NITRATE DYNAMICS OF GRASS-LEGUME PASTURES

By

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Submitted in partial fulfillment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia

in co-operation with

Nova Scotia Agricultural College
Truro, Nova Scotia

December 2010

DALHOUSIE UNIVERSITY

NOVA SCOTIA AGRICULTURAL COLLEGE

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DALHOUSIE UNIVERSITY

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NOVA SCOTIA AGRICULTURAL COLLEGE

DATE: December 2, 2010

AUTHOR: Terri MacPherson

TITLE: NITRATE DYNAMICS OF GRASS-LEGUME PASTURES

DEPARTMENT OR SCHOOL: Department of Plant and Animal Sciences

DEGREE: MSc CONVOCATON: May YEAR: 2011

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Signature of Author

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ABSTRACT

In response to environmental concerns about NO_3^- leaching research has shifted toward the increased incorporation of nitrogen-fixing legumes, such as red clover, into agroecosystems to promote tighter cycling of nitrogen (N). Although more sustainable than fertilized systems, red clover still has the potential to contribute to leaching. The objective of this study was to ascertain the contribution of red clover to soil NO_3^- when grown in mixture with bluegrass. Soil solute samples were collected at 15 and 45 cm depth using ceramic suction lysimeters from two experimental pastures in Nova Scotia in 2009. The concentration of NO_3^--N in the soil solute of bluegrass-red clover mixtures was 10 to 25 times higher in Truro, and 5 to 16 times greater in Nappan, compared to the corresponding unfertilized pure bluegrass stand. Neither sub-surface irrigation nor two distinct red clover cultivar mixtures were found to significantly alter NO_3^- leaching patterns.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ADF	Acid detergent fiber
ANOVA	Analysis of variance
B	Boron
BNF	Biological nitrogen fixation
Ca	Calcium
CEC	Cation exchange capacity
cm	Centimeter
Cu	Copper
DMY	Dry matter yield
Fe	Iron
g	Gram
h	Hour
ha	Hectare
HPLC	High pressure liquid chromatography
Irr	Irrigated treatment
K	Potassium
Kg	Kilogram
L	Litre
m^2	Square meter
MAC	Maximum acceptable concentration
Mg	Magnesium
mg	Miligram

Mix	Mixed bluegrass / red clover treatment
mL	Milliliter
mm	Millimeter
Mn	Manganese
N	Nitrogen
N_2	Dinitrogen gas
NH_4^+	Ammonium ion
NO	Nitric oxide
NO_2^-	Nitrite ion
N_2O	Nitrous oxide
NO_3^-	Nitrate ion
NO_3^--N	Nitrate-Nitrogen
Na	Sodium
NDF	Neutral detergent fiber
noIrr	Non-irrigated treatment
NSAC	Nova Scotia Agricultural College
OM	Organic matter
P	Phosphorus
PO_4^{3-}	Phosphate ion
PO_4^{3-}-P	Phosphate-Phosphorus
psi	Pound per square inch
ppm	Part per million
Pure	Seeded pure bluegrass treatment
PVC	Poly-vinyl chloride

SDI	Subsurface drainage / irrigation
SEM	Standard error of the mean
SNY	Sward nitrogen yield
t	Tonne
TDR	Time-domain reflectometer
TP	Total phosphorus
yr	Year
Zn	Zinc
°C	Degrees celsius
%	Percentage

ACKNOWLEDGEMENTS

I would like to express my gratitude to the many individuals whose support and expertise made this project possible primarily Yousef Papadopoulos, Thomas Bouman, Alan Fredeen, Matthew Crouse, Vernon Rodd, and Sherry Fillmore.

I would like to thank Mom, Dad, Grampy, Wendy and Scott for their enduring love, support and patience.

And last but certainly not least, thank you to all the Master's students (especially Pam) who were there to listen and share in our multifaceted MSc experience. I feel incredibly fortunate to have crossed paths with so many fantastic people during the two plus years spent cultivating my Masters degree at NSAC.

CHAPTER 1: INTRODUCTION

The flourishing, fossil fueled lust for ever increasing food production and subsequent population growth on this planet is reaching unsustainable limits. The realization that there is a finite, dwindling supply of fossil fuels remaining, and our species cannot continue to prosper at the rate we have enjoyed is beginning to coalesce in the collective consciousness of our society. A nitrogen (N) supply is essential for plant growth but when the amount of applied N exceeds plant metabolic needs or the capacity of the soil to immobilize it, N may be lost to the atmosphere or leached into ground and surface water systems, contaminating them (Di and Cameron 2002). Oversupply of N is due in large part to the application of synthetic N fertilizer that requires substantial inputs of natural gas. Conversely, legumes are used in agriculture to naturally fix atmospheric N using solar power.

As excess NO_3^- leaches out of agroecosystems and into nearby aquifers the question being asked is whether or not the short-term economic benefits of N fertilizer outweigh the cost to our environment and dwindling resources. Seventy percent of the worlds' freshwater is supplied from groundwater, and NO_3^- losses from agriculture have been identified as one of the dominating threats to the groundwater supply that sustains life on this planet (Power and Schepers 1989; Addiscott 1996). Future agricultural systems that seek equilibrium between the maintenance of a level of food production, sufficient for humanity, without compromising the integrity of the environment must be sought and implemented before we compromise our drinking water, and capacity to grow food at the end of the oil era. Due to increasing concerns about water contamination and a gap in pasture sustainability research there has been a shift to target optimal utilization of N fixing legumes into agroecosystems to promote more efficient utilization of N through synchronous supply and uptake of N between legume and recipient, therefore minimizing N losses (Goulding 2000; Jarvis 2000; Crews and Peoples 2004).

CHAPTER 2: OBJECTIVES

The inclusion of leguminous plant species to fix N for grassland species is a more ecologically friendly alternative to the application of industrially synthesized mineral fertilizer. However, mixed legume systems still bear some potential to leach NO_3^- into groundwater (Scherer-Lorenzen *et al*.2003). Also, some seasonal accumulation of NO_3^- in the soil and a pronounced seasonal increase of NO_3^- leaching have been observed in pastures (Estavillo *et al.* 1996; Bouman *et al*. 2010). This investigation seeks to provide information pertinent to the development of future pasture management plans that improve the efficiency of N cycling by synchronizing the supply of N with plant demand. Three field experiments were established across Nova Scotia to:

(i) ascertain the contribution of red clover (*Trifolium pratense*) to NO_3^- leaching when grown in mixture with bluegrass (*Poa pratensis*),

(ii) examine how sub-surface drainage and irrigation may affect soil NO_3^- leaching

(iii) and discern the impact of two differing red clover cultivars on NO_3^- leaching when grown in mixture with bluegrass.

CHAPTER 3: LITERATURE REVIEW

3.1 The Nitrogen Cycle

Nitrogen exists in a multitude of forms in the environment with the largest reservoir of N found in our atmosphere, which is 78% dinitrogen gas (N_2). The atmospheric reservoir may appear to be a bountiful source of N for agriculture but the triple bonds between these two N molecules makes it largely unusable for plants and animals and it must be transformed. The transformation and flux of N species in the biosphere is referred to as the Nitrogen Cycle. The N cycle is comprised of continual biochemical exchanges that occur between three main compartments: the atmosphere, the soil and water environment, and the biomass. The main transformations of N among compartments in the soil environment occur via the following mechanisms: immobilization, mineralization, ammonification, nitrification, and denitrification (Canter 1997; Brady & Weil 2002). Pertinent inputs and exports in this system will also be discussed in the context of the agroecosystem.

Immobilization is the process whereby inorganic N becomes incorporated into complex biomolecules and becomes unavailable to growing plants in these organic forms. The inverse process that converts unusable organic N into inorganic N that becomes plant available is known as mineralization. Most agricultural plants make use of N in its inorganic form, such as NO_3^- and NH_4^+ (Korsaeth *et al*. 2003). Mineralization is a biotic process whereby bacteria and fungi break down organic matter, feces or senescent life forms, and N is subsequently released. There are two main processes embedded within mineralization: ammonification and nitrification (Schimel & Bennett 2002). Ammonification is a biotic process carried out by soil microbes that break down complex organic compounds into NH_4^+ in the presence of O_2. Nitrogen fixing organisms that reduce organic N to NH_4^+ are aided by the enzyme complex *Nitrogenase*. Nitrification is carried out by another group of soil microbes that oxidize NH_4^+ to NO_2^- to NO_3^- in a two step process often (but not exclusively) mediated by *Nitrosomonas* and *Nitrobacter* bacteria in the soil, respectively. Denitrification is the inverse microbial complement to nitrification where bacteria convert NO_3^- into: N_2, N_2O and occasionally NO depending upon soil conditions and O_2 availability (Brady & Weil 2002). These immobilization and mineralization processes occur simultaneously in the soil but the net effect of their processes has been found to be dependent upon the C:N ratio of senescent organic residues as well as temperature, soil characteristics, and moisture conditions (Drinkwater *et al.* 1998).

3.2 Sources of Nitrogen

Nitrogen is imported to the agroecosystem from a variety of interconnected sources. Anthropogenic activities import N through application of synthetic fertilizer, the loitering of excess fertilizer in soil organic matter, and other industrial activities that introduce N into the atmosphere. Wastes such as manure, crop residues and compost are incorporated into agricultural soils to improve soil N content. Nitrogen is also made available in the natural environment from biological N fixation, mineralization of soil OM and lightning events.

Anthropogenic Nitrogen Sources

In the early twentieth century humans profoundly altered the global N cycle when Fritz Haber discovered a reaction that could break the strong triple bonds of atmospheric N_2 to produce NH_4^+. Following Haber's break through, Carl Bosch made this reaction viable on an industrial level. The Haber-Bosch process made it possible to synthesize large amounts of N fertilizers, causing agriculture to shift from the use of extensive legume rotations to more intensive monoculturing of the land to increase production, boosting the amount of food farmers could produce (Smil 2001; Crews & Peoples 2004).

Smil (2001) estimates that the N contained in protein and DNA in 40% of humans worldwide was derived via the Haber-Bosch process. Crews and Peoples (2004) project that by 2050 up to 60% of people could attribute their existence to synthetic fertilizer. The Haber-Bosch process injects approximately 100 x 10^6 t of NH_4^+ into the N cycle annually. In the United Kingdom in 1970 the average application of N fertilizer was about 80 kg N ha^{-1} yr^{-1} but as land costs have increased, the economic imperative to increase output resulted in an increase in fertilizer use to an average of 160 kg N ha^{-1} yr^{-1} (Addiscott 1996). Upon reflection, Addiscott (1996) notes that it was in the late 1970`s that applications of fertilizer began to exceed crop assimilation. This trend has continued worldwide, as thirty years later the application of N fertilizer to some agroecosystems often exceeds 300 kg N ha^{-1} yr^{-1} (Ledgard 2001; Smil 2001).

Increased food production permitted by the Haber-Bosch process does not come without tradeoffs, both economic and environmental. The cost of N fertilizer has quadrupled in the last decade due to an increase in the price of the non-renewable natural gas generally utilized to produce it. The synthesis of fertilizer is an energy intensive process requiring 33 X 10^6 Btu of natural gas per ton of ammonia (Huang 2007). When fertilizer is applied beyond plant requirements, excess N exits the system via volatilization, runoff or leaching causing a multitude of adverse environmental effects. Excess N fertilizer can also manifest itself as another N source through residual inorganic N that can build up in the soil. Cuttle and Scholefield (1995) postulated that the reason NO_3^- leaching increased in a grassland trial from year four onwards, despite consistent nutrient management, was attributable to a build up and subsequent mineralization of soil organic matter. It would represent a challenge for farmers to incorporate this into their nutrient management plans as mineralization activity is influenced by highly variable weather conditions that will not release a predictable amount of N from season to season.

Due to industrial activity the deposition of atmospheric N as NO_3^- or NH_4^+ has also been on the rise in many countries around the world. Atmospheric N can be deposited as N dioxide, nitric acid and particulates or it can be dissolved in vapor and deposited via precipitation. Goulding *et al.* (1998) estimates that from 28 to 46 kg N ha^{-1} yr^{-1} was deposited on arable land across the United Kingdom in 1996. This estimate quantifies the net atmospheric deposition from both wet and dry forms on sites downwind of industrialized areas. In a less industrialized area, Ledgard (2001) estimates atmospheric deposition to be much lower on arable land in New Zealand, from 5 to 10 kg N ha^{-1} yr^{-1}. Power and Schepers (1989) indicate that atmospheric N deposition is greatest in the summer and that twice as much ammonium is found in precipitation deposits compared to NO_3^-. In Atlantic Canada, grasslands are generally located in rural areas away from industry so it is assumed that in this research experiment that atmospheric deposition is low.

Waste Sources of Nitrogen

Disposal of the 15 million tons of nitrogenous waste produced annually in the United States is a major concern for both environmental protection as well as the maintenance of health standards. Sources of this waste are: 40 % from animals; 30 % from crop residue; and 20 % from municipal waste (Power & Schepers 1989). If properly managed, a large portion of this N waste can be recycled back into agricultural production systems. Animal wastes can be returned to pasture via direct excretion from grazing cattle, or collected from confined production units and redistributed mechanically. Crop residues contain N that can be recycled back into the soil. The majority of municipal nitrogenous waste is destined for the landfill, but the inception of organic diversion programs has created compost that can be used to safely return N to the soil.

Grassland production systems have long made use of livestock wastes to improve the nutritive quality of the soil. Manure is a desirable nutrient import because it is inexpensive and high in organic matter. Addiscott (1996) estimates that cattle in the UK excrete upwards of $600 million worth of N per year. Similar to the organic N discussed in the context of the N cycle, the N contained in the organic matter of manure must be mineralized and nitrified before it becomes readily plant available. This means that the N from manure is more slowly available and will improve the N availability of the soil years after its initial application, albeit unpredictably. The availability of the N from manure is highly variable depending upon its composition and prevailing soil conditions, both of which will influence microbial activity (Brady & Weil 2002). For example, when Di & Cameron (2002) reviewed the percentage of N made readily available from the total N in a range of effluents they found that from 60-85 % of the N in hog wastes became plant available, 25 % from dairy sheds and 15 % from dairy sludge treatment pond. The authors estimated that up to 90 % of the N in the pig slurry was in a readily mineralizable form, whereas most of the N in the dairy effluent was in a recalcitrant form.

A number of studies have highlighted the N contribution of grazing animals to pasture (Cuttle & Scholefield 1996; Jabro *et al.* 1997; Ledgard 2001; Di & Cameron 2002; Wachendorf *et al.* 2004). Ruminant animals in particular make poor use of the N they ingest. For every 100 kg of N applied as fertilizer as little as 5-10 kg N would be recovered in meat or milk products. This means that grazing ruminants, cows for example, return 90-95 % of the N they ingest back on to pasture (Addiscott 1996; Di & Cameron 2002). Jabro *et al.* (1997) estimates that, on average, cows excrete 3 L of urine and 2 kg of manure per defecation in pasture. Approximately 70% of the N deposition upon pasture is in the form of urine, and it is estimated that a cow may urinate 10-12 times a day on a 0.5m^2 area. The deposition of urine across the pasture is highly spatially variable, and intensifying N loading concerns, cows often urinate in a specific area of pasture habitually (Addiscott 1996).

There are approximately 3-4 metric tons of N found in crop residues annually in the United States and farmers recycle the majority of this N back into the soil (Power & Schepers 1989). Leguminous crop residues are decomposed and mineralized more rapidly to utilizable N by the microbial community versus non-legume crop residues. Nitrogen from legume residues becomes available quicker and some N can be utilized by the plant community in the following season. Non-legume crop residues such as corn stalks or straw decompose at a slower rate and during the decomposition process this N may be immobilized in the microbial biomass. As a result non-legume crop residues are not as likely to contribute excess concentrations of N in the soil (Loiseau *et al.* 2001). The technique used to incorporate crop residues into the soil will also dictate the release of N to the plant community. In a wheat production system in the UK reviewed by Di & Cameron (2002) it was observed that when the straw was mechanically incorporated into the soil an extra 43 kg N ha^{-1} was returned to the soil organic N pool.

Brady and Weil (1996) define compost as a mixture of organic residues and soil that have been piled, moistened, and stored under conditions amenable to aerobic decomposition. The humus-like end product of this decomposition is rich in nutrients such as N that are required for crop growth. The content of compost is highly variable depending upon its source composition and compost management, such as aeration. Compost can contain from 1-5% total N which can be stored until the crop requires it. Application of municipal compost is not a common in large scale agriculture.

Naturally Occurring Sources of Nitrogen

Biological N fixation has the ability to create large amounts of plant available N using solar energy and symbioses instead of the non-renewable natural gas currently utilized to synthesize N chemical fertilizers. Sprent & Sprent (1990) postulated that bacteria in the soil, water and air on earth convert approximately 100 x 10^9 kg of atmospheric N annually into life sustaining compounds. A selected number of microbes including bacteria, actinomycetes, and cyanobacteria catalyze the breakdown of N_2 using the enzyme *Nitrogenase*. One of the more important sources of natural N in agroecosystems is produced from the symbiotic relationship between leguminous plants, such as clover, and *Rhizobium* bacteria. These bacteria invade the root hairs and cortical cells of the legume causing the formation of root nodules that become the site of N fixation. This coupling is a mutually beneficial symbiosis as the bacteria supplies the legume with a source of N compounds and the legume in turn supplies the bacteria with carbohydrates for energy (Brady & Weil 2002). Pasture legumes such as red and white clover, are estimated to have the capacity to fix between 100-200 kg N ha^{-1} yr^{-1}, depending upon soil and climate conditions (Ledgard 2001). The importance of biological N fixation from clover in pasture and its benefit to adjacent species will be further explored in this document.

The power of natural force is put on display during extreme weather events such as thunderstorms. Lightning events during thunderstorms can generate enough energy to break the powerful bonds in atmospheric N_2 molecules, leaving elemental N sufficiently isolated as to combine with oxygen changing it to a useable form. Sprent and Sprent (1990) estimate that lightning events can fix as much as 30 kg N ha^{-1} yr^{-1}, but caution that amounts of N fixed from this source are spatially and temporally variable. Many of the experiments that estimate the amount of N fixed during atmospheric combustion events are conducted in the lab and may not be representative of the atmosphere at large.

The mobilization of N from soil OM by mineralization is a major natural N contributor into agroecosystems. Gerwing and Gelderman (1990) estimated that in a South Dakota study an average of 45 to 67 kg of N ha^{-1} yr^{-1} was mineralized to plant available NO_3^-. The amount of soil OM and its subsequent release of N varies with site specific conditions and management practices. Soil disturbances such as tillage have been associated with triggering an increase in the rate of net mineralization which acts to increase soil available N. This variability also introduces an increased risk for N losses (Cuttle & Scholefield 1995). The structure and characteristics of the soil itself can play a role in the N cycle as an N storage compartment. Positively charged ammonium ions strongly adsorb to the surface of negatively charged soil colloids such as clay (Brady & Weil 2002). Due to a reduction in water holding capacity of some soils, concentrations of NO_3^- ions in the soil have been found to increase with decreasing clay content. This seems to be a result of less opportunity to be immobilized or de-mineralized in a sandy soil due to rapid drainage (Cuttle & Scholefield 1995).

3.3 Fate of Nitrogen in Agroecosystems

Nitrogen is a vital nutrient required for plant growth and development. Nitrogen is essential for the engine of photosynthesis – chlorophyll, as well as the synthesis of amino acids, one of the basic building blocks of life on this planet (Di & Cameron 2002; Brady & Weil 2002). When the agroecosystem is functioning as intended the primary removal of available N is via plant uptake that will stimulate lush green biomass production of the crop. Depending upon the species of plant and its stage of development, healthy foliage contains between 2.5 to 4.0 % N. Crops take up inorganic soil N predominantly in the forms of NO_3^- and NH_4^+. Predominantly, farmers apply synthetic fertilizers to supply their crop with N. However, due to economic pressure to maximize biomass production N fertilizers are often oversupplied, reducing the N use efficiency. Common fertilization regimes can result in only 20-50 % crop N utilization (Brady & Weil 2002). Excess N not taken up by the crop nor immobilized into soil OM is reallocated to other N compartments in the agroecosystem which can result in gaseous or leaching losses. Where the excess N ends up is determined largely by edaphic conditions. Crop uptake is a function of the species composition of the plant community. There is growing evidence that functionally diverse plant communities will make better use of available N as a result of niche complementarity (Scherer-Lorenzen *et al.* 2003; Palmborg *et al.* 2005). The dynamics of biotic control in agroecosystems will be discussed later in the context of NO_3^- leaching.

When the soil pore volume is filled with water, contains less than 10% oxygen, temperatures are around 25-30°C and there is a decomposable N substrate readily available - conditions are ripe for microbial mediated denitrification and subsequent volatilization of N. If oxygen levels are minimal the soil microbes will seek the oxygen atoms contained in the NO_3^- ion, resulting in the release of non-reactive N_2 gas (Addiscott 1996; Brady & Weil 2002). If soil acidity, NO_2^- and NO_3^- concentrations are elevated, O_2 availability is not too low and soil carbon is low, reactive N_2O gas will be produced. Large increases in N_2O emissions over the past 50 years are likely attributable to agricultural fertilizers. Gaseous losses from denitrification have been observed to range from 10 to 60 kg N ha^{-1} yr^{-1} (Goss *et al.* 1995; Addiscott 1996; Kramer *et al.* 2006). When N is lost via denitrification this may lessen the amount of N lost via leaching. However, gaseous losses of N_20 molecules are nearly 300 times more effective than CO_2 at radiative warming in the atmosphere, contributing significantly to global warming (Kramer *et al.* 2006).

Consequences of N loss from Agroecosystems

Nitrate is the main form of N present in the soil environment that is readily taken up by plants. Unlike positively charged ammonium ions that are adsorbed by negatively charged soil colloids, NO_3^- ions are not. When there is an influx of water into the soil system, NO_3^- moves freely with the percolating water (Brady & Weil 2002). Nitrate is a desirable form of N for plant uptake but when N is supplied in excess of plant demand, NO_3^- can end up in unwanted places such as the groundwater or outside ecosystems. Groundwater is the primary source of water for half of the inhabitants of North America and over 90 % of rural North Americans rely upon this water source (Power & Schepers 1989). Elevated levels of NO_3^- in drinking water have been controversially linked to two medical afflictions: Methemoglobinemia in infants and stomach cancer in adults.

Methemoglobinemia, also known as "blue baby syndrome", occurs when the water for infant formula comes from NO_3^- contaminated wells. Consumed NO_3^- can combine with hemoglobin to form methemoglobin which reduces the oxygen carrying capacity in the blood, resulting in cellular anoxia. In the U.S. the maximum acceptable contaminant limit for NO_3^- in drinking water is 10 mg L^{-1} of NO_3-N based upon human case studies where methemoglobinemia poisonings in infants occurred following ingestion of drinking water that contained between 10-20mg L^{-1} of NO_3-N (Addiscott 1996; Canter 1997).

Several studies have also linked NO_3^- in groundwater to the incidence of gastric cancers in Colombia, England and Chile. Nitrate has been found to react with amine groups in the stomach to form carcinogenic N-nitroso compounds (Power & Schepers 1989). Some recent articles such as the one published by Powlson *et al*. (2008) question whether there is conclusive scientific evidence to link elevated NO_3^- to these medical afflictions. Many of these studies have failed to take into account baseline levels of NO_3^- produced by the human body, or that wells providing the formula mixture for babies contained bacteria as well as NO_3^-. One must also proceed with caution when it comes to interpreting the results of epidemiological studies. Correlation does not equal causation and there are many environmental and dietary factors that may influence gastric cancer (Van Leeuwen *et al.* 1999). However, certain sub-populations may be more susceptible to the adverse effects of elevated NO_3^- than the population at large (Powlson *et al.* 2008). There are individuals in the general population that have increased rates of endogenous formation of these carcinogenic N-nitroso compounds. The risk posed to these people by lax governmental NO_3^- limits in groundwater should be taken into consideration as well.

The issue not being disputed is the notion that NO_3^- levels in the environment have risen over the past number of years due to increased fertilizer usage. It is becoming accepted in the scientific community that this excess N is disrupting ecosystem function causing such adverse effects as aquatic eutrophication. Terrestrial plants are not the only plants that require N for growth, marine plants do as well. Aquatic ecosystems are generally nutrient poor or oligotrophic (Aber & Melillo 2001). When there is an influx of N into an N depleted aquatic ecosystem primary production and associated phytoplankton biomass increases rapidly until O_2 becomes limited, resulting in anoxic conditions that are fatal to other desirable aquatic life forms which either leave or perish. This influx of N is suspected to originate predominantly from agricultural runoff (Addiscott 1996; Beman *et al*. 2005). Annually a dead zone up to 22,000 km^2 forms in the Gulf of Mexico that coincides with seasonal fertilization and subsequent agricultural runoff from the Mississippi River (Diaz & Rosenberg 2008). Beman *et al.* (2005) have also found a close correlation between the amounts of N lost from Yacqui Valley agroecosystems during fertilizer/irrigation events and the N requirements of 600 km^2 phytoplankton blooms that form in the Gulf of California.

As of 2008, dead zones were reported in 400 locations around the world totaling 245,000 km^2 (Diaz & Rosenberg 2008). Dead zones are projected to increase in size as developing countries (many near N limited ecosystems) increase fertilizer use to boost production to feed their burgeoning populations (Janzen *et al*. 2003). Also, if the U.S. continues to press on promoting corn ethanol as a primary alternative biofuel this would act to exacerbate the runoff and subsequent marine hypoxia issues (Beman *et al*. 2005). Recently, NO_3^- leaching from agriculture has also been found to also increase phosphate mobilization, provoking phosphate and sulfate eutrophication in wetlands (Smolders *et al*. 2010). Wetlands and the diversity of plants and microbes that inhabit them act as a filter for surface and groundwater keeping our water clean. The loss of these unique ecosystems would eliminate one of nature's internal bioremediation processes currently acting to keep our coastal waters clean.

When NH_4^+ is oxidized to NO_3^- by soil microbes, hydrogen ions are released. A release of hydrogen ions during nitrification causes an increase in soil acidity and a drop in pH. Crop growth alone can cause the soil environment to slightly acidify, and this action is worsened by high availability of NH_4^+ fertilizers and an enhancement of crop growth. Many soils at risk for acidification are limed with calcium carbonate to neutralize their pH. Poorly buffered freshwater streams adjacent to agricultural operations are also at risk for acidification from influxes of NH_4^+ and NO_3^- (Rabalais 2002). The trophic structuring of freshwater streams and lakes are critically altered during such nutrient influxes. An experimental lake acidification study conducted by Mills *et al.* (2000) in Northwestern Ontario demonstrated the detrimental effects of acidification on lake biodiversity. At a pH of 5 five species of fish had recruitment failures and two of these died off entirely and were unable to recover when the pH was again neutralized. Due to such trophic alterations lake trout (*Salvenlinus namaycush*) abundance and recruitment started dropping during the pH recovery stage. The authors attributed this to an absence of early and late life stage prey items from zooplankton to sculpin fish species that disappeared when the pH first dropped.

Strong scientific evidence is accumulating that identifies agriculture as the force that is introducing the most excess NO_3^- into the environment. Therefore, it is at the agroecosystem level that we should start to seek solutions by exploring and implementing management practices that decrease NO_3^- losses while maintaining production. To limit NO_3^- leaching, we must first understand the multifaceted conditions that dictate NO_3^- mobilization in the soil environment. Key factors influencing the leaching of NO_3^- in Atlantic Agroecosystems are expanded upon in Chapter 3.4.

3.4 Factors Influencing Nitrate Leaching

There is little doubt that nutrient inputs into large scale agroecosystems are greater than ever before and that the buffering capacities of soil and water ecosystems are reaching their limit to utilize more. This limit is determined by a number of factors, both internal and external. These factors influence how an agroecosystem copes with NO_3^- excesses and ultimately determines the degree to which excess nutrients will be lost via leaching. Seasonality and climate, soil characteristics, hydrology, the form and amount of added N as well as the plant community are all important modulators of NO_3^- leaching in agroecosystems.

3.4.1 Seasonality and climate

Nitrate leaching shows some seasonal variability associated with certain months of the year. In temperate regions grasslands are prone to leaching following grazing in the autumn, and winter (Estavillo *et al.* 1996; Bouman *et al*. 2010). Weather conditions affect the supply of NO_3^-

to be lost via leaching by affecting plant uptake, mineralization, nitrification and denitrification (Scholefield *et al.* 1993; Addiscott 1996). Variable weather conditions can drive changes in the N cycle in a number of ways. For example, Scholefield *et al.* (1993) state that an extended period of hot, dry weather followed by moist conditions could act to further enhance N mineralization. The authors attribute this effect to the rejuvenation of soil microbes after dry conditions acted to partially sterilize the soils.

Soils appear to be least at risk in Atlantic Canada during the growing season because soil N leaching is largely offset by plant N uptake mid-season. Conversely, when plant growth slows in the fall and halts in the winter there is a decrease in the amount of N removed from the soil (Di &Cameron 2000; Scherer-Lorenzen *et al.* 2003; Bouman 2008). In the fall temperatures are still relatively high and there is an increase in soil moisture due to increased precipitation. These conditions create an ideal environment for the soil microbes that produce NH_4^+ and NO_3^-, in this instance just in time for when plants need it least. As seasonal rainfall moistens the soil, it also eventually saturates it, resulting in a net flow of water downwards, carrying available NO_3^- with it. This effect is especially pronounced in well drained sandy soils (Scholefield *et al.* 1993; Addiscott 1996; Conrad & Föhrer 2007).

With the knowledge that NO_3^- leaching is seasonally influenced, we can reduce fall losses with sward management strategies that synchronize the spatiotemporal growth patterns of grass species to seasons associated with NO_3^- losses. The best way to reduce seasonal N leaching losses is to ensure that there is a paucity of NO_3^- available at these risky times. The inclusion of a diversity of forage grass species provide consistent production, improved late season growth and associated N uptake patterns. Reed canary grass (*Phalaris arundinacea*) or Kentucky bluegrass (*Poa pratensis*) in mixtures, for example, could help reduce the availability of soil NO_3^- and henceforth, the risk of fall NO_3^- losses (Schuster & Garcia 1973; Barnhart 1999;

Barnes *et al.* 2007). There would also be less stored N available to leach during the winter when growth ceases entirely. Conversely, including a grass species in mixture whose dry matter production peaks early in the season, such as timothy (*Phleum pretense*), and goes dormant toward the fall would do little to alleviate late growing season leaching and a seasonal accumulation of stored N (Rode & Pringle 1986; McKenzie *et al*. 2005; Bouman *et al.* 2010). Plant phenology in the context of sward N conservation will be further discussed in Chapter 3.4.5.

3.4.2 Soil Characteristics

Soils vary greatly in water transmitting ability depending upon their texture. Henceforth they also differ in their ability to transmit dissolved NO_3^- in soil solution. Addiscott (1996) describes the different behavior of three main soil types: sandy soils, aggregated soils and heavy clay soils. Sandy soils have large soil particles, homogenous textures and large spaces between each of these particles. Water and dissolved NO_3^- percolates freely through sandy soils. Aggregated soils has a heterogeneous distribution of soil material. If fertilizer is applied to moist soil, much of the negatively charged NO_3^- will be immobilized by positively charged soil aggregates. However, if the soil is dry when fertilizer is applied, a precipitation event will wash NO_3^- through the soil taking the path of least resistance around the soil aggregates and into the groundwater. In heavy clay soils the tiny soil particles strongly adhere to one another. This dense structure inhibits the movement of water through the soil matrix. This clay structure shrinks when it is dry and swells when it is wet. When drying and shrinking of clay soil occurs, cracks can form preferential flow channels. Water can flow rapidly through these small channels when it rains, carrying NO_3^- with it (Conrad & Föhrer 2007).

Concentrations of NO_3^- in soil percolate have been found to vary five-fold depending upon soil type. Cuttle and Scholefield (1995) noted that leaching decreased with increasing clay content of the soil. The authors postulate that the presence of clay particles reduced soil water holding capacity and degree of preferential flow. Korsaeth *et al.* (2001) looked at the impact of two soil types, silty sand of morainic origin and coarse sand of sedimentary origin, on NO_3^- leaching. The authors found that the two soils had similar total porosity but the coarse soil had greater air capacity, saturated hydraulic conductivity and lower moisture retention than the silty soil. Grassland yields were lower on the coarse sand compared with silty sand. The authors concluded that the coarse sandy soil leached more NO_3^- than the silty soil.

Wachendorf *et al.* (2004) also studied forage production and NO_3^- leaching on a sandy soil deposited from glaciations in Germany. The authors indicated that it was important to study NO_3^- leaching here because 80 % of dairy cattle in Germany are kept on these soils. The N surplus observed on dairy farms was found to be a good indicator of NO_3^- leaching in sandy soils. The sandy soil had decreased water retention, compared to the clay and loam soils that were studied. Decreased water retention of sandy soils was found to exacerbate the annual precipitation surplus because it took a smaller volume of water to displace soil solution below the root zone. Unlike other studies, NO_3^- leaching on sandy, deep clay, and loam soils was found to be inconsistent. The authors also caution that in lysimeter studies, a delay in sampling on sandy soils may result in an underestimation of NO_3^- losses because the percolating water drains very quickly once the soil reaches field capacity.

Gaines and Gaines (1994) conducted an *in-vitro* study that examined NO_3^- leaching through four soil types: sandy, Greensmix of sand and peat, loamy sand and sandy clay loam. Two main tests were conducted on these soils: the retention of a measured amount of NO_3^- and a permeability test that looked at how long it took 500mL of water to percolate through the soil.

The retention test show that sandy soils retained 119 ppm of NO_3^- < Greensmix 125 ppm < loamy sand 149 ppm <sandy clay loam 173 ppm. The permeability test showed that it took water 24 min to travel through a sandy soil, 33 min through Greensmix, 20 h through loamy sand and 26 h through the sandy clay loam. Sandy soils retained less water as a result of having few silt and clay particles that also gave rise to a low CEC. Cation exchange capacity is defined as the total amount of exchangeable cations that a soil can adsorb. A lower CEC tends to lead to accelerated leaching. Variability in NO_3^- leaching according to soil characteristics indicates that approaches to mitigate NO_3^- leaching cannot be generalized across soil types and any approach to reducing leaching losses should be site specific.

3.4.3 Hydrology

Hydrological dynamics play a strong role in NO_3^- leaching from agroecosystems. The literature suggests that the risk of NO_3^- contamination is elevated in areas of pasture where the hydrological dynamics have been altered through drainage or irrigation practices (Power & Schepers 1989; Addiscott 1996). Water has been found to influence nutrient transport, uptake and transformation. These changes influence plant N use and consequently NO_3^- leaching (Scholefield *et al.* 1993; Yuan & Li 2007). The biological processes of mineralization and nitrification of organic N are dependent upon soil temperature and moisture (Brady & Weil 2002; Bouman 2008). Nitrate is a challenging nutrient to manage in a number of respects, one of which is that soil NO_3^- is highly mobile and moves freely with percolating rain or irrigation water.

Initially it was thought that high N use efficiency (NUE) was an adaptation to N poor habitats. Nitrogen Use Efficiency is described by Aber and Melillo (2001) as a measure of how a plant species responds to soil N and is generally defined as the amount of organic matter produced in a plant per amount of N used. Additionally, Yuan and Li (2007) discovered that water supply had a large impact on plant N use strategies. Their results supported the hypothesis that plant NUE strategies are indeed influenced by hydrology. Since plant uptake is a major fate of N in the agroecosystem any influence on plant uptake will change the potential amount of N remaining to be lost via leaching or volatilization. Nitrate can build up in the water of irrigated systems and the addition of N applied in irrigation water represents further N deposition into pasture that often is unaccounted for in nutrient management planning (Power & Schepers 1989).

Pakrou and Dillion (2000) examined processes of the N cycle in both surface irrigated and un-irrigated pasture on a sandy loam soil in Australia. The irrigated pasture was comprised of a mixture of perennial ryegrass and white clover. The un-irrigated pasture was comprised of mixed ryegrasses and subterranean clover. The results of this study showed that N leaching losses were higher in an irrigated section of mixed pasture, which leached 210 kg N ha^{-1} yr^{-1} on average. In the un-irrigated section of mixed pasture leaching losses were significantly lower, leaching an average of 81 kg N ha^{-1} yr^{-1}. Three times as much biological N fixation was estimated to occur in the irrigated pasture compared to the un-irrigated pasture (294 and 100 kg N ha^{-1} yr^{-1}, respectively). Atmospheric N fixation by clover was a significant part of the total accumulated N. Irrigation also boosted biomass production – the irrigated paddock produced 42% more herbage than the un-irrigated pasture. Nitrogen content in the forage was 50% higher in the irrigated pasture as a result of the increased clover content.

There are a number of sites in Atlantic Canada that possess imperfectly drained shallow soils and these sites receive an excessive amount of moisture in the fall and spring. The installation of drainage systems can create more favorable conditions for a successful growing season, boosting biomass production and increasing trafficability during seeding and harvest

(Addiscott 1996; Gordon *et al.* 2000). However, they also create a more favorable environment for soil microbes that mineralize organic N to produce NO_3^- (Addiscott 1996). Drainage increases the movement of water through the soil matrix carrying mobile NO_3^- along with it and increasing leaching. A study by Gordon *et al.* (2000) compared a number of drainage systems, revealing that there is variability in the degree to which different drainage systems influence N loading. Shallow drainage systems were found to reduce NO_3^- losses by 4.4 – 6.0 kg N ha^{-1} yr^{-1}. This is logical when one considers the water volume difference and NO_3^- solubility – less water removed equals less NO_3^- removed.

