: oats	12.48	5.35	5.78	5.34
HV – reference: oats	9.94	4.52	7.04	5.25
RC – reference: weeds	7.36	3.11	5.71	4.52

CV = common vetch (*Vicia sativa* L.)
HV = hairy vetch (*Vicia villosa* Roth)
RC = red clover (*Trifolium pretense* L.)
GrM = green manure

A. Table 10. Nitrogen derived from atmosphere estimates from first sampling date using isotopic methods in Nova Scotia.

GrM	2013		2014	
	pNDFA	kg Ndfa ha^{-1}	pNDFA	kg Ndfa ha^{-1}
CV	94.3	31.7 B	94.0 AB	25.1 B
HV	92.0	91.4 A	89.0 B	106.2 A
RC	92.7	68.5 A	95.3 A	17.9 B
STE	1.93	10.23	1.68	8.41
p-value	0.7058	0.0102	0.0824	0.0022

Values followed by the same letter are not significantly different.
CV = common vetch (*Vicia sativa* L.)
HV = hairy vetch (*Vicia villosa* Roth)
RC = red clover (*Trifolium pretense* L.)
STE = standard error
Ndfa = nitrogen derived from the atmosphere
pNDFA = percent nitrogen derived from the atmosphere
GrM = green manure

A. Table 11. Estimates of nitrogen fixation in Nova Scotia using the difference method.

GrM	Sampling date	N_{dfa} (kg N ha^{-1}) 2013		N_{dfa} (kg N ha^{-1}) 2014	
CV	1	-0.7	F	-10.7	CD
HV	1	59.5	D	86.6	B
RC	1	50.0	DE	-15.0	D
CV	2	95.9	BC	-16.5	D
HV	2	116.5	B	258.1	A
RC	2	65.8	CD	23.7	C
CV	3	20.6	EF	-1.3	CD
HV	3	184.5	A	246.3	A
RC	3	21.6	EF	20.9	C
sd*GrM	STE	11.65		11.66	
sd*GrM	p-value	<.0001		<.0001	

Values followed by the same letter are not significantly different.
CV = common vetch (*Vicia sativa* L.)
HV = hairy vetch (*Vicia villosa* Roth)
RC = red clover (*Trifolium pretense* L.)
STE = standard error
Ndfa = nitrogen derived from the atmosphere
sd = sampling date
GrM = green manure

A. Table 12. Range of δ15N measurements in Québec.

GrM	δ15N (‰) 2013		2014	
	high	low	high	low
HV	0.29ϕ	-0.28	2.37ϕ	-0.36
RC	-0.58	-0.85	-0.48ϕ	-1.00
HV-reference: oats	3.76	-0.29ϕ	2.78ϕ	1.01
RC-reference: oats	1.74	-0.89ϕ	2.48	1.20ϕ

ϕ Values are from blocks that were removed to induce normality in the data set as the blocks contained extreme outliers.
HV = hairy vetch (*Vicia villosa* Roth)
RC = red clover (*Trifolium pretense* L.)
GrM = green manure

A. Table 13. Nitrogen derived from atmosphere estimates from first sampling date using isotopic methods in Québec.

GrM	2013		2014	
	pNDFA	kg Ndfa ha^{-1}	pNDFA	kg Ndfa ha^{-1}
RC	82.2 A	57.7	92.5 A	10.2 B
HV	54.2 B	42.8	73.8 B	54.4 A
STE	4.88	6.76	1.9	4.56
p-value	0.021	0.211	0.000	0.002

Values followed by the same letter are not significantly different.
HV = hairy vetch (*Vicia villosa* Roth)
RC = red clover (*Trifolium pretense* L.)
STE = standard error
Ndfa = nitrogen derived from the atmosphere
pNDFA = percent nitrogen derived from the atmosphere
GrM = green manure

A. Table 14. Estimates of nitrogen fixation in Québec using the difference method.

GrM	Sampling date	N_{dfa} (kg N ha^{-1}) 2013	N_{dfa} (kg N ha^{-1}) 2014
HV	1	-1.2 D	49.8 C
RC	1	*	-12.6 E
HV	2	65.7 B	76.3 B
RC	2	17.6 CD	-3.6 E
HV	3	108.6 A	100.7 A
RC	3	27.1 C	13.7 D
sd*GrM	STE	9.26	4.34$^{\phi}$
sd*GrM	p-value	0.0822	0.0258

Values followed by the same letter are not significantly different.
Values replaced by an * denote a missing value.
$^{\phi}$ STE for RC*sd3 = 4.70 due to the removal of an extreme value
HV = hairy vetch (*Vicia villosa* Roth)
RC = red clover (*Trifolium pretense* L.)
STE = standard error
Ndfa = nitrogen derived from the atmosphere
sd = sampling date
GrM = green manure