Assessment of Nitrate Export in Agricultural Sub-Catchments of the Grand River Watershed:

An Isotope Approach

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.
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Thomas Fraser Cummings

ABSTRACT

Nitrate (NO_3^-) is one of the most widespread contaminants in freshwater systems globally. This is largely due to high fertilizer application rates that are required to maximize crop yield. Elevated NO_3^- concentrations can cause detrimental health effects to both humans and aquatic ecosystems. While the drinking water standard for NO_3^- in Canada is 10 mg N/L, this level is often surpassed in small headwater agricultural streams and tile drainage. Inorganic (synthetic) and organic (manure) fertilizers are the greatest non-point sources of NO_3^- to freshwater and groundwater systems in Canada. Inherent isotope fractionation during production of inorganic and organic fertilizers allows these N sources to be differentiated using the isotopic ratios of nitrogen and oxygen.

Small agricultural catchments play an important role in nutrient export to larger freshwater systems, however the source and timing of export is often unknown. The main objective of this thesis is to better understand the sources and processes governing NO_3^- concentration and export in four small agricultural catchments where large changes in nutrient concentration can occur over the course of a year. Land-use within these catchments is predominantly agriculture (>77%).

Geochemical and isotopic analysis of the four creeks, the Conestogo River, the Grand River as well as tile drain and shallow groundwater piezometers located along one creek (Boomer Creek) was undertaken. NO_3^- concentration varied seasonally, concomitantly with stream discharge, with increased concentrations and export occurring during the non-growing season at scales from small watershed to the Grand River. Peak concentrations (>10 mg N/L) occurred in late fall and early winter and lowest concentrations (<5mg N/L) occurred during the summer months. Median NO_3^- concentrations from tile drains (5.9 mg N/L) were much higher than that of groundwater samples (0.1 mg N/L). Further, groundwater NO_3^- concentrations remained consistently low even at times of high discharge and high NO_3^- concentrations in both the tiles and the creek, indicating that the creek is more influenced by water discharging from tile drains and not that of groundwater.

The studied creeks NO_3^- export varied seasonally with the greatest export occurring in all creeks during spring melt on both a per day basis and over the duration of the melt compared to other seasons. Summer low flow NO_3^- concentrations in the Conestogo River is a function of the NO_3^- concentration within the Conestogo Reservoir. Annual precipitation amounts as well as reservoir residence time are believed to be the largest factors governing reservoir NO_3^- concentrations and thus summer Conestogo River NO_3^- concentrations.

$\delta^{15}N$ and $\delta^{18}O$ analysis of NO_3^- was used to examine NO_3^- sources as well as seasonal changes in N-cycling processes. Both $\delta^{15}N$ and $\delta^{18}O$ were lower (+ 6 to + 8‰ and - 2 to + 1‰) in the fall and winter months with higher values (> + 10‰ and > + 4‰) during the summer for all creeks with the exception of the $\delta^{18}O$ of Swan Creek. Increased isotopic values occurred at lower NO_3^- concentrations and concomitant increases in both $\delta^{18}O$ and $\delta^{15}N$ in an expected ratio (approx. 1:2) providing strong evidence of the occurrence of denitrification. Annual fluctuations in concentrations of N_2O and CH_4 are also consistent with active denitrification. $\delta^{15}N$-NO_3^- values corrected for the isotopic effects caused by denitrification were consistent with that of values reported for manure (+ 8‰). Despite the $\delta^{15}N$ being within that of manure, the source of NO_3^- to these watersheds is inconclusive as a result several NO_3^- sources also falling within the same range. Isotopic analysis suggests that Boomer Creek stream water is similar to water discharging from tile drains along Boomer Creek. NO_3^- concentration and isotope data from tile drains and Boomer Creek indicate a strong influence from tile drains on annual stream NO_3^- concentration and export. The influence of tile drains will only become greater as 30 million meters of new tiles are installed annually in Ontario.

Understanding nutrient dynamics in small watersheds is vital to understanding and predicting water quality in larger surface water systems. This study re-emphasises the fact that understanding the seasonality in annual NO_3^- export requires knowledge of the N sources and processes that govern N transformation. Only with this knowledge can effective best management practices be implemented to successfully maintain surface and ground water quality.

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To begin, I would like to dedicate this thesis to my late grandfather Thomas Walter Cummings whose passion for knowledge inspired me as a child. I would like to thank my supervisor Dr. Sherry Schiff who first allowed me to work on this project, and then provided me with support and excellent feedback that helped me get my research to where it is today. In addition, I would like to thank my committee, Dr. Sherry Schiff, Dr. Will Robertson, Dr. John Spoelstra and Dr. Jason Venkiteswaran for their comments and suggestions.

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Chapter 1− Introduction

1.1 Introduction

Nitrate (NO_3^-) is one of the more wide spread contaminants found in groundwater and surface waters globally (Socolow 6001-6008). Its presence has largely resulted from the intensification and industrialization of agriculture in the second half of the 20th century (Novotny 1-13). Post 1950's, there was a shift in global farming practices towards large scale, intensively managed, monoculture farms. In order to maximize crop yields, fertilizer application to crops was greatly increased, leading to a large increase in nitrogen (N) loading to the local environment (Smil 569-601; Socolow 6001-6008; Novotny 1-13). Nitrogen in excess of crop and microbial requirements remains in the soil as residual soil nitrogen (RSN), most of which is in the form of NO_3^-, a highly mobile form of nitrogen that can be leached into the local groundwater (Janzen et al. 85-102; Novotny 1-13; Hill 696-702). Subsequently, NO_3^- leaches and contaminates surface waters through groundwater discharge to streams (Vitousek et al. 737-750), posing both an ecological and drinking water threat.

During the second half of the 20th century anthropogenically created nitrogen exceeded that derived naturally from the terrestrial environment; this caused a shift in the global nitrogen cycle (Galloway and Cowling 64-71). It is estimated that from 1890 to 1990 the amount of NH_3 emissions increased approximately 9 Tg N/yr to 43Tg N/yr as a consequence of food production and that river dissolved inorganic nitrogen fluxes to the ocean increased from 5 Tg N/yr to 20Tg N/yr. The increase in synthetic fertilizer production to ensure food production would meet increasing global demand has greatly affected the global nitrogen cycle.

Recently, Canada has made an effort to estimate the change in RSN between 1981 and 2006. A report conducted by the Agriculture and Agri-Food Canada (Eilers et al.) found that there had been an statistically significant ($p < 0.05$) increase in the amount of RSN in Canadian but not Ontario soils in this time frame (Fig. 1.1a and Fig. 1.1b). Although RSN in Ontario soils are not increasing significantly over time inputs are three times as high, resulting in a much larger amount of RSN. The peak in RSN in 2002 is thought to be a result of a drought year, which decreased crop yield and therefore nitrogen output. In 2006, a decrease in RSN was observed as a result of an increase in nitrogen output and better weather conditions (Eilers et al.).

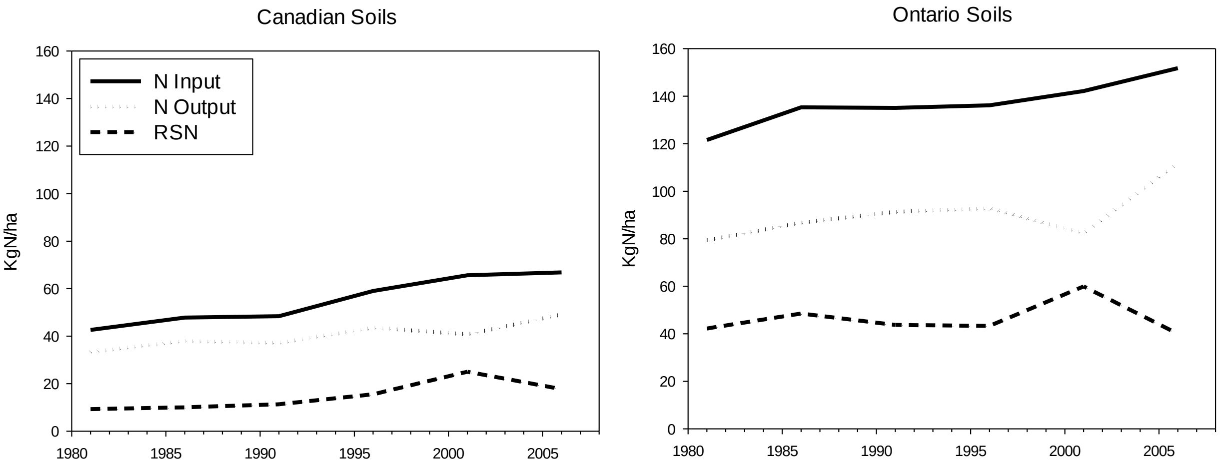


Figure 1.1a & 1.1b: Figure 1.1a: Nitrogen input, output and residual soil nitrogen (RSN) in Canadian agricultural soils between 1981-2006. Figure 1.1b: Nitrogen input, output and residual soil nitrogen (RSN) in Ontario agricultural soils between 1981-2006. Adapted from (Eilers et al.).

Greater amounts of RSN increase the threat of nitrogen leaching into local groundwater. Using a simplified equation, where nitrogen loss equals the difference between nitrogen outputs and inputs, Janzen et al. (2003) roughly estimated that, in 1996, 0.43 Tg N/yr ± 30% was leached from Canadian agroecosystems. Nitrate contamination often persists in agricultural watersheds even after better management practises (BMPs) have been put in place (Tomer and Burkart 2158-2171). As a result nitrogen in excess of crop and microbial requirements creates a legacy of contamination that poses a direct risk to the quality of the groundwater, and it's receiving waters.

High concentrations of NO_3^- leached from agricultural catchments can be detrimental to human health. The Ontario Safe Drinking Water Act, (O. Reg. 169/03, Sched. 2; O. Reg. 268/03, s. 1; O. Reg. 248/06, s. 2; O. Reg. 242/07, s. 1.) states that the maximum acceptable concentration of NO_3^- is 10 mg N/L and concentrations above this can cause adverse health effects to humans. Infants under 3-months of age are at a greater risk of adverse health effects, as the reduction of NO_3^- to NO_2^- in the gut has been shown to cause methaemoglobinaemia, also known as blue baby syndrome (Adam 79). Methaemoglobinaemia converts haemoglobin to methemoglobin, a haemoglobin form that cannot carry oxygen, leading to cyanosis and suffocation. Methaemoglobinaemia can also occur in fish species at elevated NO_3^- to NO_2^- concentrations. Health Canada has also listed NO_3^- /NO_2^- as possible carcinogens but stipulate that it is a weak relationship, with little reliable evidence. For this reason, understanding NO_3^- concentration and export from agricultural catchments into surface waters is important for drinking water supplies. Nitrate is also directly toxic to some aquatic life; the Canadian water quality guideline for that protection of aquatic life is 2.95 mg N/L (Canadian Council of Ministers of the Environment). Nitrate concentrations need to be minimized and monitored in order to ensure water quality for both drinking water and aquatic ecosystems.

1.2 Eutrophication

Eutrophication occurs as a result of excess nutrients entering water bodies and can lead to detrimental ecosystem effects, such as excess algal growth. Inorganic nitrogen and phosphorus (P) are the two principal nutrients that govern algal growth in aquatic ecosystems, and elevated levels of either of these nutrients can potentially cause eutrophication (Smith, Tilman, and Nekola 179-196). Eutrophication can cause several undesirable effects such as: the depletion of dissolved oxygen concentrations (harmful particularly to fish species), the proliferation of potentially harmful cyanobacteria, and the aesthetically displeasing appearance of excess algal growth (Smith 7-49). In temperate lakes, phosphorus is almost always the limiting nutrient to algal growth, and undesirable algal growth may be controlled by limiting phosphorus inputs (Schindler 260-262; Schindler et al. 11254-11258). In contrast, many coastal and estuarine systems have been observed to be sensitive to both phosphorus and nitrogen (Paerl 1154-1165; Howarth and Marino 364-376).

Agriculture represents a large non-point source of nutrients into the environment. The management and application of fertilizers must be well understood to avoid undesirable effects in both ground and surface waters.

1.3 Nitrate and the Nitrogen Cycle

The two major biological reactions that govern the amount of nitrogen as NO_3^- in the environment are nitrification and denitrification (Fig 1.2). In simplest terms, nitrification creates NO_3^- by the oxidation of reduced N, while denitrification breaks NO_3^- down by reduction.

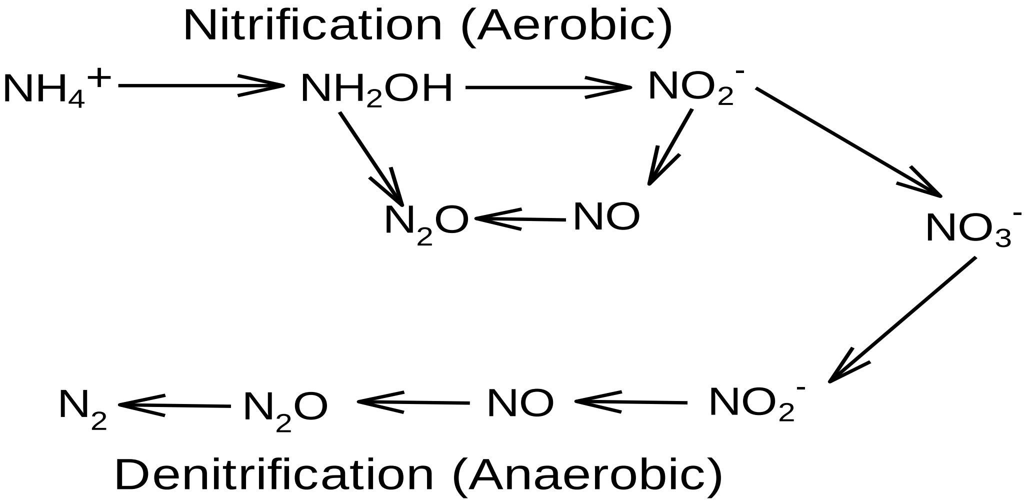


Figure 1.2: Nitrification and denitrification in the nitrogen cycle

Nitrification

In soils, NO_3^- is primarily created from nitrification, where ammonium (NH_4^+) is aerobically oxidized to NO_3^- through a series of biologically mediated reactions. For organic fertilizers such as manure, the organic nitrogen must first be transformed to an inorganic form (NH_4^+) through mineralization. In many agricultural soil environments chemolithoautotrophic bacteria and archaea are responsible for the conversion of NH_4^+ to NO_3^- (Schepers and Raun). Zhongjun et al. (2009) found that bacteria were the dominant nitrifiers in several agricultural soils when compared to archaea. The first step in nitrification involves ammonia oxidizing bacteria or archaea while the second involves nitrite (NO_2^-) oxidizing bacteria (Hayatsu, Tago, and Saito 33-45). The first step in nitrification is termed nitrosification, in which NH_4^+ is oxidized to NO_2^- through a hydroxylamine (NH_2OH) intermediate, NO_2^- is then further oxidized by microorganisms in a process called nitrite oxidation to produce NO_3^-. Bacterial NH_4^+ oxidation is predominantly performed by three genera of bacteria (*Nitrosomonas*, *Nitrosospira* and *Nitrosococcus*) whereas archaeal NH_4^+ oxidation is believed to be carried out by members of the phylum Crenarchaeota. Bacterial oxidation of nitrite is performed by four genera of bacteria (*Nitrobacter*, *Nitrospina*, *Nitrococcus* and *Nitrospira*).

Nitrification will occur in environments where there is sufficient NH_4^+ and oxygen. The optimal pH conditions for nitrifying bacteria is between 7 and 9, but nitrification will still occur at a pH of 6 (Allison and Prosser 935-941). A study conducted by Maag & Vinther (1996) on the effect of temperature on nitrification found the optimal nitrification temperature was 20°C, with decreasing efficiency at lower temperatures. In addition, N_2O production increased with a decrease in temperature. Another study conducted by Saad & Conrad (1993) used a broader temperature range and found that optimum nitrification occurred around 25-30°C. In highly acidic agricultural soils (pH 3-5) nitrification has been observed, suggesting that some species of *Nitrosospira* may have the ability to oxidize NH_4^+ (Hayatsu, Tago, and Saito 33-45). Nitrification has also been shown to occur throughout the year and contribute significant amounts of NO_3^- to the groundwater (Savard et al.).

Denitrification

Denitrification occurs when bacteria, in the absence of oxygen, use NO_3^- as the electron donor in order to produce energy. Denitrification is a series of reactions in which NO_3^- is reduced by microbial reactions to NO_2^- and further reduced to NO, N_2O, and finally to nitrogen gas (N_2). Denitrification is primarily carried out in nature by heterotrophic bacteria, i.e. *Pseudomonas* and *Paracoccus* (Knowles 43;

Hayatsu, Tago, and Saito 33-45). Heterotrophic bacteria will actively denitrify in anaerobic conditions when there is a water-extractable carbon source (Knowles 43; Bremner 7-16) or in an environment with an available source of pyrite (Tesoriero, Liebscher, and Cox 1545-1559). In addition, fungal species such as *Fusarium oxysporum* and *Fusarium solani* have been observed to reduce NO_3^- in low O_2 conditions (Hayatsu, Tago, and Saito 33-45). Recently several archaea have also been found to be capable of denitrification but there importance in the natural environment is currently unknown. Denitrification is temperature dependent and occurs optimally at temperatures of 25-30°C (Saad and Conrad 21-27). Maag & Vinther (1996) found that the ratio of N_2 to N_2O increased with increasing temperature from 5 to 20°C.

1.4 Sources of Nitrogen in the Environment

In general agricultural NO_3^- derived from nitrification receives a nitrogen atom originating from one of three main sources: the atmosphere, inorganic fertilizer, or manure/sewage. Reactive or fixed nitrogen in the terrestrial environment is naturally derived from the atmosphere in two ways; lighting in conjunction with precipitation, and biological fixation from cyanobacteria or leguminous plants (Vitousek et al. 737-750). According to a study conducted by Ro et al. (1988) the mean annual wet deposition of NO_3^--N between the years of 1981-1985 in southern Ontario was estimated to be 0.52 kg/km^2.

In the early 1900s, the Haber-Bosch process was created, a process by which nitrogen from the air is combined with hydrogen gas to synthesize NH_4^+. This process has allowed for cheap and efficient manufacturing of fertilizers, and revolutionized the agricultural industry by the middle of the century (Frink, Waggoner, and Ausubel 1175-1180).

Often referred to as the recycling of nitrogen because no new nitrogen is fixed, crop residues, manure and sewage also contribute large amounts of nitrogen to the environment by providing an organic nitrogen source that can be mineralized to NH_4^+. Both inorganic and organic fertilizers are typically applied in early spring prior to or during seeding to ensure nutrients are available during plant/crop growth. However, under the Nutrient Management Act, (O. Reg. 267/03, s. 60 (2); O. Reg. 338/09, s. 44,) stored manure must be removed at least once per year, or more frequently if the accumulated manure may produce adverse effects. Farmers must dispose of stored manure once they can no longer safely store it. Since manure is an organic form of nitrogen there is also time associated with the process of mineralization (organic nitrogen in the manure converted into an inorganic form, NH_4^+). As a result, manure residues from past years (as a result of exceeding the biological needs of the plants) are likely to increase the NO_3^- pool in the soil (Angle et al. 141-147). Although the effects were not quantified in the current study, tillage practices can also affect NO_3^- leaching to groundwater. Angle et al. (1993) found that soil NO_3^- concentrations under no-tillage fields were consistently lower when compared to conventional tillage. This was believed to result from increased denitrification and increased N uptake by crops under no-tillage soils. Some wastewater treatment plants are equipped with the capacity to nitrify their wastewater, decreasing the amount of NH_4^+ but increasing the amount of NO_3^- in their discharge.

1.5 Stable Isotopic Ratios of Nitrate within the Nitrogen Cycle

The analysis of stable isotope ratios can aid in NO_3^- source identification and provide information on N-cycling. Stable isotope analysis can be carried out on both the nitrogen ($^{15}N/^{14}N$) and oxygen ($^{18}O/^{16}O$) atoms that compose NO_3^-. Nitrogen has two stable isotopes ^{15}N and ^{14}N with relative abundances of 0.37% and 99.63%, respectively (Junk and Svec 234-243). Oxygen has three stable isotopes ^{18}O, ^{17}O and ^{16}O with relative abundances of 0.20%, 0.04% and 99.76%, respectively (Nier 789). Stable isotopic ratios are often reported as a delta (δ) value with the units in per mil (‰) in accordance to (Equation 1.1)

$$\delta = \left(\frac{R_{Sample}}{R_{Standard}} - 1\right)$$

Equation 1.1

Where R_{sample} is the isotopic ratio of the sample and $R_{standard}$ is the isotopic ratio of the standard. The isotopic standard for nitrogen is N_2 in atmospheric air ($^{15}N/^{14}N = 1/272$) and the isotopic standard from oxygen is the Vienna Standard Mean Ocean Water (VSMOW) ($^{18}N/^{16}N = 1/499$).

There are several factors that influence the isotopic ratios of both N and O. Fractionation is the preference of one isotope (reacts faster) over the other (reacts slower) in the products of chemical, physical or biological reactions (Kendall and Aravena 261-297). Often it is the case that the lighter isotope reacts faster and is more quickly incorporated into the products compared to the heavier isotope (Kendall and Aravena 261-297). In order to accurately estimate the source of NO_3^- the fractionation factors associated with biological and or chemical reactions involving NO_3^- must be known. In kinetic reactions, the fractionation factor (α) is defined as the isotopic ratio of the products relative to the substrate (reactants) (Equation 1.2):

$$\alpha_{p-s} = {}^{R_p}\!/_{R_s} \qquad \textbf{Equation 1.2}$$

Where R_p is the isotopic ratio of the products and R_s is the isotopic ratio of the substrate. Isotope enrichment factors (ε) are modelled as (Equation 1.3)

$$\varepsilon_{p-s} = (\alpha - 1) \qquad \textbf{Equation 1.3}$$

In 1st order reactions within closed systems with respect to the reactants, where there is a constant fractionation factor, the progression of the isotopic composition of the residual reactant (substrate) can be modelled by the formulation of the Rayleigh equation as (Equation 1.4).

$$\delta \approx \delta_0 + \varepsilon_{p-s}\ln(f) \qquad \textbf{Equation 1.3}$$

Where δ_0 is the initial composition (isotopic abundances) of the substrate, f is the fraction of the substrate remaining.

Fractionation is most likely to be observed during the rate determining step, or the slowest step of a reaction, when a large pool of substrate is able to develop because the amount of material used is small by comparison (Kendall and Aravena 261-297). In reactions where the substrate is quickly used, the development of a large pool is prevented, and observed isotopic fractionation of the substrate is minimal.

Volatilization

In context of the nitrogen cycle, volatilization is the loss of ammonia gas to the atmosphere from soils or organic waste reservoirs (Fig 1.3). Most of the nitrogen in manure is in the form of urea, which is hydrolysed to NH_4^+. During hydrolysis a hydrogen atom disassociates from NH_4^+ increasing the pH, which in turn favours the loss of ammonia gas by volatilization. This causes a kinetic fractionation in which the lighter isotope is preferentially lost in the gaseous products, resulting in the remaining NH_4^+ to become more enriched in ^{15}N (Kendall and Aravena 261-297). NH_4^+ remaining after volatilization can have $\delta^{15}N$ values in excess of 20‰ (Kendall, Elliott, and Wankel 375-449).

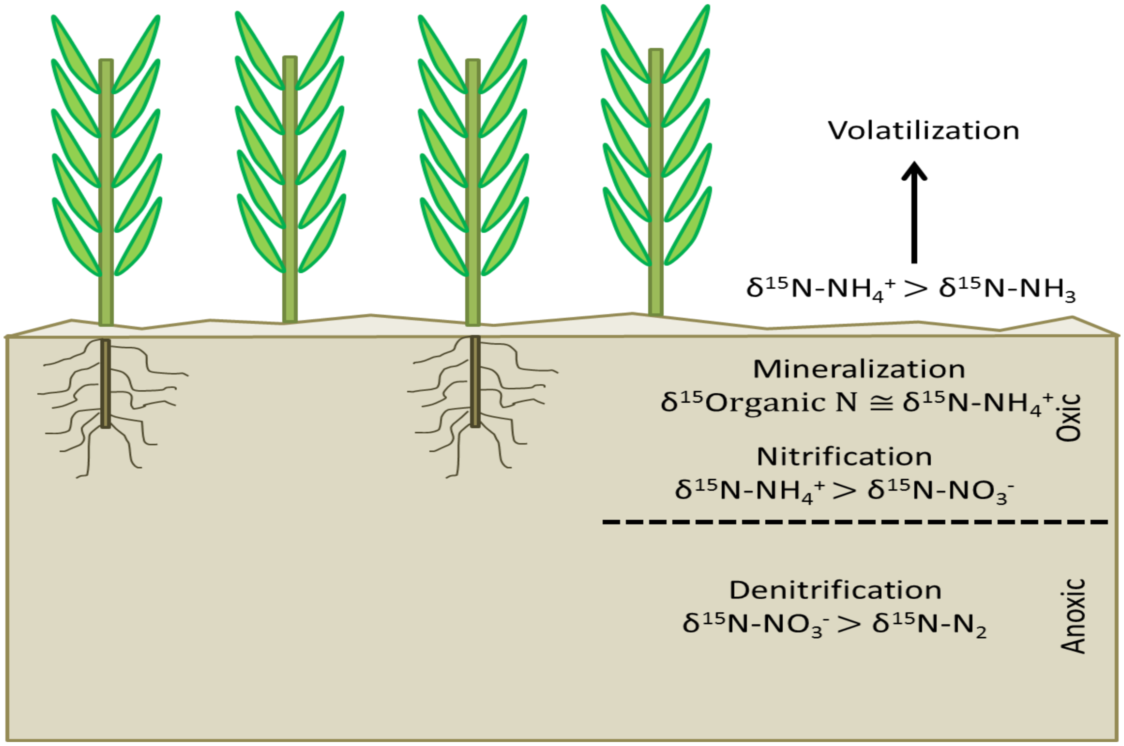


Figure 1.3: Simplified diagram of the isotopic fractionation of nitrogen within an agricultural environment, and the hypothetical division between the oxic and anoxic zone (not to scale). Mineralization: results in very little change in the $\delta^{15}N$ value of the resulting NH_4^+. Volatilization: causes the remaining NH_4^+ to have a greater $\delta^{15}N$ value. Nitrification: results in the remaining NH_4^+ having a greater $\delta^{15}N$, unless it is completely nitrified in which case the $\delta^{15}N$ will be similar to that of the initial $\delta^{15}N$ of NH_4^+. Denitrification: causes the remaining NO_3^- to have a greater $\delta^{15}N$ value.

