Quantification Of Gross Nitrogen Transformation Rates Within A Conventional Potato Rotation Using Stable Isotopes

by

Kyra L. Stiles

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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled “Quantification Of Gross Nitrogen Transformation Rates Within A Conventional Potato Rotation Using Stable Isotopes” by Kyra L. Stiles in partial fulfilment of the requirements for the degree of Master of Science.

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Co-Supervisor: _____

Co-Supervisor: _____

Readers: _____

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Signature of Author

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ABSTRACT

This study used the isotope pool dilution method to estimate gross rates of mineralization, nitrification, NH_4^+ and NO_3^- consumption, and denitrification emissions over two growing seasons within a conventional barley-red clover-potato crop rotation on Prince Edward Island. Gross rates within the 2010 season were, in most cases, not significant across crop species or sampling date. In comparison, gross nitrification, NH_4^+ consumption, and NO_3^- consumption rates in 2011 were greatest within the potato crop following planting and hilling. However, rates were highly variable within both seasons. Error analysis indicated that variation in soil mineral nitrogen concentrations between duplicate cores was the greatest source of error. The use of the isotope pool dilution method to estimate gross nitrogen transformation rates using intact cores was not viable within this production system due to high and variable soil mineral nitrogen concentrations, particularly following fertilizer application.

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Chapter 1.0 Introduction

Nitrogen (N) is commonly the most limiting nutrient required for all living plants (Marschner 1995). This limitation appears counterintuitive considering the large abundance of N found on the earth, where nitrogen gas comprises 78% of the total atmosphere (Brady and Weil 2001). However, only a small fraction of N is found in plant available forms within the soil. Unlike plants within natural systems that often use N in its organic forms, agricultural systems utilize N in its inorganic forms, primarily as nitrate (NO_3^-) and ammonium (NH_4^+) (Subbarao et al. 2006). These inorganic forms of N are only introduced naturally into the soil from the atmosphere in a limited supply, through either N_2-fixation or atmospheric deposition (Galloway 1998).

In order to overcome N limitations and optimize yields, N often needs to be added within agricultural systems through synthetic fertilizers, animal and green manures, or the introduction of N_2-fixing legumes within crop rotations (Zebarth et al. 2009). If sufficient soil N is available to plants, this can stimulate root development and activity, and aid in uptake of other essential nutrients, which can promote rapid plant growth and optimize crop yields (Stevenson 1986). However, over-application of N through fertilizers and manures, even within organic potato production systems, can occur (Lynch et al. 2012), which can pose environmental risks.

Excess amounts of N within soils accumulate as soluble nitrates that can contaminate groundwater sources through leaching, or can be released as greenhouse gas emissions in the form of nitrous oxide (N_2O) (Zebarth et al. 2009). The primary sources of N_2O within agricultural soils are the processes of denitrification and nitrification

(Mosier et al. 1998). All of these outcomes are potentially harmful to the environment and to human health (Di and Cameron 2002). When NO_3^- concentrations within the soil are high, there is a greater risk that NO_3^- can be moved through erosion or runoff into nearby surface waters, causing eutrophication (Kundu et al. 2007). High concentrations of NO_3^- can also leach into groundwater and contaminate drinking water, which can cause methemoglobinemia (also known as blue baby syndrome) and has been linked to carcinogenic activity (Health Canada 1987).

Nitrate leaching is considered the main loss of N within potato crops, often resulting in groundwater contamination (Zebarth and Rosen 2007). The rate of N applied as mineral fertilizer is one of the main factors influencing the rate of NO_3^- leaching that occurs. Potato crops require high amounts of N inputs to maintain high tuber yields (Stark and Porter 2005). The increased rate of N fertilizer increases the nitrate leaching potential and estimates of NO_3^- leaching from commercial potato fields can range from 10 to 171 kg N ha^{-1} (Zebarth and Rosen 2007). Potato crops have a very shallow root-system depth, and are also frequently grown in sandy soils with low water holding capacities; both result in soil with a high nitrate leaching potential (Shock et al. 2007). The impact of increased cultivation within agricultural crops, as seen in potato crops, has also been linked to NO_3^- leaching as cultivation can increase the rate of mineralization of soil organic N (Di and Cameron 2002).

Groundwater NO_3^- contamination is of particular concern in Prince Edward Island (PEI), a province that acquires all drinking water from groundwater sources (Savard et al. 2010), and is also known for its intensive agricultural production. In 2009, over 40% of land in PEI was under agricultural production (Commission on Nitrates in Groundwater

2008), with 34 400 ha of this land cropped to potatoes (Statistics Canada 2009). The decline in water quality of both surface water and groundwater sources in PEI in the last thirty years has been attributed to both an increase in intensive agriculture as well as residential and commercial development (Commission on Nitrates in Groundwater 2008). Concern over the future water supplies on PEI spurred the creation of the Commission on Nitrates in Groundwater (Commission on Nitrates in Groundwater 2008).

This study will examine the key processes controlling the availability of NO_3^- within the soil root zone of a conventional potato crop rotation within PEI soils. A better understanding of the controls on NO_3^- production in soils will aid in addressing this aforementioned NO_3^- contamination issue.

Chapter 2.0 Literature Review

2.1 Soil N Cycling and Nitrate Availability

The soil N cycle is a highly integrated system with many inputs and losses. There are a number of processes that influence the availability of soil NO_3^-. Nitrogen can be added to the soil in mineral fertilizers, crop residues, manures, atmospheric deposition or through biological fixation of N_2 gas by N-fixing bacteria (Stevenson 1986). Inorganic N can be removed from the soil through assimilatory NO_3^- reduction (plant and microbial assimilation), dissimilatory nitrate reduction (chemodenitrification and respiratory denitrification), NO_3^- leaching (Sylvia et al. 2005), surface runoff and erosion, and can also be bound within clay particles through NH_4^+ clay fixation (Stevenson 1986).

2.1.1. Sources of Mineral N in Soil

Nitrogen is initially incorporated into soil through N fixation, a process that converts atmospheric N_2 gas into an inorganic form, ammonia (NH_3) (Schulten and Schnitzer 1998). This process can occur naturally through biological fixation, or industrially under high temperatures and pressures through the Haber-Bosch process, which produces mineral fertilizers (Marschner 1995). Since development of the industrialized NH_3 synthesis process, inorganic fertilizers have become the largest input source of N for agricultural crops globally. The annual N fertilizer use within Canada was 1 758 000 t of N from 2006 to 2007 (International Fertilizer Industry Association 2009).

Atmospheric deposition is the addition of NO_3^-, nitrite (NO_2^-), and NH_3 from volatilized gases within the atmosphere, electrical discharge from thunderstorms, and combustion of fossil fuels and natural fires, into the soil fraction from precipitation

(Stevenson 1986). Although the amount of N found within atmospheric precipitation is considered too small to have a significant effect on crop production (i.e. approximately 2.5 kg N ha^{-1} deposited yr^{-1} was measured by Munger et al. (1996) in a forested region in central Massachusetts), the additional N from deposition can replenish denitrification and leaching losses of N within natural plant communities (Stevenson 1986).

Ammonium ions can also originate from organic N compounds in decaying crop residues or green manures through mineralization (Janzen and McGinn 1991; Fillery 2001). Unlike mineral fertilizers which are immediately plant available upon application, crop residues must be degraded into inorganic N before they are available for plant uptake (Groffman et al. 1987). Estimates of 25-100 Tg of N $year^{-1}$ supplied to agricultural crops globally through the mineralization of crop residues show the substantial role crop residues occupy in soil N availability (Kumar and Goh 2000).

Ammonium from all of the above N inputs may be transformed to soil NO_3^- through the process of nitrification, producing nitrite (NO_2^-) as an intermediate. Ammonium is oxidized by autotrophic nitrifying bacteria in two different stages: the conversion of NH_4^+ to NO_2^- and the conversion of NO_2^- to NO_3^-, both achieved through bacterial metabolism (Schulten and Schnitzer 1998). Although this is the main source of NO_3^- within the soil, soil NO_3^- can also originate from applied NO_3^- based mineral fertilizers and through small amounts of atmospheric deposition.

2.1.2. Mineral N Removal from Soil

Nitrate is removed from the inorganic N pool through active plant and microbial assimilation in a process referred to as assimilatory NO_3^- reduction (Sylvia et al. 2005).

Organic matter that has been decomposed into NH_4^+ is quickly taken up to be assimilated into microbial tissue into the form of organic compounds, such as amino acids, amines, enzymes and proteins (Stevenson 1986). Since energy is required to reduce NO_3^- to NH_4^+ before incorporation into plant tissue, plants should favour NH_4^+ uptake, however preference between NO_3^- and NH_4^+ can depend on NH_4^+ and organic-N availability, energy sources (Sylvia et al. 2005), as well as the availability of exchangeable protons and organic acid anions within the plant shoots, which are required to maintain charge balances during NH_4^+ and NO_3^- uptake, respectively (Marschner 1995).

Dissimilatory NO_3^- reduction can be subdivided into two main processes: chemodenitrification and respiratory denitrification (Sylvia et al. 2005). Chemodenitrification is the production of NO from the dismutation of NO_2^- or the reaction of NO_2^- with amino-groups of organic-N compounds, and is a relatively minor contribution to N loss, as this process occurs in acidic soils of pH 5 or less (Sylvia et al. 2005). Respiratory denitrification (referred to as denitrification) is the reduction of NO_3^- to form N_2O and N_2 gases, by forming NO_2^- and NO as intermediates, and is a main contributor to N_2O emissions (Stevenson 1986). During anaerobic respiration, some bacteria have the ability to replace O_2 with NO_3^- as the terminal electron acceptor (Stevenson 1986). This reaction is catalyzed by the presence of nitrate/nitrite reductases (Payne 1981). Denitrification rates vary greatly depending on the presence of denitrifying bacteria, anaerobic conditions, the supply of NO_2^- and NO_3^- as terminal electron acceptors (Stevenson 1986), the amount of soil organic carbon (Miller et al. 2008), temperature, water content (Chantigny et al. 2002) and soil pH (Cuhel et al. 2010). Many of these factors are increased within conventionally grown potato crops when taking into

consideration the high rate of N applied to crops at planting (Burton et al. 2012), tillage practices (Buchkina et al. 2010), and irrigation events (Hyatt et al. 2010), in comparison with other arable crops.

Nitrate leaching can have a significant impact on soil N availability. Due to its high solubility, the NO_3^- anion is very mobile and is often lost with downward water movement within soil (Sylvia et al. 2005). The amount of leaching that occurs is influenced by both the amount of nitrate present in the soil, and the amount of water movement through the soil profile from rainfall or irrigation events, which will transport NO_3^- below the root zone (Shrestha et al. 2010). In Atlantic Canada, most NO_3^- is leached during the fall and winter seasons when the amount of water within the soil exceeds water holding capacity, when the occurrence of evapotranspiration due to drier soils is very low (Shrestha et al. 2010), and for the colder months, NO_3^- leaching is often enhanced by the absence of crop N uptake. Crop N uptake, despite high precipitation and large N inputs in the form of chemical fertilizers during the growing season, is a significant factor in reducing summer NO_3^- leaching, however often variable amounts of this retained N can be lost during the winter months as crop residues decompose (Savard et al. 2010). Large rainfall events during the summer can also move substantial amounts of NO_3^- through the soil especially in sandy soils immediately following fertilizer application (Di and Cameron 2002). Using simulation modelling, Jiang et al. (2011) found high soil NO_3^- concentrations in combination with high soil water infiltration, due to precipitation and/or spring snow melt, were the main factors affecting NO_3^- leaching in a barley-red clover-potato crop rotation in PEI. Annual NO_3^- leaching losses, although quite variable depending on environmental conditions, were predicted to range from 72-

91 kg N ha^{-1}. Significant NO_3^- leaching due to rapid snow thawing events has also been documented in the literature (Zhang et al. 2004), especially within regions with humid soil moisture regimes such as in PEI which are already prone to leaching.

Nitrogen may also be lost physically through surface runoff or erosion. Often within agricultural systems, large volumes of organic N can be removed and transported to nearby water bodies in sediments (Stevenson 1986). Crop residues can provide a means of physical resistance against wind erosion, and can also amend the soil quality to provide better soil stability to counteract future erosion (Kumar and Goh 2000). The presence of crop residues has been found to reduce water erosion of soil by 27-90%, with a greater effect seen on crops with greater volume of residues added (Kumar and Goh 2000). The timing and method of tillage within potato production has also been found to reduce nutrient loss through surface runoff as well as leaching events. A recent study comparing basin tillage to conventional hilling tillage found a 53-94% reduction of runoff within a potato crop rotation in Prince County, PEI, a major contributor to nutrient contamination of surface waters (Gordon et al. 2011).

Ammonium ions can also be fixed within the lattice of clay minerals through clay fixation (Stevenson 1986). The shape and size of the NH_4^+ cation enables it to fit precisely into voids within 2:1 lattice type clay minerals (for example: vermiculite, illite, and montmorillite). Once a suitable cation fills the void within the clay mineral lattice, the lattice layers contract around the cation to prevent hydration and expansion, thereby reducing the overall availability of NH_4^+ within the soil to microbial organisms and plants (Stevenson 1986).

2.1.3. Mineralization-Immobilization Turnover

Mineralization-immobilization turnover (MIT) refers to the simultaneous processes of mineralization (the transformation from organic N to inorganic N) and immobilization (the transition from inorganic N to organic N, i.e., the reverse process of mineralization; Powlson 1993; Schulten and Schnitzer 1998). Net mineralization refers to the difference between gross mineralization and gross immobilization. Plants and microbes can take up these inorganic forms of N and convert them into simple-N compounds, such as amino acids (Sylvia et al. 2005). The MIT rate depends on numerous edaphic factors including soil pH, soil temperature, soil water content, and the availability of additional nutrients such as P, K, Mg, Ca, and S (Kumar and Goh 2000). These factors also influence microbial growth and population size, and overall can increase the rate of microbial activity (Kumar and Goh 2000).

Whether MIT results in net mineralization or net immobilization is determined by the C:N ratio of soil amendments, such as crop residues and manures (Stevenson 1986). If the C:N ratio is low (for example below 20), N mineralization is favoured, leading to a net flux of inorganic N into the system. If the C:N ratio is high (for example above 30), the utilization of inorganic N by microorganisms occurs, reducing the amount of inorganic N within the system (Powlson 1993). Within the range of 20 and 30 of the C:N ratio, there is commonly neither a net gain nor loss of inorganic N (Stevenson 1986). Depending largely on the timing (early spring or fall) and source (organic fertilizers, crop residues) of inputs, mineralization or immobilization can be favoured (Powlson 1993).

2.2 N Isotope Studies of Mineralization-Immobilization Turnover in Soil Systems

Measuring changes within soil mineral N over time by directly sampling soil mineral N is a useful approach for estimating net rates of soil mineralization and nitrification, and in predicting fertilizer requirements for crops (Olfs et al. 2005; Zebarth et al. 2009). However, this approach only provides a measure of the size of the soil mineral N pool at that time. Other methods using a combination of plant N uptake and residual soil mineral N concentrations in unfertilized plots are more comprehensive estimators of the amount of nitrogen passing through the mineral N pool over the season, and are better indicators of soil N supply (Zebarth et al. 2005). Using the size of the soil mineral N pool as the only means of estimating available N does not account for the gains and losses of N between competing soil microbiological processes, for example, microbial immobilization, nitrate leaching, denitrification or plant uptake (Hart et al. 1994). Although estimates of plant-available N within the system can be valuable, soil mineral N tests do not show the total amount of N that is cycling between soil organic N and inorganic N pools (Neill et al. 1999), and therefore, since the rates of mineralization and immobilization are occurring simultaneously, the measurement of net immobilization or net mineralization can only determine the dominant process at that point in time (Kumar and Goh 2000). The calculation of gross mineralization rates instead allows insight into the total amount of soil mineral N that is cycling between mineralization, immobilization and nitrification, regardless whether N is in a mineralized or organic form.

Stable isotope labelling provides a means of measuring nutrient cycling and has been growing as the method of choice for studying the N cycle within soil science (Davidson et al. 1991; Bengtsson et al. 2003; Savard et al. 2010). The three main uses of stable isotopes for nutrient studies within soil have been through natural abundance measurements, the application of ^{15}N as a source/sink tracer, and the pool dilution method. Natural abundance measurements are often used to estimate plant fixation of atmospheric N_2, since the differentiation in isotope enrichment between atmospheric N and soil N is significant (Oberson et al. 2007; Kurdali and Al-Shamma'a 2010). However, given that the natural abundance ratios of soil N pools is of similar isotopic enrichment, their use in measuring N cycling rates within soil is relatively difficult unless paired with soil amendments of varying isotopic signatures (Chen et al. 2011). The addition of tracers using a source to sink approach is useful for experiments looking to trace the fate of additional N added to a system by labelling, for example, mineral fertilizers, plant residues or manures with ^{15}N, and observing the recovery of the applied isotope (Harmsen and Moraghan 1988). Often tracer methods are used to follow the ^{15}N source through systems by matching the isotopic signature of the sink N to the isotopic signature of input N, for example in surface and groundwater sources (Mulholland et al. 2004), plant tissues (Azam et al. 1985), and soil systems (Tran and Giroux 1991). There is a possibility that an additional source of material can enter the substrate pool, and the representative amount of isotope recovered at the final experiment period will not reflect the initial amount of isotope added due to the contamination of new material (Hart et al. 1994). Also, this method could overestimate the cycling rates, as the addition of the isotope-labelled ions can increase the substrate pool being used, and if any of the product

pool was consumed during the process, the amount of ^{15}N-labelled substrate that was converted to product (i.e. NO_3^-) will be underestimated (Hart et al.1994).

The pool dilution method allows the measurement of gross cycling rates of mineralization, nitrification, denitrification, and immobilization without affecting the size of the substrate pool. Within agricultural systems, gross mineralization and nitrification rates are useful to monitor the magnitude and efficiency of N cycling within a system, rather than only presenting the current amount of inorganic N available at that time when measuring net N transformation rates (Davidson et al. 1992). For example, negligible rates of net mineralization may not mean that the system is not producing mineralized N, but rather that both mineralization and microbial immobilization are occurring at a similar rate (Sparling et al. 1995).

The pool dilution method measures gross N rates by measuring the isotopic dilution of product pools (i.e. NH_4^+ or NO_3^- pools for mineralization and nitrification, respectively), which have been enriched with ^{15}N in the form of $^{15}NH_4^+$ or $^{15}NO_3^-$ respectively, with ^{14}N from the mineralization or nitrification of natural abundance soil N sources. This approach reduces the possibility of overestimation of the N transformation process due to any priming effect if the substrate pool were labelled (Murphy et al. 2003). Through this process, the ^{15}N abundance of the "product" pools is increased many fold and its dilution with ^{14}N from mineralization and/or nitrification can be measured (Bedard-Haughn et al. 2006). When N from the NH_4^+ or NO_3^- pools is consumed, over a specified period of time, an increase of ^{14}N entering the product pools decreases the amount of ^{15}N, and the rate of dilution of ^{15}N can be measured (Murphy et al. 2003). Since steady-state production and consumption of NH_4^+ or NO_3^- changes only the ^{15}N

enrichment, not the size of the pools, gross mineralization and nitrification rates can be calculated as the proportion of ^{15}N enrichment declines (Davidson et al. 1991).

There are three main assumptions made when using the pool dilution method, that if are not met, can introduce biases and errors within the calculated gross mineralization and nitrification rates (Kirkham and Bartholomew 1954). First, that there is no discrimination between using ^{15}N or ^{14}N during uptake by microbial processes. Second, that the rates of immobilization and mineralization are constant throughout the incubation period. And third, that once added, the ^{15}N is uniformly distributed within the inorganic N pool and immobilized N will not be remineralized (Kirkham and Bartholomew 1954). The final assumption can only be met when incubation periods are short.

The pool dilution method is often used within an agricultural context when accurate gross measurements of microbial processes are desired. For example, Cookson and Murphy (2004) used the pool dilution method on soils from a ryegrass/white-clover pasture and a wheat-lupin rotation crop that were pre-leached with water to simulate 22 mm of rainfall, in order to quantify the amount of N supplied to microbial processes from dissolved organic matter. Bedard-Haughn et al. (2006) quantified gross mineralization and nitrification rates, as well as estimates of N_2O emissions related only to denitrification sources, using this method to examine different cropping systems on cultivated ephemeral wetlands that will reduce nitrous oxide emissions in Saskatchewan. Coyne et al. (1998) tested the viability of a reclaimed coal surface mining site as farmland by measuring gross nitrification, mineralization, and immobilization rates to determine the influence of microbial activity within organic waste amended and unamended sites. These studies represent only a small sample of the uses attributed to the

pool dilution method, an area that is seeing an increase in application since the advancement in continuous flow mass spectrometers, allowing greater number of samples to be processed within a day, at a greater accuracy, and at a lower cost (Bedard-Haughn et al. 2003).

The isotope dilution method has been more commonly used in forest soils (Pedersen et al. 1999; Burton et al. 2007; Lang et al. 2010; Lteif et al. 2010; Wanek et al. 2010), however, there has also been more recent use of this method in agricultural and grassland soils (Accoe et al. 2004; Huss-Danell et al. 2007; Herrmann and Witter 2008; Robson et al. 2010; Huygens et al. 2011). Studies have used the pool dilution method within the root zone of different crop species including annual ryegrass (Whalen et al. 2001), wild oats (Khan et al. 2002), and some legume species (Herman et al. 2006). However, no studies examined gross N transformations within the root zone of a potato crop rotation; nor has this method been employed on soils under humid soil moisture regimes within the Atlantic Canadian region. Using the isotope pool dilution method within agricultural crops, such as potato rotations in PEI, may be of particular benefit within this region where the potential for environmental losses of N through leaching and denitrification is high.

2.3 Applications Used in the Isotope Pool Dilution Method

The main applications of the isotope pool dilution method with soil is through the addition of ^{15}N-labelled solutions, the addition of ^{15}N-labelled gases, or the application of silica flour- ^{15}N-labelled NO_3^- mixtures (Murphy et al. 2003). Some studies (Barraclough and Puri 1995; Sparling et al. 1995; Stark and Firestone 1995; Willison et al. 1998) found that the addition of enriched ^{15}N-labelled solutions can overestimate the amount of gross

mineralization occurring, specifically in dry soils, as the rapid wetting of dry soils can produce a surge in microbial activity and consequently in mineralization rate. The use of the dry methods, i.e., gases and powders, can be beneficial for use in wet soils, in order to avoid promoting anaerobic conditions that will enhance denitrification, and dry soils, to avoid a change in the soil water content and associated induction of rapid mineralization as previously stated (Willison et al. 1998).

In contrast with the solution technique, isotope-labelled gas enters intact soil cores passively and variably, which can result in underestimation of the amount of gross mineralization and nitrification rates (Murphy et al. 1999). To avoid this, injection of ^{15}N-labelled gas can be done directly into the soil cores, to assure a more uniform distribution, however there is still a low recovery of ^{15}N using this method and it is assumed that it is lost during injection (Murphy et al. 1997).

The use of silica flour ^{15}N-labelled mixtures is typically done when soil has already been disturbed, and so incorporation into the soil will not affect the soil to a greater extent. By using intact core samples, it is assumed that there is minimal disturbance to the soil, reducing the probability that mixing of the soil will alter microbial processes that could affect the gross rates calculations (Davidson et al. 1991).

Therefore, of all three labelling methods, enrichment using a ^{15}N-labelled solution allows the volume of applied substance to be the most precise, the distribution of the labelled solution is assumed to be generally uniform, and disturbance of the soil is reduced, making it the most practical method for the pool dilution method in the field for measuring gross mineralization using intact cores (Murphy et al. 2003).

