ASSESSMENT OF COVER CROP SELECTION ON NITROGEN CYCLING IN A THREE-YEAR POTATO ROTATION

by

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ABSTRACT

Alternative potato rotations are increasingly being examined that can supply N to potato crops and mitigate nitrate (NO_3^-) leaching from the root zone. This study examined a legume (RC), grass (T) or legume/ grass mixture (M) on soil N supply, and soil solution N in a subsequent potato crop. The below-ground N contribution of cover crops was also examined using ^{15}N labeled fertilized. Total N uptake in unfertilized potatoes was greater under RC and M than T but did not significantly affect total tuber yield. Overall, NO_3^- susceptible to leaching was lowest under T. From steel lysimeters, at 80 cm below-ground, the M and T treatments resulted in a 66 and 86% reduction in NO_3^- respectively from 83 mg NO_3-N L^{-1} from unfertilized potato crops grown subsequent to RC. Labeled root N accounted for 24% of total ^{15}N recovered from residues into a subsequent unfertilized potato under RC and M.

LIST OF ABBREVIATIONS AND SYMBOLS USED

≈	Approximately equal
0N	0 kg N ha^{-1} fertilizer
190N	190 kg N ha^{-1} fertilizer
AEM	Anion exchange membrane
AG	Above-ground ^{15}N labeled tissue (whole plant)
AG_{only}	Above-ground ^{15}N labeled tissues only
ANOVA	Analysis of Variance
BG	Below-ground (soil + root) ^{15}N labeled tissue (whole plant)
BMP	Beneficial management practice
BNF	Biological nitrogen fixation
C	Carbon
C:N	Carbon to nitrogen ratio
GLM	General linear model
M	Red clover-timothy mixture
N	Nitrogen
N_2	Dinitrogen
N_{dff}	^{15}N fertilizer recovery in crop residues from labeled fertilizer sources
N_{dfr}	^{15}N fertilizer recovery in crop from labeled residue sources
NH_3	Ammonia
NH_4^+	Ammonium
NH_4-N	Ammonium-Nitrogen
NO_3^-	Nitrate
NO_3-N	Nitrate-Nitrogen
NO_3NH_4	Ammonium nitrate
NPK	Nitrogen-phosphorous-potassium
PEI	Prince Edward Island
PK	Phosphorous-potassium
PVC	Polyvinyl chloride
R-NH_2	Organic N compound
RC	Red clover

ROOT	Labeled ^{15}N recovered roots only
S:R	Shoot to root ratio
USA	United States of America

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CHAPTER 1. INTRODUCTION

Potatoes are the most economically viable crop in Prince Edward Island (PEI). However, intensive potato production with high nitrogen (N) inputs, has been linked with elevated groundwater nitrate (NO_3^-) contamination (DesRoches et al. 2008; Zebarth et al. 2015). Strategies to improve N management such as alternative crop rotation that can maintain economic and environmental viability of potato production are therefore receiving increased attention (Zebarth et al. 2009b; Nyiraneza et al. 2015).

In PEI, potatoes are commonly planted in a three-year barley-red clover-potato rotation (Jiang et al. 2012; Nyiraneza et al. 2015). Fall-ploughed red clover can provide N to the subsequent crop as N released from decomposing residues, however, much of this N is lost in the fall and winter (Zebarth et al. 2009b). Alternatively, grasses have been shown to reduce NO_3^- leaching compared to legumes due to a generally greater C:N ratio and extensive root system compared to legumes (Ranells and Wagger 1997a; Stark and Porter 2005). Legume/ grass mixtures are thought to reduce the risk of NO_3^- leaching after the growing season (Ranells and Wagger 1997c) and influence the decomposition rate and subsequent N release from decomposing crop residues by generally lowering the C:N ratio of crop residues compared to a pure grass stand. There is, however, limited information on how a pure grass stand or legume/ grass mixtures affect soil N supply to a subsequent potato crop in PEI. In addition, there is little information on the root contribution of preceding crop residues to a subsequent crop. A cropping system that synchronizes N release from both above- and below-ground crop residues with N uptake by a subsequent potato crop would improve N management in potato crop rotations and decrease the amount of N lost from the plant root zone (Wivstad 1999; Sincik et al. 2008).

CHAPTER 2. LITERATURE REVIEW

2.1. Sources of Nitrogen in Agricultural Production

In the atmosphere, 78% of N is in the stable N_2 form that is unavailable for plant use. Nitrogen can be incorporated into the soil and become available to plants through biological N fixation (BNF) from legume crops. Legumes can fix N_2 from the atmosphere through a symbiotic relationship with N_2-fixing bacteria inside root nodules (Stark and Porter 2005). These bacteria contain enzymes that break the N_2 bond and react with hydrogen to create organic N compounds. Legumes were commonly used to increase N availability within crop rotations prior to the availability of chemical fertilizers.

In the early 20th century the Haber-Bosch process was discovered that artificially fixed N by splitting the N_2 bond and reacting with hydrogen to create ammonia (NH_3). This rapidly increased the availability of NH_3 and subsequently of industrial fertilizer products, and ushered in an age of unprecedented agricultural production. Between the 1940s and the 1950s the use and production of fertilizer N had doubled from 5 million tons to 10 million tons (Smil 1997). Today, an estimated 70 million tons of N applied to crops is from synthetic fertilizer sources (Smil 1997; FAO 2015).

In conventional agricultural systems, the majority of plant available N comes from synthetic fertilizer N but this has also created the potential for increased environmental impact. The increased use of synthetic fertilizer N along with intensive management practices including shorter crop rotations, increased tillage and minimal crop diversity has decreased overall soil health and productivity (Nyiraneza et al. 2015). Additionally, excess N as NO_3^- remaining in the soil after crops are harvested can be easily lost from the root zone and can be leached into groundwater sources (Jiang et al. 2012).

With increasing agricultural productivity, it has become critical to maintain environmental integrity and to develop more sustainable agricultural practices. Legumes are increasingly being re-examined as a means of increasing N availability within cropping systems as a means to reduce synthetic fertilizer N use (Lynch et al. 2012).

2.2. Soil Mineral N and Nitrate Availability

Nitrogen is considered the most limiting nutrient in determining potato crop yield (Zebarth and Rosen 2007). Plants take up most N from the soil in inorganic forms such as NO_3^- or NH_4^+. Organic forms of N in the soil, from soil organic matter (SOM) and crop residues, must first be converted into inorganic N via mineralization in the soil before becoming plant available. Mineralization of SOM is a biological soil process and is largely controlled by heterotrophic microbial decomposers (Zebarth et al. 2009a; Jiang et al. 2012). Inorganic NH_4^+ is released as a by-product of microbial activity and is further nitrified to NO_3^- that can also be used by plants (Sainju & Singh 1997; Dabney et al. 2001).

Immobilization is a complementary process to mineralization, and is the conversion of inorganic N to organic N. Immobilization occurs as a result of microbial biosynthesis when organisms are metabolizing organic substrates with wide C:N ratios. Mineralization and immobilization occur simultaneously in the soil and the relative rate of each process will dictate whether there will be net N mineralization (i.e., increase in plant available N) or net N immobilization (i.e., decrease in plant available N). The relative quantity of the C and N substrates (as indicated by the C:N ratio) is a reliable indicator of whether net mineralization or net immobilization occurs as soil decomposers require both C and N from decomposing materials for protein assimilation and energy, respectively (Jarvis et al. 1996). Generally a lower C:N ratio (< 20) will result in a net

mineralization and a greater C:N ratio (> 40) will result in a net immobilization (Cabrera et al. 2005; Zebarth et al. 2009a). This range reflects the influence of the quality of C and N containing residues (e.g. polyphenol, cellulose, or lignin compounds) on residue decomposition and N release (Cabrera et al. 2005; Crews & Peoples 2005). When immobilization is greater than mineralization (i.e., net immobilization), plants can become N deficient as organisms compete for available N in the soil (Jarvis et al. 1996).

Net N mineralization from preceding crop residues and SOM can account for a significant portion of crop N demand throughout the growing season (Sharifi et al. 2008). The mineralization rate of organic N is strongly dependent on the factors and their interactions that influence microbial activity including soil type, water content, temperature, and tillage management (Cookson et al. 2002; Zebarth et al. 2009b; Dessureault-Rompré et al. 2010). Dessureault-Rompré (2010) reported that soil texture class could affect the results of a temperature-based function for predicting soil N mineralization. Such complex interactions highlight the difficulty in predicting net soil N mineralization, and therefore soil N contribution to crop N uptake.

In Atlantic Canada, there is currently no pre-season N soil test that can accurately determine in-season soil N mineralization. Uncertainty in seasonal soil N supply makes providing accurate fertilizer N recommendations difficult, with both environmental and economic implications (Dessureault-Rompré et al. 2010). In addition, net soil N mineralization can continue in the fall after the period of crop N uptake has ended (Jiang et al. 2015; Zebarth et al. 2015). After the growing season, any NO_3^- present in the soil, whether from N mineralization or N fertilizer, is susceptible to loss.

In situ N availability determination can be done in the field with anion exchange membranes (AEM). Thin positively charged membranes are inserted into the soil and adsorb anions, including NO_3^-, diffusing to a given area of the membrane over time (μg cm^2 day^{-1}). Membranes act as a sink simulating plant roots and provide a way to estimate availability of NO_3^- to plants (Sharifi et al. 2009; Zebarth et al. 2009b). The advantage of measuring NO_3^- with AEMs over traditional soil measurements of KCl extractable NO_3^- is that membranes are left in the soil, integrating NO_3^- supply over a period of time, and thereby providing a nutrient flux estimate (Ziadi et al. 2006). As nutrient supply rate varies with soil type, soil management and cropping practices, AEMs can also be used to compare varying cropping systems or soil types (Qian and Schoenau 2005).

2.3. Nitrogen Removal from Agricultural Production

In agricultural systems, NO_3^- in the soil can be removed by plants or via gaseous loss or leaching pathways. Gaseous losses can occur when denitrifying bacteria are stimulated in anaerobic or poorly drained soil conditions (Galloway et al. 2004). Nitrate is a highly mobile nutrient that is not held by the soil cation exchange capacity so when NO_3^- migrates below the plant root zone, it will eventually flow to groundwater sources. Factors that affect soil NO_3^- leaving the root zone over time include climate, soil type, management practices, amount of N fertilizer applied, and the interaction of soil and climatic factors that affect soil mineralization (Macleod and Sanderson 2002, Zebarth & Rosen 2007; Jiang et al. 2012).

In PEI, potatoes are grown on predominantly sandy soils with low organic matter above shallow unconfined aquifers that are susceptible to NO_3^- contamination (Jiang et al. 2012). The risk of NO_3^- leaching increases when high concentrations of NO_3^- coincide with soil water infiltration events (Jiang et al. 2012), and as a result NO_3^- that remains in

the soil after the growing season is susceptible to loss (Zebarth et al. 2009a). In cool, humid climates such as that of PEI, the short growing season limits the use of fall cover crops with deep root systems that can scavenge residual soil NO_3^-. As a result, most residual NO_3^- is lost from the root zone over winter, or in response to heavy spring snow melt, when plant N uptake is minimal and increases in water movement enhance NO_3^- leaching through the soil profile (Jiang et al. 2012).

In PEI, the main source of N for potato crops is from synthetic fertilizer in order to achieve industry tuber yield and size requirements. The apparent recovery of fertilizer applied N in the potato crop, however, ranges from only 40 to about 60% (Zebarth and Rosen 2007; Jiang et al. 2012). In addition, N returned to the soil in potato vines and N from other N sources such as in-season net N mineralization may contribute further to NO_3^- leaching (Zebarth et al. 2015). Additionally, according to LEACHN modelling of a three year barley-red clover-potato rotation with 150 to 200 kg N ha^{-1} fertilizer N application, 50 to 60% of N losses were within barley and red clover phases of the rotations, indicating that N leaching can be high outside of the potato production year (Jiang et al. 2012; Zebarth et al. 2015).

There are several methods to estimate or quantify NO_3^- leaching. The excess N which can contribute to leaching can be estimated by comparing N inputs (e.g., N fertilizer, mineral N from soil organic matter and crop residues, initial soil mineral N) with N outputs (e.g., crop N uptake, volatilization, denitrification, residual soil mineral N) (Prasad and Hochmuth 2013). Field based measurements of NO_3^- leaching are difficult to quantify, yet are indispensable to calibrating model-based estimates of N leaching. A combination of LEACHN modelling and a tile-drain lysimeter experiment in

PEI predicted N leaching under potato, barley and red clover to be 81, 54, and 35 kg N ha^{-1} with 200, 60 and 0 kg N ha^{-1} fertilizer respectively (Jiang et al. 2011).

To measure NO_3^- concentration in soil solution, suction lysimeters can be used to collect the solution from the water-filled pore spaces in soil. Ceramic suction lysimeters under vacuum require continuously wet pores creating points of contact in a ceramic collection cup to draw in water from the soil (Soil Moisture 2008). When lysimeters are placed under vacuum, soil water that is bound by soil under lower pressure than that of the lysimeter will be drawn into the ceramic cup then pulled up into a connected sample bottle above-ground (Djurhuus & Jacobsen 1995). If used correctly, suction lysimeters are suitable in sandy loam or coarse textured soils with little clay content (< 10%) to monitor soil solution NO_3^- concentration (Wang et al. 2012).

To calculate NO_3^- leaching using suction lysimeters, additional information on water drainage (flow) is required to determine NO_3^- flux. Zotarelli et al. (2007) used the trapezoidal approximated integration rule (Lord & Shepherd 1993) and drainage lysimeters to determine total mass of NO_3^- leached (kg ha^{-1}) by calculating NO_3^- concentration and integrating the cumulative drainage obtained by drainage lysimeters over the area under the plot.

Despite the limitations of such an approach, suction lysimeters are useful as they are relatively cheap and easy to install. Additionally, management practices, such as crop rotations, can be compared using suction lysimeters for field-based experiments. Vos & Putten (2004) compared flux-weighted NO_3^- concentration in suction lysimeters in Wageningen, Netherlands at 80 cm below the surface, in a field experiment with various catch crops cultivated after potato. Averaged across three years, there were lower NO_3^-

concentrations from catch crops of winter rye and forage rape (9.9 mg NO_3-N L^{-1}) compared to fields left fallow, with no catch crop (20.9 mg NO_3-N L^{-1}).

2.4. Crop Rotation Effect on N Availability in Potato Production

2.4.1. Potato Crop Rotations

Beneficial management practices (BMPs) have been explored to improve the sustainability of potato production in PEI, including reducing NO_3^- loading to groundwater. For example, BMPs include improvements to plant and soil based N tests, the development of new potato cultivars, the implementation of novel cropping systems, and changes in land use (Zebarth et al. 2015). Crop rotation is generally considered advantageous over continuous potato systems. The choice of crops within a rotation often depends on economic considerations, but generally rotation crops can beneficially impact SOM, nutrient replenishment and soil productivity (Ranells & Wagger 1997; Sainju & Singh 1997; Dabney et al. 2001). Diversification of crops influences microbial activity in the soil, which can subsequently affect nutrient availability or release from organic materials (Stark and Porter 2005).

In PEI, potatoes are typically grown in a one in three-year rotation (barley-red clover-potato). Barley (*Hordeum vulgare* L.) is generally planted for livestock feed, followed by red clover which can be harvested mid-season for forage and is normally ploughed under in the fall (Jiang et al. 2012). Modelling by Jiang (2012) comparing a two-year rotation (barley-potato) with a three-year rotation (barley-red clover-potato) estimated that the three-year rotation would result in 15-22% less losses from N leaching. A longer rotation can reduce the impact of nitrogen leaching, however Nyiraneza et al. (2015) concluded that the three-year rotation including red clover would not add sufficient organic matter to soil to maintain sustainable potato production. There is an

increased effort in recent years to identify crop rotations that will improve SOM and reduce N leaching in intensive potato systems in Atlantic Canada. The most effective cropping system will consider both costs and benefits to the farmer as well as site-specific soil and climatic conditions.

2.4.2. Cover Crop Selection on N Cycling

Nitrogen mineralized from legume residues, such as red clover, can be considered as an N credit to a subsequent crop. Nitrogen credits from legume crops are difficult to quantify due to variations in timing of release relative to subsequent crop N uptake and can vary widely across regions and management practices (Stark and Porter 2005). In Atlantic Canada, approximately 10 to 40 kg N ha^{-1} is suggested as a credit for red clover grown in a previous year (Zebarth et al. 2007 2009b). However, fields in which potatoes have been grown subsequent to pure legume stands have been shown to have high residual N values after potato harvest, which in turn increases the amount of NO_3^- susceptible to leaching (Crews & Peoples 2005; Dabney et al. 2010). This could be the result of asynchrony between timing of mineralization of crop residue N and crop N demand over the growing season, or the result of overestimating N fertilizer requirements without considering N credits. In addition, previous reports indicate that fall ploughed cover crops grown in conventional potato rotations can have high residual NO_3^- in the soil after the cover crop growing season, increasing the potential for N loss from the system and reducing potential N credits to subsequent crops (Macleod and Sanderson 2002; Jiang et al. 2015).

As an alternative, grasses can be planted with legumes to increase total biomass production, increase SOM content, and also affect the quantity and timing of N release from crop residues (Sainju & Singh 1997; Dabney et al. 2010). For example, timothy is a

perennial grass that grows well in cooler climates with short growing seasons (Holmstrom et al. 2001; Kunelius et al. 2006). The generally high C:N ratio of grass residues may result in net immobilization following incorporation of the crop residue, reducing the availability of N to a subsequent crop within the first year of inclusion. As high C:N ratio materials will decompose more slowly, N from grass residues may contribute to stable N pools rather than readily available labile N pools, and therefore become available in later years as net mineralization increases over time (Kumar & Goh 2002; Sharifi et al. 2008). It is suggested that the addition of a grass with a legume may improve the synchrony between the mineralization of N from crop residues and N uptake of the subsequent potato crop (Sharifi et al. 2008).

2.5. Tracing Nitrogen Cycling in Crop Rotation

2.5.1. ^{15}N-Enriched Fertilizer to Quantify Crop Residue N

As a way to quantify N cycling in crop rotations, the use of stable isotopes in agricultural research has proved to be a useful approach for field based experiments (Hauck and Bremmer 1976; Follett 2001; Delgado et al. 2009). ^{15}N-Enriched fertilizer in the form of $^{15}NH_4^+$ or $^{15}NO_3^-$ can be used to measure ^{15}N fertilizer recovery in crop residues from labeled fertilizer sources (N_{dff}), or to quantify the transfer of ^{15}N assimilated in crop residues (N_{dfr}) in a subsequent crop (Follett 2001). The use of ^{15}N fertilizer to study N cycling is possible due to its stable nature and known atmospheric concentration (0.366 atom%).

To measure N_{dff} it has to be assumed that crops will absorb ^{14}N and ^{15}N indiscriminately, despite some studies that show a slight discrimination against the heavier isotope (Delwiche and Steyn 1970; Focht 1973). This discrimination, however, appears to be negligible in field level studies (Hauck and Bremmer 1976). After

application of labeled N, the amount of labeled N found in the plant will correspond to N taken up from the labeled N pool. This does not necessarily indicate whether N was taken up as NO_3^- or NH_4^+ or the pathway in which N reached the plant (Barraclough 1995).

Field level tracer studies are normally conducted on a microplot scale or even at the scale of a single plant due to the cost of tracer materials and the number of samples required. Additionally, the difficulty in homogenous application and distribution of ^{15}N fertilizer means that the majority of field level studies are done in microplots, single row plots, or microplots with physical barriers such as hollow cylinders (Follett 2001).

An advantage of tracer studies over non-tracer studies is that they provide a direct measurement of N uptake from labeled fertilizer. In the case of labeled crop residues, direct comparisons can be made of N contributions from residues not just of different crops but also of different residue components (e.g., above- vs. below-ground biomass). Additionally, labeled fertilizer provides a direct way to account for the translocation of N within plants from labeled soil material (Follett 2001).

2.5.2. Above- and Below-ground Crop Residue N Contribution to a Subsequent Crop

Many studies have examined the N contribution from above-ground crop residues to a subsequent crop using labeled ^{15}N fertilizer techniques. For example, Ranells and Wagger (1997b) used ^{15}N ($K^{15}NO_3$, 50 kg N ha^{-1}, 10 atom%) applied to fall planted cover crops to label the crop residues. Cover crop residues were harvested in spring prior to corn planting and re-applied to new plots where the same unlabeled cover crops were harvested and removed. The ^{15}N accumulation in the non-N fertilized corn crop was then measured. As much as 35% of the residual ^{15}N from above-ground crimson clover portion of a crimson clover (*Trifolium Incarnatum* L.)-rye (*Secale Cereale* L.) mixture

was recovered in corn, compared to 4% from a pure stand of rye. Delgado et al. (2004) reported that the ^{15}N accumulated in the potato crop was the equivalent of between 6.4 and 12.8% recovery of ^{15}N fertilizer applied to three varieties of wheat grown in the preceding year. Collins et al. (2007) used ^{15}N labeled fertilizer to determine ^{15}N recovery in a subsequent potato crop from previously labeled above-ground mustard residues to be between 30 to 40 kg N ha^{-1}, totalling 29% of total N applied as residue. Approximately 66% of the N from crop residues was found in the soil in the year following labeled fertilized application, indicating that a significant portion of N remained in the soil (Collins et al. 2007).

Many studies have disregarded or underestimated below-ground contributions of total crop residue N to a subsequent crop (Russell and Fillery 1996; Khan et al. 2002; Bolger et al. 2003). The below-ground residue contribution is a challenge to quantify due to the difficulty in collecting, separating, and classifying root fractions from soil (Khan et al. 2002b; Gardner and Sarrantonio 2012). Methodological differences across studies also make it difficult to compare results as there is limited knowledge on root exudation and root turnover throughout the plant growing season (Bolinder et al. 2002; Dabney et al. 2010). Some studies have suggested that below-ground biomass can significantly impact N supply, and therefore increase total N credit estimates or impact N losses (Kumar & Goh 2002; Bolger et al. 2003).