A study conducted by Scholefield *et al.* (1993) investigated N leaching losses in drained and un-drained fertilized (400 kg N ha^{-1} yr^{-1}) grassland agroecosystems. The effects of drainage were examined in established grassland and ploughed, re-seeded grassland. The primary finding of this study was that NO_3^- leaching was much higher in the drained sections of pasture, whether it was established or renovated. The established pasture drained and un-drained plots leached 199 and un-drained 80 kg N ha^{-1} yr^{-1}, respectively. In the renovated pasture drained plots lost 85 kg N ha^{-1} yr^{-1} compared to 27 kg N ha^{-1} yr^{-1} from un-drained plots. The impact of drainage on NO_3^- leaching was significant in all years of this U.K. experiment that ran from 1983-1990.

Hack-ten Broeke (2001) observed leaching losses in drier and wetter sites of naturalized grassland in the Netherlands. On the drier fields he found that there was no significant increase in leaching losses despite changes to irrigation management. However, in the wetter fields where there was an excess of water (versus water being in limited supply on the dry fields) solute leaching became evident. Hack-ten Broeke (2001) contributes that a sustainable irrigated system that the agricultural industry ought to seek should work toward a reduction in water use, and a minimization of N leaching. As stated in the project background for the Truro experimental site, the novel irrigation system being established at the NSAC aims to recycle drainage water from pasture, and in concert with biotic control this project intends to minimize nutrient losses from the agroecosystem through tighter cycling of N.

As our views shift in favor of enhancing environmental sustainability through use of legume based pastures, it must be demonstrated that the N benefits of legumes can be increased by managing the compositional dynamics of the sward. It is expected that the biological N supply by legumes in excess of NO_3^- uptake from grasses would increase NO_3^- collection in the drainage tiles. The recycling of this NO_3^- enriched drainage water should boost the growth of grasses in mixture rendering them more competitive in mixture with red clover. Therefore, a combination of innovative irrigation technology and agronomic management is expected to synchronize N flows between legumes and grasses and reduce nutrient losses due to soil leaching in the face of shifting climate conditions.

3.4.4 Nitrogen Source

Early on, grassland agriculture was considered to be low risk for environmental pollution, but this benign label was assigned principally because available data up until the mid 1980`s pertained to cut, not grazed grassland. As more data became available it was realized that NO_3^- leaching from grazed grasslands can exceed 150 kg of N ha^{-1} yr^{-1} (Hooda et al. 1998; Di & Cameron 2002). The use of chemical fertilizers also exceeded the capacity of grasslands to immobilize N. The three main N sources that have been found to influence NO_3^- leaching in temperate agroecosystem literature are the use of synthetic fertilizer, animal wastes and biological N fixation.

Across Atlantic Canada approximately 80,000 hectares of agricultural land is comprised of grassland and many farm owners opt for high fertilizer inputs to maintain biomass production throughout the growing season (Papadopoulos *et al.* 2001). When crops are undergoing rapid growth they can take up N swiftly (5 kg N ha^{-1} d^{-1} in some instances). Farmers ensure there is a generous supply of N in the soil by applying chemical fertilizers in the range of 200-400 kg N ha^{-1} yr^{-1}. Crop production has been prosperous with such a hearty supply of nutrients, but research has begun to indicate that when the total N input to the pasture increases, so will the proportion lost to the environment through leaching and volatilization (Scholefield *et al.* 1993).

Annual leaching losses of 30-200 kg N ha^{-1} yr^{-1} have been recorded from grazed pastures that make inefficient use of the 300-400 Kg of fertilizer they receive (Cuttle & Scholefield 1995). An experiment by Scholefield *et al.* (1993) on a clay/shale soil in the Southwest U.K. measured leaching in an established grassland/beef production system where NH_4NO_3 fertilizer was applied at 200 and 400 kg N ha^{-1} yr^{-1} in nine split applications of fertilizer. The results of this experiment showed that doubling the amount of fertilizer, tripled NO_3^- leaching losses. For example, in the 1985 year of the experiment 200N and 400N pastures leached 44 and 144 kg N ha^{-1} yr^{-1}, respectively. This effect was significant from 1983-1990 with the exception of the 1988 season. Nineteen percent of the 200 kg N, and 33% of the 400 kg N fertilizer applied to pasture was lost to the environment, on average.

A study by Shepherd and Lord (1996) on a loamy sandy soil in the U.K. measured NO_3^- leaching, using porous ceramic cups, after the application of NH_4NO_3 fertilizer on spring and winter wheat rotations. When fertilizer was applied to spring wheat at a rate of 200 kg N ha^{-1} yr^{-1} NO_3^- losses ranged from 17 - 87 kg N ha^{-1} yr^{-1}. When NH_4NO_3 was applied to winter wheat at a rate of 175 kg N ha^{-1} yr^{-1} NO_3^- leaching losses ranged from 4 - 45 kg N ha^{-1} yr^{-1}. These results corroborate Addiscott (1996) and Scholefield (1993) postulated that the more fertilizer applied, more will be lost via leaching. Bjorneberg *et al.* (1996) inspected NO_3^- leaching when anhydrous NH_4^+ was applied to a continuous corn and a corn-soybean rotation on a sub-surface drained loam soil in Iowa, U.S. At the soybean stage of the experiment fertilizer was not applied but leaching was still measured. In the continuous corn treatment 200 kg of anhydrous NH_4^+ was applied. Nitrate leaching losses in this treatment ranged from 11 - 107 kg N ha^{-1} yr^{-1}. During the corn phase of the corn-soybean rotation 170 kg of anhydrous NH_4^+ was applied and NO_3^- leaching varied from 5 - 52 kg N ha^{-1} yr^{-1}. During the soybean phase of the corn-soybean rotation NO_3^- leaching measured 5 - 51 kg N ha^{-1} yr^{-1}, despite there being no anhydrous NH_4^+ applied during this period. This indicates that when the application of chemical fertilizer is ceased, pastures are still at risk for leaching whether from a buildup of excessive soil N.

In a five year study undertaken on a loamy soil in Denmark, 300 kg N ha^{-1} yr^{-1} fertilizer was applied to a monoculture ryegrass pasture. Leaching losses were compared to those from an unfertilized grazed perennial ryegrass/ white clover stand (Eriksen *et al.* 2004). Inputs in this experiment included dairy cattle excreta, chemical fertilizer and biologically fixed N. Nitrate leaching was generally low from the grazed grass/ clover mixture fluctuating between 4 – 21 kg N ha^{-1} yr^{-1}. Nitrate leaching was much higher under fertilized ryegrass ranging from 60 - 119 kg N ha^{-1} yr^{-1}. The authors estimated that the fertilized ryegrass experienced a 240 kg N surplus, while the grass-legume system had an average surplus of 50 kg N. It was further hypothesized that if fertilizer inputs were reduced from 300 to 100 kg N this would reduce NO_3^- leaching to similar levels as observed in the grass/ clover system.

It is not only the amount of fertilizer supplied that influences leaching but also the temporal application pattern. In a grass/ clover mixture experiment on sandy soils in New Zealand, Di and Cameron (2002) examined the effect of applying 200 and 400 kg N ha^{-1} yr^{-1} dairy effluent on pasture in two or four split applications. The N supplied in four split applications leached 6 and 17 kg NO_3-N ha^{-1} yr^{-1}, at rates of 200 and 400 Kg of N, respectively. The N supplied in two split applications leached 13 and 49 kg NO_3-N ha^{-1} yr^{-1}, at rates of 200 and 400 Kg of N, respectively. The results of this study show that the risk of NO_3^- leaching is increased when fertilizer is administered in infrequent, concentrated applications.

With the knowledge that NO_3^- leaching is high in grazed systems, Jabro *et al.* (1997) designed a fertilizer experiment that examined the impact of the form of N from the animal as well as the time in the season it was applied on an orchardgrass pasture. The experiment had five treatments: Control (0 N), urine applied in spring, urine applied in summer, urine applied in the fall and feces applied in the summer (Figure 3.1). The experimental site was located in Pennsylvania, U.S. on a well drained, silt loam soil from 1993-1995. Nitrate leaching was found to be low in both the control and Feces – Summer treatments. Two year average NO_3^- leaching losses varied from 150 to 255 kg N ha^{-1} yr^{-1} under the urine treatment depending on what time in the season it was applied. Leaching losses were lower when urine was applied in the summer when plant uptake was competitively removing N from the soil. Nitrate leaching was highest overall when urine-N was applied in the fall. The results of this experiment indicate that the risk of leaching is elevated when fertilizer is applied out of synch with the seasonal growth patterns of the crop.

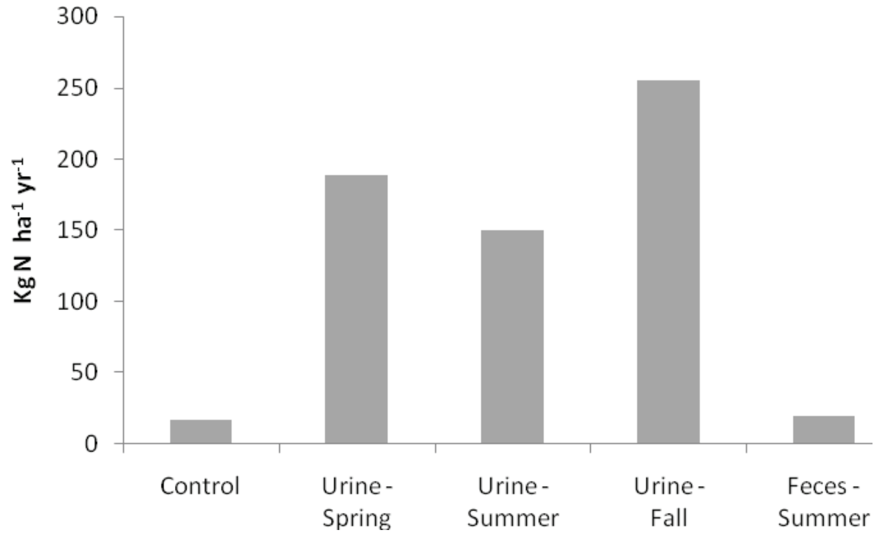

Figure 3. 1. Losses of NO_3-N from grazed grassland fertility treatments in Pennsylvania, U.S. (Adapted from Jabro *et al.* 1997).

Grass that is intensively fertilized is of high nutritive value, but this N surplus is not as valuable as it may seem because the forage often contains double what the ruminant is able to utilize successfully. As noted previously, grazing animals deposit their N concentrated excreta in a highly heterogeneous fashion across a pasture. This also means that leaching in a grazed pasture will be spatially variable as well. Cuttle and Scholefield (1995) discuss a fertilized pasture in Britain on a clay loam soil that was grazed by beef cattle. In this study, when 200 kg of fertilizer was applied NO_3^- leaching varied from 18 – 59 kg N ha^{-1} yr^{-1}, and when 400 kg of fertilizer was applied, NO_3^- leaching losses were in the area of 74 - 194 kg N ha^{-1} yr^{-1}, which is quite high. Cuttle and Scholefield (1995) infer that if a large proportion of NO_3^- leaching losses are emerging from intensively managed systems then it stands to reason that extensifying the farming operation and limiting inputs such as chemical fertilizer would help to ameliorate leaching losses.

In 1994 to 1996 Hooda *et al.* (1998) conducted a NO_3^- leaching field experiment in Southwest Scotland on a silty clay loam soil. The two forage treatments were ryegrass/white clover mixture and monoculture ryegrass. In 1991 the recommended fertility for grassland pastures in this area was 240 kg N ha^{-1} yr^{-1}. This amount of fertilizer was applied to monoculture ryegrass in three split applications: one from urea, the remaining two from chemical fertilizer. The mixture did not receive chemical fertilizer but did receive two slurry applications. All treatments were grazed at a stocking rate of two cows per hectare. In the fertilized monoculture grass treatment concentrations of NO_3-N in the soil percolate varied from 1.1 to 62.5 mg L^{-1}. The average concentration of NO_3-N in the soil percolate from the mixed sward was much lower at 1.5 to 16.3 mg L^{-1}. When NO_3^- leaching was volume weighted for two years of the experiment it was found that N leaching losses were significantly lower from the mixed sward treatment in 1994/95 season, 24.3 versus 30.2 kg N ha^{-1} yr^{-1} in the grass monoculture.

Nitrate leaching increased 2.5 fold in the 1995/96 season; this eliminated a significant treatment difference (Hooda *et al.* 1998). The authors attributed elevated leaching losses in the second season to an increase in the white clover content of the sward from 20 to 27%. Although it seems more likely that the narrowing of the leaching gap between pure and mixed swards was attributable to the interplay between slurry N release and weather conditions. The authors mention that there are dry spells followed by heavy rains in the second summer. This would have resulted in enhanced mineralization of accumulated soil organic N from the previously applied slurry. The results of this study also serve to impart upon us that because NO_3^- leaching is heavily influenced by the weather, it will vary greatly from year to year. The authors conclude that intensively managed grass-clover systems can have leaching patterns approaching those of monoculture grass. This study indicates that leaching losses from grass-legume systems can be of moderate leaching concern when managed intensively. Hooda *et al.* (1998) noted that existing studies dealing with NO_3^- leaching from clover systems are generally of short duration and the findings are inconclusive, therefore, further research efforts are required to characterize and quantify the contribution of clover to NO_3^- leaching.

Loiseau *et al.* (2001) conducted a five year study in France comparing leaching losses from four cover types: pure unfertilized ryegrass, pure white clover, mixed grass-clover and a bare soil control. No N fertilizer was applied in any treatment, just non limiting levels of P and K and what ever grazing sheep returned to the soil. Leaching losses under pure unfertilized ryegrass were low at 1-5 kg N ha^{-1} yr^{-1}, relatively low in grass-clover at 1-19 kg N ha^{-1} yr^{-1}, elevated under pure white clover at 28-140 kg N ha^{-1} yr^{-1}, and highest under the bare soil control at 84 - 149 kg N ha^{-1} yr^{-1}.

To further understand how the environment aboveground influences that belowground, it is useful to further examine what is occurring in each treatment. In the pure ryegrass that did not receive any fertilizer, leaching was low, plant uptake of inorganic N was likely high and due to a scarcity of available nutrients there was low biomass production. Low biomass production also means there is less for sheep to ingest, and less N to return to pasture via excreta. Moreover, the elevated C:N ratio may have allowed for increased N immobilization, especially beneath urine patches. In the mixed grass/ clover treatment, leaching was low - only slightly higher compared with unfertilized pure ryegrass, despite the improved sward yield and subsequent increase in NH_4^+ returns by way of urea and undigested nitrogenous compounds from grazing ruminants. The authors attribute a reduction in inorganic N to regulation of BNF due to grass competition. Nitrogen taken up by the grass was not only immobilized in grass tissue, but in doing so increases the C:N ration in its tissue, triggering further immobilization upon senescence. Loiseau *et al.* (2001) suggest that 32% of leaching was attributable to urine N deposits.

Under the pure white clover, elevated NO_3^- leaching is likely due to an increased rate of BNF, no grass competition or transfer affiliate, high N % in the tissue selected by sheep and then returned in excreta, low soil N immobilization and increased rates of soil N mineralization due to the lower C:N ratio of clover. The authors reckoned that 78% of leaching was linked to urine inputs. Nitrate leaching was likely high under bare ground because soil aeration during tillage increased soil N mineralization but unlike the other treatments there was no plant compartment to reduce soil available N. Overall, N leaching in this experiment seemed to be driven primarily by grazing management and the soils capacity to immobilize concentrated N urine applications. The authors also indicate that soil percolate concentrations below 20 mg L^{-1} and the associated characteristics of the mixed grass-clover system make such a sward more sustainable.

Ledgard *et al.* (2001) contrasted 0 kg N versus 400 kg N fertilizer grass/ clover pasture production systems in New Zealand located on silty loam soils. The pastures were grazed by dairy cattle at a stocking rate around four cows ha^{-1}. Leaching losses were measured for a five year period. Nitrate leaching from the 0 N pasture was relatively low with a five year average of 30 kg N ha^{-1} yr^{-1}. Clover content in this sward remained relatively high at around 15% clover by dry mass. Pasture receiving 400 kg N ha yr^{-1} contained less clover and leached 130 kg N ha^{-1} yr^{-1} on average. This and other studies seem to indicate that to reduce leaching it would make sense to improve plant uptake of soil N (through synchronous supply and demand), manipulate soil N supply so that it remains at low levels during high leaching risk periods, and improve N use efficiency of grazing ruminants. Some research has suggested that by altering the microflora of the rumen (potentially through the diet), more N would end up in the dung than the urine, possibly decreasing the risk of rapidly mineralized N loss from excreta overall (Van Der Meer *et al.* 1987; Ledgard *et al.* 2001). Cuttle and Scholefield (1995) also echo the three recommendations to minimize soil NO_3^- leaching. It would appear that grass/ legume systems have the potential to fulfill one, if not two of the criteria.

It appears that NO_3^- leaching is reduced under legume systems, but what would the leaching profile look like if a system were to switch from intensive fertilizer to an extensive grass/ legume mixture? Two experiments by Owens (1990 and 1996) describe the leaching characteristics when a farm transitions from an intensively fertilized system to an extensive grass/ clover system. The experimental sites were located in Ohio, U.S. on a well drained silt loam soil. In the first experiment, NO_3^- leaching concentrations in soil percolate from a well fertilized corn system measured 15-40 mg L^{-1}. When the same site was converted to alfalfa/orchardgrass over a two to three year period, concentrations of NO_3^--N in soil solution dropped to below 5 mg L^{-1} (Owens 1990). The second experiment measured NO_3^- leaching losses from grazed orchardgrass and tall fescue pastures that were fertilized with 224 kg N for five years. In the sixth year no fertilizer was administered but alfalfa was interseeded into the grass swards. The leaching from these stands was monitored for another 10 years. After only three years of legume based fertility researchers found that soil NO_3^- concentrations decreased from 17.7 to 9.3 mg L^{-1} in one watershed and from 20.4 to 13.4 mg L^{-1} in another. When chemical fertilizers were replaced with alfalfa as the source of N in this experiment, the NO_3^- in soil percolate decreased by 30% on average. The authors indicate that especially in sites with vulnerable aquifers, farmers may find that interseeded legumes can fulfill much of the N needs of the grass without leaching too much to the groundwater below (Owens *et al.* 1994).

Moderate to high levels of production can be maintained in systems solely reliant on fixed N, but NO_3^- leaching from clover/ grass pastures is not frequently quantified in the literature. Ledgard *et al.* (2001) indicates that leaching is generally low from pastures that utilize fixed N, ranging on average from 6 - 23 kg N ha^{-1} yr^{-1}. We know from literature referenced previously that in temperate regions leaching losses more than double this amount are common in fertilized grassland production systems. Scherer-Lorenzen *et al.* (2003) caution that without complementary N recipient forage species in mixture low diversity clover pastures can leach up to 33 kg N ha^{-1} yr^{-1}, and clover monocultures have the potential to leach up to 155 kg N ha^{-1} yr^{-1} if accompanying grasses are lost, as corroborated by Loiseau *et al.* (2001).

Ruz Jerez *et al.* (1995) conducted a study on a diverse pasture grazed by sheep on a sandy loam soil in New Zealand that examined NO_3^- leaching from clover in pasture. They contrasted N fixation, sward yield and NO_3^- leaching losses under three treatments: monoculture ryegrass with 400 kg urea-N fertilizer; white clover/ryegrass low diversity mixed sward; and a high diversity herbal sward. The high diversity treatment included 11 grasses, 7 legume and 5 distinct herbal species of plants. The diverse herbal system sward yields were greater than the yields from white clover/ryegrass and on par with the yields of the intensively fertilized ryegrass monoculture. This may have been accomplished, in part, because plant species in the diverse herbal sward were 42% more efficient at utilizing available N than even the grass/legume mixture. In addition, clover fixation was higher in the diverse herbal sward (144 kg N ha^{-1} yr^{-1}) when compared the fixation occurring in the white clover/ryegrass treatment (123 kg N ha^{-1} yr^{-1}).

Nitrate leaching was highest beneath the fertilized ryegrass (41 kg N ha^{-1} yr^{-1}). Nitrate losses were much lower in the more extensive treatments, and were roughly equivalent and between grass/ clover and the herbal sward treatments (6 and 7 kg N ha^{-1} yr^{-1} respectively). The studies reviewed in this chapter are summarized in Table 3.1 and 3.2. As more accumulates on a diversity of agroecosystems it becomes clear that the plant community, and the processes existing within, profoundly influences nutrient cycling and leaching. The influence of the plant community on NO_3^- leaching is further explored in the next chapter of this document.

Table 3. 1. Examples of NO_3-N leaching losses (Kg N ha^{-1} yr^{-1}), in ascending order, under grazed and arable pasture systems (adapted from the following list of publications).

Reference	**Location**	**Soil type**	**Crop**	**Farming system**	**N applied (kg N ha^{-1} yr^{-1})**	**N Leaching**
			Ryegrass		Ammonium nitrate 400	41
			Ryegrass / white clover		None	6
Ruz Jerez *et al.* 1995	N.Z.	Sandy loam	High diversity grass/ legume/ herb mixture	Sheep	None	7
					Dairy excreta-N 200	17 - 87
				4 split applications	Dairy excreta-N 400	4 - 48
					Dairy excreta-N 200	6
Di & Cameron 2002	N.Z.	Sandy soil	Grass/clover	2 split applications	Dairy excreta-N 400	17
			Spring wheat		Ammonium nitrate 200	13
Shepherd & Lord 1996	U.K.	Loamy sand	Winter wheat	Cereal rotation	Ammonium nitrate 175	49
Bjorneberg *et al.* 1995			Corn	Continuous corn	Anhydrous ammonia 170	11 - 107
			Corn		Anhydrous ammonia 200	5 - 52
	U.S.	Loam soil, Drained	soybean	Corn-soybean rotation	None	5 - 51
			Ryegrass		Ammonium nitrate 300	60 - 119
Eriksen *et al.* 2004	Denmark	Loam soil	Ryegrass/white clover	Dairy cattle	None	12 - 4
					None	30
Ledgard 2001	N.Z.	Silty loam soil	Grass/clover	Dairy cattle	Ammonium nitrate 240	130
					Ammonium nitrate 200	44
Scholefield *et al.* 1993	U.K	Clay/ shale	Ryegrass	Beef	Ammonium nitrate 400	144
			Ryegrass		None	1 – 5
			White clover		None	28 - 140
			Ryegrass/white clover		None	1 – 19
Loiseau *et al.* 2001	France	Clay silt sand	Bare soil	Sheep	None	84 - 149

Reference	Location	Soil type	Crop	Farming system	N applied (kg N ha^{-1} yr^{-1})	N Leaching
Cuttle & Scholefield 1996	U.K.	Clay loam	Mixed grasses	Beef cattle	Ammonium nitrate 200	18 – 59
					Ammonium nitrate 400	74 – 194
Jabro *et al.* 1997	U.S.	Silt loam soil	Orchardgrass	Nil	None	17
				Spring	250 - Urine N	189
				Summer	250 - Urine N	150
				Fall	250 - Urine N	255
				Summer	250 - Feces N	20

Table 3. 2. Examples of NO_3-N leaching losses (mg L^{-1} NO_3-N in soil solution) under grazed pasture systems (adapted from Owens *et al.* 1994 and Hooda *et al.* 1996).

Reference	**Location**	**Soil type**	**Crop**	**Management**	**N applied** (kg N ha^{-1} yr^{-1})	**N leaching**
Owens *et al.* 1994	U.S.	Silt loam	Orchardgrass/ Tall fescue	Cattle	Ammonium nitrate 224	17 - 20
			Grass/interseeded alfalfa		None	9 - 13
Hooda *et al.* 1998	Scotland	Silt clay loam	Ryegrass	Cattle	Ammonium nitrate 240	1 - 63
			Ryegrass/ white clover		Slurry 250, 2 applications	2 - 16

3.4.5 Plant Community

There is a variety of factors that control NO_3^- losses from grassland systems and naturally occurring plant species are one of these factors that play a role in watershed protection. As such, there is a growing consensus that vegetation exerts control over NO_3^- leaching in grasslands (Loiseau *et al.* 2000; Ledgard 2001; Scherer-Lorenzen *et al.* 2003; Palmborg *et al.* 2005; Bouman 2007). When Schlapfer and Eriksen (2001) discuss the role of the plant community in reducing NO_3^- leaching losses, they refer to this as a “biotic control system”. Further research on the utility of these biotic controls is especially important in areas that are at risk for NO_3^- leaching, such as intensively managed agricultural sites. This section will review key studies that connect the structure, and interactions of the aboveground plant community to the modulation of NO_3^- leaching characteristics belowground.

Interactions between Legumes and Non-legumes

As pasture management strategies evolve it becomes apparent that the productive function and environmental sustainability of a pasture is reliant upon the maintenance of a floristic balance between leguminous plant species and grass species (Bouman 2008). The inclusion of legumes in grassland pastures have been shown to sustain herbage production throughout the growing season compared to monoculture grass plots, increase protein concentration, digestibility and maintain a more balanced mineral composition of the herbage for grazing animals (De Klein 2001; Spehn *et al.* 2002; McKenzie *et al.* 2005; Kunelius *et al*. 2006). Mixed grass/legume swards have a number of productive benefits to confer as a result of belonging to two distinct functional groups. Ecosystems containing such mixtures benefit from their species distinctiveness because they capitalize on different terrestrial resources.

As mentioned previously in Section 3.2, we know that few plants can access the abundance of dinitrogen gas in our atmosphere, and due to this many terrestrial plant ecosystems are N limited (Aber and Melillo 2001). Over the last century agricultural systems have combated nutrient limitations on crop production predominantly through the utilization of industrially synthesized N. Intercropping experiments show that legumes also increase the availability of soil NO_3^- due to the release of symbiotically fixed N (Scherer-Lorenzen *et al.* 2003). Many species in the Family *Fabaceae* have access to this pool of atmospheric N due to a unique symbiotic relationship between the leguminous plant and the *Rhizobium* bacteria hosted in their root nodules.

A number of leguminous plants, including Birdsfoot trefoil (*Lotus corniculatus*) and alfalfa (*Medicago sativa*), have been included in forage mixtures to augment the existing N supply and to improve forage quality and productivity (Papadopoulos *et al.* 1993). One group of leguminous species in particular that has been observed to fix atmospheric N at a high rate is from the Genus *Trifolium*. In a cross-European grassland study conducted by Spehn *et al.* (2002), *Trifolium* species were found to have significant impact on the plant community by virtue of their N_2 fixation capabilities. Additionally, *Trifolium* was efficient at transferring excess N to other plant functional groups, such as grasses and herbs, in mixtures. The presence of legumes, such as *Trifolium*, in mixture was found to significantly increase N in above and belowground pools in Sweden, Ireland and Portugal. This effect was even more pronounced in Germany and Switzerland. Spehn *et al.* (2002) postulate that fixed N from legumes were made available to grasses through rhizodeposition, mycorrhizal hyphae or through the mineralization of legume litter. It was estimated that 8-39% of the N contained in non-fixing grasses was transferred from an adjacent legume.

Paynel *et al.* (2001) describes the transfer of N from legumes to recipient grasses belowground as occurring via short or long term transfer. Some nitrogenous compounds released by legumes can be readily used by the recipient but often the N must be mineralized before it becomes plant available. In the short term, leguminous plants can exude N into the rhizosphere predominantly as NH_4, but other exudates include amino acids and proteins. However, nitrogenous compounds can also be transferred more directly via the arbuscular mycorrhizal fungi that can connect the roots of two plant species. In the long term, N can be released from senescent leguminous plant tissue or through the sloughing off of root cortical cells which can be high in N.

Broadbent *et al.* (1982) conducted an isotope dilution experiment in a field that included Ladino clover (*Trifolium repens*) and Wimmera ryegrass (*Lolium rigidum*) grown together in mixture as well as individually. The monoculture clover was found to obtain about 85% of its N from atmospheric fixation. After six months of growth in a clover/ryegrass mixture the increase in percent total N and N^{15} in plant tissues revealed that substantial transfer of atmospherically fixed N had occurred. The authors calculated that up to 79% of the N in ryegrass came from biologically fixed N. Research by Scherer-Lorenzen *et al.* (2003) also confirmed the detection of the transfer of N symbiotically fixed by clover to non-fixing grasses and herbs.

Research experiments that study N fixation by a variety of leguminous species indicate that potential N fixation amounts range from 200-400 Kg N ha^{-1} yr^{-1}. Typically actually fixation is well below this estimation due to nutrient limitations, disease, pests and drought. Ledgard (2001) estimates that the actual total N fixation in grass/ legume swards in temperate zones is around 100 kg N ha^{-1} yr^{-1} on average. The fertilization value of legumes in plant communities have become widely recognized, but it still remains difficult to quantify the leguminous contributions to mixed swards. One example by Palmborg *et al.* (2005) revealed that the biomass production from mixed plots containing legumes outperformed corresponding monoculture biomass over a five year period. *Trifolium pratense* and *Trifolium repens* had significant positive effects on community biomass. Their litter has been found to decompose at a greater rate than other forage species resulting in a high net mineralization of N. The discussion in Loiseau *et al.* (2001) also supports this supposition.

Zemenchik *et al.* (2001) compared the N replacement values of Kura clover (*Trifolium ambiguum*) and Birdsfoot trefoil (*Lotus corniculatus*) when grown in mixture with Kentucky bluegrass (*Poa pratensis*), smooth bromegrass (*Bromus inermis*) or Orchardgrass (*Dactylis glomerata*) on a silt loam soil at two sites in Wisconsin, U.S (Arlington and Lancaster). The authors define the fertilizer N replacement value (FNRV) as the "amount of N fertilizer required for grass monoculture to yield as much dry matter as the same grass grown in mixture with a legume". Some researchers have found that non-fertilized mixtures containing legumes have forage yields on par with grass monocultures that receive 200-300 Kg N fertilizer annually (Carter & Scholl 1962). Other authors suggest that the yields of mixtures will be on par if the area of the grass/ legume pasture is increased by 10% (Cuttle & Scholefield 1996).

Zemenchik's (2001) study showed that the highest estimates of FNRV were reported for Kura clover or Birdsfoot trefoil when the legume was grown in mixture with Kentucky bluegrass. In Arlington, mixtures containing Birdsfoot trefoil out yielded Kura clover/grass mixtures in 1995 and 1996. In Lancaster, the Birdsfoot trefoil mixture yields exceeded the Kura clover mixtures in 1995 but the reverse was true in 1996. The authors attribute this difference to the improved productivity of Kura clover in 1996. The FNRV of both species was proportionate to the legumes dry matter production. The FNRV of Birdsfoot trefoil was high early on when its yields were high (1995), and dipped as the Birdsfoot trefoil dry matter production decreased in 1996. Average

FNRV's from both sites and across years of this experiment can be found below in Table 3.3. Birdsfoot trefoil mixtures had greater FNRV values on average than Kura clover mixtures, but it is likely the reverse would become true if the experiment was conducted over a longer time period. Kura clover is a rhizomatous species that often has low yields in the first two production years as a result of allocating resources belowground, but is hardier and more persistent in subsequent production years than Birdsfoot trefoil.

Table 3. 3. Fertilizer N replacement values (kg N ha^{-1} yr^{-1}) of Kura clover and Birdsfoot trefoil averaged across years and locations when grown in mixture with cool season grasses (Adapted from Zemenchik *et al.* 2001).

	FNRV (kg N ha^{-1} yr^{-1})	
Companion grass	Kura clover	Birdsfoot trefoil
Kentucky bluegrass	252	269
Smooth bromegrass	186	231
Orchardgrass	93	113

Birdsfoot trefoil and Kura clover both showed greater FNRV's when grown in mixture with Kentucky bluegrass. FNRV's were moderate when legumes were grown with smooth bromegrass and considerably lower when grown in mixture with orchardgrass. Orchardgrass is highly competitive in mixtures with shorter, slower growing leguminous species, shading them out early in the season. Non-complementary growth patterns between legumes and associated grasses were found to negatively impact the ability of legumes to fix N in this treatment.

Eriksen and Vinther (2004) found that NO_3^- leaching from grazed fertilized grass/clover swards was always considerably lower than from grazed fertilized ryegrass. Ledgard (2001) also showed that NO_3^- leaching was generally low in pastures that rely on legumes to fix N. These grass/ legume pastures exhibited an improved efficiency of N cycle that Ledgard (2001) attributed to the synchronicity of N supply with grass demand. The N_2 fixation by legumes in mixtures seems to be regulated by some sort of inherent feedback mechanism driven by soil inorganic N levels. Notions of such a feedback mechanism developed during observations recorded during reduced and elevated soil N. Under conditions of low soil N legumes dominate the sward and fix N_2 actively, whereas in soils with high inorganic N grass species dominate over legumes. High inorganic soil N conditions have been found to significantly inhibit nodulation and therefore fixation by legumes. This biotic control mechanism acts to limit excess N input from legumes, regulating the potential for losses. Henceforth, it appears that the productivity of the plant community is dependent not only upon the sum of species functional traits contained within, but the overall interactions between plants and the environment around them. Pertinent literature regarding plant community interactions, and their influence on NO_3^- leaching, will be addressed in the next chapter.

Plant diversity, composition and nitrate leaching

The intensification of pasture production permitted by pervasive use of chemical fertilizers has reduced the species diversity in pastures, creating a more monocultured agricultural landscape. Realizing our dependence on fossil fuels and fertilizer presents us with a unique opportunity to return to an ecological approach to diversify pasture that will enhance their multifunctionality, productivity and sustainability (Sanderson *et al.* 2007). The plant community plays an integral role as primary producers in agroecosystems. Changing the composition of plant communities is one of the easiest ways to alter overall pasture biodiversity, because plant community dynamics possess a multifaceted sphere of influence that includes the microbial community.

McKenzie *et al.* (2004) indicated that more diverse pastures improve herbage production, distribution, persistence as well as improving pasture carrying capacity for grazing ruminants. Mixtures had improved yields both through quantity of biomass produced as well as quality of forage for cattle. Kunelius *et al.* (2007) also indicate that diverse swards had increased protein concentration and digestibility of forage because of reduced fiber and balanced mineral composition. Mixed pastures are productive but their characteristics are not synergistic. In McKenzie's mixtures bluegrass and meadow fescue were components of both the highest and lowest yielding mixtures. Therefore it seems that the performance of a species in mixture is not dictated by how well they perform in monoculture, but by the sum of the inter- and intra-specific competition between other species with which they are grown. Plants with diverse phenologies grown in mixture with one another had more consistent herbage distribution throughout the growing season compared to low diversity swards. This results in a more consistent, reliable yield across the growing season for grazing ruminants.

Phenology can be defined as the study of seasonal timing of life cycle events in relation to seasonal variations in climate conditions (Rathcke & Lacey 1985). For example, bluegrass can grow slowly when it is establishing because of allocating resources belowground due to its rhizomatous growth pattern. However, once this root is established it produces a productive amount of biomass whilst effectively intercepting N passing through the soil system, leaving less stored N which could be lost in the fall and winter (McKenzie *et al.* 2004; Kunelius *et al.* 2007). Conversely, a grass species whose dry matter yield peaks early in the season and goes dormant in the fall, such as orchardgrass, would do little to alleviate fall leaching (McKenzie *et al.* 2004; Rode & Pringle 1986). Mixtures of bluegrass have been observed to enhance the performance of other species in mixture; this makes sense because the phenology of bluegrass was different than other species in mixture (McKenzie *et al.* 2004). Most of the other species in mixture with bluegrass likely grew well early in the season, during which time bluegrass would not have been competing as actively for nutrients.

In another experiment that looked at forage systems in cold winter regions Kunelius *et al.* (2007) found that timothy grown together with red clover increased total herbage yields over red clover monoculture. This study also noted that mixtures of species tended to have greater yields than their component species grown in monoculture. Perennial ryegrass has desirable characteristics in this region, such as high digestibility and high energy, but it needs to be grown in mixture because it had poor persistence in monoculture. Kentucky bluegrass in this trial was found to increase in mixture as other species declined, likely attributable to its growth habits discussed previously. Overall, mixtures maintained better ground cover, productivity and yield stability. Ultimately, despite evidence demonstrating the productivity of diverse pastures there is a dearth of information available that indicates how an individual species or cultivar will perform within a mixture.

Diverse plant communities confer greater resistance to weed invasion (Kunelius *et al.* 2004; Sanderson *et al.* 2007). Weed abundance has been found to decrease as the number of plant functional groups increase. The abundance of invasive weedy species in 28 sites across Europe was lower in forage mixtures compared to monocultures (Sanderson *et al.* 2007). How a mixture responds to weed invasion is highly variable and will have to do with the composition of the plant community, soil disturbance and nutrient availability. Diverse plant communities likely keep weeds out through increased niche occupation, and decreased nutrient availability. Combinations of forage species in a mixture should complement one another phenologically, as well as being suited to the pasture management regime (e.g., the pressures of the grazing ruminant).