2.4. Denitrification and Nitrous Oxide Emission Estimation Techniques

The use of stable isotopes has been shown to be beneficial in estimating denitrification emissions. Measuring denitrification rates in terrestrial environments has proven to be quite difficult in the past; however, the introduction of stable isotopes into denitrification methodology has greatly improved denitrification estimation (Groffman et al. 2006). Typically, field based N_2 and N_2O flux estimation techniques include the use of the "acetylene blockage" or inhibition method, and the ^{15}N gas flux method; whereas laboratory incubation studies can also incorporate the use of ^{13}N-gas flux, modified inert gas headspace and ^{15}N enriched N_2 experiments (Stevens and Laughlin 1998). The "acetylene blockage" method uses acetylene (C_2H_2) to inhibit the reduction of N_2O to N_2, and the denitrification rate is then estimated as the rate of N_2O production in the presence of C_2H_2 using soil in a sealed incubation jar or flask (Groffman et al. 2006). The acetylene blockage method generally underestimates denitrification rates as this method has been found to contribute to NO scavenging (McKenney et al. 1997). The C_2H_2 blockage method has also been found to inhibit fermentation, which can lead to less competition between denitrifiers and fermentative bacteria for C and NO_3^-, which can affect the availability of these substrate pools to bacterial consumers (Stevens and Laughlin 1998). The presence of C_2H_2 can also inhibit NO_3^- production through nitrification, although this is usually less of an issue in agricultural systems where soil NO_3^- concentrations are relatively high (Groffman et al. 2006). However, the use of C_2H_2 with the ^{15}N gas-flux method has been proven useful for source partitioning the contributors of N_2O emissions. Bateman and Baggs (2005) used the ^{15}N gas-flux method to detect and estimate N_2O emissions generated by simultaneous production from

denitrification, autotrophic nitrification and heterotrophic nitrification in a silt loam agricultural soil at differing water-filled pore space (WFPS), by using a modified version of the acetylene blockage method. By using only small concentrations of C_2H_2, only the process of autotrophic nitrification is blocked, which leaves labelled-$^{15}NO_3^-$ incubations with C_2H_2 treated soils with denitrification or heterotrophic nitrification derived N_2O, and the incubations with only labelled-$^{15}NO_3^-$ soils can be used to determine N_2O from the denitrification process only.

Use of the ^{13}N gas flux method is usually restricted to soil systems with extremely low fertility as it is assumed that the amount of ^{13}N required for the method is small enough that it will not influence or promote N cycling processes when added, nor will the amount of time for completion of the experiment be an influencing factor either (Speir et al. 1999). Although this method can give reliable results, the process of labelling soil with ^{13}N can be very labour intensive. Since the beam labelling the radioisotope can only penetrate into 2 cm depths of intact soil at a time, often cores must be cut into small (1-4 cm) slices prior to analysis (Speir et al. 1999). Estimations of N_2 and N_2O emissions using inert gases such as helium (He) to act as an inert atmosphere for denitrification processes within the soil has also been conducted (Swerts et al. 1995; Scholefield et al. 1997), however, concerns that the oxygen supply is largely altered giving artificial air conditions, as well as the complex nature of employing the He-O_2 purging process, make this method impractical for field studies.

Stable isotope techniques for measuring denitrification overcome many of the limitations stated above, and have the added advantage of being able to partition N_2O production from nitrification and denitrification sources through ^{15}N-labelled fertilizers,

manures, and residues (Baggs 2008). The use of ^{15}N-labelled fertilizers applied to agricultural soils has been widely used to measure the source and magnitude of N_2 and N_2O emissions from cultivated areas (Blackmer and Green 1995; Hood et al. 2000; Wagner-Riddle et al. 2008). However, one limitation to this method is that when paired with closed permanent fixtures or temporary chambers, the greenhouse gases within the headspace of the chamber is a combination of all soil depths within the profile, therefore the ability to detect the magnitude of emissions generated specifically from one soil depth, i.e., the root zone of a particular crop (Goldberg et al. 2008), is not possible. Although this is not an issue for studies interested in estimating total N_2O emissions from the soil surface, it may be important to note that N_2O emissions from specific soil layers can be variable due to extremely high spatial and temporal variability within soils because of varying "hotspots" of gas production and consumptive processes of N_2 and N_2O within soil microsites (Heincke and Kaupenjohann 1999).

The addition of ^{15}N for estimation of N_2 and N_2O emissions has been commonly used on a variety of soils (Delaune et al. 1997; Li et al. 2002; Bateman and Baggs 2005; Bedard-Haughn et al. 2006; Mathieu et al. 2006; Wan et al. 2009; Yang et al. 2011). A recent study using the pool dilution method for gross denitrification estimates used direct injection of labelled-$^{15}N_2O$ gas into *in situ* cores (Yang et al. 2011). In this study, Yang et al. (2011) injected a volume of $^{15}N_2O$ (98 atom %) into the chamber headspace of a fine silty soil in order to estimate net and gross N_2O emissions, and investigate the depth of diffusion of gases into the intact soil core. The labelled $^{15}N_2O$ gas diffusion method was found to be comparable with net N_2O emissions for the C_2H_2 inhibition method and the $^{15}NO_3^-$ tracer technique, however the gross N_2O emissions were significantly lower

within diffusion method cores in comparison with replicate cores using the $^{15}NO_3^-$ tracer technique, most likely due to a priming effect of the addition of $^{15}NO_3^-$ tracer (Yang et al. 2011). However, Yang et al. (2011) concluded that the gas diffusion method provided a reasonable estimate of N_2O emissions, despite consistently underestimating N_2O emissions in comparison with the other techniques. The differences were attributed to variable ^{15}N tracer distribution in the core and the inability of the ^{15}N tracer to reach all N_2O consumptive microsites. This was explained by low diffusion of the tracer to all depths of the soil cores when only one-tenth of the headspace concentration was found within the first 60 cm depth of the soil cores with the highest potential of diffusion (Yang et al. 2011). Within this study it is suggested that gas from the air is not entirely integrated into the soil during the small time period of pool dilution, which violates the assumption that complete gas integration between soil and air occurs within this method. Therefore, although pool dilution using a direct injection of labelled $^{15}N_2O$ gas would, in theory, be the ideal method to calculate gross denitrification estimates within *in situ* soil cores, there are significant issues still to be resolved with the current methodology. Therefore, the addition of labelled $^{15}NO_3$ solution as the isotope source for the pool dilution method can still be considered the most viable method of estimating gross denitrification emissions, despite concerns over the priming effect of added substrate.

Chapter 3.0 Objectives

The overall objective of this research was to examine how soil N processes influence soil NO_3^- availability within the root zone throughout the growing season for a conventional potato crop rotation. This was accomplished by studying a conventionally managed, three year barley-red clover-potato rotation throughout two growing seasons. The specific objectives of this research were to:

1. Quantify the rates of gross mineralization, nitrification, NO_3^- and NH_4^+ consumption, and total denitrification emissions at different times during the growing season within each phase of a conventional potato crop rotation using the isotope pool dilution method; and
2. Quantify the temporal variability of soil mineral N concentrations over the growing season within each phase of a potato crop rotation within both a conventional and reduced N input fertility management treatment.

Chapter 4.0 Materials and Methods

4.1 Experimental Site

This study was conducted utilizing an existing experiment that was established in 2009 at the AAFC Harrington Research Farm, Prince Edward Island. This study involves data collection during the growing seasons of 2010 and 2011. The soil type was a fine, sandy loam. Topography of the experimental site is close to flat, with a gentle slope in the northern direction. Prior to establishment of the experiment in 2009, the site was cropped to a barley-red clover-soybean rotation, with red clover present during the 2008 growing season. Organic C concentrations averaged 0.19 g kg^{-1} and 0.18 g kg^{-1}, and total N concentrations averaged 0.017 g kg^{-1} and 0.010 g kg^{-1} for 0-15 and 15-30 cm depths, respectively (from dry combustion analysis using an Elementar VarioMax Carbon and Nitrogen analyzer). Total organic matter content, estimated from organic C concentrations, averaged 0.33 g kg^{-1} and 0.3 g kg^{-1} for 0-15 and 15-30 cm depths, respectively.

The experimental design was a randomized complete block design with three treatments and four replications. Treatments were three phases of a conventional potato rotation: potato (*Solanum tuberosum* L.); barley (*Hordeum vulgare* L.); and red clover (*Trifolium pratense* L.), where the red clover was under-seeded into the barley crop. In 2009, all crops were under conventional fertility treatments. Beginning in 2010 for the current study, each phase of the rotation was grown following a conventional (CON) or a reduced N input (RN) fertility practice as described in Table 4.1. Plots were 9 m x 9 m in size, with a 12 m fallow region between experimental blocks.

Table 4.1**.** Variety and fertility treatments for all phases of the potato crop rotation. Note, due to patchy field conditions in 2011, all red clover plots were over-seeded at a rate of 14 kg ha^{-1} of 'AC Christie' red clover.

Crop species	Variety	Row spacing (m)	Inter-row Spacing (m)	Seeding rate (kg ha^{-1})	N fertility (kg N ha^{-1})	
					CON	RN
Potato	Russet Burbank	0.91	0.381	N/A	191	112
Barley	'Island' (2010), 'Queen' (2011)	0.15	N/A	168	86 (2010) 69 (2011)	57 (2010) 46 (2011)
Red clover	'AC Christie'	N/A	N/A	5.6	0 (fall plow-down)	0 (spring plow-down)

N/A not applicable

Each phase of the crop rotation, for both fertility treatments, was managed according to normal grower practice on PEI, including typical insecticide, fungicide and herbicide treatments (Table A.1). No irrigation was applied, which is typical grower practice in this area with rain-fed potato production. Under-sown red clover within the barley plots was not harvested during the barley year, and was left unmanaged to allow good crop establishment until the following year when red clover management practices began.

4.2 2010 Growing Season

In 2010, the first tillage of all barley plots occurred on May 5, and the plots were seeded at a rate of 168 kg seed ha^{-1} on May 18 (Table 4.1). Pre-mixed fertilizer (10-10-10) with ammonium sulphate as the N source was surface broadcast and incorporated prior to seeding at a rate of 86 and 57 kg N ha^{-1} for CON and RN barley plots, respectively. Barley was harvested mechanically using a Hegge® combine on August 28 and separated into grain and straw, with the intact straw returned uniformly to the plot's surface.

The CON and RN red clover plots from the 2009 growing season were plowed down on November 5, 2009 and April 15, 2010, respectively. Red clover for the 2010 growing season was planted by under-seeding, at the same time as the planting of the barley plots on May 5, 2010, at a rate of 5.6 kg seed ha^{-1}, using a Great Plains seeder. Red clover that was planted by under-seeding in the spring of 2009 was cut three times during the 2010 growing season (June 21, August 10, and September 28, 2010) using a bush mower and clippings were returned to the surface of the plot. Fall plow-down of 2010 CON red clover plots was on October 12, 2010, whereas the plow-down of the 2010 RN red clover plots occurred on May 4, 2011.

As noted previously, all plots were established in 2009, meaning all 2010 potato plots followed a red clover plow-down event, occurring either in the fall or spring for CON or RN plots, respectively. The 2010 potato plots were tilled on May 5, and the potato crop, cultivar Russet Burbank, was mechanically planted on June 10 with 0.91 m row spacing and 0.38 m within-row spacing. Pre-mixed fertilizer (17-17-17) with ammonium sulphate as the N source was banded during planting. Potatoes were pre-hilled by cultivation on July 5, and hilled on July 19. Potato vine desiccation was achieved by application of Reglone 240® approximately three weeks before final harvest of potato plots on October 15.

4.3 2011 Growing Season

All barley plots were cultivated on May 24. Barley was seeded on May 27 at a rate of 168 kg ha^{-1}. Final harvest of barley was on August 25, with similar harvest practices as the 2010 growing season.

Red clover was under-seeded at the same time as barley on May 27, at a rate of 5.6 kg ha^{-1}. Red clover that was planted within the 2010 growing season was cut three times over the 2011 growing season using a bush mower on June 22, August 17 and October 13, 2011. Red clover clippings were returned as mulch on top of the plot surface. Due to patchy growth of red clover from the 2010 season, all red clover plots for the 2011 season were re-seeded at a rate of 5.6 kg ha^{-1} using a Brillion™ seeder on May 19. Fall plow-down of conventional red clover plots occurred on October 27.

In 2011, RN clover plots from 2010 season were plowed down on May 4. All 2011 potato plots were cultivated again on May 24, and potatoes of the cultivar, Russet

Burbank, were mechanically planted on May 25 with a 0.91 m row spacing and 0.38 m within-row spacing (Table 4.1). Potatoes were hilled on July 14. Final potato harvest was on September 21.

4.4 Isotope Pool Dilution Method

The isotope pool dilution method was used to estimate gross nitrification, mineralization, and NO_3^- and NH_4^+ consumption throughout the growing season of all phases of the crop rotation during both the 2010 and 2011 seasons for the CON plots. Gross denitrification estimates were estimated only within the 2011 growing season. Soil sampling was conducted five times each growing season at approximately five week intervals beginning in early May. On each sampling date, undisturbed soil samples were taken with aluminum cores (5 cm diameter x 10 cm long for barley and red clover; 5 cm diameter x 15 cm long for potatoes). To accommodate reaching the potato root zone when potato hills were present, all core samples from the potato phase were taken using longer core lengths throughout the whole season.

Five adjacent core samples were taken from each plot on each sampling date. Cores within the potato plots were taken within the hill, lengthwise within the centre of the hill and therefore parallel to the direction of the plant row. The cores within the barley and red clover plots were taken among crop plants, with bulk vegetation removed from the top of the core. Of the five cores from each plot, two cores were injected with $^{15}(NH_4)_2SO_4$ (99.1% ^{15}N-enriched), and two were injected with $K^{15}NO_3$ (99.5% ^{15}N-enriched) solutions, while the fifth core did not receive any additions and was used for background NH_4-N and NO_3-N analyses as explained below. Both ^{15}N solutions contained 72.35 mg of $^{15}N\ L^{-1}$ and volumes of 7 or 10.5 ml (for 10 or 15 cm long cores,

respectively) were injected into each core. The volume of solution to be injected was chosen to limit the increase in gravimetric water content to no greater than approximately 0.04 g g^{-1} and to achieve an addition of approximately 2 mg N kg^{-1} of moist soil, although this varied throughout the season. For equal dispersal of the solution throughout the core, the solutions were injected into the core in seven locations (six times around the perimeter of the core, once in the centre) using a 3 ml spinal port needle on a 3 ml Luer-Lok® syringe on the bottom (or lower soil depth) side of the core. The solution was injected evenly from the bottom of the core to the top by injecting the solution while pulling the syringe upwards out of the core. Following injection, the cores were placed upright to reduce pooling of solution on the top of the core, and a cap was placed on the bottom to eliminate the possible loss of solution through the bottom of the core.

For the two cores injected with the same solution (either $^{15}(NH_4)_2SO_4$ or $K^{15}NO_3$), soil from one core (T_0) was immediately mixed and a 25 g subsample was added to 100 ml of 2 M KCl solution on site and placed on ice. The other injected core (T_{24}) for cores receiving $^{15}(NH_4)_2SO_4$ or $K^{15}NO_3$, was field incubated for approximately 24 hours, and the exact time of incubation was noted. A shallow hole was dug to mimic the depths of the soil cores used, and the cores were placed into the hole, and held in place with soil surrounding it, without covering the top of the core. A fine covered mesh was placed on the top of each of the incubated cores and held in place with an elastic band to ensure the soil was aerated, but that no foreign materials could enter and influence the soil environment while it was incubated. When precipitation overnight was probable, a table covered with a tarp was placed on top of the core incubation area to provide cover from moisture addition, but to allow airflow. At the end of the field incubation period, soil

from each core was mixed and a subsample of approximately 25 g moist soil was placed into 100 ml of 2 M KCl solution and left on ice as described for the T_0 cores. All soil extracts were shaken for 1 hour on a horizontal shaker, and then filtered using draining crucibles and Whatman 93-4AH Whatman filters. All KCl solutions were then returned to the lab and filtered using a vacuum extraction system. Extracts were stored in 120 ml Nalgene® bottles at -20°C until the diffusion disk process.

Isotope labelled extracts were analyzed using the diffusion disk process as described by Hart et al. (1994), with the exception of using polytetrafluoroethylene (PTFE) packets, rather than wire-suspended filter disks (Stark and Hart 1996). To create the PTFE packets, Whatman No. 3 filters were pre-leached with 2 M KCl and deionized water, and then cut using a paper hole-punch to obtain filter disks approximately 6 mm in diameter. The disks were then acidified by pipetting 10 µL of potassium hydrogen sulphate (K_2HSO_4) onto the disk's surface. The disk was then sandwiched in between two PTFE strips, and an airtight seal was created by pressing together the two strips of PTFE tape using the end of a dram vial. The restrictions of the airtight seal required that the disk must be free to move within the air pocket created. Following variable results from the 2010 season, all 2011 samples were analyzed using PTFE packets sealed using a modified arbor-press to ensure a complete seal. An error analysis comparing the hand-press sealed method and the arbor-press sealed method was conducted using five samples of each seal, for both $^{15}(NH4)_2SO_4$ or $K^{15}NO_3$ (10% atom percent excess) solutions through the NO_3-N and NH_4-N diffusion processes.

All isotope-labelled extract samples were weighed out to approximately 46 g ($\pm$ 0.5 g) of solution into clean 120 ml sample cups. Weights for each sample were recorded.

For $^{15}NH_4$- labelled extracts, approximately 0.2 g of magnesium oxide (MgO) was added to each pre-weighed KCl extract to increase the pH of the solution to convert NH_4^+ to NH_3 and allow gaseous movement of labelled N into the disk packet. Following MgO addition, a disk packet was added and the sample cups were covered immediately. The $^{15}NH_4$-labelled extracts were then gently shaken for at least one hour each day for six days on a horizontal shaker. The $^{15}NO_3$- labelled extracts were also given 0.2 g of MgO and shaken for six days; however, the sample cups were not fully covered during this time period, nor were any disk packets inserted into them. This process ensured that all NH_4^+ present in these extracts would be removed through gaseous diffusion prior to NO_3^- capture on the disk packet. Following six days, approximately 0.4 g of Devarda's alloy was then added, along with a disk packet to convert NO_3^- to NH_4^+. These $^{15}NO_3$-labelled extracts were then covered and shaken for an additional six days.

Following the individual NO_3-N and NH_4-N diffusion processes (in total six days for the $^{15}NH_4$-labelled extracts, and twelve days for the $^{15}NO_3$-labelled extracts), the extract containers were uncovered, and the diffusion packet was removed, dipped in distilled water, and gently patted dry with a Kimwipe®. Each PTFE packet was then dried at 25°C within a desiccator with an open jar of concentrated H_2SO_4 until the filter was dry, then each packet was opened using needle nose tweezers and the filter disk was enclosed in an 8 mm x 5 mm tin capsule. Tin capsules were sent to the University of California Davis Stable Isotope Facility (stableisotopefacility.ucdavis.edu), where samples underwent ^{15}N analysis using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Total N diffused, atom percent excess and % ^{15}N was calculated.

Gross N transformation rates were calculated according to the equations provided by Davidson et al. (1991). Gross mineralization rates (M_g) were calculated by:

$$M_g = \frac{A_0 - A_t}{t} \cdot \frac{\log\left(\frac{^{15}A_0 \cdot A_t}{^{15}A_t \cdot A_0}\right)}{\log\left(\frac{A_0}{A_t}\right)} \quad [1]$$

where M_g = gross mineralization rate (mg N kg^{-1} soil d^{-1}); A_0 = total NH_4^+ concentration at time 0 (mg N kg^{-1}); A_t = total NH_4^+ concentration at time t (mg N kg^{-1} soil); $^{15}A_0$ = $^{15}NH_4^+$ pool at time 0 (mg ^{15}N kg^{-1} soil); $^{15}A_t$ = $^{15}NH_4^+$ pool at time t (mg ^{15}N kg^{-1} soil); and t = time (d). Ammonium consumption rates were calculated as:

$$A_{con} = \frac{A_0 - A_t}{t} \cdot \frac{\log(^{15}A_0/^{15}A_t)}{\log(A_0/A_t)} \quad [2]$$

where A_{con} = NH_4^+ consumption rate (mg N kg^{-1} soil d^{-1}) and where A_0, A_t, $^{15}A_0$, $^{15}A_t$ and t are as defined above. Ammonium consumption refers to the total NH_4^+ used by all possible NH_4^+ consuming processes, including immobilization, autotrophic nitrification, NH_4^+ clay fixation and NH_3 volatilization. Assuming all NH_4^+ depleting processes stated above are negligible, with the exception of immobilization, then NH_4^+ consumption can be calculated as the sum of gross NH_4^+ immobilization and gross nitrification, as described by Davidson et al. (1991).

Gross nitrification (N_g) and NO_3^- consumption (N_{con}) rates were calculated similar to the above equations but with the substitution of NO_3^- for NH_4^+ concentrations. Thus, N_g was calculated as:

$$N_g = \frac{N_0 - N_t}{t} \cdot \frac{\log\left(\frac{^{15}N_0 \cdot N_t}{^{15}N_t \cdot N_0}\right)}{\log\left(\frac{N_0}{N_t}\right)} \quad [3]$$

where N_g = gross nitrification rate (mg N kg^{-1} soil d^{-1}); N_0 = total NO_3^- concentration at time 0 (mg N kg^{-1}); M_t = total NO_3^- concentration at time t (mg N kg^{-1} soil); $^{15}N_0$ = total $^{15}NO_3^-$ pool at time 0 (mg ^{15}N kg^{-1} soil); $^{15}N_t$ = total $^{15}NO_3^-$ pool at time t (mg ^{15}N kg^{-1} soil); and t= time (d). Similarly, N_{con} was calculated as:

$$N_{con} = \frac{N_0 - N_t}{t} \cdot \frac{\log(^{15}N_0/^{15}N_t)}{\log(N_0/N_t)} \quad [4]$$

where N_{con} = NO_3^- consumption rate (mg N kg^{-1} soil d^{-1}) and where N_0, N_t, $^{15}N_0$, $^{15}N_t$ and t are as defined above. Gross NO_3^- consumption can be assumed to be the sum of all NO_3^- consumptive processes including microbial assimilation (immobilization), dissimilatory NO_3^- reduction and denitrification; although dissimilatory NO_3^- reduction is considered negligible within this soil environment.

A fifth core was extracted along with the isotope-labelled cores for determination of microbial biomass carbon (MB-C) and N (MB-N).

Mineral N concentrations were acquired through 2 M KCl extractions with a 1:5 soil to KCl ratio. Extracts were shaken for approximately one hour on a horizontal shaker until filtration using a vacuum filtration system. All extracts were frozen at -20 °C until NO_3-N and NH_4-N analysis colorimetrically using a Technicon® flow injection auto-analyzer following the Technicon® Industrial methods 487-77A and 791-86T for NO_3-N and NH_4-N concentration determination, respectively (Technicon Industrial Systems 1977b; Technicon Industrial Systems 1986a).

Microbial biomass was analysed using the chloroform fumigation method as described by Voroney et al. (2008), using 0.5 M K_2SO_4 as an extractant with a 1:2 soil to K_2SO_4 ratio. The K_2SO_4 extracts were analysed for concentrations of dissolved organic carbon (DOC), NO_3-N and NH_4-N using a Technicon® flow injection auto-analyzer (Technicon Industrial Systems 1976; Technicon Industrial Systems 1977b; Technicon Industrial Systems 1986a). The MB-C (μg C g^{-1} soil) was calculated using a correction factor of 0.25, whereas the MB-N (μg N g^{-1} soil) was calculated using a correction factor of 0.18 (Voroney et al. 2008). All extracts were frozen at -20 °C until laboratory analysis. Gravimetric water content was calculated for all soil core samples using a 10 g sub-sample of moist soil and oven-dried at 105°C for 24 hours. Soil bulk density and water-filled pore space (WFPS) were also calculated using the fifth core for each plot on each sampling date.