Reeves et al. (1993) observed below-ground biomass and N accumulation in crimson clover to be on average 19 and 16% of the total plant respectively. Other studies have reported that 31 and 50% of the total plant N in legumes and perennial/ annual pastures, respectively, were found in below-ground fractions (Peoples and Baldock 2001;

Dabney et al. 2010; Arcand et al. 2014). Estimates of belowground N based on physical recovery of roots have generally been considered an underestimate of root N (Khan et al. 2002a). Recoverable roots in McNeil et al. (1997) accounted for only 30 to 60% of total belowground N recovered with ^{15}N fertilizer (Khan et al. 2002a). The use of ^{15}N fertilizer provides a quantitative method to analyze crop root N accumulation and subsequent N transport into subsequent crops.

2.6 Objectives and Hypothesis

The overall objective of this thesis was to determine how crop selection of three preceding non-N fertilized crops would affect soil N availability to a subsequent potato crop at two N rates (0 and 190 kg N ha^{-1}). Specifically, a pure legume stand [red clover (RC)], pure grass stand [timothy (T)] and legume/ grass mixture [red cover/ timothy (M)] were compared in addition to the N contribution of their residue components (i.e. above- vs. below-ground components). The overall objective was achieved through a small plot field experiment (Experiment 1) and a field microplot experiment (Experiment 2). Specific objectives of Experiment 1 were to:

1. Assess N contribution from three non-N fertilized cover crop treatments (RC, M, and T) to soil N supply in a subsequent non-N fertilized potato crop as measured by N uptake and by tuber yield and quality parameters.

2. Evaluate temporal changes in soil NO_3^- availability from non-N fertilized cover crop treatments in the plant root zone (0-15 cm depth) using anion exchange membranes during the cover crop and potato phases of the rotation and at two N rates in the potato phase (0 and 190N).

3. Evaluate temporal changes in soil solution NO_3^- availability from cover crop treatments using suction lysimeters located in the root zone (30 cm depth) and below the root zone (50 and 80 cm depth) cover crop and potato phases of the rotation and at two N rates in the potato phase (0 and 190N).

Hypotheses for Experiment 1 are:

1. Red clover grown as a pure stand (RC) or with timothy (M) will increase soil N supply compared to a pure timothy stand (T) as measured by plant N uptake in non-N fertilized plots in a subsequent potato crop.

2. Potatoes grown subsequent to M or T treatment will have a reduced concentration of NO_3^- in soil and in soil solution after potato harvest compared with the RC treatment.

Specific objective of Experiment 2 was to:

1. Use ^{15}N labeled fertilizer to evaluate the transfer of N from three labeled cover crops (RC, M, and T) and source of cover crop residues (labeled soil, recoverable roots or above-ground biomass) into a subsequent potato crop by measuring ^{15}N accumulation and partitioning in tubers, vines and roots of the potato crop.

Hypothesis for Experiment 2 was:

1. The relative importance of above- and below-ground crop residues in supplying N to a subsequent potato crop will vary depending on whether the crop is a pure stand of legume, a pure stand of grass, or a legume/ grass mixture.

CHAPTER 3. THE EFFECT OF COVER CROP SELECTION ON N CYCLING IN POTATO CROPPING SYSTEM (EXPERMINET 1)

3.1. INTRODUCTION

For potatoes, N is commonly the nutrient most limiting for achieving adequate tuber yield, size and quality (Zebarth and Rosen 2007). Nitrogen inputs commonly include synthetic N fertilizers and mineralization of N in organic sources such as soil organic matter, decomposing plant residues or organic amendments. Nitrogen is, however, highly susceptible to losses, mainly leaching of NO_3^- past the crop root zone and eventually into groundwater sources (Zebarth et al. 2009b; Nyiraneza et al. 2015). In PEI, intensive potato production has been linked to elevated groundwater NO_3^- concentrations, and subsequently to anoxic events in estuaries (DesRoches et al. 2008).

To reduce NO_3^- leaching, the PEI "Commission on Nitrates in Groundwater" recommended a mandatory three-year crop rotation for potatoes (DesRoches et al. 2008). In PEI, a common three-year rotation is barley-red clover-potato. Legumes such as red clover in crop rotations can supply organic matter and supply N through biological nitrogen fixation (BNF) (Sanderson et al. 1999; Stark and Porter 2005). Fall-ploughed red clover has, however, also been associated with greater overwinter N losses compared to non-leguminous crops, reducing their potential N credit to a subsequent crop (Macleod and Sanderson 2002).

Legume-grass mixtures grown before cash crops have been explored as an alternative to pure legume stands while maintaining cash crop yields (Ranells & Wagger 1997; Sainju & Singh 1997; Snapp et al. 2003; Tonitto et al. 2006). Grasses, with their ability to form a deep fibrous root system may scavenge residual NO_3^- in the soil thereby reducing NO_3^- movement in soil after cover crop plough-down (Ranells and Wagger

1997c; Stark and Porter 2005). Grasses additionally affect N availability due to their generally higher C:N ratio that can reduce soil mineralizable N as a net microbial immobilization occurs during residue decomposition (Ranells and Wagger 1997c; Nyiraneza et al. 2015).

The economic importance of potatoes in PEI and the fact that all island drinking water comes from groundwater sources (Zebarth et al. 2015) necessitates a better understanding of N cycling from rotation crops in potato cropping systems. This study will examine a red clover/ timothy mixture (M) in its ability to supply N to a subsequent potato crop in PEI compared with a red clover (RC) or timothy (T) pure stand. This study will also compare the effects of RC, M and T treatments within sub-plots of two N fertilizer rates (0 and 190 kg N ha^{-1}) on tuber yield, soil and soil solution NO_3^- availability during the potato growing season after potato harvest.

3.2. Methods

3.2.1. Site Description and Establishment Year

The field plots were established in 2013 at the Agriculture and Agri-Food Canada Harrington Research Farm (46°21'N, 63°90'W), Prince Edward Island, Canada. Before the establishment of the experiment in 2013, the field was cropped to soybean with no fertilizer addition. In 2013, the experimental site was cropped to barley under-seeded with designated cover crops (Table 3.1) and cover crops were left to regrow in 2014 with no additional N fertilizer. Potatoes were planted in 2015. This study used data collected between May and December in 2014 and 2015. The soil is characterized as a fine sandy loam and classified as an Orthic Humo-Ferric Podzol (MacDougall et al. 1988). Based on a soil test conducted on soil sampled in fall 2012 by the PEI Analytical lab, the soil organic matter content was 3.3%, with a pH of 6.4.

The Harrington Research Farm is located in a humid continental climate with a mean annual air temperature of 5.7 °C and annual precipitation of 1158 mm (mean 30-year average between 1981-2010; Environment Canada 2014). Meteorological conditions were monitored from the Environment Canada weather station in Harrington, PE.

The experimental design was a split plot arrangement of treatments in a randomized complete block design with main plots (12 x 8 m) of three potato cropping systems replicated four times and split plots (6 x 8 m) of 2 N rates (0 or 190 kg N ha^{-1}) during the 2015 potato phase. The potato cropping systems included rotations of (2013-2014-2015): barley (*Hordeum vulgare* L.)-red clover (*Trifolium pratense* L.)-potato (*Solanum tuberosum* L.) designated as RC; barley-red clover/ timothy (*Phleum pratense* L.)-potato designated as M; and, barley-timothy-potato designated as T.

In spring 2013, barley (var. Island at 150 kg ha^{-1}) was seeded in all plots with an Aerosum 3000 Seeder (Poettinger, Grieskirchen, Austria). Each crop was then under-seeded in the barley with a Brillion Seeder (Brillion Iron Works, Brillion, WI, USA) and fertilized (ammonium nitrate [NH_4NO_3]) by hand as listed in Table 3.1. Herbicide (MCPA 450) was applied in June 2013 (1 L ha^{-1}).

Table 3.1. Cultivar, seeding rate and N fertility of crops underseeded with barley in 2013.

Crop species	Cultivar	Seeding rate	2013 N fertility
		kg ha^{-1}	kg N ha^{-1}
Red clover (RC)	Endure	11	20
Red clover/ Timothy (M)	Endure/ Climax	6 / 3	40
Timothy (T)	Climax	6	60

3.2.2. 2014 Cover Crop Phase

Cover crop treatments that were under-seeded in 2013 were left to re-establish in 2014. No additional fertilizer application was made to the cover crops in 2014 in order to determine soil N supply in subsequent non-N fertilized potato. No irrigation was applied as is common in PEI rain-fed potato production systems. All cover crop treatments were managed in the same way and according to conventional practices for PEI. Weed and insect controls were applied as per commercial practice for PEI. All treatments were cut twice (17 June and 8 August 2014) throughout the growing season using a bush mower (Appendix, Table A.1). Cover crop residues were left as is on the surface of the plot. The section of each plot where ceramic lysimeters were located was cut using a STIHL grass trimmer (STIHL, Waiblingen, Germany) to avoid damaging the lysimeter sampling equipment. Fall plough-down of all treatments occurred on 3 December 2014.

3.2.3. 2015 Potato Phase

Potatoes were planted on 28 May 2015 (Appendix, Table A.2). Potato sets, cultivar Russet Burbank, were cut by hand and machine planted with 0.91 m row spacing and 0.31 m within-row spacing. Main plots were each split into subplots with 6 rows each. Subplots were randomly assigned and received either 0 kg N ha^{-1} fertilizer addition (0N) or a recommended rate of 190 kg N ha^{-1} as ammonium nitrate (NH_4NO_3). All subplots received 190 kg ha^{-1} of P_2O_5 and K_2O.

Potatoes in all treatments were managed according to normal growing practices in PEI and fungicide and herbicide was applied accordingly. There was no supplemental irrigation throughout the growing season as is common in PEI where potato production is generally rain fed.

Whole plant samples were collected on 3 September 2015 and separated into roots, vines and tubers to determine dry matter and N accumulation. Potato vine desiccation was initiated with an application of Reglone 240® on 29 September (with Bravo) and 5 October. Potatoes were harvested on 20 October 2015.

3.2.4. Plant Biomass Sampling

Plant tissue samples were taken at the first and second cut and before field was to be ploughed on 17 June, 12 August and 8 October 2014, respectively. A 1 m^2 section of each plot was manually cut with hedge clippers to 5 cm above the soil surface and analyzed for above-ground dry matter yield and total N concentration. A sub-sample of tissues (approximately 500 g) was weighed for determining dry matter content by weighing fresh samples, drying overnight at 55 °C and reweighing dry samples. The remaining biomass was brought back to each plot and spread evenly.

In the potato phase, four adjacent potato plants were dug by hand from the middle rows before vine senescence on 3 September 2015. Potato plant parts were separated into tubers, vines and readily recoverable roots (Zebarth and Milburn 2003) to determine dry matter and total N accumulation. Vines were weighed, chopped with scissors and a subsample of approximately 800 g dried at 55 °C for 48 hours. Tubers were washed and weighed, and a subsample of 6 tubers was sliced using a French fry cutter, weighed and dried, and reweighed as described for vines. Recovered roots were washed on the top of a 2-mm soil sieve to eliminate all soil particles, weighed, and also dried. Dried tubers, vines and roots were ground to pass a 1-mm screen and analyzed for total N by dry combustion method on a Vario Max Elementar analyzer (Elementar, Hanau, Germany).

To estimate the contribution of cover crop N to a subsequent potato, apparent % fertilizer N uptake was calculated as N uptake in 190N treatment – N uptake in 0N

treatment divided by fertilizer application for each cover crop treatment. To determine the ratio of N remaining in the field after the potato harvest, the N harvest index (tuber N/ total plant N) was determined for cover crop treatments and N rate treatment.

When potatoes were harvested, two internal rows were harvested first to measure total and marketable tuber yield, tuber specific gravity and tuber size categories. Tuber size categories were determined as follows: Culls: < 38 mm; Can 1 small: 38 to 51 mm; Can 1: 5.1 to 89 mm; Can 1 large: 89 to 114.3 mm; Jumbo: >114.3 mm. Marketable yield consisted of Can 1 and Can 1 large (Nyiraneza et al. 2015).

3.2.5. Soil Mineral N

Soil samples were taken to measure point in time NO_3^- and NH_4^+ availability in the soil at depths of 0-15 and 15-30 cm and in both the hill and furrow during the potato phase. Samples were collected with a Dutch auger, monthly from April to November 2014. In each plot, sampling location was chosen randomly and a composite sample was made from two samples to make one composite sample for each depth within each plot and for each subplot in the potato phase. In the spring (May) and fall (November), three cores were taken from each plot to create a composite sample per depth and plot with a Giddings hydraulic soil sampler (Giddings Machine Company Inc. CO, USA) to measure soil NO_3^- and NH_4^+ availability below 30 cm (30-45 and 45-60 cm). In the potato phase, soil samples were taken from each main plot in the spring sampling, and from each subplot in October 2015.

Soil samples were stored at 4 °C until analysis. Soil was passed through a 2 mm sieve, and a 10 g subsample was extracted with 50 mL of 2M KCl solution and shaken for 1 hour (Maynard et al. 2007), and the extract filtered with a vacuum system through Whatman #42 glass filters. Filtrate was stored at 4 °C until analyzed for concentration of

NO_3^- and NH_4^+ by flow injection analysis with a Lachat Quickchem 8500 Series 2 (Lachat Instruments, Loveland, CO, USA). The soil gravimetric moisture content was calculated by taking a separate 10 g subsample of soil and drying at 105 °C overnight and reweighing the dry sample.

3.2.5.1. Anion Exchange Membranes

Anion exchange membranes (AEM) were used to estimate NO_3^- flux from the soil and crop residues (Qian and Schoenau 2002). Membranes were used throughout the cover crop growing season, overwinter and during the subsequent potato phase. In May 2014, AEM strips (5.2 x 6.0 cm) were inserted with a trowel at a depth of 15 cm, and attached fishing wire was wrapped around a marking flag (Ziadi et al. 1999). Two membranes were inserted in the soil at the center of each main plot for a total of twenty-four membranes. Approximately every two weeks, membranes were removed from the field and replaced with fresh membranes as close to the original sample location as possible. Two membranes in each plot were left over-winter from before the first snowfall, and were removed before spring tillage on 27 May 2015 to determine winter NO_3^- flux (Ziadi et al. 1999). During the potato phase, membranes were inserted following the same method as in 2014, but with four membranes (two in potato hill and two in furrow) in each subplot (Ziadi et al. 2006). Potatoes in rows with membranes were not sampled for other analyses.

Membranes were transported in labeled plastic bags to the lab, carefully rinsed with distilled water to remove loose soil, and each membrane was placed in individual 50 mL centrifuge tubes with 40 mL 1M KCl. In the potato phase, membranes that were from the same sampling location were placed in centrifuge tubes together (2 per tube). Tubes were shaken for 1 hour to release bound NO_3^- from the membrane, and the solution was

filtered through Whatman #42 filter paper before the eluate was analyzed by flow injection analysis. Units are reported as μg cm^{-2} day^{-1}.

3.2.6. Nitrate in Soil Solution

Two types of lysimeters were installed to measure soil solution NO_3^- concentration. Ceramic lysimeters were installed at 30 cm (crop root zone), and 50 cm (below the root zone) depth in both cover crop and potato phases. Ceramic lysimeters were removed and replaced before and after tillage operations. Permanent steel lysimeters were installed in summer 2014, at 80 cm and left in place to allow samples to be collected throughout the year, including in spring before and after planting and fall before and after cover crop and potato harvest.

3.2.6.1. Ceramic Lysimeters

Ceramic lysimeters were constructed as described by Macpherson (2010). The lysimeter consisted of a polyvinyl chloride (PVC) pipe with an outside diameter of 5 cm that was cut to the appropriate length. At one end, the inner pipe was reamed out and epoxy resin was used to secure a 7 cm long porous round-bottomed ceramic cup (2 cm inner diameter). On the other end of the pipe, a rubber stopper was used to seal the pipe. A hole in the middle of the rubber stopper was made for a smaller plastic tube (0.2 cm diameter) to fit inside the pipe and extend down into the ceramic cup for solution extraction. On the outer side of the rubber stopper, a plastic 0.4 cm diameter connector was attached to the plastic tube. This piece was connected to a 1 L Boston Round amber glass bottle by rubber tubing. On the glass bottle, a two-hole rubber stopper was used to seal the bottle. One hole was connected to the lysimeter. The other hole was connected to rubber tubing with a metal stopper. This tubing was used to connect the vacuum pump for preparing samples. All lysimeters were tested before being placed in the field by

submerging them underwater and applying pressure to the system with the vacuum pump. If air bubbles were released anywhere along the length of the PVC pipe or the ceramic cup, they were not used.

In spring 2013, two ceramic suction cup lysimeters were installed at each depth (30 and 50 cm) in each main plot. To install lysimeters, an opening was drilled in the ground with a powered Stihl auger (STIHL, Waiblingen, Germany) to make a hole with diameter slightly wider than that of the lysimeter. Plain tap water was added to create a slurry at the bottom of the opening to ensure good contact of lysimeters and soil. The lysimeters were then carefully inserted and tapped into place with a mallet. Bentonite (aluminium phyllosilicate) was placed around each lysimeter at the ground surface to prevent preferential water flow along the length of the PVC pipes. In fall 2013, bentonite was removed and lysimeters were pulled out, barley was harvested, and straw was removed from fields (Cambouris et al. 2008). Before the first snowfall, lysimeters were reinserted to ensure early spring samples could be taken in the following year, but no samples were taken in that fall. RV antifreeze was injected into lysimeters over the winter to prevent ceramic cups from cracking due to frost (MacPherson 2010). In spring 2014, cracked lysimeters were replaced and others were flushed with deionized water to remove antifreeze. In October 2014, lysimeters were removed before cover crop harvest and not re-installed before the spring. In 2015, lysimeters were installed after potatoes were hilled on 30 June 2015 with two in each subplot (0N and 190N) at 30 and 50 cm depths from the soil surface of the hill. All ceramic lysimeters were removed before tubers were harvested. Due to the short duration of lysimeter installation and dry conditions in August there were only four dates between August and September in 2015. After each

installation, no samples were taken for approximately 14 days to establish proper soil contact with lysimeters before preparation and sampling (MacPherson 2010).

Leachate from suction lysimeters was collected between May and October 2014 and July and September 2015. Lysimeters were prepared for sampling approximately bi-weekly or before a known large rainfall event was to occur (Cambouris et al. 2008). A partial vacuum/ negative pressure of approximately 0.8 bar (~10 psi) was applied with a UMS portable vacuporter (UMS, Munich, Germany). Unsaturated water surrounding the ceramic cup was drawn into the cup and moved up the inner lysimeter tube through capillary action into the amber glass collection bottle. After approximately 48 hours, collection bottles were emptied and the volume of water collected was measured. A 15 mL sub sample was collected for determination of NH_4^+ and NO_3^- concentrations by flow injection analysis as described above.

In August 2014, samples were taken as normally done but there was not enough solution in lysimeters for collection so August sample dates were not included. In total, 10 and 18 samples were excluded and 181 and 198 samples were taken for 30 and 50 cm depth, respectively, in 2014.

3.2.6.2. Steel Lysimeters

The steel lysimeters were used because they could be placed permanently in the field and would not affect machinery in plots. Steel lysimeters consisted of porous steel cups (260 mL capacity) that were connected to flexible PVC tubes (6.0 cm outer diameter) of approximately 250 cm in total length. Two plastic tubes (1.5 cm diameter) extended from inside of the PVC through an opening in the top. One plastic tube was connected to either a bicycle pump or a portable vacuum pump to create positive pressure, and the other tube was used to collect solution. In each plot, a hole was created

in the soil with a Dutch auger to approximately the compact layer (~80 cm). The lysimeters were installed so that the porous cups were placed vertically into the soil, being careful to ensure contact between soil and the porous cup. The soil around the installed lysimeter was excavated and the PVC tube was then laid horizontally to create an elbow at approximately 60 cm depth across the soil floor until it was approximately 100 cm outside of each plot. The soil was shovelled back into the plot. The PVC tube was curved 90° angle again to create another elbow so that it would be vertical outside of each plot and then was secured to a wooden stake for stability.

One stainless steel lysimeter was installed at 80 cm depth in August 2014 on the section of the main plot that would become the 0N subplot in 2015. Lysimeters were only installed in plots that would become the 0N subplots due to the limited availability of lysimeters. Samples were collected on a bi-weekly basis between August and December 2014 and weekly between May and December 2015. A bicycle pump was first connected to the inlet plastic tube to push water out of the lysimeter from the collection tube. If water could not be collected with the bicycle pump, a portable vacuum pump was next set up to collect the remaining water. Pressure was applied for approximately 20 minutes or until 50 mL of solution was collected. The pump was left in place until water was emptied from the lysimeters. Samples were collected in a 50 mL centrifuge tube, brought back to the lab and immediately sent for analysis of NO_3^- and NH_4^+ by flow injection analysis as described above.

The total number of samples for each treatment was inconsistent during the sampling period depending on whether soil solution could be extracted from lysimeters.

3.2.7. Statistical Analysis

Statistical analysis was done with SAS (SAS Institute 1997) and R Studio Suite (V. 3. 2. 4 – Very Secure Dishes). In R Studio, the *nlme* package was used for Analysis of Variance (ANOVA) and the *lsmeans* package was used to determine least significant differences ($p < 0.05$) between significant groups to assess the effects of cropping system on measured parameters. The main effects (main plots) were the cover crop treatments (RC, T or M treatments) and the secondary effects (subplots) were the two levels of mineral N fertilizer (0N or 190N). Output for ANOVA tables are listed in the appendix. Treatment means were compared using the protected Fischer's LSD when the main effects were significant ($p < 0.05$). Assumptions of normality and equal variances were checked and data were transformed as appropriate. Graphs were made using the *ggplot2* package in R.