In summary, stable isotopic ratios can allow for separation of NO_3^- sources and can aid in identifying the processes governing nitrogen transformation. In the context of this study, there were two probable sources of NO_3^- to these intensively agricultural watersheds: inorganic (synthetic) or organic fertilizer. The typical $\delta^{15}N$- $\delta^{15}N$ NO_3^- for synthetic fertilizers range from – 2 to + 4‰ (Gormly and Spalding 291-301; Kendall and Aravena 261-297; Wassenaar 391-405) whereas the typical range of $\delta^{15}N$-NO_3^- derived from manure is + 8 to + 22‰ (Kreitler and Jones 53-62; Wassenaar, Hendry, and Harrington 4626-4632). It is also possible that a small portion of NO_3^- is derived from precipitation, and although $\delta^{15}N$ NO_3^- can be similar to that of inorganic and organic fertilizers, precipitation has a distinctly higher $\delta^{18}O$ value (+ 28‰ - +75‰) making it distinguishable from other sources.

Assimilation

Assimilation is the transformation of inorganic N to organic N compounds through biological reactions. Mariotti et al. (1982) found that the fractionation of nitrogen from incorporation of NO_3^- into pearl millet (*P. americanum*) was dependent on NO_3^- concentration and plant nitrate reductase, with increased fractionation occurring at higher NO_3^- concentrations and lower nitrate reductase activity. During plant growth, isotopic fractionation decreased from day 12 (ε=-4.5‰) to day 25 (ε=-2.5‰). Nitrate reductase activity was also observed to increase with increasing plant age causing a decrease in the nitrogen fractionation.

Nitrification

The mineralization of organic N to inorganic N is associated with very little fractionation (Kendall and Aravena 261-297). In areas with large pools of NH_4^+, such as agricultural fields that have had organic or inorganic fertilizer applied, there can be significant fractionation (Kendall and Aravena 261-297). There are two steps in nitrification, the oxidation of NH_4^+, and the oxidation of NO_2^-. The rate-determining step in this reaction is likely the oxidation of NH_4^+as the oxidation of NO_2^- is rapid and prevents significant fractionation from occurring. In a closed system the instantaneous NO_3^- produced will be depleted compared to the NH_4^+. As the NH_4^+ pool decreases and becomes completely nitrified it becomes more enriched in $\delta^{15}N$, causing the $\delta^{15}N$ of the NO_3^- pool to approach that of the original $\delta^{15}N$-NH_4^+ pool values. Many studies have found that the $\delta^{15}N$ of NO_3^- closely reflects the $\delta^{15}N$ value of the NH_4^+ source (Kendall and Aravena 261-297). Prior models used to calculate the $\delta^{18}O$ value of NO_3^- produced by nitrification state that NO_3^- derived from nitrification contained two oxygen atoms from water and one oxygen atom from oxygen (O_2), however recent experiments conducted by Snider et al. (2010) indicate that other isotopic effects as well as oxygen exchange with H_2O must be taken into consideration. If not, this may lead to inaccurate estimation of the $\delta^{18}O$ value of NO_3^- produced from nitrification. Bacterial nitrification generally results in the $\delta^{18}O$ of NO_3^- having a value between 0‰ and + 20‰ (Snider et al. 5358-5364). However, several field experiments have found $\delta^{18}O$ values higher than this, possibly resulting from evaporation or a change in O source apportionment (Kendall and Aravena 261-297; Kendall, Elliott, and Wankel 375-449).

Denitrification

In anoxic environments the reduction of NO_3^- becomes thermodynamically favourable and NO_3^- remaining in the soil, groundwater, or stream may undergo denitrification. Denitrification can be an effective way of reducing NO_3^- concentrations in groundwater under the proper conditions. Denitrification is the step-wise sequential reduction of NO_3^- to N_2. As with nitrification, the kinetic nature of the process allows for significant fractionation to occur, whereby the remaining NO_3^- has elevated $\delta^{15}N$ and $\delta^{18}O$ values (Kendall and Aravena 261-297; Kendall, Elliott, and Wankel 375-449). The approximately 1:2 relationship between $\delta^{18}O$ and $\delta^{15}N$ values of the residual NO_3^- that results from denitrification has been observed in many groundwater studies (Aravena and Robertson 975-982; Mengis et al. 448-457; Cey et al. 45-67). A $\delta^{18}O$:$\delta^{15}N$ ratio of 1:1 has been observed for lab incubations of marine denitrifying bacteria (Granger et al. 2533). Denitrification enrichment factors for soil denitrifies which convert $NO_3^- \rightarrow N_2$ range from -19‰ (Snider, Schiff, and Spoelstra 877-888) to -38‰ (Tilsner et al. 249-267). A denitrification ^{15}N enrichment value ($\varepsilon^{15}N$) of -27.6‰ will be used for calculations, as this was found to be the enrichment factor in a similar catchment (Strawberry Creek) within the Grand River Watershed, with the assumptions that Strawberry Creek is of comparable size, land-use, soil type and climate (Mengis et al. 448-457).

Nitrate Source Determination

If the stable isotopic ratios are known for NO_3^- it is possible to determine the source of NO_3^- by using a NO_3^- dual isotope source plot (Fig 1.4). Several authors have made compilations of $\delta^{15}N$-NO_3^- (Fogg et al. 418-426; Kendall and McDonnell) and $\delta^{18}O$-NO_3^- (Kendall and McDonnell) of varying NO_3^- sources, allowing for the estimation of a NO_3^- source based on the $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- values. The NO_3^- source plot is broken down into different boxes, the location of which has been determined empirically. Each of these boxes is an approximate range of $\delta^{15}N$ and $\delta^{18}O$ of potential sources of NO_3^-. As similar $\delta^{15}N$ values can come from several sources; e.g., atmospherically derived NO_3^- and nitrified NH_4^+, $\delta^{18}O$ values are often used in conjunction to differentiate sources. NO_3^- derived from fertilizer NH_4^+ can be subdivided into fertilizers having an inorganic source and fertilizers that have an organic source, such as manure.

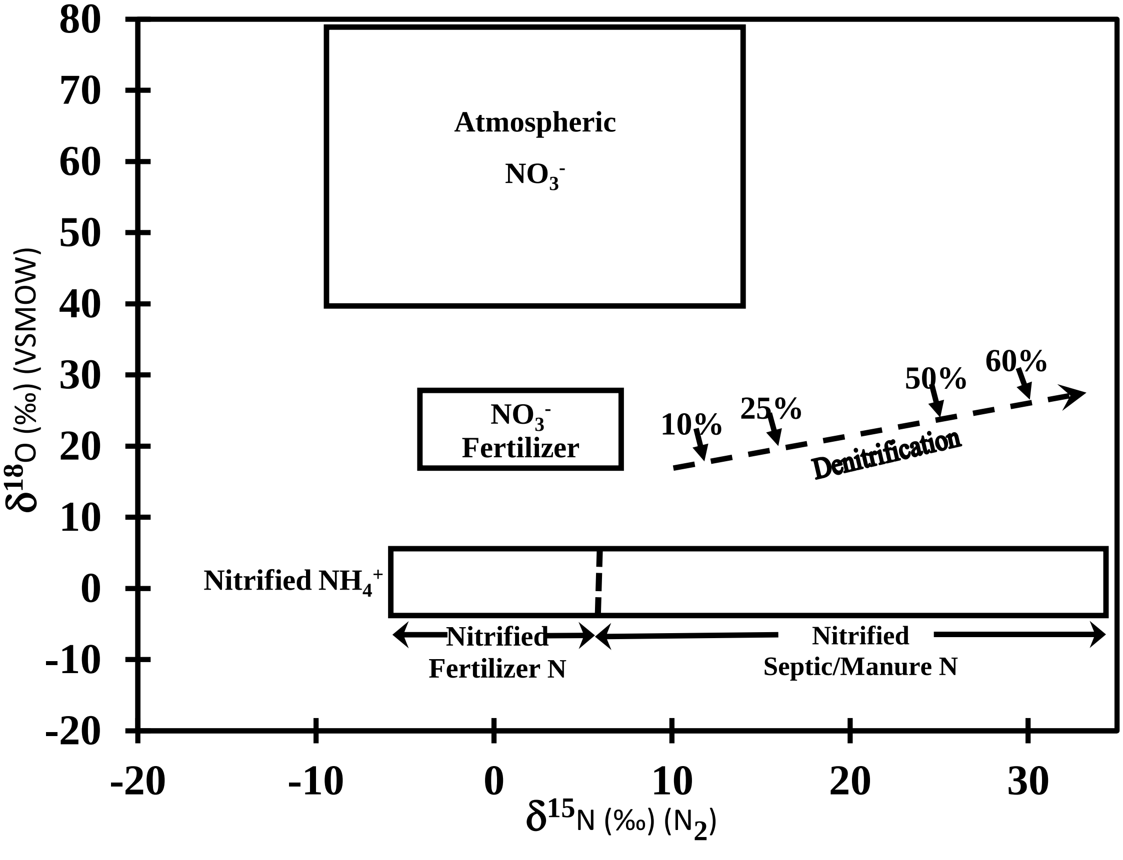


Figure 1.4: NO_3^- source plot. Boxes indicate empirical $\delta^{15}N$ and $\delta^{18}O$ of different NO_3^- sources. The denitrification line (dashed line) represents the relationship between $\delta^{15}N$ and $\delta^{18}O$ values of the residual NO_3^- that results from denitrification

and has been observed in many groundwater studies to typically be regarded as a 2:1 ratio (Kendall and McDonnell). The percentages on the denitrification line correlate to the fraction of NO_3^- that has been denitrified. The location of the percentages on the denitrification line is dependent on the enrichment factor.

1.6 Tile Drains

Tile drains are porous pipes installed within the subsurface of farm fields that work to ensure excess water is quickly drained from the soils, often into a ditch or nearby creek. This is advantageous as it allows farmers to till and plant their crops earlier during the spring, under what would normally be saturated soil conditions. Seasonality plays a large role in controlling the saturated conditions of the soil as temperature and precipitation cause the water table to fluctuate. Tile drains will flow during times when the water table is above the tile depth. During the fall and spring, precipitation and/or snowmelt increases, and evapotranspiration due to plant growth is minimal, causing the water table to rise and the tile drains to flow. In the winter, depending on air temperatures and soil conditions, tile drains may flow at certain times such as during mid-winter melt events. During the summer, a combination of increased plant evapotranspiration, decreased precipitation, and increased air temperature cause the elevation of the water table to decrease, and as a result there can be very little to no flow coming from the tile drains (Fig 1.5). Due to the high solubility of NO_3^-, groundwater directly below agricultural fields can be easily contaminated by excess NO_3^- not taken up by crops in the growing season. Water in the absence of tile drains will follow the local natural shallow groundwater flow system, slowly making its way to natural discharge areas. NO_3^- within the water that follows this natural flow path has the possibility to denitrify or mix with other groundwater sources, both of which can result in a decrease in the NO_3^- concentration of the groundwater. As a result of tile drains, groundwater transit time between agricultural fields and nearby streams is drastically reduced, and elevated concentrations of NO_3^- can be discharged directly into local surface water features. This becomes a large factor in creek NO_3^- concentration during melt events or large precipitation events occurring after crops have been harvested.

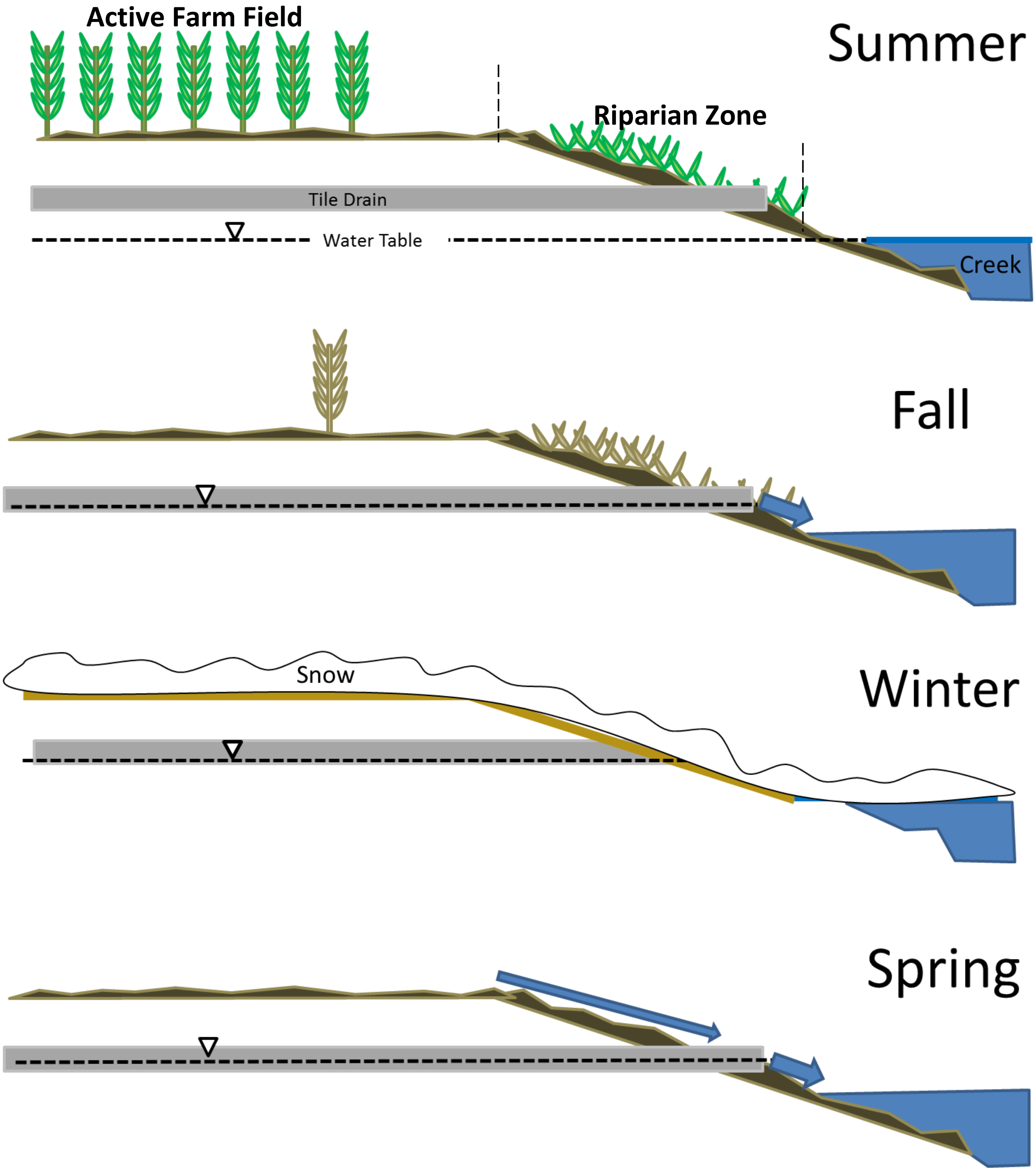


Figure 1.5: Seasonal schematic cross-section of a generalized active farm field, riparian zone and adjacent creek. Arrows indicate water flow coming from either tile drains (fall and spring) or surface run-off (spring).

1.7 Research Objectives

The overall objective of this thesis is to try to identify the sources and processes governing NO_3^- concentration and export in small agricultural catchments within the Grand River Watershed. The importance of this research is that several communities use the Grand River as their source of drinking water and in order to prevent pre-treatment of drinking water, sources and processes governing NO_3^- concentrations must be identified so that surface water and therefore drinking water contamination can be minimized. Ecologically this research is important as excess nutrients in fresh water aquatic ecosystems often causes various undesirable effects and by understanding NO_3^- sources and processes, better management strategies can be put in place to better aquatic ecosystem health. This thesis contains four chapters: Chapter 1 is an introduction to the nitrogen cycle and the use of stable isotopic ratios of NO_3^-, Chapter 2 describes an investigation of annual NO_3^- fluctuations in four agricultural catchments within the Grand River watershed as well as the Conestogo and Grand River, Chapter 3 contains the applicability of NO_3^- stable isotopic ratios in source and process identification of NO_3^- from four agricultural catchments within the Grand River watershed and Chapter 4 is a summary of the key findings and recommendations.

The objective of Chapter 2 was to investigate both inter and intra annual fluctuations of NO_3^- concentration in four small agricultural catchments and compare this to historical data. This information will better allow for drinking water treatment managers to have improved predictions for when increased NO_3^- concentrations will occur, and may also help dictate hydrological conditions when fertilization of agricultural fields should be conducted. Seasonal concentrations were then used in conjunction with flow data to estimate export of NO_3^- to downstream water bodies. The relationship between land-use and NO_3^- concentration was also investigated, as well as the change in NO_3^- concentration on receiving water bodies caused by these agricultural catchments. Finally, possible causes for the seasonal fluctuations in NO_3^- concentration seasonality were examined.

Chapter 3 investigates the applicability of using stable isotopic ratios to aid in NO_3^- source and processes identification. Isotopes were also used to investigate the processes governing NO_3^- concentration.

Finally stable isotopic ratios were used as a tool to assist in identifying the cause of NO_3^- concentration seasonality.

Chapter 2 – Role of streams in small agricultural catchments on NO_3^- concentrations and export

2.1 Introduction

The addition of excess nutrients to water systems can pose a risk to both drinking water resources and downstream water bodies. The influx of excess nutrients can lead to eutrophication and nutrient concentrations that are above the drinking water limit. Agricultural catchments can be a major source of nutrients to nearby surface water bodies; exporting large amounts of nitrogen downstream. Increased inputs of nutrients, such as nitrogen and phosphorus, have proven to lead to eutrophication in temperate lakes (D. Schindler, Armstrong, Holmgren, & Brunskill, 1971). In order to protect surface drinking water and ecosystem health, NO_3^- concentrations and export in small agricultural catchments must be understood to ensure that drinking water and aquatic ecosystem quality can be protected and maintained.

Nitrate (NO_3^-) is a contaminant in many fresh water systems globally. This is due in most part to the intensification of agriculture leading to increased fertilizer application during the 20th century. As a result of nitrogen in excess of crop and microbial requirements large amounts of nitrogen often remain in agricultural soils. This nitrogen can leach into local groundwater and eventually discharge to surface water bodies. The majority of water that enters into agricultural creeks originates from tile drains or groundwater discharge. Several studies have examined groundwater NO_3^- contamination in southern Ontario watersheds (Cey, Rudolph, Aravena, & Parkin, 1999; Robertson, Russell, & Cherry, 1996). Streams and creeks that drain agricultural catchments often have elevated NO_3^- concentrations (Omernik, 1976). These creeks are highly susceptible to large scale increases in nutrient export during significant hydrological events such as large precipitation events and snow melt events as nutrients are quickly flushed out of the soils into the creeks (Poor & McDonnell, 2007).

There are two forms of inorganic nitrogen that cause concern for drinking water, NO_3^- and nitrite (NO_2^-). The Ontario water quality standards for NO_3^- and NO_2^- are 10 mg N/L and 1.0 mg N/L respectively.

NO_3^- concentrations in excess of 10 mg N/L can cause adverse health effects, particularly to infants. The Grand River is the primary source of drinking water to the residents of Brantford and the Ohsweken community, with populations of approximately 93000 and 6000 as of 2011 (Statistics Canada). In addition NO_3^- is also directly toxic to some aquatic life; the Canadian water quality guideline for the protection of aquatic life is 2.95 mg N/L (Canadian Council of Ministers of the Environment). The Conestogo River and the agricultural creeks examined in this study enter the Grand River upstream of these communities. The Conestogo River is divided between its upper and lower reaches by the Conestogo Reservoir, whose primary purpose is to prevent flooding of downstream communities and augment downstream river flows during the summer. Conestogo Dam management practices typically store water in the spring months and slowly release it over late spring and summer months to maintain downstream river flows. This general pattern is followed but may differ from year to year in response to weather.

The Grand River also dilutes the wastewater coming from two major wastewater treatment plants located in Waterloo and Kitchener. Prior to 2010, the Kitchener wastewater treatment plant released nitrogen primarily as ammonium (NH_4^+) to the Grand River (Earth Tech). Starting in 2010, the Kitchener wastewater treatment plant underwent upgrades to their treatment system to nitrify the wastewater (converting NH_4^+ to NO_3^-) before it is released to the river. This combination of NO_3^- sources to the river could pose serious water quality problems to aquatic ecosystems and communities downstream.

The Grand River Watershed represents approximately 25% of the drainage for Lake Erie, therefore knowledge of sources of potentially harmful nutrients such as nitrogen and phosphorus within the Grand River Watershed are important as these nutrients will eventually flow into Lake Erie. During the 1960's and 1970's excess phosphorus from point and non-point sources from Canada and the United States caused large algal blooms and decreased water quality in Lake Erie (Michalak et al. 6448-6452). As a result Canada and the United States implemented phosphorus reduction strategies to mitigate the phosphorus pollution from point sources. These reduction strategies resulted in a decrease in large scale harmful algal blooms until the mid-1990's when there was a resurgence of annual large scale algal blooms. Since the mid-1990's there has been an upward trend in the amount of phytoplankton biomass in Lake Erie (Scavia et al., 2014). 2011 saw the largest bloom on record covering an estimated area of 5000 km^2 (Michalak et al. 6448-6452). Increases in the magnitude of algal biomass in recent decades is thought to partially result from increasing non-point sources such as agriculture. High NO_3^- concentrations in groundwaters as a result of agriculture may indirectly cause the release of phosphorus from soils. In addition to organic carbon NO_3^- can also be reduced by ferrous iron, ferrous iron bearing carbonates (siderite) and sulphides (pyrite) (Smolders et al. 1-7). Of particular interest is the reduction of NO_3^- by iron sulphides which results in an increased concentration of sulphate (SO_4^-) in the groundwater. The reduction of SO_4^- yields sulphide which interferes with the phosphorus sulphur cycle, which in turn decreases a soils capacity to retain phosphorus which can ultimately result in the increased mobility of phosphorus. As a result downstream receiving bodies may have increased phosphorus concentrations leading to a greater chance of eutrophication, which was indirectly caused by high NO_3^- concentrations.

The overall objective of this thesis chapter is to gain a better understanding of 1. NO_3^- concentration and export with season, and 2. the physical or chemical processes and sources controlling NO_3^- export in small agricultural catchments in the Grand River Watershed. This study focused on NO_3^-, both a current and legacy contaminant in groundwater in southern Ontario, which will continue to be a nuisance in the future. The effect of land-use on annual stream NO_3^-concentration was also investigated. Presented here are the NO_3^- concentrations and export estimates from the Conestogo River and four agricultural creeks within the Grand River watershed.

2.2 Study Sites

The Conestogo River is a 6th order river that drains a total area of 819 km^2 and is a major tributary to the Grand River (Fig 2.1). The 66 km Conestogo River flows from its headwaters in Arthur, Ontario down to Conestogo Ontario where it enters into the Grand River. The Conestogo River Watershed land-use is characterized as 83% agricultural (cultivated annual and mixed crops) (The Ontario Ministry of Natural

Resources). In addition to the Conestogo River, four small agricultural catchments were also examined as part of the present study (Fig 2.1; Table 2.1).   .