4.5 Gross Denitrification Estimation

During the 2011 field season only, gross N_2O flux from denitrification events was measured as described by Sangster (2010) by taking an additional soil core from each plot (for a total of six cores from each plot) on each sampling date. This core acted as a duplicate $^{15}NO_3$-labelled T_{24} core, with the only exception being that this core had 2.5 cm diameter holes approximately every 5 cm in the aluminum core to enhance gas exchange between the soil core and jar headspace during the incubation period. Approximately 15 minutes following $K^{15}NO_3$ injection, two T_0 gas samples were taken from the soil core surface using a 20 ml syringe. The core was then placed into a 1.5 L Mason jar, a lid fitted with a rubber septa firmly sealed into place with silicon vacuum grease onto the jar, and the soil cores were incubated for an additional 24-hour period in-field. Two T_{24} gas

samples were collected following the incubation period from each jar using a new 20 mL syringe through the rubber septa fixed to the lid. All samples were injected into previously evacuated 12 mL Labco® Exetainer vials, and were sent to the University of California Davis Stable Isotope Facility for stable isotope analysis. Samples were analyzed for total N_2 and total N_2O, as well as for $^{15}N_2$ and $^{15}N_2O$, using a SerCon Cryoprep trace gas concentration system interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Atmospheric gas samples were taken throughout the sampling dates to calculate background N_2, N_2O, $^{15}N_2$ and $^{15}N_2O$ concentrations at the time of sampling.

Gross N_2 emissions (N_2) were calculated as:

$$N_2 = \frac{(NTR_{24} - NTR_0)*200}{(APE_0 + APE_{24})*(t)*(DW)*(MM)} \quad [5]$$

where N_2 = gross N_2 emissions (μg N g^{-1} dry soil d^{-1}); NTR_{24} = total $^{15}N_2$ derived from NO_3^- per jar at T_{24} (μmoles); NTR_0 = total $^{15}N_2$ derived from NO_3^- per jar at T_0 (μmoles); APE_0 = atom percent excess of ^{15}N at T_0 (%); APE_{24} = atom percent excess of ^{15}N at T_{24} (%); t = incubation time (days); DW = total dry soil weight of soil core (g); and MM = molar mass of N within N_2 (moles). Similarly, gross N_2O emissions (N_2O) were calculated as:

$$N_2O = \frac{(NTO_{24} - NTO_0)*200}{(APE_0 + APE_{24})*(t)*(DW)*(MM)} \quad [6]$$

where N_2O = gross N_2O emissions (μg N g^{-1} dry soil d^{-1}); NTO_{24} = total $^{15}N_2O$ derived from NO_3^- per jar at T_{24} (μmoles); NTO_0 = total $^{15}N_2O$ derived from NO_3^- per jar at T_0 (μmoles); APE_0 = atom percent excess of ^{15}N at T_0 (%); APE_{24} = atom percent excess of

^{15}N at T_{24} (%); t = incubation time (days); DW = total dry soil weight of soil core (g); and MM = molar mass of N within N_2O (moles).

All gross N transformation rates (nitrification, mineralization, NO_3^- consumption, and NH_4^+ consumption), as well as N_2 and N_2O emissions were included within the 'unfiltered' dataset. Negative transformation rates (i.e., negative nitrification, mineralization, NO_3^- consumption and NH_4^+ consumption rates), as well as any transformation rates that demonstrated isotope enrichment values that did not dilute over the 24 hour incubation time period, were considered to have exceeded the assumptions of the method and were omitted from the reported 'filtered' dataset. Small negative rate values may reflect inherent variability in measurements, however in many cases, large negative rates were calculated which were difficult to interpret, as negative rates are not biologically possible. For the N_2 and N_2O emission rates, only negative rates were omitted from the 'filtered' dataset, as isotope dilution is not a requirement for the denitrification estimation method.

4.6 Gross N Transformation Rate Error Analysis

Three sources of variability were examined for their effect on estimates of gross mineralization and nitrification rates, selected from the 2010 pool dilution data. The three sources of error examined include atom percent enrichment error (ENR-ERR), segmented flow analyzer error (SFA-ERR) and soil core variability error (CV-ERR). A range of values representing two units of standard deviation (SD) from the mean, approximating a 95% confidence interval (C.I.), were calculated for each source of error (ENR-ERR, SFA-ERR, and CV-ERR) by addition or subtraction to the appropriate factor, for gross mineralization and nitrification. Atom percent enrichment error was calculated based on

values obtained from the isotopic mass spectrometer during isotope analysis from samples with known enrichments from five $(^{15}NH_4)_2SO_4$ (10% a.p.e.) and five $K^{15}NO_3$ (10% a.p.e.) standards diffused onto filter disks using the diffusion method. The second error, SFA-ERR, was the standard error obtained by determining the nitrogen concentrations from the 2 M KCl soil extracts. The SFA-ERR values were calculated from the SFA for ten NH_4-N and ten NO_3-N standards at 2 and 5 mg/L, respectively. Soil core variability error (CV-ERR) was calculated from the difference in soil mineral N concentrations from all pairs of cores from the same plot for each phase of the rotation on each of five sampling dates (n = 20). All estimated errors from each potential error source were then used (by addition or subtraction to the appropriate factor) within the gross mineralization or nitrification formulas as described by Davidson et al. (1991) above.

4.7 Diffusion Method Test for N Recovery

Error analyses were conducted for assessing potential sources of error within the diffusion method. To assess error contributed by variation within the isotope analysis method, five samples of both $(NH_4)_2SO_4$ or KNO_3 solutions (at natural abundance) containing 50 µg of N was directly pipetted onto a filter and sealed in tin capsules. To analyze error attributed to amount of N diffused and its isotopic composition, samples with 100 µg of either natural abundance $(NH_4)_2SO_4$, 10% a.p.e. $^{15}(NH_4)_2SO_4$, natural abundance KNO_3 or 10% a.p.e. $K^{15}NO_3$, were added to pre-weighed 46 g of 2M KCl solutions to calculate the amount of N diffused onto the filter during the 6 and 12 day shaking diffusion time periods for NH_4^+ and NO_3^- labelled solutions, respectively. Filters for all error analyses were then dried at 25°C within a drying oven until dry, enclosed in

tin capsules in the same procedure as described above for experimental samples, and sent for isotope analysis at the UC Davis Stable Isotope Facility.

4.8 Soil Mineral N Sampling

Additional soil samples were collected a total of nine times from early May to the end of October throughout both 2010 and 2011 growing seasons at depths of 0-15 cm and 15-30 cm. Soil sampling was timed to coincide approximately with isotope pool dilution sampling dates, as well as plant harvest dates, to estimate soil NO_3-N and NH_4-N at those times. Each sample consisted of a composite of two samples taken with a sampling auger (or a composite of five samples taken with a soil sampling probe, diameter of 2.54 cm) to make a single composite sample for each depth within each plot. Soil samples were passed through a 4.75 mm sieve. A 10 g sub-sample was then extracted with 50 ml of 2 M KCl (1:5 soil to KCl ratio), shaken for one hour and filtered using Whatman 94-3AH glass filters. All extracts were frozen at -20 °C until further analysis. Concentrations of NO_3-N and NH_4-N were determined colorimetrically on a Technicon® II flow injection auto-analyzer following the Technicon® Industrial methods 487-77A and 791-86T for NO_3-N and NH_4-N concentration determination, respectively (Technicon Industrial Systems 1977b; Technicon Industrial Systems 1986a; Maynard and Kalra 2008) . Gravimetric water content was also calculated as described for the core soil samples.

Soil characterization was conducted using soil collected at the beginning of the 2011 field season. One composite sample for each depth (0-15 cm, 15-30 cm, and 30-45 cm) was collected for each replicate, by collecting four representative soil samples around the perimeter of each replicate plot. Soil pH was analyzed using a 1:1 soil to water ratio (Hendershot et al. 2008). Organic C and total N analyses were conducted

using the dry combustion method on an Elementar VarioMax Carbon and Nitrogen analyzer (Skjemstad and Baldock 2008). A soil test analysis was completed in 2011 by the PEI Department of Agriculture Analytical Laboratories, on sub-samples of soil from both depths of each phase of the rotation, for both conventional and reduced N input treatments, giving a total of 12 samples.

Climatic data (total monthly precipitation and mean monthly air temperatures) used within this study were acquired from the Environment Canada Weather Station which is approximately 2 km from the plots (Environment Canada 2011).

4.9 Plant Biomass Sampling

Plant samples were taken from all crop species at selected times during their growth period to measure plant N uptake throughout the season. Mineral N soil samples were approximately timed with each plant harvest sampling date, and soil samples were taken from the harvested area.

Above-ground barley plant tissue samples were collected at the initial change in coloration near early August when plant N uptake is at a maximum, and again at harvest stage at the end of August for both growing seasons. On each date, plant tissues were manually collected from five rows (0.6 m wide by 1 m long). Following subsample collection at time of harvest, barley was harvested using a Hegge® combine as described above. Plant tissues were dried, weighed, and ground, and approximately 250 mg of subsample was analyzed by dry combustion for total N concentration using an Elementar VarioMax Carbon and Nitrogen analyzer.

Red clover was mowed two to three times seasonally, between mid-June to early October for both growing seasons. Above-ground plant tissue samples were collected manually at each mowing date from an area of 1 m^2. Plant tissues were cut, dried, weighed, ground and 250 mg of subsample was analyzed by dry combustion for total N concentration using an Elementar VarioMax Carbon and Nitrogen analyzer.

Potato plants were harvested during early tuber bulking phase (early to mid-August), and again just prior to vine desiccation (September) when plant N uptake is at a maximum. Four adjacent whole plants in one row were uprooted and partitioned into tubers, vines and stolons plus readily recoverable roots as described by Zebarth and Milburn (2003) to determine dry matter and N accumulation in each plant component where tissue total N concentration was determined using an Elementar VarioMax Carbon and Nitrogen analyzer. Potato total tuber yield was estimated by determining total tuber fresh weight from one full row at time of harvest.

4.10 Statistical Analyses

Data was analyzed for normality and homogeneity of variance prior to statistical analyses, and a $\log_{10}$ transformation was performed if necessary. Analysis of Variance (ANOVA) with repeated measures (for comparison among sampling dates) was performed using the Mixed Procedure Model of Statistical Analysis Software (SAS) on all soil NO_3^- and NH_4^+ concentration data, as well as gross N transformation rates. Comparisons among treatment means were performed using the protected Fisher's Least Significant Difference test. Values are considered statistically significant when *P*-value is < 0.05.

Chapter 5.0 Results and Discussion

5.1 Climate Data

Mean monthly air temperature during the growing season (May to October) averaged 14.3 and 14.1°C in 2010 and 2011, respectively (Table 5.1). Mean growing season air temperatures in both years were similar to the 30 year average of 14.2°C. The mean air temperature during the months of May and June for both 2010 and 2011 were approximately 3°C lower than the 30 year average, whereas air temperatures during the months of September and October for 2010 and 2011 were approximately 3°C higher than the 30 year average.

Table 5.1. Mean monthly air temperatures in 2010 and 2011, compared with the 30 year average (1971-2000), measured from the Environment Canada Weather Station on the Harrington Research Farm, PEI (Environment Canada 2011).

	Air temperature (°C)		
Month	2010	2011	30 year avg.
April	5.6	4.0	4.4
May	9.0	9.5	12.0
June	14.2	12.9	16.4
July	19.8	18.0	19.2
August	18.9	18.7	18.0
September	15.3	15.6	12.8
October	8.7	9.8	6.6
November	3.4	4.6	0.0
May-October	14.3	14.1	14.2

Total precipitation during the growing season in 2010 was 559 mm which was 11% lower than the 30 year average of 628 mm (Table 5.2). In comparison, total

Table 5.2. Total precipitation in 2010 and 2011, compared with the 30 year average (1971-2000), from the Environment Canada weather station on the Harrington Research Farm, PEI (Environment Canada 2011).

	Total precipitation (mm)		
Month	2010	2011	30 year avg.
April	48	68	78
May	25	113	95
June	156	51	118
July	88	150	101
August	89	126	108
September	68	21	104
October	133	284	103
November	157	97	101
May-October	559	745	629

precipitation during the 2011 growing season (745 mm) was 19% greater than the 30 year average. Total precipitation during May 2010 was 70 mm below the 30 year average, whereas total precipitation in June 2010 was 38 mm above the 30 year average. In comparison, total precipitation in 2011 was 18 mm above and 57 mm below the 30 year average in May and June, respectively. Fall precipitation was not consistent with the 30 year average for either growing season. In 2010, total monthly precipitation was 38 mm below average in September and 30 mm above average in October. In 2011, total precipitation in October (284 mm) was approximately 3 times greater than the 30 year average, and 2 times greater than during October 2010.

Soil water-filled pore space (WFPS), averaged across sampling dates, was 0.49 and 0.53 m^3 m^{-3} in 2010 and 2011, respectively (Figure 5.1). The maximum value of WFPS in 2010 (0.58 m^3 m^{-3}) was measured on September 27, whereas in 2011, the

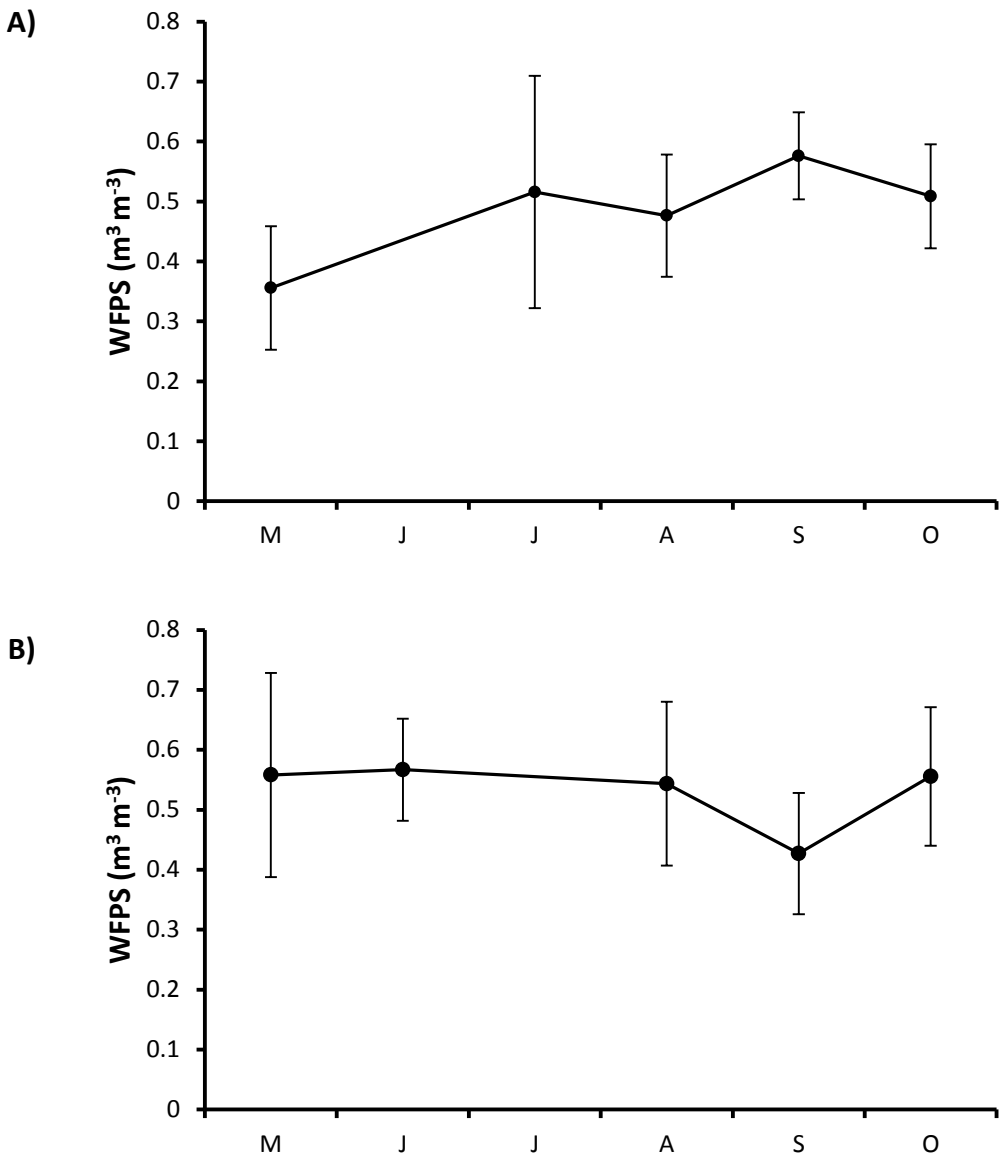

Figure 5.1. Soil water-filled pore space (WFPS) for five sampling dates in the 2010 (A) and 2011 (B) growing seasons for 0-10 cm depth of the barley and red clover plots. Error bars represent ± 1 SD.

maximum value of WFPS (0.57 m^3 m^{-3}) was measured on June 21. In 2010, the minimum value of WFPS was 0.36 m^3 m^{-3} on May 18, whereas in 2011 the minimum value of WFPS was 0.43 m^3 m^{-3} on September 27, being the only point during the season when WFPS < 0.55 m^3 m^{-3}.

Overall, climatic conditions during the growing seasons of the current study were similar to or somewhat wetter than climate normals. Although temperature was similar on average, both field seasons were slightly colder at the beginning of the growing season and slightly warmer during the fall months. Total precipitation during the 2010 growing season was comparable with long-term normals, although the 2011 field season was generally wetter than normal, particularly in July, August, and October.

When water availability is limiting, plant N uptake and transformation of N throughout the soil and plant root system is reduced (Zebarth et al. 2009). This is particularly prevalent on PEI where evapotranspiration is typically very high throughout the growing season, and drainage is only great following large precipitation events (Jiang et al. 2012). When large precipitation events do occur, such as those observed in the 2011 growing season, increased rates of mineralization and nitrification, and subsequently drainage, can be expected due to greater water availability. Microbial activity is strongly influenced by environmental conditions, particularly soil temperature and water content, and maximum microbial activity has been found to occur at approximately 60% WFPS (Paul 2007). Since water content was >55% WFPS for most of the 2011 growing season, with the exception of the month of September, microbial activity would likely have been increased under all crop species. Increased precipitation can also result in greater N losses through denitrification and NO_3^- leaching. Increased water movement caused by large precipitation events can induce greater and more rapid soil NO_3^- movement below the root zone, an effect magnified when soil NO_3^- concentrations are high (Jiang et al. 2012).

5.2 Crop Yield Data

Barley grain yields under CON management were approximately 3.4 and 2.9 t ha^{-1} in 2010 and 2011 (Table A.3), respectively, which were consistent with the annual averages of 3.0 t ha^{-1} and 3.2 t ha^{-1} of PEI barley crops within the same years (P.E.I. Agricultural Census 2011). Total potato tuber yields under CON management were approximately 40.0 and 45.5 t ha^{-1} in 2010 and 2011 (Table A.4), respectively, which were greater than the average total tuber yields within P.E.I. for both field seasons, which were 33.6 t ha^{-1} and 31.4 t ha^{-1} for 2010 and 2011, respectively (P.E.I. Agricultural Census 2011). Red clover biomass yields in 2010, totaled over three harvests during the growing season, were 7.8 and 8.1 t ha^{-1} on a dry weight basis under CON and RN management, respectively (Table A.5). Red clover biomass yields in 2011, for two harvests during the growing season, were 4.2 t ha^{-1} on a dry weight basis for both N managements (Table A.5).

Overall, crop growth among all species was considered representative of grower fields, and crop yields were comparable with or exceeded the average yields within P.E.I. for both years. Although poor over-wintering of red clover stands during winter 2010 necessitated re-seeding of red clover in May 2011, re-seeding did not appear to have an overall effect on the total yield of the red clover stand, with exception of later harvest dates compared with the 2010 field season.

5.3 Microbial Biomass

Soil MB-C concentrations did not vary by crop species or sampling date in either 2010 (Table 5.3) or 2011 (Table 5.4). Mean MB-C concentrations in 2010 were 261 μg C

Table 5.3. Microbial biomass carbon (MB-C) and nitrogen (MB-N) concentrations determined using the chloroform fumigation method from all three phases of the barley-red clover-potato crop rotation during the 2010 field season. Values shown in parentheses represent ± 1 SD.

Sampling date	Crop species	MB-C ($\mu g\ C\ g^{-1}$ soil)	MB-N ($\mu g\ N\ g^{-1}$ soil)
May 18	Potato	277 (± 259)	19 (± 7)
	Barley	184 (± 114)	10 (± 2)
	Red clover	313 (± 228)	16 (± 8)
	Mean	258 (± 190)	15 (± 7)
July 7	Potato	185 (± 60)	13.8 (± 1)
	Barley	254 (± 135)	11 (± 7)
	Red clover	391 (± 224)	16 (± 6)
	Mean	276 (± 162)	14 (± 6)
Aug. 23	Potato	261 (± 139)	7 (± 5)
	Barley	194 (± 82)	10 (± 3)
	Red clover	308 (± 115)	21 (± 9)
	Mean	254 (± 115)	13 (± 9)
Sept. 27	Potato	79 (± 63)	7 (± 5)
	Barley	236 (± 71)	15 (± 14)
	Red clover	368 (± 233)	23 (± 6)
	Mean	228 (± 181)	15 (± 11)
Oct. 25	Potato	350 (± 175)	7 (± 3)
	Barley	326 (± 182)	13 (± 2)
	Red clover	184 (± 153)	15 (± 4)
	Mean	287 (± 172)	12 (± 4)
Mean	Potato	230 (± 166)	11 (± 7) b
	Barley	239 (± 120)	12 (± 7) b
	Red clover	313 (± 186)	18 (± 7) a
P-Value			
Crop [C]		NS[z]	<0.001
Date [D]		NS	NS
C X D		NS	NS

[z] NS- not significant ($P \geq 0.05$)

g^{-1} soil and in 2011 were 737 µg C g^{-1} soil. Microbial biomass C throughout the 2010 field season was highly variable, and ranged from 79 µg C g^{-1} soil to 391 µg C g^{-1} soil for

Table 5.4. Microbial biomass carbon (MB-C) and nitrogen (MB-N) concentrations determined using the chloroform fumigation method from all three phases of the barley-red clover-potato crop rotation during the 2011 field season. Values shown in parentheses represent ± 1 SD.

Sampling date	Crop species	MB-C ($\mu g\ C\ g^{-1}$ soil)	MB-N ($\mu g\ N\ g^{-1}$ soil)
May 3	Potato	639 (± 120)	17 (± 4)
	Barley	1152 (± 322)	20 (± 10)
	Red clover	630 (± 281)	17 (± 7)
	Mean	807 (± 241)	18 (± 7) b
June 21	Potato	513 (± 291)	19
	Barley	1049 (± 667)	16 (± 8)
	Red clover	986 (± 539)	22 (± 7)
	Mean	849 (± 499)	18 (± 7) ab
Aug. 2	Potato	290 (± 116)	13 (± 6)
	Barley	540 (± 88)	16 (± 2)
	Red clover	1097 (± 185)	22 (± 8)
	Mean	642 (± 129)	17 (± 6) b
Sept. 27	Potato	744 (± 469)	18 (± 16)
	Barley	636 (± 259)	19 (± 7)
	Red clover	555 (± 401)	26 (± 7)
	Mean	645 (± 376)	21 (± 10) ab
Oct. 25	Potato	648 (± 235)	20 (± 6)
	Barley	794 (± 241)	24 (± 9)
	Red clover	781 (± 384)	39 (± 8)
	Mean	741 (± 287)	28 (± 11) a
Mean	Potato	567 (± 278)	17 (± 8) b
	Barley	834 (± 404)	19 (± 7) b
	Red clover	810 (± 396)	25 (± 10) a
P-Value			
Crop [C]		NS[z]	0.012
Date [D]		NS	0.014
C X D		NS	NS

[z] NS- not significant ($P \geq 0.05$)

all crop species. Microbial biomass C concentrations throughout the 2011 field season were similar to the 2010 field season in that they were highly variable (ranging from 290

μg C g^{-1} soil to 1152 μg C g^{-1} soil), however MB-C concentrations within the 2011 field season were significantly greater on average in comparison to the 2010 field season (*p*-value < 0.001; Table 5.4).