3.3. Results

3.3.1. Climate

Mean monthly air temperature between May and October averaged 14.5 °C in both 2014 and 2015 compared to the 30-year (1981-2010) normal of 13.9 °C (Table 3.2). From May to October, total precipitation in 2014 (522 mm) and 2015 (456 mm) was lower than the 30-year normal of 568 mm. In 2014, the average temperature in July of 21 °C was not only 2 °C warmer than the 30-year normal but total precipitation of 41 mm was 49% less compared to the 30-year normal of 80 mm.

Table 3.2. Mean monthly air temperature in 2014 and 2015 between May and October, compared with the 30-year (1981-2010) normal measured at the Environment Canada Weather Station on the Harrington Research Farm, PEI (Environment Canada 2016).

	Air Temperature (°C)		
Month	2014	2015	30 year normal
May	8.3	10.7	9.2
June	14.5	13.0	14.5
July	20.9	18.0	18.7
August	18.1	21.0	18.3
September	14.4	16.4	14.1
October	10.8	8.0	8.3
May to October	14.5	14.5	13.9

Table 3.3. Total monthly precipitation in 2014 and 2015 between May and October, compared with the 30-year (1981-2010) normal measured at the Environment Canada Weather Station on the Harrington Research Farm, PEI (Environment Canada 2016).

	Total Precipitation (mm)		
Month	2014	2015	30 year normal
May	68.2	11.2	87.2
June	79.3	111.5	98.8
July	40.6	36.7	79.9
August	120.6	115.6	95.7
September	88.7	67.6	95.9
October	124.4	112.9	110.3
May to October	521.8	455.5	567.8

3.3.2. 2014 Cover Crop Phase

3.3.2.1. Above-ground Yield and N Accumulation

Total dry matter yield was greater in RC and M treatments (6.92 t ha^{-1}) than the T treatment (2.75 t ha^{-1}). Dry matter yield for unfertilized cover crops was significantly greater from the RC and M treatments on June (average of 4.6 t ha^{-1}) and August

sampling dates (average of 1.7 t ha^{-1}) than for the T treatment (1.42 and 0.71 t ha^{-1}, respectively) (Table 3.4). There were no significant differences in dry matter yield among cover crops for the October sampling date.

Table 3.4. Cover crop total dry matter yield, N accumulation and C:N ratio of unfertilized above-ground biomass from red clover (RC), timothy (T), and red clover/ timothy (M) on three collection dates in 2014.

	Dry matter yield				Plant N accumulation				C:N		
Cover crop (C)	Jun	Aug	Oct	Total	Jun	Aug	Oct	Total	Jun	Aug	Oct
	t ha^{-1}				kg N ha^{-1}						
RC	4.55a[z]	1.54ab	0.58	6.66a	94a	29a	15	138.1a	20b	23b	21
M	4.72a	1.80a	0.66	7.18a	83a	35a	13	131.5a	25b	23b	25
T	1.42b	0.71b	0.64	2.75b	15b	8.0b	11	33.61b	42a	40a	28
ANOVA					Pr<(F)						
C	***	*	NS	***	***	**	NS	**	***	***	NS

[z]Values followed by different letters within the same column are significantly different. ($p<0.05$ = *, $p<0.01$=**, $p<0.001$=***, NS=not significant).

There was no significant difference between total N accumulation in RC and M treatments (average of 135 kg N ha^{-1}), but both treatments were significantly greater than for the T treatment (34 kg N ha^{-1}). Similarly, cover crop N accumulation was greater for the RC and M treatments for the June and August sampling dates, whereas there was no significant difference in plant N accumulation among cover crop treatments in October. Cover crop N accumulation was greatest in the first cut, ranging from 15 to 94 kg N ha^{-1} in T and RC, respectively (Table 3.4).

In June and August, there was a significant effect of cover crop selection on C:N ratio in which RC and M treatments were significantly lower (average of 23) than the T treatment. In the T treatment, the C:N ratio was lower in October (28) compared to biomass measured in June (41).

3.3.2.2. Soil – Mineral N

Soil mineral KCl-extractable NO_3^- was significantly greater in RC and M treatments for 0-30 cm depth than the T treatment for all sampling dates after July (Figure 3.1). There was a low (< 4 mg NO_3-N kg^{-1}) concentration of KCl-extractable NO_3^- early in the growing season but subsequently increased in all cover crop treatments after the June sampling date. For the RC and M treatments, KCl-extractable NO_3^- concentration continued to increase at a comparable rate in July to a peak of 11 mg NO_3-N kg^{-1} in August before decreasing over the remainder of the growing season. At its peak, KCl-extractable NO_3^- concentration in the RC and M treatments (average of 11 mg NO_3-N kg^{-1}) was over three times greater than in the T treatment (3.1 mg NO_3-N kg^{-1}). In the T treatment, KCl-extractable NO_3 concentration had decreased to 3.1 mg NO_3-N kg^{-1} by August from a peak in July of 6.5 mg NO_3-N kg^{-1}. In October, KCl-extractable NO_3 was comparable to that measured in the spring before crop emergence.

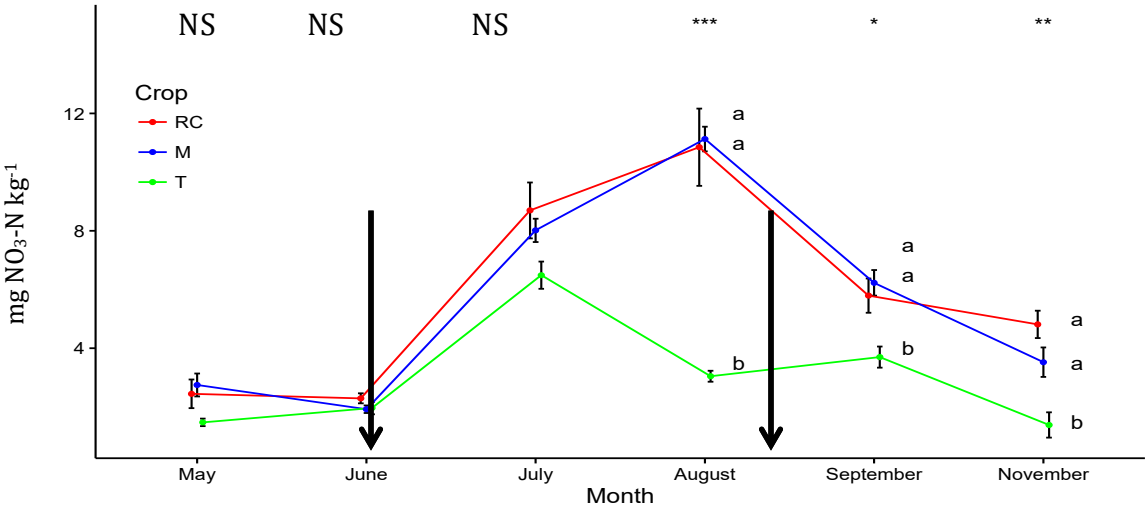


Figure 3.1. Monthly KCl-extractable soil NO_3-N concentrations for 0-30 cm depth from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M). Arrows indicate dates cover crops were cut in field. Values followed by different letters within the same month are significantly different ($p<0.05$ = *, $p<0.01$=**, $p<0.001$=***, NS=not significant)

The concentration of KCl-extractable soil NH_4^+ remained low throughout the growing season, and never exceeded an average of 0.44 mg NH_4-N kg^{-1} (Figure 3.2). The seasonal average concentration of NH_4^+ was 0.19 mg NH_4-N kg^{-1}. The greatest peak of NH_4^+ for was found in May for the M and T treatments and June for the RC treatment. On the first sampling date (May), the concentration of KCl-extractable NH_4^+ in M was approximately twice as high as those found in RC and T.

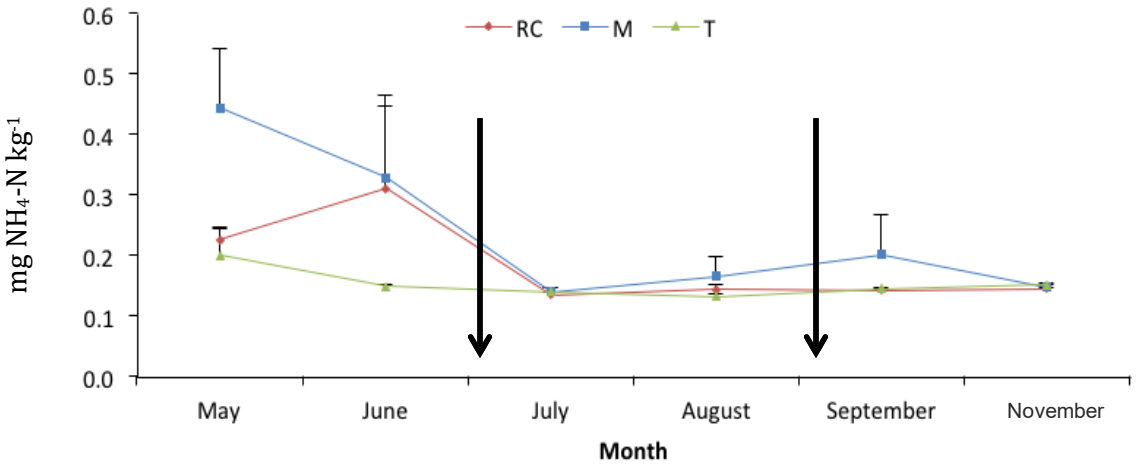


Figure 3.2. Monthly KCl-extractable soil NH_4-N concentrations for 0-30 cm depth from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M). Arrows indicate dates cover crops were cut in field.

3.3.2.3. Soil – AEM

Anion exchange membranes were used in the field to assess NO_3^- release from soil under unfertilized cover crops throughout the 2014 growing season. Nitrate measured from AEMs was low in the spring (0.05 to 0.09 µg NO_3-N cm^{-2} day^{-1}) and comparable among cover crop treatments. Throughout the growing season there was a trend towards increased AEM NO_3^-, reaching a peak NO_3^- release in September of 0.26, 0.72 and 0.98 µg NO_3-N cm^{-2} day^{-1} for T, RC and M treatments respectively (Figure 3.3). The sum of AEM NO_3^- between May and October ranged from 18 and 59.4 µg cm^{-2} $season^{-1}$.

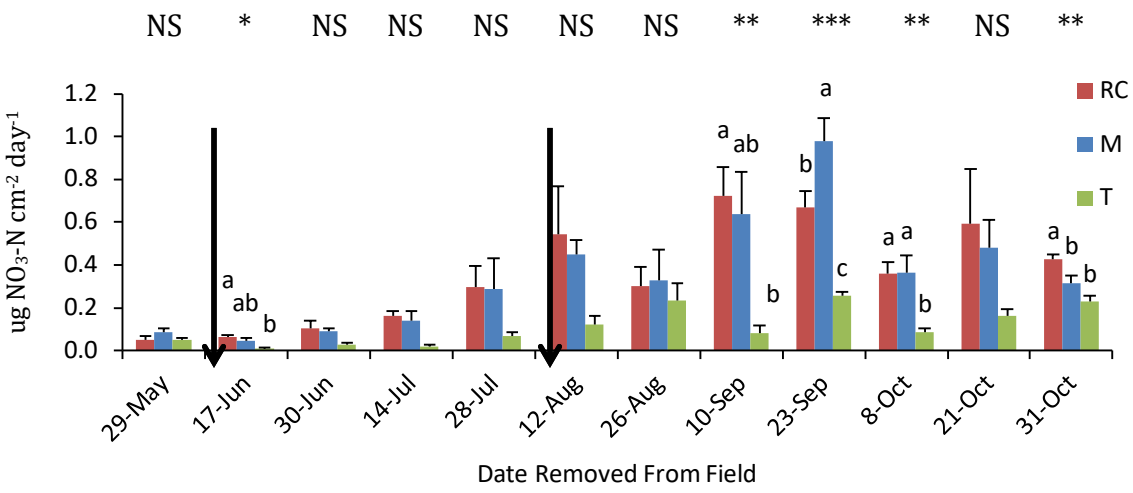


Figure 3.3. Seasonal change in NO_3-N flux (ug NO_3-N cm^{-2} day^{-1}) from unfertilized red clover (RC), timothy (T) and red clover/ timothy (M) as measured by anion exchange membranes over the 2014 cover crop growing season. Arrows indicate dates cover crops were cut in field. Values followed by different letters on the same sampling date are significantly different ($p<0.05$ = *, $p<0.01$=**, $p<0.001$=***, NS=not significant)

On sampling dates between May and August, there was an effect of cover crop treatment on AEM NO_3^- only on 17 June where AEM NO_3^- was significantly greater in the RC treatment than in the T treatment. In September and early October, the amount of N released from the RC and M treatments was significantly greater compared to the T treatment. On 31 October, AEM NO_3^- for the M and T treatments (average of 0.28 µg NO_3-N cm^{-2} day^{-1}) was significantly lower than from the RC treatment (0.43 µg NO_3^- cm^{-2} day^{-1}).

Approximately four weeks after cover crops were cut in the field, N release from AEMs increased in the RC and M treatments. For the T treatment, AEM NO_3^- increased

marginally approximately six weeks after each cut in the field, but on a smaller scale than for either the RC or M treatments.

3.3.2 4. Nitrate in Soil Solution

The mean soil solution NO_3^- concentration from all unfertilized cover crop treatments was low and ranged from 0.11 and 0.33 mg -N L^{-1} in 2014 for 30 and 50 cm depths respectively and was consistently greater in 50 cm (Figure 3.4 and 3.5). Throughout the cover crop growing season both RC and M treatments showed comparable values and peak soil solution NO_3^- concentration occurred in July. The greatest concentration of soil solution NO_3^- was measured on 18 July and it decreased slightly thereafter and remained higher than the concentration of soil solution NO_3^- measured any day before 18 July. For most sampling dates, soil solution NO_3^- from T for either depth were found to be below the detection limit (< 0.03 mg NO_3-N L^{-1}).

At 30 cm the soil solution NO_3^- never exceeded the maximum acceptable concentration limit of 10 mg NO_3-N L^{-1} for drinking water in PEI (Figure 3.4). There was a high variability in sample results from all sampling dates. There was less variation in samples measured in June but variation increased as the concentration of soil solution NO_3^- in samples increased (September and October).

The concentration of soil solution NO_3^- increased in July then again beginning in September. The results were comparable to the fluctuations measured of AEM NO_3^- over two week burial periods. Results from 30 cm lysimeters were also comparable to KCl-extractable soil NO_3^- results found in soil in July and August and decreased by September. There were no significant cover crop treatment effects at 30 cm on any sampling date but treatments with legumes were consistently greater than from the T

treatment. The high degree of variability made it difficult to determine statistical differences among cover crop treatments.

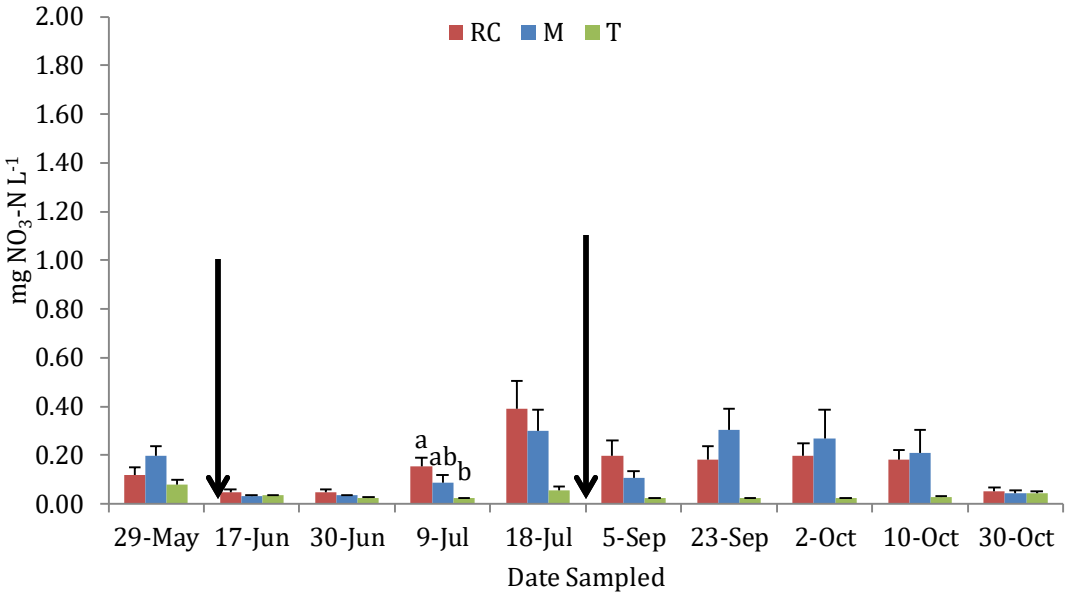


Figure 3.4. Seasonal change in soil solution NO_3-N concentration at 30 cm depth below of the soil from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M) as measured by ceramic lysimeter over the 2014 cover crop growing season. Arrows indicate dates cover crops were cut in field.

At 50 cm, it is assumed that NO_3^- is susceptible to loss as it is too deep for most N accumulation from crop roots. The level of soil solution NO_3^- in T was below the detection limit (0.03 mg NO_3-N L^{-1}) for most of the samples from any sampling date except for 18 July (Figure 3.5).

As the measured concentration of soil solution NO_3^- in 30 cm decreased by the end of October for all cover crops, the concentration increased in the corresponding 50 cm samples in all cover crop treatments.

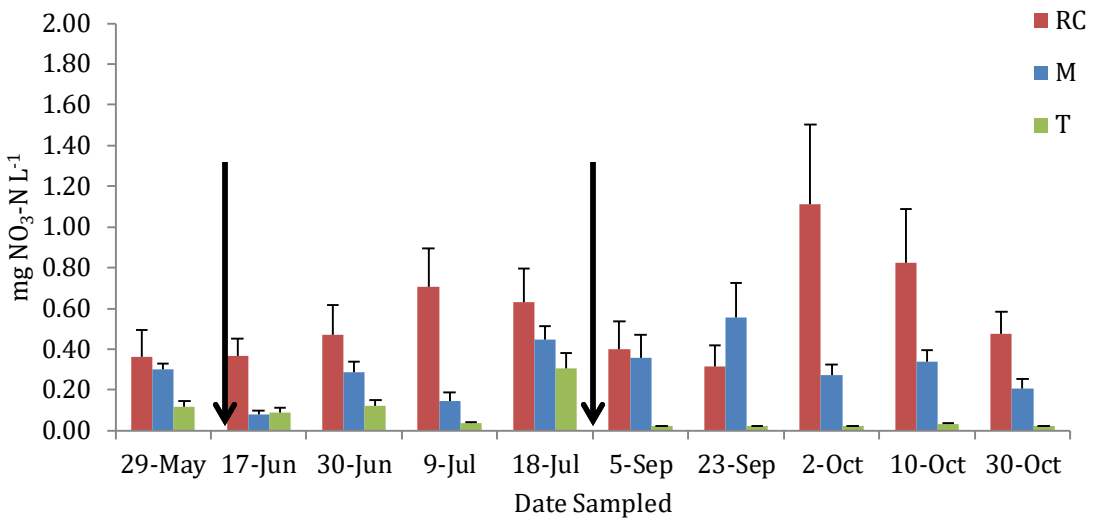


Figure 3.5. Seasonal change in soil solution NO_3-N concentration at 50 cm depth below of the soil from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M) as measured by ceramic lysimeter over the 2014 cover crop growing season. Arrows indicate dates cover crops were cut in field.

The mean concentration of soil solution NO_3^- at 80 cm was 10.73 mg NO_3-N L^{-1} across all treatments. In 2014, the values for mg NO_3-N L^{-1} showed comparable within treatment trends (RC ≈ M > T) to results measured from ceramic lysimeters despite a much greater magnitude (Figure 3.6). Overall the soil solution NO_3^- concentration from the T treatment was low and never exceeded 5 mg NO_3-N L^{-1}. Samples were not taken until October to give the lysimeter system time to settle after installation and values recorded during the cover crop phase of rotation should be assessed with caution and may explain the high standard error recorded for almost all treatments on all dates.

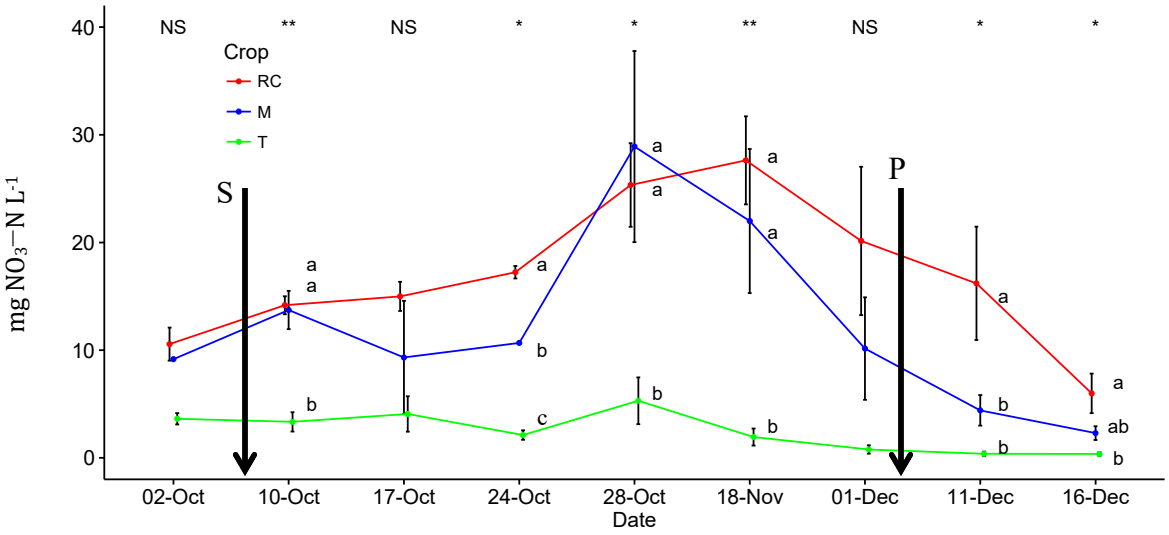


Figure 3.6. Seasonal change in soil solution NO_3-N concentration at 80 cm depth below of the soil from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M) as measured by steel lysimeters measured in cover crop main plots at the end and after the 2014 cover crop growing season. The arrow "S" indicates the date of the last biomass sampling, the arrow "P" indicates cover crop ploughdown date. Values followed by different letters on the same sampling date are significantly different ($p<0.05$ = *, $p<0.01$=**, NS=not significant)

In the first month of sampling, the concentration of soil solution NO_3^- did not fluctuate greatly among cover crop treatments until 24 October. The RC and M treatments showed comparable results to other measured NO_3^- results and were not significantly different except on 24 October and 11 December. Between 17 October and 28 October there was an increase in soil solution NO_3^- in 80 cm and comparably there was a decrease in soil solution NO_3^- at 50 cm for the RC treatment. The peak concentration of NO_3^- occurred on 28 October for the RC and T treatments and 18 November for M treatment. Thereafter soil solution NO_3^- decreased in all treatments but was significantly greater in RC compared to the red clover/ timothy cover crop treatment

or pure grass stand. By 16 December, there was no significant difference between the RC or M treatments but RC was still significantly greater than from the T treatment.

