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Plant Isotopes as Indicators of N Cycling Processes in Agricultural Fields

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By

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Abstract

When nitrogen (N) availability exceeds biological demand, excess N, especially nitrate (NO_3^-), may subsequently pollute ground and surface water. Agricultural practices in Southern Ontario typically supplement soils with organic and inorganic nutrients to aid in crop development, and employ various management techniques to limit nutrient loss. Excess N has several potential fates, which are controlled by the net effects of numerous N cycling reactions in the soil that are often difficult to measure directly. N cycling in soils is controlled in large part by soil moisture, as it affects microbial activity and soil redox conditions. Stable isotope geochemistry is a powerful tool that provides information on N sources and processes. This study uses crop N and carbon (C) isotope ratios to provide insights into the net effects of soil N cycling and N fate. This research was conducted at the Strawberry Creek Watershed (SCW), an agricultural research watershed located between Kitchener-Waterloo and Guelph, Ontario. A subsequent lab-based grow experiment was conducted at Wilfrid Laurier University in Waterloo, Ontario.

The SCW exhibits elevated NO_3^- concentrations in groundwater, tile discharge, and the stream itself. Previous isotopic work revealed that this NO_3^- is largely derived from chemical fertilizer and manure applications. Field-scale hydrological processes lead to areas where the fate of applied N differs, which has an isotopic effect on the residual N that is available to plants. Results of this study indicate significant patterns in the isotopic signature of plant tissue, in both temporal and spatial scales. At the plot-scale where soil conditions are similar, there is little to no variation in foliar isotope values, but at the field-scale there appears to be a large amount of variability related to soil moisture and N loss. However, that variability is reduced when soil moisture values are grouped. There exists a significant difference between crop productivity and soil moisture, as the medium moisture range (%VWC 30-40) produced corn yields almost 10% greater than wet (%VWC >40) and dry (%VWC <30) soil moisture areas. This relationship can potentially provide insight into ideal conditions for N uptake availability.

The simulated crop growing experiment provided a simplified and controlled system for investigating the relationship between soil and plants. As the soil from all the barrels came from the same field location, and all N inputs were quantifiable, the differences in foliar $\delta^{15}N$ provided meaningful insight into N-cycling. The combination of enriched foliar $\delta^{15}N$ and soil N_2O production in the relatively wet barrels provides reasonable conclusions about the value of using plant isotopes in the investigation of soil processes. This investigation is critical in furthering the efficiency of N application on crops; as well as, the subsequent decrease in the ecological impacts of farming. Reducing agricultural N leaching to ground and surface water requires a better understanding of N fate in the soil zone, and will result in more effective agricultural nutrient management.

Acknowledgements

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1.0 Introduction

Nitrogen (N) is an essential nutrient for plant growth. Food production, especially intensive agricultural crop activity, typically requires supplementary N application, in either organic or inorganic form. However, when N availability exceeds biological demand, excess N, especially nitrate (NO_3^-), may subsequently pollute ground and surface water. Nitrous oxide (N_2O) production also contributes to atmospheric N_2O, which is 300 times more effective as a greenhouse gas than carbon dioxide (CO_2) (Agriculture and Agri-Food Canada 2008). Conversely, a lack of N will limit plant growth, which does not maximize the economic potential of the crop. Therefore, the management of N in agriculture is important in ensuring high crop yields while simultaneously limiting the detrimental impacts of excess N to the environment. Proper N management requires a detailed understanding of the linkages between crop nutrients and N availability in the soil.

Stable isotope analysis can be utilized to gain a more detailed understanding of agricultural N cycling. Stable isotopes, specifically ^{15}N, can provide unique insights into sources and processes controlling concentrations of various forms of N in the environment (Nadelhoffer and Fry 1994; Ostrom et al. 1998). These insights may be gained because the isotopic composition of plant N ($\delta^{15}N$) reflects the isotopic composition of N taken up by the plant, primarily NO_3^- and ammonium (NH_4^+). Soil $\delta^{15}N$ is controlled by the net effects of soil cycling processes, including mineralization, nitrification, denitrification, and N-fixation (Robinson 2001). Therefore, in a given field, areas with different soil N cycling conditions should result in different $\delta^{15}N$ values for plant-available N, and therefore different $\delta^{15}N$ values. It could then be hypothesized that crop $\delta^{15}N$ values may be reflective of changing soil N cycling conditions (soil N status). Soil N cycling conditions are controlled in large part by soil moisture, as it affects microbial activity and soil redox conditions (Nadelhoffer and Fry 1994). Therefore, soil moisture plays a major role in soil N

cycling conditions, which in turn indicates areas of the field prone to specific N fates (e.g. denitrification, N leaching, and N uptake). N management solutions can be based on these indicators. These management solutions could potentially include variable-rate fertilizer application, which would reduce the volume of applied nutrients to areas of the field more susceptible to N loss. As well, the exploratory practice of altering tillage activity to promote N retention in the upper horizons of the soil most accessible to plants would be enhanced by further insight into the uptake tendencies of crops.

1.1 Nitrogen Cycle

N cycling in agricultural systems contains unique inputs dependent on the type of farming and the corresponding management strategies employed by that farm (Figure 1-1). Nutrient management practices employed by the farmer involve the introduction of inorganic or organic nutrients, as well as the management of crop residue and tillage methods. Crop type can also dictate nutrient management strategies. The complexity of multiple N inputs and land management options ultimately increases the difficulty in tracing N cycling in agricultural systems (Tate 2000). N fate is typically dictated by a combination of soil conditions (moisture and temperature) and the changes of those conditions through the various land management practices (Addiscott 2005). Organic and inorganic fertilization of agricultural fields may potentially result in nutrient leaching and the subsequent contamination of ground and surface water; perhaps through over-application or application at inappropriate times in the year, when conditions are too wet to effectively utilize the nutrients.

Agricultural soils are primarily supplemented with N in three distinct forms. The decomposition of plant organic matter, which is often cultivated into the soil, introduces organic N and increases mineralization rates. The second method of N addition is the application of inorganic, or chemical, fertilizers. These fertilizers are typically an inorganic N combination of

both NO_3^- and NH_4^+, which is readily available and utilized by the crop (Choi et al. 2003). These fertilizers are also applied in two different forms, at various points in the plant growing season. Granular fertilizer, which is spread evenly across corn rows at approximately six weeks, is the third method of supplying additional N to the plants. Various crops require varying degrees of inorganic fertilization dependent on soil available N and the N requirements of the crop (Addiscott 2005). For example, popular inorganic fertilization for corn involves the application of starter liquid fertilizer directly to the seed when planted, and the addition of similar liquid fertilizer at between six and nine weeks, which is knifed into the ground near the base of the corn plant. Finally, organic fertilizers, such as livestock and poultry manure, can be applied to fields to enhance soil N levels. This type of N application is popular, as organic fertilizer is relatively inexpensive, and most agricultural practices involve livestock or poultry production with the fields serving as a facilitator of waste management.

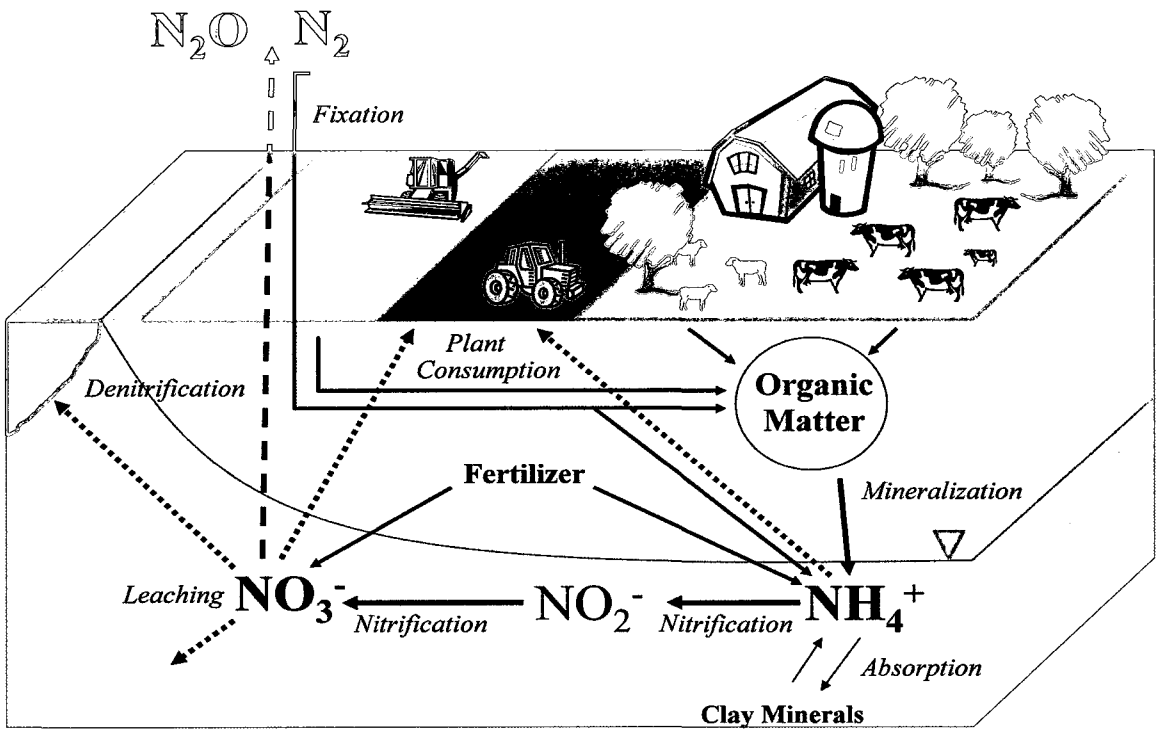

Figure 1-1: Simplified model of fates and processes associated with N cycling in an agricultural system. Typical inputs include organic matter from livestock and poultry manure and crop residue, inorganic fertilizer application, N fixation, and atmospheric deposition. Significant outputs include leaching to ground and surface water, denitrification to the atmosphere, and plant uptake. The dominate processes are driven by a variety of soil condition factors.

1.1.1 Mineralization

Mineralization is a key process that determines the amount of available N for field crops, and therefore affects the amount of fertilizer required by the farmer. In the most simplistic terms, N mineralization involves the process of converting organic N to inorganic N (Tate 2000). Net N mineralization can be predicted by microbial biomass, as it represents the actual release of inorganic N from gross N mineralization and microbial incorporation (Bonde et al. 2001). Mineralization is the only biological soil driven means of producing soil-derived N that can be utilized by green plants (Tate 2000). The mineralization process involves the release of NO_3^-, NH_4^+, carbon dioxide (CO_2), sulphate, and phosphate from soil organic matter as a result of the activity of the soil microbial biomass, and includes both ammonification (Eq. 1-1) and nitrification (Eq. 1-2, 1-3) (Addiscott 2005).

$$Organic\ N \rightarrow NH_4^+ + OH^- \qquad \textbf{Equation 1-1}$$

The process of ammonification involves the conversion of organic N compounds such as proteins and nucleic acids into NH_4^+, and can be performed by a wide variety of bacteria and fungi. It is influenced by temperature and moisture, as increases in both temperature and moisture increase activity and efficiency (Addiscott 2005). Since NH_4^+ is rapidly oxidized in soils to NO_3^- by nitrifiers, net N mineralization in agricultural systems is typically quantified by determining the change in both the soil NO_3^- and NH_4^+ concentrations over time (Tate 2000).

The bacteria that implement mineralization are facultative anaerobes whose activity depends on the temperature and the degree of anoxia in the soil. The movement of oxygen in soil is frequently impeded by water in its pores, because oxygen diffuses about 10,000 times more slowly in water than in air (Addiscott 2005). Mineralized N is stored in soil as either NH_4^+ or ammonia (NH_3), it is subject to either the processes of assimilation, nitrification or volatilization.

1.1.2 Assimilation

The assimilation of N involves the microbial or plant incorporation of mineral N compounds, NH_4^+ and NO_3^-, into plant biomass (Tate 2000). The assimilation process begins when the plant roots uptake available NO_3^- and NH_4^+ from the soil, which is subsequently transported through the plant to the cells of the leaf (Solondz 2005). In soils where there is insufficient mineral N to compensate for C or energy deficiencies, the microbial growth rate is reduced by N limitation. The result of a reduction in mineral N by immobilization is the loss or limit in plant biomass productivity (Addiscott 2005).

1.1.3 Nitrification

The nitrification of NH_4^+ to NO_3^- is implemented in two stages by chemoautotrophic bacteria, which obtain their energy exclusively from oxidizing NH_4^+ ions (Addiscott 2005). The first stage of this process converts NH_4^+ to nitrite (NO_2), and is illustrated in Eq. 1-2.

$$NH_4^+ + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^- \qquad \textbf{Equation 1-2}$$

Recent molecular biology techniques have determined that it is the *Nitrosospira*-type bacteria that predominates in arable agricultural soils, but it is the *Nitrobacter*-type of bacteria that is responsible for the second stage of the nitrification process, described in Eq. 1-3.

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^- \qquad \textbf{Equation 1-3}$$

The process of nitrification involves directly incorporating oxygen atoms into the nitrogen oxide products, as well as the production of hydrogen ions by NH_4^+ oxidation (Addiscott 2005). Under typical soil conditions, NO_2^- does not accumulate in the soil, and any NO_2^- existing in the soil was produced from NH_4^+ oxidation (Tate 2000). Therefore, in most soils the limiting factor in the overall oxidation of NH_4^+ to NO_3^- is the conversion of NH_4^+ to NO_2^- because the NO_2^- is usually converted to NO_3^- as rapidly as it is formed. In soils with elevated soil moistures, nitrification is limited primarily from the indirect control of availability of molecular oxygen by its slow

diffusion rate in water (Pate and Farquhar 1988). In dry soils, specifically when the thin film of water on the soil particles is absent, the nitrification process does not occur. As the soils wet up and the thickness of the film on soil particles increases, the soil nitrifier bacterial population is activated and nitrification is re-initiated (Addiscott 2005).

Once created, the NO_3^- present in the soil can realize several different outcomes, which include denitrification, assimilation, or removal from the system through leaching and erosional losses (Macrae 2003).

1.1.4 Nitrogen Fixation

Nitrogen fixation by plants is the process of converting atmospheric N_2 to NH_3, and is displayed in Eq. 1-4.

$$N_2 + 6H^+ + 6e^- \rightarrow 2NH_3 \qquad \textbf{Equation 1-4}$$

Sites with significant accumulation of organic matter are sustained by the cycling of N between organic N and plant-available inorganic forms, while the establishment of the fixed N pool is a requirement for continuous crop productivity in agricultural soils due to the high N requirement of intensive agriculture (Tate 2000). N-fixing crops, which include legumes, ensure that N fixation is a major contributing N input source in agricultural soils. Legumes contain rhizobia, which is symbiotic bacteria located within nodules of the root systems (Addiscott 2005). This bacterium produces NH_4^+ that the plants utilize for growth. The most common agricultural legume crops include soybeans, clover, and alfalfa. Typical crop rotations in Ontario include one or more seasons of legume crops to maintain soil N for more intensive crops, such as corn or other vegetables.

1.1.5 Denitrification

The denitrification process involves the reduction of NO_3^- to N_2O and N_2 by denitrifying bacteria, which is illustrated a sequence of reductive chemical reactions (Eq. 1-5).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \textbf{Equation 1-5}$$

Denitrification requires a low level of dissolved oxygen in the soil, a source of NO_3^--N, and finally an electric donor, which is most commonly organic carbon (Harris 1995). Pinay et al. (1993) determined that the lowest rates of denitrification occur during the summer and fall seasons, as the water table is lower, which provides a thicker aerated vadose zone. Denitrification is enhanced by frequent wetting and drying cycles, as well as in agricultural settings by irrigation, which is attributed to an increase in anaerobic sites due to higher soil moisture contents (Harris 1995).

As molecular oxygen diffuses more slowly in water as compared to air, the effect of aeration on the rates of denitrification in soil is significantly correlated to soil moisture (Pate and Farquhar 1988). This relationship is caused by the reduction in soil oxygen tension, which concurrently increases the rate of NO_3^- reduction to N_2O and N_2. Generally, lower denitrification rates are observed in fields with continuous drainage tile networks, but dramatic denitrification increases still occur and are associated with increasing soil moisture due to precipitation events (Addiscott 2005). Kroeckel and Stolp (1988) observed the overall relationship between soil moisture and rates of denitrification; which is, as soil moisture increases, losses of NO_3^- through denitrifier activity increases. As the soils dry out, denitrification rates slow down, and typically completely stop below 60% soil water holding capacity (Ostrom et al. 1998).

Soil temperature also plays a role in determining rates of denitrification, although not as significant of an influence as soil moisture. Generally, just as rates increase with increasing soil moisture, rates also tend to increase with increasing soil temperatures (Tate 2000).

The topography of a field has also been shown to influence rates of denitrification. Pennock et al. (1992) examined denitrification across fields of varying topography. The conclusions drawn from the study were that the most important factor affecting denitrification rates on level ground was volumetric water content (%VWC) of the soil, while on the slopes it was soil bulk density that was most strongly correlated with denitrification. A study conducted on a farming catchment area in France determined that riparian sites exhibit the highest rates of denitrification, based primarily on increased soil moisture levels and NO_3^-; however, hillslope denitrification is still very relevant because it represents the largest portion of the catchment area (Oehler et al. 2006).

1.2 Surface/Ground Water Contamination

While the N processes within agricultural soils are highly complex, so is the relationship between fields and the hydraulic network throughout an entire stream basin. Water quality is adversely affected by excessive NH_4^+ and NO_3^- contamination of surface water, and NO_3^- contamination of ground water (Zebarth et al. 1999). N inputs to the aquatic components of agricultural ecosystems typically promote eutrophication, which involves the excessive growth of algae and aquatic plants and subsequent loss of oxygen from the water. This process has several negative consequences; including the reduction of drainage efficiency in agricultural streams, and the reduction of species diversity. Excessive N contribution to stream courses as indirect nutrient contamination is referred to as non-point source pollution, which is caused by irrigating, fertilizing, ploughing, planting, and harvesting activities undertaken during typical crop growing seasons (US EPA 2006). These activities alter soil structure and N cycling processes, permitting transport of various forms of N into ground water, surface water, and the atmosphere. The export of N from agricultural fields into the atmosphere in the form of N_2O is responsible for over 75% of total anthropogenic N_2O emissions (Mummey et al. 1998). The

addition of N into surface and ground water as a result of agriculture activity is also significantly high relative to other sources (Table 1-1). While intensive agriculture is likely to increase in scope, the implementation of nutrient management plans and other programs will likely have a positive impact on limiting nutrient loading into ground and surface waters.

Nutrient Source	Nitrogen (10^3 t/yr)	Phosphorus (10^3 t/yr)
Municipal WTPs	80.3	5.6
Municipal sewers	11.8	2.3
Septic systems	15.4	1.9
Industry	1.8	2.0
Agriculture	293.0	55.0
Aquaculture	2.3	0.5
Atmospheric deposition	182.0	-

Table 1-1: 1996 comparison between nitrogen and phosphorus contributions from various sources into ground and surface waters in Canada (Chambers et al. 2008).

Water quality and N contamination issues are potentially exacerbated in watersheds with predominately tiled fields. Increasing the draining efficiency in a field ensures that water introduced to the soil has a shorter residence time, meaning N has less time to be immobilized and retained, and is more susceptible to be quickly drained. Decreasing surface runoff would result in lower NH_4^+ concentrations in surface water, as NH_4^+ is bound to soil particles and is typically lost through erosion. However, tile drains serve as rapid and direct N conduits to water systems, as these drains bypass the riparian zone N-removal mechanisms that are effective in filtering natural surface and groundwater flow. Greenland (1981) concluded that, under most common conditions, N leaching is unavoidable in prolonged rainfall activity. Higher soil moisture content leads to lower nitrification rates and higher NH_4^+ concentrations. Lower soil moisture content improves conditions for aerobic soil microbes, and nitrification becomes the dominant microbial process; the result of which is higher NO_3^- concentrations (Zhang and Wienhold 2002). Therefore, the wetter areas of the basin, typically those areas low-lying and located near the water source, are more susceptible to NO_3^- leaching and denitrification.

Tile drained fields also add another element to basin hydrology, as N movement is very different compared to non-tiled fields. N concentration in tile drainage water is highly dependent on the form and amount of inorganic N in the soil; the inorganic N pool in the field being greater when demand from crops are lower (Gentry et al. 1997). The difference in annual N export from tiles depends on several factors, including: amount and intensity of precipitation, timing and area of N fertilization, crop uptake of soil derived N, and inorganic N pools that remain after crop harvest (Gentry et al. 1997).

1.3 Stable Isotopes

The study of biogeochemical cycles and soil-water-air processes are increasingly reliant on the use of environmental isotopes, which serve as tracers within these complex systems. Environmental isotopes are the isotopes of abundant, naturally occurring elements in the environment; hydrogen (H), carbon (C), N, oxygen (O), and sulphur (S) (Clark and Fritz 1997). The stable isotopes of these light elements have relatively large mass differences, which makes it possible to identify physical and chemical reactions through fractionation. In soil-water and soil-plant interactive systems, the most common isotope ratios used as tracers include $^{15}N/^{14}N$, $^{18}O/^{16}O$, and $^{13}C/^{12}C$. Specifically, soil-plant system studies rely heavily on ^{15}N tracers, as the N cycle is incredibly dynamic and complex involving several transformation processes (Nadelhoffer and Fry 1994; Ostrom et al. 1998; Choi et al. 2003) (Figure 1-1). During the transformation progression microbial processes tend to favour reacting the lighter ^{14}N-compounds, which results in ^{15}N-enriched residual reactant and a ^{15}N-depleted product. As well, the various sources of N compounds occasionally exhibit distinct isotopic ratios, which increase the compound's value as a tracer.

Numerous studies have used N isotopes to trace the sources and movement of N in surface and ground water (Kendall and McDonnell 1998, Lefebvre et al. 2007, Diebel and Zanden 2009).

Organic and mineral fertilizers have distinct N isotope signatures. Inorganic fertilizers tend to have $\delta^{15}N$ values ranging between -4 and 4‰ while organic manure ranges between 2 and 30‰ (Lefebvre et al. 2007).

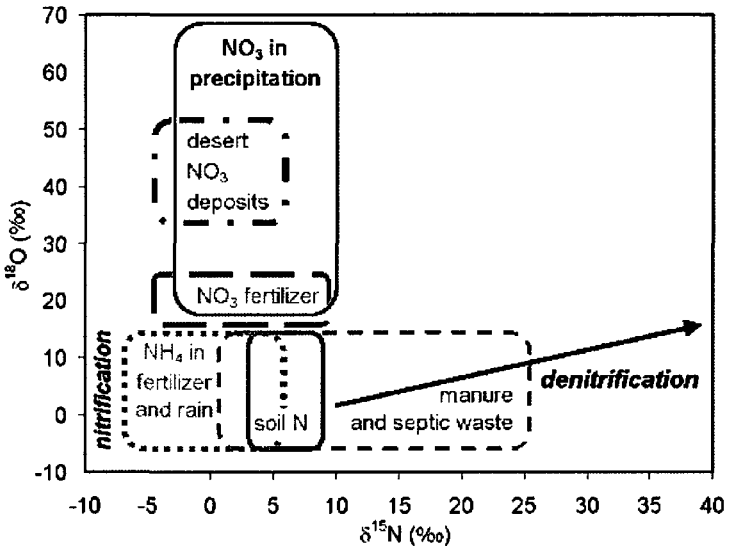

Figure 1-2: $\delta^{15}N$ values of sources and transformation processes common to agricultural areas (Kendall and McDonnell 1998).

1.3.1 Isotopic Fractionation

During the various processes in the N cycle, N isotopes undergo fractionation, which changes the ^{15}N:^{14}N ratio (Clark and Fritz 1997). This occurs because micro organisms preferentially use the light isotope (^{14}N) over the heavy isotope (^{15}N), which means the product created by the organism is isotopically lighter than the reactant not used (Högberg 1997).

Since changes in stable isotope ratios are small, isotopic values are calculated relative to a recognized standard and reported in parts per thousand deviations from that standard (Sulzman 2007). The calculated deviation is expressed in delta notation through the formula shown in Eq. 1-6.

$$\delta\,(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000 \qquad \textbf{Equation 1-6}$$

The international standard for $\delta^{15}N$ is atmospheric N ($\delta^{15}N$ = 0‰), having a ^{15}N abundance of 0.365%. In terms of $\delta^{13}C$, the international standard is Vienna Pee Dee Belemnite (VPDB; ^{13}C = 1.108) (Sulzman 2007).

Fractionation is quantified as the ratio of heavy to light isotopes in the product and substrate. As isotopic effects are relatively small, the deviation of the fractionation factor is expressed as ε (Eq. 1-7) (Sulzman 2007).

$$\varepsilon_{a-b} = (\alpha_{a-b} - 1) * 1000 \qquad \textbf{Equation 1-7}$$

ε is an indicator of enrichment ($\varepsilon > 0$) or depletion ($\varepsilon < 0$) of the heavy isotope in the product as related to the substrate (Tate 2000). The various cycling processes produce varying degrees of $\delta^{15}N$ fractionation, and in turn are affected by soil conditions; specifically moisture and temperature.

The soil conditions driving N cycling processes and determining N fate is reflected through $\delta^{15}N$ fractionation, which subsequently appears in plant $\delta^{15}N$. The most significant driver of cycling processes is moisture and therefore investigating the relationship between soil moisture and $\delta^{15}N$ of crops may provide insights into soil N-cycling processes.

1.3.2 Isotopic effects during mineralization

Mineralization is typically responsible for only a small fractionation (1‰) between the soil organic matter and the soil NH_4^+ (Kendall 1998). Therefore, $\delta^{15}N$ values for NH_4^+ would be expected to closely reflect that of the soil organic N. However, the difference in soil organic N between soil horizons causes variability in mineralization rates and $\delta^{15}N$ values of the product. Increased mineralization rates within the upper horizons of agricultural soils, due to an abundance of plant residual and increased microbial activity, results in high NH_4^+ and NO_3^- concentrations (Koba et al. 2003). Organic soil $\delta^{15}N$ is lower than mineral soils because it more closely reflects the $\delta^{15}N$ signature of plant residual, which is the major contributor of organic

matter and N (Oberson et al. 2006). Typically, lower horizon mineral soils contain the highest $\delta^{15}N$ signature because mineralization rates decrease with depth due to the decrease in organic N.

1.3.3 Isotopic effects during nitrification

During the nitrification process, due to organisms preferring the light isotope over the heavy isotope, the NO_3^- formed has lower ^{15}N isotopic signature than NH_4^+ left behind (Falkengren-Grerup et al. 2004). As well, under aerobic conditions, nitrification causes most significant difference in $\delta^{15}N$ between NH_4^+ and NO_3^- (Yun et al. 2006). Fractionation during nitrification is most prevalent when NH_4^+ is plentiful in the soil, and can be negligible when NH_4^+ is not readily available (Ostrom et al. 1998). Fractionation associated with nitrification is dependent on the rate determining step. As the oxidation from NO_2^- to NO_3^- is generally rapid, the rate determining step is typically the slow oxidation of NH_4^+ (Kendall 1998). This produces a usual fractionation range for nitrification of -12 to -29‰ (Kendall 1998). Unfortunately, the fractionation range for nitrification specific to agricultural soils is unknown.

1.3.4 Isotopic effects during denitrification

Denitrification involves the reduction of NO_3^- to N_2O and N_2, and combined with leaching is responsible for significant N loss in agricultural systems. Denitrification causes the $\delta^{15}N$ of residual soil NO_3^- to increase exponentially as soil NO_3^- concentrations decrease (Kendall 1998). High soil N mineralization rates can often be coupled with denitrification or leaching of inorganic N, as both processes result in losses of ^{15}N-depleted N_2O and N_2 or NO_3^-, respectively (Kahman et al. 2008). The remaining inorganic N pool is left enriched in $\delta^{15}N$. The typical fractionation range for denitrification is -40 to -5‰ (Kendall 1998). As with nitrification, the fractionation range for denitrification specific to an agricultural system is unknown.

1.3.5 Isotopic effects during plant assimilation of N

The plant uptake of N from the soil is a process that exhibits very little fractionation, and is not likely to be a dominant control on the $\delta^{15}N$ of NO_3^- (Ostrom et al. 1998). The documented range of fractionation by plants is -2.2 to 0.5‰ with an average value of approximately -0.25‰ (Kendall 1998). The shift in plant N uptake strategy between NO_3^- and NH_4^+ can also result in a change in foliar $\delta^{15}N$, even if the $\delta^{15}N$ of the NO_3^- and NH_4^+ remain approximately the same (Kahmen et al. 2008).

1.3.6 Plant/Soil Nitrogen Interactions

Agricultural systems experience significant disturbances, especially in the upper soil horizons, which impact N cycling dynamics between the soil and the plant. In soil systems that experience dramatically fluctuating moisture levels, the effect of N cycling processes on the $\delta^{15}N$ signature is pronounced. Aranabar et al. (2004) concluded that spatial and temporal variability of precipitation play a key role on N cycling and isotopic signatures in the plant-soil system. Specifically, $\delta^{15}N$ enrichment as a result of aridity was enhanced during wet years, which was the result of increased mineralization of old organic N pools that produced available N with high $\delta^{15}N$. Coinciding with increased precipitation is increased soil moisture, which reduces oxygen and increases denitrification processes. Falkengren-Grerup et al. (2004) found that plant species utilizing primarily NO_3^- in ecosystems without significant NO_3^- leaching or denitrification have lower $\delta^{15}N$ values in their tissues than species growing equally well, or better, on NH_4^+. On the other hand, increased tree foliar $\delta^{15}N$ coincides with increased stream-water NO_3^- concentration, which suggests that the increased nitrification responsible for elevated stream-water NO_3^- may also cause enrichment of the plant-available NH_4^+ pool in a forested watershed (Pardo et al. 2002).

In agricultural studies, isotopic analysis has focused primarily on the identification of source nutrients from the soil in plants (Choi et al. 2003; Petersen and Sorensen 2004; Yun et al. 2006). The complexity is increased when attempting to utilize plant isotopes to identify soil processes. N is a crucial crop growing nutrient, and is typically added during the growing season in either an organic or inorganic form. The added N, as well as the rest of the soil N pool, is subject to various cycling processes dictated by a variety of conditions. Handley et al. (1998) concluded that the use of foliar $\delta^{15}N$ to identify N fractionation is obscured by a variety of factors, including intra-plant isotope partitioning, discrimination during N uptake, mycorrhizal status, and nodulation. Therefore, the N uptake and partitioning strategies of the plant must be explored, as well as the available nutrients and microbial status.

The fractionation processes induce enrichment of the ^{14}N isotope of NO_3^- during nitrification and an enrichment of the ^{15}N isotope of the remaining NO_3^- during denitrification (Diebel et al. 2009). $\delta^{15}N$ of NO_3^- can be coupled with other isotope tracers and utilized as an indicator of not only the source N, but also of processes such as nitrification and denitrification in soil and water (Figure 1-2).

1.3.7 Plant Carbon

Plant carbon isotope values, specifically foliar $\delta^{13}C$, are a product of the isotopic composition of atmospheric CO_2 and fractionation during the specific photosynthetic pathway utilized by the plant. Corn utilizes the C4 photosynthetic pathway, in which the first stage of CO_2 assimilation is the carboxylation of phosphoenolpyruvate (PEP) and the first product is oxaloacetic acid (Lambers et al. 2008). In comparison, oat crops rely on the C3 photosynthetic pathway, whereby the initial stage of CO_2 assimilation is the carboxylation of ribulose 1,5-bisphosphate and the first product is phosphoglyceric acid (Lambers et al. 2008). The result is vastly different $\delta^{13}C$ values between C3 and C4 plants. Typically, $\delta^{13}C$ values of atmospheric CO_2, foliar C3, and foliar C4 are approximately -8, -27, and -13‰ respectively. Soil conditions can influence plant $\delta^{13}C$ values primarily through moisture stress, and to a less significant extent nutrient stress. A plant subjected to water stress will tend to close the leaf stomata (Figure 1-3). The stomata are leaf epidermis pore structures that are responsible for the diffusion of atmospheric CO_2 and the transpiration of plant moisture.

The higher the stomatal conductance the less $^{13}CO_2$ ends up in the photosynthates, which results in plant biomass depleted in ^{13}C. Stomatal conductance is the rate that moisture evaporates from plant pores, and is affected primarily by plant moisture stress. Plants that are not water stressed have a higher stomatal conductance. In C4 plants the fractionation during photosynthesis is dominated by fractionation during diffusion, and the isotopic composition of the plant leaves will approach that of the process that most strongly limits photosynthesis (Lambers et al. 2008). The limiting process in water stressed plants is diffusion, which controls CO_2 availability when the leaf stomata are closed. As $^{12}CO_2$ diffuses faster than $^{13}CO_2$, decreased stomatal conductance or stomatal closure results in plant organic matter that is depleted in ^{13}C. In plants that are not water stressed, the stomatal conductance is higher and atmospheric CO_2

diffuses into and out of the crop leaves. The open diffusion of atmospheric CO_2 maintains higher foliar $\delta^{13}C$ values. The nutrient status of the soil also significantly affects the plant stress, which directly relates to foliar $\delta^{13}C$. Clay et al. (2001) concluded that supplementing N to N deficient C3 and C4 plants will decrease and increase $\delta^{13}C$ in C3 and C4 plants, respectively. This reaction to nutrient stress has the same effect on foliar $\delta^{13}C$ as water stress response, as stomatal conductance decreases and diffusion is limited. Therefore, plant $\delta^{13}C$ can be utilized as an indicator of water and nutrient stress, and provide insight into plant response to changing soil moisture conditions.