There was a significant effect of crop species of MB-N concentrations in 2010, with greater MB-N averaged over sampling dates for red clover (18 μg N g^{-1} soil) than for potato and barley (average of 12 μg N g^{-1} soil; Table 5.3). However, there was no significant effect of sampling date, or sampling date by crop species interaction, on MB-N in 2010 (Table 5.3). Similar to the 2010 growing season, there was a significant effect of crop species of MB-N concentrations in the 2011 growing season, with greatest MB-N averaged over the season for red clover (25 μg N g^{-1} soil), than for barley and potato (average of 18 μg N g^{-1} soil; Table 5.4). However, a significant effect of sampling date was also observed within the 2011 field season, with significantly greater MB-N concentrations on October 25 (28 μg N g^{-1} soil), than on May 3 (18 μg N g^{-1} soil) or August 2 (17 μg N g^{-1} soil).

Soil MB-C and MB-N concentrations were highly variable for both field seasons, with SD values often greater than 50% of the mean value. Consequently, determining the effect of crop species and sampling date across the growing season was problematic due to the high degree of variation within MB-C and MB-N concentrations. Typically, changes in the size of the microbial community, as shown by MB analyses, are used to identify the response of the microbial community to varying crop species or environmental factors, and reflect how the microbial population reacts to these stresses (Voroney et al. 2008). The MB-C concentrations under the potato crop in the current study, within the 2010 season, were within $\pm$ 1 SD of other published seasonal averages

within fine sandy loam soils on P.E.I. (Angers et al. 1999; Carter and Noronha 2007; Carter et al. 2009). However, MB-C concentrations under the potato crop within the 2011 season were generally 1.5 to 2.5 times greater than the other published seasonal averages listed above (Angers et al. 1999; Carter and Noronha 2007; Carter et al. 2009).

The lack of statistical significance among crop species was unexpected. Within potato crop rotations, MB-C concentrations were commonly lower under potato production in comparison with forage and grain rotational crops (Angers et al. 1999; Nelson et al. 2009). Within a 9 year potato rotation study, Angers et al. (1999) found maximum MB-C concentrations averaged across the growing season within the potato rotation with the lowest frequency of years cropped to potatoes (specifically, a 3 year barley-red clover-potato rotation similar to the one used within the current pool dilution study). When using the barley-red clover-potato rotation, Angers et al. (1999) found MB-C concentrations, on average, over two times greater than the continuous potato rotation, due to higher concentrations of soil organic C (SOC) and less tillage events than the continuous potato rotation alone.

The growth and incorporation of crop residues of grains and forages within potato crop rotations have often been found to increase SOC, and consequently MB-C (Carter et al. 2009). Using the same barley-red clover-potato rotation as used within the current study, Carter et al. (2009) found that MB-C generally increased from year to year from potato to barley, and barley to red clover, with potato acting as a significant sink of SOC. Since there were no significant differences between the crop species, the current study does not demonstrate potato as a significant source of SOC, however, the significantly greater MB-C concentrations within the 2011 growing season in comparison to the 2010

growing season may suggest that SOC concentrations are increasing since the crop rotation establishment in 2009 (Table 5.3).

Studies on MB-N have reported conflicting results, including both a lack of seasonal effects of MB-N (Puri and Ashman 1998) and significant seasonal effects on MB-N (Burton and McGill, 1992; Tu et al. 2006). In a study conducted by Tu et al. (2006), MB-N showed seasonal fluctuations that were dependent on seasonal cropping events such as planting, mid-season crop uptake, and harvest, on various conventionally and organically managed vegetable crops. However, in a review by St. Luce et al. (2011), it is stated that within humid and temperate soils, MB-N can remain relatively constant throughout seasonal temperature and moisture variations, regardless of management practice or nutrient availability within crops. This was an outcome similar to the current study where there were no significant differences in MB-N among sampling dates in the 2010 field season; however this was not consistent with the significantly different sampling date values within the 2011 growing season. Significantly greater MB-N concentrations were found within the red clover crop, in comparison to the potato and barley crops. This may reflect higher nitrogen concentration in the red clover crop residues.

5.4 Soil Mineral N

5.4.1. 2010 Growing Season

For the barley crop, maximum soil NH_4^+ and NO_3^- concentrations were measured on May 25, shortly after planting (Tables 5.5, 5.6). Soil NH_4^+ concentrations on this date were greater for the CON management (51.0 mg N kg^{-1} dry soil) than the RN

Table 5.5. Mean soil NH_4^+ concentrations for 0-30 cm depth from the 2010 growing season under conventional (CON) and reduced N (RN) fertility management for nine sampling dates, and averaged across sampling dates, for all three phases of a barley-red clover-potato crop rotation. Statistical differences among treatment means are indicated only when the main effect or interaction are significant.

		Sampling date									
Crop	N Management	May 25	June 9	June 28	July 20	Aug. 10	Aug. 16	Aug. 25	Sept. 28	Oct. 25	Mean
		(mg NH_4-N kg^{-1} dry soil)									
Potato		2.8b [Z]	1.2b	77.3a	15.7a	1.1	1.1	3.7a	1.0	0.8	11.6a
Barley		38.5a	7.9a	0.9b	0.7b	0.4	0.4	0.7b	2.7	1.1	5.9b
Red clover		0.8c	0.9b	1.6b	1.2b	0.4	0.9	1.1b	2.2	1.3	1.1c
	CON	17.9	1.7	34.6a	7.6a	0.5	0.7	2.1	2.2	1.2	7.6
	RN	10.1	4.9	18.6b	4.0b	0.7	0.8	1.6	1.7	0.9	4.8
Potato	CON	1.9cd	1.0	101.3a	21.1a	0.7	1.2	4.6	0.8	0.8	14.8
	RN	3.7c	1.4	53.2b	10.3b	1.4	1.0	2.8	1.1	0.8	8.4
Barley	CON	51.0a	3.4	0.8c	0.7c	0.4	0.3	0.7	4.3	1.3	7.0
	RN	25.9b	12.3	1.0c	0.6c	0.3	0.4	0.7	1.0	0.8	4.8
Red clover	CON	0.8d	0.8	1.6c	1.0c	0.4	0.8	1.0	1.4	1.5	1.0
	RN	0.8d	0.9	1.6c	1.3c	0.4	1.0	1.2	2.9	1.1	1.2
P-Value											
Crop [C]		<0.001	0.029	<0.001	0.002	NS [Y]	NS	0.005	NS	NS	<0.001
Treatment [N]		NS	NS	0.025	0.024	NS	NS	NS	NS	NS	NS
C X N		0.034	NS	0.004	0.006	NS	NS	NS	NS	NS	NS

[Z] Means within a column, and for an individual main effect or interaction, with the same lowercase letter are not significantly different based on a Fisher's Least Significant Difference test

[Y] NS- not significant ($P \geq 0.05$)

Table 5.6. Mean soil NO_3^- concentrations for 0-30 cm depth from the 2010 growing season under conventional (CON) and reduced N (RN) fertility management for nine sampling dates, and averaged across sampling dates, for all three phases of a barley-red clover-potato crop rotation. Statistical differences among treatment means are indicated only when the main effect or interaction are significant.

		Sampling date									
Crop	N Management	May 25	June 9	June 28	July 20	Aug. 10	Aug. 16	Aug. 25	Sept. 28	Oct. 25	Mean
		(mg NO_3-N kg^{-1} dry soil)									
Potato		13.4b[Z]	6.2a	67.5a	91.3a	55.3a	46.6a	48.8a	13.6a	12.0a	39.4a
Barley		26.0a	12.0a	7.5b	0.5b	4.2b	2.5b	7.0b	7.6b	5.4c	8.1b
Red clover		0.4c	0.3b	0.4c	1.5c	3.7b	3.5b	3.2b	5.9b	9.2b	3.1c
	CON	14.7	4.8	27.5	32.9	16.2	16.8b	21.3a	8.8	13.1	17.3
	RN	11.8	7.6	22.7	29.2	25.8	18.2a	18.0b	9.2	12.3	17.2
Potato	CON	13.6	5.0	77.8	97.0	41.1b	45.5	49.1a	12.9	1.0	38.1
	RN	13.1	7.4	57.1	85.6	69.4a	47.6	48.5a	14.3	23.0	40.7
Barley	CON	29.8	8.9	4.4	0.5	4.2c	2.5	11.3b	8.1	5.6	8.4
	RN	22.2	15.1	10.5	0.4	4.1c	2.4	2.7c	7.0	5.2	7.7
Red clover	CON	0.6	0.4	0.2	1.3	3.4c	2.5	3.5c	5.4	9.6	3.0
	RN	0.2	0.2	0.5	1.6	3.9c	4.5	2.8c	6.3	8.7	3.2
P-Value											
Crop [C]		<0.001	<0.001	<0.001	<0.001	0.007	<0.001	<0.001	0.015	<0.001	<0.00
Treatment [N]		NS[Y]	NS	NS	NS	NS	0.046	0.024	NS	NS	NS
C X N		NS	NS	NS	NS	0.002	NS	0.030	NS	NS	NS

[Z] Means within a column, and for an individual main effect or interaction, with the same lowercase letter are not significantly different based on a Fisher's Least Significant Difference test

[Y] NS- not significant ($P \geq 0.05$)

management (25.9 mg N kg^{-1} dry soil) (Table 5.5), whereas soil NO_3^- concentrations were not significantly different between N managements and averaged 26.0 mg N kg^{-1} dry soil (Table 5.6). Soil mineral N concentrations subsequently decreased, presumably due to nitrification and plant uptake, and were generally low (< 2.7 mg NH_4^+- N kg^{-1} dry soil; < 7.6 mg NO_3^--N kg^{-1} dry soil) throughout the remainder of the growing season beginning in July (Tables 5.5, 5.6). The exception was an increase in soil NO_3^- concentrations on August 25 under the CON management, just prior to barley harvest, at 11.3 mg N kg^{-1}soil (Table 5.6).

Under red clover, soil NH_4^+ concentrations were consistently low (average of 1.1 mg N kg^{-1} dry soil) throughout the growing season and did not differ between N managements (Table 5.5). Soil NH_4^+ concentrations were lower under red clover than under barley on May 25, due to the fertilization of barley during planting on May 18, however for the remainder of the season soil NH_4^+ concentrations were comparable under both the barley and red clover crops (Table 5.5). Soil NO_3^- concentrations under red clover were also low (average of 3.1 mg N kg^{-1} dry soil) throughout the growing season and did not differ between N managements. Soil NO_3^- concentrations generally increased over the growing season from 0.4 mg N kg^{-1} dry soil on May 25 to 9.2 mg N kg^{-1} dry soil on October 25 (Table 5.6). Soil NO_3^- concentrations under red clover were lower than under barley in May and June, whereas concentrations under the two crops were comparable on most sampling dates throughout the remainder of the growing season. Low soil mineral N concentrations under red clover were expected since external additions of N through fertilizer were not a factor, and crop uptake would be expected to maintain low soil mineral N concentrations. Fall plow-down of CON managed crops

occurred on October 12, but this did not result in a significant difference between N managements on October 25 for either soil NO_3^- or NH_4^+ concentrations (Tables 5.5, 5.6).

Under potato production, soil NH_4^+ concentrations on May 25 for both N managements were low, most likely due to negligible amounts of residual N left within the soil following the winter (Table 5.5). However, soil NH_4^+ concentrations on this date were slightly greater under RN management (3.7 mg N kg^{-1} dry soil) than CON management (1.9 mg N kg^{-1} dry soil), which may reflect the red clover plow-down that occurred on the RN managed plots on April 15. Maximum soil NH_4^+ concentrations occurred on June 28, when concentrations in potato hills under CON management (101.3 mg N kg^{-1} soil) were 2 times greater than under RN management (53.2 mg N kg^{-1} dry soil). Soil NH_4^+ concentrations in potato hills were also greater under CON management (21.1 mg N kg^{-1} dry soil) in comparison with RN management (10.3 mg N kg^{-1} dry soil) on July 20 (Table 5.5). Throughout the remainder of the season, soil NH_4^+ concentrations in potato hills generally decreased until harvest on October 15 and were not significantly different between N managements. The maximum soil NO_3^- concentration for the potato crop occurred on July 20 (Table 5.5), and was likely due to rapid nitrification of the applied NH_4^+ from the fertilizer banding prior to potato planting, as soil NH_4^+ concentrations were greatly reduced at this time (Table 5.6). Soil NO_3^- concentrations were significantly greater under RN management (69.4 mg N kg^{-1} dry soil) in comparison with CON management plots (41.1 mg N kg^{-1} dry soil) on August 10 (Table 5.6).

When averaged across all sampling dates, soil NH_4^+ and NO_3^- concentrations were greatest under potato production (11.6 and 39.4 mg N kg^{-1} dry soil, respectively),

intermediate under barley (5.9 and 8.1 mg N kg^{-1} dry soil, respectively) and lowest under red clover (1.1 and 3.1 mg N kg^{-1} dry soil, respectively) (Tables 5.5, 5.6). Within individual sampling dates, soil NH_4^+ concentrations were greatest under potato production only on June 28 and July 20 whereas soil NO_3^- concentrations were greatest under potato production for all sampling dates from July 20 to October 25.

5.4.2. 2011 Growing Season

Under barley production, soil NH_4^+ concentrations for 0-30 cm depth were consistently low (<1.2 mg N kg^{-1} dry soil) over all sampling dates and for both N managements (Table 5.7). Soil NO_3^- concentrations were at a maximum (20.5 mg N kg^{-1} dry soil) on June 22 (Table 5.8). This likely reflected rapid nitrification of fertilizer N applied at planting on May 27. The N managements had no significant effect on soil NH_4^+ or NO_3^- concentrations for any individual sampling date nor when averaged over the whole growing season (Tables 5.7, 5.8).

Under red clover, soil mineral N concentrations in 2011 (Tables 5.7, 5.8) were generally similar to those reported in 2010 (Tables 5.5, 5.6). Soil NH_4^+ concentrations for 0-30 cm depth were no greater than 2.1 mg N kg^{-1} dry soil on any sampling date, and were not significantly different between N managements or among sampling dates (Table 5.7). Soil NO_3^- concentrations were slightly greater (3.1 mg N kg^{-1} dry soil) during May to July compared with August to October (1.0 mg N kg^{-1} dry soil), with maximum concentrations (average of 5.0 mg N kg^{-1} dry soil) measured on July 13 (Table 5.8). Soil NO_3^- concentrations differed between N managements only on August 16, when concentrations for the RN management (2.8 mg N kg^{-1} dry soil) were approximately two times greater than for the CON management (1.6 mg N kg^{-1} dry soil; Table 5.8). Soil

Table 5.7. Mean soil NH_4^+ concentrations for 0-30 cm depth from the 2011 growing season under conventional (CON) and reduced N (RN) fertility management for nine sampling dates, and averaged across sampling dates, for all three phases of a barley-red clover-potato crop rotation. Statistical differences among treatment means are indicated only when the main effect or interaction are significant.

		Sampling date								
Crop	N Management	May 5	May 25	June 22	July 13	Aug. 10	Aug. 25	Sept. 28	Oct. 25	Mean
		(mg NH_4-N kg^{-1} dry soil)								
Potato		0.7a[Z]	2.6	17.4a	9.5	1.4a	1.1	1.1	1.6	4.4a
Barley		0.3b	0.3	1.2b	0.4	0.2b	0.9	0.8	1.4	0.7b
Red clover		0.4b	0.5	2.0b	0.4	0.4b	0.5	1.0	1.4	0.8b
	CON	0.4	0.5	7.7	6.4a	0.8	0.9a	1.0	1.5	2.4
	RN	0.4	1.8	6.0	0.4b	0.5	0.7b	0.9	1.5	1.5
Potato	CON	0.6	0.9	20.1	18.5a	1.8b	1.2	1.3a	1.6	5.8
	RN	0.7	4.3	14.6	0.4b	0.9a	0.9	0.8b	1.6	3.0
Barley	CON	0.3	0.3	1.1	0.4b	0.2c	0.9	0.7b	1.2	0.6
	RN	0.3	0.3	1.2	0.4b	0.2c	0.9	0.8ab	1.5	0.7
Red clover	CON	0.3	0.3	1.8	0.3b	0.3bc	0.6	1.0ab	1.6	0.8
	RN	0.4	0.7	2.1	0.5b	0.5bc	0.3	0.9ab	1.2	0.8
P-Value										
Crop [C]		0.019	NS[Y]	0.019	NS	0.015	NS	NS	NS	0.004
Treatment [N]		NS	NS	NS	0.041	NS	0.042	NS	NS	NS
C X N		NS	NS	NS	0.010	0.020	NS	0.015	NS	NS

[Z] Means within a column, and for an individual main effect or interaction, with the same lowercase letter are not significantly different based on a Fisher's Least Significant Difference test

[Y] NS- not significant ($P \geq 0.05$)

Table 5.8. Mean soil NO_3^- concentrations for 0-30 cm depth from the 2011 growing season under conventional (CON) and reduced N (RN) fertility management for nine sampling dates, and averaged across sampling dates, for all three phases of a barley-red clover-potato crop rotation. Statistical differences among treatment means are indicated only when the main effect or interaction are significant.

		Sampling date								
Crop	N Management	May 5	May 25	June 22	July 13	Aug. 10	Aug. 25	Sept. 28	Oct. 25	Mean
		(mg NO_3-N kg^{-1} dry soil)								
Potato		3.8a[Z]	4.6a	68.5a	72.1a	16.1a	40.1a	28.0a	2.4a	29.4a
Barley		2.9a	1.9b	20.5b	3.1b	2.2b	1.0b	5.5b	0.9b	4.7b
Red clover		1.7b	2.0b	3.7c	5.0b	2.0b	0.5b	1.1c	0.3b	2.0c
	CON	3.3a	2.8	28.9	30.6	5.8b	18.0a	12.8a	1.3	12.9
	RN	2.3b	2.8	32.9	22.8	7.8a	9.7b	10.2b	1.1	11.2
Potato	CON	5.4a	4.1ab	61.2	82.9	13.4b	52.4	26.9a	2.5	31.1
	RN	2.1cd	5.1a	75.7	61.3	18.8a	27.8	29.0a	2.3	27.8
Barley	CON	2.6bc	1.7c	21.8	4.3	2.5c	1.2	10.2b	1.1	5.7
	RN	3.2b	2.1bc	19.1	1.9	1.9cd	0.8	0.8b	0.6	3.8
Red clover	CON	1.9cd	2.5bc	3.6	4.5	1.6d	0.5	1.2c	0.3	2.0
	RN	1.5d	1.4c	3.7	5.4	2.8c	0.5	0.9c	0.3	2.1
P-Value										
Crop [C]		0.017	0.023	<0.001	<0.001	0.001	<0.001	0.001	0.002	<0.001
Treatment [N]		<0.001	NS[Y]	NS	NS	<0.001	0.024	0.003	NS	NS
C X N		<0.001	0.049	NS	NS	<0.001	NS	<0.001	NS	NS

[Z] Means within a column, and for an individual main effect or interaction, with the same lowercase letter are not significantly different based on a Fisher's Least Significant Difference test

[Y] NS- not significant ($P \geq 0.05$)

NH_4^+ concentrations were not statistically different between barley and red clover crops on any sampling date nor when averaged across the growing season (Table 5.7). Soil NO_3^- concentrations were significantly greater under barley than under red clover on May 5, June 22, September 28, and when averaged over the whole growing season (Table 5.8).

In the potato hill, soil NH_4^+ concentrations were at a maximum (17.4 mg N kg^{-1} dry soil) on June 22 following planting and prior to hilling (Table 5.7). Soil NH_4^+ concentrations decreased rapidly under the RN management to 0.4 mg N kg^{-1} dry soil on July 13, in comparison with 18.5 mg N kg^{-1} dry soil under the CON management. By August 10, soil NH_4^+ concentrations under CON management decreased ten-fold to 1.8 mg N kg^{-1} dry soil, which was significantly greater than under the RN management (0.9 mg N kg^{-1} dry soil; Table 5.7). Maximum soil NO_3^- concentrations for the RN management occurred on June 22 following planting, and prior to hilling, whereas the greatest soil NO_3^- concentrations for the CON management occurred on July 13 following hilling (Table 5.8). Following hilling, soil NO_3^- concentrations were elevated for the remainder of the season prior to a sharp decline in soil NO_3^- concentrations to < 2.5 mg N kg^{-1} dry soil for both N treatments by the final sampling date on October 25 (Table 9). Soil NH_4^+ and NO_3^- concentrations in the potato hill were greater when averaged over all sampling dates, and were frequently significantly greater on individual sampling dates, compared with barley or red clover production (Tables 5.7, 5.8).

5.4.3. Discussion

Temporal fluctuations in both soil NH_4^+ and NO_3^- concentrations within the potato crop phase, i.e., high NH_4^+ concentrations directly following planting in response to fertilizer application, and high NO_3^- concentrations occurring prior to hilling in

response to nitrification of added fertilizer, followed by rapid declines in both soil NH_4^+ and NO_3^- concentrations by the end of the growing season, were expected within this study. These results are consistent with the soil mineral N temporality on potato rotations reported by Zebarth and Milburn (2003) and Belanger et al. (2001) on medium textured soils in New Brunswick. Elevated soil NO_3^- and NH_4^+ concentrations within the potato hills were measured between planting and hilling, prior to timing of rapid crop N uptake. Rapid decline of soil NH_4^+ concentrations within August of the 2011 season were likely affected by increased nitrification due to above average precipitation events, (as observed by Cambouris et al. 2008), and likely caused the high soil NO_3^- concentrations observed within the current study at potato harvest. However, although seasonal temporality was similar to previous studies, soil NO_3-N concentrations for both the 2010 and 2011 growing seasons within the current study were typically lower than those reported at mid-season and at harvest by Belanger et al. (2001; 2003).

The higher soil NH_4^+ concentration observed in the RN treatment in comparison with the CON treatment, in the spring of 2010 under the potato crop, was likely caused by mineralization of red clover crop residues from the recent RN plow-down. Since the CON plow-down occurred on November 5, 2009, in comparison to the RN plow-down on April 5, 2010, much of the added N within the crop residues of the CON managed plots may have been lost during the overwintering period. Legume incorporation, like the red clover plow-down observed within the current study, can supply enough N to the current potato crop that reduction of the amount of N required as fertilizer within the subsequent potato crop may be possible. To be effective, timing of incorporation must be predicted appropriately to account for the loss of N during the overwintering period or to

accurately estimate the timing of N release relative to peak potato N demand (Stark and Porter 2005, Lynch et al. 2012).Many other studies have also shown the beneficial effects of incorporating cover crops within cash crop rotations not only by increasing soil N, but by also reducing NO_3^- losses during the over-wintering phase (Thorup-Kristensen 1994; Hogh-Jensen and Schjoerring 1997; Tonitto et al. 2006; Askegaard and Eriksen 2008; Askegaard et al. 2011; Lynch et al. 2012). As mentioned, it is important to note that soil mineral N dynamics are largely dependent on the timing and incorporation of the cover crop plow-down (Thorup-Kristensen et al. 2003). Plow-down of catch crops has shown to be a significant source of potentially mineralized N when plowed-down prior to crop establishment (Askegaard et al. 2011), but, if plowed down in the fall, much of this N may be lost during over-wintering due to NO_3^- leaching (Hogh-Jensen and Schjoerring 1997). Sanderson et al. (1999) concluded from a study using three different plow-down dates of red clover (early September, mid-October and early spring) that the early September plow-down resulted in the greatest soil NO_3-N concentrations in late November and the lowest residual soil NO_3-N concentrations in early spring. This suggests that a majority of the mineralized N from the cover crop within the experiment conducted by Sanderson et al. (1999) did not remain within the soil over the winter into the spring sampling, if the plow-down of red clover occurs early in the fall. Sanderson et al. (1999) concluded that incorporation of red clover should be conducted later on in the fall (at least mid to late October), or ideally early spring when soil temperatures are colder, and mineralization and nitrification of decomposing red clover residues and the potential for NO_3-N loss due to leaching are much reduced. Generally when soils are below 5°C, N mineralization and immobilization are reduced, and consequently the late

fall plow-down would be expected to result in increased soil mineral N concentrations during the spring when soil temperatures are warmer and soil conditions are more viable for microbial activity and growth (Thorup-Kristensen et al. 2003). However, this effect is also influenced by many other factors including catch crop species, the quantity and structure of plant material, the C/N ratio of the plant tissues and within the soil, and plant cellulose content (as reviewed by Thorup-Kristensen et al. 2003). Within the current study, the small effect of spring plow-down of red clover between managements was only observed within the 2010 growing season, when soil mineral N was significantly greater within the RN treatment (spring plow-down), in comparison to the CON treatment (fall plow-down), and suggests that the loss of mineralized N within the CON treatment may have occurred over winter.