3.3.3. 2015 Potato Phase

3.3.3.1. Potato Yield and N Parameters

In the 0N treatment, there was a trend towards greater total yield from the RC and M treatments (29 t ha^{-1}) compared to 25 t ha^{-1} from the T treatment (Table 3.5). Though there was no significant difference in total tuber yield among cover crops in fertilized plots, T treatment was approximately 25% greater (40 t ha^{-1}) than from the RC and M treatments (average of 32 t ha^{-1}).

There was a significant cover crop and N rate interaction on marketable tuber yield (Table 3.5). In the 0N treatment, marketable yield was comparable among cover crop treatments with an average of 21 t ha^{-1}. In the 190N treatment, marketable yield was greatest from the T (29 t ha^{-1}) treatment compared to from the RC and M treatments (average of 17 t ha^{-1}). In addition, marketable tuber yield (Can 1) represented 72% of total tuber yield in T treatment compared to an average in 53% in RC and M treatment (Table 3.6). For these results, marketable yield consisted of mostly Can 1 tubers only as Can 1 large tubers were found in only one treatment (unfertilized T treatment) and accounted for 3% of total tuber yield in that treatment (sum of four replicates) and 4% of marketable tuber yield (data not shown).

Table 3.5. Plant dry matter accumulation at vine senescence (vine, root and tubers), and total and marketable tuber yield at potato harvest, in 2015 as influenced by N rate and previous cover crop treatments

Cover crop (C)	N rate (N)	Dry matter accumulation	Total tuber yield	Marketable tuber yield
			t ha^{-1}	
Red clover (RC)		6.97	30.5	19.4b[z]
Red clover/ timothy (M)		6.49	29.8	19.2b
Timothy (T)		7.31	31.9	24.1a
	0N	6.36b	27.2b	20.8
	190N	7.48a	34.3a	21.1
RC	0N	7.38ab	28.5ab	21.1a
M	0N	6.75bc	28.9ab	22.5a
T	0N	4.97c	25b	19.5ab
RC	190N	6.56bc	32.6ab	17.7b
M	190N	6.24bc	30.9ab	15.9b
T	190N	9.65a	39.5a	28.7a
ANOVA			Pr(>F)	
C		NS	NS	**
N		**	**	NS
C x N		***	0.06	**

[z]Values followed by different letters within the same treatment are significantly different ($p<0.01$=**, $p<0.001$=***, NS=not significant).

Table 3.6. Size distribution and specific gravity of tubers at potato harvest, in 2015 as influenced by N rate and previous cover crop treatments

Cover crop (C)	N rate (N)	Cull	Can 1 small	Can 1	Specific gravity
			%		
Red clover (RC)		2.44	33.14a	64.42	1.082
Red clover/ Timothy (M)		2.02	33.15a	64.83	1.078
Timothy (T)		1.44	23.90b	72.47	1.085
	0N	1.47b	21.67b	75.40a	1.088a
	190N	2.46a	38.45a	59.09b	1.075b[z]
RC	0N		23.15a	75.25a	
M	0N		20.57a	78.02a	
T	0N		21.29a	72.92a	
RC	190N		43.13b	53.60b	
M	190N		45.73b	51.65b	
T	190N		26.51a	72.03a	
ANOVA			Pr(>F)		
C		NS	*	NS	NS
N		*	***	***	**
C x N		NS	*	*	NS

[z]Values followed by different letters within the same treatment are significantly different ($p<0.05$ = *, $p<0.01$=**, $p<0.001$=***, NS=not significant)

Total N uptake was measured from whole potato plants (vines, roots and tubers) taken from the internal plot row before vine senescence. In 0N plots, there was no significant difference in total N uptake between RC and M (average of 107 kg N ha^{-1}), though RC was significantly greater than from the T treatment of 60 kg N ha^{-1} (Table 3.7). By subtracting the total N uptake from the RC and M treatments from that of the T treatment, it was determined that treatments with legumes provided an average of 47 kg N ha^{-1} to the subsequent potato crop grown with no additional fertilizer addition. In 190N treatments, an inverse trend of the 0N trend treatment was found where the greatest N uptake was found in the T treatment (218 kg N ha^{-1}) compared to in the M treatment (155

kg N ha $^{-1}$) or the RC treatment (174 kg N ha $^{-1}$). The apparent % fertilizer uptake was significantly affected by cover crop selection and was greater following the T treatment compared to RC and M treatment. The N harvest index was greater under the 190N potato treatment but was not affected by cover crop selection (Table 3.7).

Table 3.7. Plant total N uptake (vines, roots, tubers), N harvest index and apparent % fertilizer N uptake in 2015 as influenced by N rate and previous cover crop treatment.

Cover crop	N rate (N)	Total N uptake	N Harvest index	Apparent fertilizer N uptake
		kg ha^{-1}		%
Red clover (RC)		129	50	34b
Red clover/ Timothy (M)		119	49	26b
Timothy (T)		127	51	83a
	0N	83.2b[z]	57a	
	190N	167a	43b	
RC	0N	109c		
M	0N	105cd		
T	0N	60d		
RC	190N	174ab		
M	190N	155b		
T	190N	218a		
ANOVA		Pr(>F)		
C		NS	NS	***
N		***	***	
C x N		***	NS	

[z]Values followed by different letters within the same column are significantly different ($p<0.001$=***, NS=not significant)

3.3.3.2. Soil Mineral N

In spring 2015, KCl-extractable NO_3^- in the soil 0-30 cm depth measured before potato planting was not significantly affected by cover crop selection and averaged 7.1 mg NO_3-N kg^{-1} (Figure 3.8). For the June sampling date, in the 0N treatments, the peak KCl-extractable NO_3^- concentration reached 38 and 34 mg NO_3-N kg^{-1} for RC and T

treatments. For M in the 0N treatment, there was a comparable peak to RC in June but the greatest concentration of NO_3^- in 0N treatment was from the M treatment (41 mg NO_3-N kg^{-1}) in September (Figure 3.7). There was a significant difference between KCl-extractable NO_3^- on only two sampling dates in June after potato planting and in August. In June, the trend for soil NO_3^- was RC > M > T where only the RC treatment was significantly greater than from the T treatment. By August, the KCl-extractable NO_3^- had increased in M and was significantly greater than from the RC and T treatments. In September, KCl-extractable NO_3^- from the M treatment had again increased but there was a high degree of variability from the M treatment compared to the RC and T treatments and there were no significant differences found on this date despite at least a 67% greater mean concentration from M treatment than from the RC or T treatments.

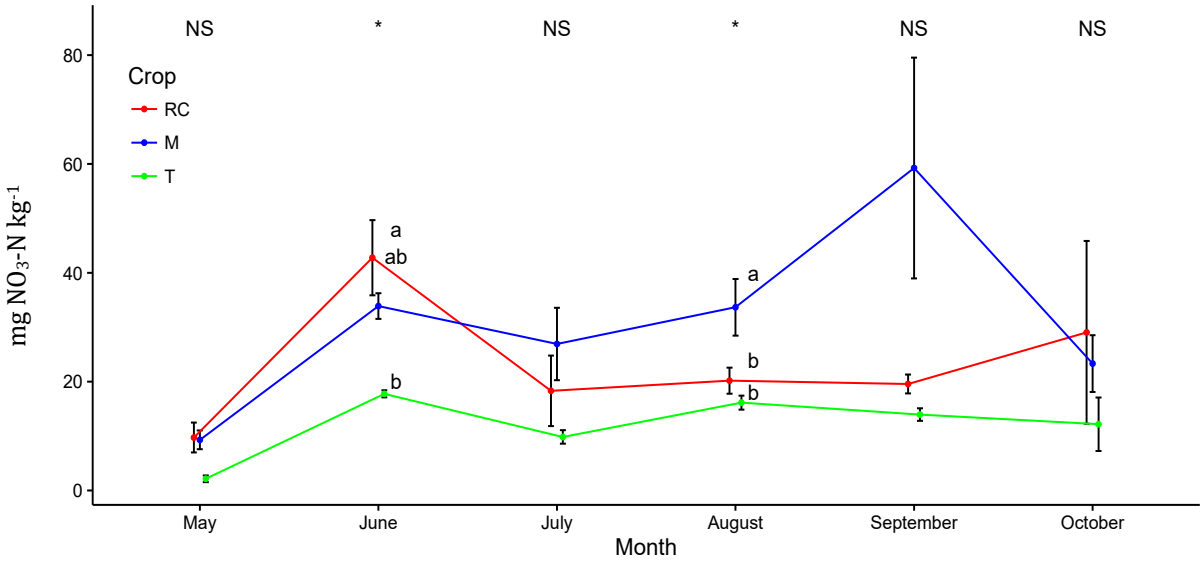


Figure 3.7. Monthly KCl-extractable soil NO_3-N concentrations for 0-30 cm depth in the potato hill from 2015 growing season from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M) under the 0N treatment. Values followed by different letters within the same month are significantly different at $p<0.05$.

In fertilized plots the availability of KCl-extractable NO_3^- over the growing season was comparable for both M and RC treatments (Figure 3.8). The peak N availability for all cover crop treatments in fertilized plots occurred in June (T and RC treatments) and July (M treatment). Treatments effects were only significant in June and RC treatment had a greater KCl-extractable NO_3^- concentration than from the T treatment. Though not significant for any other sampling date, there was a trend towards less KCl-extractable NO_3^- from T treatment compared to RC or M treatments. The concentration of KCl-extractable NO_3^- in the soil decreased over the summer from the initial peak after fertilizer application.

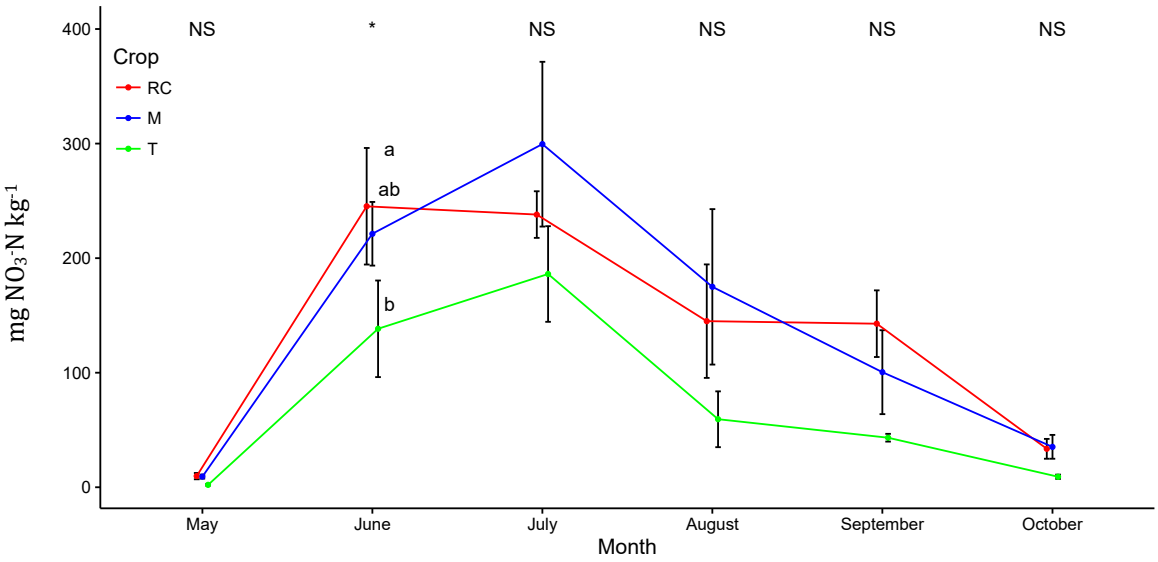


Figure 3.8. Monthly KCl-extractable NO_3-N concentrations for 0-30 cm depth in the potato hill from 2015 growing season from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M) under the 190N treatment. Values followed by different letters within the same month are significantly different at $p<0.05$.

There was no significant difference between KCl-extractable soil NH_4^+ in any cover crop treatment in 0N or 190N plots (Figure 3.9, 10). In 0N plots, KCl-extractable soil NH_4^+ did not exceed 3.10 mg NH_4-N kg^{-1} from the RC treatment in June. After July for both 0N and 190 kg N ha^{-1} plots, soil NH_4^+ dropped below 1 mg NH_4-N kg^{-1}. In 0N plots, there was a small increase in soil NH_4^+ in T in October however it was still below 1 mg NH_4-N kg^{-1}.

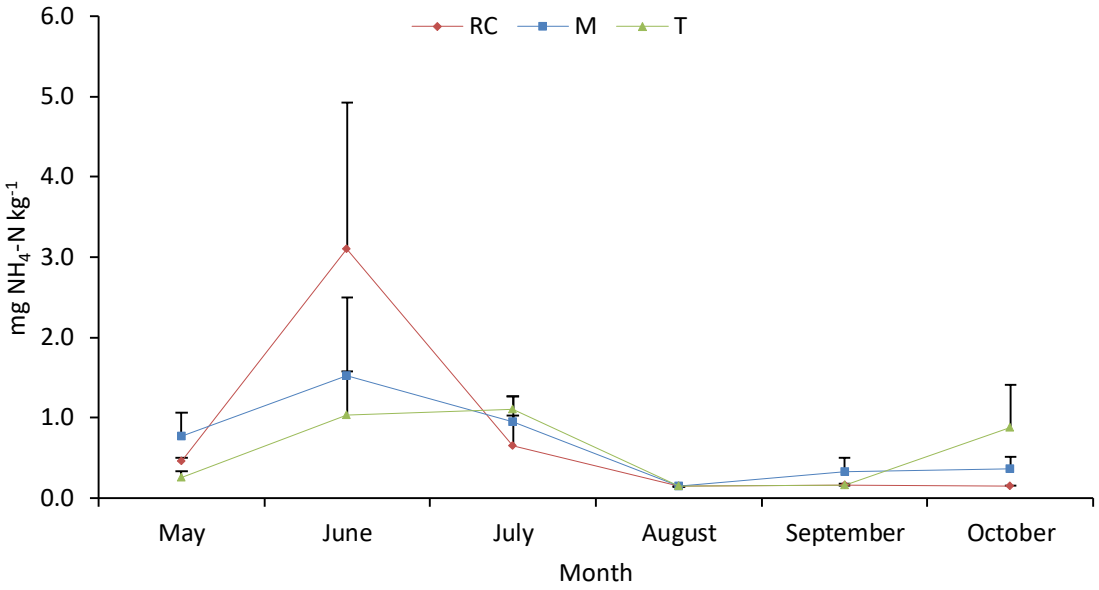


Figure 3.9. Monthly KCl-extractable soil NH_4-N concentrations for 0-30 cm depth from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M) in the potato hill from 2015 growing season under the 0N treatment.

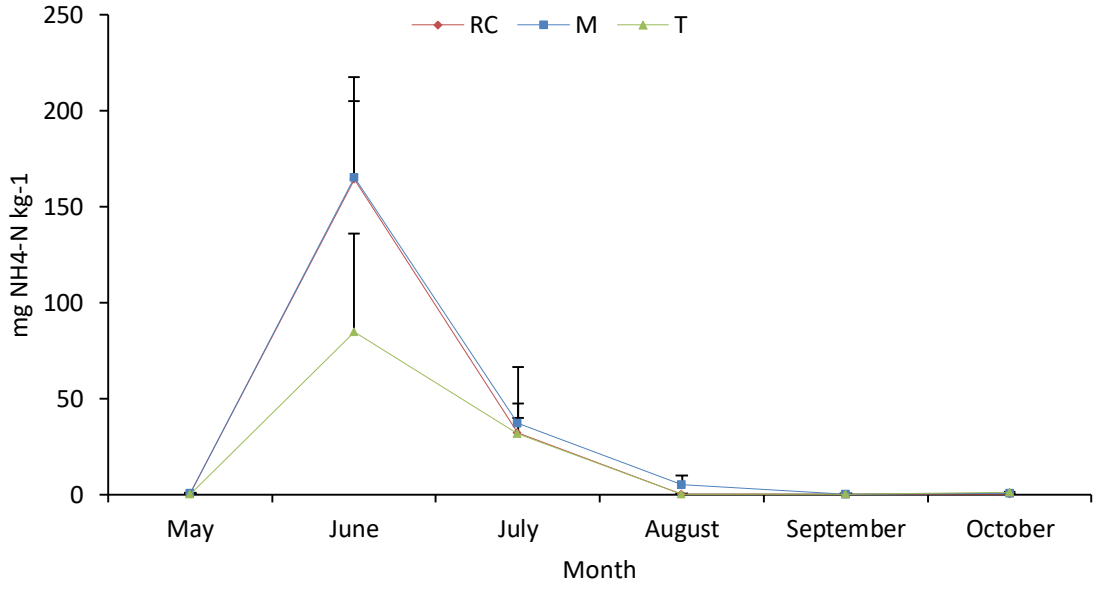


Figure 3.10. Monthly KCl-extractable soil NH_4-N concentrations for 0-30 cm depth from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M) in the potato hill from 2015 growing season under the 190N treatment.

3.3.3.3. Soil – AEM

To determine overwinter N mineralization activity, the AEMs were inserted after cover crops were ploughed and left overwinter and removed on 27 May 2015 after 162 days before potatoes were planted (Figure 3.11). This resulted in a total NO_3^- flux of 97.8, 119.8, and 169.3 μg NO_3-N cm^{-2} burial $period^{-1}$ for M, T and RC respectively. The value of AEM NO_3-N remained below 4 μg cm^{-2} day^{-1} on all sampling dates. Throughout the potato growing season, in 0N plots, soil AEM NO_3^- was between 0.07 and 3.11μg NO_3-N cm^{-2} day^{-1} (Figure 3.11). There was no significant difference between in activity of AEM NO_3^- in any cover crop treatment except for on the last sampling date (30 September). On 30 September, there was no difference between M treatment and other treatments but AEM NO_3^- in the RC treatment was approximately three times significantly greater than from the T treatment. The highest peaks of AEM NO_3^- were in June and September.

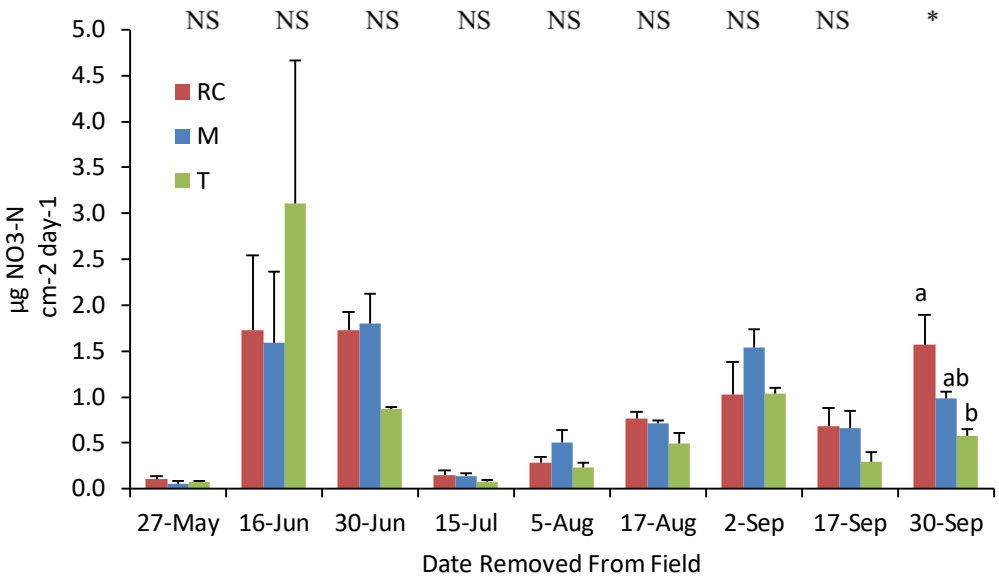


Figure 3.11. Seasonal change in NO_3-N flux (ug NO_3-N cm^{-2} day^{-1}) from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M), as measured by anion exchange membranes in the potato hill from 2015 growing season under the 0N treatment. Values followed by different letters within the same date are significantly different at $p<0.05$.

In fertilized plots, the trend of NO_3^- flux over the growing season was much more difficult to ascertain. Overall, there were no significant differences among cover crop treatments on any sampling date (Figure 3.12). The highest peak of NO_3^- flux occurred immediately after fertilizer application (June) where AEM NO_3^- was comparable in all cover crop treatments (10.11 to 11.56 µg NO_3-N cm^{-2} day^{-1}). After fertilizer application, the pattern of NO_3^- flux was comparable among cover crop treatments throughout the season except for the first sampling date in September. On 2 September, AEM NO_3^- increased in M and treatments but decreased in RC. Nitrate supply in the M treatment was more than twice as high as that measured in the RC or T treatment.