Sanderson *et al.* (2007) mentions that dairy cattle that grazed more diverse pastures had increased milk production compared to those grazing less diverse paddocks. Beef steer gain has been found to be greater on grass/legume pastures compared to grass/fertilizer pastures.

Papadopoulos *et al.* (2001) found that the incorporation of white clover into an orchardgrass pasture improved the production of grazing lambs but no significant increase in herbage was detected. Adding another layer of complexity to the diversity trifle, certain combinations of species have been found to perform better on some soils than others. This indicates that mixtures should be tailored to site-specific conditions. Despite this specificity, diverse plant communities also have the potential to sustain consistent growth even on heterogeneous soils because if one species performs poorly in certain microclimates, there may be another species in the mixture that does. Monocultures are not afforded such a buffer (Sanderson *et al.* 2007).

The "Diversity-Productivity Hypothesis" integrates the above stated interactions by indicating that "interspecific differences in the use of resources by plants allow more diverse plant communities to utilize more fully limiting resources and thus attain greater productivity". An offshoot of this theory is the "Diversity-Sustainability Hypothesis" that states that the "sustainability of soil nutrient cycles and thus of soil fertility depends upon biodiversity" (Tilman *et al.* 1996; Page 18). Humans have built up a landscape of artificial monocultures due to the convenience of fertilizer, but as the climate changes, due to global warming these monocultures become less equipped to deal with abiotic stresses and are more likely to result in massive crop failures than mixed pastures. Diverse pastures are more sustainable because they are more resistant to stress, such as drought, and result in fewer leaching losses into groundwater (Tilman *et al.* 1996; Scherer-Lorenzen *et al.* 2003; Sanderson *et al.* 2007).

To test the sustainability of diverse pastures, Tilman *et al.* (1995) undertook a 147 plot experiment on N limited soil where each plot was planted with 1, 2, 4, 6, 8, 12, or 24 species. It was discovered that plant productivity and resource utilization were significantly greater in high diversity grassland plots. Greater exploitation of available nutrients in diverse plots resulted in decreased NO_3^- in the root zone, as well as past the root zone. This suggests that niche complementarity within the more diverse plant communities allowed them to intercept more N before it could be lost via leaching. Conversely, NO_3^- leaching was found to be higher in monoculture and low diversity plots. Tilman *et al.* 1995 calculated effective species richness in these plots as a function of the Shannon-Weiner Diversity Index. Figure 3.2 represents the graphical superimposition of the NO_3^- leaching and effective species richness results across the treatments in this experiment. Tilman *et al.* (1996) calculated ESR as a derivative of the Shannon-Weiner Index of species diversity. When studied in a mature grassland pasture, similar relationships between diversity, sustainability, productivity and stability held true. This indicates that these desirable characteristics come about as a result of natural interactions, and that these natural functions can be improved through pasture management.

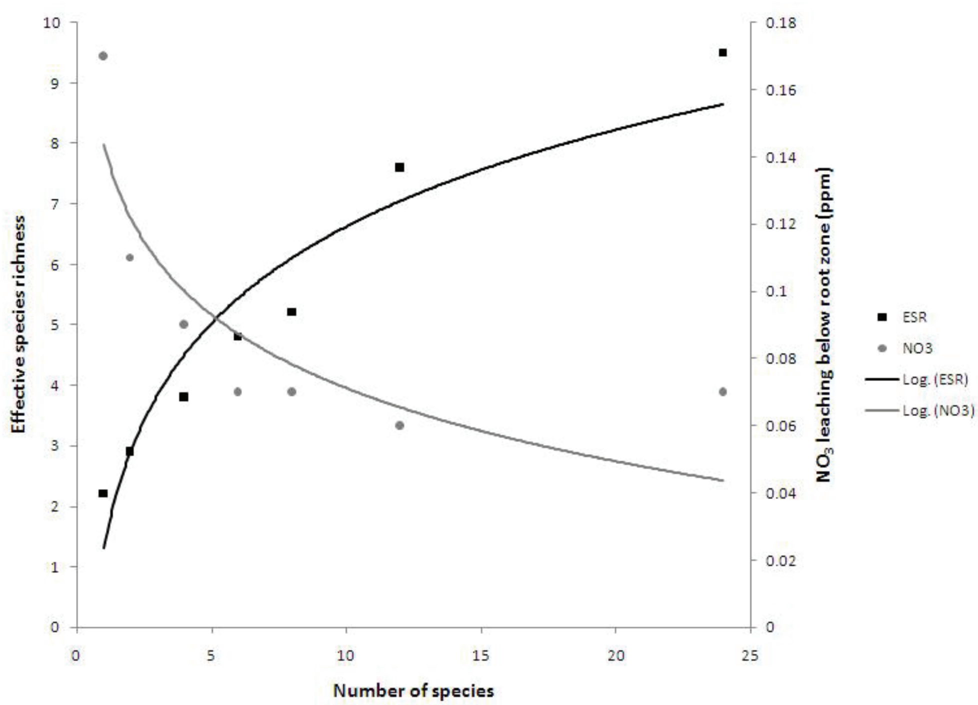

Figure 3. 2. Effective species richness (ESR) versus soil nitrate leaching (NO_3) beyond the root zone in mg L^{-1} (Adapted from Tilman *et al.* 1996).

The relationship between diversity and sustainability is a complicated and still somewhat controversial one, as noted by Hooper and Vitousek (1998), whom undertook an experiment in California similar to Tilman *et al.* (1996). This study had four forage treatments: early season annual forbs, late season annual forbs, N fixers and perennial bunchgrass. These mixtures were selected because each group possessed differing qualities such as phenology, morphology and litter C: N, all of which are relevant to nutrient cycling. Similar to Tilman *et al.* (1996), researchers found that diverse plots had greater relative resource use and greater ecosystem N retention. Unlike Tilman *et al.* (1996), Hooper and Vitousek (1998) did not observe a decrease in NO_3^- leaching with increasing plant diversity. They concluded that functional group composition of mixed pastures explained 35-40% more variance than did the quantity of species present. Hooper and Vitousek (1998) generally concluded that it was knowledge of “WHO” the functional groups are in the mixture, rather than “HOW MANY” that explained most about nutrient cycling processes in these ecosystems.

There appeared to be differences in rooting depth, phenology and total plant N use in this experiment however, leaching did not increase with decreasing diversity. Climate may be one explanation for this. Soil NO_3^- may have been reduced through complementary uptake in diverse plant communities but leaching only occurred in the month of January in this experiment (it was conducted in California).This was the only time there was enough precipitation to induce water percolation to the 75 cm soil depth where soil percolate was sampled from. Thus, evidence of the modulation of NO_3^- in the soil profile by the plant community was masked the remainder of the year. The authors also suggest that phosphorus may have been limiting, or that different plant communities induced changes in microbial immobilization. For example, annuals may encourage immobilization by providing a less recalcitrant substrate (plant litter) to the microbial community (Hooper & Vitousek 1998).

Prompted by Hooper and Vitousek (1998) findings Niklaus *et al.* (2001) conducted an experiment on a loamy soil in Northwestern Switzerland that measured NO_3^- leaching at high, medium and low levels of plant diversity. The high diversity treatment was comprised of 31 species and included red clover. The medium diversity treatment contained 12 species and the low diversity treatment consisted of five grassland species. One unique trait of this study is that members of each of the three main plant functional groups, grasses, legumes and non-leguminous forbs, were equally represented in each mixture. Researchers found that plant community diversity had a significant effect on soil NO_3^-. Soil NO_3^- was elevated at low species diversity and decreased with increasing diversity in the medium and high diversity plots. Sward yields were always reduced in low diversity communities. This study yielded data that suggests that the composition of the microbial community was altered by the plant community. There was a significant increase in nitrification potential with decreasing plant diversity and a non-significant though apparent trend toward increased mineralization at high diversity.

A grassland diversity experiment undertaken in Sweden by Palmborg *et al.* (2005) used sixty-eight plots with 1, 2, 4, 8 or 12 species in twenty-eight combinations on a silty sandy soil. Results of this experiment showed that species diversity and legume presence had a significant positive effect on biomass production during both years of sampling. *Trifolium* species had an especially significant impact upon forage yields. Legume presence, species richness, and functional group diversity had a significant impact upon soil NO_3^-. Biomass production and soil NO_3^- in mixtures were negatively correlated with one another. This makes sense because swards producing larger amounts of biomass will deplete soil nutrients to do so. After five experimental seasons, elevated plant species richness and functional diversity were found to reduce soil N pools while maintaining higher biomass production. There were considerable NO_3^- leaching losses from low diversity legume communities. It can be concluded from these results that biodiversity influenced ecosystem function but the effect diversity had on soil N pools and community biomass through resource use complementarity is reliant upon the functional traits of key species, such as N fixing *Trifolium* species.

Scherer-Lorenzen *et al.* (2003) in conjunction with Palmborg, conducted a grassland study in Germany on a site that had nutrient poor soils ranging from loamy sand to sandy clay. There were five levels of vegetative species richness in this trial: 1, 2, 4, 8, and 16. These were replicated in several mixtures in order to distinguish between species identity and number. The manipulated plant communities were also compared to a naturalized grassland control. Based on previous grassland diversity experiments Scherer-Lorenzen *et al.* (2003) postulated that plant communities must influence NO_3^- leaching physically through altering seepage patterns, and indirectly by modulating soil NO_3^- concentrations via N uptake, supply through biological N fixation, as well as plant mediated changes in the microbial community.

In monocultures, NO_3^- leaching was highest with legume, intermediate with herb and lowest in grass communities (Scherer-Lorenzen *et al.* 2003). A maximum value of 350 mg L^{-1} NO_3-N was detected in clover monocultures in the winter of 1998, whereas NO_3^- leaching from grass monocultures was virtually non-existent (<1 mg L^{-1} NO_3-N on average). Less diverse plots containing legumes lost greater amounts of NO_3^- (8-16 kg N ha^{-1} yr^{-1}). Nitrate leaching was found to be positively correlated with the above ground biomass of legumes in mixture. Patterns of belowground resource partitioning also seemed to influence leaching in this study. For example, NO_3^- leaching losses were negligible from grass plots possessing dense root systems, but higher in herbal plots that had one-third the root mass. The herb species appeared to be unable to fully exploit available NO_3^- due to its smaller rooting volume. Researchers note that “nitrate losses may be higher if the associated grass species are poorly established in their root structure or possess short periods of uptake”.

In review, researchers found a trend of decreasing NO_3^- with increasing species richness and increased number of functional groups but these effects were convoluted and non-significant statistically. The assemblage of species in the plant communities did have a strong, significant influence on NO_3^- leaching, the logistics of which this study was unable to sort out. Researchers concluded that the abundance of N fixers primarily determined NO_3^- leaching, but that co-occurring non-leguminous species with complimentary uptake patterns provided insurance against substantial NO_3^- losses that were observed in legume monocultures (Scherer-Lorenzen *et al.* 2003). Therefore, if producers were to increase the diversity of non-legume species with complementary resource use patterns to one another this should act to promote sward productivity and resilience while reducing the risk of NO_3^- leaching. Like Scherer-Lorenzen *et al.* (2003), Spehn *et al.* (2002) also identify legumes as a "keystone" species for nutrient cycling in pasture mixtures. The results of the plant diversity study they conducted in Switzerland showed that forty-four percent of the variation in aboveground N pools was attributable to species composition, and a third of this variability was attributable to the presence of N fixing legumes such as *Trifolium*. When the influence of plant assemblages on N concentration in plant tissue was examined half of this effect could be attributed to the presence of legumes.

In conclusion, it appears as though our water resources are threatened by a variety of inputs into the intensive, monocultured agroecosystems that cover our landscape, but that a shift toward more extensive management of diverse plots could harness the conservation function of natural ecosystem processes and reduce losses of N via leaching. Our reliance on chemical fertilizers can be offset by the incorporation of N_2 fixing species, such as *Trifolium*, into grassland swards. These mixed pastures appear to be able to maintain biomass production while reducing N leaching losses into groundwater. Information continues to emerge concerning the benefits of species diversity and the keystone like role that clover can play in grassland mixtures. However, more research is required that will address the impact of incorporating N_2 fixing legumes into agroecosystems. To this author's knowledge there has been no other study published that measures NO_3^- leaching in grassland mixtures made up of Bluegrass (*Poa pratensis*) and red clover (*Trifolium pratense*). The majority of the studies currently available measure leaching losses from White clover (*Trifolium repens*) mixtures.

CHAPTER 4: IMPACT OF SUB-IRRIGATION AND SWARD MIXTURE ON NITRATE LEACHING IN GRAZED BLUEGRASS AND RED CLOVER STANDS

4.1 INTRODUCTION

A nitrogen supply is essential for plant growth. However, in the pursuit of optimized production the amount of applied N can come to exceed plant metabolic requirements or the capacity of the soil to immobilize it, adversely impacting the environment and disrupting ecosystems. Negatively charged NO_3^- ions are highly soluble in percolating water and when there are influxes of water, NO_3^- will be carried in the water and may end up in nearby aquifers, contaminating them (Scholefield *et al.* 1993). Nitrate leaching losses from well fertilized, intensively managed production systems often exceed 150 kg N ha^{-1} yr^{-1} (Hooda *et al.* 1998; Di & Cameron 2002).

Elevated levels of NO_3^- (> 10 mg L^{-1}) in groundwater have been linked to methemoglobinemia in infants and stomach cancer in adults (Addiscott 1996; Canter 1997). Many in the scientific community are not satisfied that a direct correlation exits between NO_3^- in drinking water and these health afflictions; however, there is little dispute that NO_3^- levels in the environment have risen due to increased fertilizer use (Janzen *et al.* 2003; Powlson *et al.* 2008). Strong evidence is accumulating that indicates excess NO_3^- is disrupting ecosystem function causing phytoplankton blooms, anoxic dead zones and marine acidification (Mills *et al*. 2000; Beman *et al.* 2005; Diaz & Rosenberg 2008). Agriculture has been shown to be a dominant point source polluter of water resources worldwide (Power & Schepers 1989; Addiscott 1996). Henceforth, it is at the agroecosystem level that we should explore and implement management practices to decrease NO_3^- losses, while maintaining production.

A variety of factors control NO_3^- losses from grassland systems including soil characteristics, climate, hydrology, management such as tillage, as well as the timing, form and amount of N applied to pasture (Scholefield *et al.* 1993; Gaines & Gaines 1994; Jabro *et al.* 1997; Yuan & Li 2007). There is emerging evidence that indicates the plant community has a profound impact upon nutrient cycling and leaching losses in agroecosystems (Hooda *et al.* 1998; Loiseau *et al.* 2001; Di & Cameron 2002; Bouman *et al.* 2010). It has been exhibited that mixed pasture swards possess greater productivity, yield, quality for grazing ruminants, and resistance to weed invasion and environmental stressors compared to monoculture pastures (Kunelius *et al.* 2004; McKenzie *et al.* 2004; Sanderson *et al.* 2007). It is suggested that mixed pastures are more sustainable because niche complementarity allows them to intercept more N before it can be lost via leaching (Tilman *et al.* 1996; Niklaus *et al.* 2001; Scherer-Lorenzen *et al.* 2003). The ecosystem services of the plant community are as varied as the combinations of species seeded into pasture, being largely dependent upon the identity and characteristics of species in mixture and the inter- and intra-specific competition within the community (Hooper & Vitousek 1998; Zemenchik *et al.* 2001).

Spehn *et al.* (2002) identified legumes such as *Trifolium* as “keystone species” for nutrient cycling in pasture mixtures. Our reliance on environmentally damaging chemical fertilizers can be offset by the incorporation of N_2 fixing species into grassland mixtures that can fix upwards of 100 kg N ha^{-1} yr^{-1} for use by the plant community (Ledgard *et al.* 2001). Additionally, legumes possess an inherent feedback mechanism that limits excess N input, as nodulation and BNF has been found to be inhibited at elevated levels of inorganic N, regulating the potential for losses (Streeter 1985; Parsons *et al.* 1993). Nitrate leaching losses are lower from systems that rely on biological fixation rather than chemical fertilizer as a source of N; however, a number of authors caution that low diversity clover mixtures still bear the potential to leach an estimated 33 kg N ha^{-1} yr^{-1} (Owens 1990; Cuttle & Scholefield 1995; Ruz Jerez *et al.* 1995).

Nitrate leaching shows some seasonal variability associated with certain months of the year. Soils appear to be least at risk in Atlantic Canada during the summer when plants are actively competing to remove N from the soil, leaving less N in the soil to be lost via leaching (Addiscott 1996). Mild temperatures, slowed plant growth, decreased evaporative losses and increased precipitation leaves the autumn season at high risk for NO_3^- leaching losses (Estavillo *et al.* 1996; Di & Cameron 2000; Scherer-Lorenzen *et al.* 2003). The risk of NO_3^- leaching in fall is especially pronounced in sandy soils due to the large pore spaces between soil particles allowing water to pass through the soil matrix rapidly, retaining little NO_3^-. As a result of containing fewer silt and clay particles sandy soils often possess a low cation exchange capacity (Gaines & Gaines 1994; Conrad & Föhrer 2007). The best way to reduce the risk of NO_3^- leaching in the fall is to ensure there is a paucity of excess N available in the soil environment. This can be accomplished by utilizing sward management strategies that synchronize the spatiotemporal growth patterns of grass species to seasons associated with NO_3^- losses. The inclusion of a forage species with superior late season growth and associated uptake patterns such as Reed canary grass or Kentucky bluegrass, in mixtures could reduce the availability of soil NO_3^- and henceforth the risk of fall leaching losses (McKenzie *et al.* 2005; Rode & Pringle 1985; Bouman 2008; Barnes *et al.* 2007).

A number of studies have highlighted the N contribution of grazing animals to pasture (Cuttle & Scholefield 1996; Jabro *et al.* 1997; Ledgard 2001; Di & Cameron 2002; Wachendorf *et al.* 2004). Ruminant animals in particular make poor use of the N they ingest. Cows for example, return 90-95% of the N they ingest back on to pasture (Addiscott 1996; Di & Cameron 2000; Wachendorf *et al.* 2004). Jabro *et al.* (1997) estimates that on average cows expel 3 L of urine and 2 kg of manure per excretion event in pasture. Approximately 70% of the N deposition upon pasture is in the form of urine, and it is estimated that a cow may urinate 10-12 times a day on a $0.5m^2$ area. The deposition of urine across the pasture is highly spatially variable, and intensifying N loading concerns, cows often urinate in a specific area of pasture habitually (Addiscott 1996). Loiseau *et al.* (2001) postulated that between 32 and 78% of NO_3^- leaching in pastures can be attributed at the local scale to urine deposits from sheep depending upon the composition of the plant community the ruminant is grazing upon. Intensively grazed pasture systems have to potential to lose significant amounts of NO_3^- via leaching, in the range of 100 kg N ha^{-1} yr^{-1} (Jabro *et al.* 1997).

As a result of NO_3^- solubility, hydrological dynamics play an influential role in NO_3^- leaching from agroecosystems. Water influences nutrient transport, transformation and plant N use patterns (Scholefield *et al.* 1993; Hack-ten Broeke 2001; Yuan & Li 2007). Research indicates that the risk of NO_3^- contamination is elevated in areas of pasture where the hydrological dynamics have been altered through drainage or irrigation practices. Pakrou and Dillon (2000) estimate that three times as much biological N fixation takes place in irrigated pastures. The irrigated pasture in this study leached 210 kg N ha^{-1} yr^{-1}, while the non-irrigated pasture leached on 81 kg N ha^{-1} yr^{-1} on average.

There are a number of sites in Atlantic Canada that contain imperfectly drained shallow soils and moisture in these soils can become excessive when precipitation increases. The installation of artificial drainage systems can create more favorable conditions for a successful growing season, boosting biomass production, and increasing trafficability during seeding, grazing and harvest (Addiscott 1996; Gordon *et al.* 2000). These systems also create a more favorable environment for soil microbes that mineralize organic N to produce NO_3^-. Drainage increases the movement of water through the soil matrix – carrying mobile NO_3^- along with it, enhancing the risk of NO_3^- leaching (Gaines & Gaines 1994). As our views shift in favor of enhancing the environmental sustainability of legume based pastures, it must be demonstrated that the N benefits of legumes can be increased by managing the composition dynamics of the sward. The recycling of water from the drainage tiles, back on to the pasture during irrigation should render grass species more competitive against legumes. A combination of innovative irrigation technology and agronomic management is expected to synchronize N flows between legumes and grasses in order to minimize nutrient losses due to soil NO_3^- leaching in the face of shifting climate conditions.

The objectives of this study were to: (i) ascertain the contribution of red clover to NO_3^- leaching when grown in mixture with bluegrass both within the root zone (15 cm) and beyond the root zone (45 cm); (ii) examine how seasonal sub-surface irrigation may affect soil NO_3^- leaching; (iii) monitor seasonal NO_3^- leaching patterns; and (iv) assess yield dynamics in mixed red clover/ bluegrass versus monoculture bluegrass plots and irrigated versus non-irrigated plots.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Site Description:

The research site was an experimental pasture located 36.0 m above sea level at the Nova Scotia Agricultural College (NSAC) in Truro, Nova Scotia (45°37.25N, 63°25.57W). The most recent soil survey conducted by Webb (1991) described the native soil as a very rapidly draining sandy soil of the Truro Association. Methodology reported by Bouman (2008) at an adjacent site confirms the coarse textured nature of this soil as well as describing an A_p horizon indicative of a long history of agricultural land use. The Truro site is located in a cool, humid, temperate climate with an average daily temperature of 6.1°C and annual precipitation of 1169 mm (averages calculated from 1971-2000; Environment Canada 2010). Meteorological conditions were monitored via Environment Canada's weather station in nearby Debert, N.S.

This field experiment was laid out in a split-split-plot design with two replicates. The main plots were: a) irrigated and b) non-irrigated. The sub-plots are separated according to forage cover type: a) pure bluegrass or b) bluegrass-red clover mixture. A control treatment was also embedded in this design, an un-drained, un-irrigated naturalized pasture to compare a recently disturbed ecosystem to one that has likely reached more of a floristic and nutrient equilibrium. This control represents a useful reference point to evaluate any fundamental changes induced by treatments in this experiment. Experimental plots were seeded in June 2008. The bluegrass cultivar, "Ginger" was seeded into all treatments at a rate of 14 kg ha^{-1}. The selected red clover cultivar for this site, "AC Christie" (diploid), was inoculated with commercially purchased peat-based *Rhizobium* and applied at a rate of 8 kg ha^{-1}. A site map is available in Appendix A.

The Truro pasture had variable topography and covered a total area of approximately 25,000 m^2. Each main plot, either irrigated or un-irrigated, covered 6,200 m^2 on average. Main plots were further sub-divided into four experimental units, according to forage type, each covering approximately 1,550 m^2. Each forage treatment appears twice (two bluegrass-red clover; two pure bluegrass plots) within each main plot. There were 16 experimental units in this project with two additional experimental units (the un-drained, un-irrigated naturalized pasture) for a total of 18 experimental units. To estimate the NO_3^- present in the soil percolate two ceramic suction lysimeters (15 and 45 cm depth) were installed in each experimental unit for a site total of 36 soil solution sampling points. Two distinct lysimeter depths were selected in order to measure soil NO_3^- dynamics: i) within the root zone (15 cm) and ii) below the root zone (45 cm) where NO_3^- has moved beyond the rooting depth and is likely to be lost via leaching.

The length of the lysimeter apparatus was made of polyvinyl chloride (PVC) pipe with a diameter of 5 cm. A round bottom porous ceramic cup was secured to the bottom of the PVC tube with epoxy and the opposite end was sealed with a one-hole stopper. A length of clear plastic tubing (2 mm diameter) was cut to length and inserted in the one-hole stopper, running from the top, down inside the porous ceramic cup. A plastic connector (4 mm diameter) was inserted into the top of the one-hole stopper projecting out of the lysimeter. A two-hole stopper with plastic connectors through each was inserted into the top of a 1 L Boston Round amber glass bottle. A rubber tube was attached to each hole in the two-hole stopper. One tube terminated with a metal stopper, this tube was for the purposes of applying the vacuum. The other tube was linked to the plastic connector emerging from the top of the one-hole stopper in the lysimeter for the purposes of collecting the water sample and drawing it into the glass collection bottle.

The suction lysimeters were installed in the Truro experimental site June 1, 2009. To install the lysimeter, a hole of slightly smaller diameter was bored into the soil using a soil probe. A small amount of water was poured into the hole created by the soil probe and mixed at the bottom to create a “slurry” with a threaded steel rod. This slurry is created to promote contact between the ceramic cup and the surrounding soil and optimize suction according to Bouman (2008) and Aparicio (2008). The lysimeter was then inserted into the soil and the collection components were assembled. Lysimeters were given a few days to establish soil contact prior to initial vacuum application and sampling.

The Truro experiment was rotationally grazed by a 35 member herd of lactating Holstein cows at variable stocking rates hovering around 10 cows ha^{-1}. The cows entered the pasture to graze when average sward height reached 25 cm and exited when the average sward height dropped between 10-15 cm. Fences were erected in the field in June 2009 to protect the suction lysimeters and other soil water monitoring instruments in the pasture from the threat of damage by the grazing cattle component of this multidisciplinary experiment. The 2 x 2 m structures were made of wooden posts with page wire wrapped around. Cows rotationally grazed sections of either irrigated or non-irrigated pasture, regardless of forage treatment. Cows were excluded from grazing the areas around the lysimeters. Grazing was simulated by clipping inside these structures at the same time the cows were grazing around them but no animal excreta was applied within the structures. Sufficiently high stocking rates (10 or more cows per ha) were in place to ensure that the entire stand, regardless of forage cover type was reduced to a 10 cm sward height on average. Ammonium nitrate fertilizer (34-0-0) was applied to naturalized and pure bluegrass treatments at a rate of 50 kg ha^{-1} on June 25, 2009 (at the end of the second grazing cycle). This was the only date fertilizer was applied during this experiment. No fertilizer was applied to the bluegrass-red clover mixed swards or inside any of the lysimeter sampling structures.

4.2.2 Sub-irrigation background

This experiment was superimposed upon another project developed by the Water Management Research, Demonstration and Training Facility. The novel Subsurface Drainage/ Irrigation (SDI) system was established at the NSAC Pasture Research Center to demonstrate the advantages of subsurface drainage and irrigation for waterlogged and drought occurrences in the pasture production of milk and meat in Nova Scotia. The SDI system was designed to feature water conservation, low environmental impact and low energy requirement.

This system can, in times of shortage supply water, or in times of excess drain water from the crop root zone. The amount of water supplied can be adjusted within the system. Drainage water is stored and re-used for irrigation during dry periods. The independent water management system of this project allowed water to be supplied to different parts of the field, so it was then possible to have both irrigated, and un-irrigated portions of the pasture. This flexibility lent itself to comparative studies on pasture growth and animal performance with different water table management schemes employed. Additional information regarding the volume and timing of sub-irrigation water is available in Appendix F.

In 2007, drainage tiles were installed 1.5 m deep in the soil and spaced 7 m apart. The tiles were placed in the pasture in a manner that paralleled the natural topography of the landscape. Control chambers were placed strategically throughout the system so that water table heights could be adjusted at different areas of the field to compensate for variable topography. The control chambers have the capability to be set to “no-restriction” so that the system operates as a conventional drainage system, or adjusted to restrict flow to a desired water table height which is referred to as “Control Drainage” or “Sub-irrigation” when operating in supplemental irrigation mode. Water was drained by gravity from each plot and pumped to an open air, above ground storage pond. The supplemental water was pumped from this water storage pond and delivered to the highest control chamber in the plot within the sub-irrigation system during dry periods. Additional information regarding sub-irrigation volumes is available in Appendix G.

The subsurface drainage lines connected to a drainage pipe at the bottom of each plot. These pipes were connected to the sampling hut located at the lowest point in the pasture, where they emptied into stainless steel, low flow tipping buckets that were wired to a data logger. There were a total of four tipping buckets altogether, one for each plot (each plot was drained independently). Every tipping bucket had a calibrated capacity, in this case, four liters. The data logger continuously recorded how many times the bucket tipped per minute and from this the total volume of water that drained from each plot could be determined (Havard 2009).

4.2.3 Data Collection

4.2.3.1 Soil

Soil cores were collected in Truro, NS on May 13, 2008 in order to characterize baseline soil nutrient and mineral status. During this process a soil probe was inserted to a depth of 20 cm below the soil surface. Eight 20 cm depth soil cores were collected from the perimeter of the equipment enclosures in each experimental unit. No soil cores were collected within the confines of the small fenced area containing the lysimeters due to the destructive nature of this process. Each soil core sample was separated into two portions: the top 0-10 cm and the bottom 10-20 cm. Each of the top eight soil core samples from every experimental unit was ground by hand in the field and amalgamated into one sample. The eight bottom soil core sections were amalgamated in the same fashion. This sampling method resulted in two stratified samples collected from every experimental unit. Due to the variable nature of sediment composition in the field, homogenizing the collected soil volume is useful to more closely characterize site conditions (Olin-Estes *et al.* 2000). Soil samples were bagged and immediately stored at 4°C until they could be submitted for analysis at the Harlow Institute and the Environmental Soil Laboratory at the NSAC. The soil characteristics from this site are summarized in Appendix B.

4.2.3.2 Soil solution

Soil solution was sampled from ceramic suction lysimeters on a bi-weekly or event based schedule. Lysimeters were sampled predominantly during rain events because NO_3^- is highly soluble in water. Therefore, NO_3^- is moving most actively through the soil system following rain events (Canter 1997). A vacuum of 0.8 bar (~10 psi) was applied to the lysimeter system just prior to, or during rain events using a battery powered Mobile Suction and Vacuum Pump (Model number MVF-120) manufactured by Umwelt-Geräte-Technik Company. Soil solution samples were collected in 125 mL Nalgene™ sampling containers 24-48 h following vacuum application. Collected samples were immediately stored in a freezer to prevent volatilization of analytes until they could be analyzed at the Water Quality Research Laboratory, Department of Environmental Sciences of the NSAC. The volumetric water content of the soil surrounding the suction lysimeters in Truro was measured during solution collection using a handheld Hydrosense TDR device from Campbell Scientific™ with 20 cm prongs.

Soil solution was continuously monitored in Truro from June 11 to November 19, 2009. A total of 18 sampling events took placed during this time (Table 4.1). The sampling season was terminated when the air temperature dropped consistently below zero and there was a reasonable threat of ground freeze. Following the final collection of soil solution in November 2009, all 15 cm lysimeters were removed from the soil, disassembled and rinsed with de-ionized water. The 45 cm lysimeters remained in the ground and were winterized. In order to winterize the 45 cm lysimeters, their internal access tubes were removed and the PVC tube was injected with a dilute antifreeze solution. A solid rubber stopper was placed in the top of the tube and the lysimeter was then covered with straw. The 45 cm lysimeters require a number of flushes with de-ionized water in the spring before sample collection can re-commence.

Monthly water samples were collected manually from tipping buckets inside the drainage hut during high flow events. The hut yielded a water sample that broadly characterized the nutrient concentration in water flowing out of each of the four drained plots. These samples were also frozen until they could be analyzed at the Water Quality Research Laboratory, NSAC. The results of the hut drainage water analysis are presented in Appendix G.

4.2.3.3 Forage

Vegetation was clipped to a height of 10 cm when the sward height reached approximately 25 cm. This was also the sward height when cows entered to graze. Sward biomass was sampled in each experimental unit using a rectangular 0.25 m^2 quadrat. Sward composition was sampled using a 1000 cm^2 hoop which was placed directly above each lysimeter in an effort to relate plant community composition to the concentration of nitrates in soil solution. The area of the sampling hoop was assumed to coincide with the catchment area of the suction lysimeter. Following manual separation by species, all samples were dried at 70°C for 72 hours in a forced air drying oven then weighed. Dried botanical samples were ground in a Wiley Mill fitted with a 1 mm sieve. Species appearing twice within the same experimental unit were pooled together in the Wiley Mill prior to analysis.

In Truro the sward reached the desired 25 cm sward height and was first sampled May 13, 2009. There were six harvest cycles in total that occurred in Truro: May, June, July, August, September, and October. Due to the grazing schedule not all plots were sampled on the same day in each month (Table 4.1). However, all plots were harvested on the same dates on May 13 and October 1, 2009. When the protective fencing was erected in June 2009 separate biomass samples were collected inside and outside of the structures for every subsequent harvest. Inside samples related directly to leaching data, but it was also necessary to collect data outside the structures due to differing N regimes (outside received fertilizer and excreta N inputs). Quality analysis of the forage was necessary for the animal component of this multidisciplinary experiment. Botanical samples were taken inside the structures, above the lysimeters during every harvest cycle. An additional botanical sample was collected outside the structures on August 3, 2009.

Table 4. 1. Sampling dates for NO_3 testing on drainage water, sward clipping and soil core collection for Truro experimental site in 2009.

	Soil solution extraction	Sward clipped		Soil core
Month	MM/DD	MM/DD	Plot	MM/DD
May		05/13	N	05/13
		05/13	1	
		05/13	2	
		05/13	3	
		05/13	4	
June	06/12	06/03	N	
	06/13	06/10	1	
	06/22	06/17	2	
	06/23	06/17	3	
		06/26	4	
July	07/07	07/02	N	
	07/13	07/07	1	
	07/23	07/07	2	
		07/21	3	
		07/21	4	
August	08/11	07/27	N	
	08/25	08/06	1	
	08/31	08/06	2	
		08/12	3	
		08/12	4	
September	09/17	09/04	N	
	09/30	09/04	1	
		09/04	2	
		09/08	3	
		09/08	4	
October	10/09	10/01	N	
	10/19	10/01	1	
	10/26	10/01	2	
		10/01	3	
		10/01	4	
November	11/05			
	11/08			
	11/19			

4.2.4 Analytical methods

4.2.4.1 Soil

Soil core samples collected in Truro on May 13, 2009 were analyzed for pH, OM, CEC P, K, Ca, Mg, Na, S, Fe, Mn, Cu, Zn, and B at Nova Scotia Agriculture Laboratory Services, Harlow Institute, Truro, N.S. Soil samples were dried at 35°C and ground to pass through a 2mm sieve. Soil pH was measured by mixing an equal volume of soil with distilled water. This combination was stirred for half an hour and allowed to stand for half an hour. Following this mixing the pH was measured with a pH meter with reproducibility of at least 0.05 units (Schofield & Taylor 1955).

Soil organic matter was determined as a function of loss on ignition. The loss on ignition methodology is based on the research report from Donald and Harnish (1993). Testing the loss on ignition involves the weight ratio of 2 grams of sample before and after it is placed in a 450°C muffle furnace for one hour. Analysis of P, K, Ca, Mg, Na, S, Fe, Mn, Cu, Zn, and B was determined using Mehlich 3 extractant solution and concentration of elements were detected using an Autoanalyzer (Mehlich 1984 and 1978). Truro soil core samples were subjected to 2.0 M KCl extraction according to Carter (2008) and analyzed for NO_3^--N and NH_4^+-N on a Technicon Autoanalyzer (Technicon Instruments Corp.) at the Environmental Soil Lab at the Nova Scotia Agricultural College.

4.2.4.2 Soil solution

Soil percolate samples collected in 2009 were analyzed for NO_3^- and PO_4^{3-} at the Water Quality Research Laboratory, NSAC. Soil solution samples were first syringe filtered with 0.45 µm nitrocellulose membrane filters in preparation for single-column ion chromatography with direct conductivity detection as indicated by Eaton *et al*. (2006). A Waters Ion Chromatography System (Waters Canada Ltd.) was the instrument utilized in the detection of NO_3^--N. This instrument comprised of a Waters Model 1525 Binary HPLC Pump, a Waters Model 717-Plus Autosampler, and a Waters Model 432 Conductivity Detector. A Waters IC-PAK Anion HC 4.6 x 150 mm was the anion-exchange column used. The detection limit of NO_3^- analysis was 0.08 mg L^{-1}. For the analysis of the soil water data set samples whose concentrations were below the detection limit were assigned a value of 0.07 mg L^{-1}, one of the options discussed by Gochfeld *et al.* (2005) and Olin-Estes and Palermo (2000). The detection limit of PO_4^{3-} analysis was 0.10 mg L^{-1} and concentrations below this detection limit were assigned a value of 0.09 mg L^{-1}.

4.2.4.3 Forage

Ground botanical matter was analyzed for total plant tissue N via the combustion method on a LECO protein/ N determinator FP-528 according to the Dumas Method at the Department of Plant and Animal Sciences Laboratory at the NSAC. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed sequentially in the same laboratory on an ANKOM200 Fiber Analyzer according to Vogel *et al.* (1999). The proportion of non-soluble ash present in sampled forages was determined by placing samples into a 500°C oven overnight.

4.2.5 Statistical methods

The experimental design was a control plus factorial experiment established in a split-split plot design with irrigation treatment as the main plot, sward type as the sub-plot along with the reference control of the naturalized, un-irrigated pasture. The GenStat® software package (Payne 2002) was used to perform the Repeated Measures Analysis of Variance tests

(RM-ANOVA) and to calculate the Standard Error of the Mean for comparison of means at a probability level of $p < 0.05$. Graphs were generated in MS Excel and SigmaPlot 10.0. RM-ANOVA tests were conducted on sward dry matter yield, sward N yield, NO_3^- present in soil solution at 15 and 45cm, and PO_4^{3-} present in soil solution at 15 and 45cm. Histograms were constructed and large residuals identified by the Genstat software were manually removed to satisfy the normal distribution and constant variance assumptions of the ANOVA model.