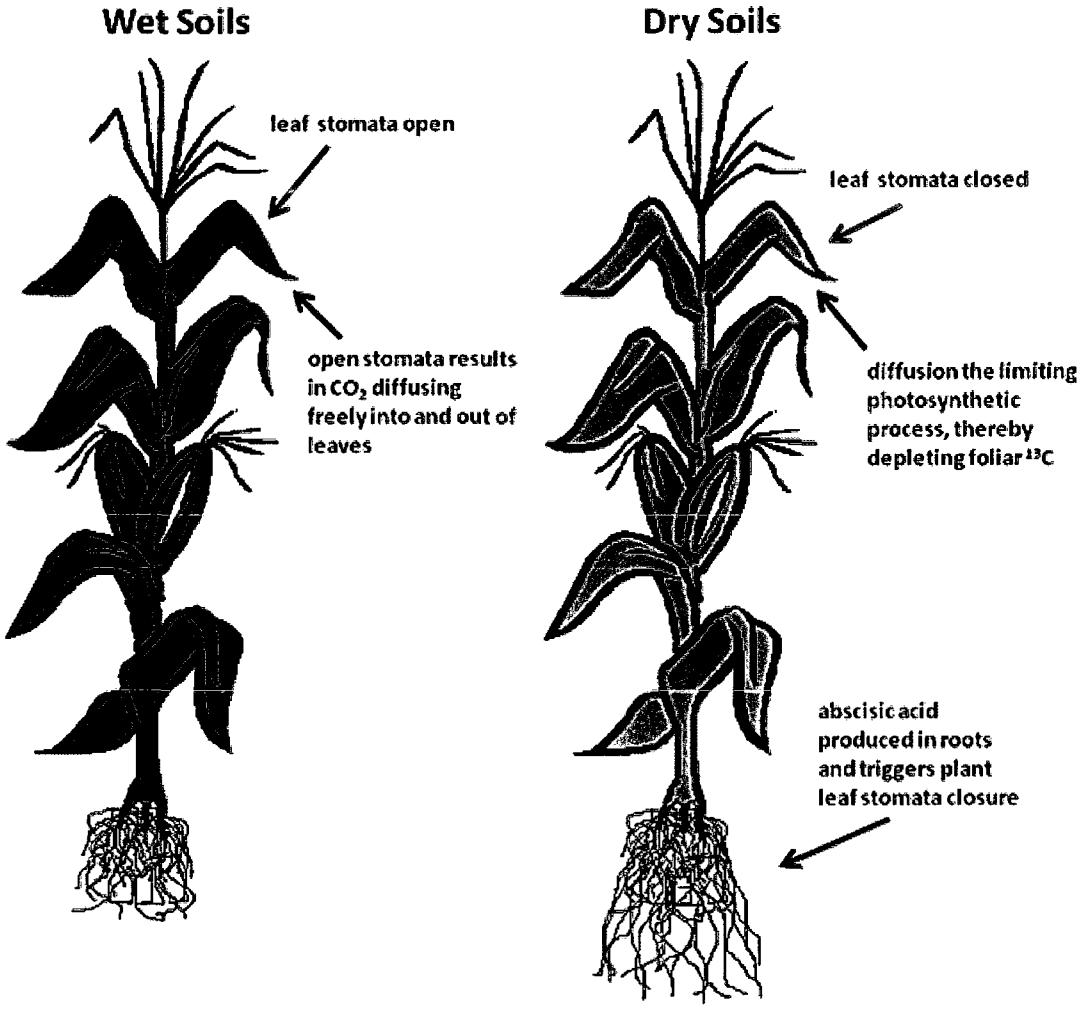

Figure 1-3: Summary of isotopic differences in ^{13}C between corn plants grown in moist and dry soil conditions.

1.4 Nutrient Management Practices

Management practices designed at fully utilizing all available N in a system without excess loss are actively employed throughout the agricultural sector. There are many factors that determine N requirements, and are all considered in best N management practices.

Precision farming techniques are necessary to minimize N losses, as NO_3^- requirements can vary to such a degree that makes it a relevant intra-field issue. Crop planting and harvesting, and fertilization (manure, fertilizer) all play a direct role, through overall nutrient management, in dictating the N processes and N availability below the soil surface. Therefore, controlling these activities through precision agriculture is important for excessive N losses to both ground and surface water, as well as to the atmosphere.

The Government of Canada identifies two primary types of best management practices; nutrient management and Integrated Pest Management (IPM). Nutrient management focuses primarily on applying to fields only the amount of fertilizer and manure that can be taken up by the crops. IPM focuses on using pesticides and herbicides in a responsible and ecologically sound manner (Hilliard and Reedyk 2003). Management practices involve the consideration of tillage, drainage efficiency, crop rotations, fertilization, cover crops, and riparian zones. The benefits of best management practices include the reduction of agricultural impacts on the environment, as well as improved economic efficiency in crop production.

1.4.1 Nutrient Use Efficiency

The proper and effective management of crop nutrients requires a detailed knowledge of the nutrient use efficiency (NUE) for each specific crop grown. The NUE refers to the effectiveness of a crop in utilizing available N in the soil because as N is taken up by plants, less is available to be lost through leaching, volatilization, immobilization, and denitrification (Agriculture and Agri-Food Canada 1997). The NUE requires consideration of the forms of N applied to crops, the

timing of application, the placement of nutrients in the soil, and overall soil and water management. In addressing the forms of N applied to crops, proper management involves employing a variety of methods to meet all the N requirements of the plant, rather than through one specific fertilizer application method. Nutrients are utilized more efficiently, and excess nutrients are limited, when a variety of methods are utilized; such as combining organic manure with inorganic fertilizer (Agriculture and Agri-Food Canada 1997). Rotating crops, as well as planting cover crops, can provide N to the soil through atmospheric N-fixation when crops are legumes or forage types, and are especially beneficial when planted before a crop with higher N requirements.

The timing and placement of added nutrients is also significant when addressing crop NUE. An example would be addition of manure to soils; which, while meeting the N requirements of the crop, also exceeds the phosphorus demands, which subsequently pollute ground and surface water. Therefore, Agriculture and Agri-Food Canada and the Ontario Ministry of Agriculture, Food, and Rural Affairs recommend that the manure application rate provides no more than approximately two thirds to three quarters of the necessary N requirements for the crop. The rest of the crop required N is expected to be supplied by the mineralization of soil organic N. However, if total N inputs do not meet the full requirements of the crops the productivity will decrease, and the long-term cropping efficiency of the soil will be reduced. Manure application should be timed when soils are dry, and application rates low enough so the liquid does not pond on the surface of the soil. Finally, the long term cropping schedule needs to be considered so that N application maintains soil fertility and crop productivity.

1.4.2 Fertilization and Tillage

Soil conditions, including moisture, temperature, and N-status, are significant when considering supplemental nutrient application. A study conducted by Schroth et al. (2000) concluded that fertilizer spread evenly between plants and plant rows, rather than exclusively at the base of the plant, will promote more even and lateral root growth. Root growth is also important in guarding against water and nutrient stress, and increases crop retention of available NO_3^- in the system (Addiscott 2005). Evenly distributed N will eliminate intensive zones of fertilizer, which when combined with proper tillage will promote more efficient N uptake and utilization. Frye (1986) developed a budget for agricultural N (Table 1-2) for silt loam by measuring N uptake and losses. At lower N fertilizer rates, more N was translocated to the grain and less was immobilized in the soil in conventional tillage systems than no-till systems. Approximately the same amount of N was lost under all tillage treatments and N application rates (Osmond et al. 2000). The latter supports studies conducted by Mitsch et al. (1999) and Smith et al. (1990), who concluded that tillage systems do not affect N losses, and have little influence on NO_3^- losses from agricultural fields.

Nitrogen Application Rate (lb/A)	Tillage Method	Fertilizer Nitrogen		
		In grain	*Immobilized*	*Lost*
		%		
75	No-tillage	23	42	29
75	Conventional	40	27	26
150	No-tillage	29	39	25
150	Conventional	28	37	27

Table 1-2: Nitrogen budget describing the effects of different tillage methods and fertilization rate on N in the soil (Osmond et al. 2000).

N application, specifically in the form of liquid manure, should be timed in accordance to soil conditions and previous field activity. The infiltration of manure can be expedited when the liquid is applied at high volumes, as the material can move down through cracks, holes, and even earthworm burrows. In fields with subsurface drainage there is an increased risk of ground water and surface water contamination (Agriculture and Agri-Food Canada 1997). Therefore, soils are typically pre-tilled before manure application, which reduces or eliminates preferential flow networks.

Tillage activity also has an effect on soil conditions and plant residuals in the field. Conventional tillage involves ploughing and multiple runs of discing and cultivation of the soil, which loosens the soil structure in the upper portions of the soil profile. However, natural flow pathways are altered, and the lower soil horizons are further compacted. This tillage method also introduces plant residue matter to lower portions of the profile, which influences the rate of decomposition and mineralization of the crop organic matter. No-till involves drilling seeds directly into the ground without any prior tillage activity. This type of tillage method is popular when planting soybeans, after a corn crop, in a standard crop rotation. No-till limits the impact and compaction of the natural soil pore spaces typically associated with increased agricultural activity. Tillage promotes a temporary decrease in soil compaction, but is quickly followed by an increase in compaction caused by the destruction of the natural soil structure. As well, the plant residual is not ploughed into lower horizons of the profile, where decomposition and mineralization may result in nutrient leaching. Conversely, higher rates of decomposition and mineralization may result in increased N availability for plants, which reduces the dependence on applied N. In tiled fields this is especially relevant as tile networks expedite the flow of water and nutrients from the soil into surface water networks. The difference in tillage methods also affects soil N cycling processes because of the effect on soil structure and soil moisture. Due to

the sensitivity of denitrifiers to changes in soil moisture, there is a significant amount of lost NO_3^- from no-till soils compared to conventionally tilled soils as N_2O and N_2. The increase in denitrification rates in no-till soils results from the formation of anaerobic microsites in the soils, which are conducive to denitrification (Tate 2000). These sites form because no-till practices limit the compacting of soil structures and prevent the destruction of natural microsites, thereby limiting impacts to ground and surface water.

The timing of tillage practices is also significant in reducing impacts to the soil structure. The cultivation of agricultural soils should not occur in the spring until the soil moisture has decreased below the plastic limit of the soil. When wet soil is cultivated, especially early in the spring, large lumps of soil form that are difficult to break apart in subsequent tilling activity, and smearing and compression often occur (Agriculture and Agri-Food Canada 1997).

1.4.3 Crop Rotation and Cover Crops

The active rotation among different crop types is necessary for proper nutrient management. Agriculture and Agri-Food Canada (1997) reports that crop yield from a growing season following a rotation typically increases between 5 to 15%. As well, legume crops can fix N in the soil and restore nutrients, if the field has become depleted from N demanding crops. Planting different crops between years also changes the tillage and fertilization timing and requirements, which keeps the soil structure in better condition than if tillage and fertilization activity was the same every year. The trend in crop rotation also serves to break disease cycles. Coinciding with the break in disease cycles, rotating crops involves applying different insecticides and herbicides, which prevents the development of pesticide resistant insects and weeds.

The primary purpose of cover crops is to protect agricultural soils from erosional processes in non-growing seasons. Cover crops maintain soil structure by holding soil particles together, especially during the winter and periods of excessive soil moisture and surface water drainage.

Specific cover crops can add N to the soil, most notably N fixing legume crops. Clover and alfalfa are common legume cover crops planted to replenish soil N immediately before or after intensive crop growth requiring significant amounts of N, such as corn or vegetable crops. Aside from N fixation, cover crops add organic material into the soil through plant residual, typically after the cover crops have been ploughed under the soil to make way for the primary crop. Additionally, the growth of cover crops can serve to retain excess nutrients in the soil, which limits the amount of N leached into ground and surface water systems (Agriculture and Agri-Food Canada 1997).

1.4.4 Riparian Zones and Subsurface Drainage

Another necessary step in reducing N leaching is the establishment of riparian zones between the agricultural fields and surface water bodies; commonly creeks, streams, and drainage ditches. These riparian areas serve as a buffer zone between field chemicals and surface water; as runoff is reduced by a zone of heavy natural vegetation, which slow the velocity of runoff water to the extent that sediment can settle out, allowing the water to infiltrate into the ground and nutrients to be taken up by the buffer vegetation (Hilliard and Reedyk 2003). The benefits of such systems have been documented, which has led to its inclusion as a standard agricultural nutrient management strategy (Table 1-3). Riparian zones can also reduce excess NO_3^- from ground water, as water tables are higher and soil moisture is greater as a result. Denitrification, uptake by plants, and immobilization make these areas an important part of conservation and management strategies (Harris 1995; Mengis et al. 1999). The desire to expand crop growing capacity must be balanced by the need to protect water resources and guard against agricultural erosion.

Crop Type	Accumulated rainfall (mm)	Diversions and grass waterways		Up and down slope cultivation	
		Runoff (mm)	Soil loss (kg/ha)	Runoff (mm)	Soil loss (kg/ha)
Grain/ryegrass	707	32	106	25	285
Potatoes	582	42	1678	203	15604
Barley	687	8	63	34	489

Table 1-3: The benefits of natural diversions and riparian zones in reducing soil loss and runoff, which in turn reduces nutrient loading into agricultural surface waters. The values are compared to traditional cultivation practices, and as expected the runoff and soil loss values are much lower when utilizing diversion systems (Chambers et al. 2008).

Subsurface tile drainage provides many potential benefits to crop growth and proper nutrient management, and warrants careful consideration. The potential issue is the rapid transfer of soil nutrients into local surface water channels, thereby increasing the negative environmental effects of agriculture on the surrounding ecosystems. However, the benefit of efficient drainage is the increased crop productivity, as effective drainage is critical for plant growth. In field soils with poor drainage, a higher water table discourages root growth, which makes crops more prone to water stress and nutrient deficiencies (Agriculture and Agri-Food Canada 1997). Efficient draining also dries soils quicker and more effectively in the spring, which has several potential benefits. Drier soils warm up quicker in the spring, which allows for enhanced microbial activity and subsequent N cycling processes (e.g. mineralization). It is also possible to access the fields earlier in the year, which reduces tillage impact and extends the crop growing season. Tile drainage also reduces overland flow during periods of heavy rainfall, which thereby reduces soil erosion and consequent nutrient loss. However, tile drainage does disrupt the natural ground water flow to surface water networks, and contributes nutrients directly into surface streams and creeks. Tile drainage bypasses the nutrient removal benefits of riparian zones and stream beds.

1.7 Knowledge Gap

The isotopic relationship between plants and soil is such that potential isotopic fractionation occurs when plants take up N from soil. Therefore, plant $\delta^{15}N$ values are a reflection of both source and transformation fractionation. However, through the investigation of multiple indictors, it should give insight into soil N cycling processes, which is beneficial because analyzing plant $\delta^{15}N$ can potentially be an easier method for qualitatively measuring the net effect of soil N cycling reactions. The use of isotopic tracers in plant - soil systems has been developed quite extensively, but the majority of the research has focused on forested ecosystems (Hogberg 1990; Nadelhoffer and Fry 1994; Pardo et al. 2002; Koba et al. 2003; Falkengren-Grerup 2004; Choi et al. 2005; Sah et al. 2006; Sierra et al. 2007). In agriculture, isotopic analysis has focused primarily on the identification of source nutrients from the soil in plants (Choi et al. 2003; Petersen and Sorensen 2004; Yun et al. 2006). The lack of understanding in using plant isotopes to indicate soil cycling processes is due in large part to the complexity of competing fractionating factors between the N source and the sink (Robinson 2001; Templar et al. 2007; Kahman et al. 2008).

The combination of fractionation due to N cycling, soil N status, and biological controls driven by changes in soil moisture and temperature makes quantitative determinations about specific N-cycling processes using plant tissue $\delta^{15}N$ a challenge. However, $\delta^{15}N$ of plant tissue may provide information on the overall N-status of soils and relative importance of N processes. As well, through the concomitant examination of specific soil characteristics, including NO_3^-, NH_4^+, and C/N, along a moisture gradient within a field with consistent treatments and nutrient sources, insights into N-status and N-availability in soils might be gained.

The issue of nutrient management in agriculture is becoming increasingly significant. N loading to surface and ground water has necessitated the need for specific crop strategies

involving cultivation, fertilization, and type rotations. The ability to gauge soil N-status and the ability of crop types to utilize soil nutrients is crucial in developing effective management strategies. This can only be achieved through careful investigation of cycling processes in various soil conditions; as well as crop specific uptake and partitioning strategies based on these conditions. Therefore, a method that effectively and efficiently assesses soil N-status and availability utilizing easily identifiable and measurable indicators, would be extremely beneficial. N-status would be characterized as the dominate soil N cycling processes occurring in an area. As soil moisture is easily measured, and is a major driver of soil N cycling process, it would be valuable to explore the linkages between differences in soil moisture and the effect on plant isotopic values. If N sources remain fairly constant throughout the field, the major difference in $\delta^{15}N$ signatures among crops in different areas of the field could be attributed to soil moisture variability and the resultant change in N cycling processes. As such, it is necessary to explore the link between plant $\delta^{15}N$ and soil N status, so that the relationship to soil moisture can be established. The examination of corn specifically is important; the various plant components will provide insight into nutrient and isotopic partitioning within plants, and how those strategies change in accordance to available N and soil conditions. Also, the ability to understand how crops partition nutrients based on N-availability may shed light on N use efficiency from organic and inorganic fertilizer inputs and final crop value and yield. These relationships should be investigated at a field-wide scale, as well as in a controlled environment with known and controlled N sources.

1.8 Study Objectives

The primary objective of this study is to investigate the relationship between plant tissue isotopes and soil conditions in an agricultural catchment. Specifically, the objective of the study is to assess the value of plant isotopes in determining N cycling processes within the soil, and whether that relationship is related to soil moisture. This will be achieved through several smaller objectives, including;

- Explore the appropriateness of plant isotopic analysis in determining dominant soil N cycling processes and the potential relationship to soil moisture conditions
    - Spatial and temporal variability in foliar and whole plant $\delta^{15}N$ at varying soil moisture locations
    - N partitioning strategies both through time and as a result of soil N-status
    - Soil inorganic N concentrations related to plant growth and uptake strategies, as well a s responses to fertilizer application
- Explore the link between soil moisture and foliar $\delta^{15}N$ and $\delta^{13}C$ at a field wide scale
    - Spatial and temporal variability in response to changing moisture conditions
    - Potential link between foliar isotopes and yield (dry cob weight)
- Investigate plant/soil relationship in a controlled environment
    - Explore the plant $\delta^{15}N$ and $\delta^{13}C$ response to extreme conditions and stress
    - Investigate soil N_2O production as evidence of denitrification

2.0 Site Description

The study was conducted at the Strawberry Creek Watershed, an agricultural research catchment located approximately one kilometre east of Maryhill, Ontario. The watershed drains approximately 2.8 km^2 of agricultural land into nearby Hopewell Creek, eventually discharging to Lake Erie via the Grand River, which at 6800 km^2 is the largest catchment basin in southern Ontario. Strawberry Creek is a two kilometre long, perennial first-order stream. The creek originates from two sources; a swampy deciduous forest stand (Figure 3-1) and a tiled field outside of the basin. Thereafter, it is fed by tile drains, ground water flow, and another forest stand in the lower reaches of the watershed (Thuss 2006). During spring melt periods and heavy precipitation events the stream can reach widths of over two metres, and during prolonged dry periods in the summer months the creek reduces to less than half a metre or becomes completely stagnant. However, typical flow produces an approximate stream width of one metre. Tile networks are situated approximately 0.75 to 1.00 metre below the ground surface, and are constructed of perforated plastic pipe; although, traces of older clay tile exist. The tiles drain the S and R Fields of the upper basin, the R and Z Fields of the middle basin, and the H-Field of the lower basin (Harris 1995).

Figure 2-1: Central portion of the SCW looking upstream of the creek (located centre of the figure). The multiple crop types, gently rolling topography, hedgerow and riparian vegetation, and farm structures are typical of the entire basin. The figure also outlines the meandering and naturalized channel pattern of the creek in the lower portion of the catchment.

The upper portion of the creek is characterized by a narrow riparian zone, tile drainage, and channelled flow between the S and R Fields, and to less of an extent in the west half of the Z-Field. The creek in the lower end of the basin, including the east portion of the Z-Field, is characterized by wider riparian zones, gentle gradients, no tile drainage excluding the Harris field tile, and a meandering flow pattern.

The upper reaches of the creek are channelized; stretching from the origin at Crowsfoot Road down past the stream's intersection with Maryhill Road. The stream's channelized flow was created for the purpose of improving drainage efficiency, and requires dredging every few years to maintain that effectiveness.

The creek flows under St. Charles Street East by means of a culvert, and drains into Hopewell Creek approximately 100 metres thereafter. The area downstream of the Lower Road is characterized by tall grass and fescue, while the stream exhibits shorter and more numerous meander lengths.

Figure 2-2: Typical channel and riparian zone characteristics, including creek flow and riparian vegetation, along Strawberry Creek. The left figure is a section of the creek looking downstream just below Upper Rd., while the right figure is looking upstream just above Middle Rd. The left figure is representative of the more natural and over-grown areas found near the origin and in the lower basin along to the outflow. The right figure is representative of the agricultural zones of the creek in the upper and central portions of the basin.

2.1 Land Use

The basin is typical for a Southern Ontario agricultural area, as it contains a combination of agricultural land, woodlots and hedgerows, and residential areas (Figure 2-3). The total area of the catchment is approximately 277 hectares, which includes over 30 individual fields with specific crop and treatment histories. The majority of the land in the basin (220 hectares; 79%) has been developed for cropping purposes. Woodlots and fencerows make up 37 hectares (13%) of the area while houses and farm yards account for 12 hectares (4%) of the total area. The Strawberry Creek, as well as the smaller drainage creek running out of the woodlot in the lower basin, and the riparian zone together account for only 2 hectares (0.07%) of the total coverage in the basin.

During the 2007 growing season, the catchment supported a variety of agricultural crops, as well as fruits and vegetables. A total of 13 fields were planted in corn during 2007, which accounted for 123.2 hectares (59.4%) of all field area. Figure 2-7 outlines the distribution of the various crop types, and it is clear that corn is fairly evenly spread throughout the catchment. Alfalfa contributed three fields and 30.8 hectares (15%) of field area. Six fields were planted in winter wheat at 25.1 hectares (12.1%) of field area, while soybeans accounted for 2 fields at 14.4 hectares (6.9%). One field at 8.6 hectares (4.1% of the total field area) contained oats. Finally, a variety of fruits and vegetables were spread across five fields and 5.4 hectares (2.6% of total field area) at the outflow area of the basin. Agricultural practices throughout the basin can be described as intensive, and employ various methods of tillage, fertilization, and nutrient management.

A poultry farm, cattle farm, and an additional cattle farm located outside of the basin mark the primary sources of organic N that, combined with synthetic non-organic fertilizer, account for an annual input of approximately 14,000 kg N across the watershed (Macrae 2003). The

upper 40% of the basin receives organic fertilizer applied at a rate of approximately 33 kg N per hectare, while the middle and lower portions of the basin receive primarily inorganic fertilizer at a rate of approximately 113 kg N per hectare (Macrae 2003).

Tillage practices are predominately conventional, which includes ploughing in either the fall or spring, followed by soil discing. No-till agriculture is not actively employed in the catchment; however, fields in the NE area of the watershed have a history of this type of practice.

Figure 2-3: Land use at the Strawberry Creek agricultural catchment. The land use is dominated by agricultural fields, but there is considerable woodlot cover. Roads, ditches, field access tracks, and the riparian zone and creek are highlighted in the figure, as well as the location of the nine intensive sites established in the area.

2.2 Soils, Vegetation, Climate

The soils within the basin can be divided into four specific types; Guelph loam, Maryhill loam, London loam, and Wauseon sandy loam (Figure 2-4). The Maryhill soil series occupies the largest spatial area across the SCW at 153 hectares, or approximately 55% of the basin. The Guelph series covers the next largest area at 67 hectares or 24%, while the London loam accounts for 10% total basin area at 28.5 hectares. The Wauseon sandy loam covers the smallest area of the major soil series found in the basin at approximately 19 hectares and 7% coverage. The Guelph loam includes soils developed over loam till, and could be overlain by potentially 30 centimetres of stone-free silt loam or loam (Presant and Wicklund 1971). This soil group is located primarily in the south of the basin, stretching to the creek in the central portion and along the western basin edge. These soils usually include clay till deposits, which underlie the loam till parent materials (Harris 1995).

The Guelph series soils are the most agriculturally significant soils in the basin; as they are capable of producing high yield corn, winter wheat, spring grain, and other crops grown in the SCW. As such, the Guelph series is considered a Class 1 agricultural soil by the Ontario Soil Survey in areas with a slope less than three degrees, which includes mostly the entire basin. Class 1 soils have no limiting factors for crop growth, and typically feature deep, well to imperfectly drained soils while maintaining good water-retaining capacity (Presant and Wicklund 1971). The London loam is closely associated with the Guelph series, as both typically form over loam till areas. However, these soils are imperfectly drained, and feature gravel pocket and sandy layer inconsistencies (Chapman and Putnam 1984). The London soils are found predominately in the central and eastern areas of the SCW; they can be prone to a seasonably high water table due to the presence of clay loam layers at depth within the till. London loam is found below approximately half of the T-Field. These soils are also rated a Class 1 soil for

agriculture by the Ontario Soil Survey because they are fertile and produce high-yield crops; however, because of the inconsistencies in the layers could require tile drainage to maximize productivity.

The dominate soil series within the basin is the Maryhill loam, which develops on stone-free sediments over loam till, and is poorly drained (Presant and Wicklund 1971). The Maryhill loam is present in the northern half of the basin, and dominates the soil class under the R-Field. The A horizons of Maryhill loam soils can be up to one metre thick with high organic matter content, while the subsoil contains clay layers at depth (Chapman and Putnam 1984). These clay layers produce high frost susceptibility in surface soil horizons, and are poorly drained due to the presence of impermeable clay deposits under the loam till. Therefore, the Ontario Soil Survey has labelled the Maryhill soil series a Class 2w, primarily due to poor drainage. The labelling suggests that this soil series is suitable for agriculture, but requires moderate conservation practices, including tile drainage. The soils have a good natural supply of nutrients, good water holding capacity, and are moderately high to high in productivity for corn, spring grain, and forage crops (Presant and Wicklund 1971). The final major soil series found within the SCW is the Wauseon sandy loam series. These soils are found at the eastern edge of the basin, and comprise some of the soils located beneath the T-Field. These soils are poorly drained, and develop on sand deposits ranging between 30 and 100 centimetres thick over clay till materials (Chapman and Putnam 1984). These soils are characterized by low fertility, while the subsoil typically has high clay content. The low fertility and poor drainage capacity of the soil combine to give the series a Class 4wf rating for agricultural, as there typically is a lack of available nutrients for crops (Presant and Wicklund 1971). Tile drained soils with added nutrients have the capacity to support grain crops, including oats and corn, while undrained soils typically can only support grass and forage crops.

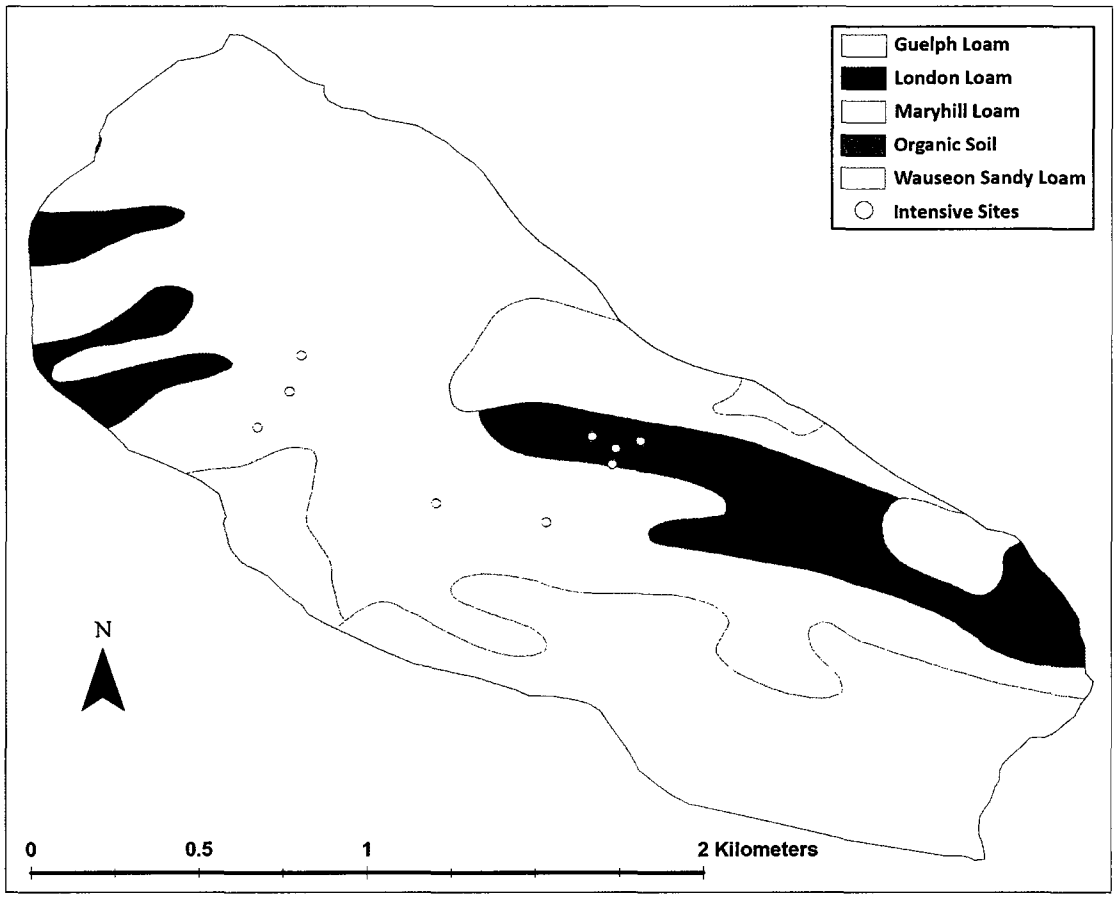

Figure 2-4: The four dominate soil types found within the Strawberry Creek Watershed. Also included is the soil type classified as organic, which is located in the swampy deciduous tree stand in the northwest corner of the basin.

The Strawberry Creek Watershed belongs to the larger Great Lakes – St. Lawrence forest system; approximately 30 hectares of the basin is forested. Dominate species include sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), white pine (*Pinus strobus*), poplar (*Populus spp.*), eastern white cedar (*Thuja occidentalis*), willow (*Salix app.*), Manitoba maple (*Acer negundo*) and alder (*Alnus spp.*) (Harris 1995). Natural vegetation is confined primarily to the four large woodlots located within the basin, the most prominent being the swampy deciduous stand in the northwest portion (Figure 2-5) and the woodlot in the south. The riparian areas along the creek, as well as road ditches and field hedgerows, contain a mix of grasses and other vegetation. Non-grass species in these areas include Canada goldenrod (*Solidago*

canadensis), common milkweed (*Asclepias syriaca*), Queen Anne's lace (*Daucus carota*), yarrow (*Achillea millefolium*), common burdock (*Arctum minus*) and thistles (*Cirsium spp.*) (Harris 1995).

The Waterloo region experiences a humid, continental climate, which produces a wide temperature range and extreme weather conditions. Figure 2-6 shows the climatic averages of precipitation and temperature between 1990 and 2005.

Figure 2-5: Typical vegetation cover within the deciduous woodlot located in the northwest portion of the basin. The figures show elevated water table and swampy characteristics of the area during the late-spring/early-summer period. The creek acts as a conduit for the drainage of this woodlot.

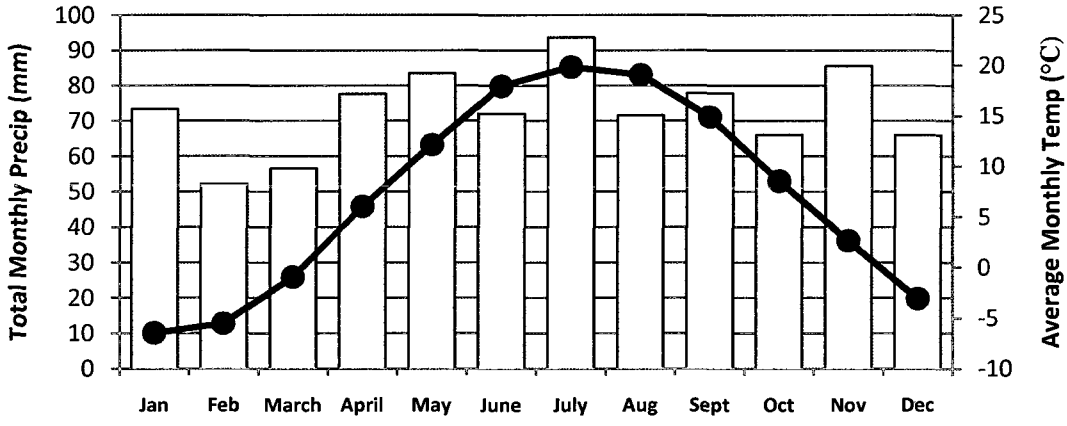

Figure 2-6: Recent climatic averages recorded in Waterloo between 1990 and 2005. Precipitation data was calculated from total monthly amounts and averaged over the 15 years, while temperature data was determined using daily averages, which was averaged for a single month (Environment Canada).