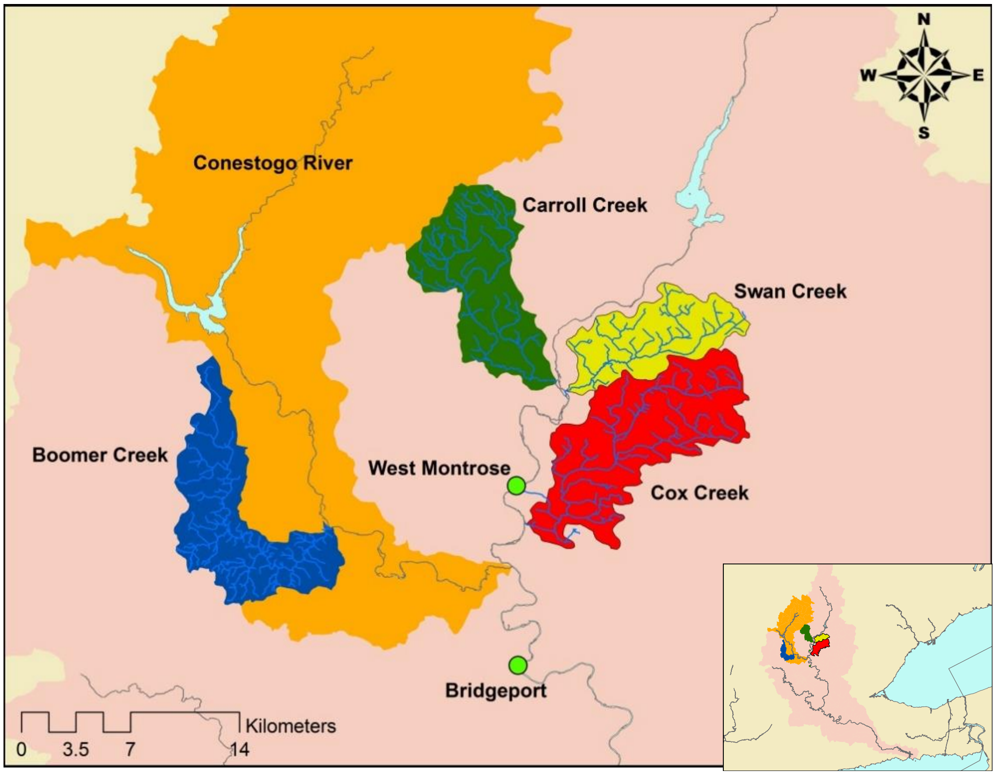


Figure 2.1: Map of the Conestogo and the agricultural catchments studied (Grand River Conservation Authority, 2014)

Table 2.1: Characteristics of the four studied agricultural watersheds. Drainage area, approximate channel Length, % land-use and mean annual flow (Ministry of Natural Resources and Forestry). Stream Order (Grand River Conservation Authority). % tile drains. N-Manure (Ontario Ministry of the Environment). N-Fertilizer and N Total (Ministry of Agriculture, Food and Rural Affairs). *Mean annual discharge was approximated by the Ministry of Natural Resources Ontario Flow Assessment Tool III.

Catchment	Drainage Area (km^2)	Approximate Channel Length (km)	% Land-use (Agriculture) of total area	Stream Order	% Tile Drains of total area	N-Manure (10^5 kg)	N-Fertilizer (10^5 kg)	N-Total (kg/ha)	*Mean Annual Discharge (m^3/s)
Boomer Creek	70	37	86	5	61	8.31	3.97	175.7	0.78
Cox Creek	87	34	79	4	29	3.97	4.89	101.8	1.0
Swan Creek	44	20	77	4	20	1.61	1.51	70.9	0.50
Carroll Creek	65	34	83	5	36	4.37	3.92	127.5	0.71

Boomer Creek is a tributary to the Conestogo River while the other creeks are small tributaries of the Grand River entering upstream of the Conestogo River. These catchments are impacted by non-point source nitrogen sources, with no municipal wastewater effluent discharges. The Conestogo River also dilutes effluent from the St. Jacobs (population 1900) wastewater treatment plant representing a point-source of nitrogen to the river (Statistics Canada). Agriculture is the dominant land-use in these watersheds, ranging from 77 to 86% (Table 2). Tile drains underlie 20-61% of the area within these watersheds. Tile drains are porous pipes installed under agricultural lands that allow excess water to be quickly drained from the field. The water from the field is often drained into a ditch or nearby creek. This is advantageous as it allows farmers to till their fields and plant their crops during the spring under what would be normally saturated soil conditions. Septic systems from local households may also be contributing nutrients to these creeks, but due to the low population density within these watersheds it is believed that septic systems would have little impact on NO_3^- concentrations within these creeks when compared to NO_3^- originating from agricultural, and thus were not separately quantified here.

Sampling of the Conestogo River was conducted at four locations beginning approximately 2.5 km downstream of the Conestogo Reservoir and ending approximately 1.5 km from where the Conestogo River enters into the Grand River (Fig 2.2). The creeks in this study were sampled at locations that were easily accessible, and close to where they discharge to the Conestoga River. Background geochemical data for Boomer and Cox Creek was obtained through the Provincial Water Quality Monitoring Network (PWQMN).

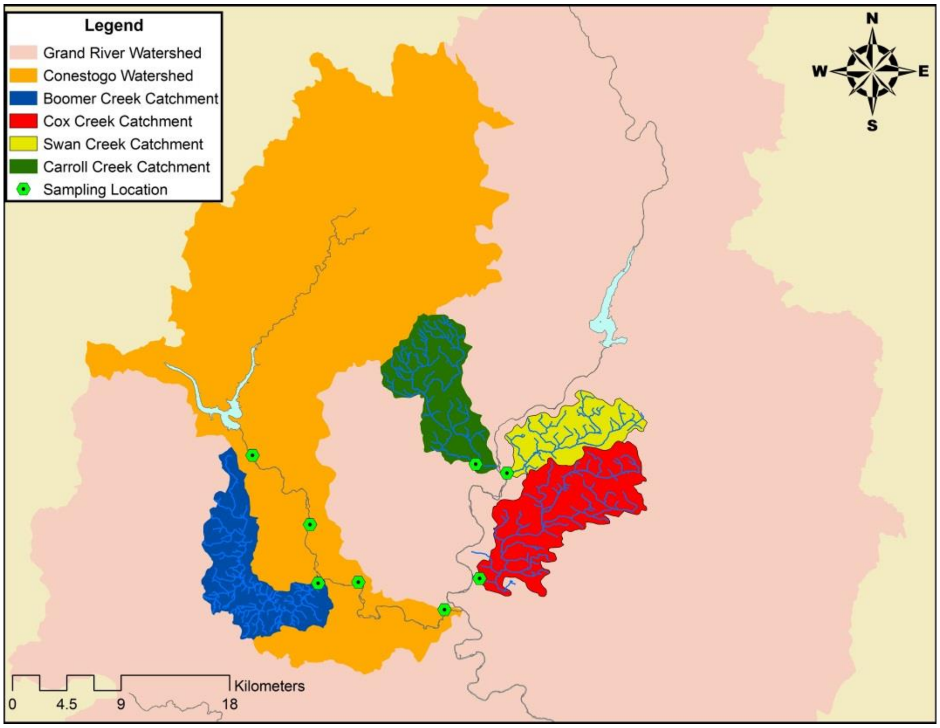


Figure 2.2: Map of field sampling locations within the agricultural catchments studied

2.2.1 Climate

The Grand River Watershed is classified in the Humid High Moderate Temperate Ecoclimatic Region (Ecoregions Working Group). The historical 30-year (1981-2010) mean temperatures and precipitations in January and July are -7.2°C, 19.8°C and 74.8, 89.8 mm, respectively (Fig 2.3). The data were taken from the Glen Allan Environment Canada meteorological station (43°41'02.1"N 80°42'37.1"W), which is located within the Conestogo watershed and is the best long-term weather station in proximity to the study sites (Fig 2.3).

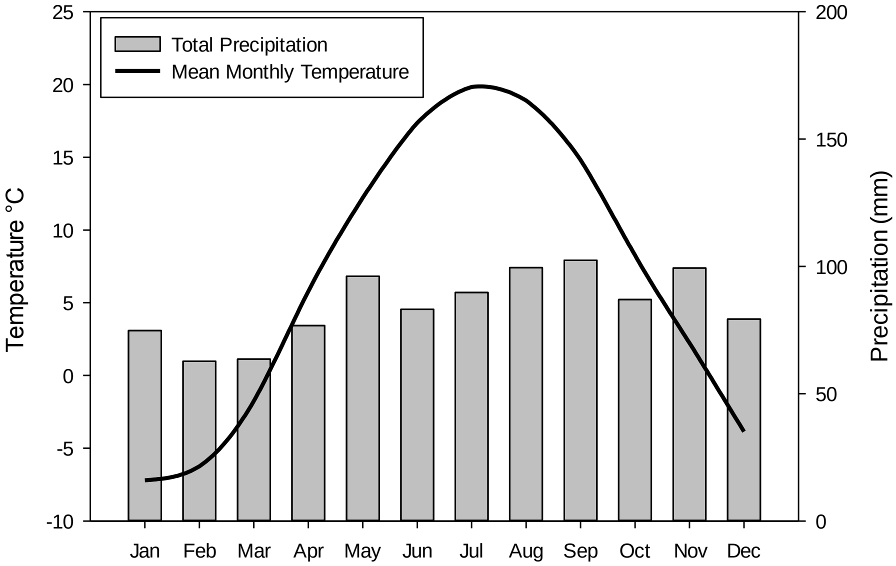


Figure 2.3: 30 year climate history as recorded at Glen Allan. Environment Canada (2014).

Temperature and precipitation data gathered during the study period were taken from the University of Waterloo’s Weather station (Seglenieks) (43°28’24.58”N, 80°33’25.95”W) as mean monthly temperature and total precipitation data were not available from Glen Allan. Both precipitation (Fig 2.4) and temperature (Fig 2.5) during the study period (August 2012 to December 2013) periodically deviated from that of the Waterloo Wellington 2 Environment Canada weather station 30-year average, which is used by the University of Waterloo weather station. Between January 2012 and August 2012, six out of the eight months had on average 45mm less precipitation than average, and 2012 was the second hottest year in the region on record with eleven months being above average (Seglenieks). The largest deviations from the average occurred between January and August with temperatures deviating above the average by 3.6°C. In contrast 2013, had mean monthly temperatures that deviated on average by only 1°C. Precipitation was remarkably different in 2013 as it was the wettest year on record with six months having above average precipitation, large amounts of precipitation were seen particularly in July and October when > 80mm above the average was recorded (Seglenieks).

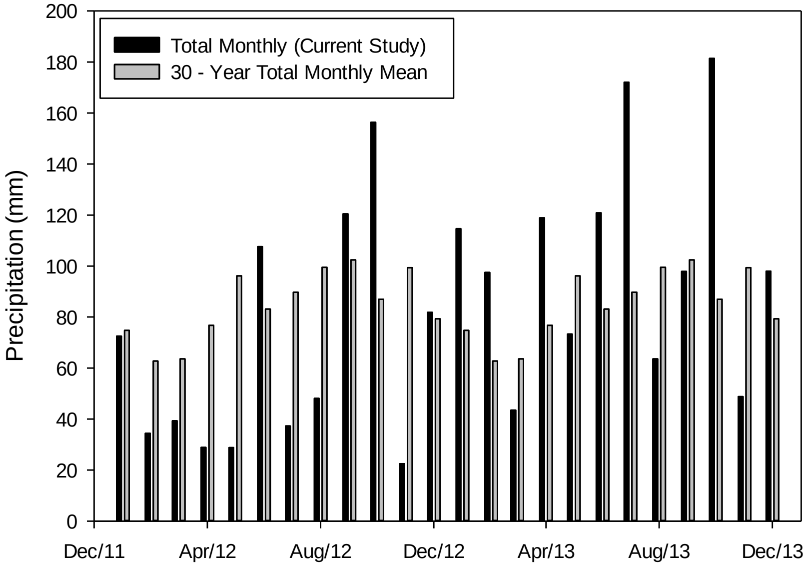


Figure 2.4: Monthly total precipitation data recorded at the University of Waterloo weather station. The 30 – year monthly mean data were collected at Glen Allan.

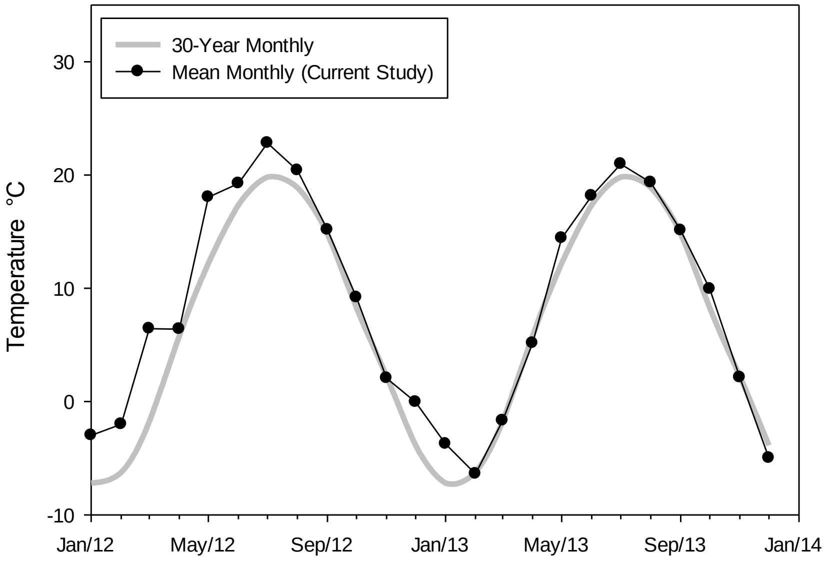


Figure 2.5: Monthly mean daily temperature data recorded at the University of Waterloo weather station. The 30 – year monthly mean data was collected at Glen Allan

Precipitation occurring between the months of February to August 2012 was below average causing a dry summer, with greater precipitation occurring during September and October. In 2013 however, the University of Waterloo weather station recorded the wettest year on record with only 3 months with below average precipitation. Eleven out of the twelve months in 2012 had mean temperatures above average. Warmer than average temperatures and little precipitation during the winter months of 2012 resulted in a low snow pack, causing only a small spring melt. Average temperatures during 2013 were much closer to the 30-year average.

The difference between “wet” (defined as above average precipitation) and “dry” (defined as below average precipitation) years on both the Conestogo River and Boomer Creek was also examined in conjunction with this study. The definition of wet and dry was based on the University of Waterloo weather station which uses the 30-year average (1971-2000) from the Waterloo Wellington 2 weather station as determined by Environment Canada to estimate average precipitation and range. For this region the average total annual precipitation is 904.0 mm with a range of 847.4 – 982.3 mm (Seglenieks).

2.2.2 Hydrology

The flow of the Conestogo River in the lower reaches is controlled by the Conestogo dam (43°40'31.3"N 80°42'57.3"W). The dam was constructed in 1958 and was primarily designed as a flood control during spring melt or extreme hydrological events and flow augmentation during low flow summer periods. Operation of the Conestogo reservoir follows a characteristic pattern of filling during the spring when river flows are high, and slowly discharging water throughout the summer months. The Conestogo dam releases water from the bottom of the reservoir.

Flow in the lower reaches of the Conestogo River follows a predictable annual pattern of higher flows during the spring and fall and lower flows during the winter and summer; which is largely regulated by the Conestogo dam. Flow data for the Conestogo River was taken at the St. Jacobs flow monitoring station (43° 32' 28" N 80° 33' 12" W) , which is approximately 8.2 km upstream from the mouth of the Conestogo River (Grand River Conservation Authority). The flow of the Conestogo River is also measured at Glen Allan but, because this gauge is located in close proximity to the Conestogo dam and complicated by macrophyte growth, the flow at St. Jacobs is more representative of flow conditions (Mark Anderson, personal communication, June 26, 2013).

2.2.3 Surficial Geology

The surficial geology of the study area is characterized by heterogeneous glacial outwash deposits summarized in Table 2.2 (Ontario Geological Survey). The Conestogo watershed is characterized by the Tavistock Till in the north, Elma Till in the northwest and Mornington Till in the southwest. The lower reaches of the watershed to the mouth of the Conestogo River also consist of glacial outwash deposits. The Boomer creek watershed consists primarily of Mornington Till with smaller deposits of gravel, sand, clay as well as Catfish Creek Till and Tavistock Till close to its mouth. Cox Creek consists primarily of

Wentworth Till with small amounts of sand and gravel. Swan Creek is dominated by the Wentworth Till as well as glacial outwash and to a smaller degree the Guelph formation (dolomite). Carroll Creek consists of Wentworth Till, Tavistock Till, gravel, sand and some Catfish Creek Till.

Table 2.2 : Characteristics of surficial Geology of study sites (Ontario Geological Survey).

Formation	Age	Lithology	Permeability
Tavistock Till	Late Wisconsian (Port Bruce Stade)	Clayey silt till	Low
Elma Till	Late Wisconsian (Port Bruce Stade)	Stoney sandy silt till	Low-Medium
Mornington Till	Late Wisconsian (Port Bruce Stade)	Silty clay till	Low
Catfish Creek Till	Late Wisconsinan (Nissouri Stade)	Sandy to silty, stoney till	Low-Medium
Wentworth Till	Late Wisconsian (Port Bruce Stade)	Sandy Till	Low-Medium

2.3 Methodology

The Conestogo River was sampled on multiple occasions between August 2012 and December 2013. Sampling of the agricultural creeks began in the fall of 2012 and continued until December 2013. Tile drains and shallow groundwater piezometers samples along Boomer Creek were collected during the fall of 2013 and spring of 2014 (Fig 2.6). Sampling of both tile drains and adjacent piezometers was done to compare NO_3^- concentrations between water coming from the tile drains and water from shallow groundwater in the riparian zone. Water originating from tile drains was collected more frequently since groundwater piezometers were not installed until late fall 2013. Piezometers were purged in spring 2014, one day before samples were taken. Sampling of the tile drains was conducted three times between October 2013 and December 2013, and four times in the spring of 2014. Paired sampling of both tile drain and shallow groundwater was conducted on three occasions. Water samples from the Conestogo River and the agricultural creeks were typically collected every two weeks.

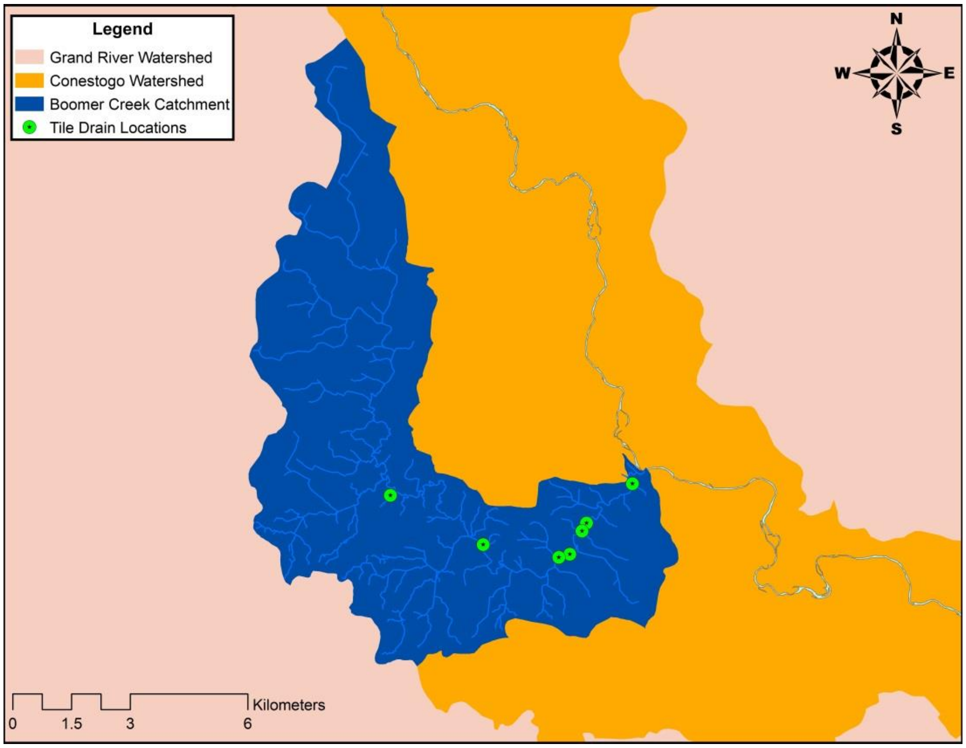


Figure 2.6: Map of tile drain sampling locations along Boomer Creek.

Increased sample frequency occurred during some major precipitation or melt events. Storm or spring melt sampling typically consisted of a background measurement, followed by measurements taken during the storm or melt event and then a post event measurement when flow had returned to background levels. Return to background flow normally occurred within a week or two after the storm/melt event had begun depending on the duration and intensity of the event. This allowed for the changes in NO_3^- concentration as a result of a storm or melt event to be accurately defined. The data collected represent at least one storm or melt event from each season with the exception of the summer.

Sampling of the Grand River was also conducted during 2013 to understand the influence of the Conestogo River on water quality in the Grand River. Sampling was conducted at two locations, upstream (West Montrose) and downstream (Bridgeport) of the Conestogo River confluence (Fig 2.1).

All water samples were collected and analysed for NO_3^-, NO_2^-, NH_4^+, nitrous oxide (N_2O), and total nitrogen (TN). When samples were taken, field parameters were also measured such as dissolved oxygen

(DO), pH, temperature and specific conductivity using a Hach HQ30d meter with attached IntelliCAL™ LDO, IntelliCAL™ PHC301 and IntelliCAL™CDC401 probes. Samples for chemical analysis were collected in 40-mL Starplex containers, 50-mL centrifuge tubes for TP and SRP, and 40-mL glass vials for DOC and TN. Samples for dissolved gases were collected in 60-mL serum bottles, capped with no headspace using a baked red Vaccutainer stopper and preserved with 0.2 mL of saturated mercuric chloride solution. Samples collected in the field were kept cool during transport back to the University of Waterloo. All water samples were filtered using a Whatman 0.45-μm syringe tip filter except for TP and N_2O. Filtering was done in the field during the summer and fall months. During the winter and spring, 1-L plastic bottles were used to collect water samples and were filtered back at the University of Waterloo Environmental Geochemistry Laboratory. Samples were subsequently frozen until analysis with the exception of DOC and dissolved gases. The concentration of organic nitrogen was found indirectly by subtracting inorganic forms of nitrogen (NO_3^-, NO_2^- and NH_4^+) from total nitrogen and assuming the difference is organic nitrogen.

Manual velocity measurements on Boomer creek were completed at various times using a Marsh McBirney® flow meter. To calculate flow the width of the stream was divided into 12.5cm transects, depth was found at the start and end of each transect and a cross sectional area was calculated from these measurements. Flow was then calculated by multiplying the velocity found at the 50% depth of each transect and multiplying by the cross sectional area. Total flow was estimated by summing all the flows calculated in each transect. This flow data was then compared to flow measurements taken from the Floradale watershed, which is of a comparable area, is located within the Grand River watershed, and has continuous flow measurements taken by the GRCA. The mouth of the Floradale watershed is located approximately 9.5 km northeast of the mouth of Boomer Creek. From this comparison a relationship was developed where past flow conditions for Boomer Creek over the time of study could be estimated based on the continuous flow data available from Floradale.

None of the other agricultural creeks studied had flow measurements taken or flow data available during the time of study (August 2012 to December 2013), as a result, the Floradale Creek flow data was used to develop flow relationships from past flow conditions so that flow during the current study could be estimated. Past (2004 to 2008) daily mean flow estimates for Carroll, Cox and Swan Creek were modelled by the GRCA using the GAWSER model. These records were then compared to historical flow records from Floradale Creek. The years with the most complete Floradale Creek flow records (2005 and 2008) were compared to flows modelled by the GRCA. A relationship comparing modelled (GAWSER) flow from each creek to historical flows on Floradale Creek was then created by performing a model 2 regression analysis. In all creeks, 2008 was found to have a better R^2 value then 2005. Boomer Creek, which did not have historical or modelled data available, was measured manually 10 times during 2013 and 2014; the relationship to Floradale Creek was then developed through model 2 regression analysis by comparing manual flow measurements on Boomer Creek to those of historical measurements taken by the GRCA at Floradale Creek. The relationship between the flow at Boomer Creek and that of Floradale Creek was found to have an R^2 value of 0.97; of the other creeks Carroll Creek had the best R^2 value of 0.33 whereas Cox and Swan Creek had values of 0.22 and 0.21, respectively (Fig 2.7). The relationship for each creek was then applied to Floradale hourly flow data from the current study period to estimate daily discharge from Carroll, Cox and Swan Creek. Seasonal discharge was calculated by summing daily discharge for a given season. Percent discharge per season was also calculated for the water year of October 18th 2012 to October 17th 2013.