Nitrate and Phosphate in Soil Solution

Nitrate leaching studies represent a unique experiment where sampling instruments are fixed and sampling is non-destructive. Sampling the exact same unit at different points in time may result in a time series data set, embedded in which there may be autocorrelation. This indicates that measurements taken closer together may likely be more similar than those samples separated by a longer period of time. That being said, time series sampling remains at odds with the regular split-plot ANOVA assumptions that: (a) every observation shares equal correlation and that, (b) the treatment a subject receives is expected to be randomized. Time defies this assumption because it is a factor that cannot be randomized (Webster & Payne 2002). As a result RM-ANOVA was utilized to best observe the patterns of change in NO_3^- and PO_4^{3-} concentrations in the soil solution over time.

Nitrate and phosphate concentrations of the soil percolate were analyzed statistically by inputting every collection date into Genstat. Initially, researchers attempted to run a RM-ANOVA using monthly averages by experimental unit. However, during unseasonably conditions in months such as September there were only two collection dates and a number of missing values. The missing values reduced the predictive capabilities of the RM-ANOVA software to the degree that the output would at best not be comprehensive, and at worst be mathematically unsound. The NO_3^- and PO_4^{3-} data sets were log-transformed to achieve normality. Nitrate and phosphate outputs are back-transformed in the results section.

The repeated measure in this analysis was sample date, for which the Julian Day is used on the x-axis. The test for significance of variability was determined using the F-test ($p < 0.05$). Analysis for fitting mathematical curves to the concentration of NO_3^- and PO_4^{3-} was conducted using linear multiple regression for PO_4^{3-} and quadratic multiple regression for NO_3^-. For the NO_3^- data set, a linear modeling equation was tested but did not provide satisfactory fit (<30% of variance accounted for). The next step to a second order polynomial equation yielded much better r^2 values. Log transformed NO_3^- and PO_4^{3-}values were regressed against sample date collection. Regressions were conducted separately by Irrigation and Sward treatment. Fitted values for each equation were expressed in (mg L^{-1}) and plotted with the observed values (mg L^{-1}) to determine degree of fit (Vittinghoff *et al.* 2005). An "Additive model regression" was used along with "smoothing splines". An additive model regression first creates a line of fit for the entire data set, second a fit is created for treatment, and thirdly a fit is created for interactions. Smoothing splines are used because they are flexible enough to link sudden changes in slope. Splines achieve this by possessing inherent constraints to ensure smoothness (Hand & Taylor 1987; Digby *et al.* 1989).

The mass loss of NO_3-N attributable to leaching was volume weighted using Environment Canada meteorological data. The estimation of drainage water volume in this experiment incorporated rainfall, potential evaporation and soil water data (Puckett *et al.* 1999). The volume weighting of NO_3^- leaching loss was possible as a result of collecting TDR soil moisture data that informed the researcher of the site field capacity (Appendix E). The drainage volume estimates represent the sum of rainfall events received that exceeded 10 mm per day when the soil water content at a 20 cm depth had reached field capacity. The quantity of NO_3-N lost via leaching was estimated by multiplication of the NO_3-N content (mg L^{-1}) and drainage water volume (L^{-1} m^2) divided by 1000, as indicated by Bouman *et al.* (2010).

Forage

Sward N yields were calculated by multiplying the dry matter yield of each species by the tissue N concentration for each species, and then expressed as a total representing all species N yields for each experimental unit. Differences between mean DMY of sward treatments were tested using RM-ANOVA. Principal Component Analysis (PCA) was utilized to depict vegetative relationships that exist for the various sward treatments over six harvests.

4.3 RESULTS AND DISCUSSION

4.3.1 Meteorological conditions

The average daily air temperatures measured by Environment Canada in nearby Debert, NS during the study did not deviate notably from climate normal air temperatures (Figure 4.1). Average monthly temperatures recorded during the growing season of 2009 for Truro, NS were 16°C (June), 18°C (July), 19°C (August), 12°C (September), 6°C (October) and 4°C (November). During the 6 month lysimeter sampling period from June until November 2009, the Truro site received an estimated 586 mm of precipitation. Rainfall in May, June, July and September was below average. August and October showed a 50% increase over rainfall climate averages. Information regarding sub-irrigation volumes that were supplied to sub-irrigated treatments in addition to recorded rainfall amounts is available in Appendix F.

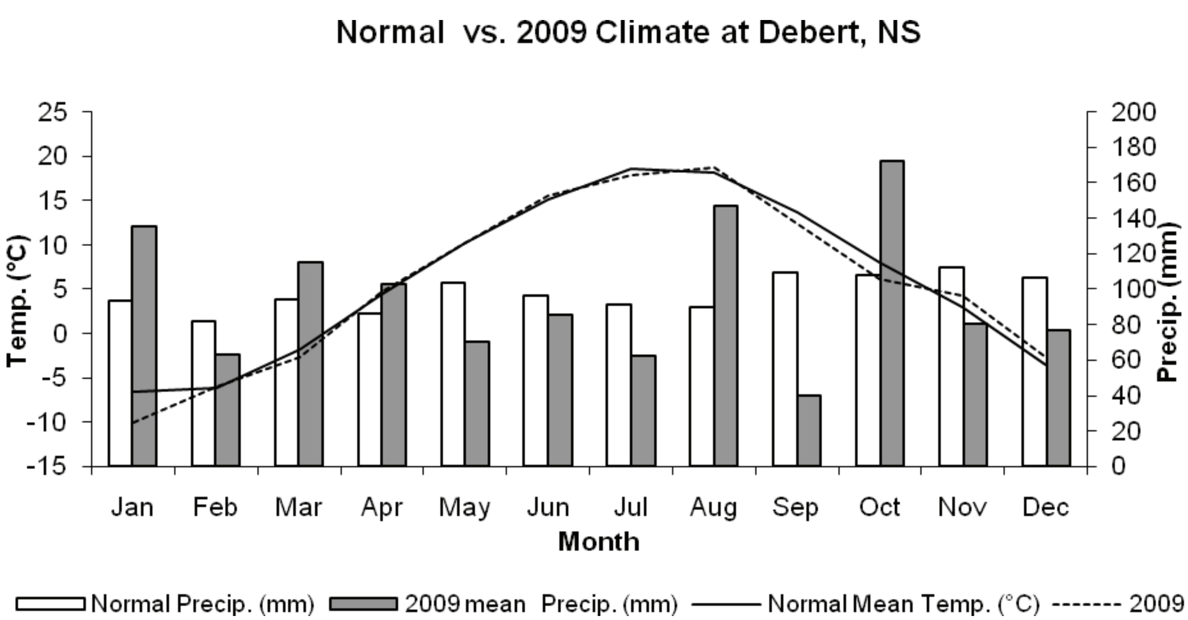

Figure 4. 1. Weather conditions in Debert, NS in 2009 compared to average Climate conditions 1971-2000 (Data obtained from Environment Canada: http://www.climate.weatheroffice.gc.ca/).

4.3.2 Nitrate in soil solution

There were 507 soil solution samples collected in total from the Truro experimental site in 2009. The mean NO_3-N concentration of soil solution extracted from all lysimeters was 3.82 mg L^{-1} and ranged from <0.08 to 46 mg L^{-1}, the latter from mixed pasture. Nitrate concentrations in soil solution (Appendix H) were similar to those observed in a previous adjacent experimental grazed pasture under organic management (Bouman 2008). Approximately 13% of all samples exceeded the 10 mg L^{-1} of NO_3-N Maximum Acceptable Contaminant Limit (MAC) for NO_3^- in drinking water. Sixty percent of exceedances qualitatively observed in the raw data occurred in the fall (September, October and November). The tendency for NO_3-N concentrations to increase in soil solution in the fall is supported by results of other extensively managed pasture experiments (Estavillo *et al.* 1996; Anger *et al.* 2002).

4.3.2.1 Nitrate in the root zone

At a 15 cm soil depth NO_3^- trends over time were best modeled by a quadratic concave regression spline that accounted for 71% of variance in the data. Nitrate concentrations in the root zone (15 cm) ranged from low to moderately-high. Nitrate levels approached, and on more than one occasion exceeded the 10 mg L^{-1} maximum acceptable contaminant limit for NO_3^- in drinking water. Over the season NO_3^- levels were found to be highest in the irrigated mixed pasture and lowest in the natural, irrigated and non-irrigated pure treatments which were similar. The results of RM-ANOVA contrasts for each sampling date by sward and irrigation treatments at 15 cm are available in Appendix H. A significant interaction was observed between sward treatment and date ($p < 0.001$), indicating that the effect sward treatment had upon concentration of NO_3^- in the root zone appeared to be modified by seasonal conditions over time. Experimental results showed that mixed swards contained 25 times the concentration of NO_3^- in soil solution at 15 cm - confirming our hypothesis that the presence of red clover would elevate N in the root zone of mixed pasture relative to monoculture bluegrass (Figure 4.2).

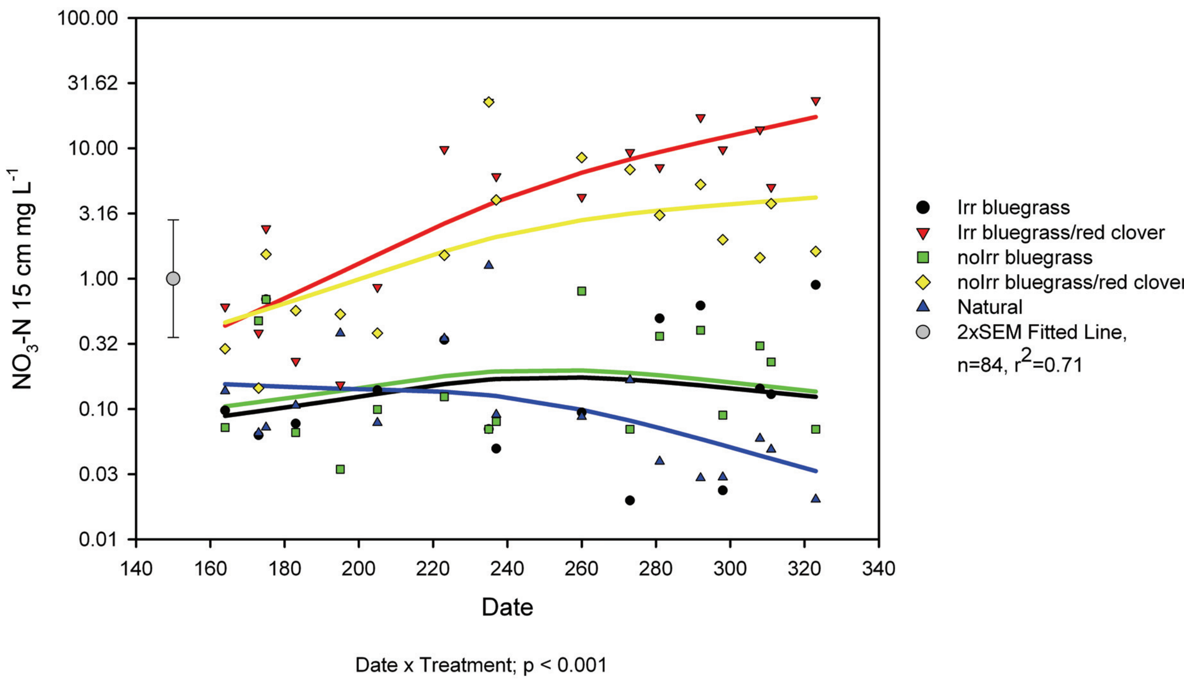

Figure 4. 2. Seasonal change in NO_3-N concentration of the soil solution in Truro, N.S. at a 15 cm depth (regression models are plotted against observed back transformed NO_3-N values).

No evidence was found that would indicate sub-irrigation affected NO_3 leaching ($P > 0.05$). Thus, the results lead us to reject our hypothesis that seasonal irrigation would boost BNF in red clover plants, as a result of being afforded a biotic advantage during dry conditions as well as improving the hydraulic conductivity of the soil media surrounding them. Significance of the irrigation treatment is largely influenced by weather conditions and will be highly variable from season to season which is why continuation of this study would be prudent. It would appear that the soil never dried out severely enough that irrigation treatments would confer a significant biotic advantage to plants growing in the irrigated sections of pasture as evidenced by rainfall (Figure 4.1) and soil moisture data (Appendix E).

Seasonal NO_3^- dynamics offer further confirmation of the keystone-like role N fixing red clover plays in pasture (Spehn *et al.* 2002). Nitrate levels in the root zone of all sward treatments were low and similar early season (Figure 4.2). Later in the season treatments diverge to reveal two discrete patterns of NO_3^- concentration: a) increased NO_3^- availability in swards containing N fixing red clover and b) decreased NO_3^- availability in swards that do not contain a N fixing species. Early in the season, NO_3 availability in the root zone was likely reduced by plant uptake (Addiscott 1996). The seasonal accumulation of NO_3^- in the root zone of leguminous pasture was also found to occur in a pasture experiment in Manitoba (Chen *et al.* 2001). These trends correspond to what is understood about red clover phenology where early season growth relies upon soil N until conditions are favorable for nodulation. Once these conditions are met red clover has the ability to release N into the soil through BNF until the end of the season, where N may continue to be contributed to soil N through the senescence of clover litter (Wu & McGechan 1998). Nitrate is removed from the root zone of grass monoculture and not replaced in any appreciable amounts by the resident white clover observed to be present in these plots. Information regarding botanical composition of pasture is available in Table 4.5.

Natural pasture begins the season with an intermediate concentration of NO_3^- relative to the other sward treatments but shows a marked decline over the season. This decline is consistent with the concept that the finite supply of soil N is being used up by the growth of grasses and not being replaced. Nitrate levels may be higher late season in the seeded grass swards compared to naturalized pasture due to past soil disturbance. Soil disturbance, such as tillage, is recognized to increase mineralization and subsequent release of soil OM in the short term. This may be one explanation as to why NO_3-N levels are higher in bluegrass swards which were ploughed under in 2007 versus naturalized pasture (Blevins *et al.* 1983; Hansen *et al.* 1997; Di & Cameron 2002). However, when this experiment was planned tillage was not a component that was incorporated into the design. The more rapid decline of NO_3^- in natural pasture may also come about as a result of high grass species diversity. All these grass species occupy the same niche and eventually fully utilize soil NO_3^- available from the mineralization of a rich layer of SOM (and small amounts of fixation) to support the robust forage growth observed in the naturalized pasture (Section 4.3.3.1; Tilman *et al.* 1996; Scherer-Lorenzen *et al.* 2003).

4.3.2.2 Nitrate leaching

At a 45 cm soil depth NO_3^- trends over time were best modeled by a quadratic convex regression spline that accommodated 79% of the variance in the data (Table 4.3). Nitrate concentrations beyond the root zone (45 cm) ranged from low in the grass treatments to moderate in the red clover treatments where NO_3^- concentrations were found to approach the 10 mg L^{-1} maximum acceptable contaminant limit. The results of RM-ANOVA contrasts for each sampling date by sward and irrigation treatments at 45 cm are available in Appendix H. Concentrations of NO_3^- at 45 cm did not exceed the MAC limit as frequently as was observed at 15 cm (Figure 4.2). This is the first indication that what was observed in the root zone is not the case deeper in the soil. Over the season the concentration of NO_3 in leachate was found to be highest in the irrigated mixed pasture and lowest in the non-irrigated pure treatment.

Overall NO_3^- concentrations from irrigated pure, non-irrigated pure and natural treatments were low, exhibiting only a modest late season increase. Nitrate levels were elevated in red clover-bluegrass mixed swards relative to the low leaching levels from pure bluegrass. The results of RM-ANOVA indicate that a significant interaction occurred at 45 cm between sward treatments and the time in the season the sample was collected (P = 0.007).

Experimental results showed that mixed swards contained 10 times the concentration of NO_3^- in soil solution late season, supporting our hypothesis that red clover grown in mixture with bluegrass may lead to an increase in NO_3^- leaching relative to monoculture grass, as influenced by other factors (Figure 4.3).

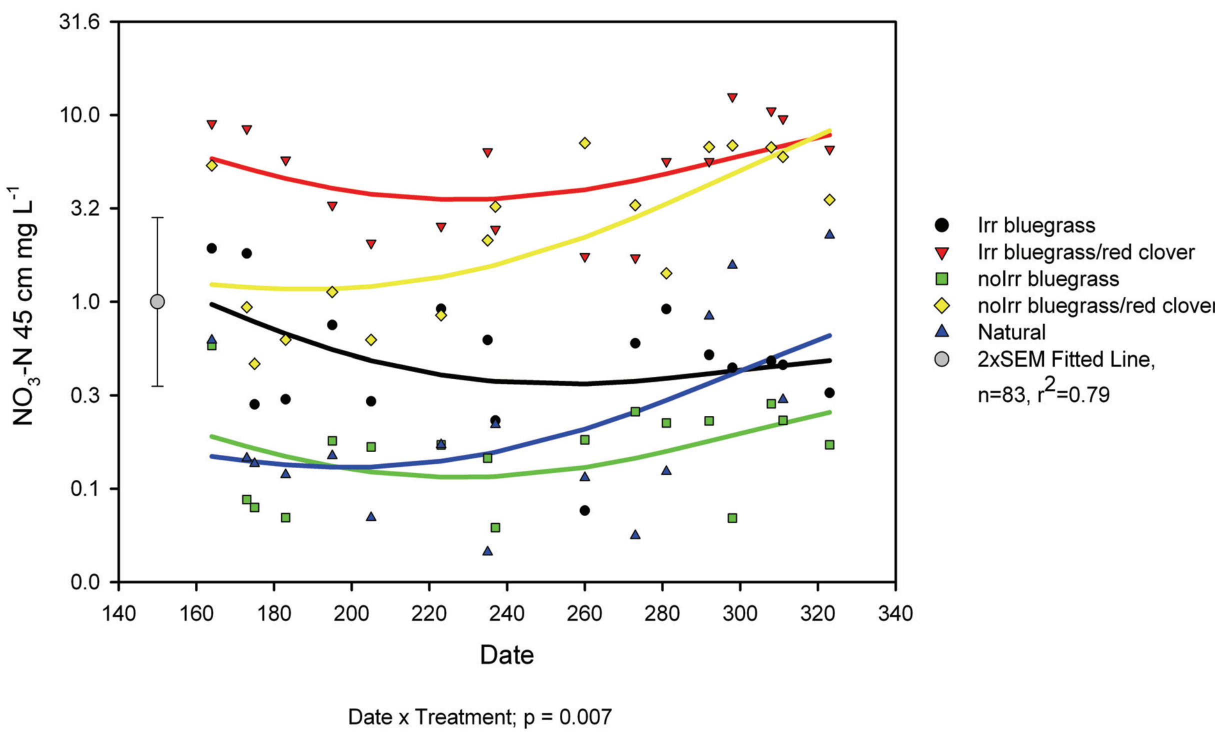

Figure 4. 3. Seasonal change in NO_3-N concentration of the soil solution in Truro, N.S. at a 45 cm depth.

Mean concentrations of NO_3^- in the soil percolate leaching from each treatment were 7.08, 1.17 and 0.38 mg L^{-1} for mixed, pure and natural swards. Average NO_3^- concentrations of soil solution from mixed pasture fit within the ranges reported by Hooda *et al.* (1998) from grazed, unfertilized grass-alfalfa mixtures and Owens *et al.* (1994) from a grazed rye grass-white clover pasture with two applications of cattle slurry. In both cases the concentrations we measured existed at the low end of these author's estimates. Volume weighted NO_3^- leaching for the six month sampling period revealed that pure swards leached only 0.5 kg N ha^{-1} yr^{-1} and mixed swards lost 7.4 kg N ha^{-1} yr^{-1} over the course of the growing season. Losses from naturalized pasture were negligible as available N was tightly cycled in this N limited sward. Nitrate leaching losses in a low input pasture that relied upon BNF from clover was significantly lower than NO_3^- leaching levels reached beneath fertilized pasture (Scholefield *et al.* 1993; Bjorneberg *et al.* 1995; Cuttle & Scholefield 1996; Loiseau *et al.* 2001). Observed leaching losses were considerably lower than those reported from similar unfertilized, red clover-grass pastures in the biodiversity study of Scherer-Lorenzen *et al*. (2003) and those described by Ledgard (2001) from grazed unfertilized grass-legume pastures. Nitrate leaching fell within the range of values reported to be leaching from two grazed ryegrass-white clover pasture studies (Loiseau *et al.* 2001; Eriksen *et al.* 2004). Field experiments undertaken by Ruz Jerez *et al.* (1995) and Di & Cameron (2002) revealed extremely similar NO_3-N leaching losses from grass-clover pastures on sandy loam soils (See Table 3.1 for values reported by the above authors).

Moreover, mineralization processes have been observed to occur during low temperature conditions in the winter during freeze thaw events. Mineralization of senescent tissue can result in localized releases of NO_3 over the winter that this study was unable to account for. Gill *et al.* (1995) noted substantial release of N from soil in the U.K. over the winter, losing 27% of the annual value during this time. However, snow cover and soil freezing during Nova Scotia winters are much more likely to impede mineralization events as pronounced as what is observed in European experiments. Following a period of heavy rainfall in a German study 33%, of total winter leaching losses was found to occur over a 3 d period (Kayser *et al.* 2010). While continual annual monitoring presents a more desirable data set, inherent limitations of the sampling equipment utilized within the context of Atlantic Canada's climate prevented this from occurring as suction lysimeters are easily damaged during the soil shifts that accompany freeze thaw events.

There was a tendency for leaching to be greater in irrigated sections of pasture in both pure and mixed swards as supported by Pakrou & Dillon (2000) and Hack Ten Broeke (2001). However, contrary to postulations supported by the literature, statistical analysis of NO_3^- leaching data over the entire sampling season did not support the hypothesis that irrigation would lead to an increase in NO_3-N produced by red clover in the root zone, to subsequently be lost via leaching. Firstly, hydrological dynamics are highly variable across pasture with changing topography, thus it was challenging to characterize the water flux occurring throughout each of the irrigation treatments. If there was more replication in the field it may have been more likely that researchers would have been able to observe irrigation differences. Due to the labor and cost intensive nature of sub-irrigated field level experiments it was not possible to have more than 2 replicates of the irrigation treatments at this site. Also, a depression in a non-irrigated plot in the second replicate appeared to have an aquifer extremely close to the surface. This resulted in this plot being at field capacity most of the spring and summer despite there being no irrigation water supplied. Volumetric soil moisture measurements are available in Appendix E.

Secondly, weather conditions have a vast influence on the water requirement of the crop and henceforth on whether or not additional water is required to maintain production through the use of sub-irrigation practices. This was only a one year excerpt of an experiment in the context of ever changing weather conditions. A longitudinal study would be required to fully realize the capacity of irrigation to influence NO_3^- leaching across a variety of seasons and weather conditions.

The results of the RM-ANOVA analysis confirm that seasonal weather conditions drive NO_3^- leaching patterns as indicated by Estavillo *et al.* (1996), Ledgard (2001) and Dinnes *et al.* (2002). Nitrate leaching was moderate in the spring as plant growth is beginning. Moisture and temperature conditions are becoming conducive to mineralization of N but not just yet for nodulation and fixation in red clover (Lipsanen & Lindstrom 1986). Even if the amounts of N mineralized from soil organic matter were small, which was not always the case (Gerwing & Gelderman 1990), whatever cannot be taken up by the plant community will be susceptible to loss via leaching. Soil disturbance may have contributed to increased mineralization of soil N that could exacerbate the risk of N losses at this time. In the spring NO_3^- levels were highest in irrigated mixture, intermediate in non-irrigated mixture and irrigated pure swards and lowest in non-irrigated pure and natural swards. It is not yet clear why non-irrigated mixed and irrigated pure swards were similar.

During the mid-summer dry period there was a decrease in NO_3^- moving through the soil profile. This was attributable to: a) a seasonal reduction of moisture that acts as a medium for NO_3^- movement and b) plants were actively removing NO_3^- from the soil through uptake (Addiscott 1996). In the fall NO_3^- leaching at 45 cm was significantly greater in mixed swards regardless of irrigation regime. Nitrate was consistently high in the irrigated mixture whereas the non-irrigated mixture had lower NO_3^- concentrations initially, but exhibited a tenfold increase across the season. By the fall NO_3^- leaching decreased from previously intermediate concentrations observed in irrigated pure swards, to similarly low concentrations as found in natural and non-irrigated pure swards as bluegrass used up the soil available N that was not replaced. Nitrate leaching in natural and non-irrigated pure swards remained low the entire season, with modest increases in spring and fall triggered by seasonal moisture levels considered favorable for the soil microbes which carry out mineralization of soil organic matter

(Gerwing & Gelderman 1990; Estavillo *et al.* 1996). Curiously, NO_3^- in the soil percolate of natural pasture increased into the fall on a similar slope to the non-irrigated mixture but at a reduced magnitude. All of the soils at this site have been utilized as farmland for some time, but unlike the re-seeded pasture, un-renovated natural pasture was not ploughed over and the microbial community may be more slowly accessing stored N (Di & Cameron 2002; Blevins *et al.* 1983; Hansen *et al.* 1997).

4.3.2.3 Nitrate dynamics within and beyond the root zone

Distinctive NO_3^- leaching patterns emerge when researchers examine the NO_3^- leaching profile at two depths: a) within the root zone and b) leaching beyond the root zone, indicating that it is pragmatically useful to observe NO_3^- dynamics at multiple levels in the soil profile. Nitrate increases from the beginning to end of the season in the root zone, reaching higher concentrations than what is observed deeper. Conversely, the NO_3^- profile at 45cm shows that leaching is higher in spring and fall with a decline in summer. It appears that the NO_3^- available at 15 cm is not what is leached through to 45 cm. Firstly, soil conductivity and aggregate distribution are known to differ with a change in depth (Gordon *et al.* 2000). Therefore, the passage and sorption of NO_3^- in solution will be influenced by changing soil conditions. Secondly, it seems that the plant community may be modulating NO_3^- leaching through their specific uptake patterns. For example, Kentucky bluegrass was selected as the companion grass species in this experiment for its consistent growth, extending into fall. It is possible that bluegrass is utilizing the NO_3^- being made steadily available in the root zone of mixed pasture so that fewer NO_3^- remains to be lost via leaching. This would be one explanation as to why NO_3^- is found to increase in the root zone of mixed pasture but is measured in diminished concentrations deeper in the soil profile.

Bluegrass architecture and phenology may play a role in the “book end” leaching increases we see in spring and fall (Figure 4.3). A warm season grass would likely have cultivated a leaching pattern skewed more toward the fall with few spring NO_3^- leaching losses. However, as a cool-season forage, Kentucky bluegrass growth and N uptake is still robust late season when NO_3^- availability is elevated due to BNF and mineralization of soil N, bluegrass growth is relatively synchronous – capturing much of the available N leaving with its dense root system leaving less to be lost via leaching. Additionally, bluegrass develops a thick mat of rhizomatous roots. Recall that denser root networks possess an enhanced capacity to intercept and remove N from the soil profile (Scherer-Lorenzen *et al.* 2003).

Scholefield *et al.* (1993) proposed that extended periods of dry weather would further enhance N mineralization when moist conditions once again prevailed. May, June and July were hot and dry relative to climate averages, and then there was an influx of 170 mm of rainfall in the month of August (Figure 4.1). However, the predicted accumulation and sudden release of N was not observed in leaching through the soil in this experiment. If there was a sudden release of N at this time its impact must have been minimized through uptake by the plant community. This postulation is supported by a corresponding increase in SNY observed in August (Table 4.5).

4.3.3 Forage

4.3.3.1 Dry matter yield

In establishment year two of the field experiment in Truro, N.S. sward treatment was found to have significantly influenced the dry matter yield (DMY) in four of the six harvests (Table 4.2). Forage treatment also significantly modified seasonal DMY (P = 0.005). Mixed swards containing red clover produced the greatest seasonal DMY of all treatments. Natural swards also produced robust yields. Pure bluegrass swards were the least productive. The forage results from this study concur with literature indicating that the productivity of plants of two complementary functional groups will yield greater than either component species in monoculture (McKenzie *et al.* 2004; Kunelius *et al.* 2005; Sanderson *et al.* 2007).

Seasonal forage yields from this experiment were similar to the yields observed in grass-legume mixtures of field experiments conducted in the Atlantic region (McKenzie *et al.* 2004; Kunelius *et al*. 2005) and Wisconsin, U.S.A. (Zemenchik *et al.* 2001). In the Kunelius *et al*. (2005) experiment a mixture comprised of timothy, bluegrass and red clover produced an average annual yield of 8.85 t ha^{-1} over three years. Forage samples in this experiment were collected within the lysimeter sampling areas. Growth of plants within the confines of this 4 m^2 area may have been hindered by the number of soil monitoring instruments, or low light conditions due to shading. Secondly, the results of this study are from only one year of data collection. Further information regarding forage quality information is available in Appendix D.

Table 4. 2. Dry matter yield (t ha^{-1}) from three sward treatments in Truro, N.S.

Treatment	n	May	Jun	July	Aug	Sep	Oct	Season
Pure bluegrass	8	0.55	1.52	1.32	1.22	0.93	0.70	6.25
Red clover mixture	8	0.92	2.42	1.63	1.47	1.24	0.84	8.68
Natural	2	1.00	2.40	1.61	0.76	1.55	0.66	7.97
SEM		0.09	0.18	0.08	0.16	0.06	0.03	0.32
P-value[1]		0.042	0.052	0.018		0.015		0.005

[1] Probability values contrast mixed red clover versus pure bluegrass sward treatments.

Table 4. 3. Dry matter yield (t ha^{-1}) by irrigation treatment in Truro, N.S.

Treatment	n	May	June	July	Aug	Sep	Oct	Season
Irr	2	0.82	1.89	1.64	1.51	1.31	0.89	8.22
noIrr	2	0.65	2.06	1.31	1.18	0.87	0.65	6.72
Natural	2	1.00	2.40	1.61	0.76	1.55	0.66	7.97
SEM		0.05	0.09	0.08	0.20	0.05	0.02	0.16
P-value[1]				0.095		0.020	0.010	0.023

[1] Probability values contrast Irrigated versus Non-irrigated treatments.

Dry matter yield was also examined as a function of both sward and irrigation treatment and the results of the RM-ANOVA are represented in Figure 4.2 and in Table 4.3. Irrigated mixed sward seasonal yield was highest, natural and non-irrigated mixture was intermediate and pure irrigated and non-irrigated swards were least productive. Yields exhibited a slight linear decline in all treatments except irrigated pure, where yields remained constant. Attributable in part to the phenology of Kentucky bluegrass, (namely consistent forage production over the course of the growing season) pure bluegrass treatments did not exhibit the late season decline observed in red clover and natural plots (McKenzie *et al*. 2005). Pure sward yields were consistently low due to N limited soil conditions.

As depicted in Table 4.2, mixed pasture containing red clover produced significantly greater amounts of sward biomass over time compared to pure bluegrass (P = 0.005), and annual total DMY (P = 0.005). Irrigation was found to have a small (approximately 1.5 t ha^{-1}) but significant impact upon annual dry matter yields (P = 0.02). The sub-irrigation treatment is significant mostly late season, which is curious because no irrigation water was delivered to pasture in September or October (Appendix G). This indicates that sub-irrigation treatment may have indirectly impacted hydraulic conductivity late season, resulting in yield differences despite the irrigation treatments no longer being implemented (Samani & Yitayew 2004). Additional information regarding forage yield is available in Appendix C.

4.3.3.2 Sward composition

To explore seasonal dynamics of the sward mixtures a PCA analysis was performed upon the mean DMY of treatments, as well as the botanical yield of component species (Figure 4.6). The first axis, which accounts for 52% of observed variation, depicts the inverse relationship between the high red clover yields and high bluegrass yields within mixtures. Analysis was structured in this way as the included forage species belong to two distinct functional groups with complementary uptake patterns (Scherer-Lorenzen *et al.* 2003). Pure mixtures yielded the greatest amount of bluegrass. Bluegrass yield remained constant over the growing season in pure swards. The greatest yield of bluegrass occurred during the second cut (Table 4.4; Figure 4.4).

Forbs appear in both natural and pure swards with the greatest proportion found in pure swards which seemed to be less competitive than clover pasture at deterring aggressive weed species (Table 4.4). Forb content was relatively constant and highest during cut four. During first cut white clover was present in natural pasture but largely disappeared following this as depicted in Figure 4.4. White clover was highest in monoculture bluegrass, concurrent with the invasion resistance – diversity relationship mentioned by Sanderson *et al*. (2007). White clover yields in pure pasture were relatively constant with a notable increase during cut three which was found to drop again for the remainder of the season. Natural pasture yielded more bluegrass than mixed pasture. Natural pasture contained a number of other grasses. Mixed pasture had high red clover yields. Red clover yields were variable over the season with the greatest red clover yield in the second and third harvest and lowest in the sixth harvest. Sward N yield was highly correlated with botanical yield in all mixture. These results seem in agreement with the composition of grass legume swards studied by McKenzie *et al.* (2004) and Kunelius *et al.* (2005).

The second axis, which accounts for 29% of observed variation, depicts the continuum between yields from renovated irrigated pasture and non-renovated natural pasture (Figure 4.4). Natural pasture exists on the low end of this continuum. Re-seeded pasture treatments exist on the high end of the continuum. Contrary to previous experiments in the literature, irrigation regime did not appear to induce large yield differences in mixed pasture swards - the values are quite similar between irrigated and non-irrigated mixed pasture (Hack Ten Broeke 2001; Pakrou & Dillon 2000). Irrigation effect was much more pronounced in pure swards. Irrigated pure sward yields were distinctively higher than non-irrigated pure sward DMY. The selected vectors accounted for 81% of the variation in yield data. The overall species composition of forage treatments over six harvests are presented in Table 4.4.

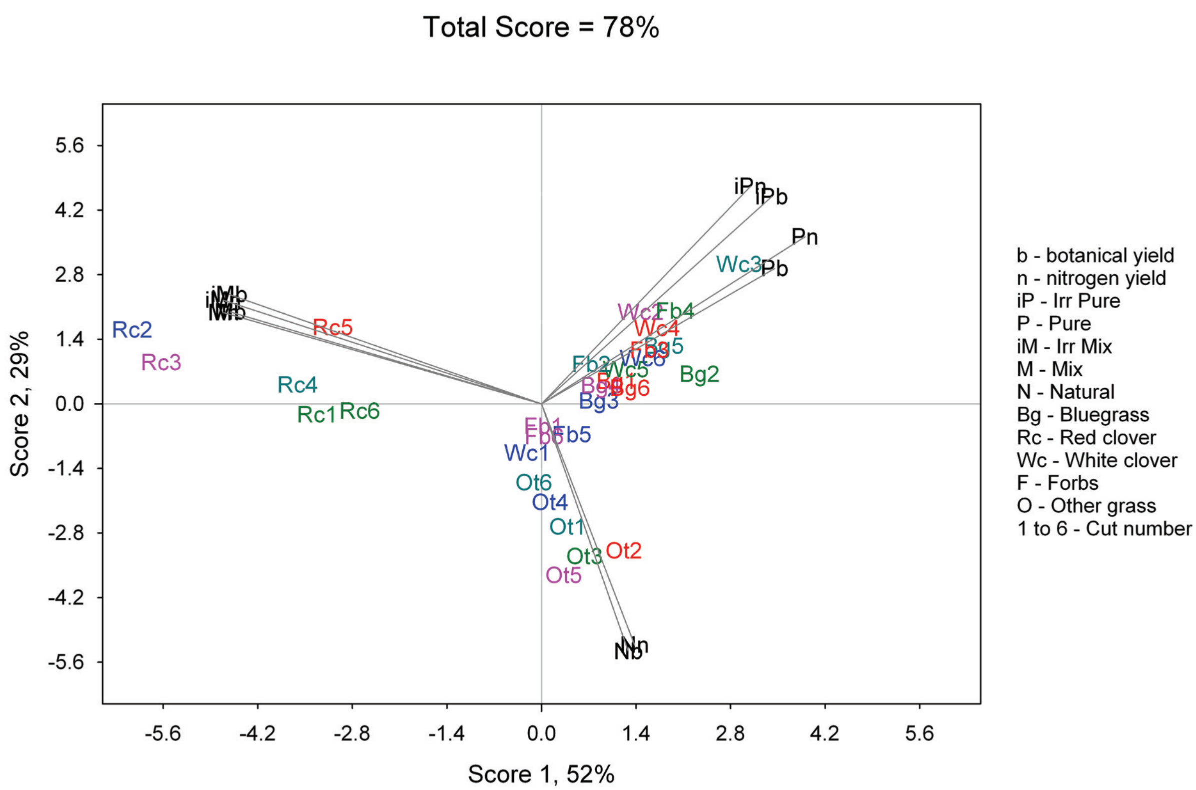

Figure 4. 4. Principal Components Analysis of sward yield and composition across six harvests.

Table 4.4 Percent species composition of forage treatments over six harvests, by mass, presented with average dry matter yield (g m^2) in Truro, N.S. 2009.