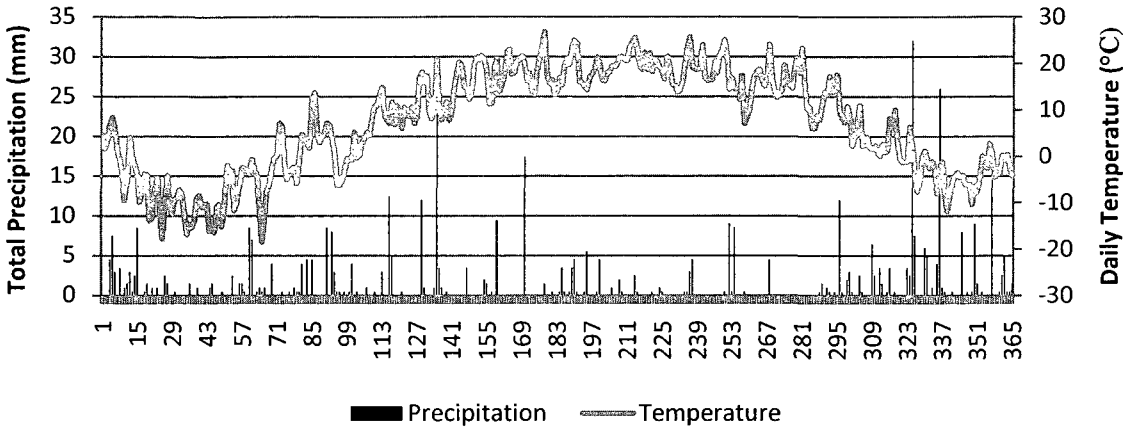

Figure 2-7: Daily total precipitation and daily average temperature recorded from the met tower within the Strawberry Creek Watershed for 2007. The spring and late fall periods received the most rainfall, while the summer months saw little precipitation.

The total precipitation recorded at the Strawberry Creek Watershed in 2007 was 464 mm, of which most fell during the spring and late fall months. Of that 464 mm of precipitation, only 143.5 mm fell during the corn growing season. As evident in figure 2-7, summer precipitation is isolated to a few intensive rain storms, while prolonged drought periods are not uncommon. A variety of chemical and climatic variables are measured and recorded directly in the basin by way of a micro-metrological tower (Figure 2-8). The tower was originally located in the riparian zone of the creek in the lower portion of the watershed, but was disassembled and relocated before the 2007 growing season. The tower was reassembled within the riparian zone in the central portion of the basin along the western edge of the R-Field. Aside from recording temperature and rainfall values, which were calculated to daily average for temperature and daily total for precipitation, the tower's other instruments took 10 readings a second and produced an average value every 30 minutes. Wind speed was measured using a CSAT 3-D Sonic Anemometer, which calculated turbulent fluctuations of wind both horizontally and vertically. A LI7500 Infrared Gas Analyzer measured CO_2 and water vapour surface flux utilizing eddy-covariance techniques, while a Kipp & Zonen CNR Net Radiometer determined photosynthetic active radiation at the ground surface. The data loggers at the tower also recorded soil moisture

within the riparian zone to a depth of 20 centimetres using a series of CS616 TDR probes. The growing season in 2007 was not as productive as usual, as there was a decline in the otherwise steady increases in yields due to increasing corn crop efficiency (Figure 2-9).

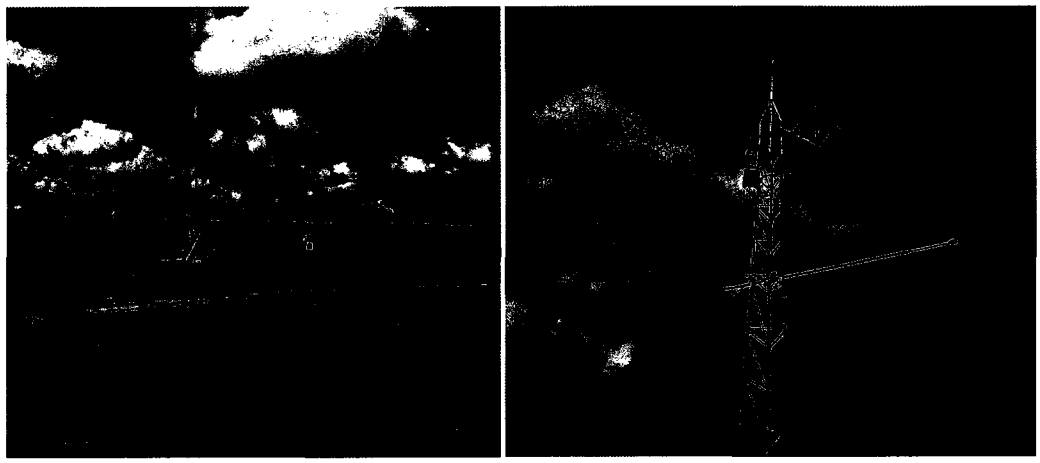

Figure 2-8: The metrological tower located within the basin. The tower is situated in the riparian zone in the middle of a large-open area lacking in significant natural vegetation (left figure), thus increasing the tower's range and efficiency. The figure on the right displays the selection of instruments installed on the tower.

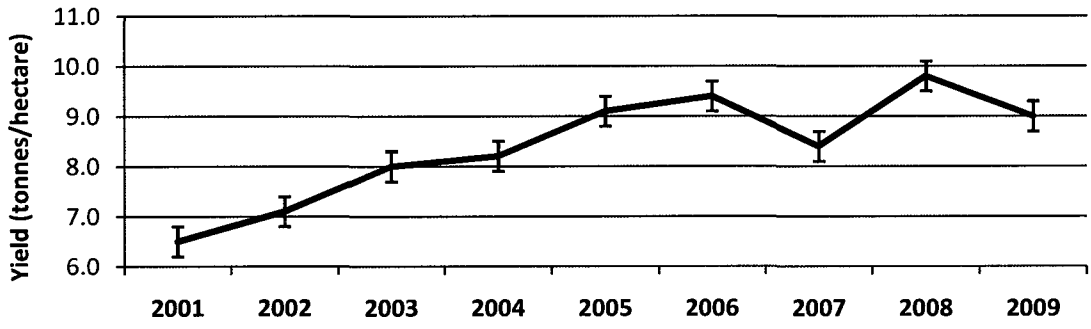

Figure 2-9: Corn yields in Ontario between 2001 and 2009 expressed in tonnes per hectare (McGee 2009). Error bars indicate ± std error.

3.0 Methodology

3.1 Site Selection and Instrumentation

The monitoring of field conditions, without interfering with regular farming activities, required the installation of permanent soil monitoring equipment. Therefore, six intensive sites were established for constant monitoring and sampling, and selected to represent the range of moisture conditions prevalent in specific fields. In both the T-Field and R-Field, a moisture survey was conducted at approximately 30 and 60 locations, respectively. Moisture survey data were utilized to determine a relative wet, medium, and dry location in each field. The intensive sites were categorized using the first letter of the field name, and numbered from one to three based on driest to wettest soil moisture conditions (Figure 3-1).

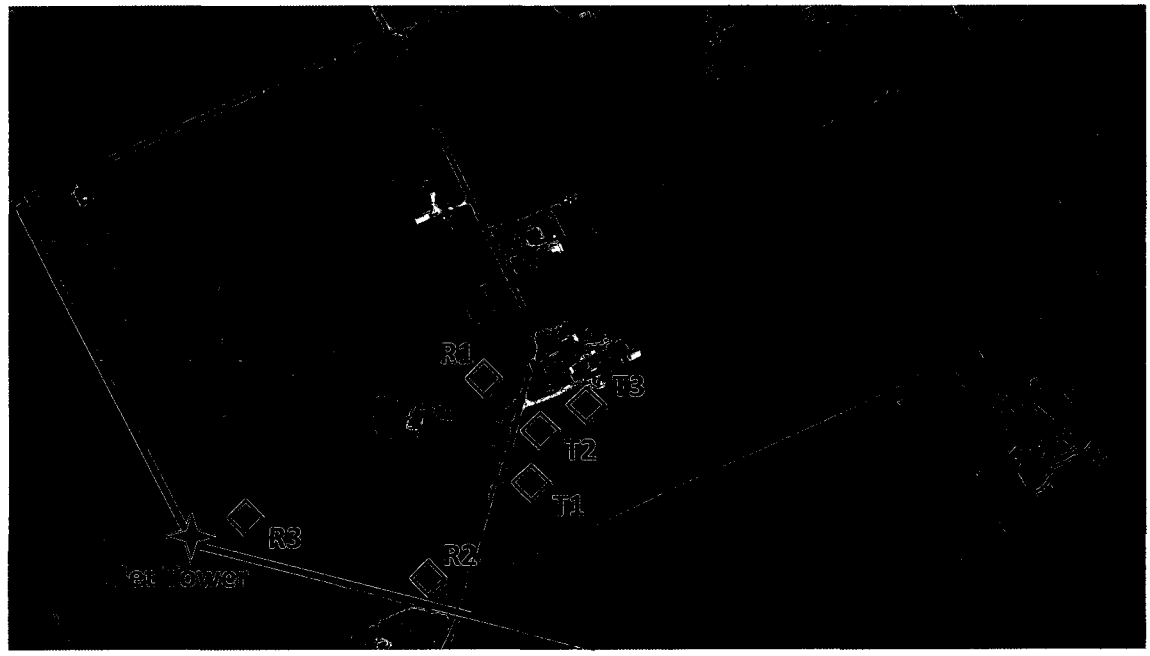

Figure 3-1: Strawberry Creek intensive agricultural research plots established in three sites in both the R-Field and T-Field. Sites were developed to maximize the range in typical soil moisture conditions experienced in both fields, and the locations were chosen in the spring of 2006.

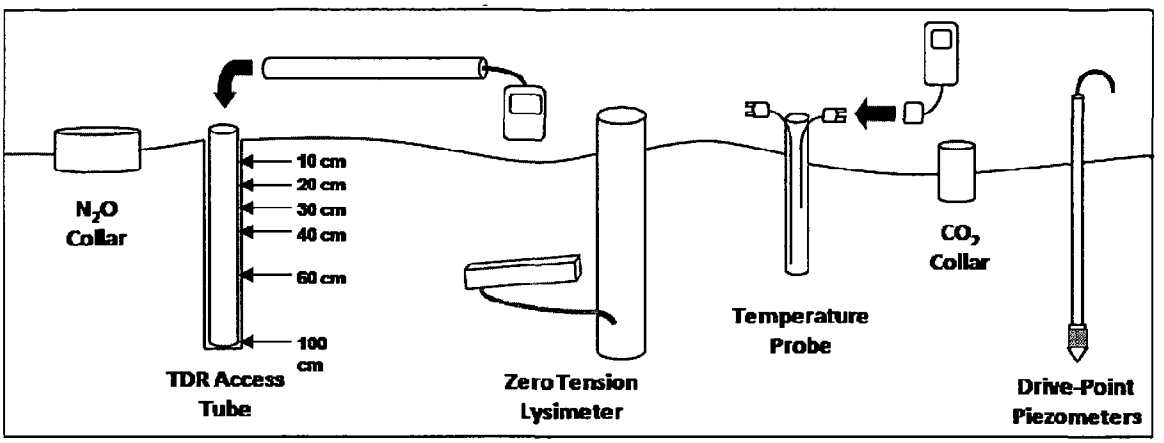

Figure 3-2: Cross sectional profile of site instrumentation installed at all intensive sites in the study fields. The diagram also illustrates the depths for soil moisture measurements within the TDR access tubes. Typically, each intensive site contained three access tubes, three temperature probes, one lysimeter, three of both the N_2O and the CO_2 collars, and two piezometers. However, the R3 site in the R-Field did not contain any piezometer installations.

The three intensive sites in both the R-Field and T-Field were equipped with a variety of soil monitoring instruments, to provide a representation of soil conditions throughout the study period (Figure 3-2).

Three randomly distributed time-domain reflectometer (TDR) access tubes were installed at each site. The tubes measured 2.54 centimetres in diameter, and were one metre in length. The holes were drilled using a gas-powered hand auger, with a slightly larger diameter than the access tubes. Caution was taken to ensure minimal disruption to the soil along the walls of the hole, as the tubes required constant contact with the soil to produce accurate measurements. Additional soil was sieved to remove larger material, and was mixed with water to create slurry that was poured into the holes in advance of the access tubes, to maximize contact and eliminate air pockets. A Delta-T PR2 Profile Probe was utilized to determine percent volumetric water content (%VWC) at six depths from ten centimetres to one metre. The probe relies on wave propagation through the soil to determine its permittivity, which directly corresponds to soil moisture, and is digitally displayed using the Delta-T HH2 Moisture Meter.

Two drive-point piezometers were installed at five of the six intensive sites; one deep and one shallow. The depths varied, but the deep piezometers were driven down approximately three plus metres at most sites, and the shallow piezometers were typically driven down between the one and two metre range. The piezometers were never properly developed as they were not required for this study.

Soil temperature profiles were measured at three or four random locations at each site, with six measuring depths at each location. This was achieved by running a thermocouple wire with an exposed end from each depth (2, 5, 10, 15, 30, 60 centimetres) to the top of the shaft, and attaching thermocouples to the end of the wires.

The zero-tension lysimeters were designed using screen mesh pulled across the top of a plastic container, and was strengthened by running thick metal wire across the mesh, which also served to disrupt water flowing along the mesh and directed it down into the collector. The interface between the mesh top and the soil profile required consistent contact, which was achieved by scraping the soil using a flat-edge to the correct angle and applying fine-grained soil and water on top of the mesh to create a workable surface. PVC pipe was installed to approximately one metre depth onto which a spout and hose was attached running back to the lysimeter. The spout allowed for sampling while eliminating prolonged exposure to the atmosphere and avoiding the risk of outside water contamination.

N_2O and CO_2 collars were constructed out of standard PVC pipe, and were installed by applying constant pressure around the rim of the collars and forcing them down to the desired depths. These depths were typically 10 to 15 centimetres, and were kept in permanent locations between cultivation and tillage activity at the intensive sites.

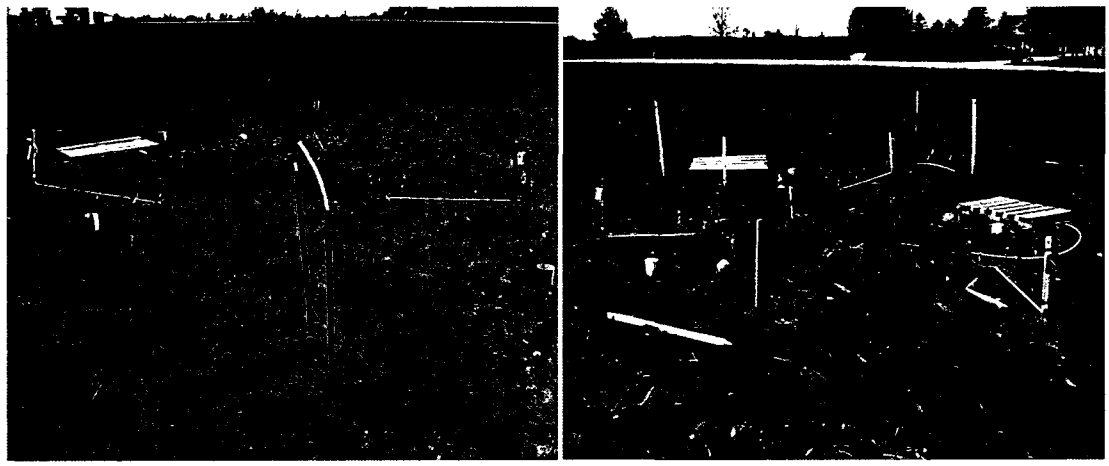

Figure 3-3: The typical instrument setup at the intensive sites in the R (R2; left) and T (T2; right) fields. Orange flags were designated to distinguish instrument locations during the winter, and small boardwalks were utilized to limit soil compaction during sampling, especially in the spring and during periods of high soil moisture.

3.2 Soil Sampling

The dominate soil characteristics were classified in the summer of 2006 using a variety of field and lab techniques and analysis. At each intensive site, three soil pits were dug to approximately one metre depth and diameter. The soil profile typically consisted of two distinct layers; an upper mineral layer high in organic content and a clay dominated layer. Also, a transition layer between the upper and lower horizons was identified and included in the sampling. Metal soil cores measuring approximately seven centimetres in length and four centimetres in diameter were carefully hammered into the soil to avoid as much disturbance as possible, thus achieving the most accurate results. To best capture natural variability, three cores were collected from each of the three layers in three pits at all nine sites across the study fields. The soil pits then underwent a detailed qualitative and quantitative assessment of the various soil profile characteristics, including particle size analysis, relative moisture and colour assessments, and horizon depth measurements. Soil samples were also removed for extractable soil NH_4^+ and NO_3^- concentrations, which assisted in establishing temporal and spatial variations in soil nutrients across fields with similar N inputs.

3.3 Laboratory Analysis

3.3.1 Soil Properties

The soil cores were weighed immediately after returning from the field, and were then placed in a container filled with water, so that the water level in the container reached the top of the core without submerging it. The cores were left in the container over a period of 24 hours, in which time the soil became completely saturated. The saturated soil cores were weighed inside collecting dishes so that all water inside the core was included in the weight. After draining for 48 hours, the samples were weighed again to determine the amount of moisture being held by capillary forces. These samples were then placed in the oven for 24 hours at 105°C until all moisture was completely evaporated. The porosity percentage of the core sample was calculated and documented (Eq. 8).

$$\boldsymbol{porosity}\ (\%) = \frac{(\boldsymbol{saturated\ weight\ (g) - oven\ dried\ weight\ (g)})}{\boldsymbol{oven\ dried\ weight\ (g)}} * \mathbf{100} \qquad \textbf{Equation 3-1}$$

It is assumed that the soil porosity within each field is consistent over an extended period of time, so soil cores were only collected in the summer, and porosity calculations were only conducted once per core sample. The bulk density of the soil was also quantified (Eq. 3-2).

$$\boldsymbol{bulk\ density}\ (\boldsymbol{g\ cm^{-3}}) = \frac{\boldsymbol{core\ dry\ soil\ weigt\ (g)}}{\boldsymbol{core\ volume\ (cm^3)}} \qquad \textbf{Equation 3-2}$$

The mass of material contained within the soil that is able to burn off through a loss on ignition test represents the organic content of that particular soil sample. Approximately 5 to 15 grams of sample was measured into a pre-weighed crucible, and placed in a 500°C muffle furnace for two hours. The sample was then weighed again, and the difference represents the organic content of that sample. Samples from all nine intensive sites collected in the summer and fall received this treatment.

The field moisture content was calculated by weighing out a portion of the soil sample into a specimen container and freeze dried for 48-72 hours. Freeze drying removed all moisture

without the use of heat, thereby not altering the chemical composition of the soil samples. The dry weight was measured, and moisture content was calculated using the equation shown in Eq. 10.

$$volumetric\ water\ content = \frac{intitial\ soil\ weigt\ (g) - freeze\text{-}dried\ soil\ weigt\ (g)}{freeze\text{-}dried\ soil\ weigt\ (g)} \quad \textbf{Equation 3-3}$$

3.3.2 Plant Preparation and Analysis

Immediately upon returning from the field, the oat plants and corn root components were removed from the bags, and the soil was washed off as thoroughly as possible using DI water. The plants and roots were allowed to dry for a period of time, and were then placed into new bags and stored frozen for future processing and analysis. The above ground corn plant components did not receive this initial treatment, but were placed in the freezer immediately after returning from the field.

Processing of the plant tissue involved several steps; the first of which consisted of sub-sampling representative material for analysis. This step was necessary because, especially with the corn components, there was simply too much material to process. The roots, lower stalk, upper stalk, and the cob were divided, while the tassel and full oat plant were included intact. One leaf was taken from the seven or eight leaves typically grown on a corn stalk and was included intact as well. Samples were subsequently placed, still frozen, in the freeze-drier for a period of 48 to 72 hours to allow for complete moisture removal. Following this step, the plant tissue was run through a grind-mill to reduce the material down into small pieces. Finally, the material was processed further by a ball-mill. The sample was placed in a metal cylinder with a ball-bearing inside and shaken for approximately 10 to 15 minutes, which transformed the plant components into a fine-powder for isotopic analysis.

3.3.3 Carbon/Nitrogen Ratio

Samples for C/N ratio analysis were prepared from the material used in the determination of soil moisture content. These freeze-dried samples were split to reduce the volume, and then placed in a two millimetre sieve shaker to remove larger stones and other objects found in the soil. The soil was then carefully picked through for 10 to 15 minutes to remove all coarse organic matter, such as fine roots. There are a large number of roots contained within each soil sample, so the time spent sorting through the sample was kept constant to achieve as much consistency as possible. After the sample was carefully sorted, it was placed in the two millimetre sieve again, so that only finer soil remained. This soil was placed in a ball miller for 15 minutes at 60% shaking capacity, which produced a fine soil powder that was placed in a scintillation vial to be analyzed. The material run for %N and $\delta^{15}N$ required no additional treatment, while a fraction of sample required for %C and $\delta^{13}C$ was acidified before analysis. A subset of 44 samples were chosen to represent the three fields, as well as give an indication of the level of spatial and temporal variability between fields, sites, individual pits, and soil layers. A complete set of 27 fall samples from site R2 were analyzed for C/N content, as well as six summer organic layer samples from R2, two fall clay layer samples from R3, and a complete set of nine fall samples from site S2.

All isotopic ratios were determined at the Environmental Isotope Lab (EIL), University of Waterloo. Samples were run for N and C on a Delta Plus Continuous Flow Stable Isotope Mass Spectrometer (Themo Fisher) coupled to a Carla Erba Elemental Analyzer (CHNS-O EA1108). Organic sample results are typically corrected to ammonium sulphate N standards IAEA-N1 ($\delta^{15}N$ = 0.36‰) and IAEA-N2 ($\delta^{15}N$ = 20.3‰) and C standards IAEA-CH6 (sugar $\delta^{13}C$ = –10.4‰), EIL-72 (cellulose $\delta^{13}C$ = –25.4‰) and EIL-70b ($\delta^{13}C$ = -19.43‰, $\delta^{15}N$ = 16.45‰). EIL denotes internal standards with values calculated using international standards. EIL-32 (graphite $\delta^{13}C$ = –25.76‰)

is utilized when sample type requires. The precision for clean ball-milled standard material is ± 0.2‰ for C and ± 0.3‰ for N. This precision can be expected to increase depending on the homogeneity, type and amount of sample used in the analysis. A more accurate representation of sample reproducibility is gained through sample repeat. The sample size for $\delta^{15}N$ and $\delta^{13}C$ was approximately one gram. Samples were run for %C and %N concentration on a Delta Plus Continuous Flow Stable Isotope Mass Spectrometer coupled to a Carla Erba Elemental Analyzer (CHNS-O EA1108). The precision for elemental composition of clean ball-milled sample material is ± 0.2% for C and ± 0.3% for N. The C and N compositions were calculated based on Carla Erba Elemental Standards B2005, B2035 and B2036 with precision of ± 1%.

3.3.4 Nitrate/Ammonium Concentration

Soil samples were taken from the freezer and stored in a cold room at 4°C until being processed for analysis, which occurred within 24 hours after the removal from the freezer. Samples were removed individually from the fridge to ensure they stayed at 4°C. Approximately 70 grams of soil was measured into a clean Nalgene bottle. A 2M KCl solution is required to free NH_4^+ bonds from the soil because NH_4^+ is a cation, and therefore necessitates a strong enough reagent to extract all fixed and mobile forms of N from the soil (Solondz, 2005). 200 mL of 2M KCl solution and the sample were mixed, six bottles at a time, in a low speed horizontal shaker for 10 minutes, and then placed in a centrifuge for 15 minutes at 6000 rpm to separate the soil from the solution. The decanted solution was then vacuum filtered to 0.45 μm and the resulting filtrate split into three scintillation vials, one each for NO_3^-, NH_4^+, and archiving, and placed immediately back into the fridge to await analysis. The archived samples were subsequently stored in the freezer for potential future analysis.

NH_4^+ concentrations of the soil samples were determined using the Technicon Auto-Analyzer utilizing a detection limit range from 0.01 mg/L NH_4-N to 0.50 mg/L NH_4-N (Department of

Geography and Environmental Studies, Wilfrid Laurier University). The samples exceeding this operating range were diluted two times, and run again. The method used for NH_4^+ detection involved Salicylate-Nitroprusside and hypochlorite solutions. The analytical precision of the NH_4^+ process using the Auto-Analyzer was 0.05 mg/L NH_4^+-N.

NO_3^- concentrations of the soil samples were determined at the Environmental Geochemistry Lab (EGL), Department of Earth and Environmental Sciences, University of Waterloo, using an Antek 945 Nitrate Reduction System, which reports NO_3^- as mg N/L. A heated vanadium solution reduced the injected sample NO_3^- to nitric oxide, which was then passed through a NaOH trap to an Antek Model 9000 Total N Analyzer. Finally, an ozonator produced NO_2, which was quantified by chemiluminescent detection.

Crop $\delta^{15}N$ as an indicator of soil N cycling processes and plant partitioning strategies

4.1 Introduction

The use of isotopic tracers in plant - soil systems has been developed quite extensively, but the majority of the research has focused on forested ecosystems (Hogberg 1990; Nadelhoffer and Fry 1994; Pardo et al. 2002; Koba et al. 2003; Falkengren-Grerup 2004; Choi et al. 2005; Sah et al. 2006; Sierra et al. 2007). In agriculture, isotopic analysis has focused primarily on the identification of source nutrients from the soil in plants (Choi et al. 2003; Petersen and Sorensen 2004; Yun et al. 2006). The complexity of utilizing isotope tracers is increased when attempting to utilize plant isotopes to identify soil processes. N is a crucial crop growing nutrient, and is typically added during the growing season in either an organic or inorganic form. The fertilizer N, as well as the rest of the soil N pool, is subject to various cycling processes dictated in significant part by changing moisture and temperature conditions. Specifically, ^{15}N isotopes can be used to trace a series of dynamic transformations, which include mineralization, assimilation, nitrification, and denitrification (Section 1.3). The ability to determine N cycling processes and fates through the examination of plant $\delta^{15}N$ is the end result of the complex nature of these soil N transformations. As such, soil N has multiple fates. Within an individual field the source N through fertilization and atmospheric deposition should remain spatially constant throughout, while leaching and crop uptake are similar to a point. Leaching and uptake are the products of experimental conditions, most notably soil moisture. Major N sources, specifically organic and inorganic fertilizer, in agriculture are significantly different isotopically, and are distinguishable in plants (Yun et al. 2006). N availability can also be a potential contributing factor. However, the relative isotopic differences in crop plant tissue throughout a field are the result of N transformations that alter the $\delta^{15}N$ values of agricultural N inputs. Gross mineralization of organic additions and in situ organic matter in the soil produces specific $\delta^{15}N$ signatures of the mineralized material, but the subsequent isotopic transformation is the product of soil

conditions. During the aerobic nitrification process, due to organisms preferring the light isotope over the heavy isotope, the NO_3^- formed has lower $\delta^{15}N$ isotopic signature than NH_4^+ left behind (Falkengren-Grerup et al. 2004). The same can be stated for both volatilization and denitrification. If the $\delta^{15}N$ signature of the soil organic matter remains temporally and spatially consistent, it would be reasonable to suggest that any changes in plant $\delta^{15}N$ are reflecting transformation processes of inorganic N in the soil. Plant $\delta^{15}N$ can also reflect N additions, which are easily quantifiable. These changes can be measured temporally by examining different plant components added throughout the growing season.

The use of foliar $\delta^{15}N$ to detect dominate N cycling processes is potentially difficult, and is more complex than attempting to identify N sources. Handley et al. (1998) concluded that the use of foliar $\delta^{15}N$ to identify N fractionation is obscured by a variety of factors, including intra-plant isotope partitioning, discrimination during N uptake, mycorrhizal status, and nodulation. Further to the point, the shift in plant N uptake strategy between NO_3^- and NH_4^+ may result in a change in foliar $\delta^{15}N$, as different $\delta^{15}N$ values for NO_3^- and NH_4^+ cause a plant $\delta^{15}N$ shift (Kahmen et al. 2008). Therefore, the N uptake and partitioning strategies of the plant must be explored. N uptake is the significant factor during the initial plant growth stage, while partitioning becomes increasingly significant in the latter stages of the growing season during cob development.

Kahmen et al. (2008) identified foliar $\Delta\delta^{15}N$ (foliar $\delta^{15}N$ – soil $\delta^{15}N_{TN}$) as a valuable indicator to characterize the soil N cycle, while other studies have also identified foliar $\delta^{15}N$ as a potential indicator of N cycling processes (Hogberg 1990; Garten 1993; Emmett et al. 1998; Pardo et al. 2002). Robinson (2001) describes the importance of examining plant tissue beyond foliage when attempting to infer N isotopic discrimination. In forest systems, recent studies have indicated that below ground plant $\delta^{15}N$ provides a more effective proxy for relative rates of soil N cycling

(Hogberg 1997; Templer et al. 2007). Pardo et al. 2006 observed that local N cycle drivers, including nitrification, mineralization, as well as soil C/N, were closely relatable to foliar $\delta^{15}N$.

With respect to nutrient partitioning, all plant components are contributing factors. In corn, the roots, stalk, leaves, and cob engage in complex intra-plant N partitioning and are influenced by available soil nutrients and moisture (Nyiraneza et al. 2009).

Aranibar et al. (2004) examined N cycling and plant-soil system isotopic signatures on a precipitation gradient, and concluded that precipitation and resulting soil moisture gradients are the major drivers of soil N cycling. Coinciding with increased precipitation is increased soil moisture, which reduces oxygen and increases denitrification. Ostrom et al. (1998) found that temporal variation in $\delta^{15}N$ indicated that the isotopic composition of NO_3^- in spring and fall is controlled primarily by fractionation during mineralization, and by denitrification in the summer. This variation is largely driven by soil moisture differences throughout the growing season. Falkengren-Grerup et al. (2004) found that plant species benefiting from NO_3^- in ecosystems without significant NO_3^- leaching or denitrification have lower $\delta^{15}N$ values in their tissues than species growing equally well, or better, on NH_4^+. On the other hand, increased foliar $\delta^{15}N$ coincides with increased stream-water nitrate concentration, which suggests that the increased nitrification responsible for elevated stream-water nitrate may also cause enrichment of the plant-available NH_4^+ pool (Pardo et al. 2002).

The overall study objective is to investigate the spatial and temporal variability in plant $\delta^{15}N$ as an indicator of N transformation processes in the soil. This insight will determine the effectiveness and appropriateness of plant isotopic analysis in determining dominant soil N cycling processes and the potential relationship to soil moisture conditions. The overall objective will be achieved through the sampling of plants at varying soil moisture locations throughout the entire growing season, and will be supported by specific examination of $\delta^{15}N$ variability within a

single plant as well as several plants within a small area. The examination of corn specifically is important, as the presence of the various plant components will provide insight into nutrient and isotopic partitioning within plants, and how those strategies change in accordance to available N and soil conditions.

4.2 Study Site

Refer to Section 2.0 for a complete Strawberry Creek Watershed description, including site reports, land use, soils, and climatic variables.

4.3 Methods

4.3.1 Plot Treatments

The study focused on the three intensive monitoring sites in both R and T-Fields (Section 3.1). These intensive sites were established to measure field conditions with permanent instruments without being disturbed by the farming activity in the surrounding field. At these sites soil moisture, temperature, soil gas and flux were regularly monitored. The six intensive sites have been excluded from regular field treatments since the beginning of the 2006 growing season, so the agricultural activity conducted on the entire field was mimicked manually by field researchers in the individual plots. The soil at each site was turned over to a depth of approximately 15 centimetres by shovels to reflect the ploughing activity done across the rest of the field. A garden sized rotor tiller was brought in to break up the turned over soil, as cultivation and discing did in the field. The same variety of crop seeds used by the farmer was planted manually to the same depth and row spacing as the corn in R-Field and the oats in T-Field. However, the planting in the plots occurred three weeks after the planting in the rest of the field. For this study plants were sampled immediately outside the plots in the field itself in order to relate the isotopic data to changes in the corn that was representative of the larger field. Fertilization of the plots involved both adding starter fertilizer in with the seeds when planting, and mechanically inserting fertilizer in the R-Field corn plots during week 8. The second application of fertilizer directed at the corn plants in the R-Field occurred the same grow week as it did in the rest of the field, so a three week leg in both growth and fertilizer application occurred. As well, the three week window between field and plot planting created a large difference in soil moistures between field and plot plants at the same stage of growth, as the field soils were wetter in the three weeks prior to plot planting. Therefore, forcing manual watering of plants in the plots for approximately two weeks during the early stages of growth,

which allowed the plot crops to survive in dryer soil conditions and to develop on pace with the crops in the rest of the field. This watering activity occurred at all three plots in both the R-Field and the T-Field. Harvesting in the plots in both fields occurred approximately three weeks after the harvesting of the crops in the respective fields. Plot crop harvesting typically involved removing the entire plant, and leaving a similar amount of plant residue on the ground as was left by mechanized harvesting activity in the field. As well, plants in a randomly selected one square metre area in each plot were cut along the stalk at ground level and removed entirely. The plants were allowed to dry and then weighed in order to approximate plot biomass. The sites in the T-Field were planted with a wheat cover crop, while oat seeds were also broadcasted to mimic the field treatment. The soils in the R-Field plots, after harvesting, were turned over and remained in that condition throughout the winter. Organic fertilizer was not manually applied to the plots, but through the indirect broadcasting nature of solid manure application the sites did receive this treatment.