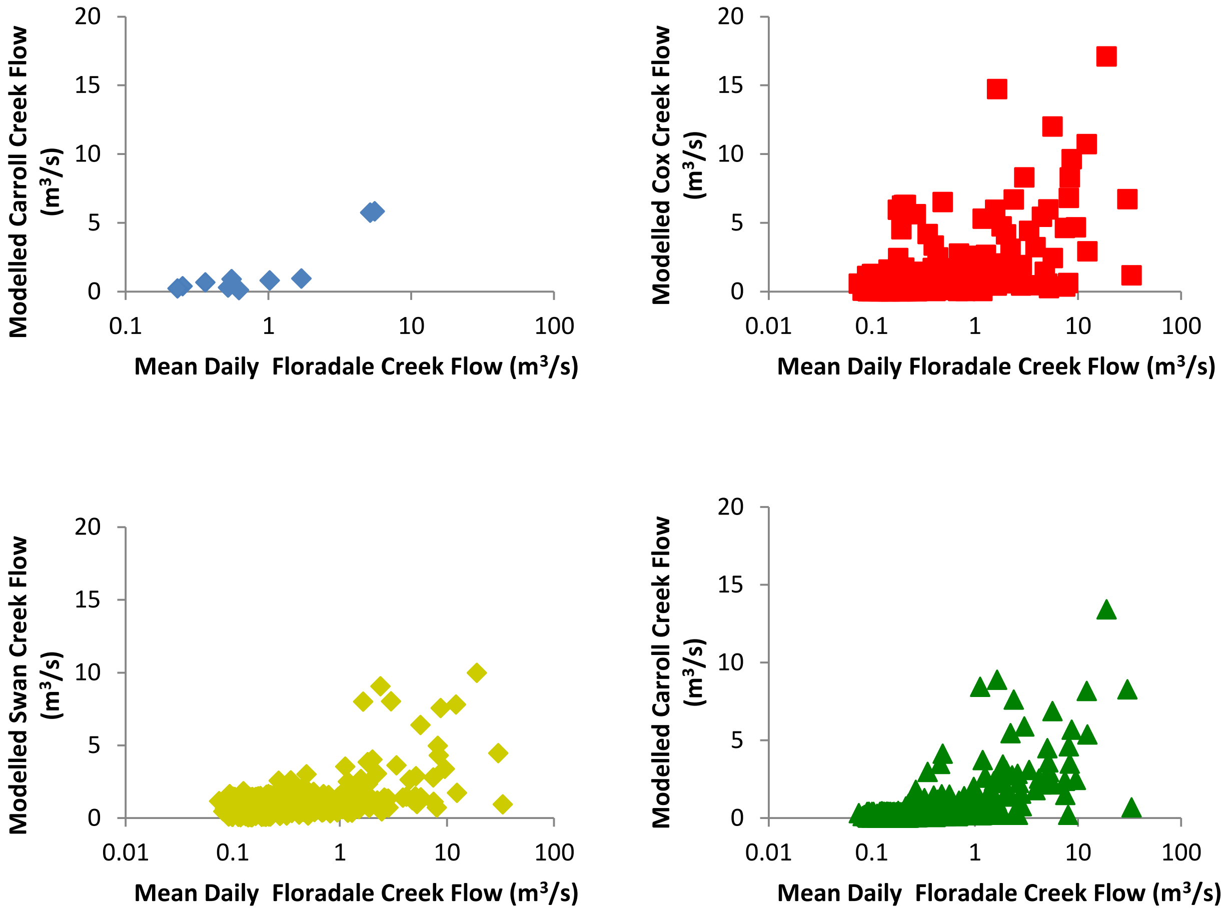


Figure 2.7: Correlation between creek flow and Floradale flow. Flow data from Cox Creek, Swan Creek and Carroll Creek is modelled (Gauser) mean daily flow for the year of 2008. Mean daily flows from Floradale Creek were taken from the year 2008. Boomer Creek flows were measured manually between November 2013 and July 2014. Floradale flow data used to compare to Boomer Creek flow data was recorded when manual measurements of Boomer Creek flow were taken.

In order to estimate NO_3^- export from each creek, NO_3^- concentration data had to be interpolated between sampling dates. Two methods were used to estimate export. The first method was a simple linear interpolation between sampling dates in which the change in concentration between sampling dates was divided equally between sampling times. This method assumes a constant change in concentration between sampling times. The second method used was based on a method created by Swistock et al. (1997) here after referred to as Method 2. In summary, this method takes the mean concentration between two consecutive sampling dates and applies this concentration to all days over the given time interval. NO_3^- concentrations were then multiplied by daily discharge from each creek.

These calculations provide an estimation of NO_3^- export from these catchments. Due to the nature of the data, there are however several inherent inaccuracies in these calculations. First, there were several assumptions that had to be made in order for these calculations to be possible. It was assumed that Floradale was a comparable catchment to all the other catchments with respect to discharge fluctuations. Also flow data, whether they were modelled or not, were assumed to be accurate and representative of flow conditions at the given time. Due to the lack of frequency of high flows, high flow data may be unrepresentative. Low R^2 values indicated there wasn't a good correlation between modelled creek flows and historical Floradale flow measurements particularly during times of high flow (storm flow). Although Boomer Creek did have an R^2 value of 0.97 the relationship between Boomer Creek and Floradale was based on only 10 manual flow measurements and only two were from times of elevated flows, making this high R^2 value likely unrealistic. Creek export from the fall of 2012 may be underestimated as a result NO_3^- concentration not being collected prior to October 31st 2012 for Carroll, Cox and Swan Creek and October 23rd for Boomer Creek, because no available concentration data was available during this time there was no export calculated for these days. Any export that occurred during this time is not known and therefore was not included.

Manure and crop data was provided by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), manure data was provided on the dissemination tract scale (resolution 10-30 km), while crop data was given on field tract scale (0.1 km resolution) (Ontario Ministry of Agriculture). Fertilizer nitrogen loads were calculated by multiplying crop type area by the recommended application of nitrogen fertilizer per hectare set out by the Ontario Ministry of Agriculture and Foods in the 2014 Field Crop Budgets. Fruits, vegetables, herbs and nursery crops which did not have a recommended fertilizer application amount together occupy < 1% of agriculture land within all the watersheds and therefore fertilizer N contributions from these crops was ignored. Land classified as "Beans" was considered coloured beans and land classified as "Other Crops" was considered flax when assigning N application rates. Land classified as either

"Hay/pasture" or "Soybeans" was assigned a value of zero as nitrogen fertilizer application is not recommended. The following assumptions were made: i) fertilizers are applied at the rate of crop requirement as recommended by the Ontario Ministry of Agriculture, Food and Rural Affairs (2014) and ii) crop N removal is zero. As a result this may lead to an over estimation in total N as N removal is ignored.

Nitrate Concentration

Water samples were analyzed for NO_3^- concentrations using a Dionex ICS-90 ion chromatography machine, equipped with an IonPac AS14A column and AS40 automated sampler. Samples were corrected to a calibration curve, created using standards that were run at the same time. The minimum detection limit (MDL) for this analysis was 0.05 mg N/L.

Nitrite Concentration

Water samples were analyzed for NO_2^- concentrations using a Unity Scientific SmartChem 200 (Discrete Analyzer) based on the method outlined by the USEPA EPA 353.2 Revision 2.0 (1993). The nitrite concentrations are measured by diazotizing the sample with sulphanilamide followed by coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly coloured azo dye which is measured colorimetrically at 550 or 520nm. The (MDL) for this method is 0.001 mg NO_2-N/L.

Ammonium Concentration

Water samples were analyzed for total ammonia nitrogen ($NH_4^+ + NH_3$) concentrations using a Unity Scientific SmartChem 200 (Discrete Analyzer) based on the method outlined by the USEPA EPA 350.1 Revision 2.0 (1993). The sample is buffered at a pH of 9.5 with a borate buffer and then distilled into a 2% solution of boric acid. Ammonia reacts with alkaline phenol and hypochlorite to form indophenol blue, the colour is intensified through the addition of sodium nitroprusside and measured colorimetrically at 630nm. The (MDL) using this method to determine total ammonia nitrogen concentration is 0.003 mg NH_4-N /L.

Total Nitrogen Concentration

Water samples were analyzed for Total Nitrogen (TN) using a Shimadzu Total Organic Carbon (TOC-L) analyzer coupled with a Total Nitrogen (TNM-L). Nitrogen is converted to NO by the combustion furnace. Using a carrier gas the sample is passed through a non-dispersive infared detector (NDIR). The carrier gas with the sample then proceeds to the nitrogen module where NO is converted to the excited state NO_x by mixing NO with ozone (O_3). Chemiluminescence photodiode detector (CLD) detects the light given off by NO_x when it returns to its ground state. The (MDL) for this method is 0.001 mg TN N/L.

2.4 Historical Data

2.4.1 Historical Nitrate Concentration Data

The Conestogo River enters the Grand River east of the town of Conestogo. Past research at the University of Waterloo has been conducted on nutrient concentrations both upstream (West Montrose) and downstream (Bridgeport) of where the Conestogo River enters the Grand River (Fig 2.8).

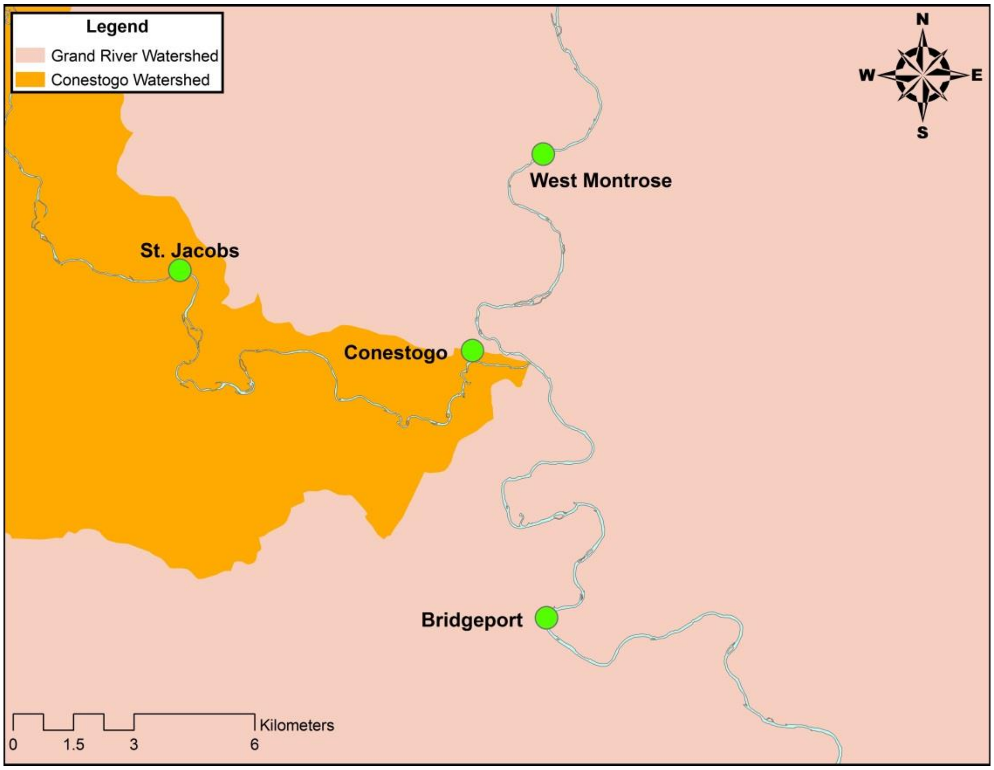


Figure 2.8: Map of the Conestogo and Grand River Watershed with sampling locations upstream (West Montrose) and downstream (Bridgeport) of the Conestogo River on the Grand River, as well as the continuous flow monitoring location on the Conestogo River (St. Jacobs).

Historical NO_3^- concentration data from these studies reveals a seasonal pattern of increased NO_3^- concentrations in the fall and winter, and decreased concentrations occurring in the summer months (Fig 2.9).

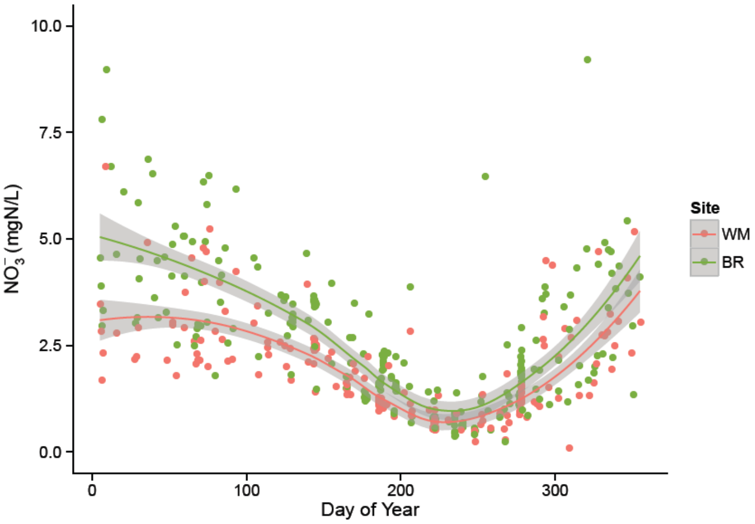


Figure 2.9: Historical (2005-2011) NO_3^- concentrations from upstream (WM) and downstream (BR) of the mouth of the Conestogo River on the Grand River (Schiff, unpublished data).

The downstream location (Bridgeport) has consistently elevated NO_3^- concentrations when compared to the upstream location of West Montrose and the difference is greatest during the winter. In addition to the Conestoga River, two wastewater treatment plants discharge to the Grand River between West Montrose and Bridgeport.

Ontario's Provincial Water Quality Monitoring Network (PWQMN) has historical nutrient concentration data available for two of the creeks studied: Boomer Creek and Cox Creek. Because of recent changes to the riparian zone by land owners to improve water quality in Boomer Creek, the geochemical historical data available for Boomer Creek is from the years 2007-2011. Cox Creek is no longer monitored by the PWQMN but historical geochemical data was available for the years of 1990-1996. The Conestogo River is also monitored by the PWQMN at St. Jacobs, which is approximately 8.2 km upstream from the mouth. Samples taken by the PWQMN are gathered typically on a monthly basis between March and November, and, as a result, storm flow and winter concentrations are typically not represented.

The historical NO_3^- concentration data of these creeks (Fig 2.10) show a pattern of increased concentrations in the winter/early spring and fall, and decreased concentrations during the summer. Data from Boomer creek displays a large seasonal range in NO_3^- concentrations with peaks of ~7.0-7.5 mg N/L occurring in the early spring and fall and minimum concentrations < 1.0 mg N/L approaching detection limits in the summer. Cox creek NO_3^- concentrations during the early spring are ~ 6 mg N/L with fall peak concentrations of 7.6 mg N/L. Summer concentrations decreased to 1-2 mg N/L.

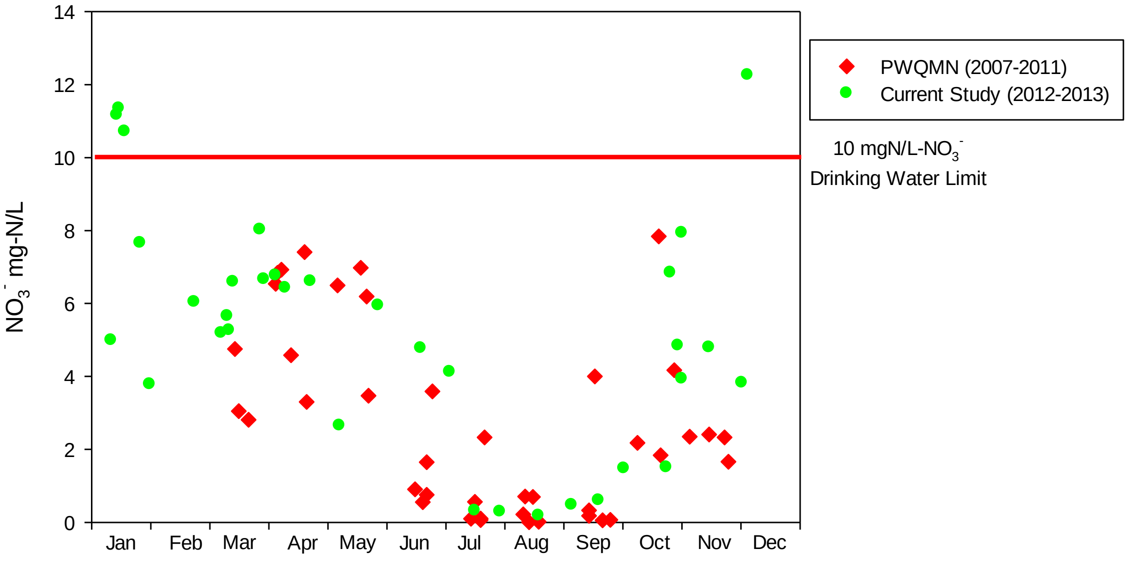


Figure 2.10: Annual NO_3^- concentration data (2007-2013) for Boomer Creek. 2007-2011 NO_3^- concentration data was collected and archived by the PWQMN. Data from 2012-2013 was collected in the current study. The red line represents the 10 mg N/L-NO_3^- provincial drinking water limit.

2.4.2 Historical Flow Data

Historical flow data was also used to divide the year into seasons based on the flow regime of Floradale Creek. This was done because dates corresponding to a certain season do not always indicate the flow conditions within the river. Flow data from the last five years (2009-2013) was taken and then normalized by dividing by the mean annual flow (Fig 2.11). Five distinct seasonal flows were then categorized based on flow (Table 2.3).

Highest flows occurred at several times during the year, including: fall with the onset of heavy precipitation, early January winter melt events coupled with precipitation, spring melt, and late spring with increased precipitation. Increased flows were also seen during the summer likely as a result of heavy, short-lived storms. For this region the average total annual precipitation is 904.0 mm with an average range of 847.4 – 982.3 mm (Seglenieks).The years 2010, 2011 and 2013 were classified as years with more than average precipitation, 2012 had below average precipitation and 2009 was classified as a year with average precipitation.

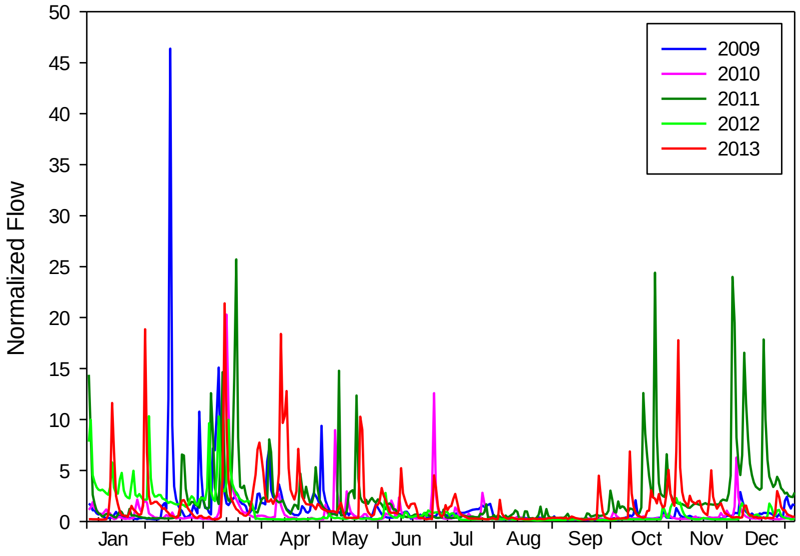


Figure 2.11: Floradale normalized flow 2009-2013. Flow was normalized to mean annual flow.

Table 2.8: Annual seasonal divisions based on flow data from Floradale Creek. Start date, end date and duration are based on patterns observed in normalized flows 2009-2013.

Season	Start Date	End Date	Duration (Days)
Winter	December 25	February 28	65
Spring Melt	March 1	April 15	45
Spring	April 16	June 14	60
Summer	June 15	October 17	125
Fall	October 18	December 24	70

Since 1999, an effort has been made by the GRCA and land owners to improve the riparian zone along Boomer Creek in an effort to improve water quality and aquatic habitat (Grand River Conservation Authority et al.). Farmers in the Boomer Creek Watershed fenced off approximately 13 km of stream, and approximately 8.5 km of treed buffers were planted in order to improve the conditions of the riparian zone along Boomer Creek. This resulted in a marked increase in fish abundance and diversity (Grand River Conservation Authority et al.). According to the report produced in 2007, the increase in fish abundance and species was attributed to an increase in water quality due to restoration of the riparian zone around Boomer Creek.

2.5 Results

2.5.1 Annual Nitrate Concentration Patterns

NO_3^- was the primary form of nitrogen within the creek and river water samples (Fig 2.12) and therefore the primary nitrogen form of interest for this study. NO_2^- concentrations did not exceed 0.2 mg N/L in all creeks with the exception of Boomer Creek on October 23, 2012 and May 8th, 2013 and Cox Creek in July and early September 2013. The highest NO_2^- concentration (0.5 mg N/L) was observed in Cox Creek on July 4th, 2013. NH_4^+ concentrations did not exceed 0.2 mg N/L, except during late February and early March and briefly in July in Cox Creek and October in the Conestogo River. Boomer Creek had the highest observed NH_4^+ concentration of all creeks of 0.9 mg N/L on March 11, 2013.

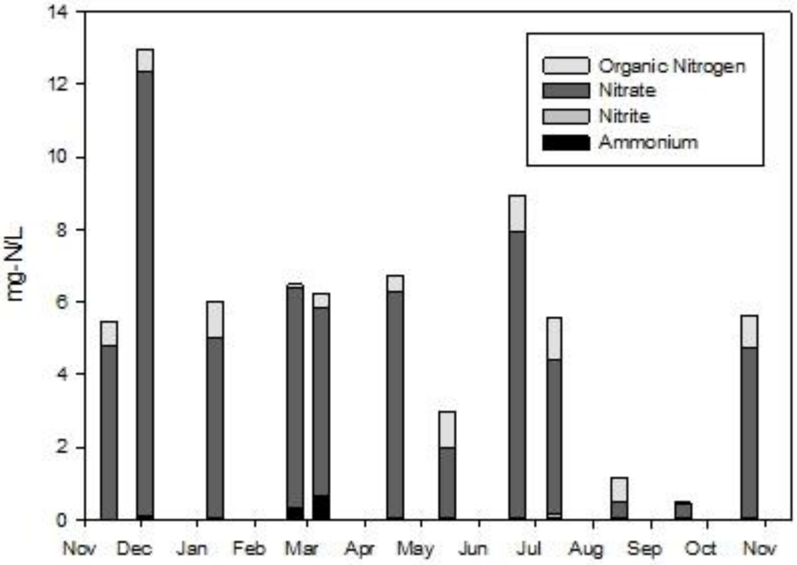


Figure 2.12: Annual nitrogen species concentrations in Boomer Creek November 2012 to October 2013. Data from November 2012 to March 2013 was collected as part of the current study. Concentration data from April 2013 to October 2014 was gathered and archived by the PWQMN.

The minimum NO_3^- concentration observed in the Conestogo River during the study period was 0.50 mg N/L on September 28th 2012, with a maximum concentration of 11 mg N/L on January 14th 2013 (Fig 2.13). The average winter NO_3^- concentration in 2013 was 6.1 mg N/L. Summer NO_3^- concentrations dropped to below 2.2 mg N/L in 2013 and below 2.0 mg N/L in 2012. Late fall concentrations were above 4.0 mg N/L with maximum concentrations above 10 mg N/L. The range in NO_3^- concentrations in the Conestogo River were ~0.5mg N/L in the fall of 2012 to 11.0 mg N/L in January of 2013. The concentration range from months with historical data are similar to years of comparable seasonal conditions.

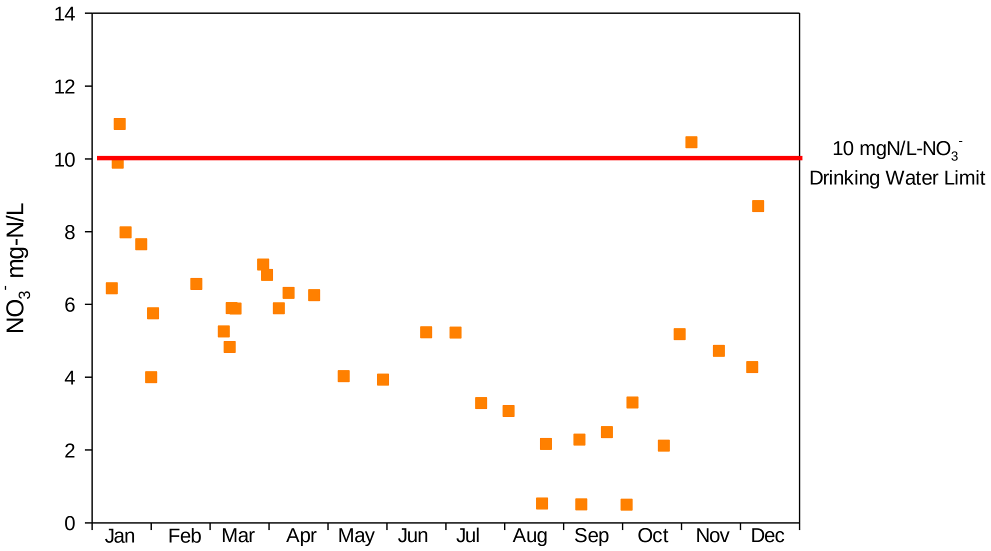


Figure 2.13: NO_3^- concentration data (2012-2013) for the Conestogo River. Maximum concentrations occur during the months of October to January with minimum concentrations occurring in August and September. The red line represents the 10 mg N/L-NO_3^- provincial drinking water limit.

NO_3^- concentrations had a much larger range in the four creeks studied then that observed in the historical data (Fig 2.14). This may result from the lack of historical PWQMN data taken during the winter and storm flow events. Minimum and maximum concentrations by season for each creek during the current research can be seen in (Table 2.4). Maximum seasonal NO_3^- concentrations for winter, spring and fall occurred during either melt or large precipitation events. Minimum NO_3^- concentrations for Boomer, Cox and Swan creek occurred during the summer months. Maximum and minimum concentrations did not necessarily occur simultaneously in all creeks. Carroll Creek's observed minimum concentration occurred during the fall of 2013 but was close to the minimum values observed in winter and summer. Annual NO_3^- concentration ranges were observed to be larger than that taken by the PWQMN. Minimum NO_3^- concentrations collected by the PWQMN agree well with the data gathered for Boomer Creek and Cox Creek during this study.