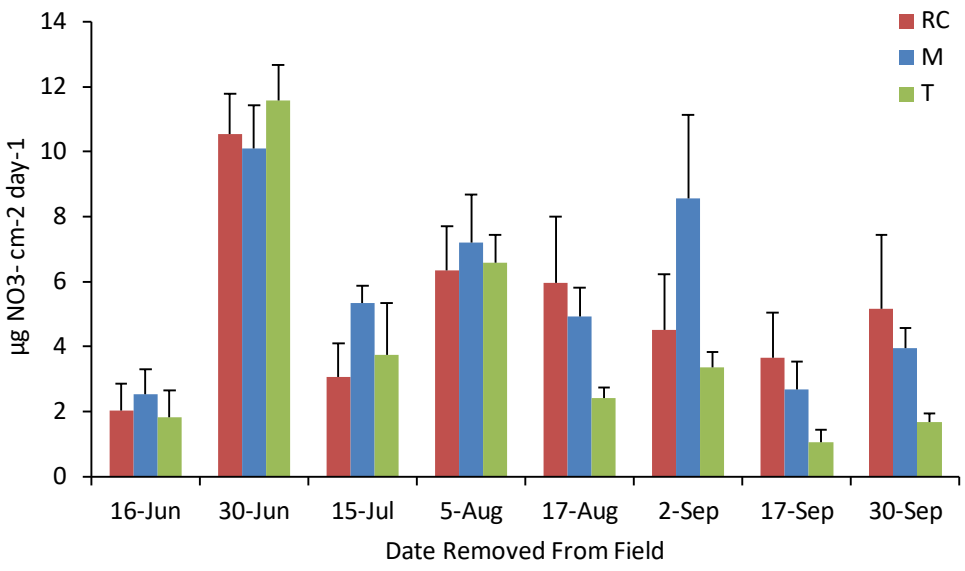


Figure 3.12. Seasonal change in NO_3-N flux (ug NO_3-N cm^{-2} day^{-1}) from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M), as measured by anion exchange membranes in the potato hill from 2015 growing season under the 190N treatment.

3.3.3.4. Nitrate in Soil Solution

3.3.3.4.1. Ceramic Lysimeter – 0N

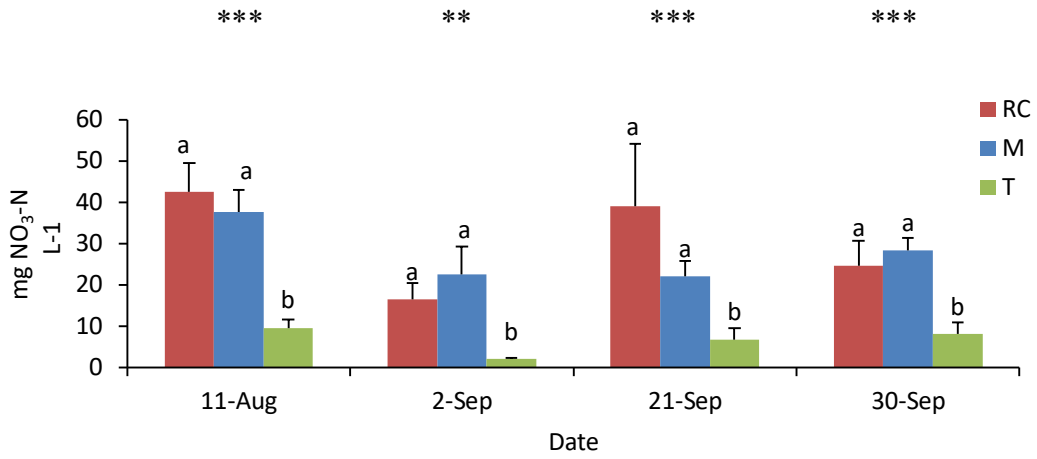
At 30 cm in the 0N treatment, there was a significant cover crop effect whereby treatments with legumes (RC and M) were found to have a greater concentration of soil solution NO_3^- compared to the T treatment on all sampling dates except one (RC ≈ M > T) (Figure 3.13A). The concentration of soil solution NO_3^- ranged between 2.03 and 42.62 mg NO_3-N L^{-1}. Results from the T treatment indicated the concentration never exceeded the drinking water guidelines of 10 mg NO_3-N L^{-1} while RC and M treatments were > 10 mg NO_3-N L^{-1} on all sampling dates.

Over the four sampling dates, there was not a large change in concentration of soil solution NO_3^- except on the second sampling date on 2 September where soil solution

NO_3^- concentration in all treatments decreased but increased again by the next sampling date on 21 September.

The concentration of soil solution NO_3^- at 50 cm (3.57 to 35.84 mg NO_3-N L^{-1}) were consistently lower than those found at 30 cm in any cover crop treatment on any sampling date (Figure 3.13B). At 50 cm, the highest concentration of soil solution NO_3^- was in the M treatment on all sampling dates compared to the RC and T treatment. Across sampling dates soil solution NO_3^- remained comparable within each cover crop treatment and there was no distinctive trend observed in such a short period between 11 August and 30 September.

A. 30 cm – 0N



B. 50 cm – 0N

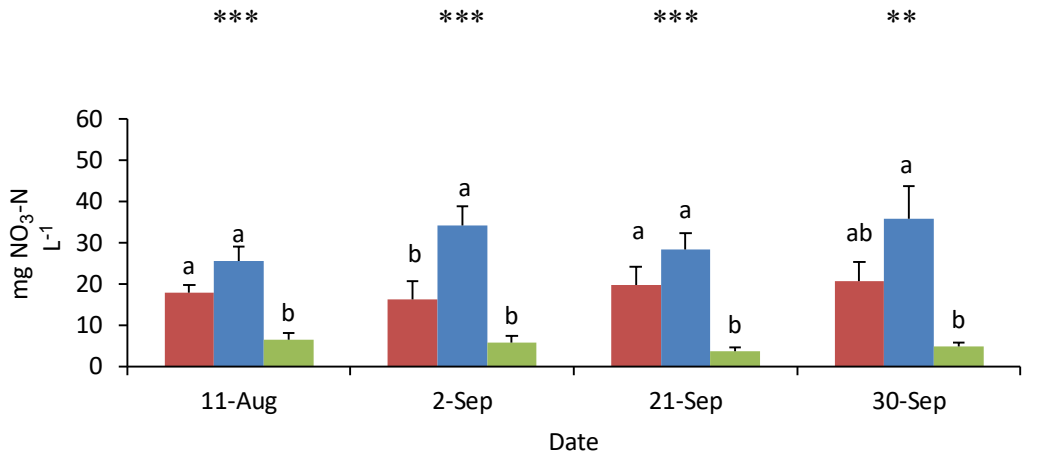


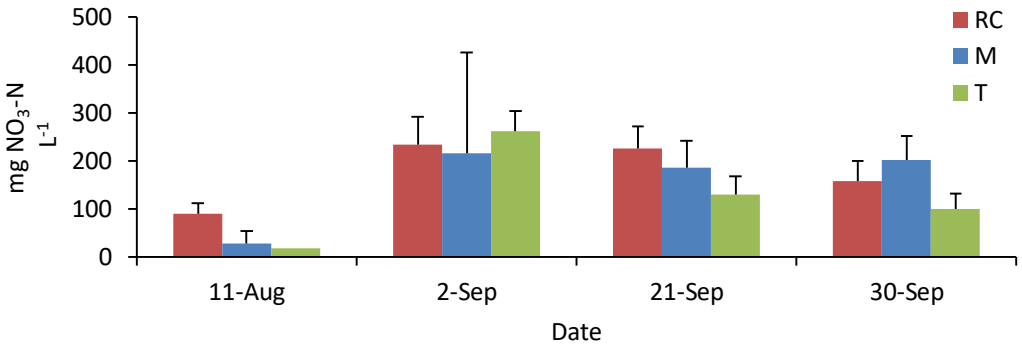
Figure 3.13. Seasonal change in soil solution concentration of NO_3-N at 30 cm (A) and 50 cm (B) from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M) in the potato hill from 2015 growing season under the 0N treatment. $^{+}$Data transformed where necessary for normality. Values followed by different letters on the same sampling date are significantly different ($p<0.01$=**, $p<0.001$=***, NS=not significant)

3.3.3.4.2. Ceramic Lysimeter – 190N

In the 190N treatments, there was a large variability in sample results within each cover crop treatment on each sampling date and there was no significant difference

among cover crop treatments (Figure 3.14). Results from the fertilized treatment were high as a result of application of 190 kg N ha^{-1} fertilizer and ranged from 44.8 to 236.9 and 26.3 to 114.7 mg NO_3-N L^{-1} in the 30 and 50 cm lysimeters respectively. At both 30 and 50 cm, the lowest concentration of soil solution NO_3^- was measured on the first sampling date in August, subsequently increased in September, and stayed relatively level thereafter.

A. 30 cm – 190 N



B. 50 cm – 190N

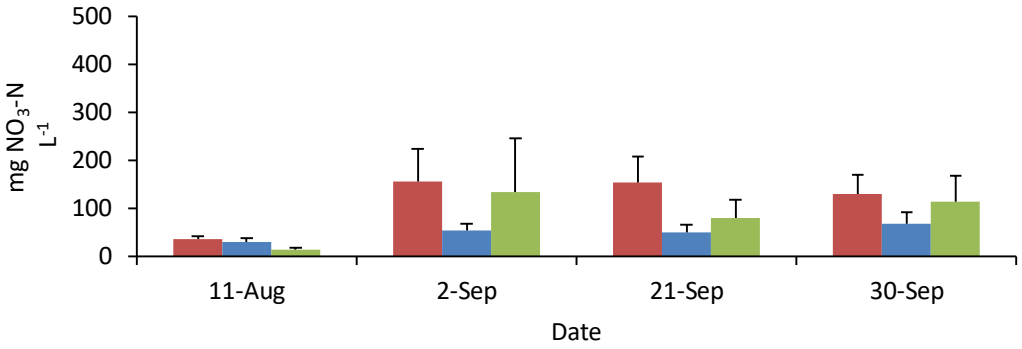


Figure 3.14. Seasonal change in soil solution concentration of NO_3-N at 30 cm (A) and 50 cm (B) from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M) in the potato hill from 2015 growing season under the 190N treatment.

3.3.3.4.3. Steel Lysimeter – 80 cm

Results from steel lysimeters are from 0N treatments only. Between the last sample in 2014 (December 16) and the first sample date in 2015 (14 May) the mean soil solution concentration within each cover crop treatment was almost unchanged (Figure 3.6, Figure 3.15). In May 2015, the RC treatment (4.7 mg NO_3-N L^{-1}) had a significantly greater concentration of soil solution NO_3^- than the T treatment (0.5 mg NO_3-N L^{-1}) and the concentration of soil solution NO_3^- increased progressively throughout the growing season.

From May until July, there was no significant difference between the T and M treatments and both were significantly lower than the RC treatment. From July to October, the trend of RC > M > T continued. The soil solution concentration in the T treatment never exceeded 12 mg NO_3-N L^{-1} but samples from the RC and M treatments were above 10 mg NO_3-N L^{-1} on 72 and 48% of sampling dates respectively. Peak soil solution NO_3^- concentration occurred in November for M treatment and December for both RC and T treatments. The biggest monthly increase in soil solution NO_3^- for all cover crop treatments occurred between August and September after vine senescence. In almost every sampling month, the concentration of soil solution NO_3^- from the RC treatment was twice as high as from the M treatment and in turn the M treatment was twice as high as soil solution NO_3^- from the T treatment indicating that at 80 cm M did have a lower concentration of soil solution NO_3^- susceptible to loss compared to the RC treatment. By November, temperature and limited number of samples created a large variability in the results from each previous crop type treatment and made it difficult to determine significant cover crop effects but the overall trend of RC > M > T remained consistent even into December.

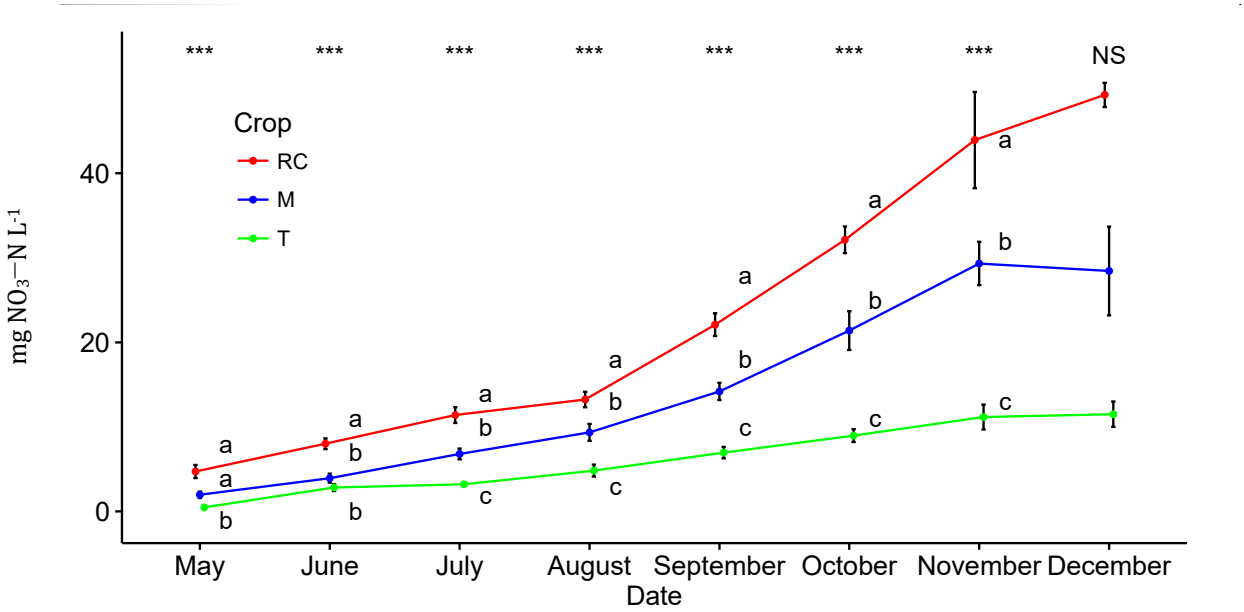


Figure 3.15. Seasonal change in soil solution NO_3-N concentration at 80 cm depth from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M) below of the soil as measured by steel lysimeters in the 2015 potato growing season under the 0N treatment. Data transformed where necessary for normality (May, June, August, November, December). Values followed by different letters in the same month are significantly different ($p<0.001$=***, NS=not significant)

3.4. Discussion

3.4.1. 2014 – Cover Crop Phase

When no fertilizer was applied to crops in the cover crop phase of rotation, red clover/timothy increased total biomass compared to a timothy pure stand by approximately three-fold. Increased dry matter yield from red clover/ timothy mixture compared to a pure timothy stand was likely the result of greater availability of N to timothy in the mixture. Nyfeler et al. (2011) demonstrated that grass legume mixtures (37 and 66% as first and second year legumes) were able to accumulate comparable rates of N (from N_2 fixation) as pure legume stands demonstrating the positive effects of growing

crops in mixture. The cover crop yields from the first cut in the RC and M treatments (average of 4.64 t ha^{-1}) were comparable to results found in Spaner and Todd (2003) for a red clover/ timothy mixture under-seeded with barley (4.99 to 5.23 t ha^{-1} depending on barley seeding rate) following the establishment year with 17 kg N ha^{-1} applied in spring forage regrowth period.

When compared to timothy grass grown with no additional N fertilizer, in soil of the same soil classification but maintained as pasture, results from this study of 2.75 t ha^{-1} were still lower than those reported in Lynch et al. (2004) of 5.17 t ha^{-1} averaged over two growing seasons. In addition to no N fertilizer addition, timothy was seeded at a lesser rate than in Kunelius (2006) of 5.6 to 15 kg ha^{-1} and 10 kg ha^{-} in Lynch et al. (2004) compared to 3 to 6 kg ha^{-1} in this study. The N content measured in this study from timothy grass tissues (10 g N kg^{-1}, data not shown) was also lower than that found in Lynch et al. (2004) of approximately 16 g N kg^{-1} in unfertilized timothy averaged over two growing seasons. Lower concentration of N in unfertilized timothy in this study suggests that there was a lower availability of soil N compared to Lynch et al. (2004), likely due to differences in field history.

Timothy grass is commonly included in rotation with potatoes and is either cut for forage once or twice in the growing season (Holmstrom et al. 2001) or if not removed from the field can be left in the field as a soil improvement measure (Carter et al. 2003). When removed as forage, N measured as part of fibre content, is an important source and indicator of nutritional quality. In this study timothy was included as an environmental management strategy to reduce residual N found in soil susceptible to N leaching compared to the traditional legumes included in potato rotation. With this consideration,

no additional fertilizer was added to timothy before planting, however. This is not the standard practice for timothy grass when managed as a forage and therefore the yield from this study may not be reflective of in-field timothy yields. In addition to management differences, timothy does well in cool conditions with consistent moisture and low timothy yield likely resulted from warmer than average temperatures in summer months, especially in July 2014 of 21 °C compared to the 30-year average of 19 °C (Table 3.1) and reduced precipitation.

Above-ground tissue N accumulation from soil available in T treatment with no additional fertilizer addition in 2014 was low at 33.61 kg N ha^{-1}. In legumes, above-ground N was substantially higher due to N accumulation from BNF. N derived from N_2 was estimated to be 98 and 104 kg N ha^{-1} in M and RC respectively (based on the N difference method of comparing a non-legume standard (timothy) to leguminous crop) and is comparable to results of apparent N derived from N_2 fixation from unfertilized red cover/ timothy mixture of 103 kg N ha^{-1} in Lynch et al. (2004). Apparent N recovered from BNF in RC and M treatments accounted for the majority of the total crop N and indicated a generally low soil N availability for crops in the cover crop phase of rotation.

The total concentration of KCl extractable NO_3^- in the plant root zone (0-30 cm) was low (< 4 mg N kg^{-1}) in the early growing season treatment but almost doubled by July in all cover crop treatments after the first cut. In Stiles (2012), a comparable but smaller increase from 0.2 to 1.3 mg NO_3^- kg^{-1} in soil 0-30 cm was seen after the first cut in conventionally grown red clover with residues returned to the plot surface. After each cut, residues were returned to the field, rather than removed, and N returned to the field after the first cut accounted for approximately 45, 63 and 68% of the total N accumulated

from T, M and RC above-ground biomass respectively. An increase in NO_3^- between July and August from RC and M treatment was likely a combination of N mineralization from readily decomposable crop materials with a low C:N ratio compared to timothy and little crop N uptake from soil N sources. Legumes capable of BNF generally have little effect on uptake of soil N compared to grasses that can only take up N from soil N sources (Ledgard and Steele 1992). Alternatively, a decrease in soil NO_3^- from T between July and August likely reflected crop N uptake from soil available N.

Legumes cover crops evaluated in this study were found to have significantly greater concentration of NO_3^- measured in the plant root zone after the major period of crop growth and N uptake compared to from T treatment. Comparatively, residual soil NO_3^- measured in September from T treatment was reduced by approximately 36 and 40% compared to 11.59 and 12.46 mg NO_3-N kg^{-1} from M and RC treatments respectively. Low soil NO_3^- in the fall period was expected from T treatment however, soil NO_3^- was greater under M than expected. It is possible that in the mixture, there was a greater proportion of red clover compared to timothy that influenced soil NO_3^- availability. This is supported by the comparable C:N ratio between RC and M above-ground biomass. In this study, the M treatment was not visibly or physically separated into red clover and timothy portions and it is possible that growth conditions that affected the pure timothy stand may have also affected the timothy portion of the red clover/ timothy mixture.

The overall results of AEM NO_3^- in all cover crop treatments were comparable to results from KCl-extractable NO_3-N and were effective to determine cover crop effects throughout the cover crop phase of rotation. Results from this study were comparable to

those found in Collins and Allinson (1999) of < 2 μg NO_3-N cm^{-2} day^{-1} from reed canarygrass tall fescue on all sampling dates between June and September. Overall there was trend of increasing concentration of NO_3^- measured from AEMs throughout May to September for all cover crop treatments is in agreement with Ziadi (2006) where there was an overall increase in NO_3^- adsorbed to AEMs in grass cover crop production. There was high variability in NO_3^- results from AEMs within cover crop treatments in August and September. Low precipitation and no fertilizer application may have had an impact on the amount of N mineralized in June by microbial activity as precipitation and temperature have an impact on the biological activity of microbes in the soil.

Two types of lysimeters were used in this experiment (tension ceramic lysimeters and zero-tension steel lysimeters). Tension lysimeters were simple to install and well suited for plant nutrition experiments to measure unsaturated water flow but could not be permanently installed in the field (Watmough et al. 2013). Zero-tension lysimeters could be placed horizontally in the soil and collect water draining freely under gravity (Watmough et al. 2013). Steel zero-tension lysimeters in this study could be left in the field without disturbance to field machinery throughout the duration of the experiment so samples could continue to be collected after cover crops were ploughed in the cover crop phase of rotation and in the spring before potatoes were planted in the potato phase of rotation. In both ceramic and steel lysimeters there was a large variability of NO_3-N concentration measured within the same plot indicative of the large spatial variability of mineral N that can occur in soil solution.