4.3.2 Plant and Soil Sampling

Plant and soil samples were collected during seven separate sampling events from all three sites in the R-Field throughout the 22 week growing season. The corn plants were sampled intact the first two times at weeks three and six, and soil was taken from around the plant roots to a depth of 10-15 centimetres. Due to the delay in planting the plots, plant extractions occurred immediately outside the plots within the field itself. Beginning in week nine, the plants were divided up into individual components and sampled that way. This required separate consideration for the roots, lower stalk, upper stalk, and leaves. As the plants developed the tassels and cob in subsequent weeks, these components were also treated separately and sampled individually. Soil variables, including moisture and temperature, were measured inside the plots during the seven extraction occasions. The R-Field sampling was supplemented with a

leaf variability survey at week 12, which involved the sampling of 10 plants within a 10 square metre area of the field that represented average soil moisture conditions, and where the plants themselves were visibly uniform. The site was chosen as representative of average moisture conditions because it was located on a mid slope area between the dry site (R1) and the wet site (R2). One leaf from each of the 10 plants was collected for analysis.

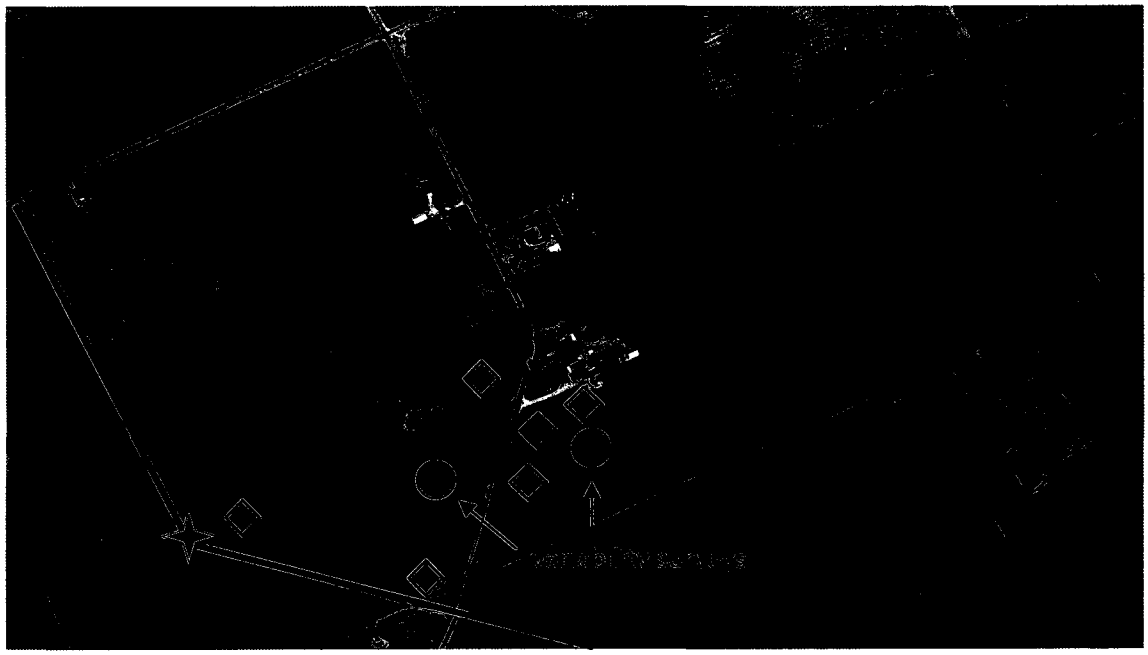

Figure 4-1: Location of the variability survey sites within the R and T Fields. The sites were chosen to closely reflect average field conditions representative of the entire area.

Plant and soil was sampled from all three sites in the T-Field as well, but in a slightly different manner. As oat plants are considerably smaller than corn plants, sampling involved extracting a cluster of three or four plants immediately beside one another in a common row. This occurred throughout the entire growing season, which at approximately 12 weeks contained four sampling events. Soil was collected from around the roots of the sampled plants to a depth of approximately 10 centimetres. The T-Field sampling was also supplemented with a plant variability survey, which involved the sampling of plants from 10 sites within a uniform 10 square metre area of the field that appeared representative of the entire field. Several plants

were sampled from each of the 10 points; however, only one of the plants was processed for analysis.

4.3.3 Sample Processing

Immediately upon returning from the field, the oat plants and corn root components were removed from the bags, and the soil was washed off as thoroughly as possible using DI water. The plants and roots were allowed to dry for 24 hours, and were then placed back into new bags and stored in the freezer for processing and analysis. The above ground corn plant components did not receive this initial treatment, but were placed in the freezer immediately after returning from the field.

Processing of the plant tissue involved several steps; the first of which consisted of measuring out material for inclusion in analysis. This step was necessary because, especially with the corn components, there was simply too much material to process. The roots, lower stalk, upper stalk, and the cob were divided, while the tassel and full oat plant were included intact. One leaf was taken from the seven or eight leaves typically grown on a corn stalk and was included intact as well. Samples were subsequently placed, still frozen, in the freeze-drier for a period of 48 to 72 hours to allow for complete moisture removal. Following this step, the plant tissue was run through a grind-mill to reduce the material down into small, rough pieces. Finally, the material size was reduced further by a ball-mill. The sample was placed in a metal cylinder with a ball-bearing inside and shaken for approximately 10 to 15 minutes, which transformed the plant components into a fine-powder.

Soil samples were also measured out into individual containers; approximately 100 grams were used. The soil was placed in the freeze-drier, typically for a period of time ranging between 48 and 72 hours, which allowed for complete moisture removal. At this point the samples were individually run through a two millimetre sieve, and the larger material was discarded (including

pebbles, plant material, and large soil clumps). The remaining soil was visually examined for ten minutes, and during that period of time smaller plant material was picked out and discarded. This material typically included small plant root fragments. The timing allowed for this step was significant, as the amount of small plant material included within the soil sample would have required hours to be completely removed. Therefore, a designated amount of time (ten minutes) was dedicated for each sample, regardless of the amount of organic material. Finally, the remaining sample was set aside for further processing and analysis, including C/N, $\delta^{15}N$, and $\delta^{13}C$.

4.3.4 Precision Calculation

The precision for the plant sample $\delta^{15}N$ and $\delta^{13}C$ values were determined using a variety of contributing factors. The analytical precision described in section 3.3.3 for both $\delta^{15}N$ and $\delta^{13}C$ combined with the determined variability within the plant, as well as within a 10 m^2 area. The 10 m^2 area is significant, as that is the area where all plants were sampled at each site throughout the growing season. These three values were combined using the standard equation for propagation of uncertainty (Eq. 4-1).

$$\boldsymbol{uncertainty} = \sqrt{\boldsymbol{a^2 + b^2 + c^2}} \qquad \textbf{Eq. 4-1}$$

Where a^2 is the analytical precision for isotopic analysis, b^2 is the standard error of the mean for intra-plant variability, and c^2 is the standard error of the mean for the site variability.

4.4 Results

The grow week, commonly referred to throughout the study, and the corresponding date are listed in the following; week 3: 24-Apr-07, week 6: 18-Jun-07, week 9: 10-Jul-07, week 12: 30-Jul-07, week 15: 22-Aug-07, week 18: 12-Sept-07, week 21: 04-Oct-07.

4.4.1 Soil Variables

Soil samples were collected in order to make comparisons with the isotopic analysis of the plants. Aside from the steady evaluation of moisture, soil was measured for $\delta^{15}N$, $\delta^{13}C$, and C/N ratios, and processed for extractable NO_3^- and NH_4^+ concentrations. Table 4-1 highlights the recorded soil moisture values measured in the upper 15 cm of the soil profile at each sampling site throughout the growing season.

	Min	Max	Mean	Std Dev	Std Err
R1	13.2	41.0	19.9	7.2	0.1
R2	11.5	58.0	27.3	13.3	0.2
R3	11.8	65.0	27.9	14.1	0.2
T1	10.0	38.0	18.7	7.1	0.2
T2	11.0	33.0	18.9	5.2	0.1
T3	15.0	43.0	21.8	7.8	0.2

Table 4-1: Soil moisture variability at all six intensive sites throughout the growing season. The averaged soil moisture values verify an increase in moisture from R1 to R3 and T1 to T3. A complete list of all soil moisture data can be found in Appendix A, Figure A-1.

		R1			R2			R3		
		$\delta^{15}N$	$\delta^{13}C$	C/N	$\delta^{15}N$	$\delta^{13}C$	C/N	$\delta^{15}N$	$\delta^{13}C$	C/N
Grow	**3**	8.1	-21.7	12.6	8.3	-19.9	13.3	5.8	-24.2	16.5
Week	**21**	8.4	-20.5	13.1	7.8	-21.5	13.1	5.5	-23.1	16.8

Table 4-2: Comparison between $\delta^{15}N$, $\delta^{13}C$, and C/N ratios for soils at all R-Field sites between weeks 3 and 21. Soil was collected from the upper 15 centimetres of the soil profile around the roots of the sampled plant.

		T1			T2			T3		
		$\delta^{15}N$	$\delta^{13}C$	C/N	$\delta^{15}N$	$\delta^{13}C$	C/N	$\delta^{15}N$	$\delta^{13}C$	C/N
Grow	**3**	9.30	-15.8	18.3	7.9	-18.4	14.2	9.2	-23.1	11.3
Week	**12**	9.0	-15.6	18.6	7.9	-16.5	15.6	9.0	-23.3	11.2

Table 4-3: Comparison between $\delta^{15}N$, $\delta^{13}C$, and C/N ratios for soils at all T-Field sites between weeks 3 and 21. Soil was collected from the upper 15 centimetres of the soil profile around the roots of the sampled plant.

Table 4-2 displays the difference in $\delta^{15}N$, $\delta^{13}C$, and C/N ratios between weeks 3 and 21 at all three R-Field sites. These two end point samples were selected in both fields as representative of the entire growing season. The $\delta^{15}N$ values increase at R1 from 8.1 to 8.4‰, and decrease at sites R2 and R3 (8.3 to 7.8‰ and 5.8 to 5.5‰) between the first and last sampling periods of the growing season. An additional sample collected in early July at the R3 site yielded a $\delta^{15}N$ value of 6.1‰, which remains fairly consistent with the end value points. The lack of change established that the bulk soil did not change over time, as the differences were not significant when considering the error.

On average, the soil becomes more depleted in ^{15}N from site R1 through to R3, while C/N ratios have the opposite effect. Soil at R1 maintains a C/N ratio of 12.6 in week 3 and 13.1 in week 21. At site R2, the C/N ratio decreases from 13.3 to 13.1, and increases at R3 from 16.5 to 16.8. In the T-Field, C/N ratios decreased from sites T1 to T3 on average (Table 4-3). Site T3 was the only site to show a C/N decrease between the two sampling events. The difference in $\delta^{13}C$ among the three sites is significant, as is the difference between $\delta^{13}C$ values in soils throughout the two fields. The difference in soil $\delta^{13}C$ is more pronounced in drier soil rather than the wet soil sites. The corn-oat rotation employed in both studied fields provides a mix in plant residue within the soil of both C3 and C4 plant tissue, which introduces significant variability in $\delta^{13}C$.

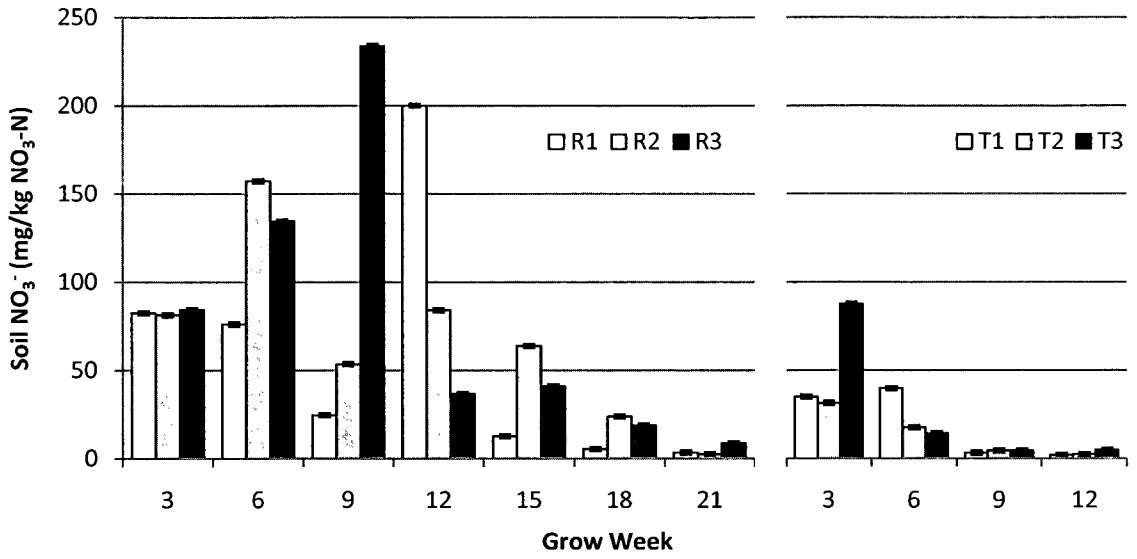

Figure 4-2: Extractable soil NO_3^- from samples taken at all three R-Field intensive sites throughout the corn growing season, as well as extractable NO_3^- from soils at the three T-Field intensive sites throughout the oat growing season. The values are based on one extraction per site per sampling period.

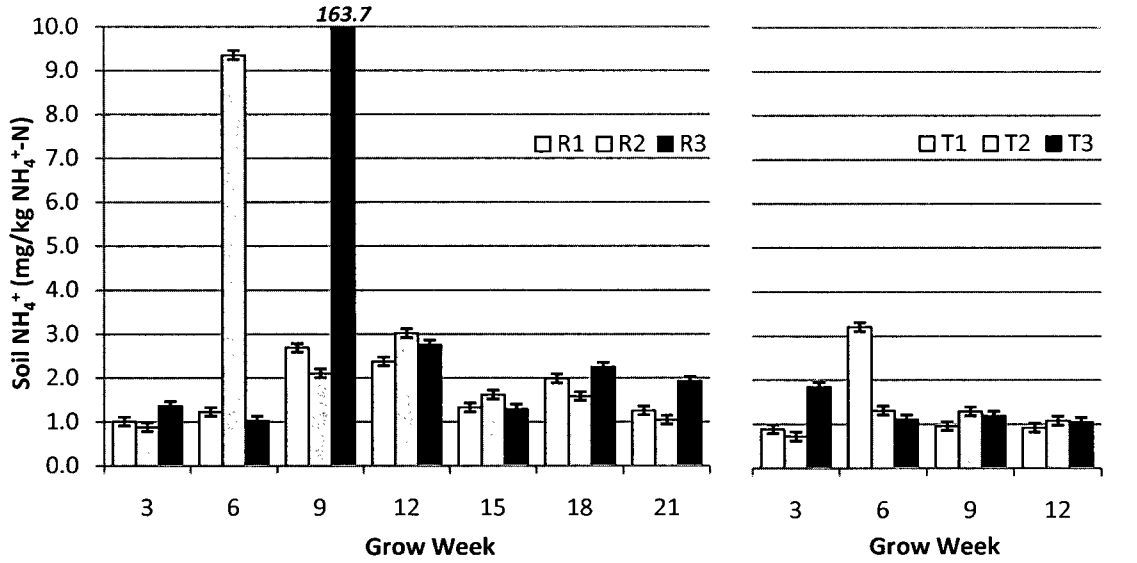

Figure 4-3: Extractable soil NH_4^+ from samples taken at all three R-Field intensive sites throughout the corn growing season, as well as extractable NH_4^+ from soils at the three T-Field intensive sites throughout the oat growing season. The values are based on one extraction per site per sampling period.

Figures 4-2 and 4-3 offer a snapshot of extractable NO_3^- and NH_4^+ respectively from the soils at all intensive sites in both fields during the respective crop' growing seasons. NO_3^- at site R1 starts at 82.41 mg/kg NO_3^--N and decreases every three weeks until experiencing a massive spike bringing the concentration in the soil to 200.06 mg/kg NO_3^--N, which could be a product of the second inorganic fertilizer treatment applied to the crops. After this large spike, the concentration dips down to 12.71 mg/kg NO_3^--N and continues to decrease until bottoming out in week 21 at 3.42 mg/kg NO_3^--N. Site R2 follows a similar trend, although the NO_3^- spike at this site (157.19 mg/kg NO_3^--N) occurs six weeks earlier than the spike at R1. Soil NO_3^- starts out at 81.34 mg/kg NO_3^--N before spiking, and beginning in week 12 experiences a steady decline from 84.09 mg/kg NO_3^--N to a low of 2.59 mg/kg NO_3^--N in week 21. R3 starts out at 84.25 mg/kg NO_3^--N, spikes in week 9 at 233.76 mg/kg NO_3^--N, and decreases to a low (8.59 mg/kg NO_3^--N) during the final sampling period.

The actual NO_3^- concentrations vary significantly, but soils at all three sites start out approximately the same and end with very low concentrations during week 21. On average, the soils in the T-Field have much lower NO_3^- concentrations than those in the R-Field, which could have something to do with the differences in fertilization or crop uptake preferences and demands. As well, corn requires more nutrients for growth, so it would be logical that those soils would contain more nutrients in the initial half of the growing season. The corn plants did receive an additional fertilizer application during that time.

Soil NH_4^+ concentrations are generally much lower than NO_3^-, as NO_3^- is the dominate N form available for crops. The highest NH_4^+ values at each R-Field site were measured the same week as the highest measured NO_3^- concentration, indicating the soil response to the second fertilizer treatment in the R-Field at nine weeks. Most notably, R2 at week 6 (9.35 mg/kg NH_4^+-N), and more significantly site R3 at week 9 (163.74 mg/kg NH_4^+-N), NH_4^+ values are significantly higher

than any other inorganic N concentrations measured at those sites, which introduces a considerable amount of variability to the soil concentrations. At R3, the mean value is 24.91 mg/kg NH_4^+ -N while the standard error of the mean is a very high 24.99 mg/kg NH_4^+ -N. If the week 9 value is removed, the average decreases to 1.77 mg/kg NH_4^+ -N and the standard error of the mean to 0.30. The trend in soil NH_4^+ is not nearly as clear or obvious as with NO_3^-; however, values typically start out fairly low, and only pick up around the time of fertilizer application. NH_4^+ concentrations in soils at the T-Field sites maintain lower values, and lack the dramatic spikes seen in the R-Field soils. However, at both sites T1 and T2 there exists an increasing trend between weeks 3 and 6, with a subsequent decrease until harvest after week 12. Extractable soil NH_4^+ at T3 does not appear to follow any apparent trend, as the values rise and fall in two six week periods. The mean NH_4^+ value is highest at T1 (1.49 mg/kg NH_4^+ -N), and is followed by T3 (1.30 mg/kg NH_4^+ -N) and T2 (1.10 mg/kg NH_4^+ -N).

	Grow Week							Mean	Std Dev	Std Error
	3	6	9	12	15	18	21			
R1	1.01	1.22	2.68	2.37	1.32	1.98	1.25	1.69	0.65	0.27
R2	0.88	9.35	2.10	3.02	1.62	1.58	1.04	2.80	2.98	1.21
R3	1.36	1.03	163.74	2.76	1.29	2.25	1.93	1.77	0.66	0.30

Table 4-4: Extractable soil NH_4^+ from samples taken at three R-Field intensive sites throughout the growing season. Site R3 variability indicators do not consider the week 9 extracted soil NH_4^+ concentration.

	Grow Week				Mean	Std Dev	Std Error
	3	6	9	12			
T1	0.88	3.20	0.95	0.92	1.49	1.42	0.57
T2	0.72	1.30	1.29	1.07	1.10	0.27	0.14
T3	1.84	1.10	1.19	1.04	1.30	0.37	0.19

Table 4-5: Extractable soil NH_4^+ from samples taken at three T-Field intensive sites throughout the growing season. Table also includes significant variability indicators; mean, standard deviation, and standard error of the mean.

	Grow Week							Mean	Std Dev	Std Error
	3	6	9	12	15	18	21			
R1	82.42	76.21	24.61	200.06	12.71	5.34	3.42	57.82	70.74	26.74
R2	81.34	157.19	53.49	84.09	63.84	23.89	2.59	66.63	49.72	18.79
R3	84.25	134.39	233.76	36.75	41.04	18.81	8.59	79.65	80.47	30.41

Table 4-6: Extractable soil NO_3^- from samples taken at three R-Field intensive sites throughout the growing season.

	Grow Week				Mean	Std Dev	Std Error
	3	6	9	12			
T1	35.02	39.81	3.27	2.06	20.04	20.16	10.08
T2	31.42	17.71	4.52	2.44	14.02	13.43	6.71
T3	87.60	14.35	4.57	4.89	27.85	40.09	20.04

Table 4-7: Extractable soil NO_3^- from samples taken at three T-Field intensive sites throughout the growing season.

4.4.2 Intra-Plant Isotopic Variability

The investigation of variability among plants at various locations within a field requires an understanding of the variability exhibited within a single plant. This is especially true if assumptions are made that the leaf represents the entire plant isotopically. As evident in tables 4-9 and 4-10, there exists only a small standard error of the mean in plants at all three sites' grow weeks 15 and 18. However, as corn plants can potentially produce more than one cob or leaf, there requires the need to understand the variability within a single component type. As leaves are the most numerous individual component on a single plant, it would seem logical to investigate its variability. Table 4-8 highlights significant variability indicators for eight leaves sampled from a plant at site R3 during week 18 of the crop growing season. In $\delta^{15}N$ there is a large range (4.9 to 9.2‰) with a mean of 8.2‰ and a standard error of 0.5. However, if leaf 2 is excluded from consideration, the range tightens up (8.1 to 9.2‰) with a mean of 8.7‰ and a standard error of 0.1. It is unknown whether the extreme difference in $\delta^{15}N$ for leaf 2 is an anomaly or the result of the growing conditions during the development of that specific leaf. Regarding $\delta^{13}C$, the range (-13.1 to -12.2‰), mean (-12.8‰), and standard error (0.1) is less than $\delta^{13}C$ values for the various components of a single plant.

	# of Leaves	Min Value	Max Value	Mean	Std Dev	Std Error
$\delta^{15}N$	8	4.9	9.2	8.2	1.4	0.5
$\delta^{13}C$	8	-13.1	-12.2	-12.8	0.3	0.1

Table 4-8: Variability in $\delta^{15}N$ and $\delta^{13}C$ between leaves of a single corn plant. Samples were taken from a plant at site R3 at crop grow week 18. The full set of data for the eight corn leaves can be found in Appendix A (Figure A-3).

		Root	Lower Stalk	Upper Stalk	Cob	Leaf	Tassel	Mean	Std Dev	Std Error
R2	$\delta^{15}N$	15.5	17.8	12.5	13.7	14.2	12.0	14.3	2.1	0.9
	$\delta^{13}C$	-13.1	-12.4	-12.7	-11.8	-13.0	-12.7	-12.6	0.5	0.2
R3	$\delta^{15}N$	7.5	8.3	7.7	8.7	8.3	7.3	8.0	0.6	0.2
	$\delta^{13}C$	-12.3	-12.1	-12.3	-11.8	-12.8	-12.4	-12.3	0.4	0.1

Table 4-9: Comparison between $\delta^{15}N$ and $\delta^{13}C$ of all plant components from sites R2 and R3 at plant grow week 15. Table also includes variability indicators; mean, standard deviation, and standard error of the mean.

A final measure of the variability within a plant is the comparison of $\delta^{15}N$ and $\delta^{13}C$ for all plant components, and an examination of the standard error of the mean (Table 4-9). As established earlier, the variability within the plant is low enough that one component can represent the entire plant. Typically, this one component has been the plant leaf. The range in $\delta^{15}N$ for components of a plant at site R3 during week 15 (7.3 to 8.7‰) is significantly less than components from a plant at R2 (12.0 to 17.8‰) during the same sampling event. That range is reflected in the standard error at the two sites (0.2 and 0.9 for R3 and R2 plants, respectively). As per usual, significant range is not demonstrated in the $\delta^{13}C$ of the plant components, as the standard error at R3 is 0.1 and 0.2 at site R2.

4.4.3 Crop Isotopic Variability

To quantify plant isotopic variability at the plot-scale, a random concentrated sampling occurred at one site in each field. The sites were selected at the mid-point between the three intensive site locations, where the plants kept uniform colour and biomass productivity. Figure 4-4 shows the plot-scale variability of leaf $\delta^{15}N$ against $\delta^{13}C$ for both crop types. The potential value for supplementing $\delta^{15}N$ analysis with $\delta^{13}C$ data is outlined in Section 1.3.7. Aside from vastly different average $\delta^{13}C$ values (-12.4 and -27.7‰) for corn and oats respectively, there exists significant differences in the variability between the two crop types (Table 4-10). The $\delta^{15}N$ standard error of the mean for the corn and oat plants is 0.2 and 0.3, respectively. The $\delta^{13}C$ standard error of the mean is only 0.1 for corn and 0.2 for oats. The reduced $\delta^{15}N$ range in corn

plants compared to oat plants is likely attributed to the larger root areas of the corn plants, which averages small-scale inorganic N variability. The small level of variability in both crops verifies that plant sampling at a single site can be related without requiring collection from the same plant every three weeks.

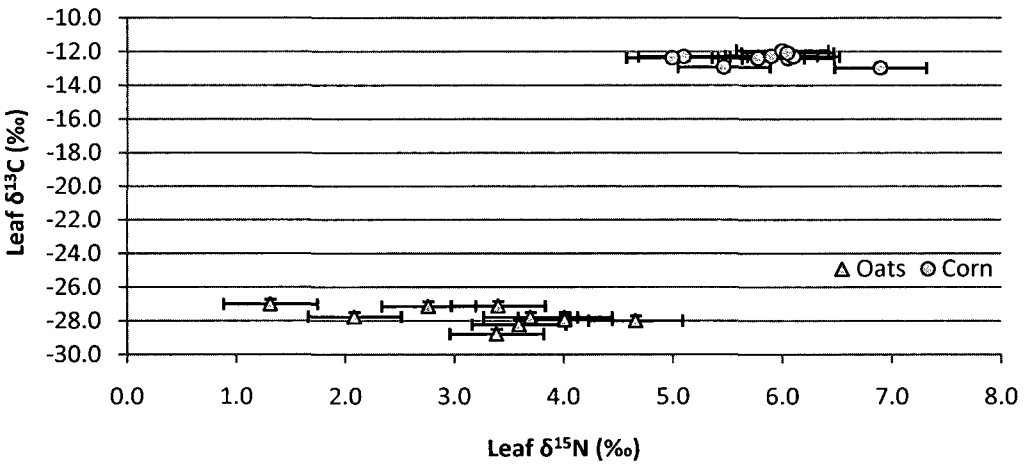

Figure 4-4: Isotopic $\delta^{15}N$ and $\delta^{13}C$ variability of 10 corn and oat plants sampled from a 10 m^2 area with visibly similar soil characteristics. Precision for corn ($\delta^{15}N$ = ±0.42, $\delta^{13}C$ = ±0.25‰), and oats ($\delta^{15}N$ = ±0.43, $\delta^{13}C$ = ±0.28‰).

		# of Samples	Min Value	Max Value	Mean	Std Dev	Std Error
Corn	$\delta^{15}N$	10	5.0	6.9	5.8	0.6	0.2
	$\delta^{13}C$	10	-13.0	-11.9	-12.4	0.3	0.1
Oats	$\delta^{15}N$	10	1.3	4.7	3.3	1.0	0.3
	$\delta^{13}C$	10	-28.8	-27.0	-27.7	0.6	0.2

Table 4-10: Significant variability indicators, including mean, standard deviation, and standard error of the mean for both corn and oats. Sampling occurred within a 10 m^2 area in each field.

4.4.4 Intra-Field Isotopic Variability

Plant tissue $\delta^{15}N$ varied significantly among the three intensive sites within the R-Field during the 2007 growing season (Figure 4-5). On average, site R1 measured the lowest $\delta^{15}N$ of plant leaf tissue (5.1‰) followed by R3 and R2 (9.7and 12.7‰ respectively) (Table 4-11). Site R3 experienced the largest range in $\delta^{15}N$ values (6.6 to 13.9‰), while plants at sites R1 and R2 remained fairly steady throughout the growing season (4.3 to 6.4‰, and 10.9 to 14.2‰ respectively). The $\delta^{13}C$ held much more consistently at all three intensive sites (R1, R2, and R2) with very low standard errors. Mean values for the leaves at each site were the lowest at site R1 (-12.9‰), followed by R3 (-12.7‰), and R2 (-12.4‰). This trend is consistent with the mean $\delta^{15}N$ values for leaves at each site, which increase from the lowest at site R1 (5.1), followed by R3 (9.7‰), and R2 (12.7‰). The similarity suggests there are linkages worth exploring in later analysis.

Plant tissue $\delta^{15}N$ also varies significantly among the three intensive T-Field sites during 2007 (Figure 4-6). However, the general trend of higher $\delta^{15}N$ values at T3 then T1 and T2 is consistent with the overall trends seen in the R-Field with respect to soil moisture. Site T3 had consistently higher soil moisture values then both sites T1 and T2 (Appendix A, Figure A-1).

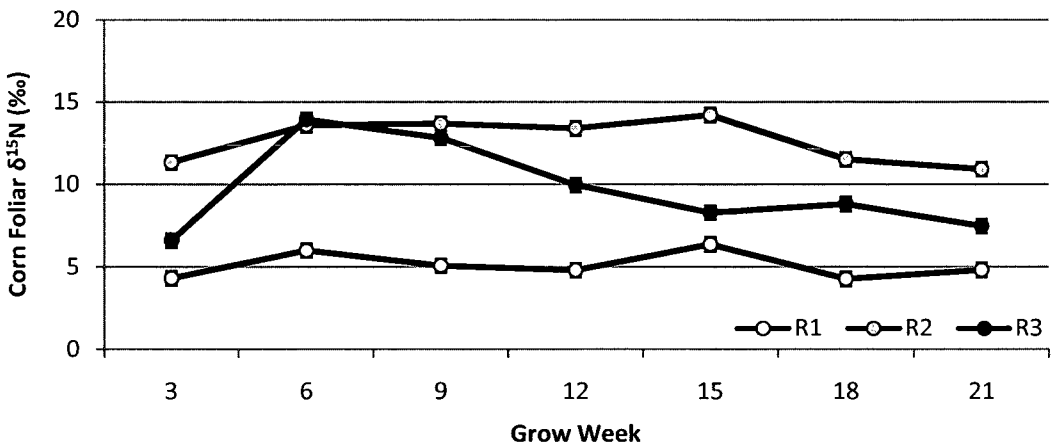

Figure 4-5: $\delta^{15}N$ of leaf tissue at all three R-Field corn intensive sites during the approximate 21 week 2007 growing season. Precision was calculated at ±0.42‰.

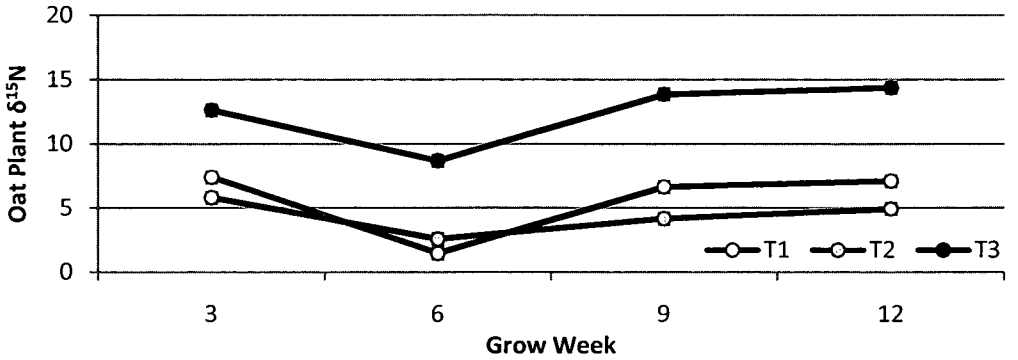

Figure 4-6: $\delta^{15}N$ of leaf tissue at all three T-Field oat intensive sites during the approximate 12 week 2007 growing season. Precision was calculated at ±0.43‰.