Within the barley crop, increased soil mineral N concentrations were observed briefly following planting followed by a gradual decline to continually low concentrations for the remainder of the growing season. This temporal pattern was consistent with other seasonal mineral N patterns within barley crops within the Atlantic Canada region (Zebarth et al. 2008, Snowdon 2010). High soil mineral N concentrations in the 2010 growing season under barley were likely caused by additional inorganic N added as mineral fertilizer during planting, however, it is important to note that the effect of the following crop has been found to be a significant factor in influencing soil N dynamics within the second year crop (Rathke et al. 2005; Alva et al. 2007; Sapkota et al. 2012). In a study by Alva et al. (2007), incorporation of potato crop residues were found to provide 72 kg N ha^{-1} over time, which is enough N to increase soil mineral N concentrations of the preceding crop. However, this may not be as relevant within this

study as the study by Alva et al. (2007) was conducted within an irrigated system under drier climatic conditions. The low concentration of soil NH_4^+ within the barley crop in the beginning of the 2011 growing season did not demonstrate the effect of planting three weeks prior, or potato tissue incorporation from the preceding year. As the study by Alva et al. (2007) was conducted under slightly different growing conditions (i.e. irrigated system with drier natural climatic conditions), it is possible that the effect of potato crop residues is not a significant factor on influencing soil mineral N concentrations in the following year. More likely the first soil sampling date soil NH_4^+ concentrations following planting (June 22) did not reflect the high inputs of broadcast fertilizer applied at planting as most of the soil NH_4^+ from the added inorganic fertilizer was rapidly transformed into soil NO_3^- due to nitrification, as seen in the significantly higher soil NO_3^- concentrations observed at this time.

There was no significant effect of N management on soil NH_4^+ or NO_3^- concentrations averaged across either growing season, however there were significant effects of N management observed on some individual sampling dates throughout the growing season. Since mineral N soil samples were taken intermittently throughout the season and specifically timed to co-ordinate with the isotope pool dilution sampling dates, the dates sampled may not always reflect times of significant contrasts between N fertility managements or times of rapid changes within soil mineral N concentrations. Soil mineral N concentrations can vary greatly throughout the season (Burton et al. 2008) and thus the timing of sampling dates can be important in interpreting the seasonality of mineral N. Soil mineral N concentrations can vary depending on different N application rates, and when sampled at various times over the growing season (Belanger et al. 2003).

Much of the excess applied N may have been lost through either leaching (Unlu et al. 1999) or denitrification (Burton et al. 2008), or used during plant uptake (Munoz et al. 2005). Timing of sampling, variability between sampled soil mineral N concentrations, and N loss mechanisms may have all contributed to a lack of effect on soil mineral N concentrations between N managements.

5.5 Gross Nitrogen Transformation Rates

5.5.1. Unfiltered Data

Mean rates of gross nitrification, mineralization, NH_4^+ consumption and NO_3^- consumption were calculated using unfiltered data for each sampling date in 2010 and 2011 (Tables 5.9, 5.10). Unfiltered data (i.e., including all negative rates, and rates in which atom percent excess values for ^{15}N were not diluted over the 24 hour incubation period within the overall mean rates) gave mean negative rates in 15 of 60 cases in 2010, and 15 of 60 cases in 2011. The frequent occurrence of negative rates was attributed primarily to a lack of dilution in the atom percent excess ^{15}N during the 24 hour incubation period, and to variation in initial soil mineral N concentrations in cores used for the T_0 and T_{24} measurements within each plot. For example, for the June 21 sampling date in 2011 under potato production, the isotope pool was not diluted, and the soil NH_4^+ concentration was approximately 8.5 mg N kg^{-1} dry soil greater for the T_0 core, compared with the NH_4^+ concentration within the T_{24} core. This resulted in a calculated gross mineralization rate of -112.0 mg N kg^{-1} dry soil d^{-1} (Table 5.10). This was a common occurrence, particularly after planting within the potato crop, due to variable soil NH_4^+ and NO_3^- concentrations resulting from banded fertilizer application. In other cases, adequate atom percent excess ^{15}N dilution occurred, however, negative rates were still

Table 5.9. Unfiltered mean gross nitrification, gross mineralization, NO_3^- consumption and NH_4^+ consumption rates for five sampling dates during the 2010 growing season. Unfiltered rates include all viable calculations including negative values and cases where atom percent excess dilution did not occur during the 24 hour incubation.

Sampling date	Crop species	Mean nitrification	Mean mineralization	Mean NO_3^- consumption	Mean NH_4^+ consumption
			(mg kg^{-1} dry soil d^{-1})		
May 18	Potato	3.13 (4)[z]	1.23 (4)	2.40 (4)	-1.22 (4)
	Barley	-0.29 (4)	1.57 (3)	0.21 (4)	2.86 (3)
	Red clover	-0.10 (4)	0.88 (3)	0.33 (4)	0.33 (4)
July 7	Potato	-41.40 (2)	-75.86 (3)	-39.11 (2)	57.17 (2)
	Barley	ND	-0.27 (4)	ND	3.39 (4)
	Red clover	0.45 (3)	0.92 (4)	0.02 (2)	0.34 (4)
Aug. 23	Potato	5.60 (4)	-0.02 (3)	2.39 (4)	-6.99 (3)
	Barley	2.45 (4)	ND	0.63 (4)	ND
	Red clover	-2.22 (4)	0.66 (2)	-0.71 (4)	-0.61 (2)
Sept. 27	Potato	-21.82 (3)	-8.99 (2)	-15.93 (3)	19.10 (2)
	Barley	4.50 (4)	4.47 (2)	1.78 (4)	1.83 (2)
	Red clover	1.20 (3)	4.11 (3)	1.59 (3)	4.45 (2)
Oct. 25	Potato	0.62 (3)	1.18 (4)	2.81 (3)	-1.22 (3)
	Barley	-3.41 (3)	1.28 (4)	-3.68 (2)	4.49 (3)
	Red clover	0.44 (4)	3.32 (4)	-1.21 (4)	3.32 (4)

[z]Values in parentheses indicate sample size (*n*).
ND - not determined; due to mathematical limitations within the calculations, some rates were not able to be calculated due to an accumulation of missing data, or data were not calculated due to missing samples or sampling error.

calculated due to large variations in soil mineral N concentrations (most often at low soil mineral N concentrations). In 2010, from a total of 120 cases, 4 cases had negative rates even though pool dilution occurred, 38 cases were not isotopically diluted over the 24 hour incubation period, and 18 cases were unable to be calculated due to operational errors, missing values or unavailable isotope data. In 2011, from a total of 120 cases, 13 cases had negative rates even though isotopic pool dilution occurred, 28 cases were not

Table 5.10. Unfiltered mean gross nitrification, gross mineralization, NO_3^- consumption and NH_4^+ consumption rates for five sampling dates during the 2011 growing season. Unfiltered rates include all viable calculations including negative values and cases where atom percent excess dilution did not occur during the 24 hour incubation.

Sampling date	Crop species	Mean nitrification	Mean mineralization	Mean NO_3^- consumption	Mean NH_4^+ consumption
			(mg kg^{-1} dry soil d^{-1})		
May 3	Potato	1.93 (4)[z]	-0.08 (2)	1.35 (4)	-1.47 (2)
	Barley	1.37 (4)	-0.10 (3)	2.05 (4)	0.32 (3)
	Red clover	1.02 (3)	-0.29 (4)	3.22 (4)	-1.80 (4)
June 21	Potato	40.76 (4)	-88.44 (3)	42.71 (4)	-82.77 (3)
	Barley	3.33 (4)	10.74 (4)	16.30 (4)	3.82 (4)
	Red clover	0.23 (4)	0.80 (3)	0.87 (4)	1.74 (3)
Aug. 2	Potato	-9.16 (3)	9.87 (4)	26.48 (3)	16.78 (3)
	Barley	0.68 (2)	1.28 (4)	0.55 (2)	2.81 (2)
	Red clover	3.10 (3)	1.03 (4)	2.50 (3)	-1.80 (2)
Sept. 27	Potato	0.78 (3)	3.38 (4)	3.86 (3)	7.55 (3)
	Barley	0.01 (4)	-1.43 (4)	2.13 (4)	-1.91 (4)
	Red clover	-3.18 (4)	0.73 (4)	-0.07 (4)	4.77 (4)
Oct. 25	Potato	0.61 (4)	-1.89 (4)	0.80 (3)	0.09 (3)
	Barley	0.38 (4)	1.14 (4)	-0.03 (4)	0.73 (4)
	Red clover	0.13 (4)	1.47 (4)	0.19 (4)	2.52 (4)

[z]Values in parentheses indicate sample size (*n*).
ND - not determined; due to mathematical limitations within the calculations, some rates were not able to be calculated due to an accumulation of missing data, or data was not calculated due to missing samples or sampling error.

isotopically diluted over the 24 hour incubation period, and 15 cases were unable to be calculated due to operational errors, missing values or unavailable isotope data. The frequent and large negative rates suggest that use of unfiltered data is problematic in estimating the gross N transformation rates; however, negative rates have been published within many other studies using this method (Murphy et al. 1997; Watson and Mills 1998; Watson et al. 2000; Wang et al. 2001; Verchot et al. 2002; Accoe et al. 2004;

Habteselassie et al. 2006). In many cases, these studies assumed the negative rates were not significantly different from zero. Watson and Mills (1998) assumed negative gross immobilization rates were a product of highly variable gross mineralization and nitrification rates, whereas Habteselassie et al. (2006) attributed the negative rates to non-uniform labeling of the NO_3^- and NH_4^+ pools at natural abundance. Watson and Mills (1998) found negative immobilization rates within their grassland study and believed it was caused by preferential consumption of applied ^{15}N, which overestimated gross mineralization and NH_4^+ consumption rates and led to non-constant rates over the 24 hour incubation period. Verchot et al. (2002) similarly found that the rates were not constant over the 24 hour incubation period and eliminated the use of negative mineralization or nitrification rates found within their study on grassland soils when calculating complete turnover times of NH_4^+ and NO_3^- pools. Given these examples, it was assumed that the calculated negative rates within the current study were likely caused by large variability within soil mineral N concentrations between soil cores, or due to non-uniform labeling of the soil mineral N pools. For this reason, the negative rates likely did not meet some of the fundamental assumptions of the isotope pool dilution method and were therefore omitted, in accordance with Sangster (2010) and Burger and Jackson (2003). Consequently, only filtered data (i.e., excluding all negative rates and where atom percent excess values for ^{15}N were not diluted over the 24 incubation period) were used for interpreting temporal variation throughout the growing season and crop rotational effects on gross N transformation rates.

Marginal isotopic dilution (i.e., extremely small changes in ^{15}N enrichment from initial ^{15}N to final ^{15}N values) has previously been identified as a potentially large source

of error (Davidson et al. 1991; Murphy et al. 2003). Davidson et al. (1991) demonstrated that small errors, of approximately 5 to 10% in the initial pool size when the final pool size is reduced through dilution by 75%, can lead to a calculated gross mineralization rate change of 10%. In comparison, when the same percentage of error occurs but the pool size is reduced through dilution by only 25%, calculated gross mineralization rates can be affected by up to 100%. Davidson et al. (1991) concluded that this method is then particularly useful in areas with low soil inorganic N pool sizes, where transformation rates are usually high enough to have complete turnover of soil inorganic N pools each day, such as the forest soil system within their study. The influence of soil mineral N variation between T_0 and T_{24} cores will be discussed in more detail in Section 5.8 below.

Data filtering resulted in exclusion of 50% of the data from 2010, and 46% of the data from 2011. This resulted in limitations in the statistical analyses that could be performed due to numerous missing data. However, broad statistical comparisons of filtered data were performed as possible. Of the filtered data from both field seasons, 2010 had a higher occurrence of non-determined data due to the lack of positive gross transformation rates on those sampling dates. There were fewer cases in 2011 than in 2010 where data had to be excluded, and there was only one case where no mean value could be calculated (Tables 5.11, 5.12).

5.5.2. 2010 Growing Season

In 2010, using only the filtered data, mean gross nitrification rates over the whole growing season were significantly different among crop species, with the greatest mean value occurring under potato (6.3 mg N kg^{-1} dry soil d^{-1}), followed by barley (2.1 mg N kg^{-1} dry soil d^{-1}) and red clover (1.7 mg N kg^{-1} dry soil d^{-1}; Table 5.11). No significant

Table 5.11. Filtered mean gross nitrification, gross mineralization, NO_3^- consumption and NH_4^+ consumption rates for five sampling dates during the 2010 growing season. Filtered rates refer to the average of calculated rates that showed atom percent excess dilution following the 24 hour incubation. All negative values were also omitted.

Sampling date	Crop species	Mean nitrification	Mean mineralization	Mean NO_3^- consumption	Mean NH_4^+ consumption
			(mg kg^{-1} dry soil d^{-1})		
May 18	Potato	4.17 (3)[Z]	4.16 (2)	1.41 (3)	1.48 (3) bcd[Y]
	Barley	0.92 (1)	2.99 (2)	2.83 (1)	2.86 (3) bcd
	Red clover	0.38 (2)	0.88 (3)	1.20 (2)	0.91 (2) bcd
	Mean	1.82	2.68	1.81	1.75
July 7	Potato	ND	13.07 (1)	ND	51.24 (1) a
	Barley	ND	0.23 (1)	ND	1.16 (3) d
	Red clover	0.70 (1)	0.92 (4)	1.09 (1)	1.36 (4) cd
	Mean	0.70	4.74	1.09	17.92
Aug. 23	Potato	9.57 (3)	2.82 (2)	7.53 (3)	3.17 (1) abcd
	Barley	2.45 (4)	ND	2.25 (2)	ND
	Red clover	3.54 (2)	6.72 (1)	2.08 (2)	0.40 (1) abcd
	Mean	5.19	4.77	3.95	0.40
Sept. 27	Potato	ND	0.72 (1)	ND	ND
	Barley	4.50 (4)	4.47 (2)	2.83 (3)	6.19 (1) ab
	Red clover	2.25 (2)	4.11 (3)	5.91 (1)	4.45 (2) abc
	Mean	3.38	3.10	4.37	8.01
Oct. 25	Potato	5.11 (2)	1.89 (3)	11.34 (1)	0.37 (1) cd
	Barley	0.41 (1)	1.28 (4)	0.46 (1)	4.49 (3) cd
	Red clover	1.52 (2)	4.48 (3)	0.63 (2)	5.91 (3) bcd
	Mean	2.35	2.55	4.14	5.20
Mean	Potato	6.28[X]	4.53	6.76	22.03
	Barley	2.07	2.24	2.09	3.68
	Red clover	1.68	3.42	2.18	2.61
P-Value	Crop [C]	0.022	NS	NS	NS
	Date [D]	NS	NS	NS	NS
	C X D	NS	NS	NS	0.046

[Z]Values in parentheses indicate sample size (*n*).

[Y]Treatment means in the same column followed by the same lowercase letter indicates means are not significantly different for the sampling date x crop species interaction.

[X]Although the main effect of crop species was significant, a means comparison was not possible as a result of excessive missing data.

Table 5.12. Filtered mean gross nitrification, gross mineralization, NO_3^- consumption and NH_4^+ consumption rates for five sampling dates during the 2011 growing season. Filtered rates refer to the average of calculated rates that showed atom percent excess dilution following the 24 hour incubation. All negative values were also omitted.

Sampling date	Crop species	Mean nitrification	Mean mineralization	Mean NO_3^- consumption	Mean NH_4^+ consumption
			(mg kg^{-1} d^{-1})		
May 3	Potato	1.93 (4)[Z] cd	0.18 (1)	2.26 (3) cde	0.37 (1) cde[Y]
	Barley	1.44 (3) cd	0.17 (1)	2.05 (4) e	0.74 (2) e
	Red clover	2.45 (2) bcd	0.24 (1)	3.64 (2) c	3.33 (1) de
	Mean	1.94 B	0.20 B	2.65[W]	1.48 C
June 21	Potato	60.93 (3) a	28.01 (2)	49.41 (3) a	37.68 (1) a
	Barley	6.67 (3) bc	15.79 (3)	19.74 (3) ab	3.82 (3) bc
	Red clover	0.23 (3) d	1.46 (2)	1.03 (3) e	3.02 (2) bcd
	Mean	22.61 A	12.08 A	23.39	9.81 A
Aug. 2	Potato	2.75 (1) bcd	21.23 (3)	57.45 (1) a	4.85 (2) b
	Barley	1.78 (1) bcd	1.28 (4)	0.55 (2) e	2.81 (2) bc
	Red clover	0.34 (1) bcd	1.98 (3)	0.21 (1) bcd	1.54 (1) bcd
	Mean	1.62 ABC	8.16 AB	19.40	3.07 B
Sept. 27	Potato	19.86 (1) ab	4.30 (3)	ND	12.49 (2) b
	Barley	2.12 (3) cd	0.57 (1)	1.34 (3) de	0.76 (2) e
	Red clover	0.05 (1) cd	1.01 (3)	0.69 (2) e	4.77 (4) cde
	Mean	7.34 AB	1.96 B	6.06	6.01 BC
Oct. 25	Potato	0.42 (2) cd	0.30 (3)	2.39 (1) cde	0.77 (1) e
	Barley	0.30 (2) cd	1.14 (4)	0.29 (2) e	1.03 (2) e
	Red clover	0.12 (2) d	2.40 (3)	0.65 (2) e	3.61 (3) bc
	Mean	0.28 C	1.28 B	1.11	1.80 C
Mean	Potato	17.18 A[X]	9.00	25.53[W]	15.41 A
	Barley	2.46 B	3.79	4.79	1.83 B
	Red clover	0.64 B	1.42	1.24	3.25 B
P-Value	Crop [C]	0.006	NS	<0.001	<0.001
	Date [D]	0.010	0.020	<0.001	<0.001
	C X D	0.008	NS	<0.001	0.001

[Z]Values in parentheses indicate sample size (*n*).

[Y]Treatment means in the same column followed by the same lowercase letter indicates means are not significantly different for the sampling date x crop species interaction.

[X]Treatment means in the same column followed by the same uppercase letter indicate that crop species are significant for the main effect of crop [C] or date [D] if the uppercase letters are the mean of the crop species throughout the whole growing season, or the mean of all crops on each individual date, respectively.

[W]Although the main effect of crop species was significant, a means comparison was not possible as a result of excessive missing data.

differences were found among sampling dates for gross nitrification rates across the whole growing season.

Gross mean mineralization rates were not significantly different among crop species or sampling dates, and averaged 3.4 mg N kg^{-1} dry soil d^{-1}. Similarly, mean gross NO_3^- consumption rates were not significantly different among crop species or sampling dates, and averaged 3.7 mg N kg^{-1} dry soil d^{-1}. There was a significant crop species by sampling date interaction on mean gross NH_4^+ consumption rates in 2010, where the NH_4^+ consumption rate in July was greater under the potato crop than under barley or red clover crops, whereas there was no significant difference among crops on any other sampling date.

The numerous missing data limited the statistical analyses of the gross transformation rates. Consequently, it is useful to consider some aspects of the numeric values of mean transformation rates in addition to the statistical analyses. Mean gross N transformation rates averaged over the five sampling dates were numerically greater for the potato crop in comparison with barley and red clover crops, particularly for gross NH_4^+ consumption rates (Table 5.11). However, this pattern was frequently not evident on individual sampling dates. Generally, similar numeric values of mean gross mineralization and gross nitrification rates, averaged across crop species, were estimated on each sampling date. The exception was July 7 when mean gross mineralization rates were approximately 5 times greater than mean gross nitrification rates, however very little data was available for calculating gross nitrification rates on this sampling date. With the exception of NH_4^+ consumption under the potato crop on July 7, numeric values of mean NO_3^- and NH_4^+ consumption rates were generally greater for sampling dates in

August to October than in May and June. Mean gross mineralization rates for red clover were consistently numerically higher than mean gross nitrification rates on all sampling dates.

It is useful to compare the calculated gross transformation rates with temporal changes in soil mineral N concentrations. For example, a high NH_4^+ consumption rate was calculated under a potato crop on July 7 (Table 5.11) which was consistent with rapid reduction in soil NH_4^+ concentrations between June 28 and July 20 (Table 5.5). In addition, rapid decreases in soil NO_3^- concentrations occurred between August 25 and September 28, and between September 28 and October 25 (Table 5.5) which is consistent with some of the highest numeric values of gross NO_3^- consumption rates under the potato crop (Table 5.11). However overall, direct comparisons of gross N transformation rates and soil mineral N data were difficult, as time periods that might be expected to have high gross rates of nitrification and NH_4^+ consumption, for example following fertilizer application at planting for barley and potato crops, were times at which gross N transformation rates could not be determined (Table 5.11). Within the red clover crop, mean nitrification and mineralization greater rates occurring on August 23, September 25, and October 25, tended to coincide with greater soil NO_3^- concentrations on those dates, however appeared to have no relationship with soil NH_4^+ concentrations (Table 5.6, 5.7, 5.11).

5.5.3. 2011 Growing Season

There was a significant crop species by sampling date interaction on mean gross nitrification rates in 2011, where nitrification rates under potato were higher than under barley and red clover on June 21 and September 27, whereas nitrification rates did not

differ among crop species on all other sampling dates (Table 5.12). There was also a main effect of crop species on mean gross nitrification rates, with rates under potato (17.2 mg kg^{-1} dry soil d^{-1}) being greater than under barley and red clover (average of 1.6 mg kg^{-1} dry soil d^{-1}). There was also a main effect of sampling date on gross nitrification rate where numerically the nitrification rate was greater on June 21 than on May 3 or October 25. There was no significant main effect of crop species or crop species by sampling date interaction on mean gross mineralization rates, however, there was a significant effect of sampling date with a significantly greater rate on June 21 (12.1 mg N kg^{-1} dry soil $d^{-}1$) than on May 3, September 27 and October 25 (average of 1.15 mg kg^{-1} dry soil d^{-1}). The higher mean mineralization rates on June 21 and August 2 primarily reflected the high calculated rates under potato and barley crops on June 21, and the potato crop on August 2. There was a significant crop species by sampling date interaction on mean NO_3^- consumption rates where rates under potato were significantly greater than under barley and red clover crops on August 2, but was not statistically different among crop species on May 3 and October 25. There was also a significant main effect of crop species on mean NO_3^- consumption, where maximum rates occurred under the potato crop (25.5 mg N kg^{-1} dry soil d^{-1}), and a significant main effect of sampling date, with greatest rates occurring on June 21 (23.4 mg kg^{-1} dry soil d^{-1}) and August 2 (19.4 mg kg^{-1} dry soil d^{-1}). There was also a significant crop species by sampling date interaction on mean gross NH_4^+ consumption rates where rates under potato were significantly greater than under both barley and red clover crops on June 21 and September 27, whereas NH_4^+ consumption rates were greatest under red clover on October 25 (Table 5.12).

Similar to the 2010 growing season, mean gross N transformation rates averaged over the five sampling dates were greater under the potato crop in comparison with barley and red clover crops, however this difference was not statistically different for gross N mineralization rates (Table 5.12). However, this difference among crop species was not evident on all individual sampling dates. Specifically, on the first and last sampling dates of the season, transformation rates under potato were often numerically lower than under red clover for all gross N transformation rates examined. Unlike the 2010 growing season, mean gross mineralization and nitrification rates were not numerically similar on each sampling date (Table 5.12). With the exception of June 21, average NO_3^- consumption and NH_4^+ consumption rates were numerically similar, although rates for both processes were not always similar for each crop species (Table 5.12). For example on August 2, mean NO_3^- consumption was 12 times greater than NH_4^+ consumption under potato, and on September 27 mean NH_4^+ consumption was 5 times greater than NO_3^- consumption under red clover.