Harvest	Treatment	Bluegrass%	Red clover%	White clover%	Forb%	Other grass%	Dead%	Total (g)
1	Mix	4	82		6	6	2	99
1	Pure	49		8	24	15	4	34
1	Natural	2			9	89		118
2	Mix	5	69	7	15	4		233
2	Pure	39	3	21	28	9		153
2	Natural	15		3	16	66		143
3	Mix	3	82	4	9	3		171
3	Pure	17		37	39	7		112
3	Natural	3		5	11	82		204
4	Mix	6	74	5	11	1	3	188
4	Pure	21	1	32	40	5	1	123
4	Natural	12		8	9	72		90
5	Mix	5	85	5	3	1		124
5	Pure	29	20	30	16	5		66
5	Natural	4		10	9	77		109
6	Mix	8	80	7	3	1	1	80
6	Pure	38	2	39	15	6		50
6	Natural	42		9	6	43		73

4.3.3.3 Sward Nitrogen yield

During the growing season mixed red clover stands produced consistently greater sward N yields (SNY) compared to pure bluegrass ($P < 0.001$) in Table 4.5. Total sward N yields measured 350 kg N ha^{-1} yr^{-1} in mixed, 291 kg N ha^{-1} yr^{-1} in naturalized and 157 kg N ha^{-1} yr^{-1} in pure swards (Table 4.5 and Figure 4.5). These numbers corresponded well with the SNY's from Boller and Nosberger's (1987) establishing grass legume mixtures in Switzerland where mixtures containing white or red clover grown in combination with bluegrass yielded 200-350 kg total sward N ha^{-1} yr^{-1} during establishing years and 435 kg N ha^{-1} yr^{-1} on average during production years – five times the observed SNY from monoculture bluegrass. During establishment year two in this Nova Scotian field experiment legume pastures produced double the SNY of bluegrass monoculture.

Table 4.5 Sward N yield (kg N ha^{-1}) from three sward treatments in Truro, N.S.

Treatment	n	May	Jun	Jul	Aug	Sep	Oct	Season
Pure bluegrass	8	19.7	46.5	42.6	40.4	37.0	29.4	215.7
Red clover mixture	8	41.4	92.1	73.7	56.4	49.0	37.2	349.8
Natural	2	38.3	88.5	52.8	28.3	57.2	25.4	290.5
SEM		4.0	8.4	4.1	7.2	1.8	2.1	15.9
P-value[1]		0.012	0.010	0.000	0.016	0.002		0.000

[1] Probability values contrast mixed red clover versus pure bluegrass sward treatments.

Statistical analysis of forage N trends over time, which incorporated both sward and irrigation treatments, showed no significant difference between the SNY of pure and mixed sward treatments (Figure 4.5). Only the naturalized, un-drained, un-irrigated pasture differed statistically from the re-seeded drained pastures ($P = 0.017$). This discrepancy is postulated to be attributable to a natural “fertigation” effect induced by nutrients present in the recycled drainage water that the irrigated plots received (Papadopoulos & Gos 1996). More information on the nutrient status of recycled drainage water from this experiment is available in Appendix G. Also, there is some evidence that tillage may enhance N mineralization and subsequently NO_3 leaching – re-seeded plots were all tilled resulting in N release conditions that did not occur in naturalized pasture (Hansen & Djuhuus 1997).

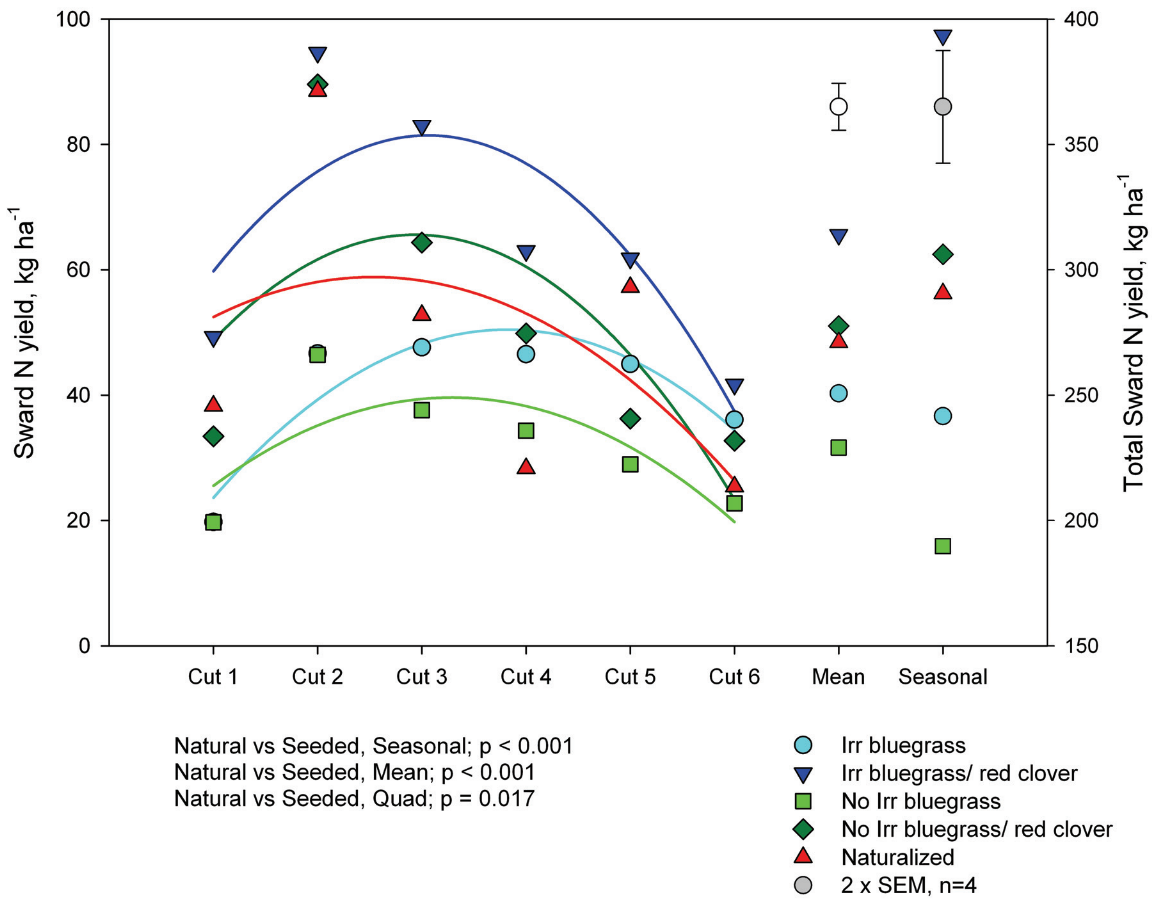

Figure 4. 5. Total sward N yield (kg ha^{-1}) by treatment in Truro, N.S.

It was previously postulated that red clover fixes atmospheric N and has the ability to transfer it to adjacent grass species (Ledgard 2001; Di & Cameron 2002; Scherer-Lorenzen *et al.* 2003). While it was not within the scope of this project to conduct a ^{15}N isotope study to confirm the transfer of fixed N as Scherer-Lorenzen *et al.* (2003) did, it is still useful to represent the N benefit grass receives when it is grown in mixture with red clover. This was accomplished by comparing the N% in bluegrass tissue grown in monoculture versus bluegrass grown in mixture with red clover (Figure 4.6). Bluegrass grown in combination with red clover was found to have significantly greater N% in its tissue compared to bluegrass grown in monoculture, on average ($P = 0.001$). This indicates that the bluegrass was indeed conferred some nutrient benefit by the presence of red clover. Bluegrass grown in all treatments continued to accumulate N in its tissue as the season progressed. This outcome is offered some support by the preliminary results of a ^{15}N study conducted in an adjacent pasture that utilized the same pasture species and cultivars. Thilakarathna *et al.* (2010) found that the percent of N in bluegrass that was derived from the atmosphere (presumably by red clover) accounted for more of the bluegrass' total N over time. Approximately 5, 15 and 25% of the total N contained in bluegrass tissue was derived from the atmosphere in Cuts 1, 2 and 3, respectively.

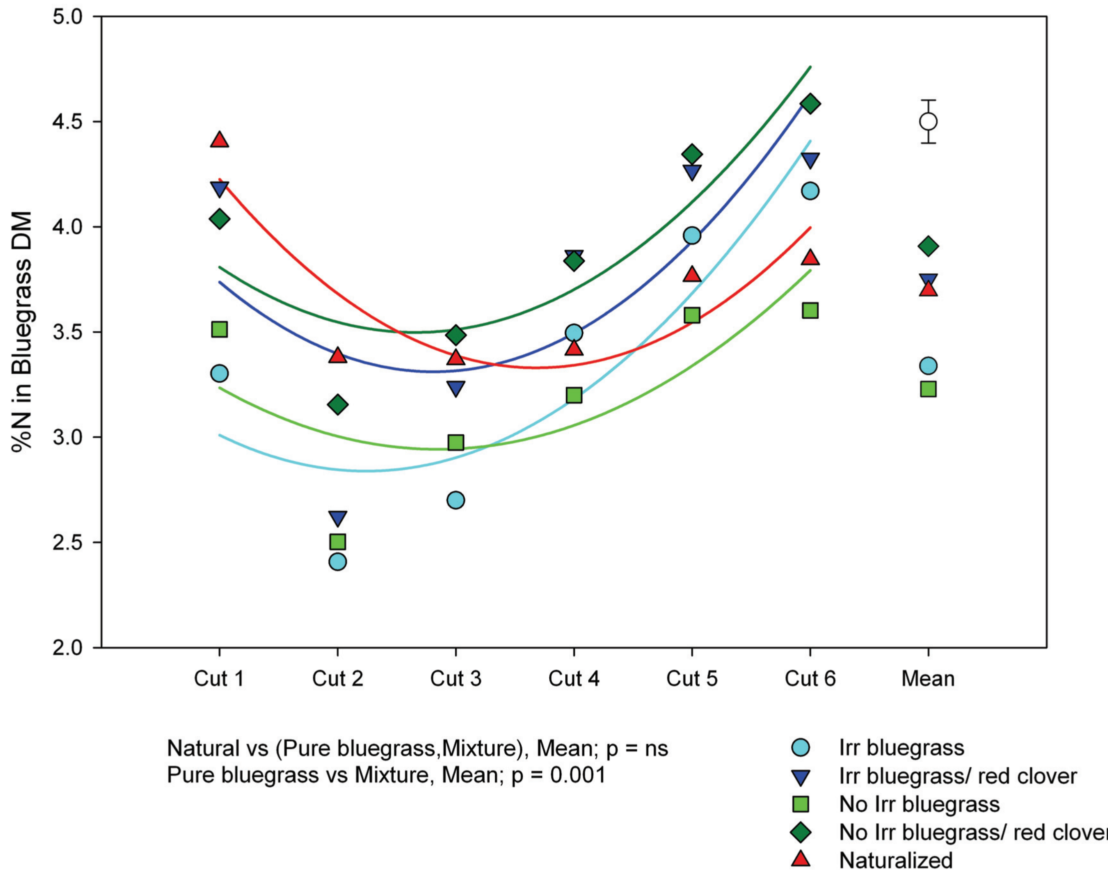

Figure 4. 6. Percent N, by mass, contained per gram of bluegrass tissue from each sward treatment in Truro, N.S.

4.3.4 Phosphate in soil solution and potential leaching losses

Phosphorus (P) is another element essential for plant growth, second only to N (Vance 2001). The results of PO_4^{3-} analysis on water samples were also included in the results section because P is commonly recognized as a limiting nutrient for red clover and BNF (Vance 2001; Government of Alberta 2002). Phosphorus does not have the same proclivity toward a gaseous phase as N, is more readily bound to soil particles and therefore is not as soluble in soil solution. However, for our purposes the availability of P is driven by similar (often microbial mediated) conditions as discussed in the N cycle such as pH, moisture, temperature, oxygen availability and agronomic management (Espinoza *et al.* 2005). A study by Hooper and Vitousek (1998) that found N-fixing species significantly reduced available P prompted this author to include the results of exploratory PO_4^{3-} analysis of soil solution in the lab.

The mean PO_4^{3-} concentration of soil solution extracted from the lysimeters was 0.20 mg L^{-1}. Values from <0.09 to 2.75 mg L^{-1} were measured in soil solution samples, the latter from natural pasture. The concentration of phosphorus acceptable, as defined by the Canadian Guidance Framework, in freshwater ecosystems varies on a continuum from 4 μg TP L^{-1} in ultra-oligotrophic sites and 100 μg TP L^{-1} for hyper-eutrophic sites (Environment Canada 2004). Phosphorus at moderate levels is not toxic to fish, livestock or humans but the introduction of phosphorus into nutrient limited aquatic ecosystems results in accelerated eutrophication (Beman *et al.* 2005; Diaz & Rosenberg 2008). Phosphate concentrations in the soil solution are generally much lower than NO_3^- even in heavily fertilized systems as they are more strongly adsorbed by soil particles, forming stable complexes with aluminum, iron and calcium (Bridgham *et al.* 2001).

Observed PO_4-P concentrations leaching from soil columns in a lab experiment by Jensen *et al.* (1999) ranged from 0.02 to 0.10 mg PO_4-P L^{-1}. Miller *et al.* (1994) conducted a lab trial exploring a similar research question and found that red clover as a cover crop leached a third less PO_4^{3-} compared to annual ryegrass. Observed concentrations leaching from three cover crops that included oilseed radish, annual ryegrass and red clover ranged from 2.0 – 15 mg PO_4-P L^{-1}. Phosphate concentrations observed in this experiment fall somewhere in between the results of these two experiments; greater than Jensen *et al.* (1999) findings and on the lower end of Miller *et al.* (1994) findings from oilseed radish.

At a 15 cm soil depth trends in PO_4^{3-} concentration were best modeled by a linear regression model that accounted for 56% of variance in the data (Figure 4.7). Similar to the results reported by Miller *et al.* (1994), concentrations of P in this experiment were lowest in the root zone of red clover pasture and highest in sward treatments dominated by grass species (Natural and Pure non-irrigated pasture). This supports the notion that red clover swards have greater P requirements than grass swards, and will remove more PO_4^{3-} from soil solution in the root zone. Statistical analysis showed that seasonal weather conditions interacted with sward treatments to induce changes in PO_4^{3-} concentration in the root zone ($P = 0.010$). Irrigation had no discernable influence on PO_4^{3-} availability. The concentration of PO_4^{3-} in the root zone increased over the course of the season concurrent with the outcome of Tischner's (1999) grassland study on a sandy soil in Germany. Tischner also postulated that transition from cultivation to fallow may have increased mobilization of previously accumulated P due to declining ionic strength brought about by the discontinuation of fertilization regime. This offers one explanation for why concentrations of PO_4^{3-} in the root zone of Natural pasture is eight times greater than the concentration found in the next closest Pure grass treatment.

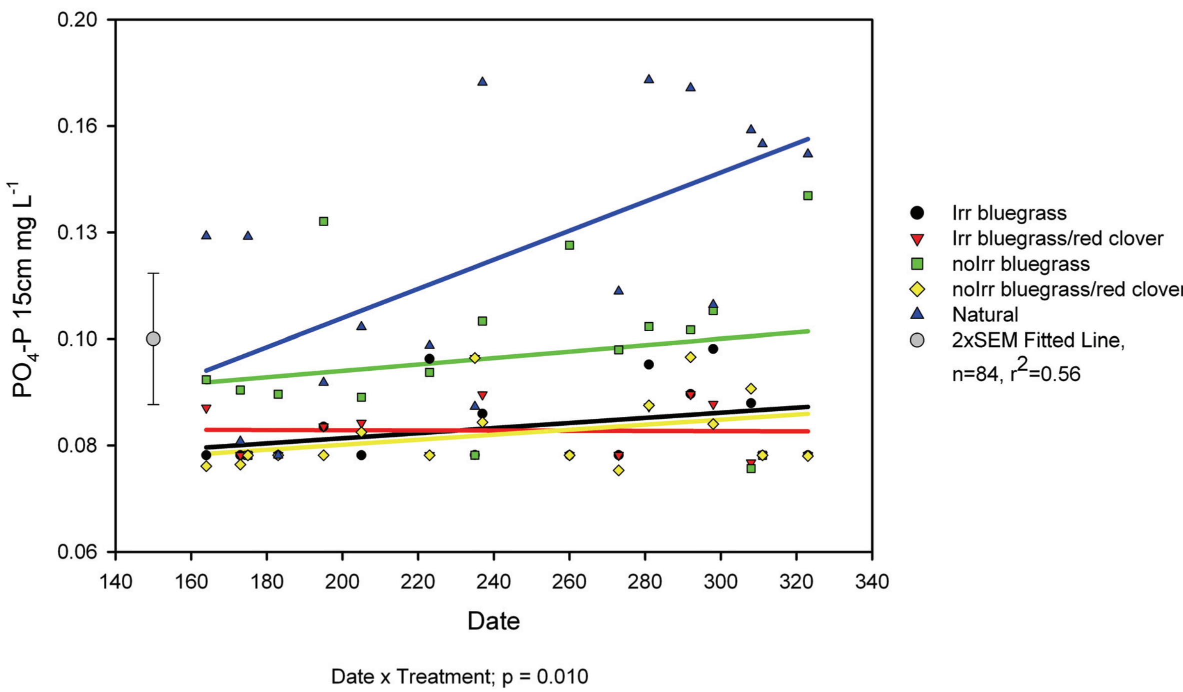

Figure 4. 7. Seasonal change in PO_4-P concentration of soil solution in Truro, N.S. at a 15 cm soil depth.

At a 45 cm soil depth PO_4^{3-} trends were best modeled by a linear regression line that accounted for 85% of the variance in the data (Figure 4.8). Contrary to the findings of Miller *et al.* (1994) and Toor *et al.* (2005) there was no observed difference in PO_4^{+} leaching between grass and clover, nor irrigation treatments. At 45 cm it emerges that the major difference is between renovated, re-seeded pasture versus un-renovated naturalized pasture. Concentrations of PO_4^{3-} were consistently low in all seeded treatments, hovering near the detection limit. Phosphate levels were 2.5 times greater in Natural pasture early in the season compared to seeded pasture. Statistical analysis showed that seasonal weather conditions interacted with sward management to induce changes in PO_4^{3-} leaching (P = 0.010). In contrast to Tischner (1999), PO_4^{3-} leaching declined over time in the current experiment. Tillage during the 2007 installation of drainage tiles in seeded pasture may account for the observed discrepancy between tilled, re-seeded and un-renovated natural pasture. As with N tillage is recognized as a mechanism influencing the mineralization of P in the soil (Hansen & Djurhuus 1997). Addiscott and Thomas (2000) indicated that tillage was also found to increase P losses. Saveedra *et al.* (2007) also found that no till pasture systems had greater concentrations of P than soils that had been tilled. The primary conclusion that can be drawn from these preliminary results is that soil disturbance has the ability to override the influence of vegetation when it comes to modulating PO_4^{3-} concentrations in pasture soils.

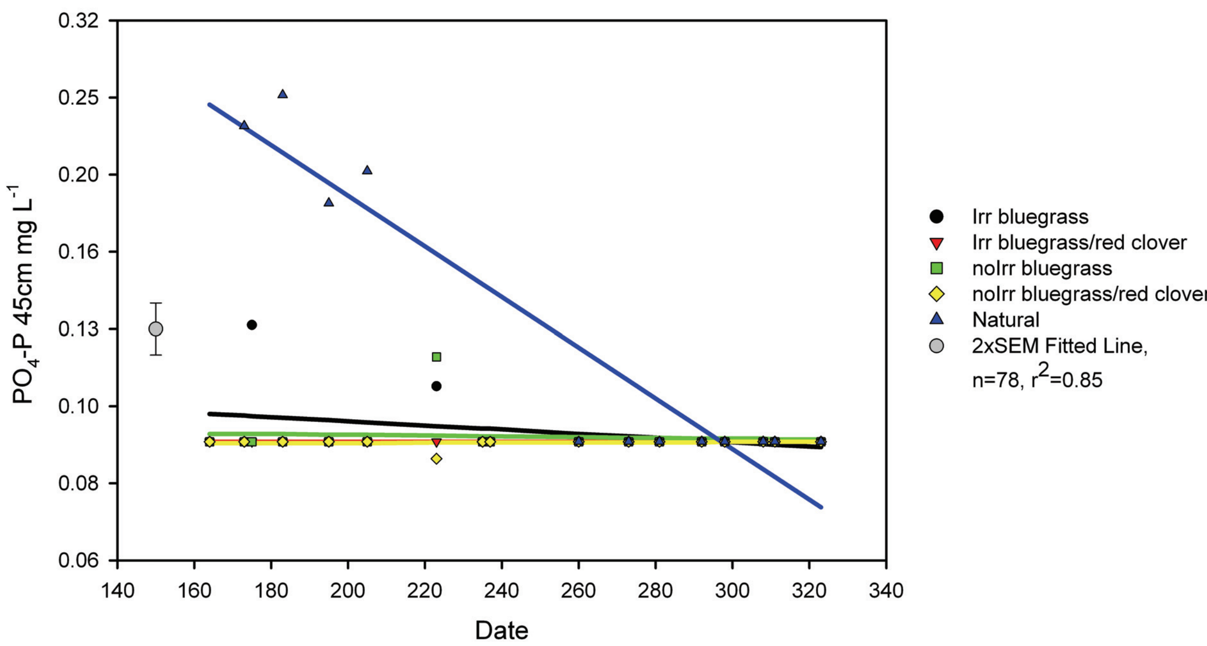

Figure 4. 8. Seasonal change in PO_4-P concentration of soil solution in Truro, N.S. at a 45 cm soil depth.

Overall concentrations of PO_4^{3-}were similar at 15 and 45 cm; however the relationship between sward management and PO_4^{3-} concentration was found to change with depth. No differences were observed in the seasonal PO_4^{3-}levels in seeded pure or mixed pasture. Natural pasture was found to contain greater concentrations of PO_4^{3-} than re-seeded pasture throughout the soil profile. However, the pattern of PO_4^{3-} availability in natural pasture was inverted from 15 to 45 cm in the soil profile. At 15 cm PO_4^{3-} is low early season and increases over the growing season in Natural pasture. At 45 cm PO_4^{3-} is highest early season and declines over the duration of the growing season.

4.4. CONCLUSIONS

The primary objective of this experiment was to determine the contribution of red clover plants to the NO_3 -N concentrations in soil solution. At 15 cm red clover showed 25 times higher concentrations of NO_3^- in the root zone relative to monoculture bluegrass. At 45 cm the concentration of NO_3^- leaching from mixed swards was 10 times greater than the concentration of NO_3^- leaching from pure bluegrass swards. The preliminary results of this one-year study support the hypothesis that the presence of red clover will increase NO_3^- availability within the root zone and subsequent leaching through deeper soil layers.

Secondly, seasonal irrigation was not found to have a significant influence upon NO_3^- leaching in this experiment. While there was a modest tendency for irrigated pasture to contain slightly greater NO_3^- levels in soil solution, the experimental results did not support the hypothesis that seasonal irrigation would potentially exacerbate NO_3^- leaching losses. Thirdly, a seasonal increase in NO_3^- leaching was observed in the spring and fall of this experiment. This confirms our hypothesis that leaching should show some seasonal variability due to weather driven N release events and asynchronous plant uptake. Although it was initially suspected that NO_3^- leaching would occur predominantly in the fall, spring conditions made soils equally vulnerable to NO_3^- losses.

Thirdly, when pasture yields from each forage treatment were assessed red clover mixtures were found to produce significantly greater dry matter and sward N yields than pure bluegrass treatments ($P = 0.005$ and $P < 0.001$). Irrigation regime was found to have a small but significant effect upon annual dry matter yields ($P = 0.02$). Forage yield results supported the experimental hypotheses that a) the presence of red clover would enhance overall sward growth through the introduction of biologically fixed N and b) irrigation should promote plant growth during dry weather conditions which would otherwise inhibit plant growth.

While not identified as an experimental objective, opportunity and scientific curiosity resulted in the analysis of PO_4^{3-} present in soil solution samples. When PO_4^{3-} dynamics in these pastures were examined it was found that PO_4^{3-} availability at 15 and 45 cm was largely influenced by both sward management and seasonal conditions ($P = 0.010$ and $P < 0.001$, respectively). Naturalized, un-tilled (for 10+ years) pasture was found to contain significantly greater concentrations of PO_4^{3-} at 15 and 45cm, compared to tilled, re-seeded bluegrass and red clover pasture. This difference is attributable to the soil disturbance experienced in 2007 when the tile drains were installed.

CHAPTER 5: ASSESSING NITRATE LEACHING IN BLUEGRASS AND RED CLOVER STANDS CONTAINING TWO DIVERSE RED CLOVER CULTIVARS

5.1 INTRODUCTION

The pursuit of ever improved agricultural production has been permitted by increased chemical fertilizer use and subsequently the concentration of NO_3^- in the environment has become elevated. In response to environmental concerns regarding NO_3^- contamination, research has shifted to target the increased incorporation of N-fixing legumes, such as red clover, into agro-ecosystems to promote more efficient utilization of N. Due to a unique symbiotic relationship with *Rhizobium* bacteria, legumes have the ability to transform atmospheric dinitrogen to plant available NO_3^- and NH_4^+ (Loiseau *et al.* 2001). Legumes such as red and white clover have the ability to fix upwards of 100 kg N ha^{-1} yr^{-1} and transfer this fixed N to nearby grass species, boosting overall sward function including biomass production and feed quality for grazing ruminants (Ledgard 2001; Kunelius *et al.* 2004; McKenzie *et al.* 2005; Sanderson *et al.* 2007).

Nitrate leaching losses from agroecosystems that rely solely upon biological N fixation are estimated to be much lower than from chemically fertilized pastures, ranging from 6 - 33 kg N ha^{-1} yr^{-1} (Ledgard 2001; Scherer-Lorezen *et al.* 2003). Conversely, NO_3^- leaching from well fertilized production systems can often exceed 150 kg N ha^{-1} yr^{-1} (Hooda *et al.* 1998; Di & Cameron 2002). Elevated NO_3^- levels in drinking water have been controversially linked to incidences of methanoglobinemia in infants and stomach cancer in adults (Addiscott 1996; Powlson *et al.* 2008). While uncertainty remains regarding the adverse impacts of NO_3^- contaminated drinking water upon health, it is becoming accepted in the scientific community that elevated nitrates are disrupting ecosystem function causing phytoplankton blooms, anoxic dead zones and marine acidification (Mills *et al*. 2000; Beman *et al.* 2005; Diaz & Rosenberg 2008).

Nitrate leaching shows some seasonal variability associated with certain months of the year. Soils appear to be least at risk in Atlantic Canada through the summer during which time plant N uptake leaves little N available to be leached. Mild temperatures, slowed plant growth, decreased evaporative losses and increased precipitation leaves the autumn season at high risk for NO_3^- leaching losses (Estavillo *et al*. 1996; Di & Cameron 2000; Scherer-Lorenzen *et al.* 2003). The risk of NO_3^- leaching in fall may be reduced in soils containing greater silt and clay molecules that drain more slowly and are capable of adsorbing more NO_3^- ions, relative to rapidly draining sandy soils (Gaines & Gaines 1994; Addiscott 1996).

The incorporation of red clover into pasture swards represents a more sustainable, alternative N source. The relationship between the *Rhizobium* bacteria and the red clover root is highly sensitive and studies indicate that nodulation, and henceforth fixation, is inhibited when there are high levels of N in the soil (Parsons *et al.* 1993). This feedback loop prevents red clover from producing N when it is not required, unlike chemical fertilizer that becomes continually released regardless of soil N levels. Due to the variability of the relationship between the host legume and the bacteria infecting the nodules, it is uncertain how differing cultivars and their ploidy levels, may impact symbiotic activity.

Ledgard *et al.* (1996) conducted an experiment that examined the productivity, N_2 fixation and transfer efficiency of nine white clover cultivars grown in a grass/clover mixture in N.Z., rotationally grazed by sheep. Researchers implemented two fertility regimes of 0 and 390 kg N ha^{-1} yr^{-1} applied as urea, and studied the effects of N fertilization upon the N_2 fixation capability of the nine cultivars. Experimental results revealed a high level of diversity among the tested cultivars. Productivity of clover cultivars was highly variable with Kopu and Pitau cultivars producing approximately 700 kg ha^{-1} yr^{-1} more clover than Aran and Blanca which were least productive. Total herbage production was less variable and Pitau mixtures remained most productive. Nesta was found to be the most effective companion cultivar in mixture - having the best transfer efficiency to grass. Kopu was the least effective companion cultivar at 0N. Kopu and Pitau fixed 152 and 159 Kg N ha^{-1} yr^{-1} at 0 N fertility and Sabeda fixed the least at 63 Kg N ha^{-1} yr^{-1}. Kopu clover best tolerated the increase to 390N fertility managing to fix 98 Kg N ha^{-1} yr^{-1}. Aran was still capable of fixing 64 Kg N ha^{-1} yr^{-1} but all other cultivars, including previously productive Pitau, N-fixation capacities deteriorated to under 43 Kg N ha^{-1} yr^{-1} at elevated levels of soil N.

Weir (1961) tested the response of a number of red clover cultivars to three diverse *Rhizobium* strains. The three *Rhizobium* strains resulted in differing nodulation success for different cultivars. Tetraploid cultivars were slower to germinate and nodulate. Early on the diploid cultivars had a greater number of nodules than tetraploid cultivars but this gap dissipated as the experiment drew on. The tetraploid cultivars generally had fewer nodules, but they were larger in size than the diploid plants' nodules. The tetraploid plants were also larger in overall habit. Thus, it appears that chromosome number may alter the clover plants response to *Rhizobium* bacteria, as tetraploid plant genotypes differed phenologically from their diploid counterparts.

Houngnandan *et al.* (2008) noted highly significant differences among seventeen soybean cultivars for nodulation, biomass production, N content and accumulation. The percentage and amount of N fixed from the atmosphere also differed significantly between cultivars. Boller and Nosberger (1994) observed significant differences in percent N_{dfa} and yield of red clover cultivars. Different clover cultivar-bacteria relationships also had a bearing upon plant competitiveness at elevated levels of N fertilization. Additional studies have been published that show the biomass yield and quality of different red clover cultivars may result in changes to rumen degradability, impacting the return of N to pasture by grazing ruminants (Broderick *et al.* 2004; Loiseau *et al.* 2001). The aforementioned experiments suggest that a high degree of genotypic variability may exist between different leguminous cultivars. If there are phenological and N fixation differences between red clover cultivars of different genetic background it stands to reason that concentrations of NO_3^- in the root zone, and leaching beyond the root zone may differ as well.

The objectives of this study were to (i) ascertain the contribution of two red clover cultivars (one diploid; one tetraploid) to NO_3^- leaching when grown in mixture with bluegrass both within the root zone (15 cm) and beyond the root zone (45 cm); (ii) monitor seasonal NO_3^- leaching patterns; and (iii) assess herbage yields from pure bluegrass, bluegrass/diploid red clover and bluegrass/ tetraploid red clover swards.

5.2 MATERIALS AND METHODS

5.2.1 Experimental Site Description: Nappan, NS

The research site was an experimental pasture located 19.8 m above sea level at the Nappan Research Farm, AAFC, in Nappan, Nova Scotia (45°45.60N, 64°14.40W). The native soil was a coarse loamy till (<18% clay) that Webb & Langille (1995) describe as a well to moderately well drained soil of the "Pugwash Association", 50 – 80 cm deep with a 2-5% slope. The Nappan site receives cool, humid climate conditions with an average daily temperature of 5.8°C and annual precipitation of 1175 mm (averages calculated from 1971-2000; Environment Canada

2010). Meteorological conditions were monitored via Environment Canada's weather monitoring station on site at the Nappan Research Station, AAFC.

Three experimental treatments were seeded in a one-way randomized block design with three replicates. The three treatments were: a) pure, unfertilized bluegrass control, b) bluegrass-red clover (AC Christie; diploid) and c) bluegrass-red clover (Tempus; tetraploid). The experimental plots were seeded in June 2008. The utilized bluegrass cultivar, "Ginger" was seeded into all treatments at a rate of 14 kg ha^{-1}. Each of the red clover cultivars, "AC Christie" and "Tempus", were inoculated with commercially purchased peat-based rhizobium and applied at a rate of 8 kg ha^{-1}. No chemical fertilizer was applied to any treatment, at any point in this experiment. Plots were sampled and clipped when the average sward height reached 30 cm. Grazing was simulated in this experiment. Forage clippings (and the N contained within them) were removed and not returned to pasture.

Each experimental unit measured 1.5 x 6 m. The entire experiment covered a total area of approximately 100 m^2. The smaller dimensions of this site allow for a more controlled, intensively sampled plot experiment that sought to estimate the contribution of biologically fixed N by two red clover cultivars. In order to estimate the NO_3^- present in the soil percolate four ceramic suction lysimeters of two depths were installed in each experimental unit amounting to an experiment total of 36 lysimeters. The suction lysimeters were installed in the Nappan experimental site October 25, 2008 according to the methods described in Section 4.2.1. Soil solution was extracted from the root zone at a depth of 15cm. These shallow lysimeters account for two of the four lysimeters in each experimental unit. Accounting for the other two lysimeter sampling points in each experimental unit, soil solution was extracted from a depth of 45cm to estimate soil NO_3^- leaching losses from the root zone and beyond. A more detailed description of the design and construction of the utilized suction lysimeters is found in Section 4.2.1.

5.2.2 Data Collection

5.2.2.1 Soil

Soil cores were collected in Nappan on September 29, 2009 using the same process as described in Section 4.2.3.1. The soil characteristics from this site are summarized in Appendix B.

5.2.2.2 Soil solution

Soil solution was sampled from ceramic suction lysimeters on an event based schedule or bi-weekly, when possible, in Nappan. The soil solution in Nappan was continuously monitored from June 8 to October 30, 2009. Lysimeters were sampled according to the protocol depicted in Section 4.2.3.2.

5.2.2.3 Forage

In Nappan the sward reached the 30 cm threshold sward height and was first sampled and harvested June 8, 2009. There were a total of four forage harvests in Nappan from June – September (Table 5.1). Instead of the forage sampling protocol illustrated in Section 4.2.3.3 each 1.5 x 6 m plot was individually harvested using a Haldrup 1500 series harvester which measured the whole plot weight in kilograms.

Table 5. 1. Sampling dates for NO_3 testing on drainage water, sward clipping and soil core collection for Nappan experimental site in 2009.

	Soil solution extraction			Sward clipped	Soil core
Month	MM/DD			MM/DD	MM/DD
June	06/08	06/22	07/03	06/08	10/29
July	07/14	07/21	07/31	07/14	
August	08/14	08/30		08/14	
September	09/29	10/08		09/23	
October	10/20	10/30			

5.2.3 Analytical methods

5.2.3.1 Soil

Soil core samples collected from Nappan on September 29, 2009 were analyzed for pH, OM, CEC P, K, Ca, Mg, Na, S, Fe, Mn, Cu, Zn, and B at Nova Scotia Agriculture Laboratory Services, Harlow Institute, Truro, N.S according to the methods described in section 4.2.4.1. The remaining soil sample was dried, ground to a fine dust and analyzed for N via the combustion method indicated by Yeomans *et al*. (1991) on a LECO FP-528 at the Department of Plant and Animal Sciences, NSAC.

5.2.3.2 Soil solution

Soil percolate samples collected from the Nappan Red clover experiment were analyzed for NO_3 and PO_4^{3-} at the Water Quality Research Laboratory, NSAC according to the methodology described in Section 4.2.4.2.

5.2.3.3 Forage

Botanical matter from the Nappan Red clover experiment was analyzed for protein N, ADF and NDF at the Department of Plant and Animal Sciences Laboratory, NSAC according to the procedures describe in Section 4.2.4.3.

5.2.4 Statistical methods

The GenStat® software package (Payne 2002) was used to perform the repeated measures analysis of variance tests (RM-ANOVA) and to calculate the Standard Error of the Mean for comparison of means at a probability level of $P < 0.05$. Data from the experiment was analyzed in a split-split-plot design with sward type as the main plot, cultivar type as the sub-plot. Graphs were generated in MS Excel and SigmaPlot 10.0. Analysis of variance tests were conducted on: sward dry matter yield, sward N yield, NO_3^- present in soil solution at 10 and 45 cm, and PO_4^{3-} present in soil solution at 10 and 45 cm. Histograms were constructed and large residuals identified by the Genstat software were manually removed to satisfy the normal distribution and constant variance assumptions of the ANOVA model. The NO_3^- and PO_4^{3-} data sets were log-transformed to achieve normality. Nitrate and phosphate outputs were back-transformed in the results section.

Sward N yields were calculated by multiplying the dry matter yield of each species by the tissue N concentration for each species, and then expressed as a total representing all species N yields for each experimental unit. Differences between mean DMY of sward treatments were tested using RM-ANOVA. Principal Component Analysis (PCA) was utilized to depict vegetative dynamics within the various sward treatments over four harvests. The Repeated Measure in this analysis was sample date, for which the Julian Day is used on the x-axis. The test for significance of variability was determined using the F-test ($P < 0.05$). The analysis for fitting mathematical curves to the concentration of NO_3^- and PO_4^{3-} was conducted using quadratic multiple regression for NO_3^- and PO_4^{3-} data. For the both data sets, a linear modeling equation was tested but the second order polynomial equation yielded improved r^2 values.