		Grow Week							Mean	Std Dev	Std Error
		3	6	9	12	15	18	21			
R1	$\delta^{15}N$	4.3	6.0	5.1	4.8	6.4	4.3	4.8	5.1	0.7	0.3
	$\delta^{13}C$	-12.4	-12.4	-12.7	-13.2	-13.2	-12.9	-13.5	-12.9	0.4	0.1
R2	$\delta^{15}N$	11.3	13.6	13.7	13.4	14.2	11.5	10.9	12.7	1.3	0.5
	$\delta^{13}C$	-11.9	-12.1	-12.3	-12.3	-13.0	-12.7	-12.5	-12.4	0.4	0.1
R3	$\delta^{15}N$	6.6	13.9	12.8	10.0	8.3	8.8	7.5	9.7	2.5	1.0
	$\delta^{13}C$	-12.4	-13.8	-12.4	-12.4	-12.8	-12.2	-12.8	-12.7	0.5	0.2

Table 4-11: $\delta^{15}N$ and $\delta^{13}C$ of corn leaves sampled every three weeks at three R-Field intensive sites during the 2007 growing season. Table also includes significant variability indicators; mean, standard deviation, and standard error of the mean.

The variability in $\delta^{15}N$ of plants at the three intensive sites located in the T-Field is slightly higher than what is seen in the R-Field. The T-Field exhibits a weaker moisture gradient with relatively little difference in average moisture values between sites T1 and T2. This also serves in increasing the difficulty in interpreting patterns and processes occurring in the soil.

Site T1 displays the largest amount of isotopic variability across the four sampling periods (Table 4-12). The larger standard error of the mean value (1.4) is almost exclusively because of the plant $\delta^{15}N$ value of 1.5‰ occurring at week six. However, as mentioned earlier, these lower values occur at all sites during this sampling time and must be taken into consideration. The reason for the lower values at week six is likely due to the fact these plants were sampled too close to the site, rather than in the surrounding field. The significance of this is that the plants in the site were planted three weeks later than the rest of the field, and were watered on several occasions. Therefore, at week six plants from sites T1, T2, and T3 all exhibit lower $\delta^{15}N$ values than expected. Site T2 maintains more $\delta^{15}N$ consistency throughout the 12 week growing period, and exhibits a standard error of only 0.7. T3 follows a similar pattern to that of T1 with substantially decreased plant $\delta^{15}N$ at week six (8.7‰) and a higher standard error of 1.3. The mean $\delta^{15}N$ values at sites T1 and T2 are fairly similar (5.6 and 4.4‰, respectively) while T3, with a higher average moisture regime than sites T1 and T2, produces a much higher plant $\delta^{15}N$ average of 12.4‰ during the growing season.

Table 4-13 highlights the $\delta^{15}N$ of all plant components at all intensive sites in the R-Field during week nine. The $\delta^{15}N$ values of the five various components, with the stalk divided into an upper and lower portion, exhibits very little variation (standard error of 0.2) at site R1. The lowest value was seen in the cob (4.5‰), highest value in the tassel (5.6‰), with an overall plant mean of 5.1‰. R2 conveys slightly more variation (standard error of 0.3), and the most enriched plant $\delta^{15}N$ with a mean of 13.6‰. The most depleted value is seen in the roots (12.2‰) while the upper stalk contains the highest value (14.2‰). The upper stalk is characterized in this study as the portion of the plant stalk above the cob section. The highest $\delta^{15}N$ signature in the plant at R3 is 12.2‰, which was found within the leaf. The lowest value (9.4‰) was found in the roots, while the plant (mean value of 11.3‰) displayed the most variability (standard error of 0.5) of plants at all three sites from week nine. The mean $\delta^{15}N$ from plants at the three intensive sites follows the same trend as the leaves exhibited from all three sites, as the lowest plant value is found at R1, followed by sites R3 and R2.

		Grow Week				Mean	Std Dev	Std Error
		3	6	9	12			
T1	$\delta^{15}N$	7.4	1.5	6.6	7.1	5.6	2.8	1.4
	$\delta^{13}C$	-28.0	-27.2	-28.0	-28.3	-27.9	0.5	0.2
T2	$\delta^{15}N$	5.8	2.6	4.2	4.9	4.4	1.4	0.7
	$\delta^{13}C$	-26.6	-27.7	-27.4	-27.9	-27.4	0.6	0.3
T3	$\delta^{15}N$	12.6	8.7	13.9	14.4	12.4	2.6	1.3
	$\delta^{13}C$	-28.2	-28.7	-26.6	-27.6	-27.8	0.9	0.5

Table 4-12: $\delta^{15}N$ and $\delta^{13}C$ of whole oat plants sampled every three weeks at three T-Field intensive sites during the 2007 growing season. Table also includes significant variability indicators; mean, standard deviation, and standard error of the mean.

	Root	Lower Stalk	Upper Stalk	Cob	Leaf	Tassel	Mean	Std Dev	Std Error
R1	5.4	5.0	5.1	4.5	5.1	5.6	5.1	0.4	0.2
R2	12.2	13.4	14.2	14.2	13.7	13.8	13.6	0.7	0.3
R3	9.4	11.1	11.3	11.6	12.8	11.8	11.3	1.1	0.5

Table 4-13: $\delta^{15}N$ of various plant components from all three R-Field sites at crop grow week 9. Table also includes significant variability indicators; mean, standard deviation, and standard error of the mean.

The $\delta^{15}N$ of individual components from plants at the three intensive sites at week 21 follow a similar trend in plant mean values with all experiencing depleted $\delta^{15}N$ values (Table 4-14). Site R1 still maintains the lowest average value at 5.4‰, followed by R3 and R2, at 6.8 and 11.8‰ respectively. The variability in $\delta^{15}N$ among the components for all three plants is opposite to the values seen in week 9, as site R1 contains the largest standard error of the mean (0.6) followed by R2 (0.5) and R3 (0.4). The lowest $\delta^{15}N$ at R1 is found within the tassel (3.5‰) while the highest (7.8‰) is seen in the lower stalk. At site R2, the lowest value is in the tassel (9.8‰) and the highest value is in the cob (13.0‰). Finally, at R3, the lowest signature is exhibited within the tassel (5.2‰) and the highest within the cob (8.0‰). The increasing variability in $\delta^{15}N$ for all components in plants between weeks 9 and 21 is likely the result of plant uptake and partitioning strategies, and may provide significant insight into soil N cycling analysis.

	Root	Lower Stalk	Upper Stalk	Cob	Leaf	Tassel	Mean	Std Dev	Std Error
R1	6.5	7.8	4.9	4.9	4.8	3.5	5.4	1.5	0.6
R2	12.5	12.5	11.9	13.0	10.9	9.8	11.8	1.2	0.5
R3	6.7	6.8	6.6	8.0	7.5	5.2	6.8	1.0	0.4

Table 4-14: $\delta^{15}N$ of various plant components from all three R-Field sites at crop grow week 21. Table also includes significant variability indicators; mean, standard deviation, and standard error of the mean.

4.5 Discussion

4.5.1 Nitrogen Uptake and Plant Partitioning

The benefit in studying corn in an agricultural system is that intra-plant nutrient and isotope partitioning is more easily identifiable than in forest or grassland vegetation due to its well defined temporal change in structure. The partitioning strategies of corn plants follow generalized patterns, except during periods of soil N deficiency or water stress (Gastal and Lemaire 2002; Nyiraneza et al. 2009). Typically, corn plants partition N from the stalk and leaves to the grain during peak production periods, but that relationship can change in respect to available soil N. Paponov and Engels (2005) examined the effect of N supply on N partitioning in corn plants during the grain production stage, and determined that excess N accumulation in the plant grain was covered primarily by depletion of N in the stalk in soils with high N availability. In soils with low availability the N accumulation within the grain was covered by the depletion of leaf N. The specific changes within plant components during the growing season at sites R1 and R3 would provide a valuable indicator of soil N status, while also providing insight into how nutrients are moved within a plant and any potential corresponding isotopic effect. The trend in plant $\delta^{15}N$ at sites R1 and R2 include overall similarities, and differ quite dramatically than the $\delta^{15}N$ from plant components at site R3 (Figure 4-7: A-C). Generally, plant components from R1 and R2 are more enriched in $\delta^{15}N$ at week 15, while plant components at R3 see the highest $\delta^{15}N$ values during week 9. The dominant N sources for the three sites would be primarily the same, as the entire field was treated with inorganic fertilizer twice during the growing season, while the soil N pool contained organic manure and crop residual matter. The addition of organic manure is known to increase plant $\delta^{15}N$ while the addition of inorganic fertilizer decreases plant $\delta^{15}N$ (Robinson 2001; Choi et al. 2003).

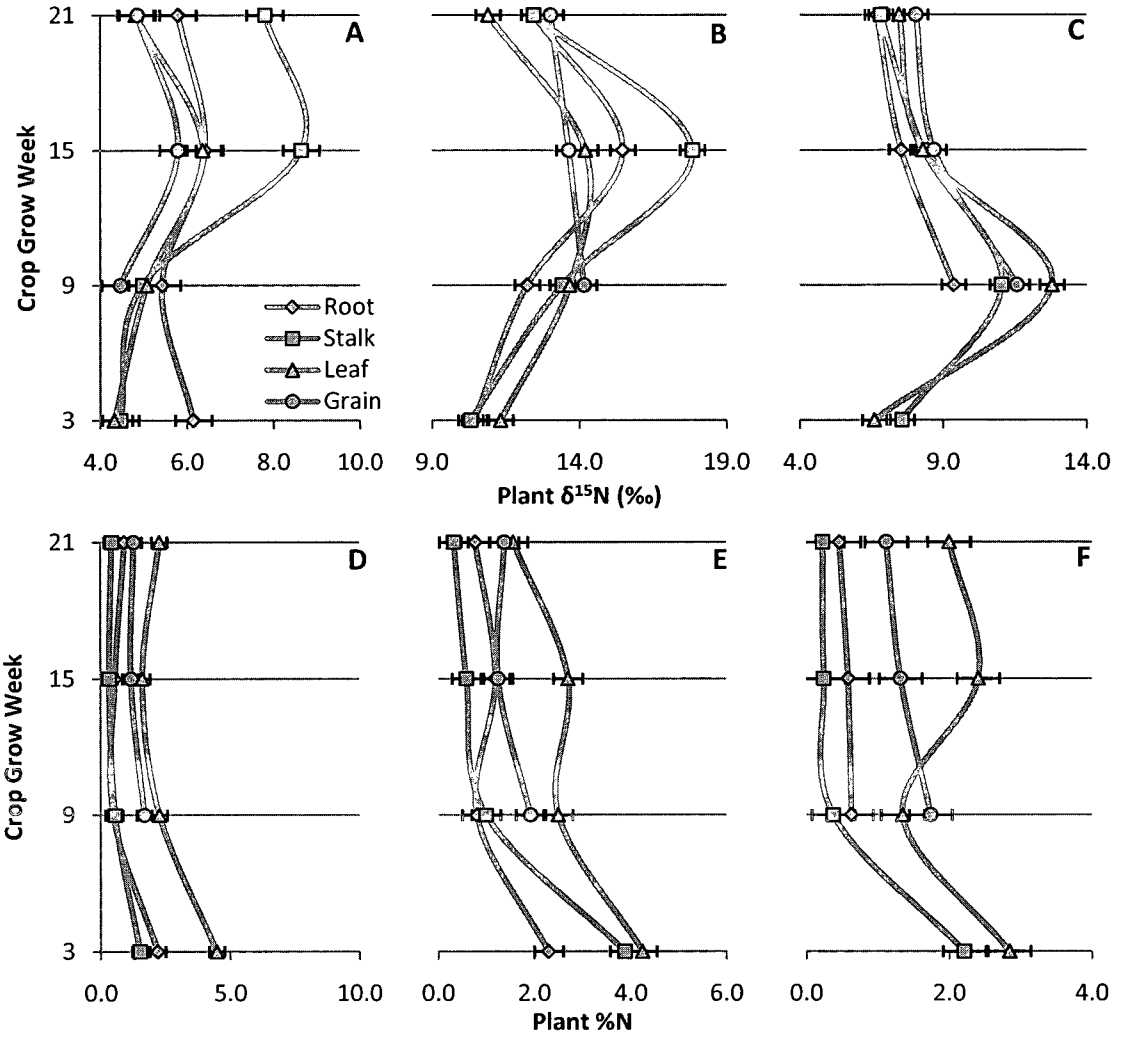

Figure 4-7: Figures A-C display the change in $\delta^{15}N$ among the various corn plant components – root, stalk, leaf, and cob – at four sampling periods throughout the growing season. Figures A through C represent whole plants from sites R1 through R3, respectively. Figures D-F display the change in total %N of the individual components. Figures D through F represent whole plants from sites R1 through R3, respectively.

As evident in Figure 4-7, the addition of inorganic fertilizer during initial planting and again in week nine appeared to have a negligible effect on plant $\delta^{15}N$. The increase in $\delta^{15}N$ of all plant components at site R1 and R2 between weeks nine and 15 suggests that the plants are relying more on the soil N pool for N uptake rather than the applied N, while the decrease in plant $\delta^{15}N$ at site R3 during that same period may indicate the crop reliance on the applied inorganic N. Sampled soil at site R3 at week nine contained an extractable NO_3^- concentration of 233.76 mg/kg NO_3^--N, which was considerably higher than concentrations at the other sites. The plants within R3 appear to have utilized that available N extensively during the subsequent grow weeks, while the remaining NO_3^- was likely lost to the atmosphere through denitrification or to ground and surface water through leaching (Addiscott 2005).

The change in %N among the various components from plants across the three sites is likely to indicate more strongly the partitioning strategies and the influence of available soil N. As corn uptakes available N continuously during plant growth, the majority of N taken up in the later stages is accumulated primarily within the plant stalk (Subedi and Ma 2005). The foliar %N appears to be a potential predictor of overall changes in plant $\delta^{15}N$, as a reduction in %N coincides with increased plant $\delta^{15}N$ values. A relative decrease in foliar %N signifies a lack of available N, as the plant is required to shift N from the leaves to the cob rather than from the stalk to the cob (Paponov and Engels 2005). The decrease in available N and potential loss could be the result of denitrification, which is reflected in the elevated $\delta^{15}N$ values of the plant. The effects of denitrification are enhanced during periods of wetter soils and subsequent decrease in O_2, which coincides here to the later stages of corn growth (Ostrom et al. 1998).

Subedi and Ma (2005) concluded that corn that was provided an adequate N supply only until plant silking provided grain with similar yields and total %N to plants grown with adequate N supply throughout the entire season. The findings suggest that plant strategies are developed

based on conditions early in plant growth, and are altered based on changing conditions and prevailing soil N availability. Those strategies also develop based on the preference and availability of NO_3^- and NH_4^+. The preference and availability of NO_3^- and NH_4^+ serves to alter the isotopic partitioning of ^{15}N within corn plants. Significant intra-plant variation is observed when NO_3^- is the primary N source, but not when NH_4^+ is the primary N source (Ostrom et al. 1998; Paponov and Engels 2005). A lack of significant intra-plant variability could suggest a crop preference for NH_4^+. However, the changes in foliar $\delta^{15}N$ from plants at all three sites are more closely related to the changes in extractable soil NO_3^- than soil NH_4^+, which combined with the overall variability visible in plant $\delta^{15}N$, indicates a crop preference and significant plant response to soil NO_3^-.

4.5.2 Plant Isotopic Analysis and Nitrogen Cycling Indicators

In the R-Field, $\delta^{15}N$ of sampled corn leaves revealed significant patterns across the range of dominate moisture condition sites. The R1 foliar $\delta^{15}N$ values were consistently lower than the more $\delta^{15}N$ enriched leaves at R2 throughout the entire growing season (Figure 4-8). The soils at site R3 experienced the greatest range in moisture contents throughout the season, and therefore produced plant $\delta^{15}N$ values that crossed into the range of $\delta^{15}N$ values seen in plants from the other two sites.

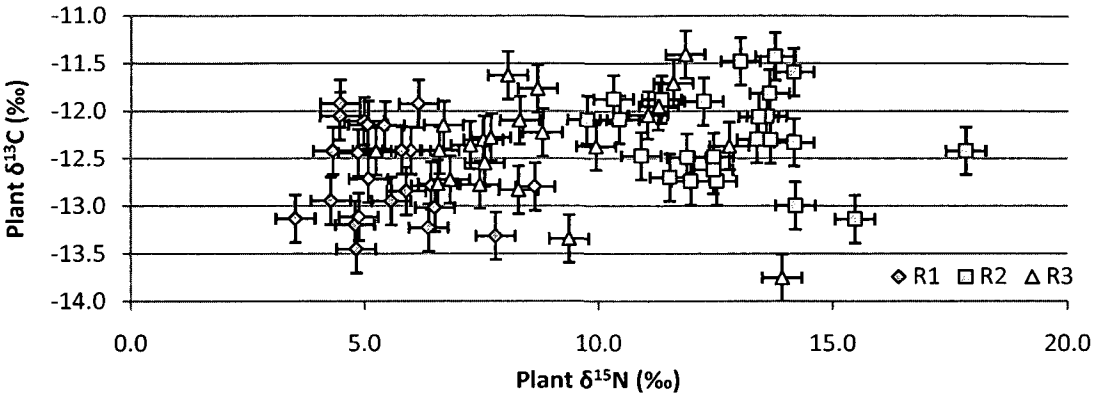

Figure 4-8: Relative comparison among all plant components at the three R-Field sites. The $\delta^{15}N$ values for components from site R2 are on average more enriched than the $\delta^{15}N$ values from the other two sites, especially R1. The $\delta^{13}C$ values also gradually increase from site R1 through R3.

As the plant $\delta^{15}N$ reflects those complex processes and is obscured by discrimination during N uptake and intra-plant N partitioning the $\delta^{15}N$ values alone cannot indicate specific N cycling processes. As well, plant $\delta^{15}N$ values reflect the soil conditions and cycles of the entire growing season before extraction, and due to the above mentioned processes the link between plant $\delta^{15}N$ and soil N becomes less distinct over time (Robinson 2001; Choi et al. 2003). The deterioration of this link occurs primarily because N fractionation interacts with the $\delta^{15}N$ of source N during plant growth (Liang and Mackenzie 1994; Choi et al. 2003; Serret et al. 2008). The examination of specific changes in plant component $\delta^{15}N$ would be a valuable tool in identifying N cycling processes and its fractionation effects. Figure 4-9 illustrates the added N during three week ranges for three specific plant components at all three intensive sites.

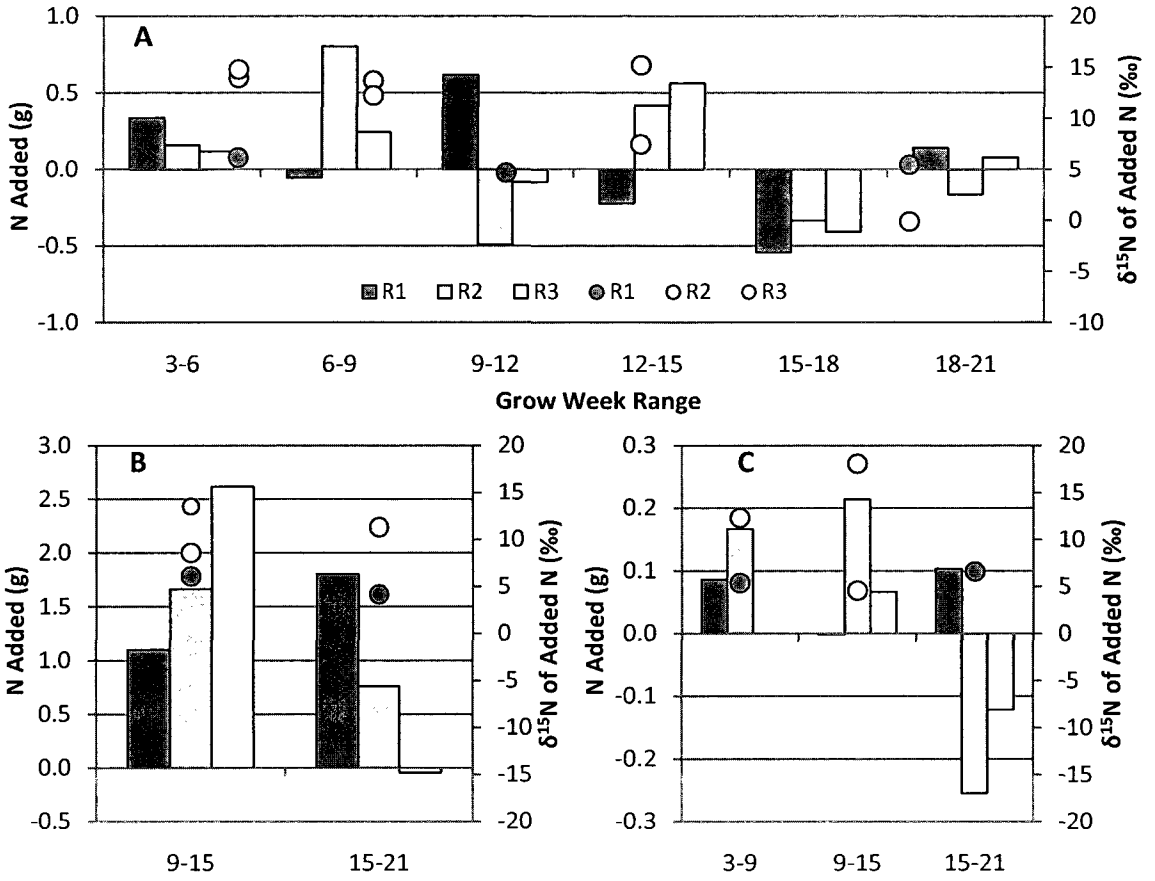

Figure 4-9: Specific $\delta^{15}N$ of N added to plant components during a specified week range. Figure A shows $\delta^{15}N$ of N added to corn leaves during six specific three week ranges, while figures B and C show $\delta^{15}N$ of N added to grain and roots, respectively.

The significance in examining the $\delta^{15}N$ of added N to plant leaves, grain, and roots is that it provides a measure of the $\delta^{15}N$ of N added during a specific time interval. Subsequently, when comparing the various plant components during similar time frames it may potentially allow for the elimination of N source influence and the identification of specific, dominate N cycling processes. However, the use of this analysis is limited in that each targeted plant component must increase in total N during the three week time windows, and due to plant uptake strategies and N partitioning, that does not always happen. During the period between weeks 15 and 21 the grain in plants from R1 gained approximately 1.8 grams of N, while the $\delta^{15}N$ value of that

specific amount of N was 4.2 ‰. Concurrently, the roots of the plants at R1 gained approximately 0.1 grams of N, while the $\delta^{15}N$ value of that specific amount of N was 6.6 ‰.

Kahmen et al. (2008) concluded that foliar $\delta^{15}N$ is the most valuable indicator of soil N cycling processes, while Templar et al. (2007) found that root $\delta^{15}N$ is the strongest proxy for relative rates of soil N cycling. Hood et al. (2002), Emmett et al. (1998), and Pardo et al. (2002) have all explored the potential for utilizing foliar $\delta^{15}N$ to indicate soil processes in some capacity. There appears to be some distinct relationships relating to plant component $\delta^{15}N$ to soil $\delta^{15}N$ at the three R-Field sites. Figure 4-10 highlights the relationship between root $\delta^{15}N$ and soil $\delta^{15}N$, as well as to soil C/N concentration. Specifically, root $\delta^{15}N$ appears to have a direct relationship with soil $\delta^{15}N$ at the three sites, as the figure includes values from the first and last sampling events at the three sites during the growing season.

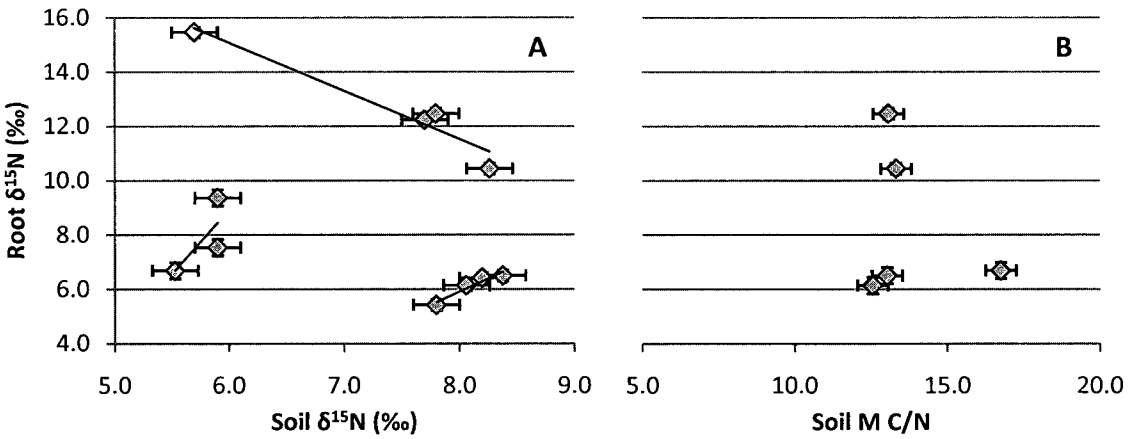

Figure 4-10: The relationship between root $\delta^{15}N$ and soil $\delta^{15}N$ from sites R1-R3 during weeks 3 and 21 (Figure A). Figure B shows the relationship between root $\delta^{15}N$ and soil C/N ratio from sites R1-R3 during weeks 3 and 21. The root $\delta^{15}N$ to soil $\delta^{15}N$ relationship appears more strongly correlated than the root $\delta^{15}N$ to soil C/N relationship. The root and soil $\delta^{15}N$ comparison includes weeks 9 and 15.

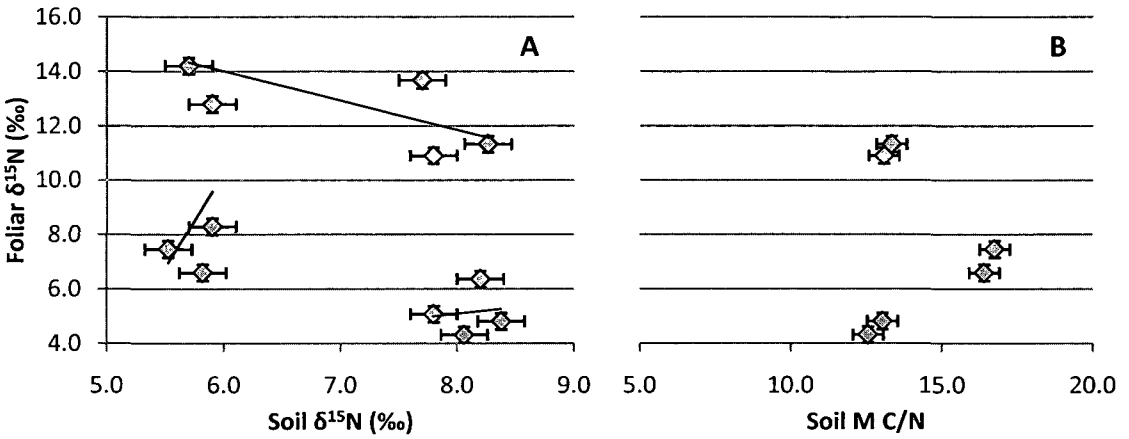

Figure 4-11: A comparison between foliar $\delta^{15}N$ and specific corresponding soil parameters, including $\delta^{15}N$ (Figure A) and C/N (Figure B). Values were taken from all three R-Field intensive sites during weeks 3 and 21. The foliar and soil $\delta^{15}N$ comparison includes weeks 9 and 15.

Qian et al. (1997) suggests that root-derived C influence soil microbial activity, which in turn regulates the N cycling and transformations within the soil. That relationship is consistent with the root – soil $\delta^{15}N$ relationship observed here. The pattern also maintains the conclusions reached by Templar et al. (2007), especially when compared to the direct relationship between foliar $\delta^{15}N$ and soil $\delta^{15}N$, as well as soil C/N (Figure 4-11). Kahmen et al. (2008) utilized foliar $\delta^{15}N$ to characterize N cycling processes largely because of the relationship between foliar $\Delta\delta^{15}N$ and plant NO_3^- to NH_4^+ uptake preference. However, those linkages were determined to be not universal, and foliar $\Delta\delta^{15}N$ was concluded to be most valuable in assessing plant N uptake patterns and to generally characterize the N cycle.

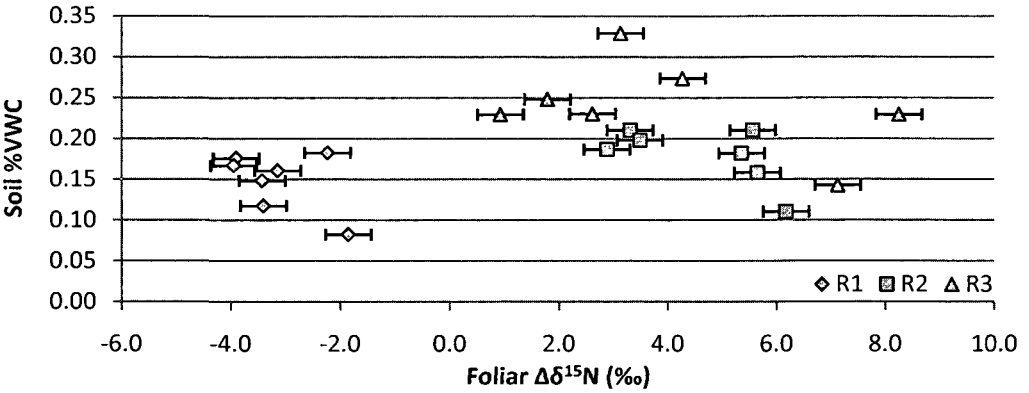

Figure 4-12: Relationship between foliar $\Delta\delta^{15}N$ and soil moisture content at the three R-Field intensive sites every three weeks during the growing season. Foliar $\Delta\delta^{15}N$ was determined by subtracting soil $\delta^{15}N$ from the corresponding site foliar $\delta^{15}N$.

The consideration of foliar $\Delta\delta^{15}N$ as a valuable indicator of relative soil N cycling processes is maintained, as it appears to indicate general N-cycling processes across the three intensive sites based on moisture conditions (Figure 4-12). The consistent differences in soil moistures between sites R1 and R2 serve to produce distinct site groupings of foliar $\Delta\delta^{15}N$, largely because of the differences in soil $\delta^{15}N$ between the sites. R3 foliar $\Delta\delta^{15}N$ varies quite considerably due to fluctuations in soil moisture at the site. While the soils at R3 maintain the highest average soil moisture of the three sites across the growing season, the soil $\delta^{15}N$ is significantly affected by the N cycling processes spurred by the larger moisture range. However, when examining individual sites, it appears at R2 and R3 trends indicate that as soil moisture increases the foliar $\delta^{15}N$ decreases. This could be a function of fertilizer application on relatively wet soils.

4.6 Conclusion

The use of plant $\delta^{15}N$ to properly assess N cycling processes is potentially viable, but several indicators must be examined simultaneously to provide any benefit. The value of plant $\delta^{15}N$ as an indicator of N status has been well documented (Robinson 2001; Templar et al. 2007; Kahmen et al. 2008). However, these studies have concluded that plant $\delta^{15}N$ is only useful in determining N sources or generally distinguishing relative N cycling processes. The assessment of specific soil characteristics, including NO_3^-, NH_4^+, and C/N, along a moisture gradient within a field with consistent treatments and nutrient sources can provide insight and valuable indicators of change in soil N.

The study highlighted the status of soil N throughout the growing season, and how that N is related to crop growth. It was evident that the majority of inorganic N within the soil was NO_3^- rather than NH_4^+, and that the NO_3^- decreased throughout the growing season until all of it was assimilated, leached, or denitrified. As well, the effect of inorganic fertilization in the early stages of the growing season was reflected in the soil N, as noticeable spikes occurred around the nine week mark.

The variability of soil and plant $\delta^{15}N$ was also established during the study. As the soil $\delta^{15}N$ is a reflection of both the organic and inorganic N, changes in NO_3^- and NH_4^+ were masked by the overwhelming amount of organic N in the sample. Therefore, little to no change in soil $\delta^{15}N$ was observed at all three sites during the season. In respect to plant $\delta^{15}N$, it was documented that the variability of plants within a 10 m^2 area was approximately the same level of variability within the leaves on a single plant. Furthermore, the difference between plants with the same soil moisture was less than the difference between plants in different soil moisture conditions.

The examination of corn exhibited differences in $\delta^{15}N$ between the wet and dry soil moisture areas. Specifically, the relatively wetter soil moisture site yielded plant tissue enriched in $\delta^{15}N$.

The higher $\delta^{15}N$ values are likely due to a combination of NO_3^- leaching and denitrification, which is more prevalent in higher soil moisture areas. The site with the largest variability in soil moisture (R3) also had the most variable foliar $\delta^{15}N$, which necessitated a more accurate reflection of soil N cycling conditions. Therefore, the study also highlighted the advantages to examining specific $\delta^{15}N$ of added N to provide more accurate snapshots of current soil status as opposed to the season long perspective. This method of isolating specific added N reaffirmed the differences between the wet and dry sites, while also showing a steady decline in $\delta^{15}N$ throughout the growing season at site R3.