Associations between calculated gross transformation rates and temporal changes in soil mineral N concentrations were also examined in 2011. High NO_3^- consumption rates under the potato crop during June 21 and August 2 sampling dates were consistent with a 6-fold decrease in soil NO_3^- concentrations from mid-July to early August (Table 5.8), likely due to rapid plant uptake of NO_3^- at this time. Under red clover, the greatest mean NO_3^- consumption rate (3.6 mg kg^{-1} d^{-1}) occurred concurrently with the highest nitrification rate on May 3, suggesting that greater NO_3^- turnover could be occurring at this time. Both nitrification and NO_3^- consumption rates decreased over the growing season ending with final rates of 0.1 and 0.7 mg kg^{-1} dry soil d^{-1} on October 25,

respectively (Table 5.12). This observation is consistent with the overall low soil NO_3^- concentrations under this crop at that time (Table 5.8). Although gross NH_4^+ consumption rates under potato and barley crops were large on June 21, only a small decrease of 1.6 mg kg^{-1} dry soil occurred within soil NH_4^+ concentrations over July to early August (Table 5.7). This may reflect simultaneous high mineralization rates that occurred at this time. High NH_4^+ consumption rates on September 27 were also coupled with high gross nitrification rates under the potato crop (Table 5.12). Low gross mineralization rates in combination with this high NH_4^+ consumption, as well as plant uptake of NH_4^+ prior to sampling, may explain the low soil NH_4^+ concentrations also found at this time (Table 5.8).

5.5.4. Discussion

Gross N transformation rates within this study were often similar to or greater than other studies also using the pool dilution method within agricultural soils (Shi and Norton 2000; Andersen and Jensen 2001; Hoyle et al. 2006; Sangster 2010). The exception was the potato crop, which often had much greater calculated gross N transformation rates compared with previous studies. However, previous studies have not calculated gross N transformation rates in heavily fertilized annual crops such as potatoes and as a result direct comparison of rates under potato production in the current study with previous studies is difficult. However, interpretations of the N cycle within this system can still be made using the gross N transformation rates within the current study, or through broader comparisons with other isotope pool dilution studies used within agricultural soils, as below.

High NH_4^+ consumption rates in late June and July, and high nitrification rates in the 2011 field season under the potato crop were likely a result of nitrification of the added ammonium fertilizer at potato planting. In heavily fertilized soils, gross nitrification rates have been found to be higher in comparison with the same soils at lower fertilization rates (Watson and Mills 1998). Watson and Mills (1998) demonstrated this within a study conducted in a heavily fertilized grassland site, where the greatest nitrification rates were often observed in the greatest fertilized treatment; which in that study was the treatment receiving 500 kg N ha^{-1} y^{-1} of calcium ammonium nitrate. A similar conclusion was made by Habteselassie et al. (2006) in a study using the isotope pool dilution method on dairy-waste compost and inorganic N fertilizer amended maize rotation, where the greatest nitrification rates were found within the greatest N input treatments of each fertilizer amendment. A relationship was found between nitrification and NH_4^+ consumption rates by Habteselassie et al. (2006) and it was implied that this relationship may show that nitrification is the main consumer of soil mineral NH_4^+. It is difficult to conclude within our study if this was the case. Gross nitrification rates were similar to but always greater than NH_4^+ consumption rates, therefore it is likely that most of the soil NH_4^+ was used for nitrification, in comparison to use by other processes such as immobilization by the microbial population. It is important to note that the timing of sampling within these systems is also essential to the calculation of gross N transformation rates. Application of fertilizers or N amendments may result in localized variation in soil mineral N, and has been shown to greatly influence variation in the gross rates of mineralization depending on the timing of sampling relative to the application of the N input (Habteselassie et al. 2006). Since all isotope pool dilution sampling dates

were taken before or at least two weeks after planting events, it is assumed that the rates calculated will better reflect temporal changes throughout the growing season rather than the immediate effects of fertilization at planting.

Within this study, the rates of gross mineralization, nitrification, NO_3^- and NH_4^+ consumption were often quite relevant to the temporal availability of soil mineral N concentrations. This effect was more prevalent within the potato and barley crops, as soil mineral N concentrations within the red clover crop were not as closely tied to the gross N transformation rates. In a review by Booth et al. (2005), gross mineralization rates were found to be positively correlated with soil mineral N, and gross nitrification rates were found to be predicted by N mineralization rates and soil NH_4^+ concentrations. The greatest nitrification and mineralization rates in 2010 and 2011 were both achieved under the potato crop during the months of June and July, when soil mineral N concentrations were at a maximum over the whole season, and higher than the other crop species. In an incubation study on a silty loam soil amended with either compost or $(NH_4)_2SO_4$ fertilizer, Shi and Norton (2000) similarly concluded that the rate of nitrification was most closely related to the concentration of soil NH_4^+.

Booth et al. (2005) also concluded that MB-C and MB-N, as well as total C and N contents, were good predictors of N mineralization and nitrification rates. In a study conducted by Accoe et al. (2004) on a grassland with a sandy loam soil, a positive correlation was found between soil organic matter C and N contents and rates of gross mineralization, nitrification and immobilization. However, in contrast to these studies, this effect was not apparent within the MB-C concentrations within the current study, as there were no significant differences in MB-C concentrations among crop species or

sampling dates in either year. Within the red clover phase of the rotation, MB-N concentrations were significantly greater than MB-N concentrations in the potato and barley phases by approximately 40-50% in both seasons (Tables 5.3, 5.4).

5.6 Gross Denitrification Estimations

For 2011 only, gross rates of total denitrification (i.e., $N_2 + N_2O$) and of N_2O emissions were estimated using the pool dilution method in conjunction with estimation of the other gross N transformation rates. Due to very low quantities of N_2 emitted in comparison with atmospheric N_2 concentrations, estimation of N_2 emissions using the pool dilution method was imprecise. Consequently, N_2 emissions were included within total denitrification calculations, but were not presented separately.

5.6.1. Total Denitrification Rates

Of the complete set of total denitrification rates, 22 of 60 rates were negative, but only resulted in two mean negative rates. Of the individual negative denitrification rates, 15 of 22 rates were marginally negative (between -5 and 0 $\mu g\ N\ kg^{-1}\ d^{-1}$). Large negative rates (lower than -5 $\mu g\ N\ kg^{-1}\ d^{-1}$) were considered to be greater than inherent variability within the method and caused by violations of the methods' assumptions (Table 5.13). For sake of consistency with the gross N transformation calculations, as well as taking into consideration the highly variable nature of the total denitrification rates, unfiltered data (all viable gross denitrification values included within total mean rates) was presented, however only filtered data (negative values omitted from total mean rates) will be interpreted and statistically analyzed.

Table 5.13. Unfiltered and filtered mean total denitrification rates (i.e., $N_2O + N_2$) from a barley-red clover-potato crop rotation on five sampling dates during the 2011 growing season using the isotope pool dilution method. Rates are as a mean ± 1 SD.

	Mean total denitrification rate ($\mu g\ N\ kg^{-1}\ d^{-1}$)					
	Unfiltered			Filtered		
Date	Potato	Barley	Red clover	Potato	Barley	Red clover
May 3	0.6 ± 1.8 (4)[Z]	1.5 ± 4.1 (4)	5.2 ± 25.1 (4)	1.8 ± 2.0 (2)	4.6 ± 3.2 (2)	20.3 ± 28.1 (2)
June 21	3.0 ± 12.8 (4)	-1.3 ± 8.4 (3)	3.6 ± 6.6 (4)	10.8 ± 14.7 (2)	7.9 (1)	8.9 ± 3.9 (2)
Aug. 2	22.5 ± 23.6 (4)	3.4 ± 3.4 (4)	3.1 ± 3.2 (4)	22.5 ± 23.6 (4)	5.0 ±1.2 (3)	4.4 ± 2.4 (3)
Sept. 27	6.4 ± 10.7 (4)	0.1 ± 2.9 (4)	10.1 ± 10.2 (4)	6.4 ± 10.7 (4)	2.3 ± 2.5 (2)	13.5 ± 9.2 (3)
Oct. 25	-1.2 ± 3.3 (4)	5.7 ± 10.1 (4)	0.4 ± 6.4 (4)	1.0 ± 0.9 (2)	13.2 ± 8.9 (2)	3.4 ± 2.9 (3)
Mean	6.3 ± 14.5 (20)	1.9 ± 6.1	4.5 ± 11.9 (20)	8.5 ± 15.7 (14)	6.6 ± 5.4 (10)	10.1 ± 11.1 (13)
	P-Value			*P*-Value		
Crop [C]	N/A			NS		
Day [D]	N/A			NS		
C X D	N/A			NS		

N/A- Not applicable

NS- not significant ($P \geq 0.05$)

[Z] Values shown in parentheses following rates represent sample number (*n*).

Interpretation of the filtered data was difficult due to low sample sizes within the filtered data set (only 34 of 60 cases were viable), and there was substantial variability within total denitrification rate means (the SD values were often similar to or greater than the mean values of denitrification rate). Total denitrification rates did not vary significantly among crop species or sampling dates (Table 5.13). The highest numeric total denitrification rate was measured in the potato hills on August 2, at the first sampling following hilling; which was approximately 8 times greater than the mean total denitrification rate measured on June 21 following planting (Table 5.13). Generally, total denitrification rates for the potato phase declined numerically from the August 2 sampling to the final sampling date on October 25 (Table 5.13).

Although total denitrification rates did not differ significantly among sampling dates or crop species, temporal fluctuations in the denitrification rates were at times consistent with variations in soil mineral N concentrations. Numerically greater rates of denitrification on August 2 were consistent with high rates of gross NO_3^- consumption and particularly high periods of precipitation at this time in the potato crop (Tables 5.12, 5.13). Similarly, above average denitrification rates for both barley and potato crops on June 21 were consistent with large concentrations of soil NO_3^-, and high gross nitrification and mean NO_3^- consumption rates, following planting of both crops on May 25 and May 27, respectively (Tables 5.12, 5.14), whereas higher than average denitrification rates under red clover on September 27 were consistent with the highest numeric rate of NO_3^- consumption for red clover just prior to the third and final cut on October 13 (Tables 5.11, 5.13).

Although total denitrification rates did not differ significantly among sampling dates or crop species, temporal fluctuations in the denitrification rates were at times consistent with variations in soil mineral N concentrations. Numerically greater rates of denitrification on August 2 were consistent with high rates of gross NO_3^- consumption and particularly high periods of precipitation at this time in the potato crop (Tables 5.12, 5.13). Similarly, above average denitrification rates for both barley and potato crops on June 21 were consistent with large concentrations of soil NO_3^-, and high gross nitrification and mean NO_3^- consumption rates, following planting of both crops on May 25 and May 27, respectively (Tables 5.12, 5.14), whereas higher than average denitrification rates under red clover on September 27 were consistent with the highest numeric rate of NO_3^- consumption for red clover just prior to the third and final cut on October 13 (Tables 5.11, 5.13).

5.6.2. Nitrous Oxide Emissions

For all N_2O emission rates, only 3 of 60 cases used for calculating mean N_2O emissions were negative, and therefore were omitted for conversion to filtered rates. Only filtered rates were presented (Table 5.14). Similar to mean total denitrification rates, variability was very high with the value of the SD often similar to or greater than the mean rate (Table 5.14). Total N_2O emissions (average of 25.6 ng N kg^{-1} dry soil d^{-1}) were on average 330 times lower in comparison with total denitrification rates (average of 8.4 µg N kg^{-1} d^{-1}), and are presented in different units.

There was a significant crop species by sampling date interaction on N_2O emissions, where N_2O emissions on August 2 were greater under red clover (243.9 ng N kg^{-1} dry soil d^{-1}) in comparison with potato (69.3 ng N kg^{-1} dry soil d^{-1}) and barley (1.8 ng

Table 5.14. Filtered mean N_2O emissions from a barley-red clover-potato crop rotation on five sampling dates during the 2011 growing season using the isotope pool dilution method. Rates are presented as a mean ± 1 SD. Note that N_2O emissions are in different units than mean total denitrification rates presented in Table 5.13.

	Filtered mean N_2O emissions ($ng\ N\ kg^{-1}\ d^{-1}$)		
Date	Potato	Barley	Red clover
May 3	0.9 ± 1.5 (4)[Z] bcd[Y]	0.5 ± 0.7 (3) bcd	0.1 ± 0.2 (4) d
June 21	7.1 ± 4.5 (4) abc	9.5 ± 14.5 (4) abcd	5.8 ± 7.5 (4) abcd
Aug. 2	69.3 ± 102.9 (4) ab	1.8 ± 2.7 (4) bcd	243.9 ± 199.7 (4) a
Sept. 27	2.4 ± 2.2 (3) bcd	0.3 ± 0.4 (4) cd	7.4 ± 8.3 (3) abcd
Oct. 25	0.6 ± 0.7 (4) cd	2.3 ± 2.0 (4) bcd	31.2 ± 47.7 (4) abc
Mean	16.1 ± 50.5 (19) AB[X]	2.9 ± 7.0 (19) B	57.7 ± 129.0 (19) A
		P-Value	
Crop [C]		0.012	
Day [D]		<.001	
C X D		0.005	

[Z] Values shown in parentheses following rates represent sample number (*n*).
[Y] Treatment means in the same column followed by the same lowercase letter are not significantly different for the sampling date x crop species interaction.
[X] Treatment means in the same row followed by the same uppercase letter are not significantly different for the main effect of crop.

N kg^{-1} dry soil d^{-1}), but there were no differences among crop species on any other sampling date. The highest N_2O emission rate under the potato crop occurred following maximum soil NO_3^- concentrations and high rates of gross NO_3^- consumption (Table 5.8), as measured on August 2 (Table 5.14). Nitrous oxide emissions averaged across the growing season were greatest under red clover (57.7 ng N kg^{-1} dry soil d^{-1}), followed by potato (16.1 ng N kg^{-1} dry soil d^{-1}) and barley (2.9 ng N kg^{-1} dry soil d^{-1}). Both barley and potato crops followed a similar trend of high and low N_2O emissions as field soil NO_3^- concentrations fluctuated, particularly following barley planting and fertilization (effect seen on June 21), and potato hilling events (effect seen on August 2), with higher than average N_2O emissions.

5.6.3. Discussion

Although there were no significant effects of crop species or sampling date observed within the total denitrification estimates, numerically the timing of maximum denitrification rates did occur when conditions were favourable for denitrification to occur. High rates of denitrification would be expected to have occurred during times of high soil NO_3^- concentrations and large rainfall events, as seen during the month of June under the potato and barley crops when total precipitation was above the long term average, and WFPS was approximately 0.6 m^3 m^{-3}. A similar result was reported by Burton et al. (2008) where denitrification was measured under a potato crop in New Brunswick. Mean N_2O emissions across the growing season under the red clover crop were significantly higher than barley, which was unexpected, and primarily reflected the above average rates of N_2O emissions under red clover observed on August 2. At this time, soil NO_3^- concentrations were quite low (approximately 2.0 mg N kg^{-1} dry soil),

although soil WFPS within this crop was elevated (0.6 m^3 m^{-3}). The possibility of "hotspots" or small pockets of above average nitrification or denitrification rates may be a possible explanation for the great variability found within denitrification rates within the current study. Small, enclosed "hotspots" within plots is not a new concept, and has been discussed in many published studies (Parkin 1987; Parkin 1993; Rover et al. 1999; Habteselassie et al. 2006). Parkin (1987) demonstrated this concept in a no-till corn trial that showed high spatial variability of denitrification can occur within intact soil cores due to active denitrification microsites dispersed unpredictably within the same plot. Parkin (1987) separated 12 intact cores into variable depths to identify if "hotspots" of denitrification were occurring within differing layers of the cores. Four of the 12 cores demonstrated areas of high denitrification activity that were inconsistent with the remainder of the cores, and these "hotspots" of activity were often several orders of magnitude greater than emissions from within other areas of the core. It was concluded that even a small number of "hotspots" within a single plot can induce great variability within mean denitrification rates, and this concept may be applied to many other soil microbial processes. Microsite variability may be an important explanation for the cause of variable total denitrification and N_2O emission rates within the red clover and potato crop phases within the current study.

Unlike total denitrification rates, N_2O emissions showed a significant crop species by sampling date interaction, and demonstrated a stronger response to abiotic soil conditions. It is likely that increased N_2O emissions observed on August 2 under the potato crop may have been a result of preferred production of N_2O from denitrification as soil conditions were favorable at this time for N_2O production, with high soil NO_3^-

concentrations and WFPS below 60%. Typically, the release of N_2O emissions within arable soils has been found to be favored when WFPS is around 60% and when soil O_2 concentrations are low (but still not completely under anaerobic conditions), in comparison with emissions as N_2 (Wrage et al. 2001, Bateman and Baggs 2005). Miller et al. (2008) also concluded that when soil NO_3^- is abundant, the NO_3^- anion has been found to be a more favorable electron acceptor in comparison with N_2O, and N_2O emissions are often produced in substitution to the usual final denitrification product, N_2, in these conditions. In their study on the effect of C and soil NO_3^- availability on gaseous emissions from denitrification, N_2O production was favored at increased soil NO_3^- concentrations (Miller et al. 2008).

Nitrous oxide emissions at this time may also have been from the nitrification process as WFPS did not exceed 60% throughout the growing season. Bateman and Baggs (2005) found that N_2O was the main product of soils within 35-60% WFPS, and was mainly produced by autotrophic nitrification. In a study conducted by Khalil et al. (2004) on an Orthic Luvisol under a maize cropping system, it was also found that at low to average O_2 availability, nitrification was the dominant source of N_2O emissions. It was also concluded within this study that the amount of N_2O derived from nitrification was positively correlated with the amount of N nitrified within the soil, but that the linear relationship was highly dependent on O_2 availability, where the greatest amounts of N_2O were produced with lower concentrations of O_2 in comparison to the soils with abundant O_2.

The influence of C additions has also been shown to increase denitrification, even at low soil moisture levels. In the same study by Miller et al. (2008), increased C addition

increased microbial respiration and depleted O_2 supply, and resulted in increased total denitrification. Numerically higher denitrification rates observed under red clover on September 27, and under barley on October 25, may have been a result of C availability from long-term degradation of the C inputs from residues created from the red clover mow-down on August 17 and barley harvest on August 25, respectively.

5.7 Diffusion Method Test for N Recovery Results

Due to variable results observed within the calculated gross N transformation rates, an in-depth examination into the methodological process of acquiring these results was conducted. Initially, the diffusion method was tested for inconsistency during the diffusion period of the KCl extracts, as well as the efficiency of N capture onto the PTFE traps. To conduct these methodological assays, approximately 100 µg of N was directly pipetted onto paper filters and analyzed for N recovery and mean atom percent enrichment (referred to as direct recovery samples). Direct recovery samples had a mean ($\pm$ 1 SD) N recovery of 91.4 % ($\pm$ 9.02) following addition of approximately 100 µg natural abundance of $(NH_4)_2SO_4$ or KNO_3 solutions (Table 5.15). Mean ^{15}N enrichments of the $(NH_4)_2SO_4$ and the KNO_3 solutions were not statistically different, with a mean ^{15}N enrichment of approximately 0.363 ($\pm$ 0.002) at natural abundance (Table 5.15).

A comparison of the amount of N recovered from the trapping of N on the PTFE packet following the diffusion period, compared with directly pipetting the same amount of N (highly concentrated within a 10 µL volume) onto the filter disk, was conducted to analyze how the recovery and enrichment of ^{15}N was affected during the diffusion process. The recovery of N from the diffusion method was much lower than that of the direct recovery method (Table 5.15). Nitrogen recoveries of natural abundance standards

Table 5.15. Total N recovery and ^{15}N enrichment of filters used for $(NH_4)_2SO_4$ or KNO_3 sample standards for analysis of methodological and instrumental errors. Four chemicals were used for standards: $(NH_4)_2SO_4$ at natural abundance (nat. ab.), KNO_3 at nat. ab., $(NH_4)_2SO_4$ (10 atm. %) and KNO_3 (10 atm. %). Polytetrafluoroethylene packets (PTFE) were sealed using a dram vial or an arbor press (AP).

Sample	Added N (µg)	Known ^{15}N enrichment (atm. %)	PTFE sealing method	Total N recovered (µg)	^{15}N enrichment measured (atm. %)	Sample size (*n*)
			Direct Recovery			
$(NH_4)_2SO_4$	100	Nat. ab.	N/A	93.19 (± 8.2)[Z] a[Y]	0.363 (± 0.001) a	5
KNO_3	100	Nat. ab.	N/A	89.69 (± 10.4) a	0.363 (± 0.003) a	5
			Diffusion Recovery			
$(NH_4)_2SO_4$	100	Nat. ab.	AP	74.61 (± 9.8) a	0.361 (± 0.001) a	5
KNO_3	100	Nat. ab.	AP	66.09 (± 7.7) a	0.362 (± 0.004) a	6
$^{15}(NH_4)_2SO_4$	100	10 atm. %	AP	64.70 (± 7.5) ab	11.80 (± 0.08) a	10
$K^{15}NO_3$	100	10 atm. %	AP	67.83 (± 11.0) ab	9.92 (± 0.24) c	9
$^{15}(NH_4)_2SO_4$	100	10 atm. %	Dram	51.71 (± 13.7) b	11.85 (± 0.07) a	5
$K^{15}NO_3$	100	10 atm. %	Dram	73.74 (± 16.2)a	11.18 (± 0.11) b	5

N/A – Not applicable.
[Z] Values shown in parentheses following rates represent ± 1 SD.
[Y] Treatment means in the same group of rows followed by the same lowercase letter indicates means within the same column are not significantly different.

used for the diffusion method were 74.6% (± 9.8) and 66.1% (± 7.7) for $(NH_4)_2SO_4$ and KNO_3 standard samples, respectively. In comparison with the direct recovery sample enrichments, enrichment values were also slightly decreased numerically for both $(NH_4)_2SO_4$ and KNO_3 natural abundance standard samples for the diffusion method at 0.361 (± 0.001) and 0.362 (± 0.004), respectively. Nitrogen recoveries of enriched standards used for the diffusion method for $^{15}(NH_4)_2SO_4$ (10 atm. %) and $^{15}KNO_3$ (10 atm. %) disks were 64.7 % and 67.9%, respectively (Table 5.15), and were not

statistically different. Enrichments for $^{15}(NH_4)_2SO_4$ (10 atm. %) and $^{15}KNO_3$ (10 atm. %) averaged 11.8% and 9.9%, respectively, suggesting that although the ^{15}N atm. % of the $^{15}(NH_4)_2SO_4$ used was listed as 10 atm. %, the enrichment of this compound may be closer to 12 atm. %.

The influence of sealing methods (arbor press method versus hand-sealed using a dram vial) on enrichment was dependent on the ^{15}N solutions used. For the $^{15}(NH_4)_2SO_4$ samples, ^{15}N enrichment was not significantly different between sealing methods (Table 5.15). However, for the $K^{15}NO_3$ samples, ^{15}N enrichment was significantly greater using the dram vial method (11.2%) when compared with the AP sealing method (9.9%) (Table 5.15). The amount of N recovered using the diffusion method did not differ between sealing methods, regardless of the N solution used. However, when using the dram sealing method, N recovery was greater for $K^{15}NO_3$ samples (73.7%) than for $^{15}(NH_4)_2SO_4$ samples (51.7%). Although both sealing methods, using $^{15}(NH_4)_2SO_4$ (10 atm. %) and $K^{15}NO_3$ (10 atm. %) standards, appeared to have an influence on enrichment values, the AP method gave the most reproducible N recovery results for both standards (Table 5.15). Consequently PTFE packets sealed using the AP method were deemed reliable for the isotope pool dilution method based on this diffusion method test.