Overall soil solution NO_3^- measured at 30 cm was low in the cover crop phase (<2 mg NO_3-N L^-1). However, there was a trend toward greater concentration of soil solution

NO_3^- from the RC and M treatments compared to the T treatment averaged across all sampling dates. Results from the T treatment were consistently low or below the detection limit (<0.03 mg NO_3-N L^{-1}) on all sampling dates. Soil solution NO_3^- from RC and M increased three and two-fold from 0.39 and 0.30 mg NO_3-N L^{-1} respectively between the 9 July and 18 July 2014 sampling dates. Subsequently, the concentration remained comparable thereafter and decreased by 62 and 53% from RC and M. MacPherson (2010) found a similarly greater soil solution NO_3^- availability in pasture treatments with legumes compared to Kentucky bluegrass. Results from this study from the M treatment at 30 cm and 50 cm (average of 0.17 and 0.40 mg NO_3-N L^{-1} respectively) were slightly lower than those found in MacPherson (2010) of soil solution NO_3-N for bluegrass and red clover mixed cover crop pasture with no fertilizer addition at 45 cm (0.27 to 9.28 mg NO_3-N L^{-1}). At 50 cm, results from the T treatment (0.03 to 0.30 mg NO_3-N L^{-1}) were on the lower range of results found in Macpherson (2010) measured in Truro and Nappan of 0.02 to 1.15 mg NO_3-N L^{-1}.

The overall trend of soil solution NO_3^- from the RC and M treatments was comparable to results from MacPherson (2010) where there was a low amount of soil solution NO_3^- in spring and summer compared to the fall sampling dates. As in MacPherson (2010), legumes were characterized by early season plant nutrient uptake from soil N until favourable conditions are met for plant N uptake via BNF. Despite the addition of a grass in the M treatment, at 30 and 50 cm there were no significant difference from the pure legume treatment for soil solution NO_3^-. This may have been related to the composition of M treatment whereby in the cover crop phase of rotation,

the legume component may have undergone rapid mineralization relative the grass component of the mixture.

A low concentration of soil solution NO_3^- from timothy grass was expected considering there was no source of N applied in this treatment, all plant N uptake would have come from soil N sources without replenishment of N from BNF. The timothy treatment represented a system with considerably reduced N. In addition to no N fertilizer applied, substantial crop N uptake (65% of total N accumulation) occurred in timothy between August and October compared to 32 and 37% from RC and M treatments.

At 80 cm, almost all results from steel lysimeters were 10 times higher than those from ceramic lysimeters even those sampled on the same dates. During the cover crop phase, peak concentration of soil solution NO_3^- was measured in October and November in RC and M treatments of 29 and 28 mg NO_3-N L^{-1} but had decreased by 93 and 79% respectively by December 2014. These results were slightly greater than predicted results from a LEACHN model simulation in Jiang et al. (2011) for a barley/ red clover/ potato rotation. Taking into account climatic and cropping conditions, Jiang et al. (2011) observed a peak concentration of solution NO_3^- measured at 90 cm in November and December, decreasing thereafter until spring after red clover for an annual loss of 28 to 45 kg N ha^{-1} in the red clover phase representing approximately 5.6 to 11 mg NO_3-N L^{-1} (volume-weighted nitrate leaching/ drainage). Greater concentration found in these results was likely the result of leaving cover crops on the field after cut compared to crop removal in Jiang et al. (2012). Jiang et al. (2012) estimated that 82% of drainage occurs outside the growing season (November to April). In addition, Jiang et al. (2011) reported that 50 to 60% of NO_3^- leaching is associated with the barley and red clover phase of

rotation suggesting that NO_3^- leaching can be significantly reduced outside the potato phase of rotation as preliminary results from the M and T treatments showed in this experiment. Results from the T treatment never exceeded 5 mg NO_3-N L^{-1} and therefore were effective in reducing the concentration of soil solution NO_3^- found in the fall period when drainage is expected to be high compared to the RC and M treatment.

The results from both 30 and 50 cm indicate the high spatial and temporal variability compared to soil solution results from steel lysimeters measured at 80 cm. It is possible that the concentration of NO_3^- measured at 80 cm represented NO_3^- previously leached below the root zone that was not captured by the shallower lysimeters. Results from steel lysimeters for the cover crop phase should be regarded with caution as the greater concentration of soil solution NO_3^- relative to ceramic lysimeters could have been affected by the effect of rapid mineralization from disturbed crop residues after lysimeter installation. Alternatively, soil solution NO_3^- at 80 cm may have reflected the concentration of NO_3^- at a depth where little to no plant N uptake is expected to occur.

3.4.2. 2015 – Potato Phase

In this experiment, timothy grass was compared to legume treatments as a novel rotational crop to determine rotations that can reduce the potential for N leaching and when grown with red clover, could supply N to a subsequent crop. When no N fertilizer was applied to cover crops, total tuber yield was mostly the result of N rate and was reduced by 21% from 34 t ha^{-1} to 27 t ha^{-1} when no N was applied to potatoes. When no N fertilizer was applied to potatoes, total tuber yield averaged 27.2 t ha^{-1} among cover crop treatments and was within the ranges previously reported of 18.4 to 27.5 t ha^{-1} following grasses or small grain and 25 to 38.7 t ha^{-1} following red clover (Porter and Sisson 1991; Zebarth et al. 2004 2009a). When no N fertilizer was applied to potatoes,

total N uptake in potato crops grown was greater when grown subsequent to a red clover pure stand compared to a pure timothy stand. Greater N uptake measured in the potato crop from the RC treatment compared to the T treatment did support the hypothesis that the RC treatment could increase soil N supply in a subsequent potato crop however, this is did not translate to greater total tuber or marketable tuber yield among cover crop treatments.

When N fertilizer was applied to potatoes, a preceding crop of red clover did not increase total tuber yield or total N uptake compared to potatoes following T treatment. In this study, the apparent % fertilizer N uptake was approximately 34 and 26% following RC and M respectively compared to 83% in potatoes following timothy. This suggests that potatoes grown subsequent to timothy was better able to utilize soil and fertilizer N throughout the growing season compared to from RC and M treatments despite the greater overall availability of N measured from soil NO_3^- from RC and M treatments. In addition, marketable tuber yield from T treatment of 29 t ha^{-1} was approximately 61% and 81% greater compared to following RC or M respectively. It is possible that the timing in N availability in the soil did not match crop N uptake demands. Small tuber size can be the result of asynchrony in timing between soil N and crop N demands. In the fertilized plots, almost half of the total tuber yield from the RC and M treatments were Can 1 small or culls (< 51 mm) and there were no tubers in the Can 1 large marketable tuber category. High early season N from fertilizer or released as soil mineral N may results in excessive above-ground growth, and can affect tuber bulking subsequently reducing tuber yield (Stark and Porter 2005; Zebarth and Rosen 2007). By contrast, late season crop N uptake can either increase tuber N uptake but not

tuber yield or if not taken up, increases the potential for N leaching from soil N released late in the growing season (Griffin and Hesterman 1991; Stark and Porter 2005).

Alternatively, it is possible that non-N effects that were not measured in this study may have affected tuber yield in the RC treatment compared to the T treatment. Carter et al. (2003) found consistently lower potato tuber yield when grown subsequent to red clover (fertilized with 25 kg N ha^{-1}) compared to Italian ryegrass (fertilized with 130 kg N ha^{-1}) in a two-year rotation measured over 9 years. In Carter et al. (2003), year-to-year variability in potato yield was high (19.7 to 41.4 Mg ha^{-1} in potatoes grown subsequent to red clover over 9 years) Some of the yield variability of was explained by earlier than usual top kill. It should be noted that Carter et al. 2003 examined a 2 year potato rotation system and in general longer rotations are recommended over shorter rotations for additional soil improvement benefits and increased disease suppression (Lynch et al. 2012). However, the importance of long-term study of multiple rotation cycles to determine cover crop effects that can affect tuber yield and quality should not be understated.

When potatoes were sampled before top kill, approximately 40% of N remained in the vine portion of the potato from 0N and 190N treatments respectively and were within the range measured from (Zebarth et al. 2004a). This corresponded to approximately 70, 62 and 87 kg N ha^{-1} from RC, M and T respectively returned to the field from potato vines. Combined with lower apparent % fertilizer N uptake from RC (34%) and M (26%) treatments compared to T (83%), an estimated 195, 203, and 120 kg N ha^{-1} from RC, M and T respectively could have remained in the soil post potato harvest. Apparent % fertilizer N uptake generally tends to decrease when there is greater

residual N from legumes or organic sources (Plotkin 2000) as was found in RC and M compared to T in this study.

In the 0N treatment, the choice of cover crop selection did not impact KCl-extractable NO_3^- measured before potato planting (May) suggesting little N from residues incorporated in the fall was carried over from the previous growing season. However, by June soil NO_3^- measured in the soil 0-30 cm was significantly greater following RC compared to from T. Greater availability soil NO_3^- likely reflected rapid in-season mineralization of RC crop residues with a low C:N ratio compared to the T treatment residues. In addition, greater soil NO_3^- availability was reflected in greater total N uptake in potato plants following RC compared to the T treatment at 0N before vine desiccation. Peak NO_3^- appeared to occur a month later than that found in previous studies (Plotkin 2000; Stiles 2012) and could be the result of 30% less precipitation between May and July 2015 compared to the 30-year average. Soil moisture can affect both N leaving the root zone and the amount of N mineralized by microbial bacteria in the soil.

In the M treatment, the concentration of NO_3^- continued to rise after the June sampling period, was significantly greater in August compared to the RC and T treatment and reached peak NO_3^- concentration in September. A delayed and more varied response from the M treatment is consistent with previous observations of mixed cover crop residues with a widening C:N ratio in residues (Nyiraneza and Snapp 2007). Increased availability later in the growing season of soil NO_3^- from the M treatment suggested that soil N mineralization continued to occur over the growing season but this late season N was likely not taken by potato crops.

The total overwinter NO_3^- flux of 129 µg NO_3-N cm^{-2} burial $period^{-1}$ averaged across cover crop treatments confirmed previous reports that N mineralization activity does continue in the winter periods (Nyiraneza et al. 2010). A trend of high early season AEM NO_3^- is consistent with results from (Nyiraneza et al. 2015) where an initially high concentration of NO_3^- will decrease as plant N uptake occurs throughout the growing season. Subsequent increase in AEM NO_3^- is generally attributed to increased soil N mineralization under ideal temperature and moisture condition as plant N uptake slows down as was seen in these results (Ziadi et al. 1999). Cover crop selection had a significant effect on AEM NO_3^- only on the last sampling date (30 September). Significantly less AEM NO_3^- in T treatment compared to the RC treatment were in agreement with the hypothesis that NO_3^- measured in the soil would be lower under the grass treatment after the potato growing season. An increased concentration of AEM NO_3^- measured from RC compared to T reflects an increased availability of soil NO_3^- at risk of leaching from the plant root zone compared to from potatoes following T and further supports a greater amount of N released from RC residues compared to the T treatment.

When no N fertilizer was applied to potatoes, NO_3^- measured in soil solution at 30 cm was significantly reduced by 69 to 90% when potatoes were grown subsequent to the T treatment compared to legume treatments (19.5 to 40.1 mg NO_3^- L^{-1}) on all sampling dates. With only four sampling dates, it was difficult to determine any seasonal pattern amongst cover crop treatments. On the 2 September sampling date, soil solution from the M treatment at 50 cm was significantly greater than from the RC and T treatments. This

was consistent with a late season peak in KCl-extractable NO_3^- and AEM NO_3^- and points to the overall effectiveness of using ceramic lysimeters to examine cover crop effects.

At 80 cm below-ground, there was an upward trend throughout the potato phase of rotation in all cover crop treatments. By the last sampling date, soil solution NO_3^- was three and seven times greater from the RC treatment (83 mg NO_3-N L^{-1}) compared to the M and T treatment respectively. As predicted, results from the M and T treatments were significantly less than from the RC treatment on all sampling dates and were consistent with results that there was a greater availability of N from red clover residues that could be used by subsequent crop but still returned high amounts residual N to the soil compared to a pure timothy stand. In addition, in the final sampling period in December, soil solution NO_3^- continued to increase in the RC treatment compared to the M or T treatment. Low levels of soil solution in the summer months compared to the fall is consistent with the seasonal pattern of water movement within the soil (Jiang et al. 2011; Zebarth et al. 2015). Additional information on drainage (water infiltration volume over a given area) that was not measured in this study would provide an estimate for the volume-weighted concentration of N loss from cover crop treatments (MacPherson 2010).

Measurements taken within the plant root zone (KCl and AEMs) had considerable variability in results compared to results from measurements taken deeper below the soil (soil solution from 30, 50 and 80 cm lysimeters). Samples taken below the root zone likely reflect cumulative cover crop effects on the subsequent potato crop that were not masked by plant N uptake and fluctuating soil microbial activity.

When 190 kg N ha^{-1} was applied to the potato crop, there was no effect of preceding cover crop observed in soil or soil solution NO_3^-. There was a small trend towards less KCl-extractable NO_3^- measured from the T treatment compared to the RC or M treatments but there was great variability within cover crop treatments on each sampling date. High spatial NO_3^- variability is generally not considered in the case of uniform fertilizer N application (Ziadi et al. 2012).

These results followed only one complete potato rotation and therefore require further investigation for long-term effects of cover crop selection on N effects and additional non-N effects.

3.5. Conclusion

This study evaluated the effects of unfertilized preceding cover crops on soil N availability in subsequent potato crop. Specifically, a red clover legume/ timothy grass mixture was compared with a pure red clover or timothy stand. This study also quantified the temporal changes of NO_3^- in the soil and soil solution after crop harvest in the cover crop and subsequent potato phase based on cover crop selection. An understanding of N cycling from these cover crops into a subsequent potato crop will help to determine beneficial management practices that balance economically viable potato production with environmental risks.

When no N fertilizer was applied in the potato phase of rotation, fall ploughed M and RC treatments resulted in a significantly greater contribution to soil N supply (average of 107 kg N ha^{-1}) compared to the T treatment (60 kg N ha^{-1}). Greater N availability from the RC and M treatments in the potato phase of rotation were likely the result of lower C:N ratio and greater quantity of red clover and mixture residues compared to the timothy residues that subsequently affect soil N mineralization. Greater

N uptake in potatoes from the RC and M treatments however did not result in significantly greater total tuber yield or marketable tuber yield compared to the T treatment and point to possible non-N effects that may have affected tuber yield overall in RC and M treatments.

Potatoes grown subsequent to timothy grass resulted in significantly greater marketable tuber yield compared to potatoes grown subsequent to red clover or red clover/ timothy mixture. Low marketable tuber yield from the RC and M treatments and significantly less potato N uptake from the M treatment compared to the T treatment was likely the result of excess N in the soil that was not supplied at the right time for adequate tuber bulking based on the greater number of smaller tubers compared to those from the T treatment. Alternatively, considering above-ground biomass, C:N ratio and N uptake were comparable in red clover and mixed cover crop treatment and it is possible that below-ground root residues, known to contribute significant quantity of biomass, may have affected the pattern of N release from red clover/ timothy mixture residues compared to red clover residues in the subsequent potato year.

No N fertilizer applied to timothy grass did result in low biomass dry matter but also reduced soil solution NO_3^- measured at 80 cm below-ground compared to a red clover pure stand after the cover crop phase of rotation. In the 0N treatment, a cover crop selection of timothy grass or a mixture of red clover/ timothy offer an alternative rotation compared to a red clover pure stand that can result in reduced soil solution NO_3^- after the potato crop harvest and therefore the potential for NO_3^- leaching. Overall, soil and soil solution NO_3^- results from fertilized potato plots suggest that data sets from more than one rotation cycle are required to determine seasonal and yearly variability.

Currently the risk of applying insufficient N fertilizer to potato crops is greater than the economic cost of applying extra N fertilizer therefore increasing the environmental risk of N leaching. Therefore, though limited by only one cycle of potato rotation, there are two considerations for improving N management in intensive potato production. Firstly, red clover is generally planted in rotation with potatoes for their ability to provide to the soil however, initial results from this study suggest that the additional N provided by legumes did not provide any additional benefit to tuber yield when fertilized with 190 kg N ha^{-1}. In addition, high N application combined with low C:N ratio crop residues like red clover resulted in greater amounts of soil NO_3-N found in field after the cover crop and potato growing season. Secondly, results from this experiment after one cycle of rotation suggest that grass and mixture cover crops provide opportunities to reduce NO_3^- susceptible to leaching outside of the potato phase of rotation in addition to during the potato phase of rotation.

CHAPTER 4. ASSESSING THE TRANSFER OF ^{15}N FROM COVER CROP ABOVE- AND BELOW-GROWND RESIDUES TO SUBSEQUENT A POTATO CROP (EXPERMINET 2)

4.1. INTRODUCTION

The N contribution of above-ground biomass of crops grown preceding potatoes has been well documented (Porter and Sisson 1991; Grandy et al. 2002; Stark and Porter 2005). The below-ground (e.g. root) portion has generally been ignored or underestimated compared to the above-ground residues (Dabney et al. 2010). However, previous work has suggested that the root system contains as much as 33 and 50% of the total plant N in pastures and legumes respectively (Peoples et al. 2001).

The objective of this experiment was to quantify the transfer of N in above and below-ground biomass of cover crop residues to a subsequent potato crop using ^{15}N enriched (labeled) fertilizer. In addition a crop residue exchange technique similar to (Delgado et al. 2004) where labeled above-ground cover crop residues were exchanged with non-labeled residues to trace the fate of the ^{15}N labeled cover crop to a subsequent crop (Fredrickson et al. 1982; Collins et al. 2007). Hollow metal cylinders installed in the field (Follett 2001) as microplots to minimize lateral movement of ^{15}N tracer.

This study examined a red clover legume (conventional grower practice) compared to a timothy pure grass stand or as a red clover/ timothy mixture. Due to differences in quality (e.g. C:N ratio) and quantity of below-ground residues compared to their above-ground counterpart, measuring below-ground N plant biomass contribution would improve the understanding of how different cover crop components contribute N to subsequent potato crops.

4.2. METHODS

4.2.1. Microplot and Cover Crop Establishment

The microplot experiment was established in the same field as experiment 1. In spring 2013, a small area of land (approximate 4 x 20 m) was ploughed and tilled immediately adjacent to experiment 1. Hollow metal cylinders (height: 0.3 m, diameter: 0.46 m, volume: 0.05 m^3) were installed. Each cylinder along with the total plant and soil matter contained within was considered as one microplot. For each microplot, an insertion channel was created in the soil using a proto-type cookie cutter saw blade cut specifically for this project for easier installation and to minimize soil disturbance. The blade was connected to a hydraulic drive attached to the prongs of a Super Boom skid steer loader (New Holland, New Holland, PA, USA). Insertions were made approximately with approximately 0.5 m spacing between cylinders. Each cylinder was inserted into the ground by hand and hammered down leaving approximately 0.1 m protruding above the ground to ensure they were buried at a uniform depth (burial depth: 0.2 m).

The experimental design was analyzed as a split-plot arrangement of treatments in a randomized (block) design with main plots of five ^{15}N labeled residue types and subplots of three cover crop treatments with four replicates each for a total of 60 microplots. The ^{15}N labeled residue type options are listed in Table 4.1.

Table 4.1. Description of ^{15}N labeled residue type for one cover crop treatment.

Treatment	^{15}N labeled residue component	Microplot composition after residue exchange
AG	Above-ground residues	Labeled above-ground residues + unlabeled roots and soil
BG	Below-ground roots and soil	Unlabeled above-ground residues + labeled roots and soil
SOIL	Soil and unrecovered roots	All cover crop biomass removed from microplot
AG_{only}	Above-ground residues	Above-ground residues placed in new microplot
ROOT	Recoverable roots	Root residues placed in new microplot

The three cover crop treatments were red clover (RC), timothy (T) and a red clover/ timothy mixture (M) with four replicates each for a total of 60 cylinders/ microplots. The microplots were hand-seeded to one of the three cover crop treatments at the time of microplot establishment. Cover crops were seeded at three times the rate seeded in Experiment 1 to ensure an adequate plant density. In 2013, cover crops were left undisturbed.

4.2.2. ^{15}N Fertilizer Application

On 28 May 2014, labeled fertilizer ($^{15}NH_4{}^{15}NO_3$, atom 98% ^{15}N) was applied to designated microplots at an equivalent rate of 20, 40 and 60 kg N ha^{-1} for the RC, M, and T treatments respectively. Other designated microplots received $^{14}NH_4{}^{14}NO_3$ fertilizer at the same rates. Pre-weighed fertilizer was dissolved in 0.5 L of distilled water and sprinkled over microplots with a watering can. An additional 0.5 L (total 1 L) of distilled water was added to residual solution in the watering can (Haws Elliott Ltd, West

Midlands, England) and applied to ensure complete application of fertilizer treatments. Metal watering cans were used to apply fertilizer due to the small area of microplots instead of by spraying with a pressurized gas cylinder as was done in previous work (Follett 2001; Delgado et al. 2004) due to the small size of the microplots.

Before fertilizer application, soil samples were taken to provide an estimate of NO_3^- distribution and background ^{15}N in the soil. Samples were taken at a depth of 0-15 cm and 15-30 cm depth. Samples were taken around the perimeter of each microplot so as not to disturb soil in microplots and to prevent N fertilizer from preferentially draining through the sample hole.

4.2.3. Cover Crop and Soil Recovery

During the 2014 growing season, cover crops in microplots were managed in the same way as crops in experiment 1 except they were only cut twice. The first cut occurred on August 8, 2014. Above-ground cover crop tissue was cut to soil level and put into labeled bags. Non-labeled cover crops were harvested before labeled cover crops to avoid cross-contamination. All below-ground roots and soil were left undisturbed. The cover crop tissue samples were weighed and a representative subsample of approximately 100 g was taken. Subsamples of above-ground biomass were dried at 55 °C for 48 hours to determine cover crop dry matter content. The remaining biomass was cut into 5 cm long segments, air dried and then stored in the freezer until crop residue exchange. Samples from the M treatment were not separated into red clover and timothy residues and only total mixed biomass and N accumulation was measured.

The final cut of above-ground biomass occurred on 29 October 2014. The sampling method was identical to the first cut except that an approximately 60 g of tissue was used to determine dry matter content because the collected biomass was low (<

400g) for all cover crop treatments. The remaining biomass was air dried and combined with biomass from the first cut to create one composite sample per microplot.