While foliar $\delta^{15}N$ have definite value in outlining generalized N cycling processes, it is evident that root $\delta^{15}N$ has the potential to be more accurate. Templar et al. (2007) found that root $\delta^{15}N$ values were correlated with mineralization and nitrification rates, and as such could be used as an indicator of relative rates of NO_3^- production. The benefit of examining both root and foliar $\delta^{15}N$ in respect to N cycling has been explored at a larger scale (Pardo et al. 2006), so this study provides a wider scope of component $\delta^{15}N$ at a much smaller scale.

The findings can be supplemented with a survey investigating foliar $\delta^{15}N$ in a variety of soil moisture regimes across an entire field. Field-wide soil moisture gradients, which are due to a variety of factors including topography, drainage efficiency, soil type, and crop type, should cause differences in soil N cycling processes. The relationship between foliar $\delta^{15}N$ and soil moisture exists between site locations with very distinct moisture regimes, and should exist throughout the field in areas with consistent moisture levels relative to the rest of the field. The effectiveness of using plant $\delta^{15}N$ as an indicator of soil N cycling processes would be strengthened further by removing as many complicating factors as possible. These factors include most notably the tendency for plants to draw on different N sources, thereby altering the tissue $\delta^{15}N$ values. The validity of the relationship between plant $\delta^{15}N$ and soil N cycling

processes should be tested using a controlled plant growth simulation where soil moisture is controlled, as well as the various N sources available to the crops. However, the initial investigation exploring the linkages between plant isotopic values and soil N processes suggests that foliar $\delta^{15}N$ may be an effective way of documenting soil N-cycling. As well, the ability and tendency for corn to partition nutrients between different plant components based on the period of growth and nutrient availability can also be documented when utilizing isotopic analysis strategies.

Foliar $\delta^{15}N$ and $\delta^{13}C$ as related to soil moisture and crop productivity at a field-wide scale

5.1 Introduction

The emphasis on nutrient management in agriculture has received noticeably more attention in recent years, as the intensification of the industry has resulted in the increased use of additional N inputs to supplement crop growth (Agriculture and Agri-Food Canada 2008). Thus, the negative impacts of cropping activity on ground and surface water systems, especially within predominately agricultural catchments, can potentially increase as well. The need to assess the ability of crops to utilize soil N efficiently is crucial in managing impacts and controlling input costs (Addiscott 2005). Soil moisture is easily measured, and would provide insight into plant N-uptake.

The suitability of investigating crop isotopic values as indicators of N cycling processes in the soil has been documented, but only among specific sites with vastly different soil moisture regimes (Robinson 2001; Templar et al. 2007; Kahmen et al. 2008). It was shown that plant $\delta^{15}N$ does reflect N transformation within the soil, and can be identified as such when soil N sources remain relatively constant. Within a field, due to a variety of natural and human-influenced factors, soil moisture levels fluctuate dramatically both spatially and temporally. The ability of crop isotopes to indicate soil N-status has been documented through a wide ranging soil moisture gradient (Emmett et al. 1998; Templer et al. 2007). The ability of the relationship to be maintained, throughout the range in conditions, reflects field conditions more closely; which would establish the method as a viable indictor of N-status and availability.

The ability to assess soil N-status and plant N uptake is one step towards minimizing N losses to ground water, surface water, and the atmosphere. Soil N-status is characterized in this study as the concentrations of extractable NO_3^- and NH_4^+. The areas of the field more prone to N losses are likely the areas with consistently higher moisture values, which may impact plant growth. The higher N losses in the wetter areas of the field have the potential to affect crop

yield if N becomes a limiting factor. However, the losses may not be significant to influence N supply to the plants, and yield will not be affected. As the value of the crop is essentially gauged by yield, the weight of the cob is the economic driver of a corn crop and may provide a useful indictor of potential N loss.

The nutrient uptake and partitioning strategies of crops is also partially dictated by conditions in the soil. Soil moisture and N-availability can alter the timing of component development, as well as the weight and nutrient composition of components (Pardo et al. 2002). As previously established (Section 4.4.3), $\delta^{13}C$ is affected by water stress. The examination of $\delta^{13}C$ may provide insight into whether yield has been influenced by soil moisture rather than N-availability or water/nutrient stress. It could be beneficial to investigate the C/N ratios of crop foliage, as it may provide information on shifts in plant growth strategy when compared to more in-depth crop isotope investigation completed in the prior growing season. Any additional insight into the linkages between field crops and soil conditions could ultimately identify potential thresholds, and conditions for maximizing crop growth while minimizing lost nutrients. Therefore, the implications for crop value are intimately linked with soil conditions.

The overall objective of the study is to examine the relationship between soil moisture, crop $\delta^{15}N$ and $\delta^{13}C$, and crop yield, at the field-scale. This insight will be gained first by determining the soil moisture gradient throughout the field, and establishing a series of sampling sites that represents the range in soil moisture conditions. Crop extractions and soil moisture measurements will occur during the growing season to establish whether the relationship between soil moisture and plant isotopes is consistent at a field-scale with what was documented at the intensive plot locations. The goal after identifying these soil moisture gradients is to investigate using foliar isotopes the N-status of the soil, and how the changes in N-status affect the crop productivity across the field. Finally, the potential link between foliar

isotopic values and soil N-status will be compared to cob weight, which will provide a final indication of crop value. The relationship between soil N-status and crop yield would allow for shifts in nutrient management, and could provide the basis for examination into variable rate fertilization or constructed drainage systems.

5.2 Site Description

The T-Field was utilized for the field scale survey because it was planted to corn, as well as its large size and variable topography. The T-Field is located in the central portion of the SCW, and occupies an area of approximately 27 hectares. A total of 14 hectares of the field are located outside of the watershed, but due to the presence of extensive tile drainage networks, the soil water throughout the entire field flows into Strawberry Creek (Figure 5-1). The majority of the field within the basin drains through the west tile network to the BMR tile outlet (A), while the area outside the basin drains through the east tile network to the Fencerow tile outlet located further downstream. The R-Field tile network drains to the AMR tile outlet (B). The topography of the R-Field itself is relatively similar to that of the entire basin, as it contains a gently rolling landscape with very little elevation change. However, it is characterized by slight downward slopes towards the creek and towards the east edge of the field.

The cropping history in the R and T Fields is similar to other fields throughout the basin, as they experience crop rotation, tillage, and fertilization activity. Specifically, the T-Field sees a two-crop rotation schedule, switching between corn and oats in bi-yearly cycles. This cycle has been maintained between 2005 and 2009. Fertilization practices also remained constant throughout the same time frame, and consisted of a mixture of both inorganic and organic applications. The applied organic fertilizer consisted of poultry manure applied annually in the spring, usually in early May when the soil was dry enough for the field to be accessed. The application rate of poultry manure was 0.5 tonnes per acre, which translated into approximately 11 kilograms N per hectare. Inorganic N:P:K starter fertilizer (9:18:09 mixture) was applied to both corn and oat crops when planted in the T-Field. These applications were applied at a rate of 20 pounds per acre, which is equivalent to 23 kilograms N per hectare. In June, the crops were supplemented with 28% N liquid NO_3NH_4 fertilizer at 90 pounds per acre in June resulting

in an additional 101 kilograms per acre of N for the crops to utilize. In the autumn following a corn crop, supplementary poultry manure was not applied to the soil. However, in the autumn following an oat crop and preceding a corn crop manure was applied in September after harvest at a rate of 1.5 tons per acre. The N content of the applied manure was approximately 32 kilograms per hectare.

Tillage activity also remained consistent throughout 2005-2009. Conventional tillage involved ploughing and discing every autumn in mid November. The ploughing turned over the soil to a depth of 10 to 12 centimetres, while the discing broke up the large soil chunks and smoothed the soil surface. During the oat crop years, discing would occur in August, followed by the harvest and conventional tillage in mid November. The corn harvest typically occurred in mid to late October. The precise timing of harvest for both crops was dependent on planting date, soil conditions, and climatic influences throughout the growing season.

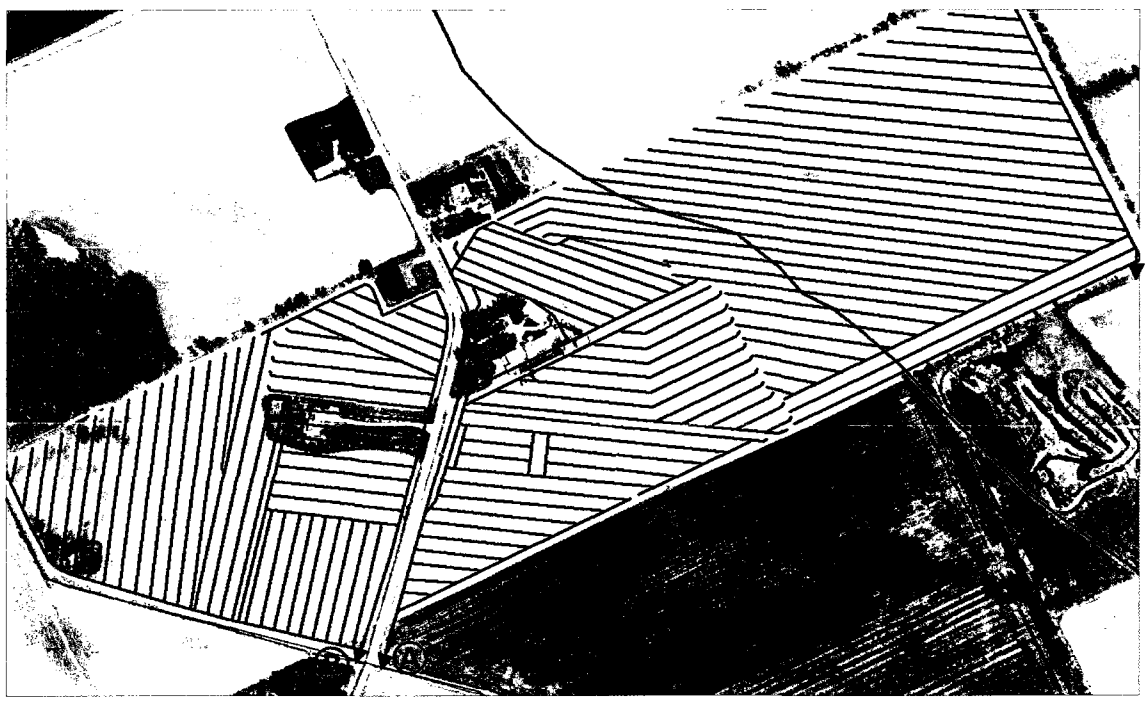

Figure 5-1: Location of tile drainage networks (blue lines) in the R and T Fields, as well as the location of the watershed boundary (black line) within the T-Field. The tile drains are divided into three separate networks across the two fields, and therefore discharge to three distinct outlets (blue arrows). The AMR and BMR tile outlets are shown, while the Fencerow tile outlet is located further downstream.

The precipitation levels recorded throughout the 2008 (Figure 5-2) growing season were consistently higher than in 2007. The month of June 2008 experienced approximately 93 mm total precipitation, while July (120 mm) and August (126 mm) were subsequently wetter months. September saw a decrease in total precipitation at 112 mm. The summer months were characterized by inconsistent precipitation events, as long periods with no rain were mixed with days with total precipitation between 20 and 40 millimetres. This pattern was especially prevalent in July and August, while June experienced more rain days with moderate intensities and lengths. The regularity in precipitation in late spring/early summer is consistent with previous years, and is largely responsible for high soil moisture levels recorded across the field in the June and July sampling periods. The average August soil moisture values are lower, which is the result of several factors including less precipitation and the increased moisture requirements of growing crops. A single corn plant transpires on average 1.25 litres a day, and as the crop grows that transpiration rate can increase to 3.8 litres a day (Agriculture and Agri-Food Canada 2008). The dates of the surveys and samplings were selected at random with no consideration of overall precipitation trends and patterns.

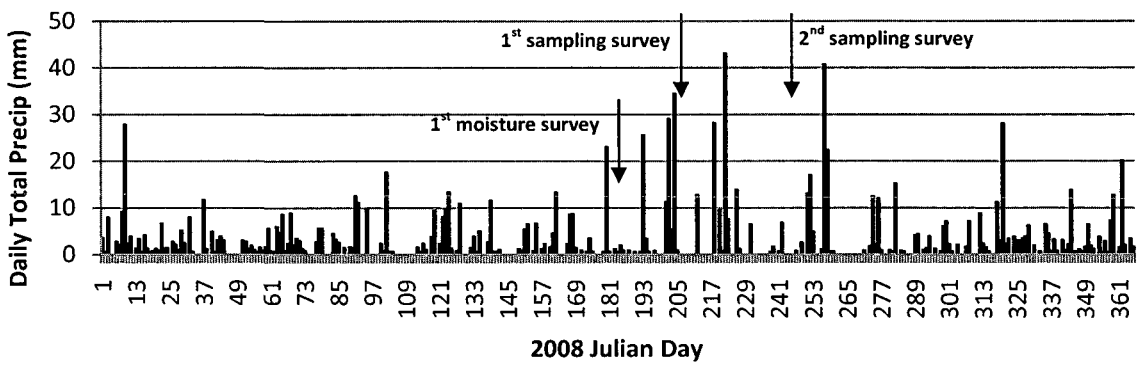

Figure 5-2: Daily total precipitation throughout 2008, as recorded from the SCW. Also indicated is the initial moisture survey (day 168), the initial leaf sampling (day 184) and the final leaf and cob sampling (day 226).

5.3 Methods

The grid survey was designed to capture the entire spatial extent of the field, while also representing the broad range of soil moisture conditions. An initial soil moisture survey was conducted early in the growing season (June) to include approximately 90 moisture locations across the field. The pattern of sampling was designed to maximize spatial coverage while minimizing damage to the crop. Therefore, measurements were taken along crop rows every 50 metres, as well as including the three intensive sites in the field. A GPS unit was utilized to record the coordinates of every sampling location (±6m), which typically ran laterally along crop rows from the front of the field towards the back. Soil moisture was quantified using a mobile Campbell Scientific HydroSense time-domain reflectrometer (TDR) device that measured moisture content in the upper 20 centimetres of the soil profile, and expressed values as volumetric water content (%VWC). At each site a 10 centimetre pit was dug and the TDR was inserted into the pit, which allowed for moisture measurements up to 30 centimetres in depth. The values were calculated to determine a weighted mean average depth to 30 cm. The VWC point data was plotted and charted using Surfer 8.0 software, which visually displayed the moisture profile of the studied field.

The 90 soil moistures sites were narrowed down to 15 sites, which were selected to represent the entire range of moisture conditions occurring in the field, and were quantitatively classified as either wet (%VWC >40), medium (%VWC 30 - 40), or dry (%VWC <30) sites relative to the other point locations. The sites were also selected to capture the spatial extent of the field. The three intensive sites were included in this study, bringing the total sampling locations to 18.

The first crop sampling occurred in July, 2008 at the 18 selected locations. The leaf located above the cob was removed from a representative (size) stalk in the immediate area. As well, soil moisture was measured at three points around the sampled plant to a 20 centimetre depth and averaged, so that one moisture value was associated with each leaf collected. The second sampling occurred in August, 2008, and followed the same procedure as in July. In addition to extracting the leaf, the cob was also harvested from the stalk. Plants with one dominant cob, rather than two smaller but more similar sized ones, were selected to maintain consistency during sampling.

The leaves and cobs were weighed immediately after returning from the field, and then placed in a freezer until the time of analysis. The frozen leaves were placed in individual specimen containers with a filter instead of the lid on top to allow for moisture removal during the freeze-drying process. After the moisture had been completely removed, the leaf samples were weighed and subsequently processed for analysis (see sections 3.3.2 and 3.3.3). The cobs were too large to place in containers, so they were wrapped with nylon screen and freeze-dried. The dry weight was recorded, and the cobs were stored for potential future analysis.

5.4. Results and Discussion

5.4.1. Field-scale variability in soil moisture

The three complete moisture surveys displayed temporal and spatial variability across the T-Field (Table 5-1). As expected, the field yielded progressively drier soils on average, from 37 %VWC in June to 35 %VWC in July, and finally 23 %VWC in August. The June sampling also saw the largest range in moisture values, with a maximum moisture value of 56 %VWC, minimum value of 22 %VWC, and a standard deviation of 10.7. July conditions were very similar to June moisture conditions, as specific measured values ranged between 50 and 22 %VWC with a standard deviation of 9.2. August experienced the driest conditions, as evident by the average %VWC value, and the smallest spatial range in values. The highest moisture value at a measured site was 30 %VWC, the lowest was 15 %VWC, and the standard deviation was 4.7.

	Max	Min	Average	Std Dev	Std Err
June	56.0	22.0	37.3	10.7	2.6
July	50.0	22.0	36.8	9.2	2.2
August	30.0	15.0	23.2	4.7	1.1

Table 5-1: %VWC at 18 locations across the T-Field during the three field scale moisture surveys during the summer of 2008. The table highlights the range in moisture conditions, while also indicating averages and statistical variability.

Figure 5-3: Soil moisture gradient in the T-Field based on approximately 90 soil moisture measurements from the upper 30 centimetres of the soil profile. The measurements were taken in June, 2008 when the corn was seven weeks into the crop growing season.

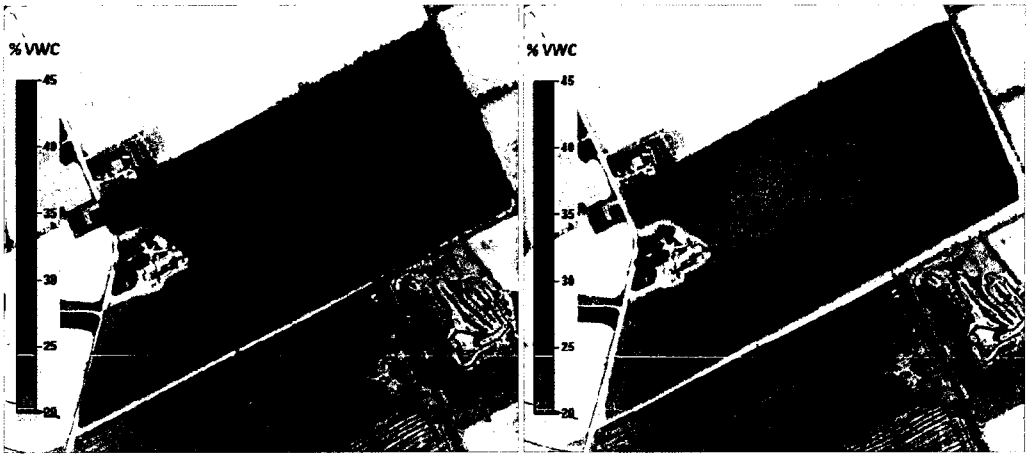

Figure 5-4: Spatial variability in soil moisture recorded in July (A) and August (B) of 2008 across the T-Field. The east end of the field was consistently moister than the central portion of the field, as well as the areas around the house and farm buildings. Topography and tile drainage efficiency may be responsible for some of the overall trends.

The highest soil moisture conditions were typically found along the edges of the field, while the drier sites were more centrally located (Figure 5-4). As well, the NE portion of the field was on average higher in moisture during both sampling events in July and August. The consistently higher moisture values in specific areas of the field are potentially the result of drainage efficiency due to topology or the tile drainage network.

The investigation into spatial soil moisture gradients exhibited the tendency of specific areas of the field to maintain higher relative soil moistures throughout the growing season. The previous chapter established a relationship between foliar $\delta^{15}N$ and soil moisture differences, while several studies explored the use of plant isotopes as indicators of soil cycling processes (Hogberg 1990; Garten 1993; Emmett et al. 1998; Pardo et al. 2002; Kahmen et al. 2008). The consensus of prior studies, within the SCW and at other locations, is that a relationship exists, but must be investigated at vastly different soil condition sites because of the presence of competing factors. These factors can be potentially responsible for all or part of the isotopic transformation between the soil and plant (Robinson 2001). However, through the careful examination of $\delta^{15}N$ and $\delta^{13}C$ in crop leaves involving consideration of both spatial and temporal variability, it may be possible to provide insight into the use of plant isotopes as indicators of N-status.

5.4.2 Investigation of foliar $\delta^{15}N$ and $\delta^{13}C$

The difference between foliar isotopic values provides a valuable comparison, as the leaves were sampled from the same location in both July and August. Therefore, any relative differences can be related to soil moisture trends to extract potential relationships. In respect to ^{15}N, there was little overall difference between leaves collected in July and leaves sampled in August (Figure 5-5). Average foliar $\delta^{15}N$ remained relatively unchanged from July (7.2‰) to August (7.7‰) while average soil moisture decreased during those same two sampling events (37 %VWC to 23 %VWC). The $\delta^{13}C$ values of the plant leaves displayed a more dramatic difference between July and August, as average $\delta^{13}C$ values decreased from -11.7‰ to -12.7‰. This change is even more significant when considering the variability in $\delta^{13}C$ values within the 18 leaf samples, as the standard deviation among the values was only 0.19 in July and 0.32 in August.

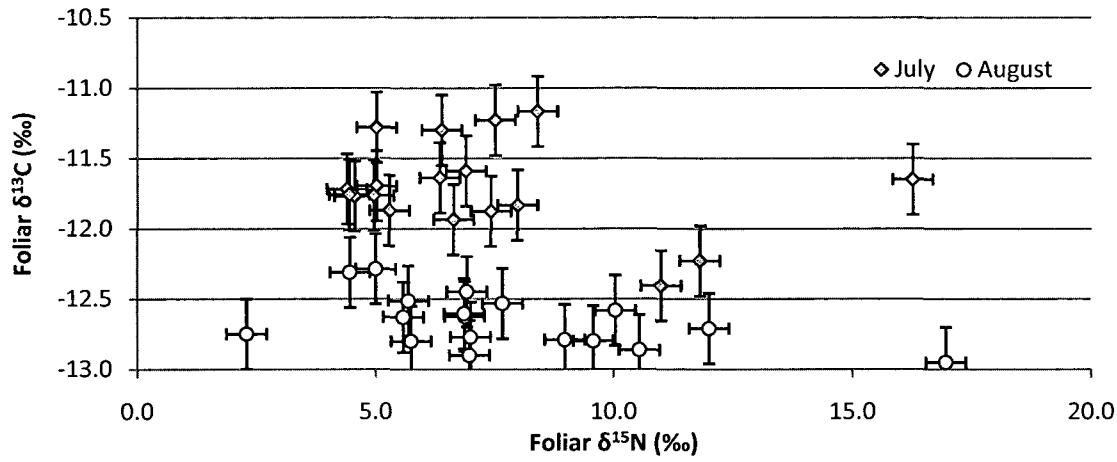

Figure 5-5: Foliar $\delta^{15}N$ vs. $\delta^{13}C$ from leaf samples collected during both sampling events in July and August, 2008 within the T-Field. Both sampling events show variability within $\delta^{15}N$ while August samples show a noticeable decrease in foliar $\delta^{13}C$ over July values.

The overall shift in foliar %C and %N from July (Table 5-2) to August (Table 5-3) showed a dramatic increase in average C/N ratio values. While the average increased from 16.0 in July to 19.5 in August, the standard deviation also increased from 2.6 to 3.1 during the same time frame. The reason for the increase is primarily due to the decrease in average %N within the leaves; 3.3% in July down to 2.8% in August. Plant partitioning strategies during plant growth, as well as N-status and availability within the soil, are possible explanations for the shift in %N. However, the increased variability in foliar C/N ratios from July to August could be caused by the lower overall soil moisture levels across the field, as conditions could dictate a change in growth and N partitioning strategies.

It is also significant to note that some sites with elevated foliar $\delta^{15}N$ values, which are predominately the wet sites, also show moisture stress through depleted $\delta^{13}C$ values. This trend indicated that sites that were wet during the early stages of plant growth when N was being added are still susceptible to moisture stress later in the growing season. The cause is potentially a lack of established root systems due to the easily available soil moisture during early growth.

Sample ID	Soil θ	$\delta^{13}C$	$\delta^{15}N$	%C	%N	C/N
TS-1	47	-11.65	16.25	44.61	3.64	14.32
TS-2	50	-11.59	6.89	44.50	3.75	13.83
TS-3	34	-11.87	7.42	43.78	3.43	14.87
TS-4	34	-11.93	6.63	44.27	3.67	14.07
TS-5	43	-11.87	5.28	44.18	3.62	14.26
TS-6	22	-11.30	6.39	44.09	2.32	22.22
TS-7	28	-11.72	4.39	44.74	3.41	15.31
TS-8	38	-11.77	4.55	44.07	3.19	16.13
TS-9	29	-11.76	4.43	44.66	3.26	15.98
TS-10	48	-12.23	11.81	44.11	4.04	12.75
TS-11	43	-12.40	10.99	44.01	4.07	12.61
TS-12	40	-11.69	5.02	44.34	3.28	15.78
TS-13	40	-11.83	7.98	44.25	3.54	14.58
TS-14	45	-11.64	6.34	44.44	3.09	16.75
TS-15	46	-11.76	4.96	44.42	2.96	17.49
TS-16	24	-11.23	7.51	44.14	2.64	19.53
TS-17	24	-11.28	5.02	44.00	3.17	16.19
TS-18	28	-11.16	8.40	44.40	2.53	20.49
AVERAGE	**36.8**	**-11.70**	**7.24**	**44.28**	**3.31**	**15.95**
ST DEV	**9.2**	**0.32**	**3.09**	**0.26**	**0.48**	**2.59**
ST ERR	**2.2**	**0.08**	**0.73**	**0.06**	**0.11**	**0.61**

Table 5-2: Summary of soil moisture (%VWC) and foliar $\delta^{13}C$, $\delta^{15}N$, %C, %N, and C/N ratios at the 18 locations across the T-Field from the July sampling event. The table highlights the range in values, while also indicating averages and statistical variability.

Sample ID	Soil θ	$\delta^{13}C$	$\delta^{15}N$	%C	%N	C/N
TS2-1	28	-12.71	12.00	45.78	2.76	19.37
TS2-2	25	-12.95	16.95	46.42	3.61	14.99
TS2-3	17	-12.62	6.87	45.72	2.65	20.14
TS2-4	19	-12.77	6.99	46.10	2.83	19.04
TS2-5	30	-12.63	5.58	45.42	2.82	18.78
TS2-6	15	-12.31	4.46	45.26	1.94	27.26
TS2-7	23	-12.51	5.69	45.87	2.51	21.29
TS2-8	22	-12.75	2.29	46.33	2.86	18.91
TS2-9	21	-12.45	6.92	45.74	2.60	20.49
TS2-10	26	-12.80	9.58	45.11	3.17	16.62
TS2-11	20	-12.86	10.54	45.96	3.32	16.17
TS2-12	28	-12.53	7.66	45.51	2.57	20.69
TS2-13	28	-12.90	6.97	45.60	3.01	17.65
TS2-14	26	-12.58	10.04	45.32	2.95	17.91
TS2-15	26	-12.80	5.75	45.30	2.88	18.36
TS2-16	14	-12.60	6.86	44.64	2.63	19.81
TS2-17	23	-12.28	5.00	44.55	2.01	25.85
TS2-18	26	-12.79	8.97	45.25	3.08	17.13
AVERAGE	**23.2**	**-12.66**	**7.73**	**45.55**	**2.79**	**19.47**
ST DEV	**4.7**	**0.19**	**3.30**	**0.51**	**0.41**	**3.07**
ST ERR	**1.1**	**0.04**	**0.78**	**0.12**	**0.10**	**0.72**

Table 5-3: Summary of soil moisture (%VWC) and foliar $\delta^{13}C$, $\delta^{15}N$, %C, %N, and C/N ratios at the 18 locations across the T-Field from the August sampling event. The table highlights the range in values, while also indicating averages and statistical variability.

The ability to explore the relationship between the composition of plant leaves and soil conditions requires an understanding of basic growth strategies and how those strategies alter based on the soil conditions. The nutrient partitioning strategies of corn plants remain fairly consistent throughout the growing season, except during periods of soil N deficiency or water stress (Gastal and Lemaire 2002; Nyiraneza et al. 2009). Typically, corn plants partition N from the outer components to the grain to supplement cob growth during peak production periods, but the N donor component changes based on available soil N. Paponov and Engels (2005) examined the effect of N supply on N partitioning in corn plants during the grain production stage, and determined that excess N accumulation in the plant grain was covered primarily by depletion of N in the stalk in soils with high N supply and availability. In soils with low supply and availability the N accumulation within the grain was covered by the depletion of leaf N. The expectation in any N-available regime is an overall decrease in foliar %N, which is potentially evident in the slight difference between July and August values (Figure 5-6).

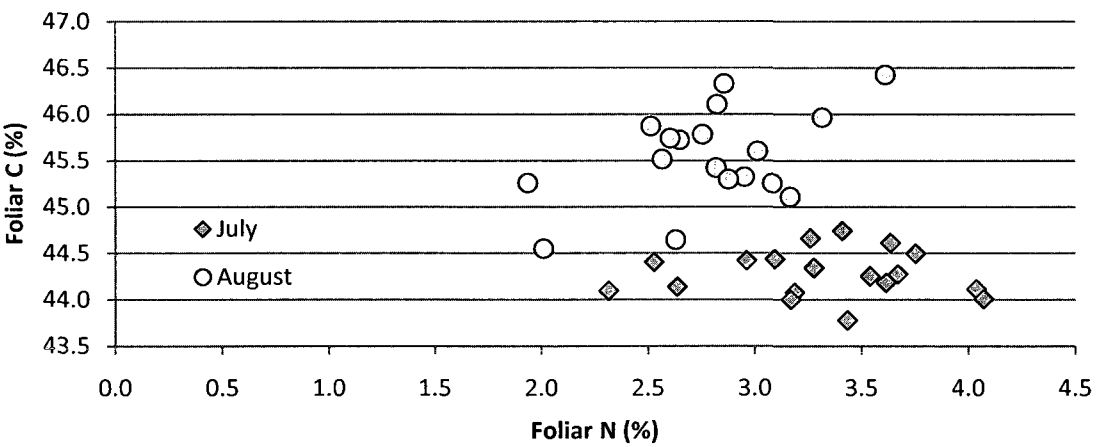

Figure 5-6: The relationship between foliar %C and %N from the July and August sampling events. The difference in values is reflective of the expected decrease in %N during the development of the cob. The degree of change is influenced by outside factors, including soil moisture.

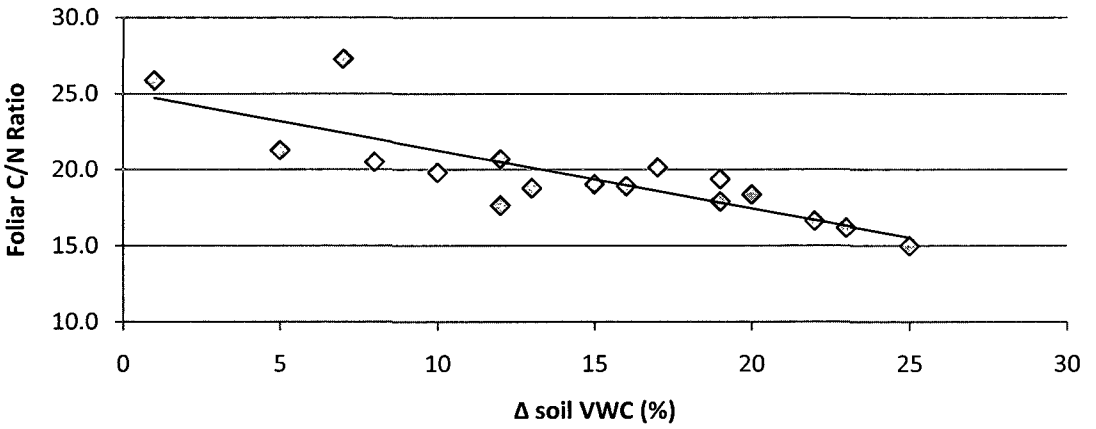

Figure 5-7: The relationship between foliar C/N from the August sampling event and the difference in recorded soil moisture values at each site between July and August. $R^2 = 0.68$.

While the natural decline in foliar %N is expected during the production phase of the growth cycle, the rate at which the N decreases is dependent on outside influences; specifically, soil water and nutrient conditions. As mentioned, the plant alters its partitioning strategy based on stress levels during growth (Choi et al. 2003; Paponov and Engels 2005; Subedi and Ma 2005). Figure 5-7 displays the C/N ratios of the 18 leaf samples collected in August in relation to the difference in soil moisture at the 18 sites between the July and August sampling events. The biggest change in soil moisture between the two sampling events at individual sites represents a higher rate of soil moisture decrease, so a change of 25% from July to August would more strongly signify water stress in August then a change of 5%. The strong correlation ($R^2 = 0.683$) indicates that as the soils dry up in late summer, the largest changes produce higher levels of stress on the growing crops, which lowers the foliar C/N ratio and alters the N partitioning strategy.