Log transformed NO_3^- and PO_4^{3-} values were regressed against sample date collection. Fitted values for each equation were expressed in (mg L^{-1}) and plotted with the observed values (mg L^{-1}) to determine degree of fit (Vittinghoff *et al.* 2005). An "additive model regression" was used along with "smoothing splines". An additive model regression first creates a line of fit for the entire data set, second a fit is created for treatment, and thirdly a fit is created for interactions. Smoothing splines are used because they are flexible enough to link sudden changes in slope. Splines achieve this by possessing inherent constraints to ensure smoothness (Hand & Taylor 1987; Digby *et al.* 1989).

5.3 RESULTS AND DISCUSSION

5.3.1 Meteorological conditions

The average daily air temperatures measured for Nappan, NS during the study period did not frequently deviate from climate normal air temperatures (Figure 5.1). The month of October was colder than climate normals. Average monthly temperatures recorded during the growing season of 2009 for Nappan were 15.4°C (June), 17.5°C (July), 18.6°C (August), 13.0°C (September) and 5.2°C (October). During the five month lysimeter sampling period from June until October 2009 the Nappan site received an estimated 521 mm of precipitation, 52% of that precipitation fell in July and August which experienced above average rainfall levels compared to climate averages. Rainfall was slightly less in September and was average in June and October.

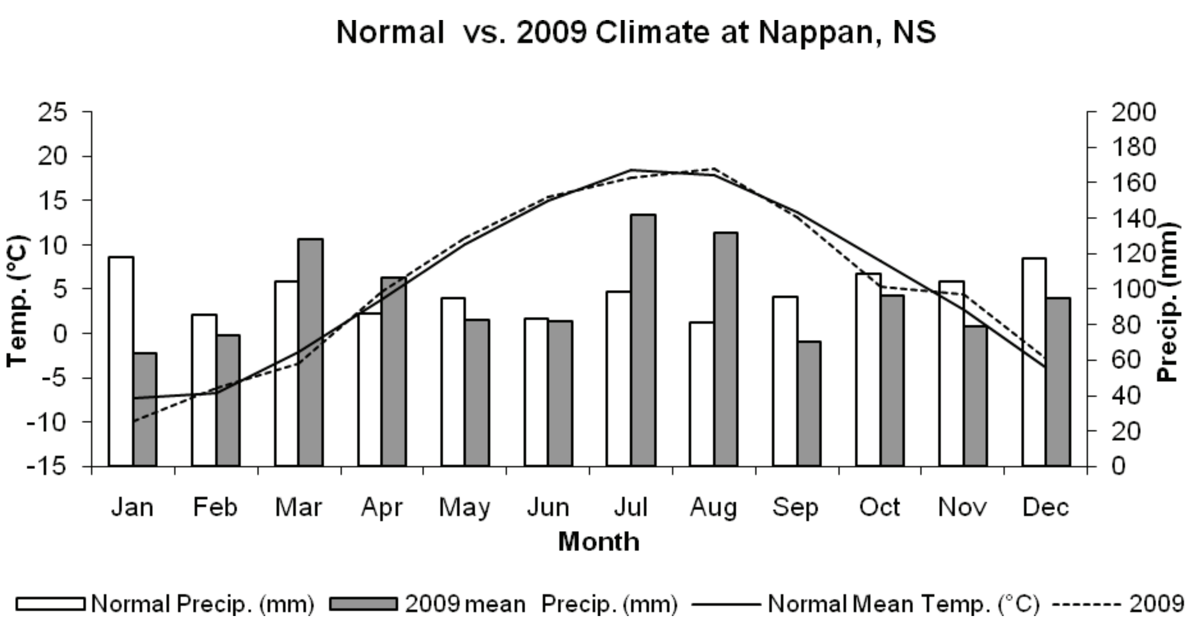

Figure 5. 1. Weather conditions in Nappan, NS in 2009 compared to average Climate conditions 1971-2000 (Data obtained from Environment Canada:http://www.climate.weatheroffice.gc.ca/).

5.3.2 Nitrate in Soil Solution

There were 419 soil solution samples collected from the Nappan experimental site in 2009. The mean NO_3^- concentration of soil solution extracted from all lysimeters was 0.88 mg L^{-1} and ranged from <0.08 to 13.32 mg L^{-1}. Nitrate concentrations in soil solution were much lower in Nappan relative to levels detected in the grazed pasture experiment in Truro (Appendix H). In this study, only 1% of collected samples exceeded the 10 mg L^{-1} of NO_3-N MAC Limit for NO_3 in drinking water, all of which were collected from mixed pasture early in the growing season. The maximum concentration of NO_3^- recorded in pure pasture was 4.15 mg L^{-1} on the first sampling date June 8, 2009.

Nitrate concentrations in soil solution were lower but similar to the levels recorded by Bouman (2008) from a grazed experimental pasture under organic management. Dissimilar from the results of the Truro experiment there was no fall increase in NO_3^- leaching in Nappan. Similar to the outcomes of a Pennsylvanian study by Jabro *et al.* (1997) this evidence suggests that leaching may be just as likely to occur throughout the grazing season depending upon site characteristics and sward management.

5.3.2.1 Nitrate in the root zone

At a 15 cm soil depth NO_3^- trends over time were best modeled by a quadratic convex regression spline that accounted for 67% of variance in the data (Figure 5.2). Nitrate concentrations in the root zone were low with NO_3^- levels far from approaching the 10 mg L^{-1} of NO_3-N MAC Limit for NO_3^- in drinking water. Over the growing season NO_3^- levels were greatest for the Tempus mixture, correspondingly high in Christie mixed pasture and low in pure bluegrass monoculture. The results of RM-ANOVA contrasts for each sampling date by treatment at 15 cm are available in Appendix H. There was a significant interaction between season and sward treatments in the root zone (P =0.005), indicating that the main effect sward type had on concentration of NO_3^- in the root zone was modified by seasonal conditions. By the end of the growing season it was apparent that the presence of red clover increased NO_3^- in the root zone to significantly greater levels than pure bluegrass – up to 16 times (Figure 5.2). This confirms our initial hypothesis that red clover plants will contribute NO_3^- into the soil solution.

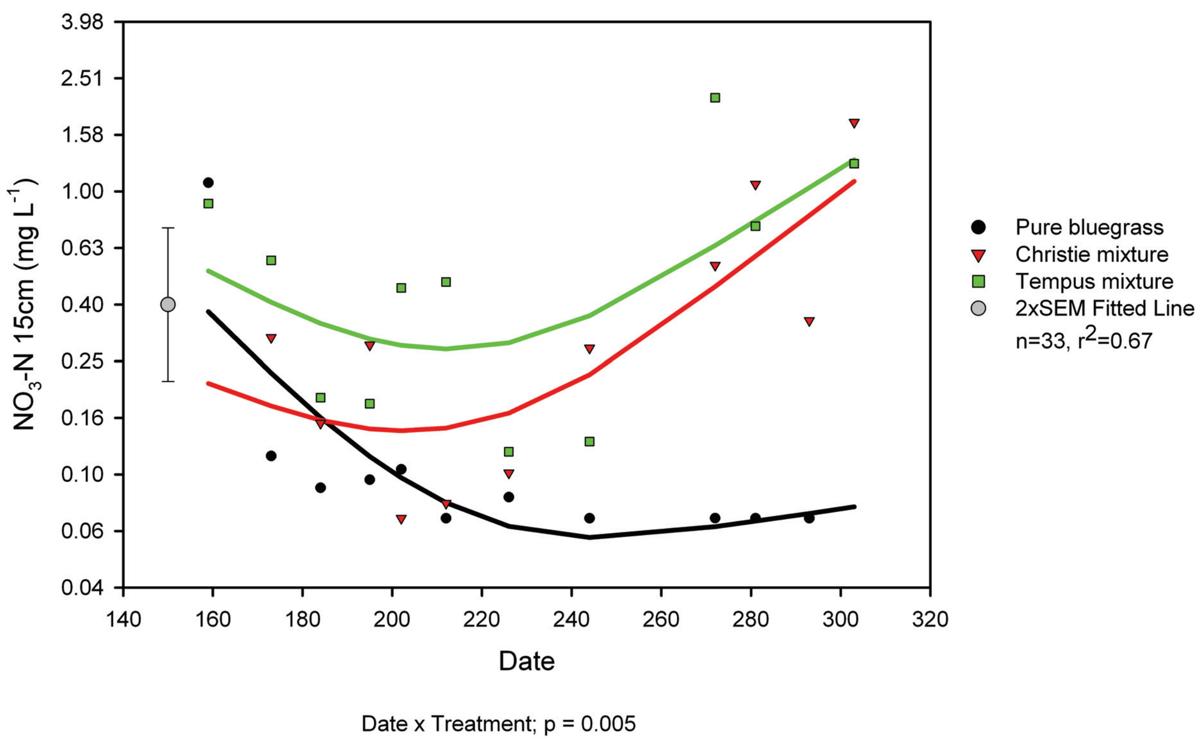

Figure 5. 2. Seasonal change in NO_3-N concentration of the soil solution in Nappan, N.S. at a 15 cm depth.

Nitrate concentrations in the soil solution of the root zone were quite similar for tested Christie and Tempus red clover cultivars. There was no evidence to suggest that the genetic differences of red clover cultivars utilized in this study will have a significant bearing upon the amount of NO_3^- red clover contributes to soil solution. Ledgard *et al.* 1996 notes that among tested white clover cultivars N-fixation and transfer abilities were largely influenced by the interaction and competition of the clover cultivar with companion grass in pasture. Physiologically diverse clover cultivars were the most likely to interact differently with the companion grass. In that particular experiment the more prostrate white clover cultivars behaved quite differently than broad leaved, upright cultivars such as Kopu, Aran and Pitau – which were most productive. The red clover cultivars utilized in this experiment, Christie and Tempus, are physically indistinguishable in the field and likely interact similarly with companion bluegrass. A phenotypic difference between these two cultivars seems most likely to manifest through unique and varying phenologies. While there was a small difference in slope observed in Figure 5.2, postulated phenotypic differences in nodulation and N-fixation were insufficient to induce significant changes in soil NO_3^- in this field experiment.

Nitrate concentrations in the root zone of all sward treatments were similar and moderately low in June. Curiously, the concentration of NO_3^- in the root zone of pure swards was intermediate early season with Tempus stands being greater and Christie stands lesser. It does not appear that red clover plants are actively introducing fixed N into soil solution at this time, and if so at levels that are taken up quickly and unavailable to percolate throughout the soil. These results are concurrent with Wu and McGechan's (1998) statement that early season growth by grass and red clover will rely upon soil N until conditions become favorable to nodulation and subsequent biological N fixation.

Nitrate levels decrease in the root zone of mixed swards in July and decline to a much greater extent in pure swards. In the Truro experiment this summer depression was partly attributed to the lack of precipitation. However, precipitation in July 2009 in Nappan exceeded climate normals. Nitrate levels were likely depleted by plant uptake in the rapidly growing sward, henceforth the observed concentrations of NO_3^- must not have been because there was no vehicle for NO_3 transport, as hydrological conditions were in fact conducive to N leaching.

Addiscott (1996) indicated that fast growing swards have the capacity to take up to 5 kg N d^{-1}. The experimental swards show robust yields at this point in the season indicating that the swards are making apt use of available N (Table 5.3). Following the observed mid-season decline, NO_3^- levels in pure bluegrass swards remain very low into the fall indicating that little to no NO_3^- is available to travel in the soil solution of the root zone. This seasonal decline is consistent with the notion that the finite supply of soil available N is being used up by the growth of grasses and not being replaced. An entirely different process was observed in the root zone of mixed pasture however, as NO_3^- levels continue to increase into the fall. These results are similar to the findings of Chen *et al.* (2001) who noted that the presence of leguminous plants in pasture was found to result in the seasonal accumulation of N in the root zone.

5.3.2.2. Nitrate leaching

At a 45 cm soil depth, NO_3^- trends over time were best modeled by a quadratic convex regression spline that accounted for 39% of variance in the data (Figure 5.3). It is unknown why variability increased at 45 cm. Nitrate leaching losses were low with NO_3^- concentrations only approaching the 10 mg L^{-1} MAC limit for NO_3 in drinking water. Over the growing season NO_3^- leaching was greatest in Tempus mixture, similar in Christie mixture and low in pure bluegrass monoculture. Overall NO_3^- losses were low in this unfertilized field experiment. The results of RM-ANOVA contrasts for each sampling date by treatment at 45 cm are available in Appendix H. Notably, there was no statistical interaction between season and sward treatment at 45 cm, indicating that seasonal conditions did not alter how sward treatments influenced NO_3^- concentrations over time to the same degree as was observed in Truro or in Nappan at 15 cm. This may be attributable to a more aggregated soil structure that acts to isolate deeper soil layers leaving them less influenced by the ephemeral environmental changes (Gaines & Gaines 1994; Wachendorf *et al.* 2004; Conrad & Föhrer 2007).

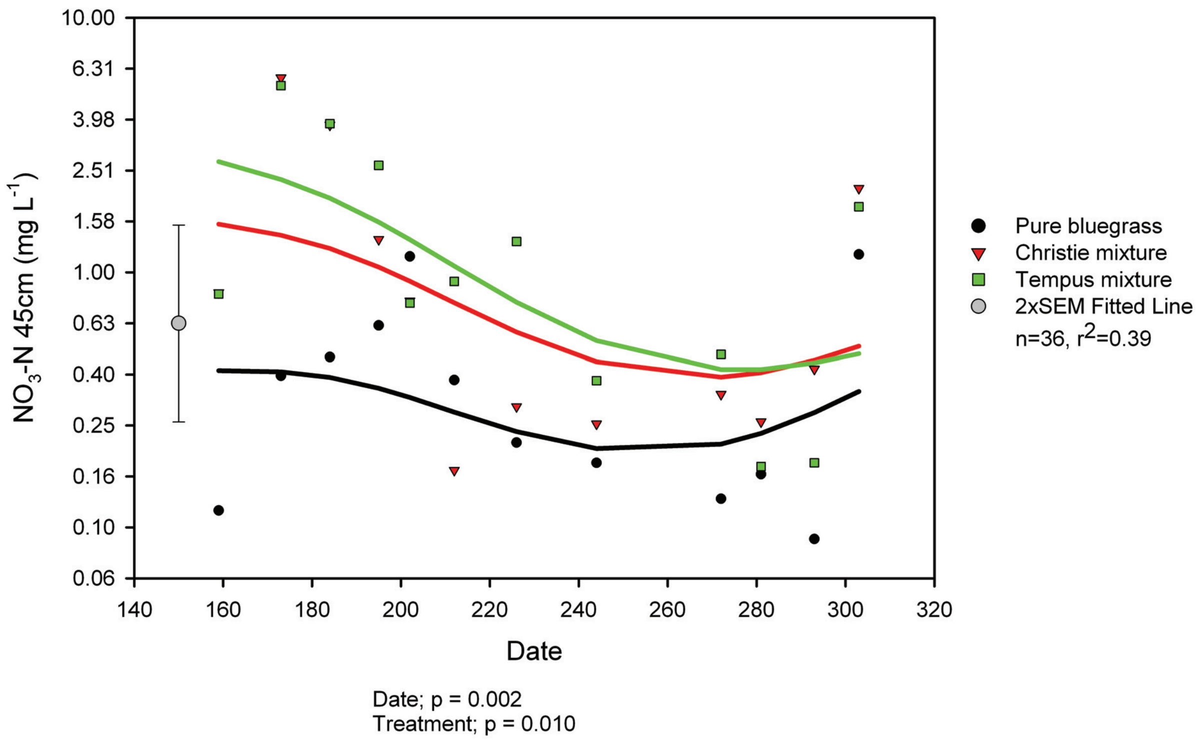

Figure 5. 3. Seasonal change in NO_3-N concentration of the soil solution in Nappan, N.S. at a 45 cm depth.

Sward treatments were found to have a significant influence upon NO_3^- leaching (P = 0.010), more clearly supporting our hypothesis that NO_3^- leaching from red clover-bluegrass swards will be greater than from pure bluegrass as a result of the contribution of N from BNF (Figure 5.3). However, there was no evidence to suggest that the differences among the two cultivars utilized in this study will have a significant bearing on NO_3^- leaching as NO_3^- concentrations in the soil solution at 45 cm were quite similar for the two red clover cultivars. Concomitant with other field leaching trials from Estavillo *et al.* (1996) and Bouman (2008), this study found that seasonal conditions significantly modified NO_3^- leaching losses (P = 0.002). This offers positive evidence endorsing the hypothesis that seasonal conditions will play a role in shaping NO_3^- leaching patterns.

Incongruent with literature indicating that the risk of NO_3^- leaching will be greatest in temperate pastures during the fall, the greatest loss of NO_3^- in this experiment took place early in the growing season - June and July (Bergstrom & Brink 1985; Addiscot 1996; Di & Cameron 2002). Early in the season the concentration of NO_3^- in red clover-bluegrass mixed swards was up to five times higher than the monoculture bluegrass plot (Figure 5.3). The concentration of NO_3^- in leguminous stands declined to half the levels observed early season. Despite this decrease leguminous stands still maintained elevated NO_3^- levels late season relative to pure swards. The seasonal distribution of NO_3^- leaching in Nappan was more concurrent with the findings of Jabro *et al*. (1997) and Bouman (2008) which indicated that, dependent on prevailing weather conditions, an increase in NO_3^- leaching can also occur earlier in the growing season.

Mean concentrations of NO_3^- in the soil percolate leaching from each treatment were 0.33, 1.25 and 1.34 mg L^{-1} for Pure, Christie and Tempus swards (Appendix D). Average concentrations of NO_3^- leaching from mixed pasture were lower than expected but still fit within the ranges reported by Hooda *et al*. (1998) from grazed, unfertilized grass-alfalfa swards and Owens *et al.* (1994) from a grazed rye grass-white clover pasture with two applications of cattle slurry. The NO_3^-concentrations from the field experiment in Nappan were likely lower from these studies (as well as the Truro experiment) because there was no import or enhanced recycling of N through the excreta of grazing ruminants (Loiseau *et al.* 2001). Wachendorf *et al.* (2004) concluded that the presence of grazing ruminants significantly impact N cycling and henceforth the amount of N leaching through the soil. Of course there are soil and climate differences between the two sites in this study, but it seems likely that grazing Holstein cattle played a role in augmenting the cycling of N in the Truro agroecosystem relative to the un-grazed cultivated field experiment in Nappan.

It was not possible to conclusively volume weight NO_3^- leaching losses as there were not sufficient soil hydrological measurements recorded from the Nappan site to determine when the soil had reached field capacity. However, for the purposes of comparison field capacity was roughly estimated and rainfall amounts greater than 10 mm were multiplied by the concentration of NO_3^- leaching through the soil solute. These volume weighted values of NO_3-N leaching losses for the five months of this experiment showed that pure swards leached 0.8 kg N ha^{-1} yr^{-1}, Christie 0.9 kg N ha^{-1} yr^{-1} and Tempus 1.7kg N ha^{-1} yr^{-1}. Nitrate leaching from all swards was extremely conservative relative to the concentrations of NO_3^- found leaching from fertilized pastures (Scholefield *et al.* 1993; Bjorneberg *et al.* 1995; Cuttle & Scholefield 1996; Shepherd & Lord 1996).

Observed NO_3^- leaching in Nappan was much lower than reported losses from a similar unfertilized, red clover-grass pasture studied by Scherer-Lorenzen *et al.* (2003) and losses described by Ledgard (2001) from grazed unfertilized grass-legume pastures (Figure 5.3). Nitrate leaching losses fell within the lowest end of the range of values described to be leaching from grazed ryegrass-white clover pastures examined by Loiseau *et al.* (2001) and Eriksen *et al.* (2004). Some of the more conservative estimates of NO_3^- leaching losses from Ruz Jerez *et al.* (1995) and Di & Cameron (2002) are similar to the NO_3^- leaching values observed in Nappan. These studies and the Truro experiment all were conducted on sandy loam soils whereas the Nappan site has a soil type described as a coarse loamy till (Webb & Langille 1995). In the literature review the impact of soil type on NO_3^- leaching was addressed and it was noted that NO_3^- concentrations can vary up to five-fold depending upon soil characteristics.

Gaines & Gaines (1994), Wachendorf *et al.* (2004) and Conrad & Föhrer (2007) indicate that the decreased water retention of sandy soils can often exacerbate seasonal precipitation surpluses because a smaller volume of water is required to displace soil solution, and any dissolved NO_3^-, beyond the root zone. Conversely, in aggregated soils clay molecules possess greater capacity to adsorb negatively charged ions, thus immobilizing some of the NO_3^- ions in solution as it passes by. Additionally, the dense structure of an aggregated soil impedes the flow of water through the soil matrix so NO_3^- is not transported as deeply or as rapidly as what is observed in sandy soil (Gaines & Gaines 1994). The conclusion to be drawn from this discussion of soil type is that the aggregated soils in the Nappan experiment, in concert with differing weather conditions, may have had some bearing upon observed NO_3^- leaching, resulting in more conservative amounts of NO_3^- to be found leaching to 45 cm.

5.3.2.3 Nitrate dynamics at two depths

Distinctive NO_3^- leaching patterns are found to emerge when one is to examine the nutrient profile at multiple depths through the soil profile. Nitrate was found in slightly higher concentrations at 45 cm relative to 15 cm measurements. This seems to indicate that much of the N made available in the root zone by red clover was quickly utilized or immobilized and thus was not available to enter the soil solution. Different soil processes appear to be in play at 45 cm, whether it was through differing aggregate distribution or differing sorption capacity. Nitrate concentration in the soil solution of pure swards was always quite low, with the exception of a modest early season increase in the root zone.

The pure sward system was representative of an N limited system where insufficient concentrations of N were leftover to percolate in soil solution - as it was likely removed and fully utilized by N hungry bluegrass. Nitrate availability in the root zone of Christie and Tempus swards was found to increase as the season progressed. However, the inverse was true at 45 cm as NO_3^- leaching decreased as the season progressed. The transformation in NO_3^- availability and dynamics from 15 to 45 cm speaks to the role that the plant community and soil characteristics play (in the context of ever changing weather conditions) in shaping NO_3^- leaching patterns from temperate pasture stands, supporting our supposition and Tilman *et al.* (1996) and Scherer-Lorenzen *et al.*'s (2003) postulations that the characteristics of the plant community influence NO_3^- leaching in pasture.

5.3.3 Forage

5.3.3.1 Dry Matter Yield

In establishment year two of the field experiment in Nappan sward treatment was found to have a significant impact upon DMY production during three of the four cuts (Table 5.3). Sward treatment was also found to significantly influence seasonal total DMY (P = 0.004). Christie and Tempus mixed swards containing red clover produced greater DMY than pure swards. Pure bluegrass swards were the least productive. There was no evidence of any difference in DMY between the two red clover cultivar mixtures. Dry matter yields were similar in this experiment compared to the yields observed in grass mixtures in other Atlantic field experiments (Kunelius *et al.* 2005; McKenzie *et al.* 2005). In the Kunelius *et al.* (2005) study a mixture comprised of timothy, bluegrass and red clover had an average annual yield of 8.85 t ha^{-1} over three years. The bluegrass red clover mixtures in this one year experiment fell just short of Truro experiment and Kunelius' (2005) study yields - with Tempus and Christie stands producing 7.43 and 8.56 t ha^{-1}, respectively. Further information regarding forage quality information is available in Appendix D.

Table 5.2 Dry matter yield (t ha^{-1}) from sward treatments in Nappan, N.S.

Treatment	n	June	Jul	Aug	Sept	Season
Pure bluegrass	6	1.14	0.67	1.05	0.83	3.68
Christie mixture	3	2.54	1.45	2.95	1.62	8.56
Tempus mixture	3	2.34	1.15	2.43	1.52	7.43
SEM		0.33	0.25	0.40	0.35	1.12
P-value[1]		0.003	0.030	0.003	0.064	0.004

[1] Probability value compares Tempus and Christie to the Pure bluegrass treatment.

When the seasonal tendencies of dry matter production in Nappan were analyzed over time, mixed swards containing Christie and Tempus red clover were found to produce significantly greater yields compared to monoculture bluegrass (P = 0.004). This result supports information provided by Kunelius *et al.* (2005) showing that grasses grown in mixture with legumes (such as red clover) will increase dry matter yield over either component species grown in monoculture (Table 5.3). Yields over the season remained consistent and low in the pure treatment. Dry matter yields from red clover stands tended to decline late in the growing season. This is potentially attributable to the red clover canopy reaching maximum possible light interception. Red clover plants may have invested readily in developing their canopy early summer to out-compete other species for light resources. In this endeavor they were observed to be quite successful. Christie produced greater DMY than Tempus swards but no significant difference was observed between these two treatments, despite some foreshadowing from the literature indicating that different red clover cultivars may exhibit different phenologies (Boller and Nosberger 1994; Houngnandan *et al.* 2008). Additional information on forage yield is available in Appendix C.

5.3.3.2 Sward composition

To explore seasonal dynamics of the sward mixtures in Nappan a PCA analysis was performed upon the mean DMY of treatments, as well as the botanical yields of component species (Figure 5.4). The first axis, which accounts for 69% of observed variation, depicts the continuum between swards that contain a large proportion of bluegrass and conversely swards containing a large proportion of red clover. This is logical considering they belong to two distinct functional groups with complementary uptake patterns (Scherer-Lorenzen *et al.* 2003). Christie and Tempus mixed swards yield large amounts of red clover. Red clover yields between Christie and Tempus swards are similar, despite differing ploidy levels (Boller & Nosberger 1994; Houngnandan *et al.* 2008). Red clover yields are highest in cuts one and three. This may be influenced by sampling interval to some extent. Bluegrass yields are highest in pure swards. Bluegrass yield is consistent across cuts, being slightly higher in cuts one and three.

The second axis, which accounts for 27% of the variation, depicts the continuum between swards that contain a large proportion of bluegrass and conversely swards containing a large proportion of white clover (Figure 5.4). The pure sward treatment yields a large volume of bluegrass. Some white clover is present in the pure swards with very low levels of forbs and other grasses. Mixed swards did not yield any appreciable amounts of white clover. White clover yields in pure stands exhibits little change in yield over the season with cut three yielding modestly greater amounts of white clover. Overall the amount of white clover, forbs and other grasses in this experiment was very low. The Nappan experiment was not a pasture experiment like Truro. The Nappan experiment remains a cultivated area where variation was able to be actively minimized. The Pure bluegrass plots were sprayed with broad leaf herbicide and hand weeded during the first production year so there is very little contamination in the seed bank of this pasture. Accordingly, the selected vectors for the Nappan experiment were able to account for 96% of the variation in botanical yield data. The species composition of forage treatments over four harvests are presented in Table 5.4.

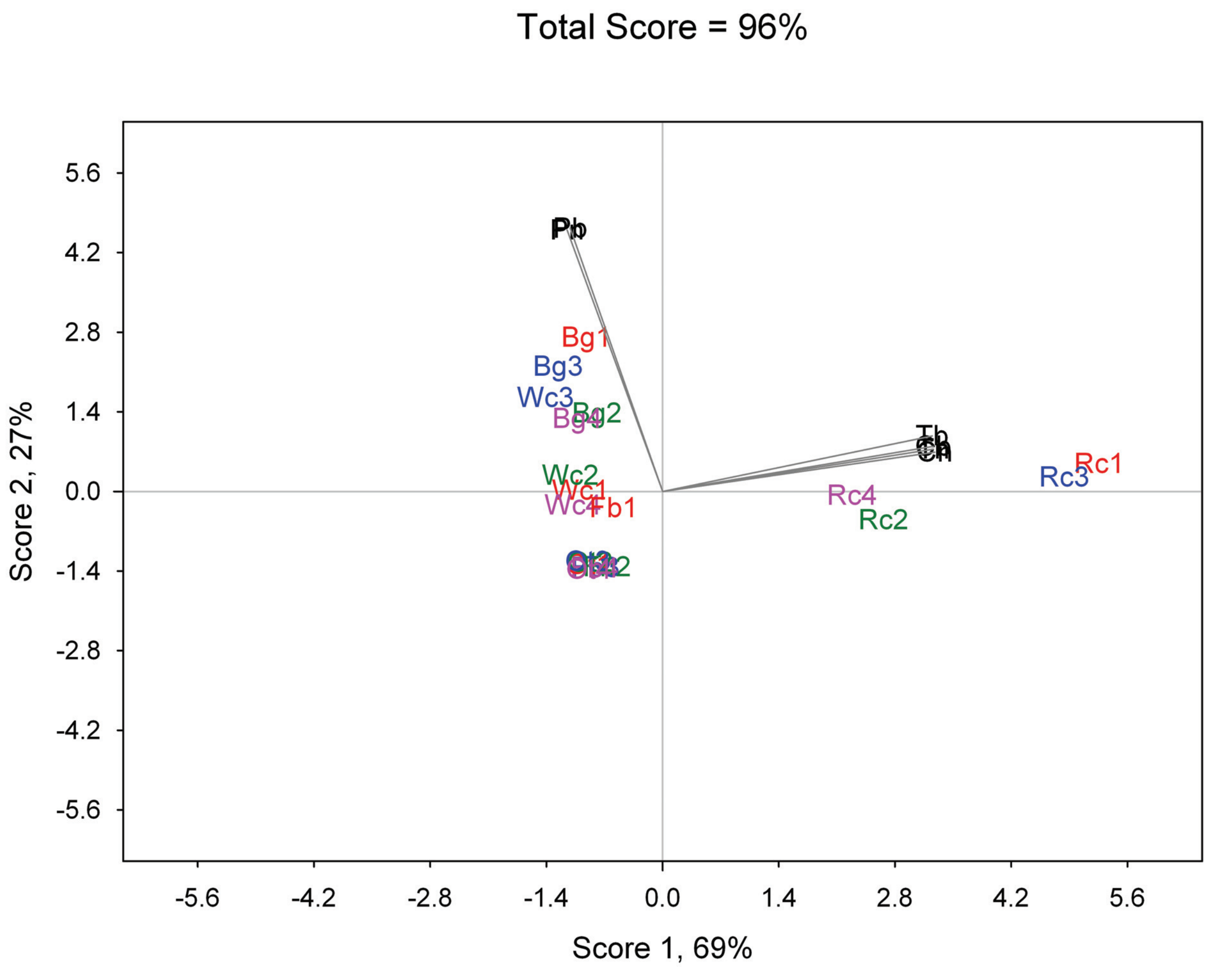

Figure 5. 4. Principal Components Analysis of sward yield and composition across four harvests in Nappan, NS.

Table 5.3 Percent species composition of forage treatments over four harvests, by mass, presented with average dry matter yield (g m^2) in Nappan, N.S. 2009.

Harvest	Treatment	Bluegrass%	Red clover%	White clover%	Forb%	Other grass%	Dead%	Total (g)
1	Pure	68	2	12	17	1		168
1	Christie	12	82		6			432
1	Tempus	15	73	2	10			374
2	Pure	72		24		1	3	106
2	Christie	18	73	3	6			221
2	Tempus	19	72	4	5			250
3	Pure	67	1	31		1		109
3	Christie	7	90	2				236
3	Tempus	9	84	4	2			233
4	Pure	75	7	17	1			58
4	Christie	14	83	2	0			143
4	Tempus	14	81	2	3			128

5.3.3.3 Sward Nitrogen Yield

Christie and Tempus red clover stands produced significantly greater sward N yields (SNY) over time, compared to pure bluegrass ($P < 0.001$). At the end of the growing season Christie stands had produced the most above ground N at 279 kg N ha^{-1} followed closely by Tempus at 257 kg N ha^{-1} and trailed by N poor bluegrass stands with 89 kg N being produced per hectare (Table 5.5). No significant difference in SNY was observed between the two cultivars. Both red clover stands possessed nearly triple the amount of N found to be present in bluegrass stands (Figure 5.5). These numbers corresponded closely with reported SNY's from Boller and Nosberger's (1987) establishing grass legume mixtures in Switzerland. Mixtures containing white or red clover grown in combination with bluegrass yielded 200-350 kg total sward N ha^{-1} yr^{-1} during establishment years. The leguminous pasture in that particular experiment also produced approximately triples the SNY of a corresponding bluegrass monoculture while establishing.

Table 5.4 Sward N yield (kg ha^{-1}) from three sward treatments in Nappan, NS.

Treatment	n	June	July	Aug	Sept	Season
Pure bluegrass	6	21.5	15.5	28.7	23.1	88.7
Christie mixture	3	76.3	48.5	97.0	56.8	278.6
Tempus mixture	3	73.9	38.5	82.5	61.6	256.5
SEM		9.9	7.5	13.6	10.6	36.0
P- value[1]		0.000	0.004	0.002	0.008	0.001

[1] Probability value compares Pure versus Clover treatments (Christie and Tempus). No significant differences ($P > 0.05$) were observed when SNY from the two red clover cultivars were contrasted.

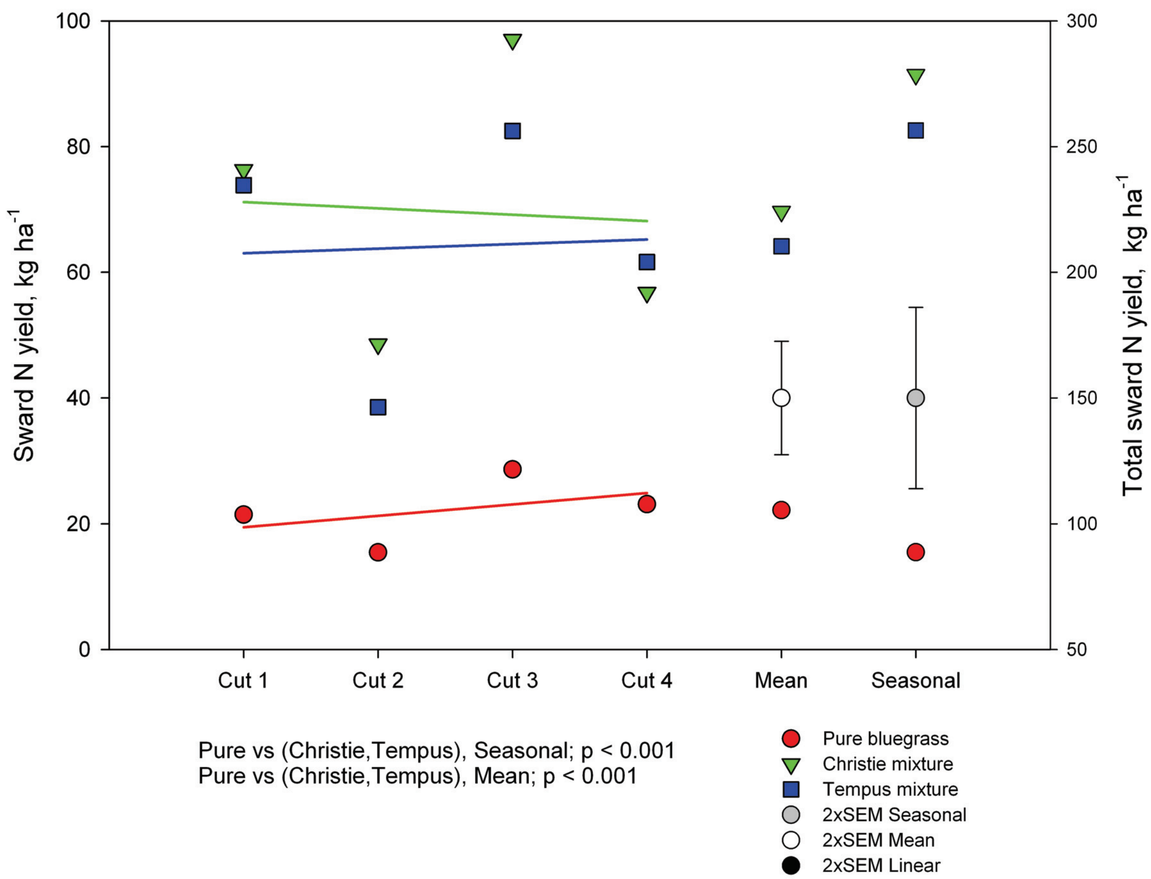

Figure 5. 5. Total sward N yield (kg ha^{-1}) by treatment in Nappan, N.S.

In order to represent the N benefit bluegrass receives when grown in mixture with red clover the N% in bluegrass tissue grown in monoculture versus bluegrass grown in mixture with the two red clover cultivars was compared (Figure 5.6). Bluegrass grown in combination with red clover was found to have significantly greater N% in its tissue compared to bluegrass grown in monoculture (P < 0.001). This indicates that the bluegrass was indeed conferred some nutrient benefit by the presence of red clover plants. Bluegrass grown in all treatments continued to accumulate N in its tissue as the season progressed. This outcome is offered some support by the preliminary results of a ^{15}N study conducted in an adjacent pasture that utilized the same pasture species and cultivars. Thilakarathna *et al.* (2010) found that the percent of N in bluegrass that was derived from the atmosphere (presumably by red clover) accounted for more of the bluegrass' total N over time. Approximately 5, 15 and 25% of the total N contained in bluegrass tissue was derived from the atmosphere in Cuts 1, 2 and 3, respectively. No significant difference in N benefit was observed between the two red clover cultivars in the current experiment.