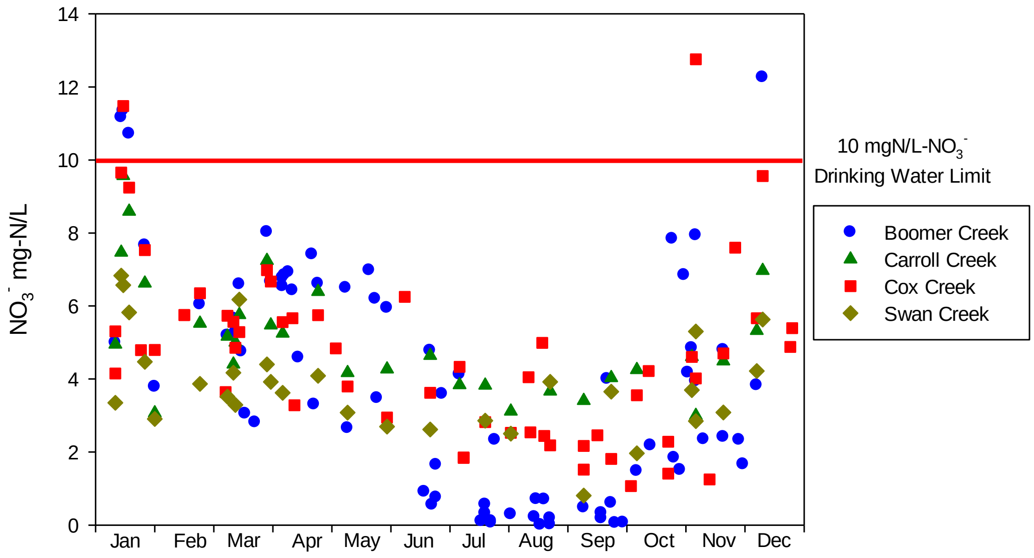


Figure 2.14: NO_3^- concentration data (2012-2013) for Boomer Creek, Carroll Creek, Cox Creek and Swan Creek catchments. The red line represents the 10 mg N/L provincial drinking water limit for NO_3^-.

Table 2.9: 2012-2013 Seasonal maximum and minimum NO_3^- Concentrations (mg NO_3^-- N/L). *2012 Data is from October 2012 forward

	*2012-2013 Seasonal Maximum and Minimum NO_3^- Concentrations (mg N/L)							
	Winter		Spring		Summer		Fall	
	Dec 22 - Mar 21		Mar 22 - Jun 21		Jun 22 - Sep 21		Sep 22 -Dec 21	
	Min	Max	Min	Max	Min	Max	Min	Max
Boomer Creek	3.8	11.6	2.7	8.0	0.2	4.1	1.5	12.3
Cox Creek	4.8	11.5	3.0	7.0	1.8	4.3	3.6	12.8
Swan Creek	2.9	6.8	2.6	4.4	0.8	3.9	2.0	5.6
Carroll Creek	3.1	9.6	4.2	7.3	3.1	4.0	3.0	7.8

During the fall of 2013 and the spring of 2014, water samples were collected from tile drains and shallow groundwater piezometers along Boomer Creek in addition to creek samples. NO_3^- concentrations for the tile drains during the fall had a large range in values from 1.3 mg N/L up to 20.7 mg N/L with a median concentration of 5.9 mg N/L. In the spring of 2014, tile drain NO_3^- concentrations ranged from 2.4 mg N/L up to 16.2 mg N/L with an median concentration of 6.7 mg N/L. Shallow groundwater obtained from piezometers from both fall 2013 and spring 2014 had a NO_3^- concentration range from below the detection limit to 1.6 mg N/L. Stream NO_3^- concentrations were between shallow groundwater concentrations and that of water from the tile drains.

Historical NO_3^- concentration data were used in conjunction with data obtained from the current study as well as precipitation data to obtain a concentration record for the years between 2004 and 2013 that were considered wet, dry or average. Regardless of the amount of precipitation, annual NO_3^- concentration follows the pattern of high concentrations during the winter months, which slowly declines toward summer minimum values, followed by a rise in concentration during the fall months (Fig 2.15). NO_3^- concentrations during wet years tend to be higher than those at the same time in dry years. The lowest NO_3^- concentration for all wet years was 1.6 mg N/L compared to the lowest NO_3^- concentration from dry years of 0.23 mg N/L. Data from years with total precipitation that fell within the average range tended to fall between wet and dry with the lowest concentration being 0.78 mg N/L.

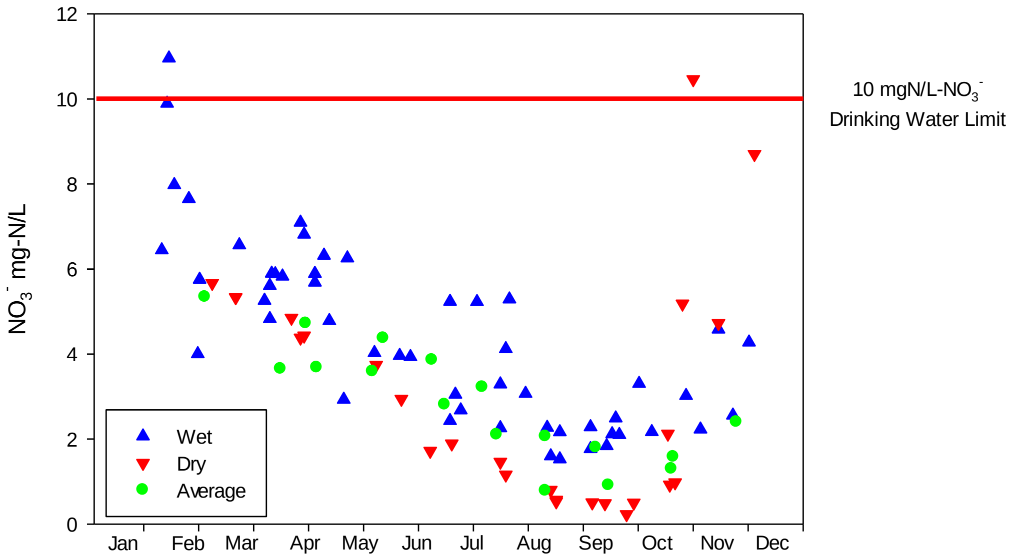


Figure 2.15: Conestogo River annual NO_3^- concentration data (2004-2013). Dry years (below average precipitation) are in red, wet years (above average precipitation) in blue and average (average precipitation) years in green. 2004-2011 NO_3^- concentration data was collected and archived by the PWQMN. Data from 2012-2013 was collected in the current study. The red line represents the 10 mg N/L-NO_3^- provincial drinking water limit.

Concentration patterns for wet and dry years were also analyzed for Boomer Creek (Fig 2.16). There was very little observable concentration difference between wet and dry years, particularly during the summer months.

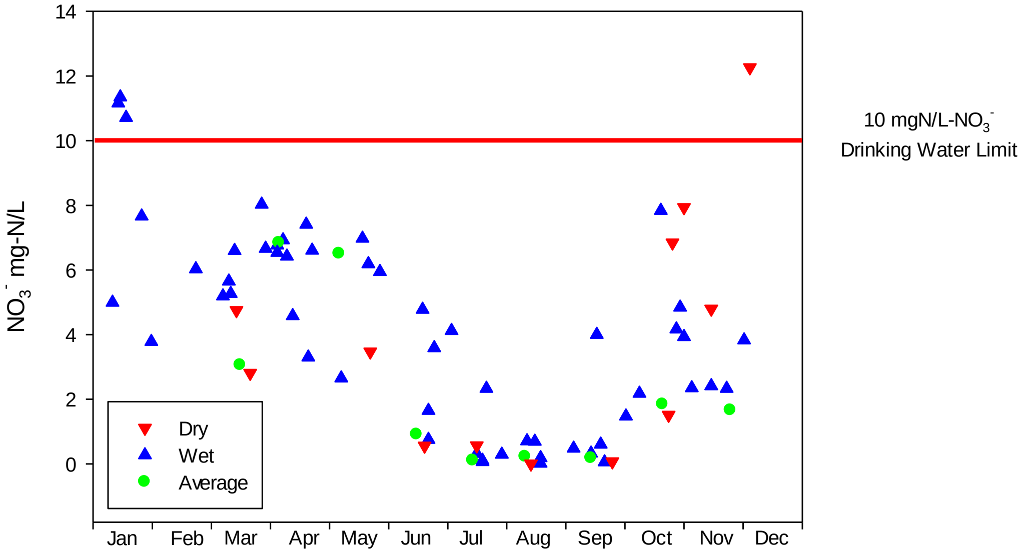


Figure 2.16: Boomer Creek annual NO_3^- concentration data (2007-2013). Dry years (below average precipitation) are in red, wet years (above average precipitation) in blue and average (average precipitation) years in green. 2007-2011 NO_3^- concentration data was collected and archived by the PWQMN. Data from 2012-2013 was collected in the current study. The red line represents the 10 mg N/L-NO_3^- provincial drinking water quality limit.

The Conestogo River has consistently higher NO_3^- concentrations throughout the year than the Grand River (Fig. 2.17a). The upstream Grand River location (West Montrose) has consistently lower concentrations than the downstream location (Bridgeport) (Fig 2.17b).

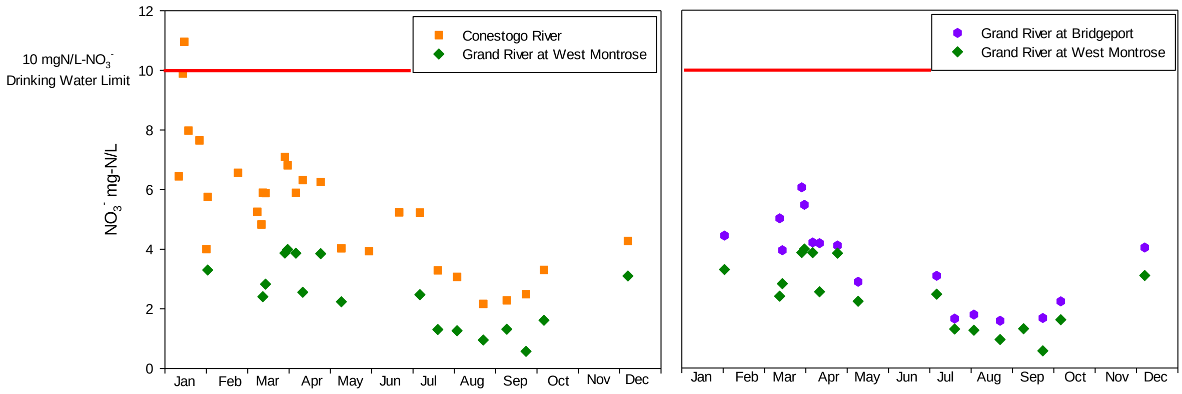


Figure 2.17a & 2.17b: Figure 2.17a: NO_3^- concentration data (2012-2013) for the Conestogo River (at St. Jacobs) and the Grand River at West Montrose (upstream location). Figure 2.17b: NO_3^- concentration data (2012-2013) for the Grand River at West Montrose (upstream location), and Grand River at Bridgeport (downstream location). The red line represents the 10 mg N/L provincial water quality guideline for NO_3^-.

Paired sampling of both groundwater and tile drains was conducted during the fall of 2013 and spring of 2014 (Fig 2.18). Tile drains show consistently elevated NO_3^- concentrations when compared to both Boomer Creek and shallow groundwater. Groundwater was found to have median NO_3^- concentrations of ~0.1 mg N/L, much lower than Boomer Creek (3.8 mg N/L) and tile drains (7.8 mg N/L).

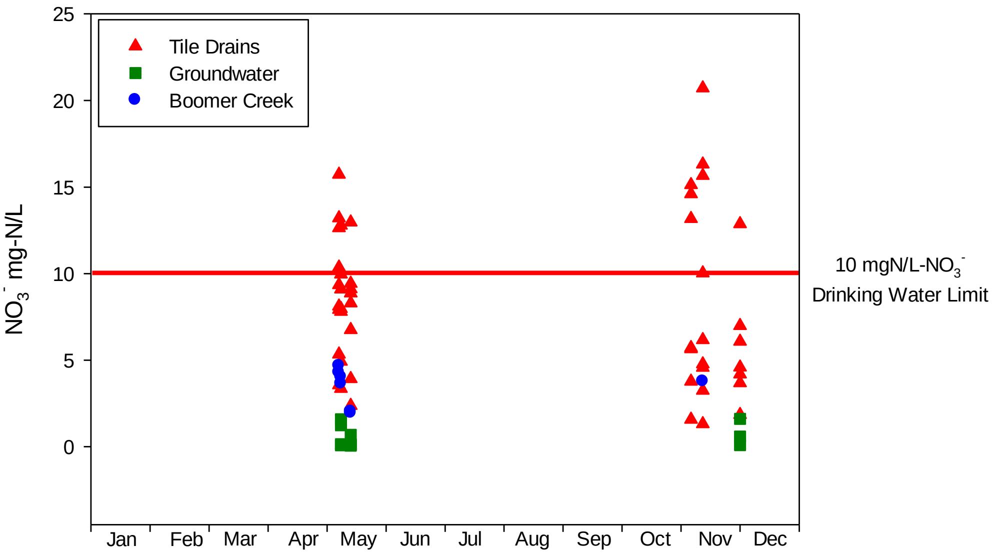


Figure 2.18: NO_3^- concentration data (2013-2014) for tile drains and shallow groundwater piezometers along Boomer Creek as well as Boomer Creek itself. Samples in the month of May were taken in 2014, samples in the month of November were taken November 2013. The red line represents the 10 mg N/L provincial drinking water limit for NO_3^-.

2.5.2 Annual Discharge

During sampling it was noted that the studied creeks and the Conestogo River experienced the lowest flows during the summer with the highest flows occurring during spring or winter melt events and during heavy autumn precipitation. Flow data on the Conestogo River discharge was continuously measured by the GRCA at St. Jacobs over the study period (Fig 2.19). In 2012 after the spring melt, flow remained low for the remainder of the year. The maximum average daily flow occurred on January 3rd with a discharge of 63 m^3/s. In 2013 flows were highly variable with several peaks occurring throughout the year. 2013 had 19 days with average daily flows higher than 63 m^3/s, with a peak average daily flow of 123 m^3/s occurring on April 10th. When comparing total annual discharge calculated using hourly discharge measurements, it was found that 2012 had approximately 36% of the total discharge of 2013.

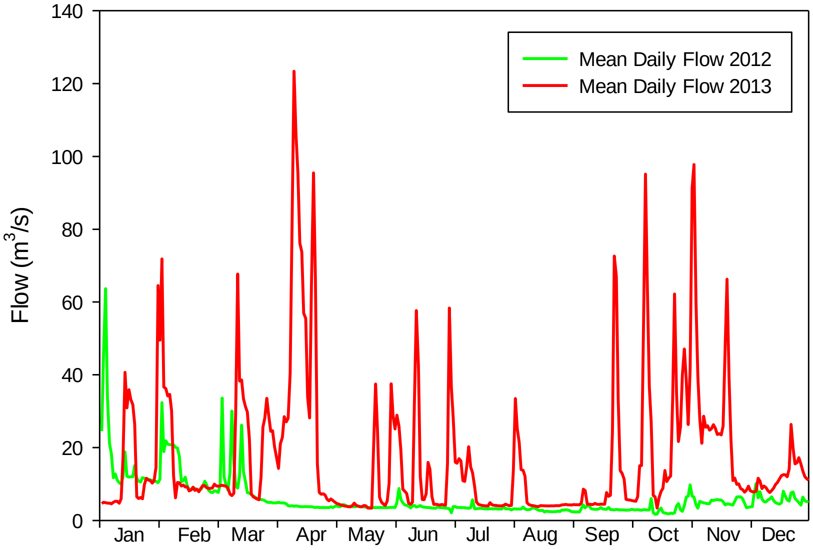


Figure 2.19: Mean daily flow data (2012-2013) for the Conestogo River (at St. Jacobs) (GRCA).

Flow data during the time of study, for the studied creeks was not available but flow data was available for another watershed of comparable size (Floradale; Fig 2.20).

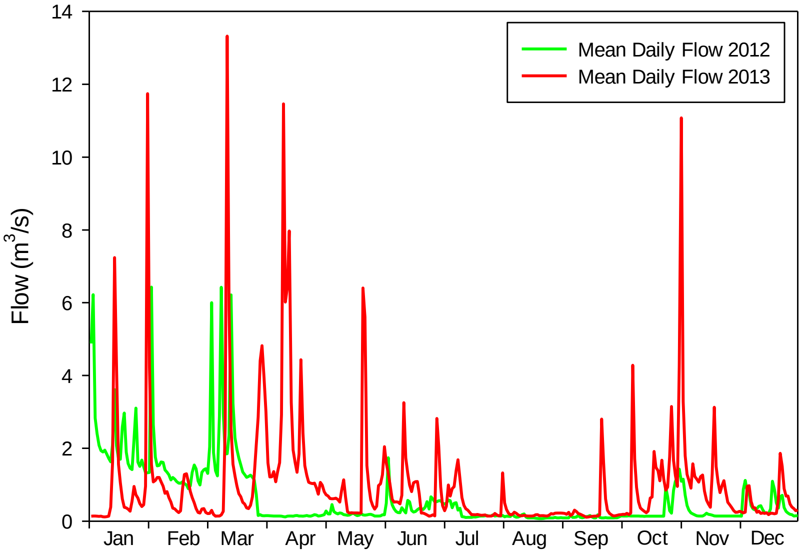


Figure 2.20: Mean daily flow data (2012-2013) for Floradale Creek (GRCA).

Discharge in the Floradale Creek followed a very similar pattern to that observed in the Conestogo River. 2013 had a larger range in flow and possessed higher and more frequent increases in flow throughout the year. Both the Conestogo River and Floradale Creek display elevated flows at some point during early winter (January), spring melt (March), spring (April-May) and fall (late October early November). Low flows occurred in the summer months (June-September) with occasional storms increasing discharge. Floradale Creek flow data (2004-2013) was also used to compare differences in seasonal discharges between years with greater, less and average amounts of precipitation (Table 2.5). There was large variability in the percentage of flow per season regardless of a year being classified as wet, dry or average. The spring melt however did represent 19-39% of the annual flow in all years. The largest difference between years in percent discharge per season was during the winter. Years that had average or below average precipitation (dry) had over 40% of the discharge occur within the winter, whereas only 10-17% occurred in the winter during above average (wet) years. Wet years had a higher percentage of discharge occur within the fall when compared to both average and dry years. Spring melt represented a similar amount (22-32%) of discharge in all years. Both wet and average years had similar percent discharge during the spring and summer with the exception of the summer of 2010 which saw 22% of annual discharge occur in the summer.

Table 2.10: Summary of % discharge per season for Floradale creek from 2004-2012. 2007 was not included as a large portion of data was missing.

Season/Year	2004	2005	2006	2008	2009	2010	2011	2012	2013
Winter	14%	27%	25%	28%	41%	17%	10%	49%	17%
Spring Melt	26%	19%	21%	39%	27%	32%	22%	25%	29%
Spring	21%	13%	26%	4%	13%	17%	16%	6%	19%
Summer	24%	24%	17%	12%	12%	22%	13%	9%	15%
Fall	15%	17%	11%	17%	8%	12%	39%	11%	21%

2.5.3 Export Estimation

NO_3^- Export estimation was done by interpolating concentration data between sampling dates and by developing relationships between past modelled flows (Carroll, Cox and Swan) or manual flow measurements (Boomer) and flow measurements taken at Floradale Creek. It was found that there was little variation in export estimation between concentration estimates based on linear interpolation or Method 2 (Table 2.6). Even though these methods for interpolating concentration data agreed well with each other it still must be stated that these export estimates are based on discharge relationships that did not have a good correlation to Floradale Creek, or as in the case of Boomer Creek had very few times to compare flow, as such these are merely rough estimates calculated for the sake of this study. Although every season with the exception of fall differed by less than 10%, the method developed by Swistock et al. (1997) (Method 2) was chosen as the best representation for N export. This was done as Swistock et al. (1997) found that this method agreed well with concentration interpolation based on regression analysis which was believed to be the most accurate estimation of unknown concentration data. The sum of NO_3^- export for days within a given season was used to estimate seasonal export from each creek (Table 2.7).

Table 2.11: Summary of seasonal NO_3^- export calculated based on concentration interpolation using linear interpolation and a Method 2 developed by Swistock et al. (1997). Seasons are from fall 2012 to fall 2013.

Creek	Season	Linear Interpolation 10^3 (kg N-NO_3^-)	Method 2 Swistock et al. (1997) (10^3 kg N-NO_3^-)	% Difference
Boomer Creek	Fall	15.3	13.5	13%
	Winter	33.7	34.3	-2%
	Spring Melt	60.8	61.3	-1%
	Spring	30.9	29.9	3%
	Summer	9.4	10.2	-7%
Carroll Creek	Fall	15.3	14.6	4%
	Winter	22.3	23.0	-3%
	Spring Melt	27.0	27.3	-1%
	Spring	19.9	19.7	1%
	Summer	22.8	22.9	0%
Cox Creek	Fall	33.0	30.9	7%
	Winter	42.2	43.4	-3%
	Spring Melt	40.0	40.2	-1%
	Spring	25.1	25.2	-1%
	Summer	28.3	28.5	-1%
Swan Creek	Fall	21.0	19.9	5%
	Winter	25.4	26.2	-3%
	Spring Melt	23.4	23.7	-1%
	Spring	18.6	18.5	0%
	Summer	24.5	25.2	-3%

Table 12: Summary of creek N-NO$_3^-$ export, discharge, % discharge and %export per year for Boomer Creek, Carroll Creek, Cox Creek and Swan Creek over all seasons from Fall 2012 to Fall 2013.

Watershed/ Total Annual Export (10^3 kg NO$_3^-$ N/ha)	Season	Discharge (10^5 m^3)	% Discharge/year	Export N-NO$_3^-$ (kg/ha)	% Export/year
Boomer Creek 27.3	Fall	17.8	7%	1.9	9%
	Winter	50.8	20%	4.9	23%
	Spring Melt	94.3	37%	8.7	41%
	Spring	57.6	22%	4.3	20%
	Summer	36.4	14%	1.5	7%
Carroll Creek 16.6	Fall	29.7	14%	2.2	14%
	Winter	41.1	19%	3.5	21%
	Spring Melt	47.6	22%	4.2	25%
	Spring	41.1	19%	3.0	18%
	Summer	58.6	27%	3.5	21%
Cox Creek 19.2	Fall	48.2	14%	3.5	18%
	Winter	63.3	19%	5.0	26%
	Spring Melt	68.8	20%	4.6	24%
	Spring	62.3	18%	2.9	15%
	Summer	94.8	28%	3.3	17%
Swan Creek 26.1	Fall	53.9	16%	4.6	18%
	Winter	62.4	19%	6.0	23%
	Spring Melt	56.0	17%	5.4	21%
	Spring	59.2	18%	4.2	16%
	Summer	105.5	31%	5.8	22%

The percentage of discharge that occurred throughout seasons varied by creek. Winter and spring represented about 20% and 19% of discharge in all creeks, while spring melt represented 17-37%. Both fall and summer had discharge that varied greatly between creeks, but fall represented the lowest discharge in all creeks. Apart from Boomer Creek, all other creeks had summer discharges of approximately 30%.

Export also varied between watersheds and season. Winter and spring melt both represented over 20% of the export per year from all watersheds. Spring represented between 15 and 20% of export, whereas summer export drastically changes depending on watershed. Fall export varied between creeks but represented less than 18% of export in all watersheds.

2.6 Discussion

2.6.1 Seasonality of NO_3^- Concentrations in Agricultural Catchments

NO_3^- concentration varied seasonally among the Conestogo River and the agricultural watersheds. During the study period both the creeks and the Conestogo River had similar patterns to that observed in the historical data; increased NO_3^- concentrations during late October to April and decreased NO_3^- concentrations occurring from late May to September (Fig 2.21). Since this pattern in NO_3^- concentration was also observed on the Conestogo River and Grand River it appears to be independent of watershed scale.

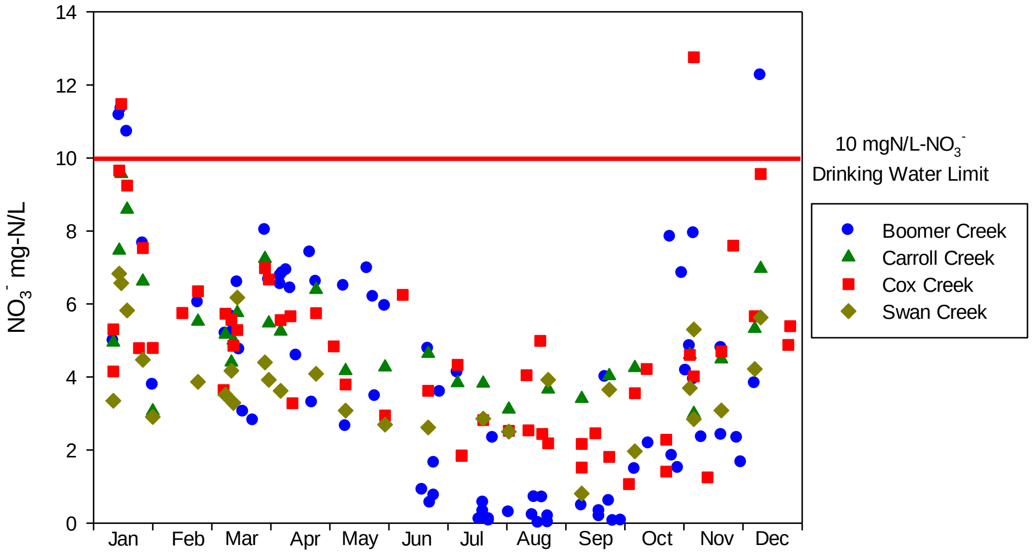


Figure 2.21: NO_3^- concentration data (2012-2013) for Boomer Creek, Carroll Creek, Cox Creek and Swan Creek catchments. The red line represents the 10 mg N/L provincial drinking water limit for NO_3^-.