It was indicated previously that foliar $\delta^{13}C$ may better represent short term fluctuations in soil moisture, while $\delta^{15}N$ is a better representation of conditions throughout the growing season. When investigating foliar isotopic values along the field-wide moisture gradient, it appears that the relationship continues to exist. Figure 5-8 outlines the value of $\delta^{13}C$ when

considering short term soil moisture changes rather than average conditions recorded over a period of time. The correlation between $\delta^{13}C$ and the difference in soil moisture between July and August at all 18 sites within the T-Field show an R^2 value of 0.665, which is considerably stronger then the relationship between foliar $\delta^{13}C$ and average soil moisture ($R^2 = 0.377$). As indicated by Lambers et al. (2008), the response in foliar $\delta^{13}C$ is likely due to the effect on plant transpiration within the leaf stomata. The fluctuation in soil moisture provides a level of water stress to the plant. As plant available soil moisture typically decreases in the summer months, due to precipitation becoming less frequent and the water needs of the crop increasing, the overall trend in foliar $\delta^{13}C$ is a decrease. In plants that are not water stressed the stomata stay open and atmospheric CO_2 diffuses into and out of the crop leaves. The open diffusion of atmospheric CO_2 maintains foliar $\delta^{13}C$ values closer to the isotopic signature of atmospheric $\delta^{13}C$ (-8‰).

The use of foliar $\delta^{13}C$ relating to soil moisture levels is complicated by the influence of soil N-status. Clay et al. (2001) concluded that supplementing N to N deficient C3 and C4 plants will increase and decrease $\delta^{13}C$ in C3 and C4 plants, respectively. Therefore, foliar $\delta^{13}C$ can be utilized in combination with $\delta^{15}N$ as an indicator of both water stress due to moisture changes and N-status and N-availability within the soil.

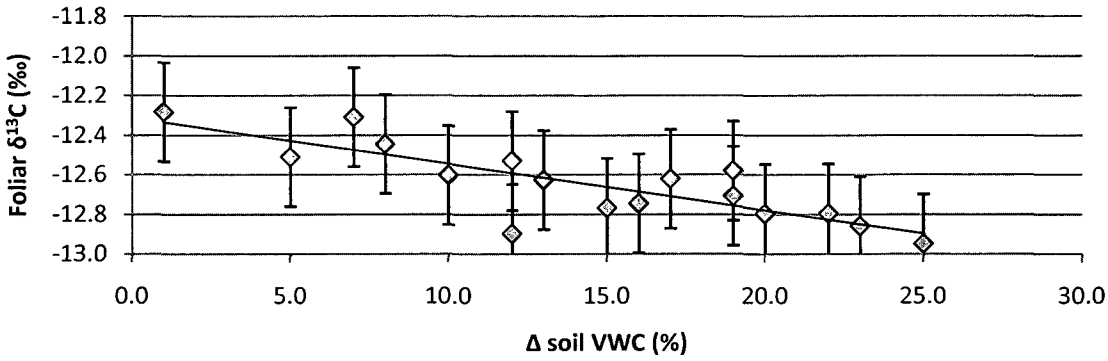

Figure 5-8: The correlation between foliar $\delta^{13}C$ and recorded soil moisture difference at all sites throughout the T-Field. The soil moisture values are the difference between July and August values at each specific site, while the foliar $\delta^{13}C$ values are from the August sampling event. $R^2 = 0.67$.

When evaluating foliar $\delta^{15}N$ at a field-wide scale the relationship becomes more complex and more obscured. Figure 5-9:A highlights the correlation between soil moisture and foliar $\delta^{15}N$ across the T-Field. As it has been established that $\delta^{15}N$ more closely reflects overall soil moisture conditions, the %VWC used is a calculation of the average moisture values of the first two moisture surveys. The foliar $\delta^{15}N$ values are from the August sampling event. While a relationship between soil moisture and foliar $\delta^{15}N$ exists, it does not appear to be as strong as the difference between individual sites within the R-Field discussed in the prior chapter.

5.4.3 Foliar $\delta^{15}N$ and plant productivity

There appears to be a correlation between foliar $\delta^{15}N$ and plant productivity, represented in this study by dry cob weight (Figure 5-9: B). As foliar $\delta^{15}N$ increased, dry cob weight decreased. The weight did not meaningfully decline until the corresponding foliar $\delta^{15}N$ values became significantly enriched, which indicated that plants can likely compensate for low N availability until a threshold is reached, at which point crop yield is dramatically reduced. Paponov and Engels (2005) reported the difference in partitioning strategies based on N-availability, and found that stress on the plant does not necessarily impact crop yield in a negative way. Figure 5-10 highlights the relationship between cob weight and foliar %N, and while the relationship is

not as definitive as what has been previously highlighted, it does show that lower total %N in the plant leaves corresponds to higher cob weights. The allocation of N between plant components, especially the cob and leaves, is highly dependent on soil N-availability (Gastal and Lemaire 2001). As such, cob weight is an essential aspect to consider when attempting to investigate relationships at the field scale, as potential partitioning strategy changes are largely based on soil N-status.

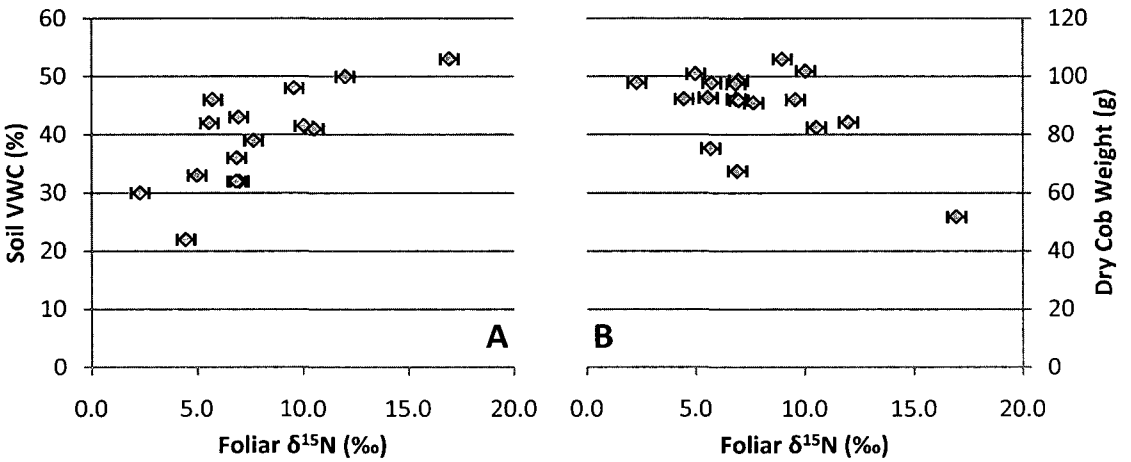

Figure 5-9: Figure A shows the correlation between foliar $\delta^{15}N$ and soil VWC at all sites across the T-Field, while Figure B shows the relationship between foliar $\delta^{15}N$ and dry cob weight at all sites. The soil moisture values are averaged from the first two moisture surveys, while the foliar $\delta^{15}N$ values are from the August sampling event. R^2 (Fig A) = 0.56, R^2 (Fig B) = 0.31.

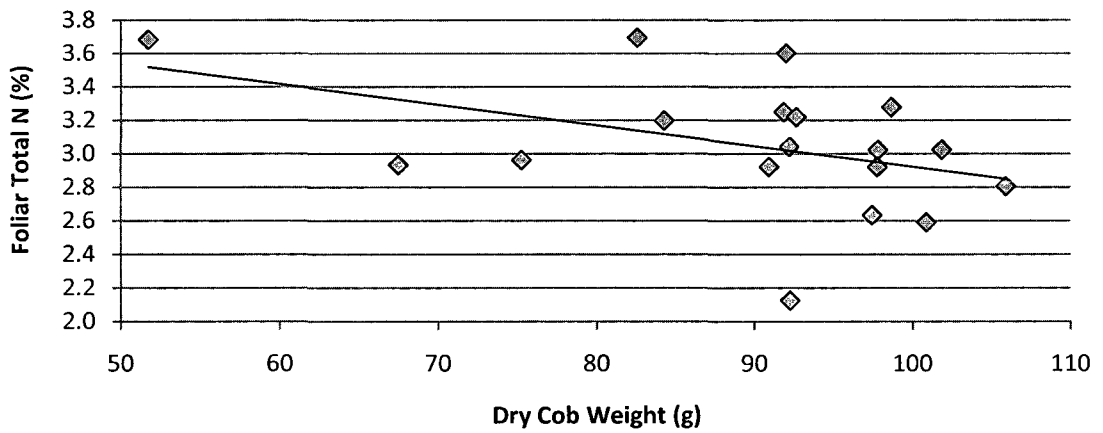

Figure 5-10: The correlation between foliar %N and dry cob weight at all sites throughout the T-Field. The foliar %N values are the averaged July and August values at each specific site, while the cobs were collected during the August sampling event. R^2 = 0.18.

5.4.4 Management Implications

The relationship between soil moisture and plant productivity is not pronounced when considering the wide range of values across the field. However, if the values are grouped together based on the three moisture categories previously established, trends emerge. Figure 5-11 shows the dominant moisture areas documented throughout the growing season.

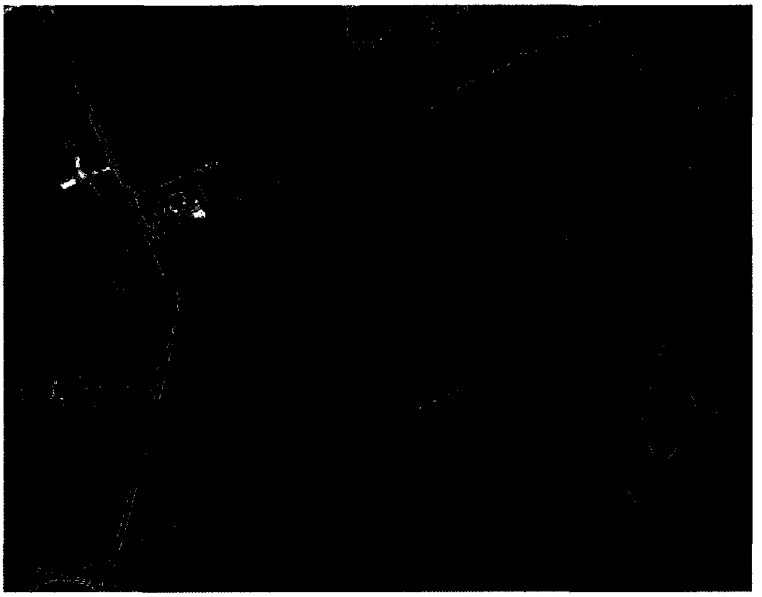

Figure 5-11: Predominant dry (red), medium (green), and wet (blue) soil moisture areas in the T-Field. These areas were established based on soil moisture values measured three times throughout the 2008 growing season.

The plants within these moisture areas had noticeably different production levels, which could be a response to the moisture conditions. The average dry cob weight from plants in both the dry and wet zones of the T-Field was 84.9 g. However, cobs sampled from plants in the medium soil moisture zone were an average of 10 g heavier. This change is likely a response to optimum growing conditions, as the plants are uptaking N efficiently and not over-emphasizing N partitioning, which can result in the underdevelopment of leaves or stalks (Plenet and Lemaire2000). The significance of the production difference is more noteworthy; in that leaf %N between plants within the different soil moisture areas follow the trend established previously in the study. The plants in the wet zone have the highest average foliar %N (3.1), followed by the

medium (2.9), and dry (2.6) zone plants. This pattern further supports the notion that the medium moisture zone plants are utilizing soil N more efficiently, as available N is utilized for cob growth instead of being stored in the leaves.

The plant production differences between soil moisture zones are significant in terms of N management practices. The decreasing N-uptake efficiency of plants in wetter soils supports the practice of tile installation to improve drainage; if only from the perspective of maximizing crop productivity. If an average of 40,000 corn plants is seeded per acre, the productivity per acre would equal 3760 kg in the medium moisture zone and 3360 kg in the wet and dry zones. As 25.4 kg of corn make a bushel based on 15.5% moisture content, 21.5 kg of corn would equal a bushel based on zero soil moisture. Therefore, the total productivity for plants in the medium moisture zone is 175 bushels per acre, while the wet and dry zones produced 156 bushels per acre. The end result is an approximately $90 difference per acre between the optimum medium moisture zone and the wet and dry areas, which is made all the more noteworthy by rising corn sale prices. The difference in productivity, even within a tiled field, is evidence of the significant impact of field-wide moisture variability and its impact on plant N-uptake.

5.6. Conclusion

The link between soil moisture and plant isotopes has been established in the previous study, as crop $\delta^{15}N$ was markedly different between sites with consistently diverse moisture conditions. As explored by Templer et al. (2007) the changes in soil moisture influence N cycling processes, which is reflected in plant isotopic values. However, those changes are more difficult to identify when there is a wider range in soil conditions. If a relationship can be established at a field-wide scale it could provide valuable indictors of soil N-status and plant available N, which could have implications for nutrient management practices and maximum productivity strategies.

The general pattern has soil moisture and N-availability correlated, as water stress and N stress are intrinsically linked. As indicated in this study, water stress can be a dramatic shift in soil moisture, as at many sites across the T-Field the shift and decrease in %VWC from July to August was enough to have an important effect on plant isotopes. The level of stress shown by the plant due to either factor does have an effect on productivity and crop yield. However, an absolute link between moisture conditions, foliar isotopic values, and dry cob weight was not identified.

The value of investigating both foliar $\delta^{15}N$ and $\delta^{13}C$ was also explored, and it appears that both methods can be used in unison to explain specific trends. The benefit and value of $\delta^{15}N$ exploration was proven to be effective in examining long-term trends, as the $\delta^{15}N$ of the leaf is dictated largely by the conditions during the growth of the component. As most leaves are added early in the growing season, the $\delta^{15}N$ more closely reflects those conditions. However, as N partitioning during cob growth based on soil conditions move around nutrients within the plant, smaller shifts in $\delta^{15}N$ occur later in the growing season. Foliar $\delta^{13}C$ appears to be better suited for indentifying short term changes in soil conditions, as water and nutrient levels are

closely related to photosynthesis and transpiration within the leaves. C assimilation of corn can be related to crop N through N distribution among leaves, which directly affects leaf and overall canopy photosynthesis (Gastal and Lemaire 2002). As well, C assimilation depends on crop N through leaf area, and the growth rate of the plant depends on the balance of N allocation between growing and mature leaves.

The examination of crop $\delta^{15}N$ along a field-wide moisture gradient has provided insight into the value of using plant isotopes as an indicator of N-status in the soil (Robinson 2001; Templer et al. 2007; Kahmen et al. 2008). However, the ability to control N inputs in a closed system would allow for a better assessment of the effects of soil moisture on N cycling processes, and how exactly those processes are reflected in plant $\delta^{15}N$. Therefore, the final strategy for assessing plant isotopes as indicators of soil N-status would be the development of indoor crop growing simulations. The reduction of potential supplementing N sources and more easily identifiable and measurable factors would increase accuracy and provide more insight on potential soil and plant isotopic relationships.

Examining plant and soil N interactions in a controlled plant growth simulation

6.1 Introduction

The examination of plant tissue as an indicator of soil N cycling is clouded by the introduction of various N sources and transformations within the soil (Robinson 2001). Applied inorganic fertilizer or organic livestock manure would expect to have a measurable range in $\delta^{15}N$ values before introduction into the soil, but would thereafter be subject to ^{15}N transformation processes and interactions with the soil N pool (Choi et al. 2003). The result of these complex interactions is an increase in difficulty in identifying the exact linkages between soil and plant N. Therefore, it would be extremely valuable to the investigation of plant $\delta^{15}N$ as an indicator of soil N status to attempt at minimizing the outside sources of N to achieve a more direct relationship between plant and soil N. The first necessary step would be to reduce the amount of soil, which would decrease the pool of available N that the plants may utilize. The most effective method of reducing the soil and N pool would be to locate the study from the field into a controlled environment. Constructed soil profiles within barrels or boxes would allow for quantification of soil nutrients, which could be transferred to the known volume of soil. This would dramatically increase the known N status of the soil, and would decrease the unknown variables between the soil N and plant uptake. Unfortunately, simulated growing conditions cannot possibly replicate natural conditions, so the focus is on meeting the basic requirements for plant growth throughout the life cycle of the crop. Simulating solar radiation, maintaining soil temperature and moisture, and providing adequate nutrients for sustained growth become the primary objectives. If these various conditions are not met the plant will change its growing strategy; altering individual component development and intra-plant nutrient reallocation. The altering growing strategies will reduce the effectiveness of the study and comparison to natural conditions. However, the examination of trends in plant $\delta^{15}N$ between soils of varying soil moisture values would offer support to the relationships documented in actual field conditions.

As determined in chapter 4, plant tissue $\delta^{15}N$ is more enriched in areas of fields that experience higher soil moisture conditions in the early stages of plant growth. The likely cause of this relationship is that wetter soils experience higher rates of denitrification, so remaining soil N is more ^{15}N enriched because the lighter ^{14}N is preferentially lost (Robinson 2001; Templar et al. 2007; Kahman et al. 2008). The relationship is complicated by the potential introduction of additional N sources specific to various areas of the field cultivated over several years. For example, plant tissue $\delta^{15}N$ may be higher at field site R2 not because of wetter soil conditions at the early stages of the growing season but because of specific soil conditions over the course of several years has cultivated a specific $\delta^{15}N$ value in the SON pool (Yun et al. 2006). Therefore, an investigation of simulated plant growth would benefit by utilizing soil from a specific area of the field so that all grow barrels would have a similar N pool at the outset of the experiment.

The use of plant $\delta^{15}N$ as an indicator of soil N status and availability is complicated by various factors, but a relationship has been previously established. The ability to reduce or eliminate a number of these complicating factors would serve to increase the accuracy and effectiveness of the study. This is achieved through the replication of natural growing conditions in a controlled environment, so that soil conditions are known from the beginning to the end of the plant growth cycle. As well, a range of moisture conditions can be established and maintained so that the relationship between soil N cycling processes and plant tissue $\delta^{15}N$ can be verified.

The study will test the controls of plant $\delta^{15}N$ and $\delta^{13}C$ values observed in the field setting by growing corn under controlled soil, moisture, and fertilization conditions. The understanding of denitrification as a mechanism of enrichment in wetter soils gained through field observations will be further verified by controlled plant growth. This knowledge will be supplemented by investigating the isotopes of NO_3^- and soil measuring N_2O production. A simulated drought will also be conducted on selected barrels to examine the effects of plant $\delta^{13}C$ values due to

moisture stress, which could not have been properly assessed in the field. The relationship between soil conditions and plant isotopes verified within the controlled growth environment can subsequently be coupled with observed field values, which will further gauge the effectiveness of plant tissue isotopes as an indicator of N cycling and status in the soil.

6.2 Methods

6.2.1 Growth chamber setup

The soil that was used to construct the soil profiles in all nine grow barrels was removed from the R-Field close to site R2. The average conditions of soils in this area were documented previously. The soil was dug up and stored by horizon, so that three distinctive layers were established and utilized in the reconstruction of the barrel profiles. Bulk densities measured for each horizon at site R2 were used during profile reconstruction, as well as the addition of flow paths, to more closely replicate natural conditions. The barrels had several holes drilled out the bottom for the purpose of drainage, and we covered with screen mesh to keep the holes clear of soil. The mesh was covered with five centimetres of gravel to further promote drainage efficiency. Each of the three soil horizons were constructed using only soil from the same horizon out in the R-Field, which produced a profile with a darker, more organic mineral A horizon, a transitional B horizon that included some clay material, and a silt/clay C horizon. The larger clumps of soil were broken up before being added to the barrels and each horizon was packed according to its specific bulk density. The top horizon was cultivated and mixed after compaction to mimic regular field cultivation and tillage activity. All soil horizons were constructed to similar depths and thicknesses. Campbell Scientific ECH2O-TM probes were utilized to monitor soil moisture and temperature values at each horizon, and recorded conditions every five minutes and averaged those values out over an hour. Soil gas probes were constructed from 6 mm OD stainless steel tubes with septa capped tops, and were attached to

tubing. The tubing consisted of 1/8 inch PE tube through the septa at one end, and a luer connector on the other end to allow for sampling by syringe. Each barrel had four gas probes; installed to depths of 10, 20, 30, and 37 cm. Sunlight was simulated by two plant grow lights, and was measured by a net radiometer that was located among the barrels. While the intensity of the lights were never adjusted to simulate dawn, dusk, or cloudy days, the length of time the lights were on was adjusted to account for longer days at the beginning of plant growth and progressively shorter days as the cycle continued. A humidifier was utilized to increase the humidity in the air to more closely reflect summer growing conditions. The water used to maintain soil moisture conditions was collected over a period of several months from natural precipitation so that N introduced through atmospheric deposition could be maintained in the soil. The water was applied to each barrel with a garden watering can in an attempt to minimize the impact of the water droplets and subsequent soil compaction.

The primary reason for maintaining a regular frequency watering schedule was because the barrel soil profiles lacked the moisture retention abilities of natural soil profiles, so more frequent and less intense watering was more conducive to maintaining a targeted soil moisture range and healthy plant growth.

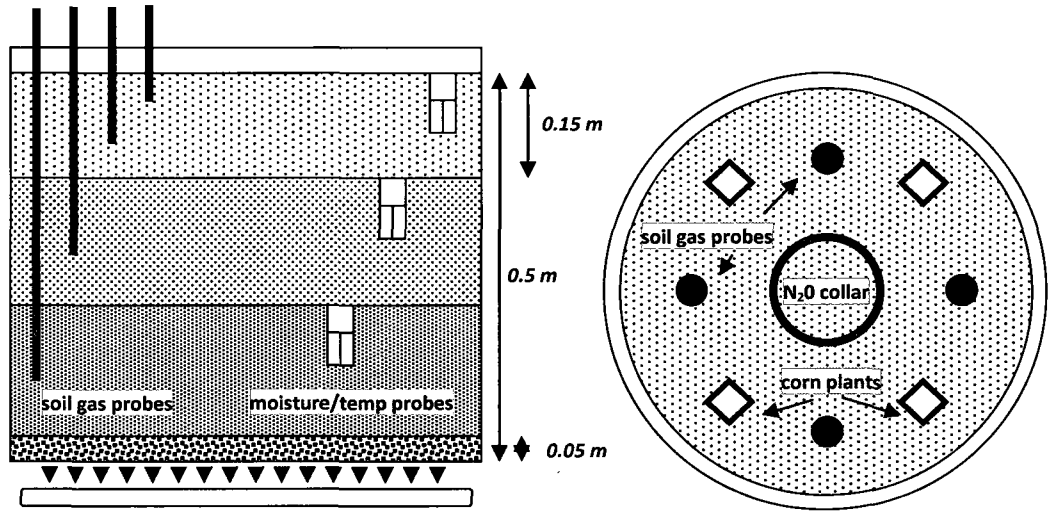

Figure 6-1: Schematic diagram of the soil barrels constructed for the experiment. The figure on the left shows the thicknesses of the individual soil layers (each layer was 15 cm), as well as the layer of gravel at the bottom. The figure also highlights the instruments installed in each barrel. The figure on the right shows the ideal distribution of plants and instruments within the barrels.

6.2.2 Growing conditions

Four corn seeds were planted in each barrel, and were evenly distributed between the four soil gas probes. The planting depth was the same as field planting depth, as was the type and rate of applied inorganic fertilizer mixed with the seed at planting.

The grow barrels were divided into relative moisture levels, consisting of dry moderate, and wet. As the constructed soil profiles retained water less efficiently it was difficult to maintain consistent relative moisture conditions within the barrels. Therefore, it was assumed that increased water inputs resulted in higher average soil moisture conditions. The wet barrels received consistently more water than the moderate barrels, while the dry barrels received the least throughout the growing season (Table 6-1).

Date	Wet Barrels	Moderate Barrels	Dry Barrels
14-Mar	4.08	2.55	1.02
22-Mar	4.08	2.55	1.02
26-Mar	4.08	2.55	1.02
01-Apr	4.08	2.55	1.02
07-Apr	5.10	3.57	1.02
15-Apr	5.10	2.04	0.51
21-Apr	4.08	1.53	1.02
27-Apr	2.04	1.02	0.51
28-Apr	2.04	1.02	0.00
01-May	2.04	1.02	0.00
06-May	4.08	2.04	0.51
09-May	2.55	2.55	2.55
20-May	2.55	2.04	1.53
23-May	3.06	2.04	1.02
28-May	2.55	0.00	0.00
04-Jun	3.06	0.00	1.02
10-Jun	3.06	0.00	0.00
16-Jun	3.06	7.14	0.51
25-Jun	3.06	2.04	1.02
01-Jul	4.08	4.08	3.06
03-Jul	3.06	2.55	2.04
06-Jul	2.55	2.04	1.02
11-Jul	3.06	2.04	0.51
16-Jul	3.06	2.04	1.02
21-Jul	3.06	2.04	0.51
25-Jul	4.08	3.06	2.04
29-Jul	3.06	2.55	2.04
05-Aug	3.06	2.55	1.53

Table 6-1: Water inputs to each barrel cluster throughout the plant growing season. The values are expressed in millimetres.

Figure 6-2: Barrel setup within the grow room. The figure on the left (18-Mar-08) shows the arrangement of instruments within the barrels, while the figure on the right (25-Jul-08) shows the stunted growth of the corn plants near harvest time.

6.2.3 Sampling and analysis

Soil samples were collected from all three layers in every barrel at the conclusion of the growing simulation. Soil samples were not collected throughout the experiment to avoid disrupting the soil profile and the upper horizon. Leaf samples were collected from a plant every three weeks throughout the season, while an entire plant was removed and divided by component in week eight. At the conclusion of the experiment the remaining two or three plants in every barrel were completely sampled and divided by component. The soil and plant samples were analyzed using the method described in section 3.3.3. Soil gas sampling involved purging three times the volume of the probe and tubing by syringe before sampling. 20 mL of sample was injected into an evacuated 12 mL Exetainer to await analysis. The gas samples in the Exetainers were run using a Varian CP3800 Gas Chromatograph, and the N_2O concentrations were calibrated against gas standards included in each run. The precision for the N_2O

concentrations is ± 10 ppbV. Water samples were routinely taken from the applied snow and water, as well as the drained sample located in the barrel water collection pans.

6.2.4 Simulated drought experiment

The moderate barrels were subjected to an additional drought simulation exercise which occurred at the end of May until mid-June. The simulation required zero water input during that period, aside from occasional plant misting to mimic morning dew conditions in the field. This misting was necessary in order to prevent the plants from dying due to lack of available soil moisture. Soil samples were collected three separate days following the end of the drought period, as well as plant samples and regular soil gas extractions.

6.3 Results and Discussion

6.3.1 Plant growing experiment

The soil moisture data was not properly captured by the instrumentation installed in the barrels, and therefore could not be included as part of the results. As a result, the range in soil moisture conditions created between the barrels is verified by the water inputs (Table 6-1). The wet barrels received a total of approximately 93 mm of rainfall throughout the growing season, while the moderate and dry barrels received 61 and 30 mm, respectively. The precipitation recorded at Strawberry Creek during the 2007 growing season was 143.5 mm. However, that value included two precipitation events that were difficult to reproduce in the barrels. If those two events are reduced to values more easily added to the barrels the total precipitation number drops from 143.5 to approximately 110 mm, which is much closer to the 93 mm applied to the wet barrels. The other consideration is that the plants grown during this simulation had a growing season two weeks shorter than the field crops, which results in lower total precipitation.

The range in water inputs between the barrels made it possible to identify potential $\delta^{15}N$ transformations brought on by N cycling processes. As well, it was also possible to investigate N movement through the soil. The barrels created a closed soil system, so that careful analysis on N inputs could be compared to outputs. The pans located below the barrels allowed for collection of excess water that filtered through the system, while the gravel layer under the soil profile served to promote that filtration. As in the field, the moisture requirements of the plant increase during growth, while the amount of water input in the form of precipitation generally decreases. Therefore, it was only possible to collect water samples towards the start of the growing season within the wet soil barrels. Figure 6-3 shows the NH_4^+ concentration of water samples taken from the pans below the three wet barrels at the onset of the growing season. The figure also highlights the NH_4^+ concentration of the snow and rain water storage reservoir at three points during those early weeks of the season. The lower concentrations observed in the collection pans is an indicator of the soil's ability to retain N from precipitation, while also potentially indicating low N concentrations in the soil. The average concentration decreases as the growing season progresses, which is the result of the continuing need for N by the plants as well as the steady decrease in soil moisture and the increased ability to retain both water and N.

The simulated growing conditions manufactured within a controlled environment produced similar trends and magnitudes in foliar $\delta^{15}N$ to what was documented in the field (Figure 6-4). The driest conditions produced depleted foliar $\delta^{15}N$ relative to the other barrel moisture regimes. The foliar $\delta^{15}N$ among all plants is very closely related at the outset of the growing season, as average values in all nine barrels at week 3 were approximately 8‰. The significance of the similar $\delta^{15}N$ in leaves in the early portion of the growing season is that the values may not only reflect N transformations in the soil but also the SON pool. The soils from all nine barrels came from a common location out in the field, and were allowed to dry out over the course of

several months before being reconstructed into the soil layers. The initial water inputs throughout the first several weeks of the growing season may only been serving to bring the moisture conditions back to normal levels, so that initially the rates of denitrification would have been low in all barrels, including the wet set. As the season progressed, and the wet and moderate barrels continued to receive higher water inputs, the soil conditions eventually balanced to the point where denitrification became a factor. This trend in N cycling is supported by foliar $\delta^{15}N$ values in the dry barrels never significantly ranging from the initial values, while those in the moderate and wet barrels continued to increase well into the growing season.

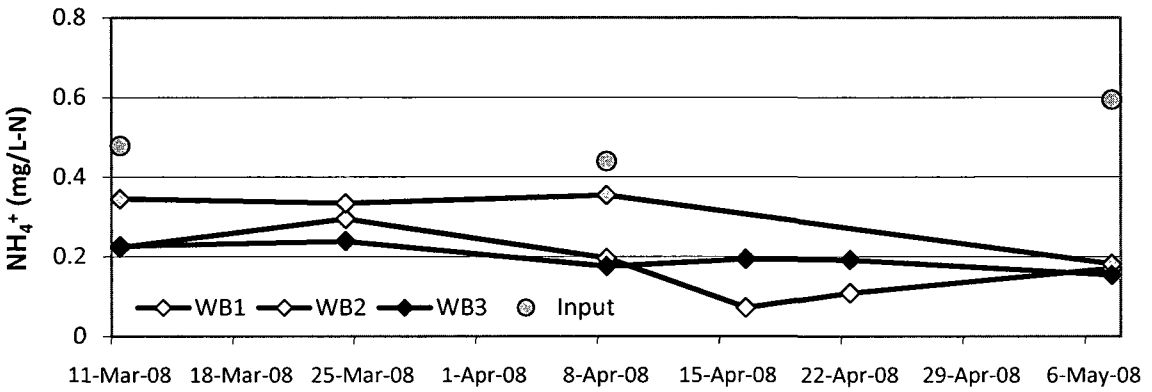

Figure 6-3: NH_4^+ concentration of water sampled from the collection pans below the barrels, as well as the NH_4^+ concentration of the snow/rain reservoir used for water input. As the moisture requirements of the plants increased during growth, and due to the soil profile's inability to retain moisture, samples were only collected in the wet barrels during the early stages of the growing season.

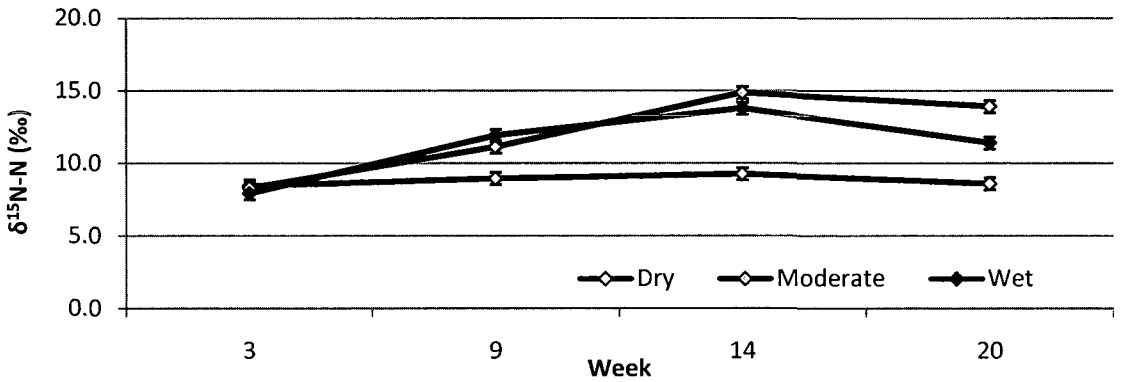

Figure 6-4: Foliar $\delta^{15}N$ values from the four sampling events throughout the growing season. The values displayed in the figure are an average of the three leaf samples collected from each of the moisture barrel clusters. Error bars indicate calculated precision.