Mean recoveries within this study were generally lower in comparison to other analyses conducted on the diffusion method; however high variability within this diffusion method can be observed, given the large ranges of percent N recovered that have been reported (Davidson et al. 1991, Stark and Hart 1996, Stephan and Kavanagh 2009). Stephan and Kavanagh (2009) received N recoveries ranging from <40% to >110% when using natural abundance blank diffusion samples to analyze error associated

within the diffusion process, and approximately 0.2 ‰ and 0.3 ‰ error was found within every 1% loss associated with complete recovery for NH_4^+ and NO_3^- diffusions, respectively. Kelley et al. (1991) found almost 100% N recoveries (ranging from 99.8% to 101.3%) using acidified glass disks that were suspended above 2M KCl solutions over a 6 day diffusion period when capturing $^{15}NH_4$, whereas N recoveries found within the methodological diffusion method study conducted by Stark and Hart (1996) showed a mean N recovery of all diffusions to be approximately 96.5 % $\pm$ 0.8.

Lower N recoveries received from diffused samples in comparison with direct recovery samples may have been produced by a number of causes. Stark and Hart (1996) suggest that some leakage of NH_3 may occur from the seal of the sample containers from pressure buildup from the oxidation process of NH_4-N. Within our study, inconsistent shaking of the mechanical shaker was an important confounding factor within the diffusion method, which may have also led to small amounts of KCl lost from the open-lid containers during the burn off of NH_3 within the $^{15}NO_3$-labelled solutions; however, in these cases an effort to account for the KCl loss due to spillage was conducted. Loss of N from the diffusion method was difficult to trace as there could be N loss during one of the many steps prior to isotope analysis (loss of N during pipetting of known amount of sample into diffusion cup, during the 6-12 days of diffusion, during the packing into Sn capsules), whereas direct recovery of N from PTFE packets had less of an opportunity to be lost as direct recovery samples only underwent one step prior to isotope analysis. Overall, small losses of N during the diffusion process were at times unavoidable. However, given that this experiment was conducted over two growing seasons, most of the methodological issues mentioned above were reduced during the second season.

Given that variable N recoveries were still reported within the diffusions of the second season, this may suggest that other unidentified factors may have caused a role in reducing N recoveries within the diffusion process within this current study. Since ^{15}N enrichment error was generally found to be negligible regardless of N recovery within the diffusion method (Stark and Hart 1996), the error associated with N enrichments and variable recoveries of N within this study was assumed to be minimal.

5.8 Gross N Transformation Rate Error and Variability Analyses

Since the technical application of the diffusion method was consistent among the standards analysis, a further examination into the sources of variability in gross N transformation rates was performed. Three different sources of inherent error, atom percent enrichment error (ENR-ERR), soil core variability error (CV-ERR), and segmented flow analyzer error (SFA-ERR), were considered in the error analysis. The error analysis was performed on nitrification and mineralization rates, using five representative sample calculations for each crop species from the 2010 data set (Figures 5.2, 5.3).

In most cases, variability in measurement of enrichment (ENR-ERR) was not a large contributor to high error rates. However, in cases where enrichments were small (i.e., close to natural abundance) and were not greatly diluted from T_0 to T_{24}, greater ENR-ERR was more likely to occur. The greatest individual values of ENR-ERR were measured under potato production at $\pm$ 5.6 and $\pm$ 5.3 (i.e., $\pm$ 2 SD) for nitrification rates of 7.3 and 9.4 mg kg^{-1} d^{-1}, respectively (Figure 5.3a). Average ENR-ERR was low for all crop species at $\pm$ 0.6 for mineralization rates, and $\pm$ 1.1 for nitrification rates (Table 5.16); however, the error calculated for most example rates was lower than this mean

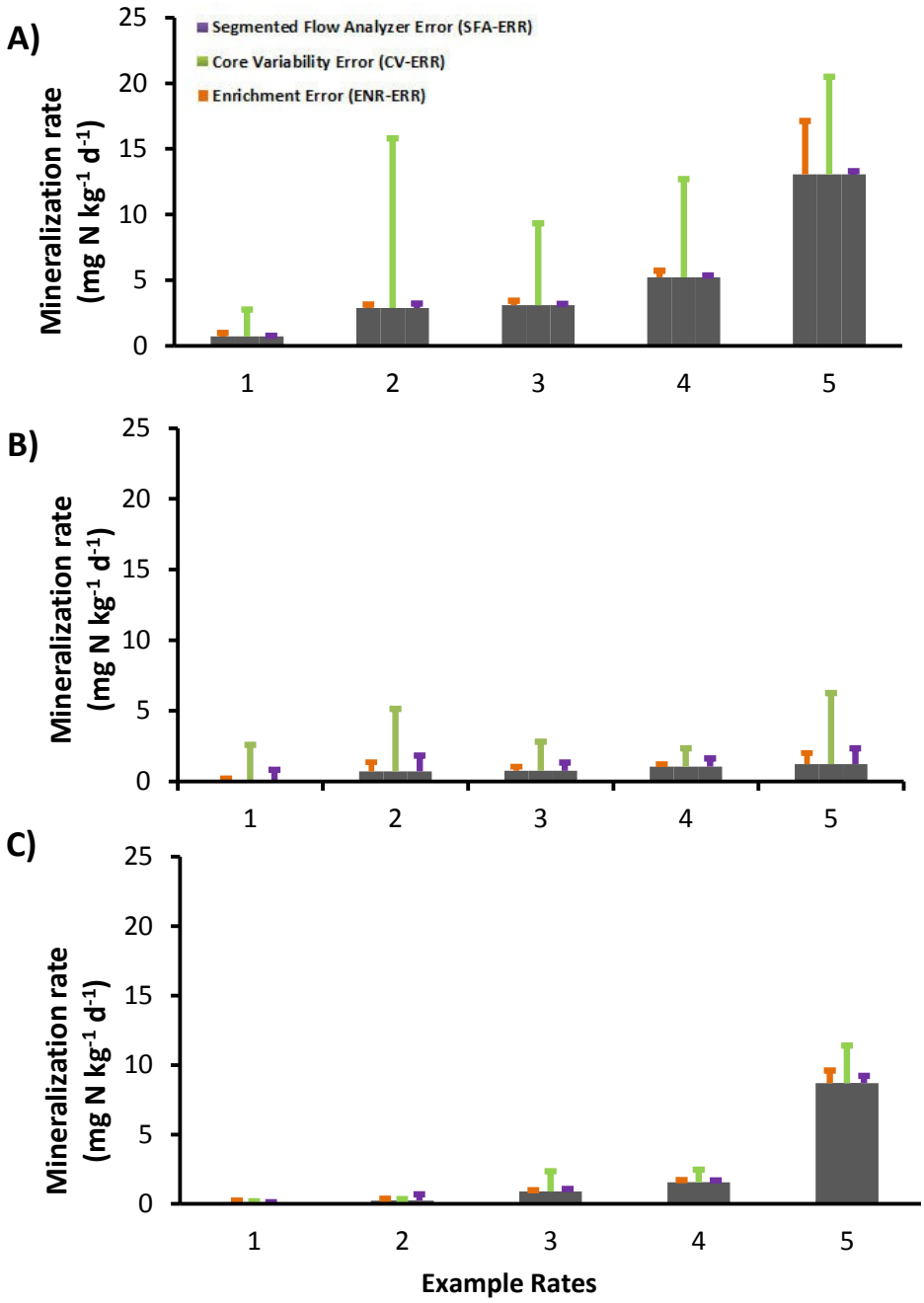

Figure 5.2. Using five representative examples of mineralization rates calculated using the pool dilution method for potato (A), red clover (B) and barley (C) phases of a conventional crop rotation in the 2010 growing season, the error bars indicate the approximate 95% C.I. of error as influenced by three sources of variation: the analysis of isotope enrichment (ENR-ERR), the variation of soil mineral N concentrations between two cores taken from the same crop phase on the same sampling date when used within the pool dilution calculation (CV-ERR), and the analysis of soil mineral N concentrations on a segmented flow-analyzer (SFA-ERR).

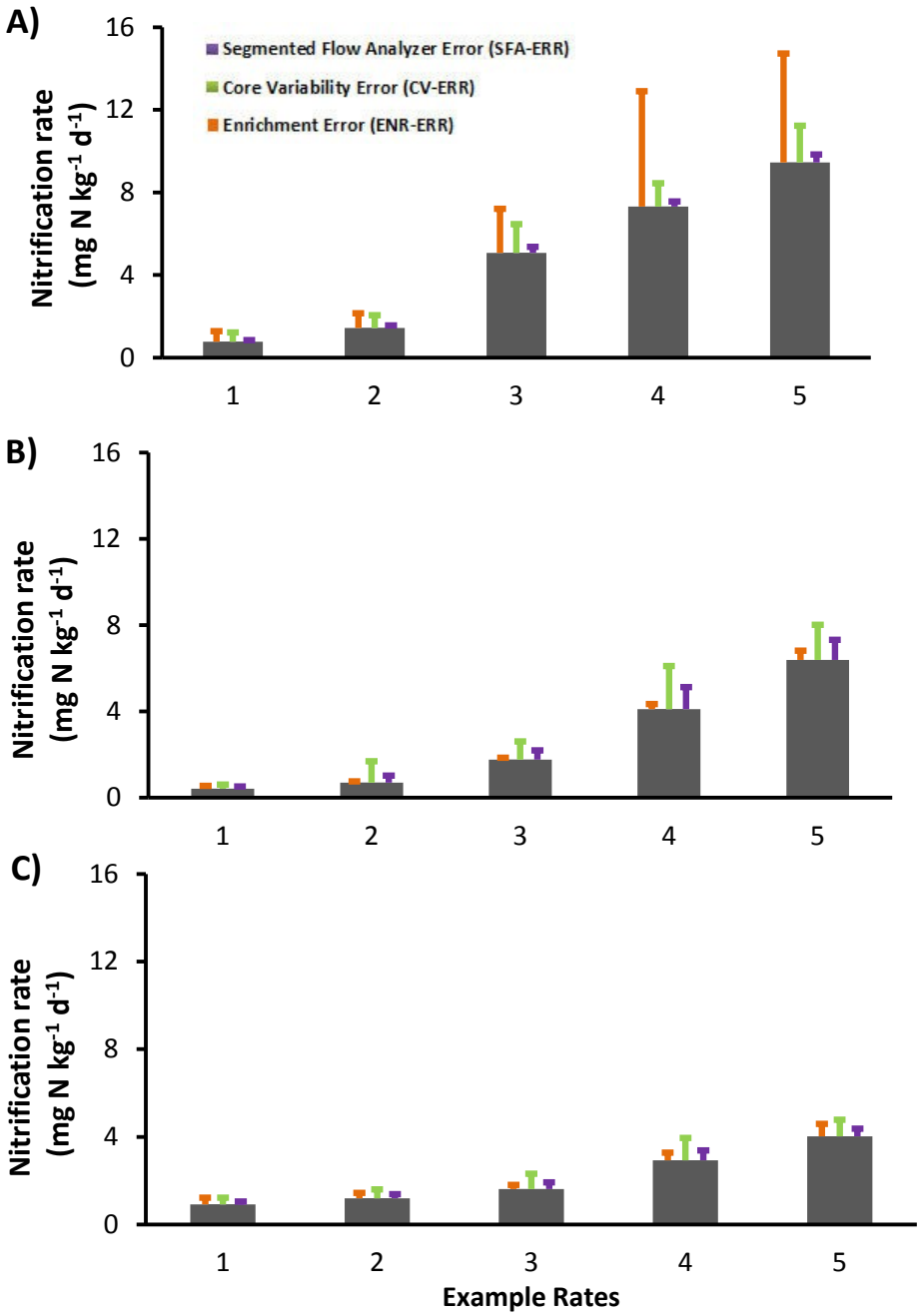

Figure 5.3. Using five representative examples of nitrification rates calculated using the pool dilution method for potato (A), red clover (B) and barley (C) phases of a conventional crop rotation in the 2010 growing season, the error bars indicate the approximate 95% C.I. of error as influenced by three sources of variation: the analysis of isotope enrichment (ENR-ERR), the variation of soil mineral N concentrations between two cores taken from the same crop phase on the same sampling date when used within the pool dilution calculation (CV-ERR), and the analysis of soil mineral N concentrations on a segmented flow-analyzer (SFA-ERR).

value because of a small number of samples with high error values such as for the nitrification rates under potato production as described above. These results suggest that ENR-ERR is commonly lower than CV-ERR (Table 5.16), however ENR-ERR can be great when enrichments were quite low and when there is very little dilution of the ^{15}N pool.

Table 5.16. Mean error for segmented flow analyzer error (SFA-ERR), atom percent enrichment error (ENR-ERR) and soil core variability error (CV-ERR) for mineralization (Min.) and nitrification (Nit.) rates from the 2010 field season error analysis using five examples for each phase of the barley-red clover-potato rotation. Values presented are the mean (± 1 SD) of the errors (i.e., SFA-ERR, ENR-ERR, and CV-ERR) for each crop species.

	SFA-ERR		ENR-ERR		CV-ERR	
Crop	Min.	Nit.	Min.	Nit.	Min.	Nit.
	(mg N kg^{-1} dry soil d^{-1})					
Potato	0.2 (± 0.1)	0.2 (± 0.1)	1.1 (± 1.7)	2.8 (± 2.4)	7.2 (± 3.9)	1.1 (± 0.6)
Barley	0.3 (± 0.2)	0.3 (±0.1)	0.3 (± 0.3)	0.3 (± 0.1)	1.1 (± 1.1)	0.6 (± 0.3)
Red clover	0.8 (± 0.3)	0.6 (± 0.4)	0.4 (± 0.3)	0.2 (± 0.2)	3.1 (± 1.6)	1.1 (± 0.7)

Soil core variability (CV-ERR), which considered variation between cores in initial values of soil mineral N, was found to be the greatest contributor to variability in the mean for this method, and often high rates of CV-ERR were present in all crop phases due to variable NH_4-N and NO_3-N concentrations between adjacent cores (Figures 5.2, 5.3). Core variability was as high as ± 12.9 for a mineralization rate of 2.9 mg kg^{-1} d^{-1} under potato production. Overall, mean values of CV-ERR were greater for mineralization rates than for nitrification rates for all crop species (Table 5.16). The lowest mean values of CV-ERR were for the barley crop at ± 0.6 for nitrification rates and ± 1.1 for mineralization rates. Consistently large individual CV-ERR were present

for each nitrification and mineralization sample rate (Figures 5.2, 5.3). With the exception of high values of ENR-ERR for nitrification rates under potato, CV-ERR represented the primary source of variability within the pool dilution method, and the greatest source of variation for this production system (Figure 5.3a).

Errors in nitrification and mineralization rates caused by the segmented flow analyzer (SFA-ERR) were negligible for most cases (Figures 5.2, 5.3). The greatest error calculated for SFA-ERR was $\pm$ 1.2 for a mineralization rate of 1.2 mg kg^{-1} d^{-1} under red clover. The lowest error calculated for SFA-ERR was $\pm$ 0.0 for a mineralization rate of 0.1 mg kg^{-1} d^{-1} under barley. Overall, SFA-ERR was the smallest source of error examined, and was not considered a significant source of error for the pool dilution method (Figures 5.2, 5.3).

The magnitude of the errors did not vary consistently with the magnitude of the rate of nitrification or mineralization. For example under potato production, a nitrification rate of 5.2 mg kg^{-1} d^{-1} had errors of $\pm$ 0.5, $\pm$ 7.5 and $\pm$ 0.1 for ENR-ERR, CV-ERR, and SFA-ERR, respectively, whereas a nitrification rate of 13.1 mg kg^{-1} d^{-1} had errors of $\pm$ 4.1, $\pm$ 7.4, and $\pm$ 0.2, for ENR-ERR, CV-ERR, and SFA-ERR, respectively. The possible error as indicated by the 95% CI was often similar to, or greater than, the calculated mineralization or nitrification rate. The greatest variability in magnitude of error within all crops was caused by CV-ERR.

When all three errors were compared, SFA-ERR showed the least important effect on either group of nitrogen transformation rates, and CV-ERR showed the greatest effect. Given that CV-ERR has the largest potential to influence error within gross

transformation rates, crops with the highest soil variability due to banded fertilizer application and N management procedures that influence soil N placement within the crops (such as potato), as well as, to a lesser degree, non-banded crops with high overall added N fertilizer inputs (such as barley) would be expected to have the greatest error within gross N transformation rates, as was observed in Section 5.5. For example, Zebarth and Milburn (2003) reported soil mineral NH_4^+ concentrations at different locations within potato hills under conventionally fertilized management (banded N application rate at planting of 180 kg N ha^{-1}) can vary from 2 to 40 mg N kg^{-1}, 1 to 19 mg N kg^{-1}, and 1 to 3 mg N kg^{-1}within the 0-30 cm depths at post-hilling, mid-growing season, and topkill stages, respectively. Soil NO_3^- concentrations were even more variable, with values ranging from 10 to 381 mg N kg^{-1}, 2 to 147 mg N kg^{-1}, and 12 to 59 mg N kg^{-1} within the 0-30 cm depths at post-hilling, mid-growing season, and topkill stages, respectively. Thus, there is a great potential for variation in soil core mineral N concentrations not only due to high fertilization rates but also to the soil variability caused by fertilizer banding. As a result, there is the potential for large errors in calculated gross N transformation rates within a crop such as potato which received banded fertilizer application.

Errors attributed to large variations in soil mineral N concentrations between replicate soil cores have been previously reported as a potentially large source of error (Murphy et al. 2003). Coefficients of variation between replicate soil core samples within a study by Stockdale et al. (1994) (as reviewed by Murphy et al. 2003) showed significant variation between soil mineral N concentrations between a total of 100 soil cores, with a coefficient of variation of 60%. In a study conducted by Habteselassie et al.

(2006), “hot spots” of rapid nitrification and mineralization due to heterogeneous spreading of dairy-waste compost within the soil was identified as the probable cause of variable soil mineral N concentrations within replicate soil cores, giving highly variable and often negative gross nitrification rates. This study concluded that of the nitrification rates presented, the lowest rates were likely to be the most accurate as these would not reflect the rapid nitrification occurring within the undistributed areas of compost. The application of banded fertilizer within the potato hills within our study were analogous with the heterogeneous distribution of dairy-waste compost, and the plots within our study likely also had similar regions of rapid nitrification within the banded fertilized areas within the soil cores. Habteselassie et al. (2006) concluded that other methods of homogenizing the soil mineral N concentrations within this method, such as soil mixing into a single composite sample or sieving, would potentially still create significantly large errors like those produced from the heterogeneity of the soil applied with dairy-waste compost. In a long-term incubation study by Burger and Jackson (2003), sieved samples re-packed into cores that were used for the isotope dilution method were found to give even higher percentages of negative nitrification rates (45%) in comparison to cores that remained intact but were only pre-leached (9%). In another study, Luxhoi and Jensen (2005) used two methods of labeling soil cores with ^{15}N to compare their effect on gross N transformation rates: intact soil cores injected with the isotope label, and soil cores injected with isotope label that were then thoroughly mixed and re-packed into the cores for the incubation period. Gross mineralization rates within this study were not significantly different from either technique; however, gross nitrification rates were two times greater within the mixed soil cores. Luxhoi and Jensen (2005) attributed this

finding to a better dispersion of both nitrifiers and NH_4^+ within the core, which allows for more interaction between both components, and they concluded that rates observed in the mixed soil core are more representative of the potential gross nitrification rather than the actual gross nitrification rates occurring during the incubation period. By mixing and sieving soil to reduce soil homogeneity within replicate cores prior to incubation, it is probable that disturbances to the soil will occur and microbial processes will be altered (Davidson et al. 1991). Although these artificial N conditions may be produced within the test soil, some studies have still found this approach useful and errors associated with soil mixing are reduced due to the effect of compositing soil cores prior to re-packing of soil cores, particularly within long-term incubation studies (Recous et al. 1999; Shi and Norton 2000; Sorensen 2001; Whalen et al. 2001).

Another approach to reducing soil variability within intact replicate cores was employed by Davidson et al. (1991) where the use of small concentric cores inside one another were used in order to receive a representative soil mineral N sample within both cores; however, in our study, this approach would not likely resolve the variability issue since different soil mineral N concentrations were found within centimeters of distance due to the fertilizer banding. To avoid the effect of fertilizer banding, Burger and Jackson (2003) sampled cores specifically outside of the range where the fertilizer banding was applied in a tomato-corn rotation, to avoid soil samples with large sources of inorganic N. Another approach to decrease potential error caused by soil variability within this crop rotation may be to further increase the amount of replicates within the sampling dates, as suggested by Murphy et al. (2003). However, a balance must be made to ensure enough replicates are conducted to reduce soil variability, but still maintain sampling protocols

that are practical and remain within reasonable time and resource constraints. Although avoiding areas of fertilizer banding for soil core sampling as done by Burger and Jackson (2003) may not be completely representative of the root zone of the banded crops, it is still a viable alternative compared with the confounding issue of heterogeneous inorganic N concentrations within soil cores. This approach, as well as implementation of collecting more replicate samples as suggested by Murphy et al. (2003), may together be the best alternatives in avoiding large fluctuations in soil mineral N due to the presence of banded fertilizers as observed in the current study.

5.9 Soil Mineral N Comparison between Field and Core Data

Comparisons between soil probe and soil core sampling dates were conducted to observe the variation between soil mineral N concentrations using the two methods, and to determine if the replicate soil core samples were representative of the variation of soil mineral N within the individual cropped plots. Soil mineral N concentrations from the soil cores showed a similar temporal pattern across the 2010 growing season for the potato crop as for concentrations measured from the soil probe sampling (Figure 5.4). Soil NO_3^- concentrations in cores were approximately 5 times greater compared with the soil probe sample concentrations within late June and early July, which was a result of potato planting occurring between soil probe sampling on June 20, and core sampling which occurred on July 7 (Figure 5.4a). Under barley, soil mineral N concentrations in soil cores also showed similar seasonal fluctuations in comparison with the soil mineral N probe data (Figure 5.4b), with exception of the (May 18) sampling date when cores were collected prior to planting and fertilizer application, whereas the soil probe samples were collected after planting and fertilizer application on May 25. More than 50% of

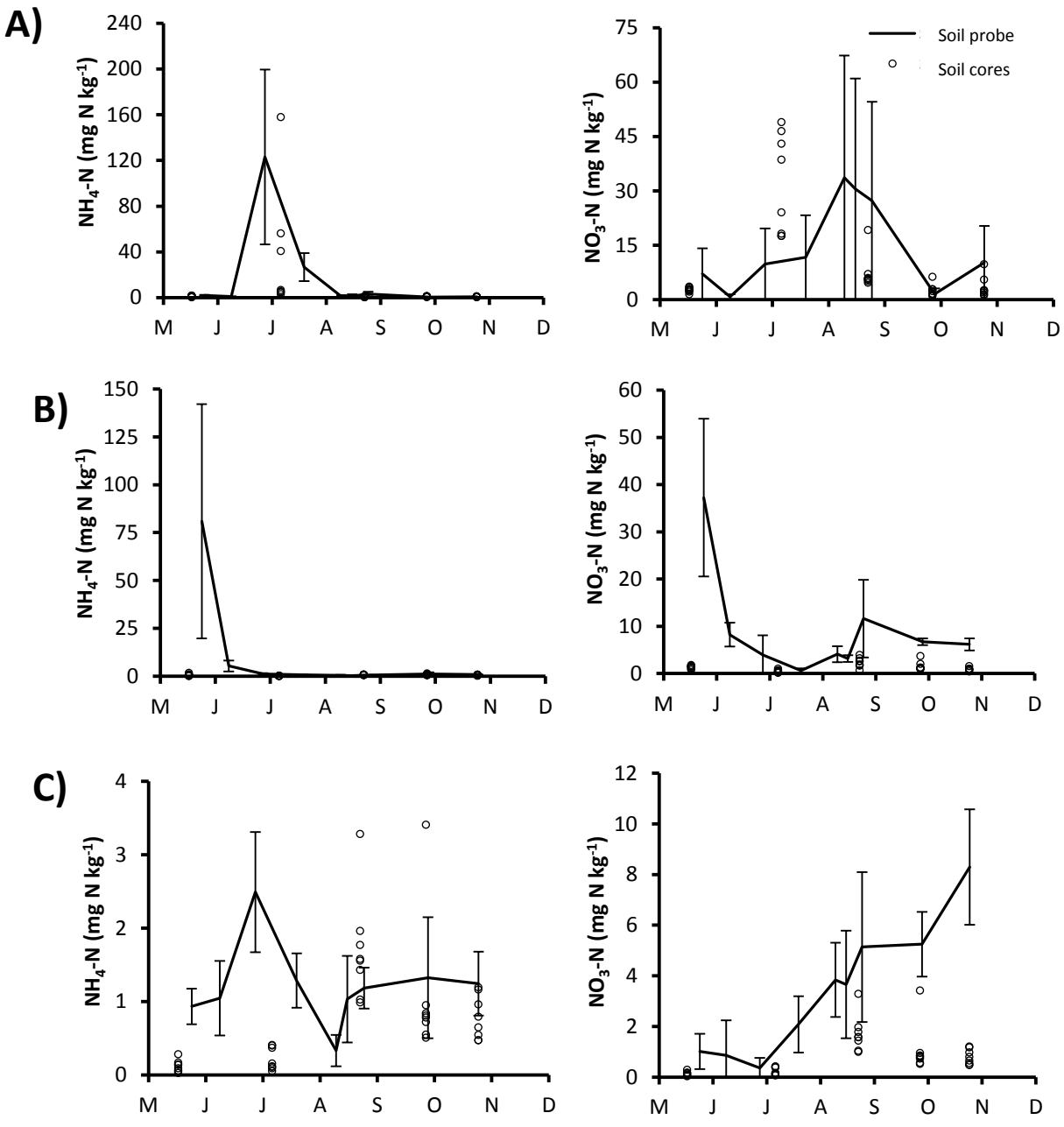

Figure 5.4. Soil NH_4^+ and NO_3^- concentrations under potato (A), barley (B) and red clover (C) crops during the 2010 growing season at the 0-15 cm depth as measured in composite samples collected using soil probes, in comparison with soil NH_4^+ and NO_3^- concentrations of individual soil cores (0-15 cm depth for potato, 0-10 cm for barley and red clover) used for the isotope pool dilution method. Error bars signify $\pm$ 1 SD. Note that the range of values on the Y axis varies among graphs.

barley soil core samples, on each date, were within ± 1 SD of soil probe samples in 3 of a total of 10 cases (5 sampling dates for both NH_4-N and NO_3-N concentrations) within 2010. Under red clover, both sampling methods showed no difference greater than 2.5 mg N kg^{-1} and 6 mg N kg^{-1} soil throughout the whole growing season for soil NH_4^+ and NO_3^- concentrations, respectively, however, this was often enough variation to be greater than ± 1 SD difference from the probe sampling soil concentrations (Figure 5.4c). Under red clover in 2010, only 3 of 10 cases had approximately 50% of soil core samples within ± 1 SD of the corresponding sampling date's soil probe mineral N concentrations (Figures 5.4, 5.5).