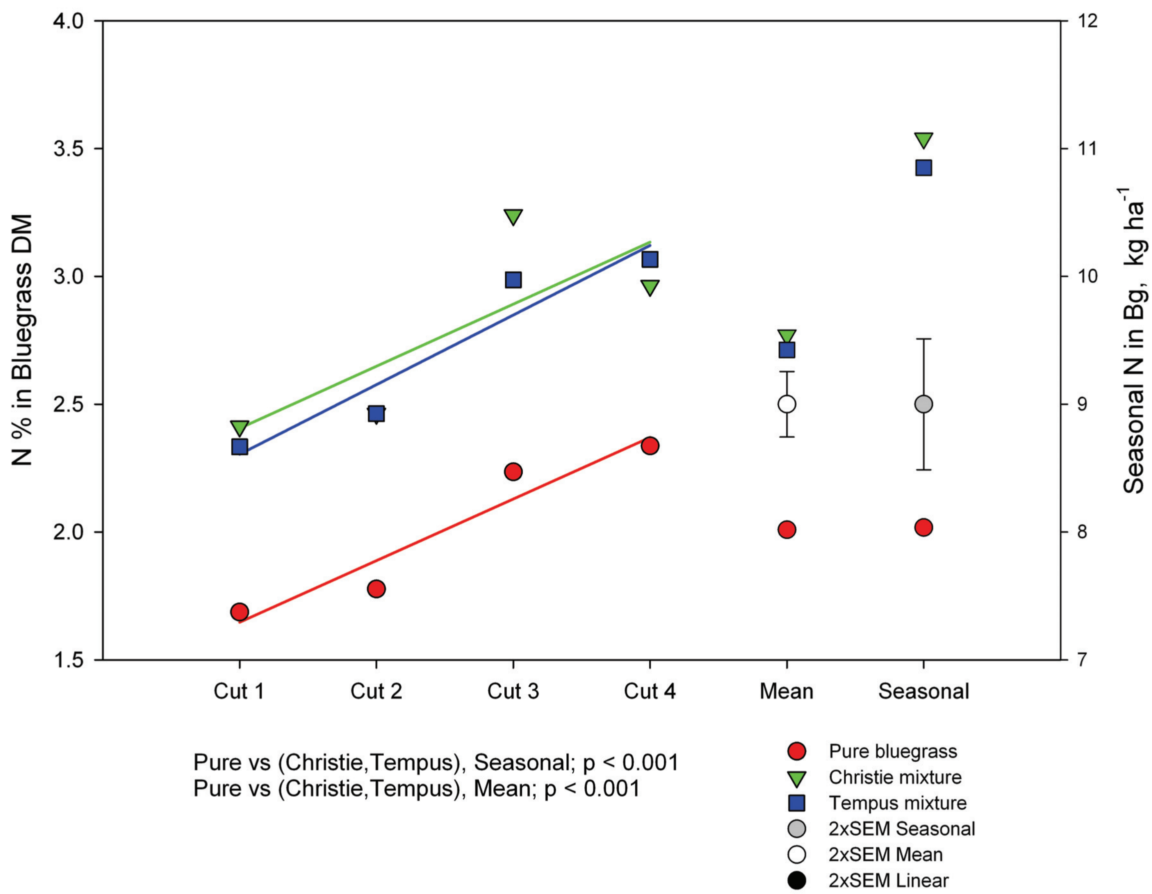

Figure 5. 6. Percent N contained per gram of bluegrass tissue from each sward treatment in Nappan, N.S.

5.3.4. Phosphate in soil solution and potential leaching losses

The mean PO_4^{3-} concentration of soil solution extracted from the lysimeters was 0.40 mg L^{-1} and ranged from <0.09 to 4.20 mg L^{-1}. The concentration of P acceptable, as defined by the Canadian Guidance Framework, in freshwater ecosystems varies on a continuum from 4 μg TP L^{-1} in ultra-oligotrophic sites and 100 μg TP L^{-1} for hyper-eutrophic sites (Environment Canada 2004). Observed PO_4^{3-}concentrations leaching from soil columns in a lab experiment by Jensen *et al.* (1999) ranged from 0.02 to 0.10 mg PO_4-P L^{-1}. A similar lab trial conducted by Miller *et al.* (1994) observed 2.0– 15.0 mg PO_4-P L^{-1} leaching from three cover crops that included oilseed radish, annual ryegrass and red clover. Phosphate concentrations observed in this experiment fall somewhere in between the results of these two experiments; greater than Jensen's findings and lower than Miller's findings from oilseed radish.

At a 15 cm soil depth PO_4^{3-} trends over time were best modeled by quadratic regression splines with varied slopes that accounted for 57% of variance in the data (Figure 5.7). Sward type was found to influence PO_4^{3-} concentrations in the root zone but not in the manner that was expected - the concentration of PO_4^{3-} in pure swards was intermediate between the two red clover mixtures. Concurrent with Harrison (1997) and Vance (2001) findings that P acquisition in red clover is under genetic control, the two cultivars in this study appeared to alter the concentrations of PO_4^{3-} in the root zone differentially. Vance and Harrison attribute these differences predominantly to changes in the mycorrhizae-red clover symbiotic relationship. Statistical analysis showed that seasonal weather conditions interacted with sward treatments (P = 0.005) to induce changes in PO_4^{3-} concentration in the root zone.

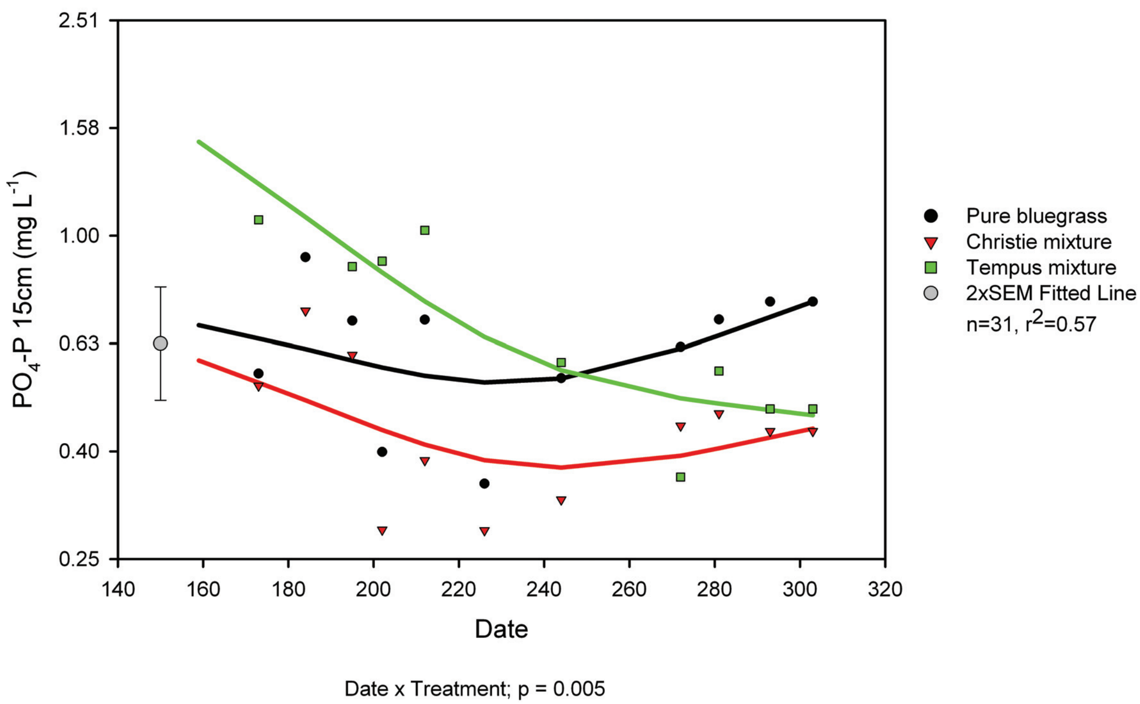

Figure 5. 7. Seasonal changes in PO_4-P concentration of the soil solution in Nappan, N.S. at a 15 cm depth.

At a 45 cm depth phosphate trends over time were best modeled by quadratic regression splines with variable slopes that accounted for 75% of the variation in the data set (Figure 5.8). Sward type was found to strongly influence PO_4^{3-} leaching. Pure swards leached much greater amounts of PO_4^{3-}compared to mixed red clover swards both of which leached consistently low amounts of PO_4^{3-} over the growing season. No cultivar induced PO_4^{3-} leaching differences were detected for Christie and Tempus red clover mixtures in this experiment (Harrison 1997; Vance 2001). The seasonal tendency for PO_4^{3-} to accumulate in the soil of grass mixtures over the course of the growing season was similar to the seasonal increase observed by Tischner (1999). Phosphate leaching from pure bluegrass plots in the Nappan experiment increased by 25% over time. Statistical analysis showed that seasonal weather conditions interacted with sward treatments (P = 0.036) to shape PO_4^{3-} leaching patterns.

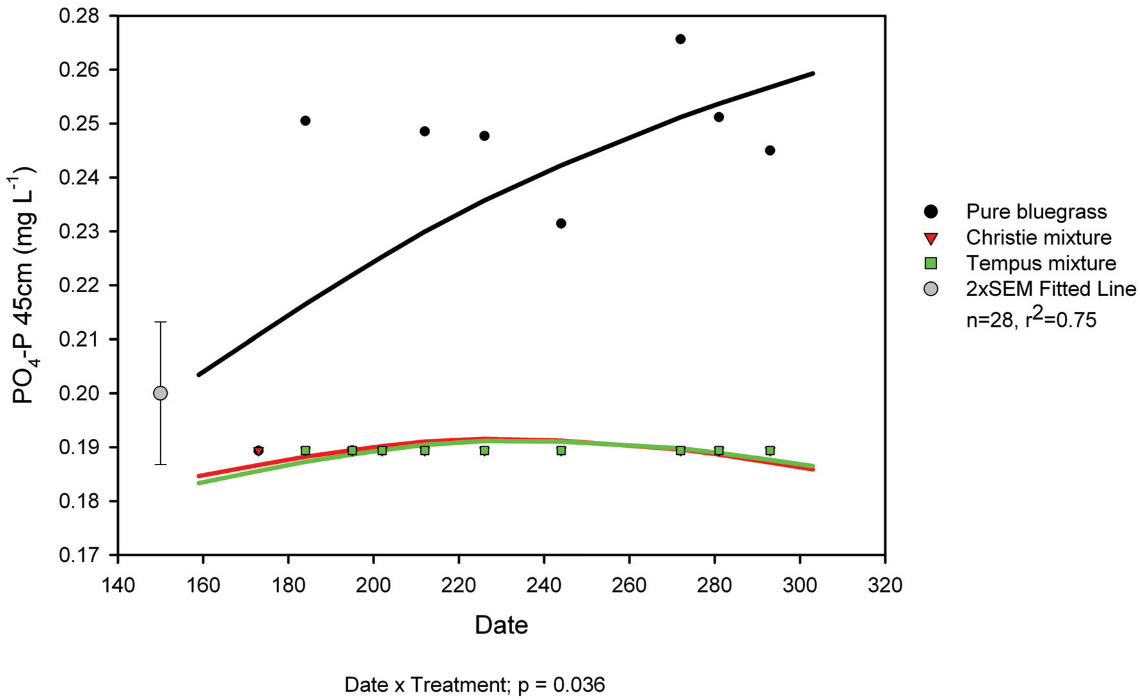

Figure 5. 8. Seasonal changes in PO_4-P concentration of the soil solution in Nappan, N.S. at a 45 cm depth.

Concentrations of PO_4^{3-} varied at different depths in the soil profile of this experiment. Phosphate was found to decrease with increasing depth as was reported by Tischner (1999). The availability of PO_4^{3-} in bluegrass swards was intermediate in the root zone, but much higher at 45 cm relative to leguminous pasture. This suggests bluegrass had reduced demand for P relative to red clover (less PO_4^{3-} was removed thus more PO_4^{3-} was available to be leached). In the mixed red clover pasture concentrations of PO_4^{3-} at 45 cm were much lower than the levels observed in the root zone. Mixed swards leached 60% less PO_4^{3-} compared to pure bluegrass. Miller *et al.* (1994) also found that red clover leached a third less PO_4^{3-} than seeded grass. Phosphate was available in different concentrations in the root zone of Tempus and Christie mixtures but both cultivars successfully utilized available P leaving only negligible amounts available to be lost via leaching. Changes in PO_4^{3-} availability in the soil between pure bluegrass and red clover mixtures seems to confirm Tilman *et al.* (1996) and Scherer-Lorenzen *et al.*'s (2003) hypotheses (and our supposition) that the plant community does play a profound role in modulating the concentration of nutrients as they percolate through the soil profile.

5.4. CONCLUSIONS

The primary objective of this experiment was to determine the contribution of AC Christie and Tempus red clover cultivars to NO_3^- concentrations in the soil solution when grown in mixture with bluegrass, compared to pure, unfertilized bluegrass grown in monoculture. Both red clover mixtures exhibited considerable increases in the concentration of NO_3-N in the root zone (15 cm) of mixed pasture by season's end, compared to the pure, unfertilized bluegrass treatment. This affirms the ability of red clover to fix substantial amounts of N from the atmosphere for use by the plant community. Sward treatment had a significant influence upon NO_3-N leaching at 45 cm ($P = 0.01$). Mixed red clover-bluegrass pasture was found to leach greater amounts of NO_3^- than pure, unfertilized pasture. However, NO_3^- losses were low overall, well beneath the MAC Limit for NO_3^- in drinking water. These results support our initial hypothesis indicating that the plant community plays a role in modulating the passage of NO_3^- through the soil profile. The research hypothesis, postulating that genetic differences between clover cultivars may impact NO_3^- fixation and subsequently leaching, was not supported by the findings of this experiment. Researchers found no significant evidence at 15 or 45 cm suggesting that diploid and tetraploid red clover cultivars differ in their contribution to soil NO_3^- in the results of this one-year experiment.

Secondly, the expected fall increase in NO_3^- leaching was not observed in this experiment. The absence of this late season increase is most likely associated with: a) the ability of the aggregated soil colloids to immobilize NO_3^- ions as they passed through the soil and b) a pattern of synchronous introduction of N from red clover and N uptake by bluegrass companion and also c) the absence of grazing ruminants. It would be desirable to have more than one year of data to better characterize the seasonality of NO_3^- leaching across a variety of weather conditions. However, the preliminary findings of this study show that researchers should seek to develop pasture mixtures with complementary functional groups and phenologies, adapted to the local climate in order to ameliorate environmentally damaging leaching losses.

Thirdly, pasture yields from each forage treatment were quantified. Red clover mixtures were found to produce significantly greater dry matter and sward N yields than pure, unfertilized bluegrass ($P < 0.001$). There was no observed difference in sward yields from AC Christie versus Tempus red clover mixtures. When PO_4^{3-} dynamics in Nappan were examined it was found that PO_4^{3-} availability was largely influenced by both sward type and seasonal conditions. The pure bluegrass treatment was found to contain significantly greater concentrations of PO_4^{3-} at 15 and 45 cm, compared to red clover mixed pastures. This difference is attributable to the increased demand for P as a consequence of BNF in red clover mixtures. Different P uptake patterns were observed between the two red clover cultivars in the root zone, but this effect was negligible at 45 cm, indicating that both cultivar mixtures successfully utilized or the soil immobilized the PO_4^{3-} before it was lost via leaching.

CHAPTER 6: CONCLUSIONS

The main objective of this project was to determine the contribution of red clover to soil NO_3^- when grown in mixture with bluegrass, compared to unfertilized, monoculture bluegrass. The results of this study illustrated that growing red clover in mixture with bluegrass will increase the amount of soil available NO_3^- and improve sward yields relative to monoculture bluegrass. Swards appeared to tightly cycle available N, as NO_3^- leaching losses were much lower from red clover-bluegrass mixtures compared to NO_3^- leaching losses from intensive fertilizer systems. In the literature, N leaching losses from intensive fertilized grass systems have been found to range from 30 - 200 kg N ha^{-1} yr^{-1}. In this study mixed grass-legume pasture at the Truro, N.S. site was found to leach approximately 7.4 kg N ha^{-1} yr^{-1}.

The introduction of water via sub-irrigation was found to increase N leaching slightly in grass-legume pasture, but this effect was not found to be significant. In order to discern the impact of sub-irrigation practices on NO_3 leaching it would be advisable to have more than two replicates in the experimental design and more than one observation year. The red clover cultivar variety utilized in mixture was found to have a small effect early season but the two selected cultivars were not found to differ significantly in their impact on soil NO_3^-. In order to determine the impact of red clover cultivar upon N supply and loss, this author would recommend that another experiment be designed that would examine the impact of a number of diverse red clover cultivars in mixture.

Seasonal conditions were found to interact with sward treatments to shape N leaching patterns in this study. A fall accumulation of NO_3^- was observed in the root zone of leguminous pasture in both Truro and Nappan, N.S. Nitrate leaching beyond the root zone was lowest mid-summer in Truro, N.S. with spring and fall increases in NO_3^- concentration in soil percolate. Nitrate leaching in Nappan, N.S. was highest in the spring and declined into the fall. Nitrate concentrations were much lower in Nappan compared to Truro, N.S. This difference was likely attributable to soil type and site management differences between the two locations. The Truro, N.S. site was observed to have a rapidly draining sandy soil which has been identified as being vulnerable to N leaching. Congruent with this assumption, Truro grass-legume mixtures leached the greatest amount of N in this study. It is our judgment that the N leaching out of this system would be an elevated estimate of potential N losses from grass-legume pastures.

The findings of this experiment elucidate the need to develop pasture management strategies that increase the synchrony of spatiotemporal growth patterns both between included pasture species, as well as to seasons associated with N losses. Further research should be undertaken to increase producers' confidence in extensive grassland systems. In order for society at large to benefit from the sustainability of extensive mixture that can rely on BNF rather than synthetic fertilizer for their N needs, these strategies must first be of benefit to the farmers who produce our food. Government should be encouraged to place emphasis on the quality of our soil and water by implementing incentive programs for farmers to reduce N loading into nearby aquifers.

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APPENDICES

APPENDIX A: EXPERIMENTAL SITE MAPS

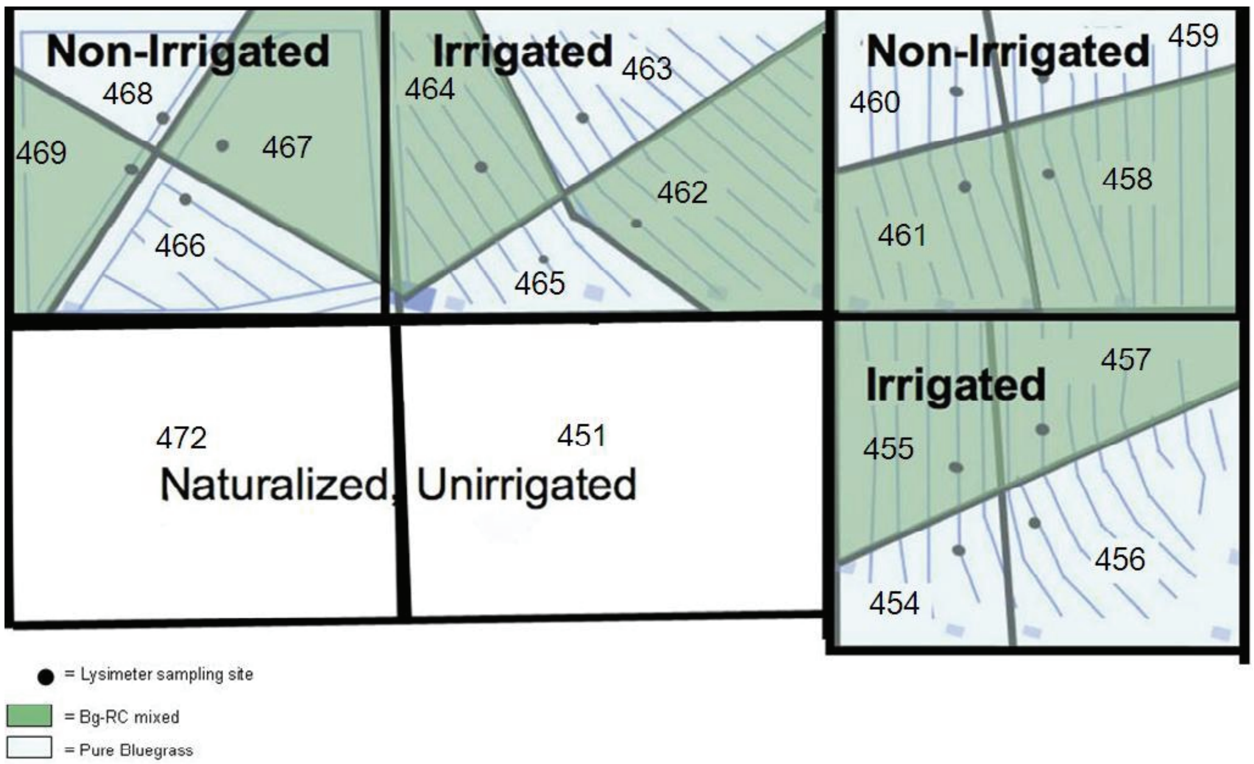

Figure A1. The Truro experimental site map. Locations of drainage tile lines, plot boundaries, drainage hut and control chambers are adapted to scale from AutoCAD GPS co-ordinates. This map covers an area approximately 25,000 m^2.

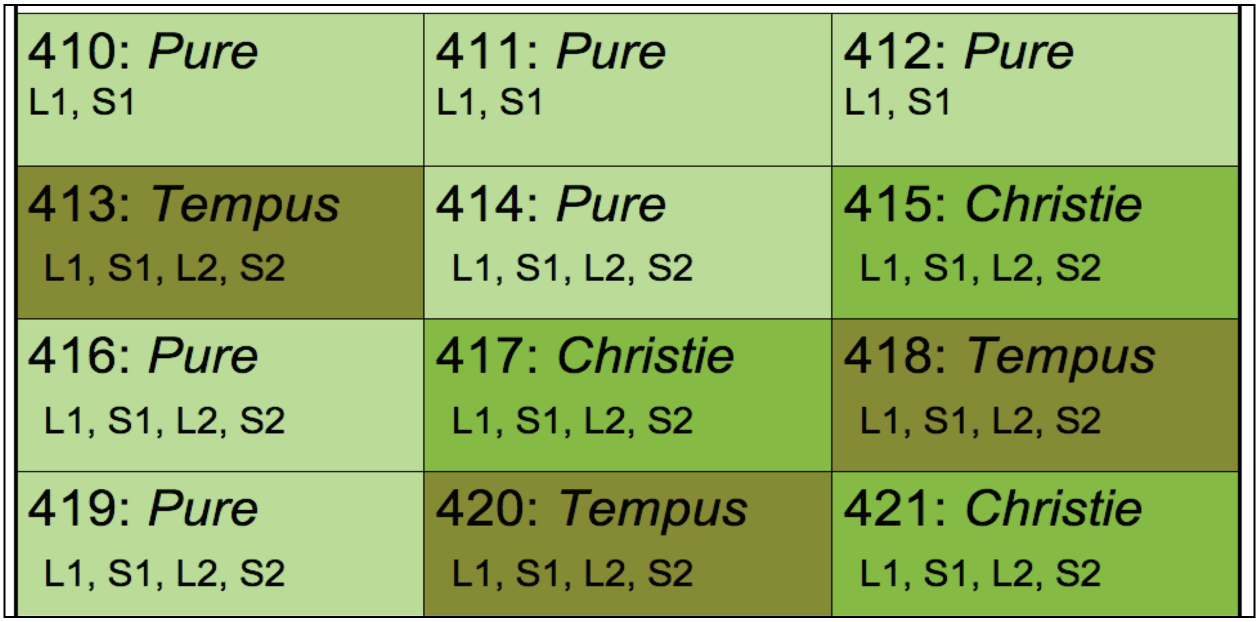

Figure A2. Field map of the Nappan red clover experiment. The entire experiment covers an area approximately 100 m^2. The dimensions of the experimental units measure 1.5 x 6 m. Letter and number combinations within plots indicate the depth and replicate of the lysimeter (ex. L1 = Long lysimeter; Replicate 1, S2 = Short lysimeter; Replicate 2). Pure indicates pure bluegrass; Tempus and Christie indicate the red clover cultivar variety that is grown in mixture with bluegrass.

APPENDIX B. SOIL CHARACTERISTICS

Table B1(a). Results of Truro soil physical analysis of the upper 0-10cm of soil cores. All units are ppm unless otherwise specified. Percent K, CA, MG, NA and H values refer to base saturation values.

eUnit	Depth(cm)	OM%	PH	CEC	P_2O_5	K_2O	CA	MG	NA	S	FE	MN	CU	ZN	B	%K	%CA	%MG	%NA	%H
451	0-10	3.3	5.4	10	506	162	957	161	31	14	254	97	1.16	3.9	0.38	3.3	46.2	13.0	1.3	36.3
454	0-10	3.6	6.3	12	520	282	1286	187	18	15	173	63	0.98	2.7	0.57	4.9	53.1	12.9	0.6	28.4
455	0-10	3.5	6.4	13	654	219	1471	232	21	14	162	79	1.07	3.5	0.71	3.5	55.7	14.6	0.7	25.5
456	0-10	3.3	6.1	12	665	141	1292	170	17	14	177	59	0.90	3.0	0.49	2.6	55.3	12.1	0.6	29.4
457	0-10	3.5	6.0	12	795	132	1338	169	16	14	164	57	1.06	4.4	0.50	2.3	55.6	11.7	0.6	29.9
458	0-10	3.5	5.9	12	863	142	1335	178	14	14	170	81	1.22	4.8	0.32	2.4	53.7	11.9	0.5	31.5
459	0-10	3.2	5.7	12	813	102	1200	175	19	14	212	93	1.16	4.1	0.26	1.9	51.7	12.6	0.7	33.1
460	0-10	3.4	6.0	12	811	204	1285	241	18	13	207	91	1.15	3.8	0.39	3.5	51.5	16.1	0.6	28.3
461	0-10	3.5	6.1	13	726	253	1322	261	16	15	157	87	0.91	3.0	0.37	4.2	51.5	17.0	0.5	26.8
462	0-10	3.8	5.7	13	957	322	1314	192	17	17	287	57	1.10	5.2	0.41	5.4	51.8	12.6	0.6	29.7
463	0-10	3.7	5.8	12	614	141	1195	169	14	15	188	41	0.87	2.7	0.30	2.5	50.9	12.0	0.5	34.1
464	0-10	3.3	5.4	11	907	198	1030	164	17	16	359	64	1.04	4.9	0.28	3.9	48.6	12.8	0.7	33.9
465	0-10	3.7	6.0	13	752	120	1479	207	24	13	339	103	1.16	5.7	0.40	2.0	58.0	13.5	0.8	25.7
466	0-10	3.5	5.8	13	725	140	1457	204	37	15	384	144	1.60	6.3	0.42	2.3	56.2	13.1	1.2	27.2
467	0-10	4.6	5.8	14	719	141	1587	238	45	17	322	100	1.62	7.0	0.48	2.1	54.8	13.7	1.3	28.2
468	0-10	4.2	6.5	15	696	97	1924	264	35	11	306	141	1.68	7.5	0.64	1.4	66.0	15.1	1.0	16.5
469	0-10	4.2	6.0	14	585	104	1775	242	26	14	356	83	1.69	5.4	0.53	1.6	63.3	14.4	0.8	20.0
472	0-10	3.7	5.8	12	895	411	1367	195	20	14	336	112	1.29	9.8	0.35	7.0	54.9	13.0	0.7	24.4

Table B1(b). Results of Truro soil physical analysis of the lower 10-20 cm of soil cores. All units are ppm unless otherwise specified. Percent K, CA, MG, NA and H values refer to base saturation values.

eUnit	Depth(cm)	OM%	PH	CEC	P_2O_5	K_2O	CA	MG	NA	S	FE	MN	CU	ZN	B	%K	%CA	%MG	%NA	%H
451	10-20	2.9	5.4	10	522	51	848	129	19	12	255	82	1.13	3.3	0.34	1.1	43.9	11.1	0.8	43.1
454	10-20	3.4	6.0	13	535	121	1388	197	21	14	182	60	1.03	2.6	0.45	2.0	54.7	12.9	0.7	29.6
455	10-20	3.2	6.2	13	650	148	1501	246	18	14	160	72	1.05	3.1	0.62	2.4	58.5	16.0	0.6	22.5
456	10-20	3.4	6.0	12	697	131	1401	189	16	14	171	60	1.03	3.4	0.49	2.3	57.0	12.8	0.5	27.4
457	10-20	3.7	6.0	12	821	67	1397	171	15	14	163	55	1.08	4.5	0.46	1.2	57.2	11.6	0.5	29.5
458	10-20	3.6	5.8	13	925	85	1608	197	18	13	172	78	1.18	5.3	0.43	1.4	60.8	12.4	0.6	24.8
459	10-20	3.4	5.7	11	816	99	1192	178	17	14	211	89	1.19	4.4	0.26	1.8	52.6	13.1	0.7	31.8
460	10-20	3.5	6.0	12	781	126	1335	254	17	14	206	79	1.20	3.4	0.37	2.1	53.4	16.9	0.6	26.9
461	10-20	3.1	5.9	12	719	199	1334	257	16	16	155	78	0.83	2.4	0.30	3.4	54.4	17.5	0.5	24.1
462	10-20	3.5	5.7	12	888	158	1249	183	17	15	259	43	1.00	4.8	0.36	2.8	51.6	12.6	0.6	32.4
463	10-20	3.6	5.8	12	573	54	1272	188	19	14	180	35	0.94	2.4	0.32	0.9	52.5	12.9	0.7	33.0
464	10-20	3.2	5.6	10	801	129	967	154	15	14	319	58	0.92	4.1	0.20	2.8	49.7	13.1	0.7	33.7
465	10-20	4.4	6.0	13	699	146	1573	226	26	16	422	87	1.29	5.7	0.52	2.4	60.3	14.4	0.9	22.1
466	10-20	3.6	5.8	13	652	131	1470	212	33	14	399	135	1.65	5.9	0.40	2.2	57.4	13.8	1.1	25.6
467	10-20	3.8	5.9	15	792	122	1750	266	41	16	316	99	1.84	7.6	0.49	1.7	57.7	14.6	1.2	24.8
468	10-20	4.5	6.3	16	754	96	2064	290	43	13	331	134	1.88	7.9	0.65	1.3	65.1	15.2	1.2	17.2
469	10-20	4.2	6.0	14	555	82	1688	222	26	12	333	89	1.58	5.1	0.41	1.3	61.3	13.4	0.8	23.2
472	10-20	3.2	5.7	12	984	216	1421	176	21	13	329	93	1.32	9.6	0.35	3.7	56.9	11.8	0.7	26.9

Table B2. Laboratory results of KCl extractable NH_4 and NO_3 ($mg\ L^{-1}$) from two depths at the Truro site collected May 13, 2008.

			0-10 cm		10-20 cm	
Block	Sward	eUnit	NH_4	NO_3	NH_4	NO_3
1	Pure	454	1.784	0.558	0.000	1.178
1	Mix	455	0.176	1.187	0.000	2.209
1	Pure	456	0.179	1.537	0.017	1.635
1	Mix	457	0.876	1.893	0.111	1.291
2	Mix	458	0.853	1.395	1.554	1.805
2	Pure	459	1.126	1.235	0.167	1.371
2	Pure	460	0.036	1.342	0.000	1.726
2	Mix	461	0.330	2.358	0.112	1.189
3	Mix	462	5.149	1.962	0.643	1.900
3	Pure	463	0.606	1.600	0.045	1.706
3	Mix	464	0.659	2.294	0.388	2.407
3	Pure	465	0.045	1.039	0.749	3.417
4	Pure	466	0.060	1.423	0.179	2.376
4	Mix	467	0.642	3.652	0.029	2.300
4	Pure	468	0.095	1.467	0.018	2.736
4	Mix	469	0.205	2.436	0.050	2.798
5	Natural	472	0.103	2.217	0.103	2.217
5	Natural	451	1.496	1.872	0.670	0.906

Table B3. Results of Nappan soil chemical analysis of soil cores stratified into two depths. All units are ppm unless otherwise specified. Percent K, CA, MG, NA and H values refer to base saturation values.

eUnit	Depth(cm)	OM%	PH	CEC	P_2O_5	K_2O	CA	MG	NA	S	FE	MN	CU	ZN	B	%K	%CA	%MG	%NA	%H
410	0-10	3.3	6.6	13	1275	187	1722	292	16	15	369	87	1.15	3.2	0.71	3.0	64.1	18.1	0.5	14.3
411	0-10	3.5	6.7	13	1244	221	1689	288	16	14	359	88	1.15	4.3	0.70	3.5	63.5	18.0	0.5	14.4
412	0-10	3.2	6.7	14	1234	194	1767	292	18	15	360	97	1.12	2.9	0.69	3.0	64.6	17.8	0.6	14.0
413	0-10	3.4	6.6	13	1219	147	1700	282	20	13	358	92	1.16	3.3	0.62	2.3	63.0	17.4	0.6	16.6
414	0-10	3.5	6.6	13	1233	193	1673	286	18	14	355	91	1.12	3.4	0.66	3.1	63.6	18.1	0.6	14.6
415	0-10	3.5	6.4	13	1145	123	1657	280	19	16	339	92	1.13	3.2	0.65	2.0	62.8	17.7	0.6	17.0
416	0-10	3.5	6.4	13	1143	201	1595	279	23	14	345	88	1.19	3.9	0.69	3.3	62.2	18.1	0.8	15.6
417	0-10	3.4	6.5	12	1116	142	1546	264	19	13	343	89	1.09	3.4	0.61	2.4	62.8	17.8	0.7	16.3
418	0-10	3.4	6.7	13	1052	158	1614	275	23	14	335	96	1.12	2.6	0.67	2.6	62.3	17.7	0.8	16.7
419	0-10	3.4	6.6	12	1083	203	1549	263	15	12	332	92	1.22	3.1	0.61	3.5	63.1	17.8	0.5	15.0
420	0-10	3.4	6.7	13	932	99	1600	283	18	12	305	89	1.09	3.1	0.76	1.6	62.5	18.4	0.6	16.9
421	0-10	3.5	6.6	13	939	119	1596	278	18	13	303	98	1.06	2.3	0.62	2.0	62.4	18.1	0.6	16.9
410	10-20	3.5	6.6	14	1196	114	1793	304	19	13	350	83	1.14	3.2	0.70	1.8	65.3	18.4	0.6	14.0
411	10-20	3.6	6.7	14	1228	119	1801	310	18	14	361	88	1.19	4.2	0.77	1.8	65.1	18.7	0.6	13.9
412	10-20	3.7	6.7	14	1144	110	1781	315	16	14	341	88	1.11	3.1	0.74	1.7	64.4	19.0	0.5	14.5
413	10-20	3.5	6.6	14	1168	100	1739	308	20	13	354	89	1.17	3.6	0.77	1.5	63.4	18.7	0.6	15.7
414	10-20	3.4	6.7	14	1180	117	1785	308	20	15	363	94	1.18	3.6	0.78	1.8	64.6	18.6	0.6	14.5
415	10-20	3.3	6.7	14	1123	88	1776	319	17	13	342	92	1.13	2.6	0.73	1.3	63.6	19.0	0.5	15.5
416	10-20	3.7	6.5	13	1109	128	1677	295	19	14	340	88	1.19	4.8	0.75	2.1	64.7	19.0	0.6	13.6
417	10-20	3.3	6.6	12	1066	99	1614	286	19	15	344	90	1.17	3.4	0.68	1.7	64.9	19.2	0.7	13.5
418	10-20	3.6	6.7	13	966	104	1603	284	16	14	319	97	1.09	2.7	0.67	1.7	62.8	18.6	0.5	16.3
419	10-20	3.1	6.7	13	1058	156	1646	288	17	14	331	94	1.14	3.2	0.69	2.6	64.0	18.6	0.6	14.3
420	10-20	3.2	6.5	13	1094	150	1654	286	16	14	339	98	1.12	3.1	0.63	2.4	62.7	18.0	0.5	16.4

eUnit	Depth(cm)	OM%	PH	CEC	P_2O_5	K_2O	CA	MG	NA	S	FE	MN	CU	ZN	B	%K	%CA	%MG	%NA	%H
421	10-20	3.5	6.8	13	874	94	1676	298	19	13	296	98	1.08	2.6	0.73	1.5	63.4	18.8	0.6	15.7

Table B4. Percent nitrogen per gram of soil in Nappan soil cores 2009 as determined by the LECO Combustion method.

			N%	
Block	Sward	eUnit	0-10cm	10-20cm
0	Pure	410	0.54	0.04
0	Pure	411	0.36	0.04
0	Pure	412	0.20	0.06
1	Tempus	413	0.15	0.06
1	Pure	414	0.11	0.00
1	Christie	415	0.11	0.07
2	Pure	416	0.10	0.04
2	Christie	417	0.08	0.02
2	Tempus	418	0.10	0.00
3	Pure	419	0.07	0.05
3	Tempus	420	0.03	0.00
3	Christie	421	0.07	0.00

APPENDIX C. SWARD DRY MATTER YIELD

Table C1. Dry matter yield (g m^2) by irrigation and sward treatment over six harvests in Truro, N.S. 2009.