Below-ground ^{15}N labeled recoverable roots from 12 microplots (4 for each cover crop treatment) were collected during the second cut at the same time as the final above-ground cover crops using a method similar to Bolinder et al. (2002). All soil and recoverable roots were excavated from designated microplots to a depth of 0.3 m (i.e. depth of cylinder) and brought back to the lab in large plastic bags. Cylinders were left in place in the field. The soil was passed through a 4 mm sieve to separate roots from the soil. The roots were then washed twice through a series of sieves (4 mm, 2 mm, 1mm) with distilled water to remove additional soil. The whole root sample was weighed (fresh weight) as collected biomass was small (~ 1000 g) and left to air dry until the date of the crop residue exchange. Recoverable roots were categorized as all the roots that could be collected through sieving and by picking them out with tweezers from the soil. Immediately after the roots were removed from the soil, the soil was returned to the field to their original microplots.

Air dried above-ground tissue and root subsamples were weighed to determine water content and biomass accumulation. Above-ground tissues were ground to pass a < 1 mm screen with a Wiley Mill grinder (Arthur H. Thomas Co., Philadelphia, USA). Due to the small sample size, it was not possible to grind root samples with the commercial grinder. Instead to ensure there was no contamination between samples, root samples were ground using liquid N inside a mortar and pestle until they formed a powder. Each above-ground and root sample (twelve total) were encapsulated in 5 x 8 mm tin capsules. A sample was taken from above-ground tissue from the first and last sampling date

before they were combined. Samples were sent for analysis at the Agriculture and Agri-Food Canada Stable Isotope Lab at the Lethbridge Research and Development Centre for total ^{14}N and ^{15}N and total C using a gas chromatograph-mass spectrometer.

After the final removal of plant biomass, soil samples were taken from all microplots with a soil probe. Three samples were taken in each microplot to form one composite sample at a depth of 0-15 cm and 15-30 cm to measure background ^{15}N in unlabeled plots and ^{15}N fertilizer recovery in labeled plots. Soil samples were passed through a 2 mm sieve. A subsample of soil was ground by hand with a mortar and pestle to pass < 1 mm sieve, weighed and encapsulated in tin capsules to be sent for analysis of total ^{14}N and ^{15}N using a gas chromatograph-mass spectrometer. Plant N accumulation was calculated as:

Eq. [1]: $$Plant\ N\ accumulation\ (kg\ ha^{-1}) = tissueN\ concentration\ g\ kg^{-1} \times \frac{dry\ matter\ yield\ kg\ ha^{-1}}{1000}$$

Labeled fertilizer N recovery in plant and soil samples was calculated based on (Nyiraneza et al. 2010) Labeled fertilizer N recovery was calculated as:

Eq. [2]: $$Plant\ N\ recovery\ from\ ^{15}N\ fertilizer\ (kg\ ha^{-1}) = 100 \times \frac{p\,(c-b)}{f\,(a-b)}$$

where p (kg N ha^{-1}) is the plant total N uptake, f is the amount of fertilizer applied (kg N ha^{1}), a is the abundance in the applied fertilizer (98 atom% ^{15}N), b is the ^{15}N natural abundance of unlabeled plants and c is the atom% ^{15}N of labeled plants. Labeled fertilizer N recovery in soil was calculated as:

Eq. [3]: $$Recovery\ of\ ^{15}N\ in\ soil\ (kg\ ha^{-1}) = 100 \times \frac{s\,(c-b)}{f\,(a-b)}$$

where s (kg N ha^{-1}) is the quantity of N in soil (as calculated by Eq. [4]), f is the amount of fertilizer applied (kg ha^{-1}), a is the abundance in the applied fertilizer (98

atom% ^{15}N), b is the ^{15}N natural abundance of soil samples collected in unlabeled plots and c is the atom% ^{15}N of labeled soil sample.

Soil total N was calculated as follows:

$$\text{Eq. [4]: Soil total N } (kg\ N\ ha^{-1}) = (A \times BD \times D \times TSN)/1000$$

where A is the microplot area (m^2), BD is the soil bulk density (kg soil m^{-3}), D is the sampling depth (m) and TSN is the soil total N concentration (kg N kg^{-1} soil).

4.2.4. Crop Residue Exchange

In fall 2014, above-ground plant biomass from the second cut was combined with biomass from the first cut. Above-ground cover crops that had been combined were brought back to the field on 21 November 2014 in large plastic bags and applied to the designated microplot to establish residue type (Figure 4.1).

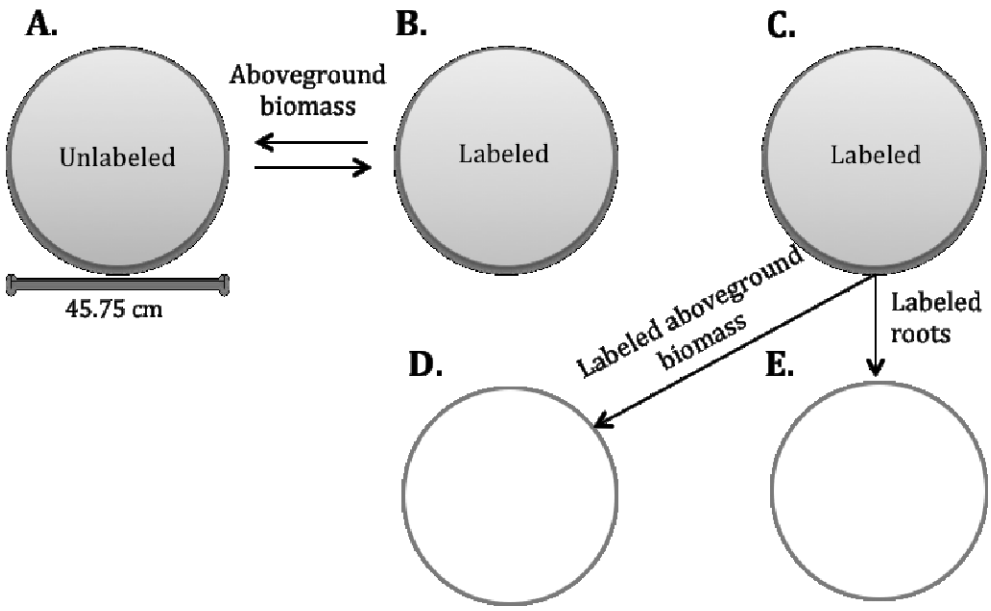


Figure 4.1. Visual description of residue type after crop residue exchange for one cover crop treatment. Shaded areas indicate microplots where cover crops were growing in 2014 before residue exchange, unshaded areas were fallow until residue exchange; A: AG - Above-ground ^{15}N labeled tissue (whole plant); B: BG - Below-ground (soil + root) ^{15}N labeled tissue (whole plant); C: SOIL - Labeled soil only; D: AG_{only} - Above-ground ^{15}N labeled tissues only; E: ROOT - Labeled ^{15}N recovered roots only.

For the AG and BG treatments, soil and roots to a depth of approximately 20 cm from the corresponding microplot were excavated with a hand shovel and carefully placed in a large plastic bag. The plant biomass was placed evenly inside the microplot, and the soil was then placed on top of the biomass where the soil was upturned to attempt to stimulate ploughing. For AG_{only} and ROOT residue types, soil from microplots previously fallow were removed in the same way as for the AG and BG treatments and placed in bags, the corresponding plant tissues were placed inside the microplots and the

soil was placed back inside the microplot upturned to simulate ploughing. After incorporation, microplots were left undisturbed until spring 2015.

In the SOIL treatment, samples to quantify ^{15}N enrichment were taken before soil disturbance (sieving to recover the roots) when the containers were still in the field using soil probes. However, another soil sample should have been taken to measure ^{15}N enrichment after soil disturbance. Soil ^{15}N enrichment from this source includes not only labeled soil but also labeled unrecovered roots as well as associated rhizodeposits (soil influenced by roots), and therefore is expected to be higher than in the soil sample taken before soil disturbance. The SOIL treatment was therefore excluded from analysis.

4.2.5. 2015 – Potato Phase

Potatoes were planted in spring 2015. Soil in microplots was cultivated by hand to mix the soil. A small hill was created within the microplot by moving the soil together towards the centre of the cylinder. Two whole seed potatoes (var. Russet Burbank) were planted in each microplot to ensure that at least one would emerge. Whole seeds were used to decrease the risk of disease (Nolte et al. 2003). As soon as the plants emerged, the smallest potato plant of the two in each microplot was carefully removed by hand.

Potatoes were managed as closely as possible to field conditions. No additional N fertilizer was applied but P and K fertilizer (equivalent to 190 kg ha^{-1} of 0-17-17) was applied to ensure they were non-limiting. The microplots in all treatments were managed according to normal growing practices in PEI and fungicide and herbicide was applied accordingly. There was no supplemental irrigation throughout the growing season as is common in PEI where potato production is generally rain fed (Jiang et al. 2012; Stiles 2012).

The potato plants were harvested from each microplot on 3 September 2015, before potato vine senescence, to determine root, vine and tuber dry matter biomass, N uptake, and ^{15}N residue recovery. The entire potato from each microplot was dug out by hand and placed in designated labeled bags. Tissues were separated into root (with stolons), tuber and vines before being washed and weighed. All the vine and root tissue was then cut by hand to 5 cm segments and dried at 55 °C for 48 hours to determine biomass. All collected tubers from each microplot were cut and weighed. A subsample of approximately 6 tubers from each microplot was dried in the same way as roots and vines for subsequent ^{15}N analysis preparation. To determine tuber yield, calculations were made based on a row spacing of 91 cm and within-row spacing of 38 cm (i.e., 28 704 tubers per ha). This value was used to determine yield in kg ha^{-1} and converted to g m^{-2}.

The dried tissue samples were ground to pass a 1 mm screen using a Black and Decker Smart Grind™ Coffee and Spice Grinder (Miramar, USA). A coffee grinder was used due to the small amount of sample collected. Once tissue was ground, vine, tuber and root subsamples were encapsulated for ^{15}N analysis as was done for cover crops and again sent away for analysis of total C content and ^{14}N and ^{15}N enrichment to determine recovery from cover crop residues. These results were also used to estimate the partitioning of ^{15}N in the potato crop. In the potato year, recovery of ^{15}N was calculated in the same way for fertilizer ^{15}N recovery (Eq.[2]) except f is the amount of ^{15}N applied from cover crop residues (kg N ha^{1}) and a is the abundance in the applied residues (98 atom% ^{15}N).

The potato N uptake from the unlabeled sources was calculated as the difference between total N uptake and ^{15}N uptake from labeled residues.

4.2.6. Statistical Analysis

Statistical analysis was done with the GLM procedure in SAS (SAS Institute 1997) and R Studio Suite (V. 3. 2. 4 – Very Secure Dishes). In R Studio, the following packages were used for analysis: *nlme* for Analysis of Variance (ANOVA) and linear regression for completely randomized design and *lsmeans* to determine least significant differences among treatment means. Significance was determined when p-value was < 0.05. Assumptions of normality and equal variances were checked before analysis. Data was transformed as necessary.

In 2014, cover crop treatments were used to determine above-ground yield (n=12), root dry matter yield (n=4), N uptake, and % ^{15}N recovery. There were no significant differences in dry matter or N uptake between labeled and unlabeled fertilized plots that received the same amount of fertilizer so cover crop biomass and N uptake results were analyzed together by cover crop treatment. In 2015, the main fixed effects were the three cover crop treatments (RC, M, and T) and four residue treatments (AG, BG, AG_{only} and ROOT) for a total of 12 cover crop and residue treatments with 4 reps. The experimental design was analyzed as a split-plot arrangement of treatments in a randomized (block) design. The effects of cover crop treatments and residue type on ^{15}N cycling and potato parameters were measured using ANOVA and linear regression.

4.3. Results and Discussion

4.3.1. 2014 Cover Crop Yield and N uptake

In the cover crop phase of rotation, cover crop selection had a significant effect on above-ground dry matter yield. Above-ground dry matter yield from the RC and M treatment with an average of 1454 and 705 g m^{-2} on the first and second sampling date respectively were significantly greater than from the T treatment of 995 and 106 g m^{-2} on

the first and second sampling dates (Table 4.2). Total above-ground dry matter cover crop yield was an average of 2159 g m^{-2} from the RC and M treatments and approximately three times higher than a yield of 637 g m^{-2} reported by Bolinder et al. (2002) of second year red clover (var. Florex) that was not underseeded with barley. High dry matter yield from cover crops may have been the result of greater seed density within microplots. The seeding rate of red clover in this study was an equivalent of 33 kg ha^{-1} and for red clover/ timothy was 18/ 9 kg ha^{-1} compared to rates of between 10 to 15 kg ha^{-1} for all cover crops observed in Bolinder et. al (2002). Total dry matter yield from the T treatment of 1101 g m^{-2} from an equivalent seeding rate of 18 kg ha^{-1} were comparable to 763 g m^{-2} from timothy (var. Champ) in Bolinder et. al (2002).

Table 4.2. Total cover crop dry matter yield from red clover (RC), timothy (T), and red clover/ timothy (M) treatments in above-ground tissues (i.e. first + second harvest) and below-ground tissues (^{15}N labeled plants only) in 2014.

	Dry matter yield			Biomass
Cover crop	Above-ground		Total	Roots
Date	8-Aug	29-Oct		12-Nov
	g m^{-2}			
RC	1372a[z]	684a	2056a	1030
M	1535a	726a	2261a	1237
T	995b	106b	1101b	1119
ANOVA	Pr>(F)			
Cover Crop	***	***	***	NS

[z]Values followed by different letters within the same column are significantly different p<0.001=***, NS=not significant)

There was no significant difference among cover crop treatments on total recoverable root biomass which averaged 1129 g m^{-2} from soil 0-20 cm from one harvest at the end of the growing season. It was difficult to objectively compare recoverable root biomass in this study compared to previous reports namely due to differences in

experimental design (green pot experiments vs. field plots vs. microplots created with physical barriers), root sampling period and depth, and root separation processes (Bolinder et al. 2002; Skuodiene and Tomchuk 2015). For example, recoverable root biomass in this study from timothy grass was within the range of those found in Bolinder et al. (2002) of 1351 g m^{-2} recovered from the soil 0-45 cm in field plots (1.5 x 4 m). In comparison, red clover root biomass was less than those found in Bolinder et al. (2002) of 794 g m^{-2} averaged between two years. In a an acidic loam soil in Western Lithuania, Skuodiene and Tomchuk, (2015) recorded root biomass recovered from the soil 0-10 cm of a second year red clover (var. Vyliai) and timothy (var. Gintara II) underseeded with barley in the previous year with no mineral fertilizer of 660 and 721 g m^{-2}. Greater root biomass from this experiment was consistent with greater above-ground tissue also recovered in this study compared to other studies.

Recoverable root biomass accounted for 35, 33 and 49% of the total dry matter collected from the RC, M, and T treatments, respectively (Table 4.2). The proportion of root biomass compared to the total cover crop collected was comparable to values reported in Li et al. (2015) of values 37, 36 and 40% from first year catch crops of red clover (var. Rajah), perennial ryegrass (*Lolium perenne* L., var. Foxtrot) /red clover mixture and perennial ryegrass roots recovered from soil at 0-18 cm depth. In addition, partitioning of root biomass as a proportion of total plant biomass was within the range found in Bolinder et al. (2002) of 15 to 34% for red clover (no N fertilizer) and 36 to 61% from timothy with 90 kg N ha^{-1} fertilizer applied Bolinder et al. (2002).

Nitrogen accumulation from above-ground tissue in the RC and M treatments (averaged 34 and 20 g N m^{-2} on the first and second sampling dates, respectively) was

significantly greater than N accumulation in the T treatment (7 and 1 g N m^{-2} on the first and second sampling dates, respectively). Nitrogen accumulation from BNF likely explains the greater N accumulation from RC and M compared to from the T treatment. Fertilizer N was only applied in a small enough amount to adequately label cover crops and the majority of N accumulated from the T treatment would have had to come from soil available N. The recoverable root portion from the RC and M treatments accounted for 26 and 28% of total cover crop N uptake and were on the lower end of results compared to 31 to 37% found in Li et al. (2015) from legume containing catch crops underseeded with barley (red clover, perennial ryegrass/ red clover mixture and winter vetch [*Vicia villosa*, var. Villana]). In this study no additional fertilizer N was applied other than tracer compared to fertilizer application in Li et al. (2015) that consisted of a total of 80 kg NH_4-N ha^{-1} (as 33 Mg ha^{-1} cattle slurry applied in spring) and 10 kg N ha^{-1} applied in three split applications as KNO_3 (11.58% atm excess ^{15}N). From the T treatment, 41% of the total plant N uptake was found in the root partition comparable to 40% in perennial ryegrass found in Li et al. (2015). In Li et al. (2015), plant measurements were taken in the planting year compared to in the second year for this experiment.

Table 4.3. Total N accumulation in above-ground tissue from red clover (RC), timothy (T), and red clover/ timothy (M) treatments (i.e. first + second harvest) and below-ground tissues (^{15}N labeled roots only) in 2014.

	N accumulation			
Cover crop	Above-ground tissue		Total above-ground tissue	Root tissue
Date	8-Aug	29-Oct		12-Nov
			g N m^{-2}	
RC	35.1a[z]	19.0a	54.1a	19.3a
M	32.4a	20.5a	52.9a	20.2a
T	7.3b	1.1b	8.5a	5.9b
ANOVA			Pr>(F)	
Cover Crop	***	***	***	***

[z]Values followed by different letters within the same column are significantly different ($p<0.001$=***, NS=not significant)

For above-ground tissues, the C:N ratio was greater in the T treatment (44) than in the RC and M treatments (average of 17, Table 4.4). The C:N ratio of plant tissues is an indicator of tissue N content where a lower C:N value generally indicates greater N content then tissues with a greater C:N ratio. The low C:N ratio from the M and RC treatments suggests that these tissues would likely decompose more quickly, and result in a net mineralization, compared to residues from the T treatment with a greater C:N ratio (Janzen et al. 1990; Ranells and Wagger 1997b; Kumar and Goh 2002).

Biomass in the mixed cover crop treatment were not separated into grass and legume tissues, but the lower C:N ratio in the above-ground M treatment compared to the T treatment was likely a reflection of the greater N concentration in red clover tissues. Ranells and Wagger, (1996) likewise observed a lower C:N ratio in above-ground legume/ grass cover crops of an average of 26 in a rye and crimson clover compared to an average of 40 in a rye pure stand in two experiments over 2 years.

Table 4.4. The C:N ratio of above-ground tissues from red clover (RC), timothy (T), and red clover/ timothy (M) treatments on two sampling dates and recovered roots collected before residue incorporation.

	C:N ratio		
Cover crop	Above-ground tissue		Recovered roots
	08-Aug	29-Oct	12-Nov
RC	18b[z]	16b	16b
M	22b	16b	18b
T	61a	37a	44a
ANOVA		Pr>(F)	
Cover crop	***	***	***

[z]Values followed by different letters within the same column are significantly different. ($p<0.001$=***, NS=not significant)

4.3.2. 2014 Recovery and Partitioning in Cover Crops Labeled With ^{15}N Fertilizer

The plant-soil recovery of applied ^{15}N fertilizer in the cover crop treatments ranged from 84 to 104% (sum of above-ground, root and soil recovery), the latter from the RC treatment (Table 4.5). Generally high recovery of ^{15}N fertilizer suggests there were minimal losses of fertilizer N throughout the growing season and sampling periods.

The total recovery of ^{15}N fertilizer in above-ground residues from two cuts was 32, 43 and 50% from the RC, M, and T treatments respectively and was significantly greater from the T treatment than from the RC treatment in the first cut. In the second cut, ^{15}N recovery in above-ground tissue from the M treatment was significantly greater than from the RC treatment and from the T treatment (Table 4.5), indicative of continued plant N uptake from the M treatment. There was an overall trend of increased ^{15}N fertilizer recovery with decreasing legume content (RC < M ≈ T). Labeled fertilizer recovery in above-ground tissues from this study were comparable to Ranells and Wagger (1997) for

fall applied ^{15}N fertilizer (spring recovery) from a pure clover stand (4%), a rye-clover mixture (19%) and rye pure stand (39%) averaged across 2 growing seasons.

Table 4.5. Percent of applied ^{15}N fertilizer recovered in above-ground plant tissues, recovered roots and soil as influenced by cover crop treatment in 2014.

Cover crop	Above-ground tissue		Total	Root tissue	Soil 0-15 cm	Soil 15-30 cm	Total soil
	8-Aug	29-Oct		12-Nov	31-Oct		
	% ^{15}N of applied fertilizer						
Red clover (RC)	29.36b[z]	2.20b	31.56b	9.57a	45.93a	17.35a	63.28a
Red clover/Timothy (M)	38.86ab	4.15a	43.01ab	8.27b	32.98a	9.69b	42.67b
Timothy (T)	48.08a	1.98b	50.06a	10.96a	16.27b	6.68b	22.95c
ANOVA				Pr>(F)			
Cover crop	*	**	*	**	***	***	***

Above-ground Tissue; n= 8, Roots; n=4, soil; n=8

[z]Values followed by different letters within the same column are significantly different.
($p<0.05$=*, $p<0.01$=**, $p<0.001$=***, NS=not significant)

The recovery of ^{15}N fertilizer in roots was lower in the M treatment (8%) compared to the RC and T treatments which averaged 10% (Table 4.5). Root recovery of ^{15}N fertilizer was comparable to ^{15}N greenhouse experiments from McNeil et al. (1997) for leaf-fed (immersed in solution of ^{15}N labeled urea, 99.6 atm% excess) subterranean clover and seradella (*Ornithopus compressus* L.) of around 3 to 12%. In McNeil et al. (1997), recoverable root recovery of ^{15}N fertilizer accounted for 8 to 10% of total ^{15}N recovered in above and below-ground root, slightly lower, compared to 23, 16 and 18% root recovery of total ^{15}N fertilizer recovery from the RC, M, and T treatments respectively. Lower recovery from McNeil et al. (1997) could be the result of ^{15}N fertilizer application method (leaf-feeding vs. broadcast spraying onto soil in this study) and subsequent translocation of ^{15}N (Khan et al. 2002a).