In 2011, comparisons between the soil core mineral N concentrations and the soil probe sample mineral N concentrations for the potato crop were generally all within ± 1 SD of the soil probe samples, with the exception of soil NO_3^- concentrations on September 28 (Figure 5.5a). The greatest variability in soil core NH_4-N and NO_3-N concentrations occurred on July 7 following planting for both sampling methods. However on July 7, all soil core mineral N concentrations were still within ± 1 SD of the soil probe concentrations (Figure 5.5a). In only 2 of 10 cases under barley were soil core mineral N concentrations within ± 1 SD of soil probe concentrations. Both sampling methods were the most variable on June 22, the first sampling event following barley planting (Figure 5.5b). For both sampling methods, soil N concentrations under red clover showed the same seasonal temporal patterns, although core soil NH_4^+ and NO_3^- concentrations were generally lower than soil sample probe data (Figure 5.5c). Soil core mineral N concentrations were only within ± 1 SD of the soil probe sampling

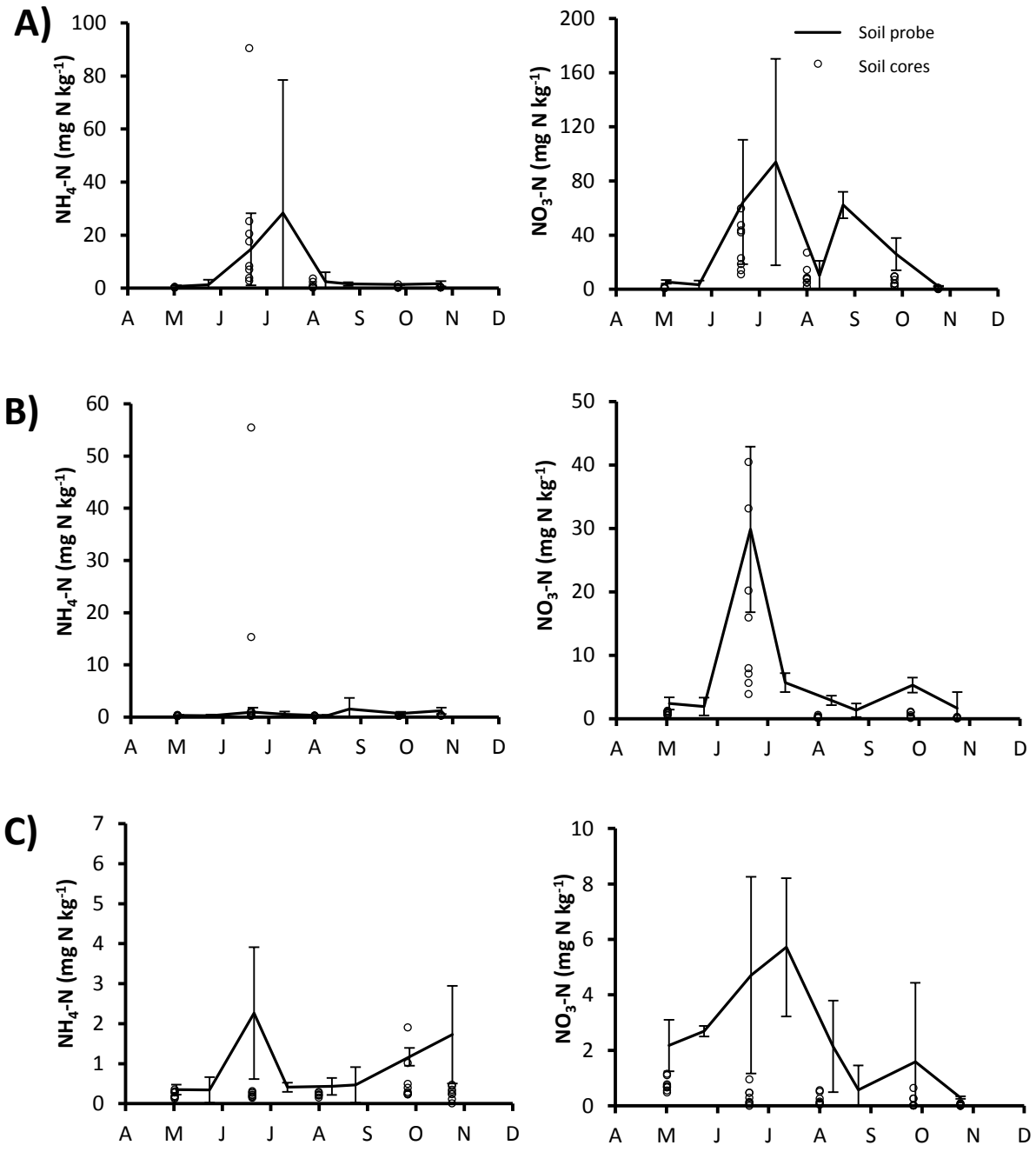

Figure 5.5. Soil NH_4^+ and NO_3^- concentrations under potato (A), barley (B) and red clover (C) crops during the 2011 growing season at the 0-15 cm depth as measured in composite samples collected using soil probes, in comparison with soil NH_4^+ and NO_3^- concentrations of individual soil cores (0-15 cm depth for potato, 0-10 cm for barley and red clover) used for the isotope pool dilution method. Error bars signify $\pm$ 1 SD. Note that the range of values on the Y axis varies among graphs.

concentrations in 3 of 10 cases, with the greatest variation observed on June 22 for both soil NO_3^- and NH_4^+ concentrations (Figure 5.5c).

Generally, soil core mineral N concentrations were often beyond $\pm$ 1 SD that of soil probe mineral N concentrations in all crop species, with exception of the potato crop in the 2011 growing season as the soil mineral N concentrations from the soil probe within the potato phase were also highly variable. Although both sampling methods were not always sampled on the same day, samples were often collected within a few days of each other, or at most one week apart. Since soil probe samples are a composite of five samples from within the whole plot, soil probe samples were considered to be the "standard" soil mineral N concentration. Soil core mineral N concentrations that were extremely variable and not representative of the soil probe mineral N concentrations might be expected following the analysis of soil core variation in the error analysis in Section 5.7. In a study by Rover and Kaiser (1999), spatial variability of soil properties was measured within arable soils at 0-15 cm depth. Soils within a 21 m x 20 m plot under barley, which were under the exact same N management and cultivation practices, averaged a 55% coefficient of variation in NO_3^- concentrations within 7 m intervals of sampling. However, it was concluded that soil NO_3^- concentrations near the end of the growing season under all fertilized crops were more homogeneous, as applied inorganic N was taken up by the crop, or transported following two months post-application (Rover and Kaiser 1999).This finding was not consistent with the variation between soil mineral N concentrations within either growing season of our study, as similarities between both methods did not improve significantly by the end of the growing season. In a different study examining N_2O emissions on the same trial, Rover et al. (1999) found that N_2O

emissions within the same plot dimensions of the barley crop, and a similar sugar beet crop, had coefficients of variation of 199 % and 237%, respectively. This high variation was attributed to the presence of numerous "hotspots" that emitted high amounts of N_2O periodically. Rover et al. (1999) concluded that the use of individual cores were not representative of the total emissions from the plot and that within these crops, a consolidated sample of N_2O emissions greater than 1 m^2 should be made to produce a representative rate. This issue of localized "hotspots" of nitrification activity is very likely to have occurred within the potato and barley crops of the current study, and may have been an important factor in largely variable soil mineral N concentrations, regardless of sampling method.

Soil core samples taken in close proximity to one another may not ensure reproducible soil mineral N concentrations (Habteselassie et al. 2006), especially within crops such as potato which receive banded fertilizer application (Zebarth and Milburn 2003), as described within the error analysis and within comparisons between both sampling methods of soil mineral N concentrations. Since soil probe mineral N concentrations were combined into one single composite sample per depth and plot, the influence of disproportionately distributed soil microsites within the plots would be reduced and equalized within the sample, an advantage not present within distinct soil cores. The use of distinct intact replicate cores for analyzing gross N transformation rates therefore may not always be appropriate for all cropping systems. It is suggested that prior to the establishment of any soil core sampling experiment, variations between replicate cores, and variations between replicate cores and soil probe mineral N

concentrations, should be examined to ensure that all sample concentrations are comparable, and soil sampling protocols can then be modified if necessary.

The feasibility of the isotope pool dilution method for the study of N transformation rates in a potato cropping system may be questioned given the previous discussion of errors associated with the method, and the high occurrence of soil variability within soil systems. Although studies have shown the relative usefulness of this method within wetlands (Bedard-Haughn et al. 2006), grasslands (Corre et al. 2002), forests (Pedersen et al. 1999), and agricultural soils (Shi and Norton 2000); a number of other studies have reported fundamental issues within the method that may cause disparaging results as encountered within the current study (e.g., Verchot et al. 2002; Burger and Jackson 2003; Accoe et al. 2004; Habteselassie et al. 2006; Griffin 2007). Typically, this method has proven useful in soils of low N content where mineral N pool turnover times are slow (Hart et al. 1994), and in incubation studies less than 1 week (when re-mineralization is not considered a significant problem; Bjarnason 1988), and in longer incubation studies using pre-mixed soil composite samples (Andersen and Jensen 2001). However, within studies of varying soil N concentrations, or long term incubations with added amendments, numerous issues may arise. As previously mentioned, the additions of animal manures and fertilizer amendments (Sorensen 2001; Burger and Jackson 2003; Habteselassie et al. 2006; Griffin 2007) have given variable gross mineralization and nitrification rates, often due to highly variable soil mineral N concentrations, which in turn give highly variable or negative gross immobilization rates.

Techniques to improve the isotope pool dilution method within these systems include mixing the numerous soil subsamples into a single composite (Muller et al.

2011), sieving samples (Sorensen 2001) and pre-leaching samples prior to incubation experiments (Burger and Jackson 2003). However, the negative effects of some of these alternative techniques have already been discussed. Recently, the FLUAZ numerical model of Mary et al. (1998) has been widely used within isotope pool dilution studies to better estimate gross N transformation rates (Recous et al. 1999; Andersen and Jensen 2001; Sorensen 2001; Luxhoi et al. 2004; Hoyle and Murphy 2006; Cheng et al. 2012). This model allows gross N transformation rates to be considered under either zero-order or first order kinetics depending on the incubation period, as well as the prediction of eight potential N fluxes occurring within the incubation: mineralization, nitrification, immobilization of NO_3^- and NH_4^+, denitrification, volatilization, humification and remineralization rates, which are dependent on the changes over time of the ^{14}N and ^{15}N pools (Accoe et al. 2005). Values are fitted to a non-linear fitting program (Mary et al. 1998), and variability within the data can be assessed by reducing the weight of specific parameters found with high coefficients of variation that may affect the calculation of rates (Andersen and Jensen 2001; Sorensen 2001). Gross immobilization rates calculated using the analytical method are more prone to errors associated with the gross mineralization and nitrification rates, whereas calculation of immobilization rates within the FLUAZ model are calculated separately from these rates and are not further biased due to these errors (Andersen and Jensen 2001). Generally, rates fitted within this model have been considered more reliable in comparison to analytically derived rates, and the use of this model has been clearly very useful within isotope pool dilution studies. Use of this model may have been beneficial to the current study; however, not enough data was

collected to fit the basic requirements of the model, including the isotopic signature of either microbial biomass or organic matter (Mary et al. 1998).

Chapter 6.0 Conclusions and Recommendations

The overall objective of this research was to examine how soil N processes influence soil NO_3^- availability within the root zone throughout the growing season for a conventional potato crop rotation. Gross rates of gross mineralization, nitrification, NH_4^+ and NO_3^- consumption and denitrification were calculated five times over two growing seasons in all phases of a conventionally managed barley-red clover-potato crop rotation. Temporal variation of soil NO_3^- and NH_4^+ concentrations were similarly studied throughout the growing season under both CON and RN management systems. Soil mineral N concentrations from the CON management system were compared with the gross N transformation rates to obtain a better understanding of soil mineral N processes within the root zone of this rotation.

The soil mineral N concentrations following fertilizer N application at planting for both the barley and potato crop species were not significantly different between N managements (CON and RN), with the exception of soil NH_4^+ concentrations in the 2010 growing season. The overall mean effect of the N fertility managements was not significantly different within both growing seasons. This may have been caused by the highly variable nature of soil mineral N concentrations observed throughout the season, and loss of excess N to gaseous emissions, leaching and plant N uptake. The lack of significant differences observed in mean concentrations across the growing season may also reflect inopportune and infrequent timing of sampling that was administered when significant effects of fertility were not as prevalent. Soil mineral N concentrations among crop species were, however, significantly greater under the potato crop, followed by barley and red clover within both growing seasons, which was likely due to the effect of

high fertilizer inputs added at the beginning of the early growing season to both potato and barley crops.

Gross nitrification rates were generally consistent with the seasonal temporality observed within the soil mineral N concentrations for the barley and potato crops. High nitrification and NH_4^+ consumption rates were generally found on the sampling dates post fertilization and planting of both potato and barley crops, with the greatest rates often occurring primarily under the potato crop, followed by barley and red clover. This was likely caused by the addition of high amounts of inorganic NH_4^+ to the potato crop during planting that was cycled throughout the season before declining to concentrations similar to red clover at the end of the growing season. Gross N transformation rates were generally not significantly different among sampling dates or crop species within the 2010 growing season, however, significantly different gross N rates were measured during 2011. Total denitrification rates were in some cases consistent with high soil NO_3^- concentrations and gross NO_3^- consumption rates, however rates were not significantly different among sampling dates or crop species. Nitrous oxide emissions were also related to soil NO_3^- concentrations and were, on average, significantly greater under red clover. Due to low WFPS values, high soil NO_3^- concentrations and high O_2 availability within the soils, it was concluded that a large proportion of N_2O emissions were a product of nitrification rather than denitrification.

Within the isotope pool dilution method, greatest potential error in gross nitrification and mineralization rate calculations were caused by large variation between soil mineral N concentrations of replicate cores. Error caused by variability within known ^{15}N enrichment values were generally negligible, however low enrichment values that

were close to natural abundance, or enrichments that were not greatly diluted from T_{24} to T_0 samples, were shown to induce potentially large errors within the gross rates. Since soil core variability was considered to be the greatest cause of variable error within gross N transformation rates, it was concluded that heavily fertilized crops with high soil mineral N variation due to banding or non-uniform application of N inputs and amendments, such as the potato and barley crops within the current study, will be prone to higher variability and errors within this method. Therefore, the use of this method within crop experiments that apply banded fertilizers is not encouraged for future studies unless alterations to the methodology are performed.

Achieving soil mineral N homogeneity within replicate cores was often unattainable and led to highly variable and negative gross N transformation rates. Elimination of negative N transformation rates was performed as they were considered to have not met some of the fundamental assumptions of the method, and filtered data (i.e., data without negative rates and replicate cores that were not isotopically diluted over the 24 hour incubation period), were slightly less variable than unfiltered data (all viable calculated data). Modeling programs like the FLUAZ numerical model, although not used within this study, were proposed to be valuable in producing more reliable and representative rates for this method by avoiding error biases that may be prevalent in analytical calculations. Future work using the isotope pool dilution method may benefit from adjusting the experimental methodology at the beginning of the study in order to fit the requirements of the model. Sampling outside of banded rows within heavily fertilized crop rotations may also give more appropriate results for this method if intact cores are desired in future isotope dilution method studies. However, due to localized areas of high

soil microbial activity (i.e., “hotspots” of nitrification and mineralization) that are prominent within many arable soils, strategic sampling of cores may not always guarantee comparable replicate soil mineral N concentrations between cores. Amalgamating numerous soil cores sampled from within the same plot and mixing to produce a representative composite sample is also suggested in future isotope pool dilution studies. However, as mentioned above, this approach also has limitations as the mixing of soil can induce microbial activity and result in overestimations of gross nitrification and consumption rates. Overall, the best approach to reducing soil variability and overall variability within the isotope pool dilution method may be dependent on the specific soil system within the experiment, and it is highly recommended that soil mineral N concentration inconsistencies are assessed prior to the execution of the experiment to ensure that the best technique is selected. Within the current study, the best approach may have been to homogenize the soil cores prior to isotope labeling and to avoid sampling of the banded fertilizer rows to ensure compatible soil mineral N concentrations were observed and to reduce the potential of producing negative rates. However, given that these techniques are not always proven effective, this method should be used with caution within similar crop rotations and fluctuating soil conditions.

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APPENDIX A: Crop Management and Yield

Table A.1. Chemical application and tillage dates for field season 2010. All potato plots (conventional and reduced N input plots) were similarly sprayed on all spray dates.

Date	Activity
June 10	Admire 240 F® application[1]
June 29	Potato plot cultivation
July 3	Sodium 300® application[2]
July 5	Potato plot pre-hilling tillage
July 12	Bravo® application[3]
July 19	Bravo® application
July 26	Bravo® application
August 3	Bravo® application
August 10	Bravo® application
August 18	Bravo® application
August 26	Bravo® application
September 7	Bravo® application
September 13	Bravo® application
October 15	Reglone® application[4]

[1]The potato insecticide, Admire 240 F® (i.e. Imadocloprid) was applied concurrently with potato planting at a rate of 800 ml ha^{-1}.

[2]Sodium 300® (application rate of 300 g L^{-1} was applied once to barley and potato crops respectively during growing season.

[3]The fungicide, Bravo 500® (i.e. Chlorothalonil) was applied approximately weekly throughout both seasons to the potato crops at a rate of 1.2 L ha^{-1}.

[4]Reglone 240® (Diquat) was applied as a potato topkill at the end of the growing period for potatoes in both seasons, at a rate of 2.5 L ha^{-1}.

Table A.2. Chemical application and tillage dates for field season 2011. All potato plots (conventional and reduced N input plots) were similarly sprayed on all spray dates.

Date	Activity
May 24	Potato and barley plots tillage
May 25	Admire 240 F® application[1]
May 27	Barley plot tillage (fertilizer worked in)
June 14	Sencor® application[2]
	Bravo® application[3]
July 7	Bravo® application
July 14	Bravo® application
July 20	Bravo® application
July 25	Bravo® application
July 30	Bravo® application
August 4	Bravo® application
August 11	Bravo® application
August 16	Bravo® application
August 23	Bravo® application
August 31	Bravo® application
September 12	Bravo® application
September 21	Bravo® application
September 29	Bravo® application
October 11	Bravo® application

[1]The potato insecticide, Admire 240 F® (i.e. Imadocloprid) was applied concurrently with potato planting at a rate of 800 ml ha^{-1}.

[2] Sencor® (application rate of 400 g L^{-1}) was applied once to barley and potato crops respectively, as a substitute to Sodium 300® to control weeds during the growing season.

[3]The fungicide, Bravo 500® (i.e. Chlorothalonil) was applied approximately weekly throughout both seasons to the potato crops at a rate of 1.2 L ha^{-1}.

Table A.3. Mean barley above-ground biomass, grain and straw dry weight yield and N contents for the 2010 and 2011 field seasons at plant ripening and harvest growth stages, under conventional (CON) and reduced N (RN) management treatments.

	Biomass Dry Wt.		Grain Yield		Straw Dry Wt.		Biomass N Content		Grain N Content		Straw N Content	
	($t\ ha^{-1}$)						($kg\ N\ ha^{-1}$)					
Date	CON	RN	CON	RN	CON	RN	CON	RN	CON	RN	CON	RN
						2010						
Aug. 9	7.00	5.80	N/A	N/A	N/A	N/A	109	90	N/A	N/	N/A	N/A
Aug. 28	4.08	3.86	3.39	3.20	0.69	0.6	88	75	79	68	9	7
						2011						
Aug. 17	2.11	1.73	N/A	N/A	N/A	N/	29	23	N/A	N/	N/A	N/A
Aug. 25	N/A	N/A	2.94	2.35	N/A	N/	N/A	N/A	55	42	N/A	N/A

N/A- not applicable

Table A.4. Total tuber yield, tuber N content, plant biomass and plant biomass N content for the 2010 and 2011 field seasons at mid-season during tuber bulking stage and at tuber harvest, under conventional (CON) and reduced N (RN) management treatments.

	Total tuber yield		Total plant biomass		Tuber N content		Plant biomass N content	
	(t ha^{-1} fwt.)		(t ha^{-1} dwt.)		(kg N ha^{-1})			
Date	CON	RN	CON	RN	CON	RN	CON	RN
				2010				
Aug. 9	12.48	12.85	3.08	3.10	N/A	N/A	N/A	N/A
Sept. 28	39.97	40.70	0.65	0.79	596	623	73	69
				2011				
Aug. 21	29.98	28.08	2.22	2.70	390	326	77	92
Sept. 21	41.62	40.54	3.27	2.56	1003	932	54	41
Oct. 25	45.52	44.34	N/A	N/A	N/A	N/A	N/A	N/A

N/A- not applicable

Table A.5. Red clover above-ground biomass and total N contents from three harvest dates for the conventional (CON) and reduced N (RN) management plots from both the 2010 and 2011 field seasons.

	Biomass Dry Wt.		Biomass N Content	
	(t ha^{-1})		(kg N ha^{-1})	
Date	CON	RN	CON	RN
		2010		
June 8	2.44	3.18	71	88
Aug. 9	4.90	4.41	116	110
Sept. 28	0.48	0.48	16	16
Total	7.82	8.08	221	232
		2011		
June 22	3.04	3.12	93	77
Aug. 17	DNR	DNR	DNR	DNR
Oct. 13	1.16	1.13	39	33
Total	N/A	N/A	N/A	N/A

DNR- Data not recorded
N/A- Not applicable