				Cut 1		Cut 2		Cut 3		Cut 4		Cut 5		Cut 6	
Block	Irrigation	Forage	eUnit	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
1	Irr	Pure	454	*	48.8	111.2	110.4	135.2	111.2	126.4	95.2	118.0	85.2	84.8	111.2
1	Irr	Mix	455	*	109.2	267.6	217.6	171.2	115.0	216.0	123.4	126.0	108.2	109.2	137.0
1	Irr	Pure	456	*	48.4	155.2	143.2	171.6	127.6	234.8	110.2	111.2	95.6	111.6	134.0
1	Irr	Mix	457	*	68.0	138.0	194.0	166.4	122.4	234.0	167.0	151.6	150.8	111.6	122.8
2	noIrr	Mix	458	*	87.6	173.6	322.0	154.8	78.8	131.6	143.4	66.8	167.0	55.2	148.0
2	noIrr	Pure	459	*	50.0	98.4	84.4	91.2	61.8	117.6	147.6	93.6	122.4	76.8	156.6
2	noIrr	Pure	460	*	34.0	130.0	158.4	160.0	60.4	182.0	111.8	90.4	158.6	64.4	61.0
2	noIrr	Mix	461	*	74.0	278.4	178.4	161.6	80.6	142.8	136.2	81.6	136.4	120.4	163.0
3	Irr	Mix	462	*	94.8	338.0	316.8	199.6	75.8	102.4	86.8	162.8	151.4	69.6	116.4
3	Irr	Pure	463	*	78.0	170.8	212.8	154.4	147.2	83.2	70.4	113.6	124.6	74.0	94.6
3	Irr	Mix	464	*	159.2	214.0	215.6	306.4	161.8	124.4	82.4	186.0	182.8	87.6	107.6
3	Irr	Pure	465	*	50.4	113.6	227.6	131.6	154.4	89.2	66.0	81.6	92.4	62.0	123.4
4	noIrr	Pure	466	*	30.4	116.0	156.8	109.2	28.2	60.4	78.6	63.6	47.2	22.4	124
4	noIrr	Mix	467	*	33.2	175.6	308.8	148.4	112.6	100.4	78.4	112.0	130.0	66.0	89.4
4	noIrr	Pure	468	*	102.8	323.2	323.2	101.6	73.2	81.2	83.6	75.6	72.2	64.4	37.8
4	noIrr	Mix	469	*	111.6	350.4	404.4	122.8	125.6	127.2	85.0	108.4	130.4	49.6	115.2
5	Natural	Natural	451	*	95.6	197.6	274.8	154.0	150.5	70.0	85.8	164.0	84.3	76.4	140.3
5	Natural	Natural	472	*	104.0	282.4	196.4	168.0	145.4	81.2	104.8	145.2	89.68	56.4	128.9
		SEM			8.1	19.8	20.0	11.0	9.1	12.9	6.9	8.5	8.6	5.9	7.4

Table C2. Dry matter yield (g m^2) by sward treatment on four harvest dates for Nappan: Cultivar experiment in 2009.

Block	Sward	eUnit	June	July	August	Sept
0	Pure	410	117.1	35.2	47.3	41.7
0	Pure	411	84.9	63.5	72.4	9.1
0	Pure	412	48.9	9.1	38.1	34.1
1	Tempus	413	181.3	134.7	227.5	142.9
1	Pure	414	104.3	58.3	143.2	79.3
1	Christie	415	194.1	137.6	176.8	100.4
2	Pure	416	187.3	158.5	190.8	121.9
2	Christie	417	232.2	121.2	373.9	166.0
2	Tempus	418	199.4	72.5	204.7	140.6
3	Pure	419	128.1	69.0	131.4	206.0
3	Tempus	420	307.4	128.3	287.8	167.7
3	Christie	421	320.8	170.3	325.3	215.5
	SEM		24.3	14.9	30.9	19.4

APPENDIX D. FORAGE DIGESTIBILITY

Table D1. Percent neutral and acid detergent fiber digestibility for five harvests in Truro, NS.

Month	Forage	NDF%	ADF%
5	Mix	36	18
5	Pure	48	21
5	Natural	35	17
	SEM	4	1
6	Mix	35	22
6	Pure	35	22
6	Natural	42	22
	SEM	3	1
8	Mix	39	24
8	Pure	37	23
8	Natural	*	*
	SEM	1	1
9	Mix	33	20
9	Pure	35	21
9	Natural	56	26
	SEM	7	2
10	Mix	30	18
10	Pure	38	22
10	Natural	44	21
	SEM	4	1

Table D2. Percent neutral and acid detergent fiber digestibility for three harvests in the Nappan cultivar experiment.

Month	Forage	NDF%	ADF%
6	Pure	42	24
6	Christie	39	24
6	Tempus	42	26
	SEM	1	1
8	Pure	54	29
8	Christie	43	28
8	Tempus	44	26
	SEM	4	1
9	Pure	53	28
9	Christie	43	24
9	Tempus	38	22
	SEM	4	2

APPENDIX E. SOIL HYDROLOGICAL MEASUREMENTS

Table E1. Average monthly soil matric potential (in centibar) from 0.5 m depth field tensiometers located in the Truro, NS, 2009 (Havard 2009).

Block	Forage	Irrigation	eUnit	May	June	July	August
1	Pure	Irr	454	21	26	24	27
1	Mix	Irr	455	19	29	25	27
1	Pure	Irr	456	16	31	21	38
1	Mix	Irr	457	17	23	19	24
2	Mix	noIrr	458	16	35	20	16
2	Pure	noIrr	459	19	18	16	21
2	Pure	noIrr	460	14	25	13	18
2	Mix	noIrr	461	18	37	27	32
3	Mix	Irr	462	18	42	25	14
3	Pure	Irr	463	16	28	27	24
3	Mix	Irr	464	8	11	17	15
3	Pure	Irr	465	9	14	16	27
4	Pure	noIrr	466	10	7	8	11
4	Mix	noIrr	467	11	11	13	14
4	Pure	noIrr	468	10	19	15	23
4	Mix	noIrr	469	11	14	14	25
5	Natural	noIrr	472	12	12	12	12
5	Natural	noIrr	451	15	19	18	18

Table E2. Percent soil moisture values from 0-20cm TDR probe depth in 2009.

				MM/DD													
Block	Forage	Irr	eUnit	06/13	06/22	06/23	07/07	07/13	07/23	08/11	08/25	09/17	09/28	10/08	10/19	10/26	11/05
1	Pure	Irr	454	*	28	10	30	31	33	29	13	28	19	37	44	38	35
1	Mix	Irr	455	*	32	*	36	24	32	19	30	25	23	32	39	34	39
1	Pure	Irr	456	28	26	*	32	32	37	20	13	30	29	42	36	32	26
1	Mix	Irr	457	*	14	*	29	24	27	22	42	24	30	34	37	35	34
2	Mix	noIrr	458	16	27	34	29	24	17	18	22	18	16	33	37	32	33
2	Pure	noIrr	459	*	33	28	31	26	32	15	24	25	28	38	39	36	36
2	Pure	noIrr	460	*	*	*	29	26	*	12	28	23	30	39	39	33	38
2	Mix	noIrr	461	36	*	28	35	23	*	17	19	27	29	39	34	35	35
3	Mix	Irr	462	16	*	*	30	21	34	17	27	25	27	38	34	34	36
3	Pure	Irr	463	24	33	*	30	27	11	11	23	23	23	37	34	39	38
3	Mix	Irr	464	14	31	13	33	25	35	13	28	20	26	40	41	34	39
3	Pure	Irr	465	43	*	*	*	35	47	29	26	23	25	42	48	46	41
4	Pure	noIrr	466	49	48	*	41	30	*	13	*	29	34	38	62	45	33
4	Mix	noIrr	467	13	39	*	43	34	11	18	*	21	32	37	45	46	35
4	Pure	noIrr	468	*	*	*	*	30	12	11	*	30	31	42	49	48	48
4	Mix	noIrr	469	14	*	*	42	39	27	42	*	26	36	44	48	45	50
5	Natural	noIrr	451	26	24	31	26	33	31	30	29	18	27	35	43	41	40
5	Natural	noIrr	472	15	34	34	36	26	28	30	24	21	25	31	52	48	39

APPENDIX F. SUB-IRRIGATION WATER VOLUME

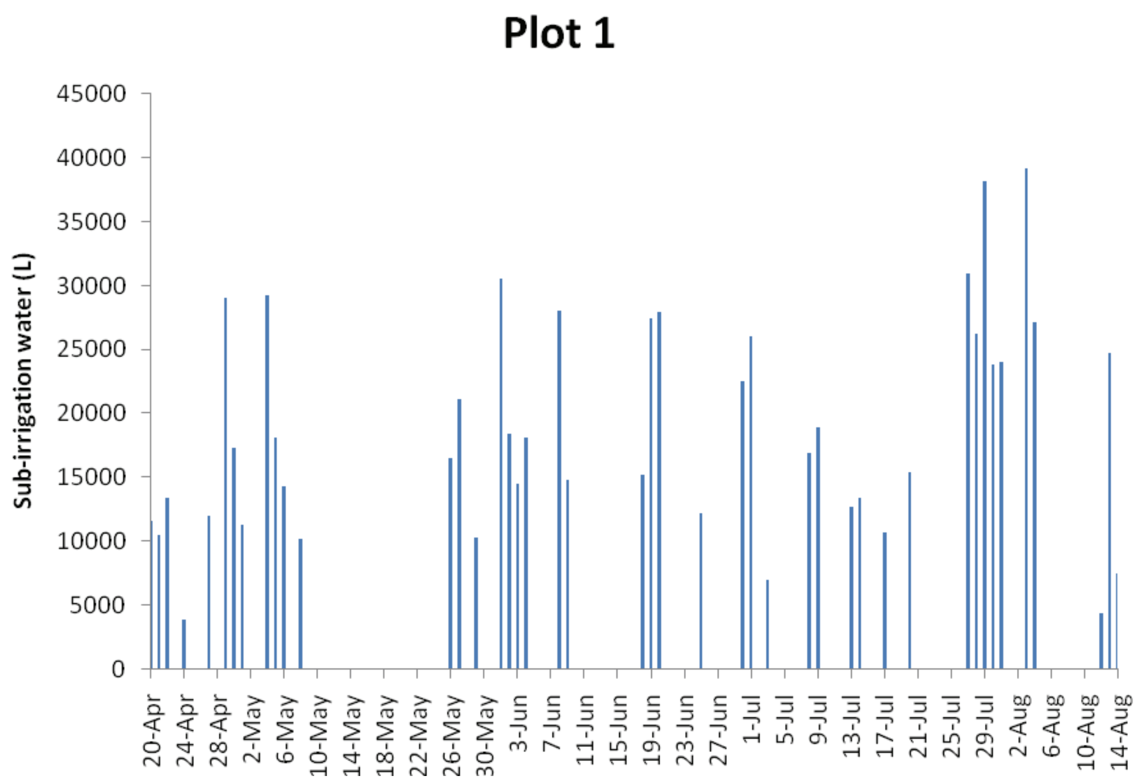

Figure F1. Sub-irrigation water volume supplied in Truro, N.S. to Plot 1 from April 20 to August 14, 2009 (Havard 2009).

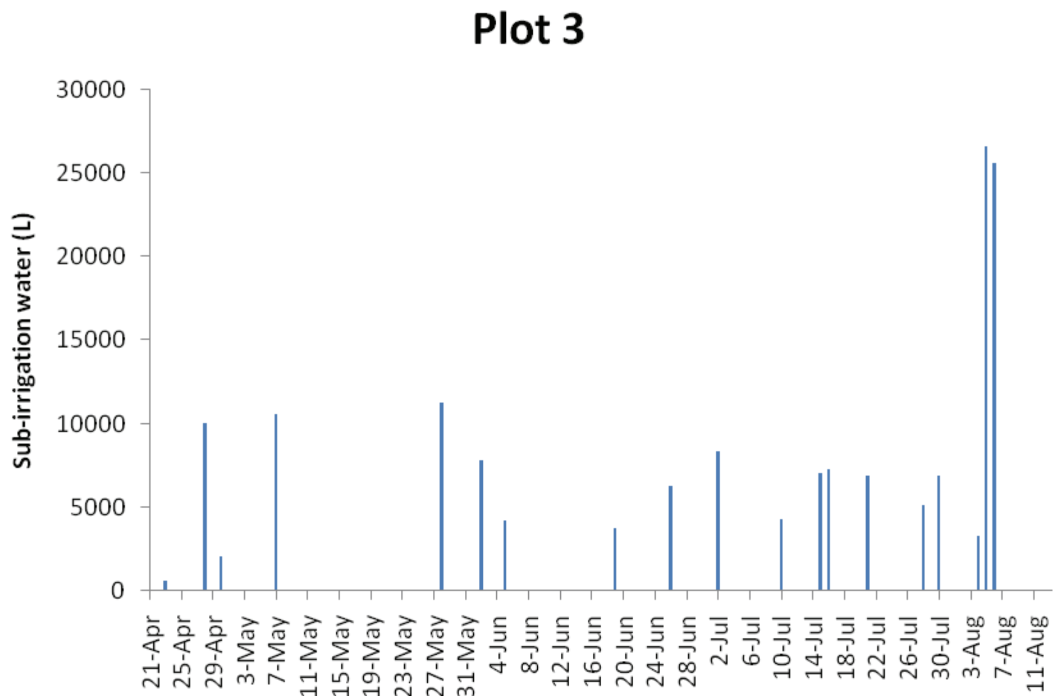

Figure F2. Sub-irrigation water volume supplied in Truro, N.S. to Plot 3 from April 21 to August 14, 2009 (Havard 2009).

Table F1. Water volumes (L) delivered to sub-irrigated pasture plots in Truro, N.S. in 2009 (Havard 2009).

Month	Plot 1	Plot 3
April	97944	15256
May	151560	21849
June	226925	23243
July	264549	46602
August	66366	63129
Total	807334	170079

APPENDIX G. DRAINAGE WATER ANALYSIS

Table G1. Results of soil water analysis from the tile lines that drained from each plot individually into the sampling hut on six different dates (Havard 2009). (NO_3^-, PO_4^{3-} and NH_4^+ values mg L^{-1})

Date	Plot	NO_3^-	NH_4^+	PO_4^{3-}	pH
16/04	1	6.44	0.2	0.19	6.95
16/04	2	5.81	<0.10	0.09	7.06
16/04	3	5.47	<0.10	0.11	6.93
16/04	4	2.08	0.31	0.15	6.90
23/04	1	3.54	<0.10	0.13	7.18
23/04	2	7.60	<0.10	0.12	7.08
23/04	3	6.43	<0.10	0.12	6.74
23/04	4	2.31	<0.10	0.17	6.77
07/05	1	7.57	<0.10	0.20	7.27
07/05	2				
07/05	3	0.87	<0.10	0.09	7.65
07/05	4	2.27	<0.10	0.20	7.42
30/09	1	6.30		<0.10	
30/09	2	2.75		<0.10	
30/09	3	6.45		<0.10	
30/09	4	4.66		<0.10	
19/10	1	1.28		<0.10	
19/10	2	1.53		<0.10	
19/10	3	6.46		<0.10	
19/10	4	2.96		<0.10	
08/11	1	3.31		<0.10	
08/11	2	2.46		<0.10	
08/11	3	5.94		<0.10	
08/11	4	2.51		<0.10	

APPENDIX H: NO_3 ANALYSIS BY SAMPLING DATE

TRURO

Table H1. Mean concentration of NO_3-N (mg L^{-1}) in forage treatments at 15 cm lysimeter depth sampled from June to November 2009 in Truro, N.S.

		Date (MM/DD)																
Forage*	n	06/14	06/23	06/24	07/08	07/14	24/07	11/08	23/08	25/08	17/09	30/09	08/10	19/10	25/10	04/11	07/11	19/11
Pure	8	0.08	0.17	0.69	0.07	0.51	0.12	0.21	0.07	0.06	0.27	0.04	0.42	0.50	0.05	0.21	0.17	0.25
Mixed	8	0.42	0.24	1.93	0.36	0.29	0.57	3.85	22.64	4.94	5.99	8.01	4.67	9.54	4.41	4.49	4.35	6.14
Natural	2	0.14	0.07	0.07	0.11	0.38	0.08	0.35	1.26	0.09	0.09	0.17	0.04	0.03	0.03	0.06	0.05	0.02
SEM		1.60	2.82	2.29	1.68	1.64	1.41	2.18	1.00	1.67	1.53	1.52	1.39	1.74	1.27	1.34	1.52	3.88
P-value		0.031			0.014		0.018	0.032	0.024	0.000	0.000	0.000	0.002	0.001	0.004	0.008	0.001	0.050

* Where "pure" = pure bluegrass, "mixed" = bluegrass and red clover mixed stand and "natural" = naturalized, undisturbed pasture.

Table H2. Mean concentration of NO_3-N (mg L^{-1}) in forage treatments at 45 cm lysimeter depth sampled from June to November 2009 in Truro, N.S.

		Date (MM/DD)																	
Forage	n	13/06	14/06	23/06	24/06	08/07	14/07	24/07	11/08	23/08	25/08	17/09	30/09	08/10	19/10	25/10	04/11	07/11	19/11
Pure	8	0.73	1.06	0.40	0.15	0.15	0.37	0.22	0.40	0.30	0.12	0.12	0.39	0.45	0.35	0.18	0.37	0.33	0.24
Mixed	8	5.86	6.95	2.81	0.27	1.90	1.92	1.13	1.47	3.68	2.81	3.52	2.38	2.83	6.17	9.28	8.40	7.55	4.80
Natural	2	0.64	0.62	0.15	0.14	0.12	0.15	0.07	0.17	0.05	0.22	0.11	0.06	0.12	0.84	1.57	0.05	0.30	2.27
SEM		1.69	1.62	1.43	1.63	1.68	1.71	1.42	1.65	1.51	1.78	1.74	1.11	1.85	1.61	1.64	1.62	1.78	1.28
P-value		0.008	0.005	0.042		0.004		0.092		0.024	0.004	0.009			0.009	0.001	0.002	0.001	0.013

Table H3. Mean concentration of NO_3-N (mg L^{-1}) in irrigation treatments at 15 cm lysimeter depth sampled from June to November 2009 in Truro, N.S.

		Date (MM/DD)																
Irr	n	14/06	23/06	24/06	08/07	14/07	24/07	11/08	23/08	25/08	17/09	30/09	08/10	19/10	25/10	04/11	07/11	19/11
Irr	8	0.24	0.16	1.30	0.13	1.07	0.35	1.82	1.26	0.55	0.63	0.43	1.88	3.27	0.48	1.41	0.81	4.57
noIrr	8	0.14	0.26	1.03	0.19	0.14	0.19	0.43	1.26	0.57	2.61	0.69	1.05	1.46	0.42	0.67	0.93	0.34
Natural	2	0.14	0.07	0.07	0.11	0.38	0.08	0.35	1.26	0.09	0.09	0.17	0.04	0.03	0.03	0.06	0.05	0.02
SEM		1.66	3.94	2.02	1.86	1.91	1.33	2.14	1.00	1.86	1.80	1.74	1.51	2.07	1.12	1.16	1.68	6.32
P-value																		

Table H4. Mean concentration of NO_3-N (mg L^{-1}) in irrigation treatments at 45 cm lysimeter depth sampled from June to November 2009 in Truro, N.S.

		Date (MM/DD)																	
Irr	n	13/06	14/06	23/06	24/06	08/07	14/07	24/07	11/08	23/08	25/08	17/09	30/09	08/10	19/10	25/10	04/11	07/11	19/11
Irr	8	2.99	4.16	3.91	0.21	1.31	1.57	0.78	1.52	1.99	0.75	0.37	1.01	2.27	1.71	2.36	2.25	2.10	1.46
noIrr	8	1.42	1.77	0.29	0.19	0.21	0.45	0.32	0.38	0.56	0.45	1.14	0.92	0.56	1.25	0.69	1.38	1.18	0.78
Natural	2	0.64	0.62	0.15	0.14	0.12	0.15	0.07	0.17	0.05	0.22	0.11	0.06	0.12	0.84	1.57	0.05	0.30	2.27
SEM		1.82	1.78	1.20	1.59	1.81	1.58	1.19	1.35	1.23	1.73	1.44	1.05	1.48	1.32	1.55	1.57	1.90	1.13
P-value				0.009				0.073	0.083	0.048									

NAPPAN

Table H5. Mean concentration of NO_3-N (mg L^{-1}) in sward treatments at 15 cm lysimeter depth sampled from June to October 2009 in Nappan, N.S.

		Date (DD/MM)											
Forage	n	06/08	06/22	07/03	07/14	07/21	07/31	08/14	09/01	09/29	10/08	10/20	10/30
Pure bluegrass	6	1.07	0.12	0.09	0.10	0.10	0.07	0.08	0.07	0.07	0.07	0.07	1.17
Christie mixture	3	3.73	0.30	0.15	0.29	0.07	0.08	0.10	0.28	0.55	1.06	0.35	1.76
Tempus mixture	3	0.91	0.57	0.19	0.18	0.46	0.48	0.12	0.13	2.15	0.75	0.12	1.25
SEM		1.72	1.76	1.32	1.45	1.58	1.29	1.29	1.66	1.71	1.57	1.38	1.15
P-value[1]							0.02			0.15	0.003	0.044	0.091
P-value[2]						0.072	0.007						

[1] Probability values contrast mixed red clover-bluegrass versus pure bluegrass sward treatments

[2] Probability values contrast Christie mixture versus Tempus mixture

Table H6. Mean concentration of NO_3-N (mg L^{-1}) in sward treatments at 45 cm lysimeter depth sampled from June to October 2009 in Nappan, N.S.

		Date (DD/MM)											
Forage	n	06/08	06/22	07/03	07/14	07/21	07/31	08/14	09/01	09/29	10/08	10/20	10/30
Pure bluegrass	6	0.12	0.39	0.47	0.62	1.15	0.38	0.22	0.18	0.13	0.16	0.09	1.17
Christie mixture	3	0.83	5.81	3.79	1.35	0.77	0.17	0.30	0.25	0.33	0.26	0.42	2.14
Tempus mixture	3	0.82	5.42	3.84	2.63	0.76	0.92	1.32	0.38	0.48	0.17	0.18	1.81
SEM		2.06	1.69	1.61	1.37	1.87	1.52	1.44	1.91	1.85	1.72	1.47	1.19
P-value[1]		0.091	0.007	0.017	0.04			0.073				0.069	0.062
P-value[2]							0.074	0.077					

[1] Probability values contrast mixed red clover-bluegrass versus pure bluegrass sward treatments

[2] Probability values contrast Christie mixture versus Tempus mixture

APPENDIX I: ANOVA TABLES

TRURO

---NITRATE---

Response variate: v[1] -> log10 15cm NO3 mg L
Fitted terms: Constant + jDate + Trmt + jDate.Trmt
Submodels: SSPLINE(jDate; 2)

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	10	44.16	4.4158	21.76	<.001
Residual	73	14.82	0.2030		
Total	83	58.98	0.7105		
Change	-4	-5.62	1.4040	6.92	<.001

Percentage variance accounted for 71.4
Standard error of observations is estimated to be 0.451.

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ SSPLINE(jDate; 2)	2	2.7684	1.3842	6.82	0.002
+ Trmt	4	35.7742	8.9436	44.06	<.001
+ SSPLINExTrmt	4	5.6158	1.4040	6.92	<.001
Residual	73	14.8171	0.2030		
Total	83	58.9755	0.7105		

Response variate: v[2] -> log10 45cm NO3 mg L
Fitted terms: Constant + jDate + Trmt + jDate.Trmt
Submodels: SSPLINE(jDate; 2)

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	10	31.253	3.12526	32.06	<.001
Residual	72	7.018	0.09747		
Total	82	38.271	0.46672		
Change	-4	-1.490	0.37249	3.82	0.007

Percentage variance accounted for 79.1
Standard error of observations is estimated to be 0.312.

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ SSPLINE(jDate; 2)	2	2.5829	1.2915	11.54	<.001
+ Trmt	4	27.1798	6.7949	60.70	<.001
Residual	76	8.5082	0.1119		
Total	82	38.2708	0.4667		

---PHOSPHATE---

Response variate: v[3] -> log10 15cm PO4 mg L
Fitted terms: Constant + jDate + Trmt + jDate.Trmt

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	9	10.853	1.20587	12.72	<.001
Residual	74	7.016	0.09481		
Total	83	17.869	0.21529		
Change	-4	-1.355	0.33880	3.57	0.010

Percentage variance accounted for 56.0
Standard error of observations is estimated to be 0.308.

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ jDate	1	1.02010	1.02010	10.76	0.002
+ Trmt	4	8.47748	2.11937	22.35	<.001
+ jDate.Trmt	4	1.35522	0.33880	3.57	0.010
Residual	74	7.01587	0.09481		
Total	83	17.86867	0.21529		

Response variate: v[4] -> log10 45cm PO4 mg L
Fitted terms: Constant + jDate + Trmt + jDate.Trmt
Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	9	0.50826	0.056473	50.13	<.001
Residual	68	0.07660	0.001127		
Total	77	0.58486	0.007596		
Change	-4	-0.28667	0.071667	63.62	<.001

Percentage variance accounted for 85.2

Standard error of observations is estimated to be 0.0336

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ jDate	1	0.050572	0.050572	44.89	<.001
+ Trmt	4	0.171017	0.042754	37.95	<.001
+ jDate.Trmt	4	0.286669	0.071667	63.62	<.001
Residual	68	0.076604	0.001127		
Total	77	0.584862	0.007596		

--DRY MATTER YIELD---

F pr. tables <= 0.1 where 0.000 = p<0.001

1 -> May09 cut 1 out/inside, t/ha
2 -> Jun09 cut 2 inside, t/ha
3 -> Jul09 cut 3 inside, t/ha
4 -> Aug09 cut 4 inside, t/ha
5 -> Sep09 cut 5 inside, t/ha

Source	d.f.	1	2	3	4	5
Rep stratum	1	.	.	.	.	.
Rep.Block stratum	.	.	.	.	.	.
NatPast	1	.	.	.	.	0.041
NatPast.Irr	1	.	.	0.095	.	0.020
Residual	2	.	.	.	.	.
Rep.Block.sPlot.fU stratum	.	.	.	.	.	.
NatPast.Forage	1	0.042	0.052	0.018	.	0.015
NatPast.Irr.Forage	1	.	.	.	.	0.092
Residual	10	.	.	.	.	.
Total	17	.	.	.	.	.

6 -> Oct09 cut 6 inside, t/ha
7 -> Total for season inside, t/ha
8 -> mean across inside cuts
9 -> lin across inside cuts
10 -> quad across inside cuts

Source	d.f.	6	7	8	9	10
Rep stratum	1	.	.	.	.	.
Rep.Block stratum	.	.	.	.	.	.
NatPast	1	.	.	.	0.063	.
NatPast.Irr	1	0.010	0.023	0.023	0.039	.
Residual	2	.	.	.	.	.
Rep.Block.sPlot.fU stratum	.	.	.	.	.	.
NatPast.Forage	1	.	0.005	0.005	.	.
NatPast.Irr.Forage	1	.	.	.	.	.
Residual	10	.	.	.	.	.

Total	17	.	.	.	.	.

--SWARD NITROGEN YIELD--

131 -> N May09 cut 1 out/inside, kg/ha
132 -> N Jun09 cut 2 inside, kg/ha
133 -> N Jul09 cut 3 inside, kg/ha
134 -> N Aug09 cut 4 inside, kg/ha
135 -> N Sep09 cut 5 inside, kg/ha

Source	d.f.	131	132	133	134	135
Rep stratum	1	.	.	.	.	.
Rep.Block stratum	.	.	.	.	.	.
NatPast	1	.	.	.	.	0.042
NatPast.Irr	1	.	.	.	.	0.009
Residual	2	.	.	.	.	.
Rep.Block.sPlot.fU stratum	.	.	.	.	.	.
NatPast.Forage	1	0.012	0.010	0.000	0.016	0.002
NatPast.Irr.Forage	1	.	.	.	.	.
Residual	10	.	.	.	.	.
Total	17	.	.	.	.	.

136 -> N Oct09 cut 6 inside, kg/ha
137 -> N Total for season inside, kg/ha
138 -> mean N across inside cuts
139 -> lin N across inside cuts
140 -> quad N across inside cuts

Source	d.f.	136	137	138	139	140
Rep stratum	1	.	.	.	.	.
Rep.Block stratum	.	.	.	.	.	.
NatPast	1	0.070	.	.	.	.
NatPast.Irr	1	0.017	0.065	0.065	.	.
Residual	2	.	.	.	.	.
Rep.Block.sPlot.fU stratum	.	.	.	.	.	.
NatPast.Forage	1	.	0.000	0.000	0.021	0.017
NatPast.Irr.Forage	1	.	.	.	.	.
Residual	10	.	.	.	.	.
Total	17	.	.	.	.	.

---N% IN BLUEGRASS---

121 -> N in Bg May09 cut 1 out/inside, %
122 -> N in Bg Jun09 cut 2 inside, %
123 -> N in Bg Jul09 cut 3 inside, %
124 -> N in Bg Aug09 cut 4 inside, %
125 -> N in Bg Sep09 cut 5 inside, %

Source	d.f.	121	122	123	124	125
Rep stratum	1	0.9203	6.492	0.9430	0.6235	0.1943
Rep.Block stratum	.	.	.	.	.	.
NatPast	1	0.7396	0.891	0.1296	0.0600	0.1320
NatPast.Irr	1	0.0036	0.394	0.2704	0.1024	0.0900
Residual	2	0.0018	0.906	0.0334	0.1124	0.1057
Rep.Block.sPlot.fU stratum	.	.	.	.	.	.
NatPast.Forage	1	1.9881	0.753	1.1025	1.0100	1.1556
NatPast.Irr.Forage	1	0.1296	0.191	0.0009	0.0729	0.2070
Residual	10	0.2106	0.425	0.1490	0.1083	0.1133
Total	17	.	.	.	.	.

126 -> N in Bg Oct09 cut 6 inside, %
127 -> N in Bg Mean for season inside, %
128 -> mean % N in Bg across inside cuts
129 -> lin % N in Bg across inside cuts
130 -> quad % N in Bg across inside cuts

Source	d.f.	126	127	128	129	130
Rep stratum	1	.	.	.	.	.
Rep.Block stratum	.	.	.	.	.	.
NatPast	1	.	.	.	.	.
NatPast.Irr	1	.	.	.	.	0.055
Residual	2	.	.	.	.	.
Rep.Block.sPlot.fU stratum	.	.	.	.	.	.
NatPast.Forage	1	0.001	0.001	0.001	.	.
NatPast.Irr.Forage	1	0.008	.	.	.	.
Residual	10	.	.	.	.	.
Total	17	.	.	.	.	.

NAPPAN

---NITRATE---

Response variate: v[1] -> log10 15cm NO3 mg L
Fitted terms: Constant + jDate + Trmt + jDate.Trmt
Submodels: SSPLINE(jDate; 2)

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	6	5.254	0.87571	11.89	<.001
Residual	26	1.915	0.07365		
Total	32	7.169	0.22404		
Change	-2	-0.945	0.47237	6.41	0.005

Percentage variance accounted for 67.1
Standard error of observations is estimated to be 0.271.

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ SSPLINE(jDate; 2)	2	2.15223	1.07611	14.61	<.001
+ Trmt	2	2.15729	1.07864	14.64	<.001
+ SSPLINE(jDate; 2).Trmt	2	0.94474	0.47237	6.41	0.005
Residual	26	1.91500	0.07365		
Total	32	7.16926	0.22404		

Response variate: v[2] -> log10 45cm NO3 mg L
Fitted terms: Constant + jDate + Trmt
Submodels: SSPLINE(jDate; 2)

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	4	3.889	0.9723	6.52	<.001
Residual	31	4.621	0.1491		
Total	35	8.510	0.2432		
Change	-2	-1.612	0.8061	5.41	0.010

Percentage variance accounted for 38.7
Standard error of observations is estimated to be 0.386.

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ SSPLINE(jDate; 2)	2	2.2768	1.1384	7.64	0.002
+ Trmt	2	1.6122	0.8061	5.41	0.010
Residual	31	4.6214	0.1491		
Total	35	8.5105	0.2432		

---PHOSPHATE---

Response variate: v[3] -> log10 15cm PO4 mg L
Fitted terms: Constant + jDate + Trmt + jDate.Trmt
Submodels: SSPLINE(jDate; 2)

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	6	0.4954	0.08257	7.50	<.001
Residual	24	0.2641	0.01100		
Total	30	0.7595	0.02532		
Change	-2	-0.1500	0.07502	6.82	0.005

Percentage variance accounted for 56.5
Standard error of observations is estimated to be 0.105

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ SSPLINE(jDate; 2)	2	0.13486	0.06743	6.13	0.007
+ Trmt	2	0.21052	0.10526	9.57	<.001
+ SSPLINE(jDate; 2).Trmt	2	0.15003	0.07502	6.82	0.005
Residual	24	0.26405	0.01100		
Total	30	0.75946	0.02532		

Response variate: v[4] -> log10 45cm PO4 mg L
Fitted terms: Constant + jDate + Trmt + jDate.Trmt
Submodels: SSPLINE(jDate; 2)

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	6	0.06135	0.0102251	14.62	<.001
Residual	21	0.01469	0.0006994		
Total	27	0.07604	0.0028162		
Change	-2	-0.00550	0.0027481	3.93	0.036

Percentage variance accounted for 75.2
Standard error of observations is estimated to be 0.0264

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ SSPLINE(jDate; 2)	2	0.0034491	0.0017246	2.47	0.109
+ Trmt	2	0.0524054	0.0262027	37.47	<.001
+ SSPLINE(jDate; 2).Trmt	2	0.0054962	0.0027481	3.93	0.036
Residual	21	0.0146870	0.0006994		
Total	27	0.0760377	0.0028162		

---DRY MATTER YIELD---

[Table]
1 -> Jun 8, DMY Cut 1, t/ha
2 -> Jul 14, DMY Cut 2, t/ha
3 -> Aug 14, DMY Cut 3, t/ha
4 -> Sep 23, DMY Cut 4, t/ha

Source	d.f.	1	2	3	4
Bg	2	0.010	0.068	0.008	.
Pure vs C,T	1	0.003	0.030	0.003	0.064
C vs T	1	.	.	.	.
Residual	9	.	.	.	.
Total	11	.	.	.	.

5 -> Season DMY 2009, t/ha
6 -> DMY, mean 2009
7 -> DMY, linear 2009
8 -> DMY, quad 2009

Source	d.f.	5	6	7	8
Bg	2	0.011	0.011	.	.
Pure vs C,T	1	0.004	0.004	.	.
C vs T	1	.	.	.	.
Residual	9	.	.	.	.
Total	11	.	.	.	.

[Figure]
Variate: v[5] -> Season DMY 2009, t/ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Bg	2	57.789	28.895	7.64	0.011
Pure vs C,T	1	55.855	55.855	14.77	0.004
C vs T	1	1.934	1.934	0.51	0.493
Residual	9	34.041	3.782		
Total	11	91.830			

Variate: v[6] -> DMY, mean 2009, t/ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Bg	2	3.6118	1.8059	7.64	0.011
Pure vs C,T	1	3.4909	3.4909	14.77	0.004
C vs T	1	0.1209	0.1209	0.51	0.493
Residual	9	2.1276	0.2364		
Total	11	5.7394			

Variate: v[7] -> DMY, linear 2009

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Bg	2	0.00001656	0.00000828	0.34	0.721
Pure vs C,T	1	0.00001631	0.00001631	0.67	0.434
C vs T	1	0.00000025	0.00000025	0.01	0.922
Residual	9	0.00021924	0.00002436		
Total	11	0.00023580			

--SWARD NITROGEN YIELD--

89 -> Cut 1, Jun 8 N kg/ha
90 -> Cut 2, Jul 14 N kg/ha
91 -> Cut 3, Aug 14 N kg/ha
92 -> Cut 4, Sep 23 N kg/ha

Source	d.f.	89	90	91	92
Bg	2	0.002	0.012	0.005	0.024
Pure vs C,T	1	0.000	0.004	0.002	0.008
C vs T	1	.	.	.	.
Residual	9	.	.	.	.
Total	11	.	.	.	.

93 -> Seasonal N kg/ha
94 -> mean N kg/ha
95 -> linear N kg/ha
96 -> quad N kg/ha

Source	d.f.	93	94	95	96
Bg	2	0.003	0.003	.	.
Pure vs C,T	1	0.001	0.001	.	.
C vs T	1	.	.	.	0.075
Residual	9	.	.	.	.
Total	11	.	.	.	.

--N% IN BLUEGRASS---

97 -> Cut 1, Jun 8 bg N%
98 -> Cut 2, Jul 14 bg N%
99 -> Cut 3, Aug 14 bg N%
100 -> Cut 4, Sep 23 bg N%

Source	d.f.	97	98	99	100
fU stratum	.	.	.	.	.
Bg	2	0.004	0.005	0.003	0.001
Pure vs C,T	1	0.001	0.001	0.001	0.000
C vs T	1	.	.	.	.
Residual	9	.	.	.	.
Total	11	.	.	.	.

101 -> Seasonal bg N%
102 -> mean bg N%
103 -> linear bg N%
104 -> quad bg N%

Source	d.f.	101	102	103	104
fU stratum	.	.	.	.	.
Bg	2	0.001	0.001	.	.
Pure vs C,T	1	0.000	0.000	.	.
C vs T	1	.	.	.	.
Residual	9	.	.	.	.
Total	11	.	.	.	.