The total amount of fertilizer recovered in the soil at 0-30 cm depth after cover crop harvest followed an inverse relationship (T < M < RC) to the recovery found in the above-ground tissues (Table 4.5). In the soil at 0-15 cm depth, there was no significant difference between RC and M (average of 40%) and was significantly greater than ^{15}N fertilizer recovery in the T treatment (16%). However, in the soil at 15-30 cm depth, ^{15}N fertilizer recovery was significantly greater from the RC treatment (17%) compared to the M or T treatments (average of 8%). Greater ^{15}N fertilizer recovery in deeper soil depths could reflect the downward movement of N that was not utilized by the red clover. Soil ^{15}N recovery from T treatment was comparable to results in Delgado et al. (2004) for spring planted small grains (barley (var. C-37), white wheat (var. Centennial) and hard red wheat (Oslo) that averaged 27% recovery of applied ^{15}N fertilizer.

4.3.3. 2015 Potato Biomass and N accumulation

There was a significant interaction among cover crop treatments and residue type (Table 4.6). Across all cover crop treatments and residue types, potato total plant biomass was low and ranged from 175 g m^{-2} from the AG and BG x timothy treatment and 696 g m from the AG and BG x red clover treatment (Table 4.7). Potato total plant biomass was significantly greater from the RC and M (average of 655, 657 and 514 g m^{-2}) residues compared to residue from the T treatment (175, 221, and 261 g $^{-2}$) in AG, BG and AG_{only} treatments respectively. When only root residues of cover crops (i.e. ROOT treatment) were applied to microplots the total plant biomass from the RC treatment (597 g m^{-2}) was significantly greater than from the T treatment (393 g m^{-2}). Greater total plant biomass following RC and M compared to T reflected the greater amount of biomass returned to the soil with a low C:N ratio compared to from T treatment. There was a trend towards decreased potato biomass in partial cover crop (i.e. AG_{only} and ROOT treatment) compared to whole plant treatments (i.e. AG and BG) from the RC and M treatments whereas the reverse trend (AG≈BG < AG_{only}≈ROOT) was observed from T treatment. In the RC and M treatment, a trend towards decreased potato biomass when above-ground biomass was removed reflects a scenario where potatoes would be grown subsequent to a forage red clover where the crop is removed from the field. Alternatively, the inverse trend when above-ground residues from T treatment are removed reflects a trend toward improved potato biomass when not competing for soil available N.

Table 4.6. Total biomass and N uptake in total plant for potatoes grown subsequent to contrasting residues from unfertilized red clover (RC), timothy (T), and red clover/ timothy (M).

Cover crop (C)	Residue (R)	Biomass	N uptake
		g m^{-2}	
RC		620a[z]	7.94a
M		561a	8.02a
T		263b	2.96b
	AG	495	7.59a
	BG	511	7.96a
	AG_{only}	429	5.66ab
	ROOT	488	4.01b
ANOVA		Pr(>F)	
C		***	***
R		NS	***
C x R		**	***

AG; Above-ground ^{15}N labeled tissue (whole plant), BG; Below-ground (soil + root) ^{15}N labeled tissue (whole plant), AG_{only}; Above-ground ^{15}N labeled tissues only, ROOT; Labeled ^{15}N recovered roots only

[z]Values followed by different letters within the same column are significantly different ($p<0.01$=**, $p<0.001$=***, NS=not significant)

(Cover crop x residue; n=4)

Table 4.7. Whole potato biomass and N uptake as influenced by cover crop treatment (red clover [RC], timothy [T], and red clover/ timothy [M]) and residue type interaction.

Cover crop	Residue							
	Biomass				N uptake			
	AG	BG	AG_{only}	ROOT	AG	BG	AG_{only}	ROOT
	g m^{-2}							
RC	631.8a[z]	695.8a	553.7a	597.1a	9.17a	10.51a	7.19a	4.89a
M	678.3a	617.4a	472.8a	473.6ab	11.11a	10.56a	6.37a	4.04a
T	175.1b	220.6b	261.3b	393.1b	2.49b	2.83b	3.42b	3.11a

AG; Above-ground ^{15}N labeled tissue (whole plant), BG; Below-ground (soil + root) ^{15}N labeled tissue (whole plant), AG_{only}; Above-ground ^{15}N labeled tissues only, ROOT; Labeled ^{15}N recovered roots only

[z]Values followed by different letters within the same column are significantly different.

There was significant cover crop treatment by residue type interaction on total N uptake in potatoes. In the AG, BG and AG_{only} treatments, both legume treatments had a significantly greater uptake of total N compared to the grass treatment whereas N uptake from the ROOT treatment was low for all cover crop treatments and did not exceed 5 g N m^{-2}. There was a trend towards decreased N uptake with decreased cover crop residue addition (AG and BG treatment compared to AG_{only} and ROOT treatments) from the RC and M treatments. In the grass treatment, there was a small trend towards increased N uptake with decreased cover crop residue application ($AG \approx BG < AG_{only} \approx ROOT$). Slightly greater N uptake as the amount of applied grass residues was decreased was likely the result of less completion between soil microbes and potato crop N demands.

4.3.4. 2015 Recovery and Partitioning in Potato From ^{15}N Labeled Cover Crop Residues

In all cover crop and residue, the pattern of recovered ^{15}N residues in total potato plant components (Table 4.6) was 50, 45 and 5% in tubers, vines and roots respectively (Table 4.8) and was comparable to the pattern of total N uptake in potato plant components (data not shown). There was a significant cover crop effect on tuber and total plant ^{15}N recovery among residue types. Overall the recovery of ^{15}N from residues averaged across residue types was significantly greater from the RC (3.5%) and M (3.4%) treatments compared to the T treatment (1.0%).

Table 4.8. Total ^{15}N recovery from applied labeled residues in potato plant components and recovery in soil after potato harvest in 2015 as influenced cover crop treatment (red clover [RC], timothy [T], and red clover/ timothy [M]) and residue type.

		Potato				Soil		Total
Cover crop (C)	Residue (R)	Tuber	Vine	Root	Total	0-15 cm	15-30 cm	
		%^{15}N Recovery from Applied Residues						
RC		1.82a[z]	1.56a	0.10a	3.48a	49.2a	14.3	66.76a
M		1.72a	1.57a	0.09a	3.38a	30.5b	13.2	46.83b
T		0.49b	0.45b	0.04b	0.99b	42.6b	14.9	58.40ab
	AG	1.10	1.15	0.06	2.31	35.9	14.8	52.98ab
	BG	1.33	1.48	0.09	2.90	43.7	12.9	58.91ab
	AG_{only}	1.70	1.18	0.07	2.95	52.2	17.4	72.29a
	ROOT	1.25	0.96	0.08	2.29	31.3	11.5	45.14b
ANOVA		Pr(>F)						
C		***	***	***	***	*	NS	*
R		NS	*	NS	NS	NS	NS	*
C x R		NS	NS	*	NS	NS	NS	NS

AG; Above-ground ^{15}N labeled tissue (whole plant), BG; Below-ground (soil + root) ^{15}N labeled tissue (whole plant), AG_{only}; Above-ground ^{15}N labeled tissues only, ROOT; Labeled ^{15}N recovered roots only

[z]Values followed by different letters within the same column are significantly different ($p<0.05$ = *, $p<0.001$=***, NS=not significant)

In this study, the percentage of ^{15}N recovered in the potato crop from cover crop residues was lower than values from other tracer studies. In addition, there were few studies that explored cycling of ^{15}N from fall incorporated cover crops in the field into

subsequent crops. The majority of the following studies examined the effect of labeled cover crops grown in the green house and applied to field (Harris et al. 1994) or followed winter cover crops labeled with ^{15}N in the fall (Ranells and Wagger 1997b; Collins et al. 2007). Harris et al. (1994) found that corn recovered 15-16% ^{15}N (equivalent to 25 kg N ha $^{-1}$) from labeled above-ground residues of red clover (grown in the greenhouse) when applied to hollow cylinders that were inserted into a previously established field plot before spring planting. In small field microplots (3.5 x 1.6 m within a larger 5.9 x 1.6 m plot), results from Collins et al. (2007) reported that 29% of ^{15}N (equivalent to 40 kg N ha $^{-1}$) was recovered from above-ground mustard grown as a winter cover crop and incorporated in spring before planting potatoes. In Colorado in sandy loam soil, Delgado et al. (2004) observed that 6 and 12% of ^{15}N (equivalent to 2.6 to 4.4 kg N ha $^{-1}$) from fall incorporated grain straw and chaff in wheat and barley, respectively, was incorporated into a subsequent potato crop. From these experiments, only Delgado et al. (2004) followed fall incorporated residues as in this study but were done in field microplots (3.7 x 2.3 m within larger 3.9 x 5.3 m plots). In this study, assuming N uptake in potatoes is comparable between ^{15}N and N, the amount of N recovered from the RC and M above-ground residues was approximately 1.89 and 1.79 g N m^{-2} (equivalent to 19 and 18 kg N ha^{-1}) respectively.

In addition to cover crop selection, cover crop management is also an important consideration. The AG_{only} treatment where roots were removed from the soil was assessed to monitor the fate of only the above-ground labeled N but was not reflective of field practices. The whole biomass, AG and BG treatments gave insight into N cycling

from green manure whereas the ROOT treatment is more reflective of a forage rotation system where above-ground residues are removed for forage, leaving behind the roots.

In this experiment, total ^{15}N recovery from the ROOT treatment was not significantly different from other residue types. On average, the subsequent potato plant recovered 2.6% of ^{15}N from labeled residues. In another experiment using hollow cylinders (0.6 m diameter, 0.6 m depth), Harris and Hesterman, (1990) found no significant difference in recovery of ^{15}N from fall incorporated labeled alfalfa shoots or roots recovery (average of 26%) in a subsequent corn crop in a sandy loam soil. In the same study, in a loam soil, recovery of ^{15}N labeled above-ground alfafa shoots was significantly greater (19.3%) than the contribution from labeled roots and crown material (14.0%) in a subsequent corn crop. Comparable results to Harris and Hesterman (1990) were found in Li et al. (2015) for spring incorporated ^{15}N labeled crimson clover tops (17%) and roots (14%) residue recovery in a subsequent spring barley using hollow metal cylinders (inserted in established field plots). Much lower recovery rates in potato plants as related to residue type from this study compared to the previously mentioned studies could be the application method of ^{15}N fertilizer (presumed to be uniformly labeled ^{15}N red clover shoots [5.5atm% excess]) at a rate of 165 kg N h^{-1} Harris and Hesterman, (1994) and spring incorporation immediately before crop planting Li et al. (2015).

Greater total recovery from AG, BG and AG_{only} compared to ROOT treatment, averaged across cover crop treatments, possibly reflected the ^{15}N recovered in soil 0-30 cm. The ROOT treatment consisted of labeled recoverable roots only and it is possible that the recovery of root ^{15}N in the cover crop phase was an underestimate of total root-derived N. McNeil et al. (1997) reported that between 30-62% of below-ground N could

be attributed to recoverable roots but that an additional 17-24% was attributed to root-derived rhizodeposits and root-derived N found in sampled bulk soil. In the BG treatment, below-ground tissues were left mostly undisturbed except for during residue exchange incorporation. No distinction was made whether% ^{15}N recovery in the subsequent potato plant from BG treatment was from soil derived ^{15}N (initial ^{15}N fertilizer) or from root or legume derived ^{15}N. It is possible the results from BG in this study underestimated the total root N (below-ground N) contribution and overestimated soil ^{15}N contribution.

Total recovery of labeled residues in a subsequent potato crop of an average of 58.72% (subsequent potato + soil 0-30 cm) from partial residue treatment (i.e. AG_{only} and ROOT) across three cover crop treatments in this study was comparable to Harris and Hesterman, (1990). Total ^{15}N recovery in Harris and Hesterman, (1990) from labeled residues averaged 53.6 and 67.7% (subsequent corn + soil 0-60 cm) on sandy loam and loam soil respectively, averaged between labeled alfafa shoot and roots/crown residues.

There was a significant cover crop effect of residual ^{15}N remaining in the soil 0-15 cm and residual ^{15}N residues from the RC treatment (49%) was significantly greater compared to M (31%) or T (43%) residues. Total residual ^{15}N recovery in soil 0-30 cm was 64, 44 and 58% from the RC, M, and T treatments respectively. Residual soil ^{15}N may become available to subsequent crops. However, previous studies have suggested that most residual N will be lost from cropping systems with the first two years (Harris et al. 1994; Kumar and Goh 2002b) so further work should continue to determine the long-term cover crop and residue type effects on N cycling from cover crops.

4.4. CONCLUSION

The purpose of this study was to quantify the above-ground residue and below-ground roots and soil contribution from three different cover crop treatments to a subsequent potato crop using a ^{15}N isotopic residue exchange technique.

In the initial ^{15}N fertilizer application year, cover crop recovery of labeled fertilizer ranged from 84 to 104% indicating minimal losses of ^{15}N fertilizer in the cover crop growing season. Total recovery of labeled fertilizer was 56% greater in above-ground biomass measured from the T treatment than RC treatment (32%). and reflected the greater ability to remove fertilizer N from the soil.

When the RC and M whole cover crop biomass (AG and BG) was incorporated, there was a trend towards greater total N uptake (average of 7.76 g N m^{-2}) in the subsequent unfertilized potato plant compared to when only partial residues were returned from AG_{only} or ROOT treatments (5.66 and 4.01 g N $^{-2}$ respectively). The potato crop recovered approximately 3.4% ^{15}N (equivalent to 25 kg N ha^{-1}) from labeled RC or M residues. Recovery of ^{15}N from labeled residues was largely related to the quantity of residue added (and quality of cover crop as related to C:N ratio). Recovery of labeled root residues was approximately 0.6 g N m^{-2} (6 kg N ha $^{-1}$) from legume treatments, accounting 24% of N contribution in comparison to the whole biomass.

There was no significant difference in ^{15}N residue recovery in the potato crop among residue treatments on total ^{15}N recovery however greater residue ^{15}N was recovered in the soil when only AG_{only} residues were incorporated compared to the ROOT treatment. The ^{15}N remaining in the soil was 60% greater in AG_{only} than from the ROOT treatment (45%) and suggested that although more N was released from above-ground residues, it was not actually taken up by the subsequent potato crop. Nitrogen

remaining in the soil will have implications for both the risk of NO_3^- leaving the plant root zone and on N availability in subsequent years.

The tracer experiment was done in microplot cylinders and may not have been representative of root growing patterns in the field. Root-derived N was not sampled in this study meaning root ^{15}N as a portion of total belowground N may have been underestimated or misattributed to soil ^{15}N in this study. Further study should continue to evaluate the quantity and quality of below-ground cover crop residues for their contribution to soil N supply. In addition, much of the labeled ^{15}N from cover crop residues was found in soil (58%) including from the T treatment in the subsequent potato phase of rotation and could potentially become available in subsequent years. This study was limited on one cycle of rotation and additional studies are needed to validate these findings.

CHAPTER 5. CONCLUSION

The main objective of this project was to examine novel potato crop rotations as a strategy to improve N management in potato production that could also mitigate NO_3^- leaching. In addition, cover crops were separated by above and below-ground biomass (roots and soil) to determine their separate N contribution to a subsequent crop with the use of ^{15}N fertilizer.

From the field and microplot experiment, biomass production from above-ground RC and M was approximately two and a half times greater and resulted in four times the N accumulation compared to under the T treatment. In addition, recovery of labeled fertilizer (experiment two) was significantly influenced by cover crop selection. Timothy was 49 and 24% more effective in removing labeled fertilizer N from the soil compared to RC (41%) or M (51%) treatments respectively, reflecting the overall greater N "scavenging" abilities of timothy compared to legumes and a legume/ grass mixture. In addition, NO_3^- measured below the root zone in T was reduced by as much as 81% compared to from RC and M treatments (approximately 28 mg NO_3-N L^{-1}). Greater soil and soil solution NO_3^- reflected N accumulation from BNF in RC and the red clover portion of M and the greater amount of N returned the field from RC and M treatment residues of 135 kg N ha^{-1} (average) with a low C:N ratio compared to from the T treatment.

In the potato phase of the rotation in the field study, total tuber yield was affected mainly by N rate but not cover crop selection. Despite greater total tuber yield when N fertilizer was applied, marketable tuber yield was significantly reduced when grown following cover crops of red clover or red clover/ timothy. When no N fertilizer was applied in the potato phase of rotation, fall ploughed M and RC treatments resulted in a

significantly greater contribution to soil N supply (average of 107 kg N ha $^{-1}$) compared to the T treatment (60 kg N ha $^{-1}$). The direct contribution measured from labeled crop residues was approximately 25 kg N ha^{-1} (experiment two) and approximately 24% of this contribution was attributed the root portion from RC and M treatments. Additionally, there was a small trend upwards of total N uptake when the whole cover crop biomass was incorporated suggesting an overall synergistic effect of incorporating above- and below-ground biomasses from RC and M treatments.

When N fertilizer was applied on potato phase in the field study, total N uptake was not significantly different between the RC and T treatments so despite the additional N contributed by the RC treatment, it was not in synchrony with potato N demands, was in excess, or there were other non-N effects that may have affected total tuber sizing in the RC treatment. In the field study, excess N resulted in yield depression following the legume treatments (RC &M treatments) which were in turn associated with higher residual soil nitrate after potato harvest. The amount of N returned to the field (potato vine N + residual soil and fertilizer N) following T resulted in a decrease of at least 38 and 41% of N remaining in the field compared to RC (195 kg N ha^{-1}) and M (203 kg N ha^{-1}). From the steel lysimeters, at 80 cm below-ground, a preceding crop of M and T resulted in a 66 and 86% reduction in NO_3^- respectively from 83 mg NO_3-N L^{-1} from unfertilized potato crops grown subsequent to RC.

The selection of cover crops within a potato crop rotation has implications for nitrogen management. Timothy has generally been included in mixture with red clover to utilize the N scavenging ability of grasses with N contribution from red clover, however in this study results from the M treatment were comparable to RC for almost all measured

parameters implying that red clover dominated timothy in the mixture even if the proportion of red clover with timothy was not measured in this study and should possibly be evaluated.

Legumes are included in potato rotation as beneficial N nitrogen management practice to reduce the use of synthetic fertilizer ultimately to reduce the risk of NO_3^- leaching. However, this practice must be combined with an accurate estimate of N credit from both above- and below-ground red clover residues in addition, N losses outside of the potato growing season should also be considered for red clover within a three-year potato rotation. The initial results from this study suggest that timothy is a good cover crop to uptake residual soil mineral N, and when grown preceding potatoes could reduce the risk for NO_3^- leaching in potato production in PEI compared to red clover. In the field, above-ground cover crop biomass will either be returned to the field or removed (as forage), however, roots will remain in the soil and therefore should also be considered as part of the cover crop N credit.

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APPENDIX

Table A. 1. Field activity and sampling dates throughout the 2014 cover crop phase of rotation.

Month	Date	Soil	Bio	Lys	AEM	Forage cut
May	29	x		x	x	
Jun	11	x				
	17			x	x	1
	24		x	x		
	30				x	
Jul	9	x		x		
	14				x	
	18			x		
	28				x	
Aug	1	x				
	8			x		2
	12				x	
	20			x		
	26		x		x	
Sep	5			x		
	10				x	
	17	x				
	23			x	x	
Oct	2			x		
	8		x*		x	
	10			x		
	17			x		
	21				x	
	22					
	30 /31			x	x	
Nov	27	x				
Dec	3					x

Soil – Soil Sample 0-15, 15-30 cm in each plot (May and Nov; 0-15, 15-30, 30-45, 45-60)
Bio – 1 m^2 squared biomass sampling. On each sampling date, a subsample from the 1 m^2 was taken for dry biomass matter samples
Lys – Ceramic or steel lysimeter samples
AEMs – Date of AEM removal in field
* On Oct 8, biomass sample was taken before cover crop ploughdown

Table A. 2. Field activity and sampling dates throughout the 2015 potato growing season.

Month	Date	Soil	Cer	AEM	Pot	STL
May	14					x
	20	x*				
	22					x
	27			x**		
	28				x	
June	4			x		x
	10					x
	12	x				
	16			x		x
	24					x
	30					x
July	15	x		x		x
	21			x	x	
	24					x
	29					x
Aug	4					x
	5			x		
	11		x			
	17	x		x		
	26/28					x
Sep	2		x	x		x
	3				x	
	14					x
	15	x				
	17			x		
	21		x			
	24					x
Oct	5					x
	9			x		
	13/15		x			
	20				x	
	21					x
Nov	3					x

May 28 2015 – Potato planting
July 21 2015 – Potato hilling
September 3 2015 – Tissue sample
October 20 2015 – Potato harvest

Table A. 3. Total % ^{15}N recovery by labeled parts of forage treatment and residue type for calculation in potato phase. The values used to calculate residue biomass contribution and potato 15N recovery from residues.

Treatment		Total Biomass	AG ^{15}N	Root ^{15}N	Soil 0-15cm ^{15}N	Soil 0-30cm ^{15}N	Total Recovery
Residue	Forage	g m $^{-2}$			%		
	RC	3067	36.04				36.04
AG	M	3593	48.99				48.99
	T	2240	56.68				56.68
	RC	3213		10.19	53.92	71.25	107.29
BG*	M	3612		8.83	36.43	47.41	96.40
	T	2136		11.69	19.20	26.80	83.47
	RC	1947	31.01				31.01
AG_{only}	M	2051	42.54				42.54
	T	1166	49.94				49.94
	RC	1023		10.19			10.19
ROOT	M	1237		8.83			8.83
	T	1119		11.69			11.69

*BG; total biomass and root values estimated from recovered roots (n=4).