Year round and increased sampling during times of increased discharge captured a wider range of NO_3^- values within the Conestogo River and the studied agricultural creeks, than that indicated by routine monitoring programs that do not include winter (e.g PWQMN). This is due to the fact that routine monitoring programs often gather data on a monthly basis to estimate trends and are less focused on detailed concentration changes. The current data reveal that, in addition to the seasonal pattern of high NO_3^- concentrations in the winter and fall, there is also an increase in NO_3^- concentration associated with spring melt events. Sampling during times of increased discharge such as during melt or large precipitation events is critical to accurate estimation of NO_3^- export.

2.6.2 Seasonal Weather Controls on Agricultural River and Creek NO_3^- concentrations (Wet vs. Dry Years)

The amount and timing of precipitation an area receives during a given year governs several important parameters within a watershed. The amount and timing of precipitation will affect the snowpack, water table depth and both groundwater and creek discharge. For instance, during a wet year, the water table would be higher and groundwater and creek discharge would be greater. Changes to these parameters could have an effect on NO_3^- concentrations and export by altering the amount and timing of NO_3^- discharge from agricultural fields.

The use of recent (2004-2012) historical NO_3^- concentration data from the Conestogo River and Boomer Creek has allowed for a comparison between wet and dry years. PQWMN data does not cover the entire year and some years only have concentration data from several months, typically the largest number of data points comes from measurements conducted during the summer and early fall months (June-October) when sampling conditions are ideal. Predominantly summer NO_3^- concentration will be discussed as there is the most available data for this time of the year and the largest variation in NO_3^- concentration between wet and dry years is observed during this time.

Due to the small amount of historical data from Boomer Creek, it is difficult to draw conclusions between wet and dry years, but the available data suggests that there may be little difference in NO_3^- concentrations between years (Figure 2.16). Conversely in the Conestogo River a difference in NO_3^- concentration between wet and dry years can be observed during the late summer/early fall (August-early October) (Figure 2.15). Even with the larger data set there were still only 9 samples taken from this time

(August-early October) in years classified as dry and 10 from years classified as wet. With the available data, wet year (August-early October) concentrations averaged 2.2 mg N/L whereas dry year (August-October) concentrations averaged 0.6 mg N/L. A Mann-Whitney-Wilcoxon Test indicated that NO_3^- concentrations in the Conestogo River between wet and dry years are significantly different ($p = 0.02$) therefore there is likely a process controlling NO_3^- concentrations in Conestogo River that is not occurring within Boomer Creek.

2.6.3 Effect of the Conestogo Reservoir on NO_3^- Concentrations in the Conestogo River

The Conestogo Reservoir has an effect on downstream NO_3^- concentrations. The effect on downstream NO_3^- concentrations may be weather dependent with dryer years leading to lower downstream NO_3^- concentrations and vice versa. The first hypothesis is that during dry years it is expected that the reservoir would have a stronger influence on river chemistry as there is little contribution from other sources. During a dry year, when there is little input and it is feasible that the reservoir as a result of little mixing could become thermally stratified, as colder water will sink to the bottom as warmer water which is less dense remains in the upper water column. As a result the sediments of the reservoir may become anoxic due to the increased microbial respiration during the decomposition of organic matter (Cooke et al.). This would create an environment favourable to denitrification and, dependent on water residence time within the reservoir, a decrease in the NO_3^- concentration could occur (Friedl and Wüest 55-65). Because the Conestogo reservoir is a bottom draw dam, any water released would subsequently have a low NO_3^- concentration under this denitrification scenario. This is an important consideration when trying to explain the Conestogo River's NO_3^- concentration, particularly during a dry summer when the reservoir is contributing a large proportion of water to the river. During a wet year there will likely be a larger input from tributaries, this may lead to a decrease in the residence time of the water within the reservoir as more water may need to be let out over the course of the year to maintain operational water levels. A decrease in the residence time would likely increase the amount of mixing and may result in the reservoir not becoming thermally stratified which would prevent the bottom of the reservoir from becoming anoxic causing increased concentrations of NO_3^- to be released to the Conestogo River. Yet increased precipitation during wet years will dilute NO_3^- concentrations within the reservoir and reduce residence time. Of particular importance is if a large amount of precipitation occurs during spring melt, which would dilute NO_3^- laden water from agricultural field runoff. A large amount of precipitation would also decrease reservoir residence time possibly allowing for highly NO_3^- concentrated water to be flushed out. In addition to precipitation factors such as timing of precipitation and residence time will affect NO_3^- concentrations within the reservoir

The Conestogo Dam begins filling during the time of spring melt when flood risk is the greatest. After this time the reservoir slowly lets out water to maintain river levels downstream throughout the rest of the year. The second hypothesis as to why there is a difference between wet and dry years in the Conestogo River is that water that fills the reservoir during the spring will continue to have an effect on the river for the remainder of the year, assuming it has undergone little biochemical changes while in the reservoir. Therefore if water entering the reservoir during the spring has high NO_3^- concentrations then for the remainder of the year the river downstream may display this legacy NO_3^- and vice versa if the water filling the reservoir has low NO_3^- concentrations.

Low NO_3^- concentrations are preferred from both an aquatic ecology and drinking water standpoint, and maintaining low concentrations during either a wet or dry year is ideal. This is a difficult management problem and is not easily solvable as current data has revealed that elevated concentrations are often associated with elevated flows. A better understanding of reservoir, nutrient, and flow dynamics must be known in order to optimize reservoir and downstream riverine health.

2.5.4 Role of Riparian Zone on Groundwater NO_3^- Concentrations

Boomer Creek experiences the largest range in NO_3^- concentration followed by Cox Creek, Swan Creek and Carroll Creek (Table 2.4). Although all creeks followed the same general annual trend, the range was quite variable between creeks. Variation in both the range and maximum and minimum concentrations can be influenced by several factors such as tile drains, size of riparian zone, crop type, seasonality, irrigation and fertilizer practises.

Tile drains can play an important role in governing annual NO_3^- concentrations and flows in agricultural creeks (Kellman and Hillaire-Marcel 87-102). To determine the extent of tile drain influence on agricultural creek NO_3^- concentrations, one of the agricultural watersheds (Boomer Creek) was studied in great detail. When tiles drains are present and flowing, NO_3^- laden water is discharged into a nearby ditch or stream, short-circuiting the natural groundwater flow path. In this study, water originating from tile drains displayed a wide range of NO_3^- concentrations, yet the majority of tile drains displayed elevated NO_3^- (> 5mg/L) when compared to both the shallow groundwater and Boomer Creek.

Had Boomer Creek only been receiving water from a groundwater source then it would be expected that the NO_3^- concentrations within the creek would be similar to concentrations within the groundwater. Thus tile drains appear to play a major role in the elevated NO_3^- concentrations of Boomer Creek. Unfortunately not all tile drains along Boomer creek could be assessed and flow measurements from the tile drains were not taken. Consequently a quantitative estimate for the contribution of tile drains to the flow of the creek is unknown.

The data in this study are consistent with the following hypothesis: a) tile drains govern the NO_3^- concentration seasonality in agricultural creeks, b) the highest concentrations occur at times when tile drains are flowing (fall – spring), and c) lowest concentrations occur at times when tile drains have minimal to no flow (summer). Tile drains flow during times of saturated soil conditions, which typically occur from late fall to late spring when there is very little to no uptake of water by crops, and increased precipitation or snow melt depending on the season. Tile drains will also flow during the winter depending on weather and soil conditions. During the summer, tile drains will have minimal flow as a result of the water table being lower because of plant uptake and decreased recharge. However tile drains may flow briefly following large precipitation events during the summer months. Times when tile drains are flowing closely reflects times of increased NO_3^- concentrations. The largest amount of precipitation typically falls during the fall in Waterloo region (Seglenieks), and this increase in precipitation leads to a rise in the water table elevation, this causes NO_3^- to be flushed out of the agricultural soils and into tile drains. Tile drain NO_3^- concentration data confirms this with a concentration median of approximately 8.0 mg N/L as well as increased NO_3^- concentrations observed in Boomer Creek without an increase in the shallow groundwater NO_3^- concentrations. This also occurred during the spring when Boomer Creek had increased NO_3^- concentrations during times of tile drain flow, whereas shallow groundwater concentrations remained consistently low (< 1.6mg N/L). The time at which Boomer creek NO_3^- concentrations drop occurs when the influence of the tile drains decrease. This occurs as crops begin to grow, the soil becomes drier, and the water table drops. Although cessation of tile drain flow is not synchronized, it was observed that over a couple of weeks in late May there was a drastic decrease in flow from all tile drains. By June, tile drain flow decreased until June 5th when no flow was detected from half of the tile drains. Three tiles remained active into the summer until the end of the current study (August 3rd). Spring rainfall was close to the average but possibly due to the colder than average winter and large snowpack there was a greater amount of water to recharge the groundwater, causing water tables to remain high well into the spring. Annual NO_3^- concentration fluctuations are likely controlled by tile drains.

Data collected from shallow groundwater piezometers during fall 2013 and spring/summer 2014, within the riparian zone of Boomer Creek indicate that riparian groundwater has very low NO_3^- concentrations. Summer NO_3^- concentrations within Boomer creek may have decreased NO_3^- to below that observed in the other creeks due to management actions taken to improve the riparian zone. Although not quantified in this study, topography, DOC and dissolved oxygen concentrations affect riparian zone effectiveness.

The hypothesis that tile drains are governing the NO_3^- concentrations within these agricultural watersheds has several management implications. Agricultural watersheds are known to be a non-point sources of nutrients to catchments, which can have detrimental effects on both aquatic ecosystems and drinking water quality. Instead of treating water downstream it may be easier and significantly more cost effective to manage nutrient concentrations and export as close to the source as possible. Agricultural catchments will continue to be a source of non-point source nutrient pollution but tile drains may act as a catalyst to this system, increasing the speed at which nutrients are transported downstream as well as the quantity of nutrients, by circumventing the riparian zone. When water draining from these agricultural fields is able to take its natural flow path, this research would suggest that it has reduced NO_3^- concentrations.

Tile drains may also play an important role during storms, particularly when the water table is already high. Storm and melt events in this study were found to correspond with the highest NO_3^- concentrations in streams, greatly increasing export. This is of particular importance if rainfall occurs close to the time of fertilizer application. Poor & McDonnell (2007) observed that rainfall events during or shortly after the time of fertilization led to a large export of nutrients into nearby streams. Tile drains have a compounding effect during storms: they allow for the relatively quick draining of water from fields, increasing discharge to creeks and rivers and also work to flush highly mobile NO_3^- out of the soil leading to increased concentration and export.

The decrease in NO_3^- concentration in both the Conestogo River and Boomer Creek occurs at a similar time of year but the decrease in NO_3^- concentration is greater in Boomer Creek. During the summer of 2013 the NO_3^- concentrations in Boomer Creek dropped to less than 0.19 mg N/L compared to the Conestogo River whose concentrations decreased to only 2.2 mg N/L. This may have resulted from a) little NO_3^- entering into Boomer Creek during the summer, b) insufficient removal of NO_3^- from denitrification within the Conestogo river, or c) due to the influence the Conestogo reservoir has on the Conestogo River NO_3^- concentrations. During the summer there is a limited supply of NO_3^- to Boomer Creek due to tile drains ceasing to flow and riparian zone denitrification may be limiting NO_3^- entering the creek. The Conestogo River is strongly influenced by NO_3^- originating from the reservoir upstream, which could cause elevated (> 1mg N/L) NO_3^-concentrations. Sampling conducted at several locations downstream of the reservoir found that there was little difference in NO_3^- concentrations downstream of the reservoir and often concentrations were higher further downstream. This indicates that there is possibly little denitrification within the river and also that additional water entering the river downstream of the reservoir has elevated NO_3^- concentrations. Although denitrification has been observed to occur within anaerobic zones in stream sediments (Garcia-Ruiz, Pattinson, and Whitton 467-476; Hill and Sanmugadas 1579-1586; Birgand et al. 381-487), denitrification may not be effective at decreasing NO_3^- concentrations as a result of unfavourable conditions or due to the small fraction of NO_3^- within the river being denitrified at the thin anoxic sediment water interface. It is also possible that denitrification is high but cannot overcome the input of NO_3^-.

The use of tile drains is becoming increasingly popular with 100 million feet installed annually in Ontario as farmers wish to maximize the growing season by ensuring there soils are dry in the spring so that they may be tilled and seeded (Pearce). There is a need for further research and a better understanding of the role tile drains play in agricultural watersheds so that management practises can be suggested or put in place to attempt to mitigate this problem. Alternatively bio-reactors have been shown to cheaply and effectively reduce NO_3^- concentrations in tile drain discharge (Blowes et al. 207-221).

2.6.5 NO_3^- Concentrations Related to Fall and Spring Wet Up

During the fall, water tables rise due to increased precipitation and decreased plant uptake, causing a wetting up of the soil. This works to quickly mobilize residual NO_3^- remaining within the upper soil which was previously unsaturated, this mechanism is described as the flushing hypothesis (Creed et al. 3337-3354). During the summer months, groundwater is less susceptible to NO_3^- contamination as a result of the water tables being lower and having less contact with NO_3^- within the pore spaces of the upper unsaturated zone. During the fall, winter and spring months, when there is little to no uptake by crops, as the water table rises it mobilizes NO_3^- within the previously unsaturated zone into groundwater and tile drains.

The timing and amount of precipitation is also an important factor when trying to understand seasonality in NO_3^- concentration. During 2012, there was very little precipitation from April onward (Seglenieks), decreasing base flows in the river and contributing to added pressure on the Conestogo Reservoir to maintain adequate base flows. In addition, this may also increase the amount of nutrients that come out of agricultural fields during the fall. During a dry year it would be expected that there would be a greater drop in the water table when compared to an average or a wet year. A consequence of this would be that, during the fall "wet up" phase, the water table would encounter a larger amount of previously unsaturated soil, which may cause a greater flushing of nutrients at that time. Wassenaar (1995) observed a flushing of NO_3^- as a result of NO_3^- being generated through nitrification of fertilizer in the summer and mobilized when the water table elevation increased. Flushing of NO_3^- resulted in increased stream and tile NO_3^- concentrations during the times of increased precipitation. Flushing of NO_3^- could also occur during the spring from nitrification occurring throughout the winter. Despite nitrification being soil moisture sensitive (Breuer, Kiese, and Butterbach-Bahl 834-844) and known to decrease during dry periods, NO_3^- generated during this time may still represent a significant source of NO_3^- to groundwater and nearby creeks and streams.

2.6.6 Storm and Melt Events

Concentrations of NO_3^- during storm and spring melt events can represent a large amount of export within a short period of time. Storm events provide large amounts of precipitation within a short period of time, often leading to a large increase in river or creek discharge. If this increased discharge is coupled with elevated NO_3^- concentrations this can lead to a large amount of nitrogen being exported out of the basin and into the river, towards Lake Erie.

Several storm events were sampled between the years of 2012-2013. During a storm event, sampling was done in rapid succession in order to capture the storm hydrograph. Storm and melt events have produced elevated NO_3^- concentrations in the agricultural creeks and the Conestogo River, but concentrations still follow seasonal trends. Spring melt peak concentrations were not observed to exceed NO_3^- concentrations seen in either the early winter or fall following the observed seasonal trend. During the spring melt, NO_3^- concentrations rose and peaked for Boomer Creek at 8.0 mg N/L whereas during the winter storm event the peak concentration was 11 mg N/L. This trend was similarly observed in the other creeks and the Conestogo River. Samples that are only taken monthly will miss these events and will most likely underestimate the range of seasonal NO_3^- concentration as well as the amount of NO_3^- exported.

2.6.7 Dynamics in Seasonal Nitrate Export

Nitrate export was found to be dependent on seasonal conditions. Although the spring melt period only represents 45 days out of the entire year it is estimated that spring melt accounts for over 20% of the annual export in all creeks and over 40% for Boomer Creek. During spring melt NO_3^- is rapidly flushed out of agricultural fields and represents a large percentage of annual export. Export estimations during spring melt are also important as the water filling reservoirs will have a high concentration of NO_3^- and could cause elevated concentrations to persist downstream for the remainder of the year.

2.6.8 Effect of Land-Use on NO_3^- concentrations

Land-use may influence NO_3^- export and concentration. In catchments where the dominant land-use is agriculture, export of nutrients to nearby water-bodies is greater than that in undisturbed catchments (Hill 696-702; Poor and McDonnell 54-68). Greater input of nutrients would result in elevated nutrient concentrations in the receiving waters of these catchments. Agricultural catchments can differ in several intrinsic factors and this thesis looked to identify and evaluate if there was a correlation between these factors and NO_3^- concentration. The factors investigated in each catchment were: percent agriculture (Fig. 2.22), manure density (Fig. 2.23), total nitrogen density (Fig. 2.24), and percent tile drained.

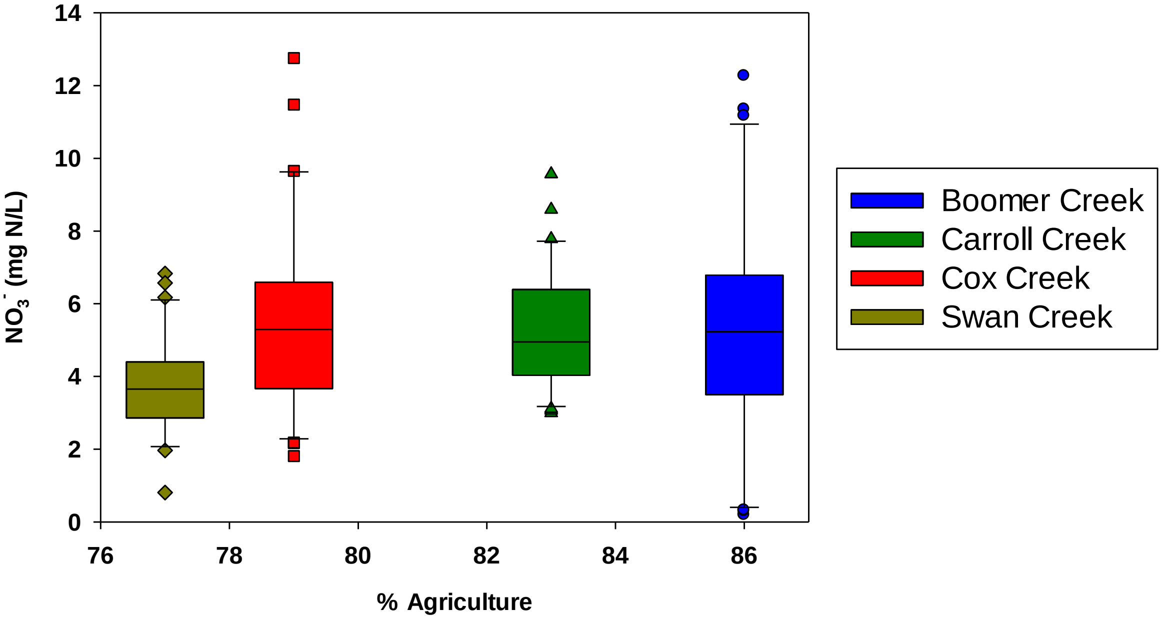


Figure 2.22: % Agriculture and NO_3^- concentration boxplot, NO_3^- concentration data (2012-2013) for Boomer Creek, Carroll Creek, Cox Creek and Swan Creek catchments.

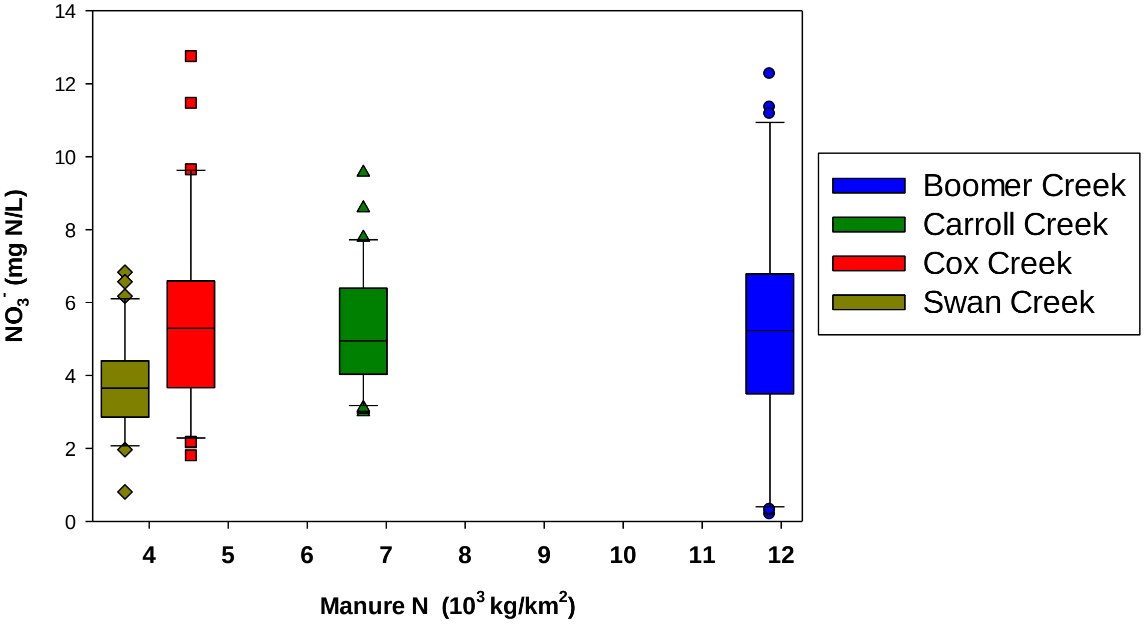


Figure 2.23: Manure density reported in 10^3 kg/watershed km^2 and NO_3^- concentration boxplot, NO_3^- concentration data (2012-2013) for Boomer Creek, Carroll Creek, Cox Creek and Swan Creek catchments.

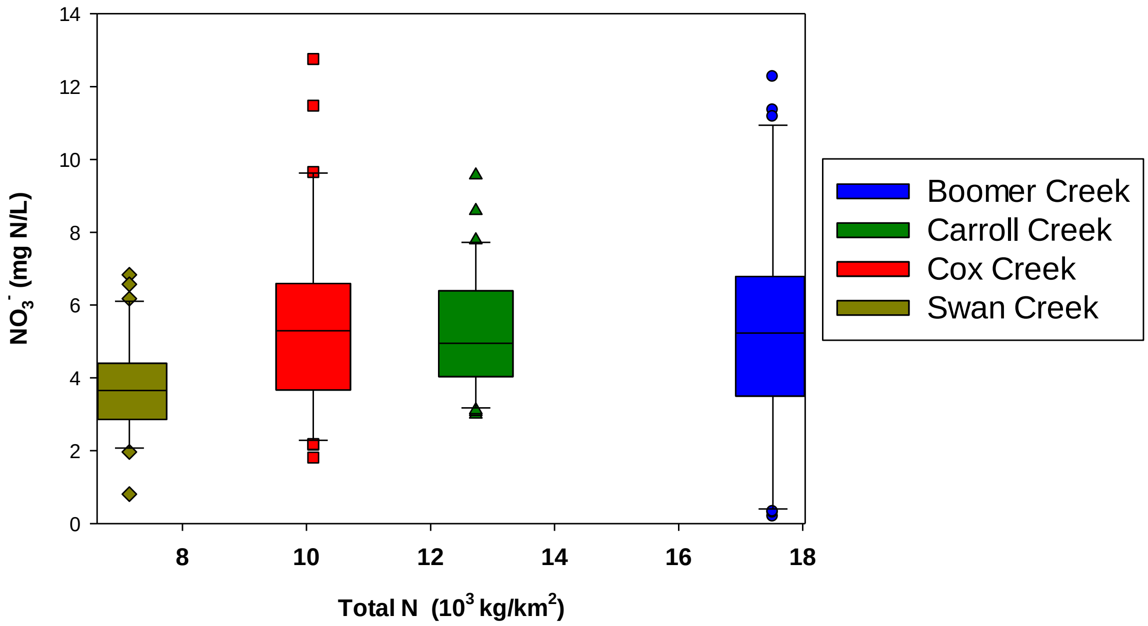


Figure 2.24: Total N (nitrogen) reported in 10^3 kg/watershed km^2 and NO_3^- concentration boxplot, NO_3^- concentration data (2012-2013) for Boomer Creek, Carroll Creek, Cox Creek and Swan Creek catchments.

The percent of land-use classified as agriculture in these catchments falls between a small range of 76-86%. Manure density ranges from 3.6 x 10^3 kg/km^2 in Swan Creek to 12 x 10^3 kg/km^2 in Boomer Creek. The total nitrogen per km^2 which is the sum of the amount of nitrogen from manure as well as the amount of nitrogen from fertilizer ranges from 7.1 x 10^3 kg/km^2 in Swan Creek to ~ 18 kg/km^2 in Boomer Creek.