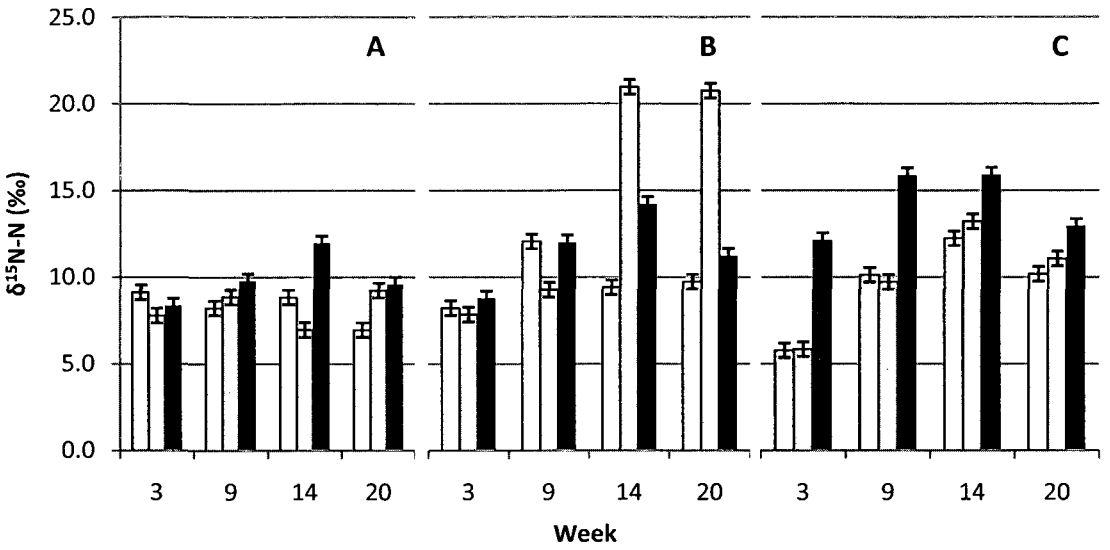

Figure 6-5: Foliar $\delta^{15}N$ values from collected leaves of the individual barrels throughout the growing season. Figure A (dry barrels), Figure B (moderate barrels), and Figure C (wet barrels) show inter-barrel variability within the same moisture classes. Error bars indicate calculated precision.

While there does appear to be a trend when examining the averaged values among the barrels, the pattern is much less defined when looking at plants from individual barrels. Figure 6-5 provides insight into that variability between barrels within the same moisture regime. While the dry barrel plants show very little variability, the moderate barrel plants show significant variability, especially MB2. The cause of the dramatic enrichment of foliar $\delta^{15}N$ between weeks 9 and 14 is potentially the influence of the simulated drought, which occurred during that time period. The foliar $\delta^{15}N$ values of the plants in the barrels also follow a similar trend to what was documented in the field; the decrease in $\delta^{15}N$ towards the end of the growing cycle.

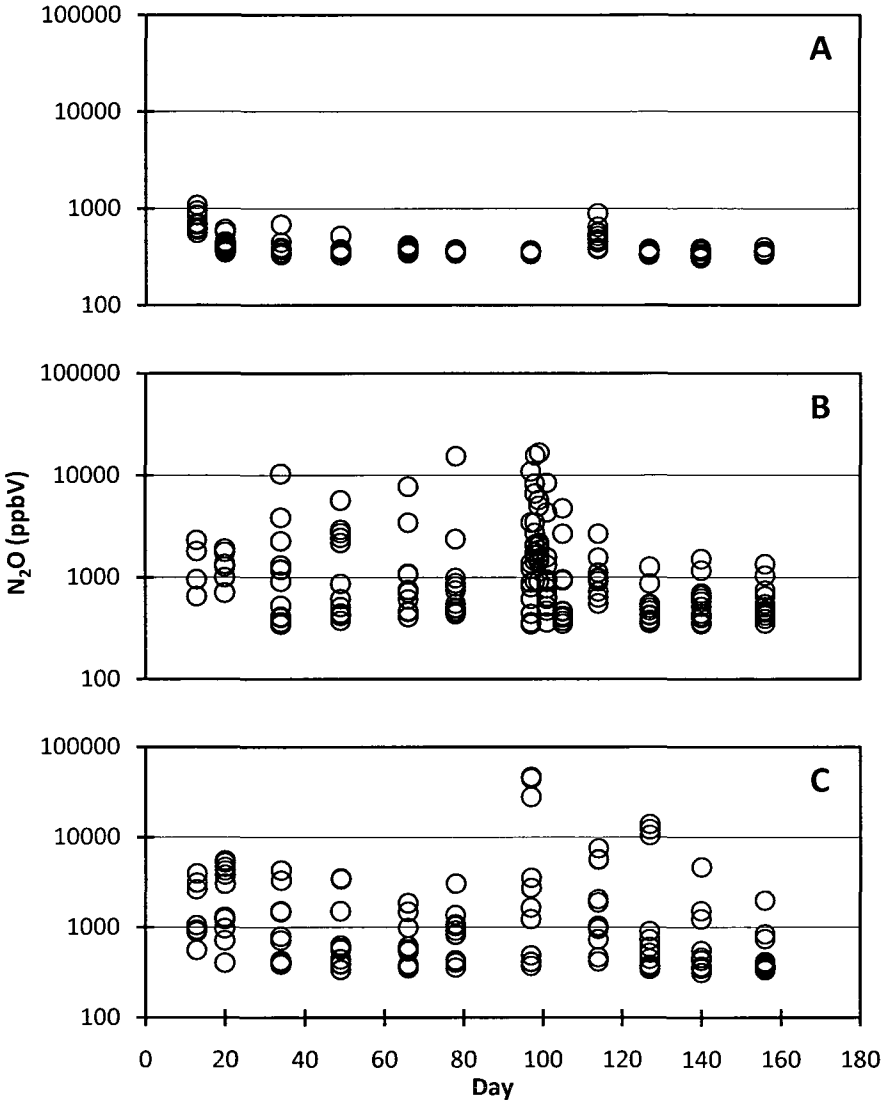

Figure 6-6: N_2O concentration in soil gas measured at all three barrels throughout the growing season. Figure A shows the data measured at the dry barrels, Figure B the moderate moisture barrels, and Figure C the wet moisture barrels. Data provided by John Spoelstra, University of Waterloo.

The foliar $\delta^{15}N$ differences between the wet and dry sites are consistent with the values measured in the field. The leaves are more enriched in $\delta^{15}N$ in the wet soils, which potentially indicates the increased presence of denitrification. Figure 6-6 highlights the N_2O concentration measured from all barrels throughout the growing season. The values seem to indicate that higher concentration values are being recorded in the moderate and wet barrels. More specifically, the moderate barrels experience the highest amount of soil N_2O production after

the drought period. These values coincide with the rewetting of the moderate barrels, which involved the highest single water input event (7 mm) to occur in any barrel during the entire growing season.

The foliar $\delta^{13}C$ values also provide a valuable comparison between those measured in the field, as patterns emerged throughout the growing season. It was discussed earlier that while $\delta^{15}N$ provides a reliable indicator of N cycling processes during the early stages of plant growth and component development, the relationship gets diluted over time if conditions change. Fortunately, foliar $\delta^{13}C$ is a reflection of the much more recent history of soil conditions, as the values respond to changes in soil moisture. Specifically, if soil moisture decreases to the point where the plant becomes water stressed the leaf stomata will close to limit transpiration and the foliar $\delta^{13}C$ will decrease as a result. However, the structural C must be taken into consideration, which would have a similar effect on foliar $\delta^{13}C$ as N has on foliar $\delta^{15}N$. The soil moisture values in the field were never low enough to decisively cause water stress in the crops, so while the foliar $\delta^{13}C$ values did decrease from July to August along with soil moisture it was difficult to address the relationship more closely. During the simulated crop growing season, the conditions in the dry barrels were such that the plants were visibly water stressed. Aside from receiving little water input, the plants also did not experience the moisture provided by daily morning dew that the field crops were subjected to. This lack of moisture had a noticeable effect on the growth and productivity of the plants. In fact, likely partially due to the limited ability of the constructed soil profiles to retain adequate moisture, the foliar $\delta^{13}C$ values of plants in all three barrel moisture regimes were lower than those values extracted from plants during the driest portions of the growing season.

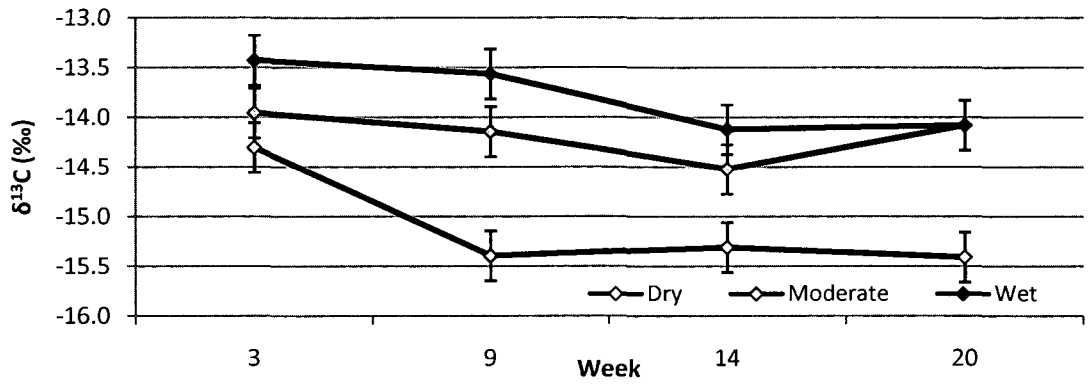

Figure 6-7: Foliar $\delta^{13}C$ values from the four sampling events throughout the growing season. The values displayed in the figure are an average of the three leaf samples collected from each of the moisture barrel clusters. Error bars indicate calculated precision.

The visible water stress incurred by the plants, specifically in the dry barrels, and the resultant decrease in foliar $\delta^{13}C$ is evident in Figure 6-7. As the wet barrels had more moisture available for plant uptake the plant $\delta^{13}C$ values were higher, but as the plants grew the water stress visibly increased and the leaf $\delta^{13}C$ decreased. Another measure of the value of foliar $\delta^{13}C$ as an indicator of plant stress was evident in the moderate barrels (Section 6.3.2). The simulated drought occurred between weeks 9 and 14, and there is a noticeable decrease in $\delta^{13}C$ between those sampling events. The barrels also received more water than usual at the conclusion of the drought, which appears to be reflected in the increase in leaf $\delta^{13}C$ between weeks 14 and 20. However, the majority of $\delta^{13}C$ values obtained from the sampled leaves were lower than the foliar $\delta^{13}C$ values from the field-wide survey, and in the case of the dry barrel samples, much lower. That discrepancy may serve as an indication of the different soil conditions between the barrels and the field, or perhaps it is just a measure of the difficulty in maintaining consistent moisture conditions in a constructed soil profile. As the effectiveness of supplementing ^{15}N analysis with ^{13}C data was one of the potential indicators in the grow simulation it appears that the combination of both isotopes provides both a long-term and short-term reflection of soil processes and conditions.

6.3.2 Simulated drought experiment

The simulated drought experiment in the moderate barrel set allowed for investigation into plant and soil response at conditions not seen in the field. In respect to soil gas, a noticeable increase in soil N_2O production was documented immediately following the rewetting of the moderate barrels at the conclusion of the drought (Figure 6-6). This immediate response is likely due to the increased rates of denitrification occurring as a result of the wetter soils, and creates flux levels not seen in the moderate barrels throughout the rest of the growing season. Regarding foliar $\delta^{13}C$ there appears to be a correlation between the drought simulation and measured values (Figure 6-7). There does not appear to be a noticeable response to the lack of water between weeks 9 and 14, as the decrease in $\delta^{13}C$ values in the moderate barrels coincides with the decrease in foliar $\delta^{13}C$ values from the wet barrels. However, there is a clear increase in foliar $\delta^{13}C$ between weeks 14 and 20 that does not correspond to an increase in the wet barrel foliar values. This increase, due to the increased availability of water and the subsequent opening of leaf stomata and diffusion of atmospheric CO_2, is consistent with field observations and is a clear indication of the late season value of $\delta^{13}C$ analysis. There does not appear to be a clear response in foliar $\delta^{15}N$ to the drought simulation, likely because the leaves were fully developed by the time the drought started (Figure 6-4). However, it is also possible that the plants were not able to uptake N due to the lack of available soil moisture for uptake, and would not alter plant $\delta^{15}N$. The fact that the dry barrels were kept at near-drought conditions for much of the growing season, especially during the early stages of leaf development, would qualify those dry barrel foliar $\delta^{15}N$ values as the closest response to drought conditions.

6.4 Conclusion

The investigation into the use of plant $\delta^{15}N$ and $\delta^{13}C$ as an indicator of N processes and soil conditions is made increasingly difficult by the numerous factors at work within a complex field system. The goal of the growth simulation was to reduce those complicating factors as much as possible, and to assess whether the trends seen in the field remained. Another potential goal of the simulation was to push soil conditions to the extreme limits to gauge plant response. These conditions can provide additional insight, and cannot be replicated in field systems.

The foliar isotopic values from the grow simulation followed similar trends to those seen in the field. The plants grown in dry soil conditions were depleted in $\delta^{15}N$ while the plants in the wetter soils were enriched in $\delta^{15}N$. The barrels with the moderate soil moisture regime closely mirrored $\delta^{15}N$ values recorded at the field site with the highest fluctuation in moisture, suggesting that deriving soil N processes from those sites are increasingly difficult. That difficulty exists because $\delta^{15}N$ of plant tissue is a reflection of the soil conditions at the time of specific component growth, so if soil moisture changes between wet and dry conditions the $\delta^{15}N$ values of the plant tissue will be equally variable. The $\delta^{13}C$ of leaf tissue was verified in the growth simulation as being a valuable supplement for $\delta^{15}N$, as $\delta^{13}C$ can more closely reflect changes in soil conditions in the latter stages of the growing season. Fortunately, $\delta^{13}C$ is also not further complicated by partitioning of nutrients within the plant between various components, especially during cob development. The combination of plant tissue $\delta^{15}N$ and $\delta^{13}C$ supports the findings in the field in that plant isotopes can be indicators of soil conditions and processes throughout the entire growing season.

7.0 Conclusion

The issue of managing nutrients is becoming increasingly important both agriculturally and ecologically. The proper management of agricultural nutrients requires that enough organic and inorganic fertilizer is applied to the field to meet the requirements of the crop while subsequently limiting losses both to ground and surface water and the atmosphere. This understanding necessitates a need to explore the soil conditions and N processes that are responsible for managing N fate within the soil. The use of plant tissue as an indicator of those conditions and processes would provide valuable insight into the relationship between plants and the soil.

The value of plant $\delta^{15}N$ as an indicator of N status has been investigated previously (Robinson 2001; Templar et al. 2007; Kahmen et al. 2008). The conclusions outlined in these studies state that plant $\delta^{15}N$ can only generally distinguishing relative N cycling processes. This study has concluded the same, except that those general processes can be further refined using additional information available. The benefit in utilizing a variety of plant and soil indicators, as well as exploring their relative correlations, is that the major factors interfering with this type of analysis can be better understood. These interferences, including discrimination during N uptake and intra-plant partitioning, can be specifically examined. The introduction of $\delta^{13}C$ further tightens the ability of isotopes to reflect what is occurring in the soil. $\delta^{13}C$ was found in this study to reflect more recent soil moisture conditions relative to sampling time, and in the more extreme conditions it provides an indicator of overall plant water stress. The relationships between soil moisture and N cycling established during the investigation into $\delta^{15}N$ can validate foliar $\delta^{13}C$ as a valuable indicator of cycling processes in the soil.

The difference between wet and dry soil locations and the effect on plant $\delta^{15}N$ was well established. Due in large part to higher rates of denitrification in the wet locations, the plant

$\delta^{15}N$ is more enriched than plant $\delta^{15}N$ at drier sites. This trend is maintained throughout the growing season. The potential for NO_3^- leaching also factored into the enriched foliar $\delta^{15}N$ values at the wetter sites. It was documented that the majority of soil inorganic N was in the form of NO_3^-, and by the end of the growing season almost all of that NO_3^- was leached, denitrified, or assimilated.

The investigation into whether the moisture/ $\delta^{15}N$ relationship exists across a wider range of soil moisture conditions showed that, while the relationship was not quite as pronounced, it still existed. As well, the field-scale investigation verified $\delta^{13}C$ as a potential measure of changes in soil moisture during specific periods of time. The combination of both isotopes could provide a detailed map of conditions during both the early stages of plant growth as well as during the latter stages of the growing season when plant components, with the exception of the cob, are finished growing. A final growth simulation was attempted to minimize complicating factors between plant and soil N. This experiment also provided opportunity to push the soil conditions past what was seen or possible in the field. The experiment further reinforced the relationships observed in the field, and through the idea of potential water stress reaffirmed the value of $\delta^{13}C$ as both a stress indicator as well as a measure of more recent changes in soil conditions. The combination of studies confirmed plant $\delta^{15}N$ and $\delta^{13}C$ as indicators of N status and soil conditions. The existence of completing and complicating fractionation factors makes it difficult to make absolute claims on the relationship between plants and the soil, but it is possible to minimize the complicating factors by investigating all plant components. As mentioned, $\delta^{15}N$ and $\delta^{13}C$ provides insight into both recent changes in soil conditions as well as conditions that existed during component development. The combination of all available indicators provides an estimation of N cycling processes, and could assist in the further development of nutrient management strategies in crop agriculture.

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Appendix A

Figure A-1: Recorded soil moisture values of the upper 20 cm of the soil profile at all six intensive sites throughout the 2007 growing season.

	R1	R2	R3	T1	T2	T3
28-Apr-07	41.0	55.0	65.0	38.0	33.0	43.0
22-May-07	19.0	36.0	42.0	22.0	15.0	21.0
30-May-07	26.0	37.0	37.0	22.0	25.0	35.0
31-May-07	35.0	58.0	30.0	25.0	26.0	29.0
05-Jun-07	17.0	33.0	36.0	-	-	-
06-Jun-07	17.0	26.0	42.0	-	-	-
07-Jun-07	19.0	29.0	33.0	-	-	-
12-Jun-07	-	-	-	18.0	17.0	29.0
15-Jun-07	18.0	22.0	27.0	15.0	20.0	15.0
18-Jun-07	18.3	21.1	23.0	29.4	21.2	21.2
20-Jun-07	20.0	32.0	40.0	19.0	18.0	17.0
28-Jun-07	16.0	31.0	22.0	10.0	11.0	19.0
05-Jul-07	15.9	16.3	13.7	13.1	17.5	15.6
10-Jul-07	16.1	15.8	14.3	12.1	16.0	16.4
19-Jul-07	-	-	-	12.0	15.3	15.7
30-Jul-07	16.9	15.3	12.8	17.5	18.2	17.9
17-Aug-07	14.2	14.1	11.8	15.0	15.8	17.7
24-Aug-07	13.2	11.5	13.2	14.4	14.1	18.0
12-Sep-07	15.1	15.7	17.3	15.9	17.4	20.1
24-Oct-07	20.8	21.9	22.2	18.7	21.5	20.2

Figure A-2: Isotopic data from 10 corn leaves and 10 whole plants sampled as a measure of inter-plant variability within the two crop types.

#	$\delta^{13}C$	$\delta^{15}N$	%C	%N	#	$\delta^{13}C$	$\delta^{15}N$	%C	%N
C-1	-12.97	6.89	44.46	1.89	**O-1**	-28.22	3.59	42.00	1.02
C-2	-12.39	5.77	45.08	2.03	**O-2**	-27.77	3.70	43.03	1.21
C-3	-12.30	5.10	44.88	1.68	**O-3**	-26.99	1.31	43.30	1.35
C-4	-12.92	5.46	45.07	1.51	**O-4**	-28.77	3.39	43.62	1.10
C-5	-12.37	4.99	44.73	1.44	**O-5**	-27.13	2.76	43.59	1.27
C-6	-12.40	6.05	45.44	1.82	**O-6**	-27.78	4.01	43.23	0.90
C-7	-12.26	5.89	44.42	1.88	**O-7**	-27.11	3.40	43.48	1.29
C-8	-12.29	6.10	44.50	1.68	**O-8**	-27.76	2.08	43.11	1.14
C-9	-11.93	6.00	44.70	2.22	**O-9**	-27.98	4.66	43.56	1.26
C-10	-12.07	6.04	44.15	1.47	**O-10**	-27.92	4.01	43.64	1.00

Figure A-3: Isotopic data from 8 corn leaves sampled from a single plant as a measure of intra-plant variability.

	$\delta^{13}C$	$\delta^{15}N$	%C	%N
R3F-1	-12.22	8.80	42.95	1.63
R3F-2	-12.85	4.93	44.79	1.02
R3F-3	-13.08	8.79	42.80	1.37
R3F-4	-13.00	8.77	42.69	1.31
R3F-5	-12.95	8.62	42.51	1.49
R3F-6	-12.68	8.64	42.34	1.43
R3F-7	-12.66	8.14	41.73	0.39
R3F-8	-12.82	9.23	41.57	0.77

Figure A-4: Isotopic data from leaves collected at each of the R-Field sites every three weeks of the 2007 corn growing season.

	Week	$\delta^{13}C$	$\delta^{15}N$	%C	%N
R1	**3**	-12.42	4.32	43.52	4.47
	6	-12.42	5.99	42.97	4.51
	9	-12.71	5.08	42.64	1.60
	12	-13.20	4.79	43.07	2.46
	15	-13.23	6.37	44.43	2.32
	18	-12.94	4.27	43.30	0.98
	21	-13.45	4.81	42.04	0.96
R2	**3**	-11.88	11.33	43.00	4.24
	6	-12.06	13.59	42.86	2.47
	9	-12.30	13.68	45.80	2.50
	12	-12.30	13.39	44.77	1.78
	15	-12.99	14.21	46.08	2.71
	18	-12.70	11.52	44.66	1.79
	21	-12.47	10.91	44.87	1.57
R3	**3**	-12.41	6.60	42.93	2.84
	6	-13.75	13.93	44.20	2.48
	9	-12.37	12.80	44.75	1.35
	12	-12.37	9.95	43.64	0.89
	15	-12.83	8.29	44.75	2.41
	18	-12.22	8.80	42.95	1.63
	21	-12.77	7.46	42.63	2.00

Figure A-5: Isotopic data from leaves sampled at each of the T-Field sites every three weeks of the 2007 oat growing season.

	Week	$\delta^{13}C$	$\delta^{15}N$	%C	%N
T1	3	-27.97	7.39	40.43	3.99
	6	-27.19	1.46	36.85	1.27
	9	-28.04	6.63	30.29	0.60
	12	-28.32	7.08	44.95	0.96
T2	3	-26.60	5.81	38.28	2.44
	6	-27.71	2.57	41.61	0.94
	9	-27.42	4.17	41.63	0.80
	12	-27.87	4.92	44.77	1.24
T3	3	-28.20	12.63	38.99	3.05
	6	-28.70	8.69	40.68	1.43
	9	-26.59	13.85	44.33	1.23
	12	-27.64	14.37	43.28	1.14

Figure A-6: Isotopic data from all corn plant components sampled at site R1 throughout the 2007 growing season.

R1	Week	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Roots	3	-11.92	6.14	35.49	2.20
	9	-12.15	5.42	24.66	0.48
	15	-12.79	6.42	35.12	0.54
	21	-13.02	6.50	39.54	0.92
Lower Stalk	3	-11.92	4.47	40.88	1.51
	9	-12.11	4.98	41.59	0.56
	15	-12.80	8.64	44.94	0.33
	21	-13.31	7.80	43.40	0.43
Upper Stalk	3				
	9	-12.14	5.07	41.40	0.69
	15				
	21	-13.11	4.87	44.34	0.47
Cob	3				
	9	-12.05	4.46	41.84	1.68
	15	-12.41	5.79	44.94	1.17
	21	-12.44	4.85	42.71	1.28
Tassel	3				
	9	-12.95	5.57	45.11	2.27
	15	-12.84	5.89	45.74	0.95
	21	-13.13	3.52	43.90	1.03

Figure A-7: Isotopic data from all corn plant components sampled at site R2 throughout the 2007 growing season.

R2	Week	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Roots	3	-12.09	10.44	36.30	2.30
	9	-11.90	12.24	30.52	0.80
	15	-13.14	15.47	34.07	1.20
	21	-12.48	12.47	42.72	0.78
Lower Stalk	3	-11.87	10.31	40.57	3.88
	9	-12.06	13.42	43.06	1.00
	15	-12.42	17.83	42.79	0.59
	21	-12.62	12.46	45.86	0.33
Upper Stalk	3				
	9	-12.33	14.18	43.28	0.84
	15	-12.74	12.53	44.64	0.58
	21	-12.49	11.88	46.31	0.31
Cob	3				
	9	-11.59	14.16	42.96	1.93
	15	-11.82	13.65	45.53	1.25
	21	-11.48	13.03	45.46	1.39
Tassel	3				
	9	-11.43	13.77	47.27	2.38
	15	-12.74	11.97	43.87	0.95
	21	-12.09	9.75	46.21	0.90

Figure A-8: Isotopic data from all corn plant components sampled at site R3 throughout the 2007 growing season.

R3	Week	$\delta^{13}C$	$\delta^{15}N$	%C	%N
Roots	3				
	9	-13.34	9.37	38.32	0.63
	15	-12.30	7.54	69.35	0.59
	21	-12.15	6.69	43.10	0.46
Lower Stalk	3	-12.55	7.57	40.80	2.21
	9	-12.04	11.05	43.85	0.38
	15	-12.10	8.31	79.71	0.25
	21	-12.72	6.83	44.12	0.23
Upper Stalk	3				
	9	-11.95	11.27	41.82	0.69
	15	-12.28	7.70	43.84	0.41
	21	-12.76	6.57	44.78	0.33
Cob	3				
	9	-11.71	11.59	42.85	1.74
	15	-11.76	8.69	45.05	1.32
	21	-11.62	8.05	45.36	1.12
Tassel	3				
	9	-11.41	11.84	47.73	2.22
	15	-12.36	7.27	45.42	0.90
	21	-12.42	5.24	44.89	0.78

Figure A-9: Isotopic data from all soils sampled at all three R-Field and T-Field sites throughout the 2007 growing season.

	Week	$\delta^{13}C$	$\delta^{15}N$	%C	%N
R1	3	-21.73	8.06	1.97	0.18
	9		7.80		0.20
	15		8.20		0.17
	21	-20.47	8.38	2.11	0.19
R2	3	-19.85	8.26	2.67	0.23
	9		7.70		0.24
	15		5.70		0.54
	21	-21.45	7.80	3.02	0.27
R3	3	-24.16	5.82	10.06	0.72
	9		5.90		0.64
	15		5.90		0.51
	21	-23.13	5.53	8.59	0.60
T1	3	-15.84	9.32	3.34	0.21
	21	-15.60	8.99	3.44	0.22
T2	3	-18.38	7.88	2.35	0.19
	21	-16.54	7.88	2.44	0.18
T3	3	-23.11	9.19	3.12	0.32
	21	-23.29	9.02	2.91	0.30

Figure A-10: Extracted inorganic N concentrations from soils sampled at the three R-Field sites every three weeks of the 2007 growing season.

	Week	NH_4^+	NO_3^-
R1	3	1.01	82.41
	6	1.22	76.21
	9	2.68	24.61
	12	2.37	200.06
	15	1.32	12.71
	18	1.98	5.34
	21	1.25	3.42
R2	3	0.88	81.34
	6	9.35	157.19
	9	2.10	53.49
	12	3.02	84.09
	15	1.62	63.84
	18	1.58	23.89
	21	1.04	2.59
R3	3	1.36	84.25
	6	1.03	134.39
	9	163.74	233.76
	12	2.76	36.75
	15	1.29	41.04
	18	2.25	18.81
	21	1.93	8.59

Figure A-11: Extracted inorganic N concentrations from soils sampled at the three T-Field sites every three weeks of the 2007 growing season.

	Week	NH_4^+	NO_3^-
R1	3	0.88	35.02
	6	3.20	39.81
	9	0.95	3.27
	12	0.92	2.06
R2	3	0.72	31.42
	6	1.30	17.71
	9	1.29	4.52
	12	1.07	2.44
R3	3	1.84	87.60
	6	1.10	14.35
	9	1.19	4.57
	12	1.05	4.89

Figure A-12: Isotopic data from corn leaves sampled in both July and August at 18 sites across the T-Field during the 2008 growing season.

July	$\delta^{13}C$	$\delta^{15}N$	%C	%N	August	$\delta^{13}C$	$\delta^{15}N$	%C	%N
1-1	-11.65	16.25	44.61	3.64	**2-1**	-12.71	12.00	45.78	2.76
1-2	-11.59	6.89	44.50	3.75	**2-2**	-12.95	16.95	46.42	3.61
1-3	-11.87	7.42	43.78	3.43	**2-3**	-12.62	6.87	45.72	2.65
1-4	-11.93	6.63	44.27	3.67	**2-4**	-12.77	6.99	46.10	2.83
1-5	-11.87	5.28	44.18	3.62	**2-5**	-12.63	5.58	45.42	2.82
1-6	-11.30	6.39	44.09	2.32	**2-6**	-12.31	4.46	45.26	1.94
1-7	-11.72	4.39	44.74	3.41	**2-7**	-12.51	5.69	45.87	2.51
1-8	-11.77	4.55	44.07	3.19	**2-8**	-12.75	2.29	46.33	2.86
1-9	-11.76	4.43	44.66	3.26	**2-9**	-12.45	6.92	45.74	2.60
1-10	-12.23	11.81	44.11	4.04	**2-10**	-12.80	9.58	45.11	3.17
1-11	-12.40	10.99	44.01	4.07	**2-11**	-12.86	10.54	45.96	3.32
1-12	-11.69	5.02	44.34	3.28	**2-12**	-12.53	7.66	45.51	2.57
1-13	-11.83	7.98	44.25	3.54	**2-13**	-12.90	6.97	45.60	3.01
1-14	-11.64	6.34	44.44	3.09	**2-14**	-12.58	10.04	45.32	2.95
1-15	-11.76	4.96	44.42	2.96	**2-15**	-12.80	5.75	45.30	2.88
1-16	-11.23	7.51	44.14	2.64	**2-16**	-12.60	6.86	44.64	2.63
1-17	-11.28	5.02	44.00	3.17	**2-17**	-12.28	5.00	44.55	2.01
1-18	-11.16	8.40	44.40	2.53	**2-18**	-12.79	8.97	45.25	3.08

Figure A-13: Soil moisture values measured in the upper 30 cm of the soil profile in June, July, and August at 18 sites across the T-Field during the 2008 growing season.

Site	June	July	August	Site	June	July	August
1	53.0	47.0	28.0	**10**	48.0	48.0	26.0
2	56.0	50.0	25.0	**11**	39.0	43.0	40.0
3	38.0	34.0	17.0	**12**	38.0	40.0	28.0
4	30.0	34.0	19.0	**13**	46.0	40.0	28.0
5	41.0	43.0	30.0	**14**	38.0	45.0	26.0
6	22.0	22.0	15.0	**15**	46.0	46.0	26.0
7	25.0	28.0	23.0	**16**	40.0	24.0	14.0
8	22.0	38.0	22.0	**17**	42.0	24.0	23.0
9	24.0	29.0	21.0	**18**	23.0	28.0	26.0

Figure A-14: Isotopic data from leaves sampled in the three dry moisture soil barrels during the simulated plant growth experiment in 2008.

	Week	$\delta^{13}C$	$\delta^{15}N$	%C	%N
DB1	3	-14.23	9.13	43.51	4.52
	9	-15.78	8.22	45.01	3.36
	14	-15.62	8.85	44.90	2.43
	20	-16.10	6.95	44.18	1.74
DB2	3	-14.57	7.80	45.20	5.01
	9	-15.76	8.85	44.93	2.51
	14	-15.69	6.97	45.45	2.96
	20	-15.47	9.23	45.23	1.70
DB3	3	-14.11	8.37	43.73	4.72
	9	-14.64	9.77	45.01	3.14
	14	-14.62	11.96	45.68	3.20
	20	-14.65	9.58	43.15	2.48

Figure A-15: Isotopic data from leaves sampled in the three moderate moisture soil barrels during the simulated plant growth experiment in 2008.

	Week	$\delta^{13}C$	$\delta^{15}N$	%C	%N
MB1	3	-14.27	8.22	43.77	3.89
	9	-14.17	12.07	42.91	2.64
	14	-13.28	9.42	44.11	2.31
	20	-13.46	9.74	43.54	2.10
MB2	3	-13.39	7.86	44.35	5.26
	9	-14.20	9.27	45.21	4.18
	14	-15.28	20.95	45.07	2.95
	20	-14.64	20.75	43.45	1.20
MB3	3	-14.20	8.78	43.54	5.11
	9	-14.06	12.01	44.87	2.50
	14	-15.00	14.21	43.63	2.57
	20	-14.14	11.23	43.47	1.41

Figure A-16: Isotopic data from leaves sampled in the three wet moisture soil barrels during the simulated plant growth experiment in 2008.

	Week	$\delta^{13}C$	$\delta^{15}N$	%C	%N
WB1	3	-13.61	5.78	45.74	5.39
	9	-13.51	10.13	45.38	3.47
	14	-13.88	12.24	44.27	3.05
	20	-14.04	10.18	41.49	1.93
WB2	3	-13.12	5.84	40.34	5.07
	9	-13.43	9.72	43.29	4.07
	14	-14.28	13.21	44.29	3.04
	20	-13.98	11.07	41.25	1.63
WB3	3	-13.55	12.13	43.10	4.66
	9	-13.75	15.88	44.21	2.83
	14	-14.21	15.90	43.70	2.95
	20	-14.22	12.95	43.30	1.54