All catchments fall within a 10% range of each other in terms of percent agriculture. This is in contrast to the range between catchments for both manure density and total nitrogen density. Boomer Creek has in excess of triple the manure density of Swan Creek and nearly double that of Carroll Creek. Boomer Creek also has the highest density of total nitrogen, accounting for almost triple that of Swan Creek. Cox Creek, which had a similar manure density to Swan Creek and slightly over half the total nitrogen density, displayed the highest NO_3^- concentration and the second highest range in annual NO_3^- concentrations. Cox Creek peak concentrations may result from rapid flushing of NO_3^- laden waters during storm events. Yet Carroll Creek, which had a higher percentage of tile drains (36%), did not have as large of range or peak

NO_3^- concentrations. Due to the lack of both historical concentration data from the time of the land-use survey and current land-use data it is difficult to interpret the results of Cox Creek as land-use and fertilizer application may have changed. Despite differences in land-use there was little variability observed in NO_3^- concentrations and ranges between watersheds.

2.6.9 Effects on Downstream Water Bodies

In addition to the seasonal variation in NO_3^- concentrations in the Conestogo River, this study also aimed to investigate the effect of the Conestogo River on the Grand River's NO_3^- concentrations. The Conestogo River was shown to possess consistently higher NO_3^- concentrations than the Grand River (2.17a & 2.17b), causing a marked increase in Grand River NO_3^- downstream of the Conestogo River confluence. This study emphasizes the importance of understanding nutrient concentrations in waterways within agricultural catchments, as these tributaries can have a strong effect on downstream water bodies. For nutrient management purposes, emphasis should be put on managing nutrients from sources such as agricultural watersheds in order to minimize the nutrient impact these watersheds have on their receiving water bodies. Greater attention should be paid to tributary water quality if the goal is to improve downstream ecosystem health and drinking water quality, such as that of the Grand River and Lake Erie.

2.7 Conclusions

The objectives for Chapter 2 were to 1. Better understand NO_3^- concentrations and export both seasonally and annually in four small agricultural catchments and compare this to historical data and 2. Investigate the physical/chemical processes and source of NO_3^- within these watersheds.

Annual NO_3^- concentrations within all the studied creeks revealed a seasonal pattern which showed good agreement with seasonal patterns observed in past research done on the Grand River as well as available historical NO_3^- concentration data on the Conestoga River, Cox Creek and Boomer Creek. The seasonal pattern is described as having the highest NO_3^- concentrations from fall to spring with lowest concentrations occurring during the summer months. Although the range in annual NO_3^- concentrations were not the same between the agricultural creeks and the larger river systems (Conestoga River and Grand River) the pattern still existed and appears to be independent of scale. Times of increased NO_3^- most often occurred simultaneously with times of increased discharge, which equates to increased export. Spring melt in particular, represents a large amount of the annual export of NO_3^-. During the 2013 spring melt >35% of the annual NO_3^- export occurred within 45 days. Although spring melt was defined as the season with the fewest number of days, it represented >20% of the export in Carroll, Cox and Swan Creek, and >40% in Boomer Creek. In contrast summer which was defined as days between June 15th and October 17th only represented between 7-22% of seasonal NO_3^- export.

In addition to the verification that a seasonal NO_3^- concentration pattern existed, the physical/chemical processes and source of NO_3^- within these watersheds was also investigated. Annual precipitation patterns were observed to influence NO_3^- concentrations within the Conestogo River but not within Boomer Creek. This is thought to be a result of the influence the reservoir has on downstream NO_3^- concentrations in the Conestogo River. Specifically in-reservoir redox conditions may differ between years with below average and above average precipitation which in turn may affect NO_3^- concentrations within the reservoir and water discharged into the Conestogo River.

Agricultural creeks primarily receive water from either groundwater discharge or discharge from tile drains. Groundwater samples taken from the riparian zone along Boomer Creek were found to contain very low NO_3^-concentrations, while water discharging from adjacent tile drains were found to have consistently elevated (~ 8 mg N/L) concentrations. High NO_3^-concentrations within tile drains are believed to occur because of the rapid movement of water from fertilized agricultural fields. Times of increased in-stream NO_3^-concentrations often corresponded to times of increased flow conditions (late fall and during melt events in winter and spring), while decreased flows (summer) related to times of decreased NO_3^- concentrations. Times of increased flows correspond to times when tile drains are discharging the greatest amount of water. Increased NO_3^- concentrations occurring in the fall and spring are therefore thought to result from the increased discharge from tile drains as a result of an increase in the water table elevation.

This increase in water table elevation allows groundwater to encounter shallow soils which have high NO_3^- concentrations, as this is where fertilizer is applied and the most active nitrogen cycling occurs. This NO_3^- is then quickly mobilized and flushed out through the tile drains into the creek. Boomer Creek had elevated NO_3^- concentrations compared to the groundwater demonstrating the influence that tile drains have on agricultural creeks.

Despite differences in various land-use categories there existed little difference in NO_3^- concentration ranges. In Boomer Creek, there was nearly triple the amount of manure density and total nitrogen in comparison to Swan Creek yet the median NO_3^- concentration differed by only 1.5 mg/L. Based on current data Boomer Creek was observed to have the greatest amount of annual NO_3^- export (27.3 x 10^3kg/ha) while Swan Creek which had the lowest manure and total nitrogen density of all the studied creeks had the second highest annual NO_3^- export (26.1 x 10^3kg/ha). There is little evidence that land-use has an effect on either NO_3^- concentrations or export in agricultural creeks.

In conclusion there is strong evidence to suggest that tile drains play an important role in seasonal NO_3^- patterns as the highest concentrations and greatest export occur during times of high tile drain discharge.

Chapter 3 – The Applicability of Stable Isotopic Ratios of Nitrate in Small Agricultural Creeks in Identifying Sources or Processes

3.1 Introduction

Agriculture represents one of the largest non-point sources of NO_3^- to the aquatic environment. Nitrogen in excess of crop and microbial requirements remains in the soil. In Canada, nitrogen remaining in the soil after crop harvest will predominantly be in the form of NO_3^-, which is highly mobile (Eilers et al.). NO_3^- can be flushed out of agricultural systems and pollute nearby and downstream water bodies. Therefore, it is important to understand the sources and fate of NO_3^- in order to mitigate any detrimental effects.

Source identification of NO_3^- can be done through the use of stable isotopic ratios. Several biogeochemical processes generate NO_3^- and without the use of isotopes it is very difficult to conclusively identify the source. Several studies have shown the applicability of stable isotope analysis of NO_3^- for source determination (Kellman 131-137; Aravena and Robertson 975-982; RW.ERROR - Unable to find reference:103). NO_3^- produced by synthetic/inorganic (anthropogenically produced) or organic (animal waste) fertilizers have distinct isotopic signitures. The typical range of $\delta^{15}N\text{-}NO_3^-$ in synthetic fertilizers is – 2 to + 4‰ (Gormly & Spalding, 1979; Kendall & Aravena, 2000; Wassenaar, 1995) whereas the typical range of $\delta^{15}N\text{-}NO_3^-$ derived from manure is + 8 to +22‰ (Kreitler & Jones, 1975; Wassenaar, Hendry, & Harrington, 2006).

Stable isotopic analysis of NO_3^- can also be used to understand the processes governing nitrogen transformation. This is particularly useful when explaining decreases in NO_3^- concentration as the occurrence of denitrification causes a change to the stable isotopic ratios of NO_3^-. The process of denitrification is predominantly a unidirectional kinetic reaction. As a result, the lighter isotopes (^{14}N, ^{16}O) react faster and the products (N_2O + N_2) become preferentially enriched leaving the remaining NO_3^- isotopically more abundant in both ^{15}N and ^{18}O (Kendall and Aravena 261-297; Kendall, Elliott, and

Wankel 375-449). The isotopic analysis of $\delta^{18}O$ of NO_3^- is particularly important as differing sources of NO_3^- can have similar $\delta^{15}N$ values and $\delta^{18}O$ values can be used in addition to separate these sources. For a more detailed explanation on the subject and the fractionation associated with certain biogeochemical processes, please refer to Chapter 1.

Isotopes analysis was used for several purposes during this study. Sources of NO_3^- were estimated based on the stable isotopic ratios of nitrate ($\delta^{15}N$, $\delta^{18}O$). Isotopic ratios were also used to assist in understanding the reasons for fluctuations in NO_3^- concentrations throughout the year.

The overall objectives of this chapter were 1) to use isotopes to assist in identifying the processes governing NO_3^- concentrations in small agricultural catchments and 2) determine the applicability of the stable isotopic ratios of NO_3^- for source determination.

3.2 Site Description & Methodology

For site descriptions, sample collection details and chemical analysis techniques aside from stable isotopic analysis, please refer to the site description and methods outlined in Chapter 2.

In brief, water samples were collected from creeks in four small agricultural watersheds during the years of 2012 to 2013. Nitrate isotopic analysis was performed on selected water samples from each creek in an effort to represent both the entire year and specific storm/melt events. Nitrate isotopic analysis was also performed on surface, tile drain, and groundwater samples collected from the Boomer Creek watershed during the fall of 2013 and the spring/summer of 2014.

$\delta^{15}N$ and $\delta^{18}O$ Isotopic Analysis

Nitrate stable isotopic analysis was conducted using two methods: the bacterial denitrification method (Sigman et al. 4145-4153) and the chemical denitrification method (Spoelstra, Kralt, and Elgood 14), this was due to a contamination issue with $\delta^{18}O$ using the bacterial denitrification method. All samples were kept frozen prior to isotopic analysis. Samples from October 23rd-31st, 2012 and January 10th-25th, 2013 were analyzed using the bacterial denitrification method at the University of California Davis Stable Isotope Facility. All other samples for isotopic analysis were analyzed at the University of Waterloo Environmental Geochemistry Laboratory (EGL), and the Environmental Isotope Laboratory (EIL) using the chemical denitrification method

Bacterial Denitrification Method

Water samples (minimum of 10mL) for isotopic analysis were filtered to 0.45µm and kept frozen until analysis. Water samples were prepared on location according to the procedure outlined by Sigman et al. (2001). Isotope ratios of $\delta^{15}N$ and $\delta^{18}O$ were measured using a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany). In brief, sub-samples containing 10-20 nmoles N (5-10 nmol N_2O) are placed into glass vials, these vials are then incubated overnight with denitrifying bacterial cultures which convert NO_3^- to N_2O. This N_2O is then extracted and is first purged from vials through a double-needle sampler into a helium carrier stream (25 mL/min). This gas sample is then passed through a CO_2 scrubber (Ascarite) and N_2O is trapped and concentrated in two liquid nitrogen cryo-traps operated in series such that the N_2O is held in the first trap until the non-condensing portion of the sample gas has been replaced by helium carrier, then passed to the second, smaller trap. The second trap is then warmed to ambient temperature and the N_2O is carried by helium to the IRMS via an Agilent GS-Q capillary column (30m x 0.32 mm, 40°C, 1.0 mL/min). This column separates N_2O from residual CO_2. A reference N_2O peak is used to calculate provisional isotope ratios of the sample N_2O peak. $\delta^{15}N$ values are calculated by adjusting the provisional isotopic ratios such that correct $\delta^{15}N$ values for an internal check are obtained. Standards are included in each batch to monitor and correct for instrumental drift and linearity. The limit of quantification is 2 µM of NO_3^- in water with a precision of ±0.4‰ for ^{15}N and ±0.5‰ for ^{18}O.

Chemical Denitrification Method

Samples were prepared according to Spoelstra et al. (2014), which is a modified version of the chemical denitrification method by (McIlvin and Altabet 5589-5595; Ryabenko, Altabet, and Wallace 545-552) in which $\delta^{15}N$ and $\delta^{18}O$ values of nitrate are measured by chemically converting NO_3^- to N_2O and measuring the isotopic composition of N_2O. Isotopic analysis following the chemical denitrification method was conducted at the University of Waterloo Environmental Isotope Laboratory (EIL) using a Trace Gas IRMS. In brief, sub-samples containing 2 µg of NO_3^--N were freeze-dried for a minimum of 48 hours then redissolved in 3 mL of sodium chloride – imidazole solution in a 3.7-mL Exetainer® vial. Following redissolution, activated cadmium is added to each vial to reduce nitrate to nitrite over a 24-hour period. Samples are then syringe filtered into a 20-ml helium-filled serum vial where a buffer solution (acetic acid and sodium azide) is added to convert nitrite to nitrous oxide. The reaction is then allowed to proceed to completion until sodium hydroxide is added to quench the reaction. Nitrous oxide from the headspace of the vial can then be either directly injected or sub-sampled into a suitable autosampler for isotopic analysis. Prior to analysis the Trace Gas IRMS stability tests are run on a reference gas, deviations are typically better than 0.05‰ for $\delta^{15}N$ and $\delta^{18}O$ after 10 cycles. Standard gas (standardized at the University of California Davis Stable Isotope Facility) is then injected to check daily peak height behaviour. From this, ideal sample volumes for analysis are chosen. The isotopic composition of the original NO_3^- is then determined by creating a correction equation using the nitrate standards (EGC 17, USGS 34, USGS 35) included in the run. The correction accounts for any isotopic fractionation that occurred during the various reactions, oxygen exchange between water and NOx, and the fact that one N atom of the N_2O comes from the original nitrate and one from azide. Isotopic analysis results for the $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios are reported using δ-notation. Duplicate samples and standards within each run are typically within ±0.3‰ and ±0.8‰ for $\delta^{15}N$ and $\delta^{18}O$, respectively.

A comparison of results (Fig. 3.1) revealed that $\delta^{15}N$ values between methods correlated well to each other ($r^2 = 0.92$) and had an average $\delta^{15}N$ difference between methods of ±0.4‰ between duplicates,

$\delta^{18}O$ did not show as strong of correlation ($r^2 = 0.65$) between methods and had a larger $\delta^{18}O$ average difference (±0.8‰) between methods. This difference is thought to have resulted from a difference in assigned reference values. Although there was a difference between methods, these methods are interpreted to be comparable because the difference in isotopic values between methods is small when compared to the range in $\delta^{15}N$ (-5‰ to + 30‰) and $\delta^{18}O$ (-5 to +5‰) of probable NO_3^- sources in these catchments (inorganic fertilizer, organic fertilizer, septic) (Ctp 1; Fig 1.4)

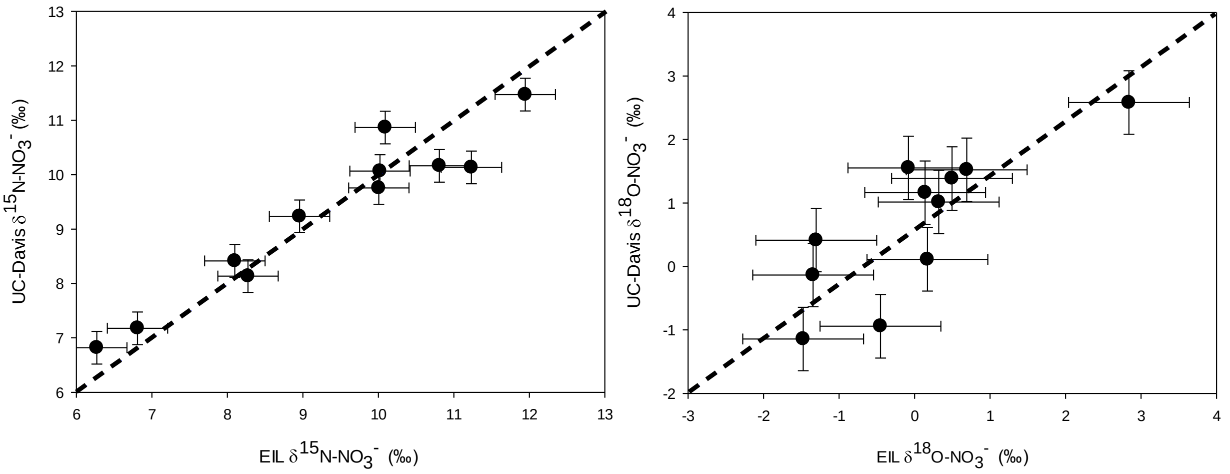


Figure 3.1: Comparison of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ run at the University of California Davis Stable Isotope Facility using the bacterial denitrification method and $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values run at the University of Waterloo Environmental Isotope Lab. The dashed line represents a 1:1 line.

N_2O and CH_4

Dissolved gasses were measured using a headspace equilibration technique. Samples were first prepared by creating 5 mL of headspace by injecting 10 mL of ultra-high purity helium. This created positive pressure allowing for headspace to be removed without causing a vacuum. Sample bottles were then put on an orbital shaker for 2 hours to equilibrate. A sample headspace of 2.5 mL was then analyzed for concentration using a Varian 3800 GC equipped with TCD, FID and ECD. Certified gas standards were run to ensure accuracy. The method precision for N_2O and CH_4 was +/- 5 %.

3.3 Results

3.3.1 Creek $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$

An annual pattern in NO_3^- concentrations was observed in all creeks (Cpt. 2; Fig 2.19), in which elevated NO_3^- concentrations (≥6 mg N/L) were observed from fall to spring, with reduced concentrations (<4 mg N/L) during summer months in all catchments

Isotopic values for $\delta^{15}N\text{-}NO_3^-$ were similar between creeks seasonally with the exception of Swan Creek which was observed to have a smaller range in $\delta^{15}N\text{-}NO_3^-$ (Fig 3.2). Lower $\delta^{15}N\text{-}NO_3^-$ values occurred during the fall and winter months with higher values during the summer. All $\delta^{15}N\text{-}NO_3^-$ values were greater than +6.5‰ (Table 3.1), which is above the values typically associated with synthetic fertilizer. The highest $\delta^{15}N\text{-}NO_3^-$ values occurred in Boomer Creek during the summer (+17.0 ‰). Boomer Creek had the greatest range (+8.6‰) in $\delta^{15}N\text{-}NO_3^-$ of all the creeks. Swan Creek had the smallest range with $\delta^{15}N\text{-}NO_3^-$ values ranging from +6.8 to +11.5 ‰. Both Cox Creek and Swan Creek have $\delta^{15}N\text{-}NO_3^-$ ranges of approximately +7.5 ‰, which is similar to that of Boomer creek. The lowest $\delta^{15}N\text{-}NO_3^-$ values occurred in Swan and Cox Creek in late October (+6.5‰).

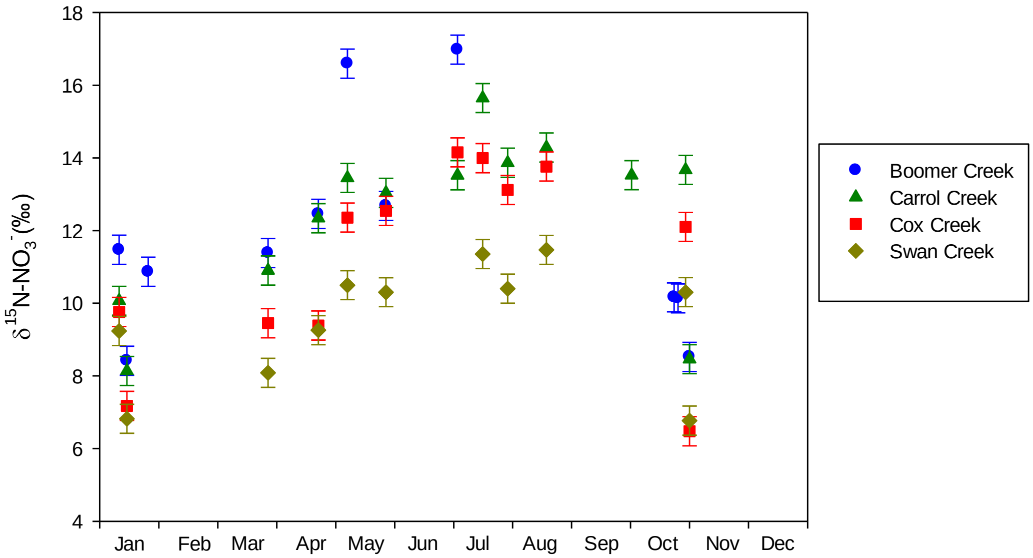


Figure 3.2: $\delta^{15}N\text{-}NO_3^-$ values from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, collected at various times of year throughout the study period.

Table 3.1: Summary of maximum, minimum and range of $\delta^{15}N\text{-}NO_3^-$ from Boomer, Carroll, Cox and Swan Creek. Samples collected at various times of year throughout the study period.

Creek	Maximum $\delta^{15}N\text{-}NO_3^-$	Minimum $\delta^{15}N\text{-}NO_3^-$	Range $\delta^{15}N\text{-}NO_3^-$
Boomer	+17.0	+8.4	+8.6
Carroll	+15.7	+8.1	+7.5
Cox	+14.2	+6.5	+7.7
Swan	+11.5	+6.8	+4.7

The values for $\delta^{18}O\text{-}NO_3^-$ follow a seasonal pattern similar to that of the $\delta^{15}N\text{-}NO_3^-$ values (Fig 3.3). Lower $\delta^{18}O\text{-}NO_3^-$ values were present during the fall and winter months with higher values during the summer. The most enriched $\delta^{18}O\text{-}NO_3^-$ values occurred in Boomer Creek in early May (+6.1‰). Boomer, Cox and Swan Creek all had $\delta^{18}O\text{-}NO_3^-$ within +7.0‰ range. Swan Creek had a notably smaller range than the other creeks with δ^{18}O-NO$_3^-$ values ranging from -1.9 to +1.3 ‰. Swan and Cox creek had the lowest δ^{18}O-NO$_3^-$ values (~ +1.8 ‰), which occurred in late October.

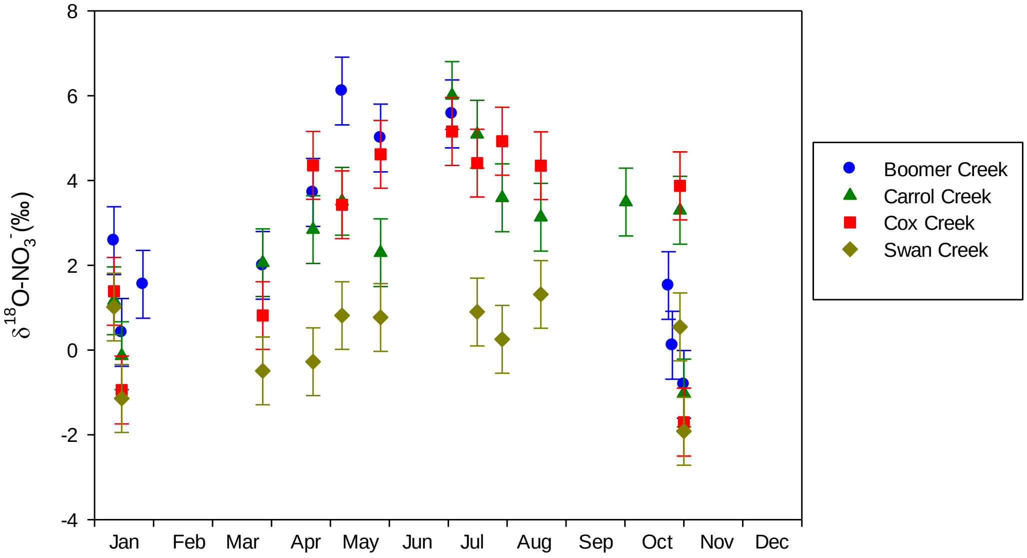


Figure 3.3: δ^{18}O-NO$_3^-$ values from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, samples collected at various times throughout the study period.

3.3.2 Creek δ15N-NO3- and NO3-

Low NO$_3^-$ concentrations in all creeks were generally associated with increased δ^{15}N values as well as an increased range in δ^{15}N (Fig 3.4). δ^{15}N-NO$_3^-$ values peaked at NO$_3^-$ concentrations of ~ + 3.5 to + 4.5 mg N/L. A further decrease in NO$_3^-$ concentration caused very little change to the δ^{15}N-NO$_3^-$ values, except at Boomer Creek where one low δ^{15}N-NO$_3^-$ value (+10.2 ‰) was observed at a low NO$_3^-$ concentration (1.9 mg N/L). It was also observed that lower NO$_3^-$ concentrations were associated with increased δ^{18}O values as well as an increased range in δ^{18}O (Fig 3.5). The highest NO$_3^-$ concentrations were associated with δ^{18}O-NO$_3^-$ values ranging from ~ -1.8 and + 0.5 ‰. Peak δ^{18}O -NO$_3^-$ values for the creeks ranged from approximately + 1.3 to + 6.1 ‰ with Swan Creek at the low end and Boomer Creek the high end. δ^{18}O-NO$_3^-$ values for the creeks peaked at ~ + 2.5 to + 4.5 mg N/L and remained fairly consistent at low NO$_3^-$

concentrations. Boomer Creek however, had a low δ^{18}O-NO_3^- (+ 1.5 ‰) that occurred at the same concentration as the low δ^{15}N-NO_3^-.

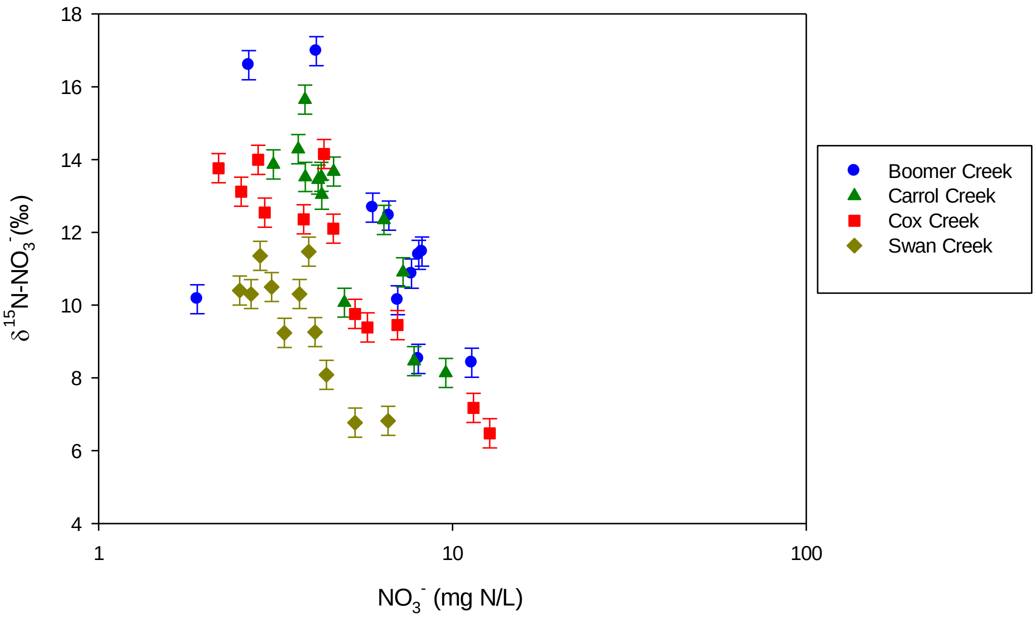


Figure 3.4: δ^{15}N-NO_3^- and NO_3^- values from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, samples collected at various times of year throughout the study period.

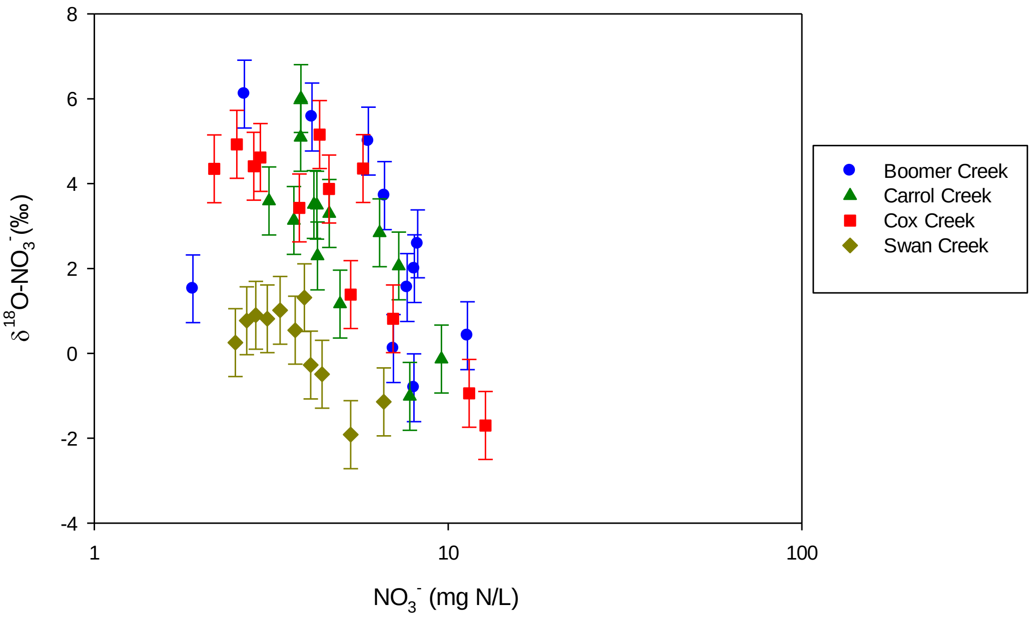


Figure 3.5: δ^{18}O-NO$_3^-$ and NO$_3^-$ values from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, samples collected at various times of year throughout the study period.

3.4 Discussion

3.4.1 Role of Denitrification in Streams and Groundwater

Denitrification in streams and groundwater plays an important role in both NO$_3^-$ concentration and isotopic abundances. The use of both δ^{15}N and δ^{18}O values together aid in the determination of the occurrence of denitrification, but isotopic abundances, particularly of δ^{18}O, can be misleading due to the rapid cycling of NO$_3^-$ within anoxic soils or stream sediments (Kool et al. 1180-1185; Wunderlich, Meckenstock, and Einsiedl 31-45).

The majority of δ^{15}N and δ^{18}O values from all the agricultural creeks with the exception of Swan Creek had $\frac{\delta^{18}O}{\delta^{15}N}$ slopes greater than that expected for denitrification in groundwater (0.5-0.7) (Fig 3.6), the $\frac{\delta^{18}O}{\delta^{15}N}$ slopes and R^2 values for which are summarized in Table 3.2.

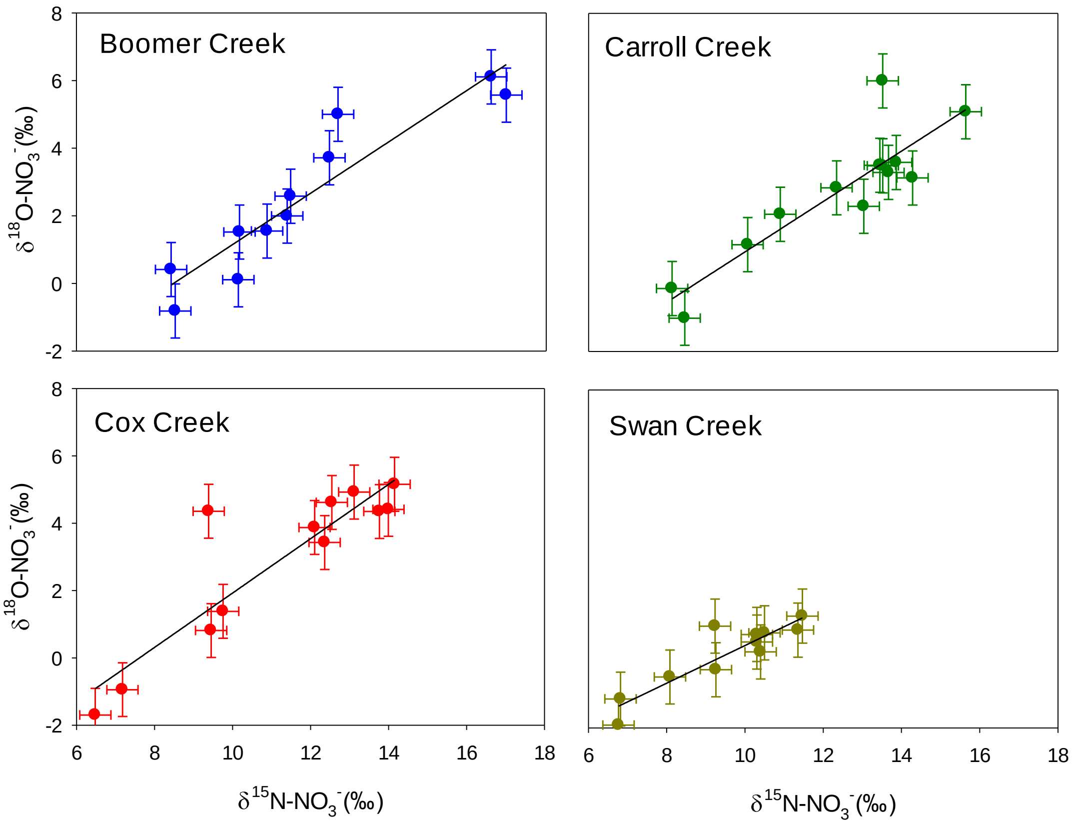


Figure 3.6: δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ values from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, samples collected at various times of year throughout the study period. The black lines represent a line of best fit, the exact slopes of which can be seen in Table 3.2

Table 3.2: Summary of $\frac{\delta^{15}N}{\delta^{18}O}$ type II linear regression analysis and R^2 values from Boomer, Carroll, Cox and Swan Creek. Analysis was conducted using RStudio R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. Model II Regression was conducted using Pierre Legendre (2014). lmodel2: Model II Regression. R package version 1.7-2. http://CRAN.R-project.org/package=lmodel2

Catchment	$\frac{\delta^{18}O}{\delta^{15}N}$ slope	R^2
Boomer Creek	0.81	0.87
Carroll Creek	0.81	0.80
Cox Creek	0.89	0.81
Swan Creek	0.59	0.83

Slopes and R^2 values varied between sites, with a range in slope of 0.59 to 0.89 and a range in R^2 range of 0.8 and 0.87. A high level of model fit was observed at Boomer Creek ($R^2 = 0.87$) despite the anomalous $\delta^{15}N$ value that occurred at a low NO_3^- . The low slope (0.59) observed at Swan Creek, which was the only creek to fall within the empirical $\frac{\delta^{18}O}{\delta^{15}N}$ denitrification slope (0.5-0.7) may be caused by the small range of measured NO_3^- concentrations.

Denitrification rates are not uniform throughout the year and are dependent on seasonal conditions such as temperature and dissolved oxygen concentrations, with greater denitrification occurring during times of warmer temperatures (summer). This not only affects the amount of NO_3^- within groundwater but also affects the enrichment factor associated with denitrification, as it is dependent on the denitrification rate and the concentration of NO_3^- within the substrate. All creeks displayed an increase in both $\delta^{15}N$ (Fig 3.7) and $\delta^{18}O$ values with declining NO_3^- concentrations, which may indicate that denitrification is occurring. Swan Creek which had a $\frac{\delta^{18}O}{\delta^{15}N}$ denitrification slope (0.59) closest to that observed in other groundwater studies, had a much smaller annual range in $\delta^{15}N$. One possible explanation for this may be a result of Swan Creek having a lower input of manure (1.61 x 10^5kg). When compared to the other creeks studied Swan Creek has less than half of the manure input as Cox Creek (3.97 x 10^5kg) which has the second lowest input of manure. Also it is estimated that Swan Creek has almost equal the amount of input from inorganic fertilizer (1.51 x 10^5kg). Inorganic fertilizer typically has lower δ^{15}N values (0‰) when compared to manure (> 8‰), this may result in δ^{15}N values of the Creek reflecting a mix of these sources.

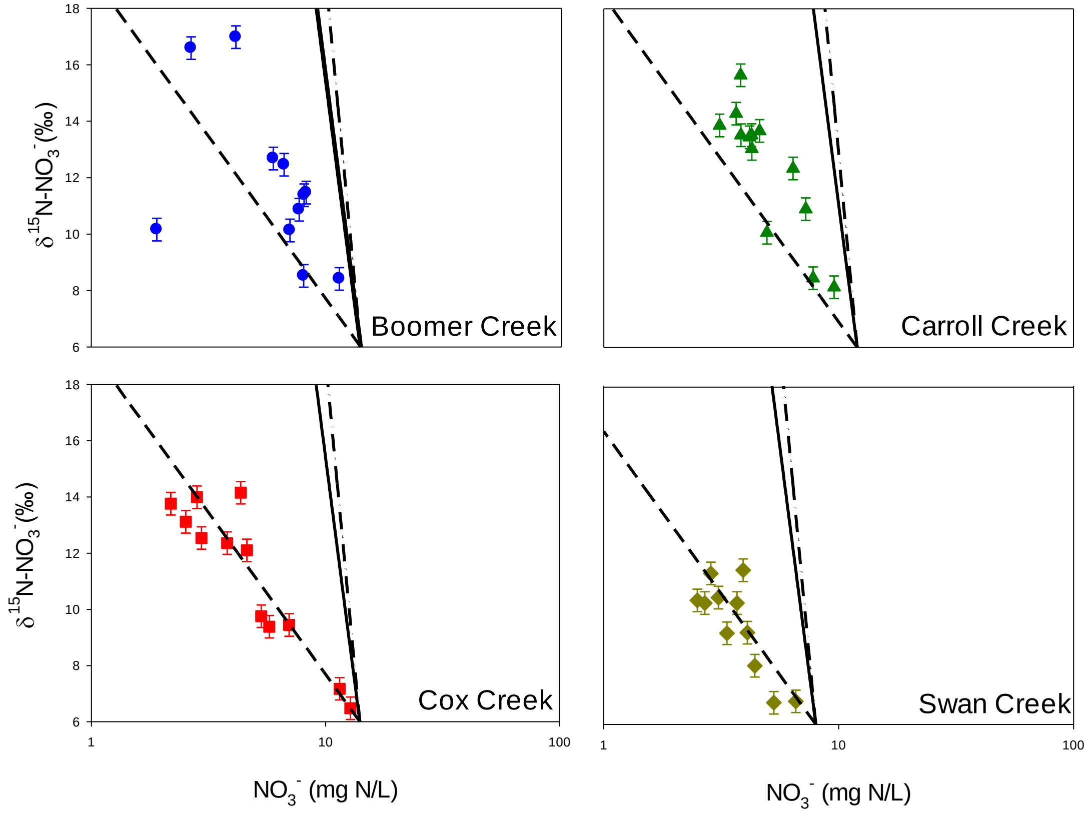


Figure 3.7: δ^{15}N-NO_3^- values and NO_3^- concentrations from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, samples collected at various times of year throughout the study period. The black line represents the enrichment in δ^{15}N-NO_3^- as a result of denitrification causing NO_3^- concentrations to decrease. This line was calculated using the Rayleigh fraction equation with a ε of -27.6‰. Since a site specific enrichment factor was not calculated the single dot dashed line represents an enrichment factor of -5‰ and the double dot dashed line represents an enrichment factor of -38‰ which represent the possible range in enrichment factors The start of this line is based on an estimation of source values from each creek: (Boomer Creek: 14‰, Cox Creek: 14‰, Swan Creek: 8‰, Carroll Creek: 12‰.

Slopes from $\delta^{15}N$ vs. NO_3^- are less than that of the modelled denitrification line. Although it has been found that groundwater denitrification can produce enrichment factors of -5‰ it is unlikely that the data observed in this study would have enrichment factors less than -10‰ as it was found that denitrification occurring in the Strawberry Creek catchment had an enrich**h**ment value of -27‰. This would suggest that denitrification as well as dilution or mixing with a low NO_3^- source is occurring. This would cause a decrease in the NO_3^- concentration but would not cause a change in the $\delta^{15}N$ (Kendall and Aravena 261-297).

3.4.1.1 N_2O as an Indicator for Denitrification

N_2O is generated through the processes of nitrifrification and denitrifrification (Yokoyama and Ohama 967-972). N_2O is created as a by-product of nitrification and is an intermediate product of denitrification. The production of N_2O during denitrification is greater than that of nitrification (Snider, Schiff, and Spoelstra 877-888). Nitrifying bacteria can create N_2O in two ways, either through nitrification or through nitrifier denitrification. Hayatsu et al. (2008) effectively summarizes these reactions; during nitrification, N_2O is produced as a by-product as a result of ammonia oxidation. During ammonia oxidation an unstable intermediate (HNO) is formed which spontaneously decomposes to N_2O. In nitrifier-denitrification NH_4^+ is oxidized to NO_2 and subsequently reduced to N_2O. N_2O produced through denitrification is an intermediate product of the reduction of NO_3^- to N_2.

The generation of N_2O through nitrification has been shown to be strongly influenced by O_2 availability (Khalil, Mary, and Renault 687-699) as well as NH_4^+ (Yokoyama and Ohama 967-972). N_2O in an agricultural system will be produced through both nitrification and denitrification. Concentrations of N_2O from the studied creeks followed a similar seasonal pattern to the NO_3^-, but maximum peak concentrations did not occur simultaneously. Specifically, higher N_2O values occurred during fall 2012, spring 2013 and to a lesser degree during the winter of 2013 (Fig 3.8a & 3.8b). Similar to the pattern observed in NO_3^- concentrations, N_2O decreased during the summer months but was still present at saturated concentrations. Unlike NO_3^- concentrations however, which were observed to have peak concentrations during the late fall and early winter when the water table is closer to the surface and tile drains are likely to be active, N_2O was observed to reach its greatest concentration during the time of spring melt. Following fertilization, which likely occurs during times of tile drain flow (fall-spring) it would be expected that a large amount of N_2O would be produced through nitrification, yet peak concentrations occur during spring melt. As a result it is likely that N_2O produced during spring melt is a result from denitrification. Increased concentrations occurring during the fall may be a combination of N_2O produced through both denitrification and nitrification as it was observed that fertilizer application took place during this time. The solubility of N_2O is a function of temperature with increased solubility occurring at lower temperatures (Weiss and Price 347-359). The range between summer and winter temperatures within the studied creeks was approximately 30°C, which results in close to three-fold increase (2.16 mol L^{-1} atm^{-}1 to 5.93 mol L^{-1} atm^{-1}) in the solubility of N_2O, even so this cannot account for the N_2O concentrations observed during spring melt, indicating that N_2O is either being produced or is being rapidly exported into the creeks during these times.

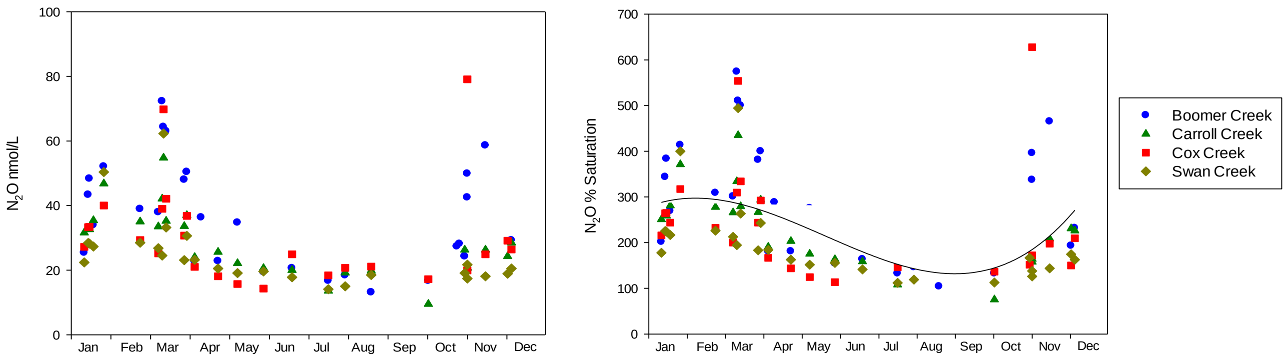


Figure 3.8a & Figure 3.8b: Figure 3.8a: N_2O concentrations from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, collected at various times of year throughout the study period. Figure 3.8b: N_2O as % saturation with respect to the atmosphere at 9°C (average groundwater temperature) for Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, collected at various times of year throughout the study period.

Since the highest N_2O concentrations did not occur at times of low NO_3^- concentrations (Fig 3.9) an alternate process may be controlling N_2O concentrations. There are two hypotheses as to why this is occurring that will be discussed in detail in this section; 1) denitrification causes the increased N_2O

concentrations, but as a result of the large NO_3^- pool even if a small percent of the NO_3^- were denitrified this would create a large amount of N_2O, or 2) nitrification occurs throughout the year, causing an N_2O build up in the soil that is flushed when the water table rises. 1) Denitrification during the spring and fall causes increased N_2O concentrations. Times of increased NO_3^- concentrations, such as those observed in the fall, spring and to a lesser extent the winter, correspond to times of increased N_2O concentrations, however there is no increase in the $\delta^{15}N$-NO_3^- indicative of denitrification. This may be due to the fact that between the fall and spring the water table rises and is exposed to a large amount of NO_3^- from the shallow soil layer. Some of the NO_3^- will likely undergo denitrification but due to the large NO_3^- pool the changes in the NO_3^- concentration, $\delta^{15}N$ and $\delta^{18}O$ values is small. This denitrification still causes an increase in the N_2O concentration, even if only a small amount of the NO_3^- pool is denitrified. During the summer, there is an observed increase in both $\delta^{15}N$ and $\delta^{18}O$ with a decrease in NO_3^-, which could be attributed to denitrification. This may be a result of a smaller NO_3^- pool, causing denitrification to have a large effect on the overall NO_3^- concentration and isotopic values.

Another possible indicator of denitrification is the presence of metheane (CH_4). In the absence of O_2, NO_3^-, manganese, iron, sulphate, redox conditions will change causing the reduction of carbon dioxide (CO_2) to become thermodynamically favourable, resulting in the production of CH_4 (Bremner 7-16). Therefore if CH_4 is being produced it is likely that the majority of the NO_3^- in the soil has been denitrified. Methane data would suggest that during late April to late May, depending on the creek, conditions become thermodynamically favourable for the production of CH_4 (Fig 3.10). A change in redox conditions making the reduction of CO_2 thermodynamically favourable would indicate that denitrification is going to completion, which would decrease the amount of N_2O produced; as denitrification approaches completion the N_2O:N_2 ratio decreases reducing the amount of N_2O produced (Van Cleemput 187-194). The production of CH_4 is strongly influenced by temperature as well as particulate organic matter (Jones Jr et al. 155-173). During the late spring, anoxic conditions in the soil due to increased water levels from spring melt, increased temperatures and likely a large source of organic carbon from fertilizer (manure) application promote favourable conditions for CH_4 production. Methane concentrations likely decrease once the water table decreases and there is no longer a large source of organic carbon. Since tile drains were observed to have consistently elevated concentrations of NO_3^-, it is likely that CH_4 is being produced in the groundwater and subsequently entering the creek through groundwater discharge. Methane concentrations decline throughout the summer, this is likely a result of a change in redox conditions whereby the reduction of CO_2 is no longer thermodynamically favourable.

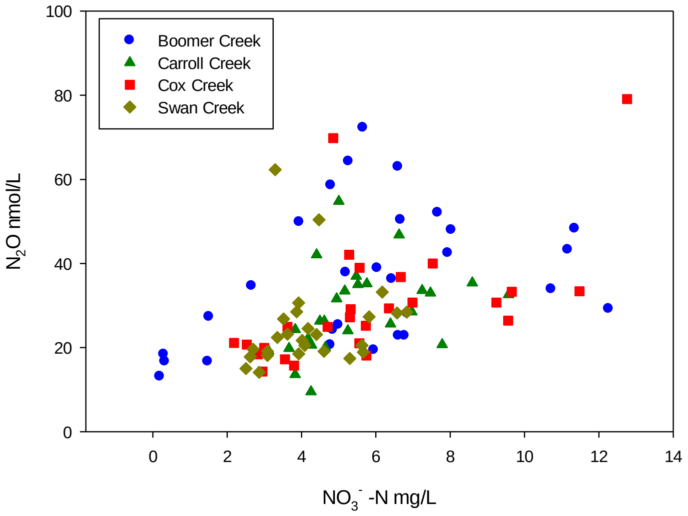


Figure 3.9: N_2O and NO_3^- concentrations from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, collected at various times of year throughout the study period.

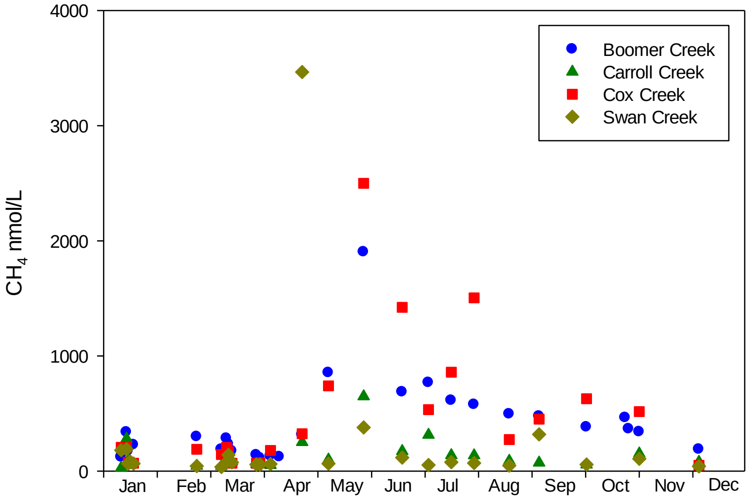


Figure 3.10: CH_4 concentrations from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, collected at various times of year throughout the study period.

A second hypothesis could be that nitrification occurs throughout the year, causing an N_2O build up in the soils that is flushed when the water table rises. Nitrification has been shown to occur in soils throughout the winter (Savard et al.). It has also been observed that at lower temperatures nitrification generates more N_2O than at higher temperatures (Maag and Vinther 5-14). Manure applied onto the fields after crop harvest will be slowly nitrified over the coming months. This nitrification will generate NO_3^- as well as N_2O. This N_2O is then subsequently flushed out during melt or large precipitation events.

It is likely that both of these factors contribute to the increased N_2O observed in the spring and fall. During the fall, when crops are harvested, precipitation increases causing the water table to rise. This would also occur in the spring when the snowpack begins to melt with the onset of spring precipitation and to a lesser degree during a mid-winter melt event. With the rise of the water table NO_3^-, as well as the N_2O, is rapidly flushed out of the soil through tile drains and into the groundwater. Studies conducted on the N_2O concentrations in the Strawberry Creek watershed by Rempel et al. (2008) and Thuss et al. (2006), found that elevated N_2O concentrations were observed in tile drain discharge during melt and storm events.

Isotopic analysis of samples collected by Rempel found that the N_2O was likely generated by denitrification. The increased rate of transport and access to previously unsaturated soil is reflected by the increased N_2O concentrations in the creeks.

3.4.2 Calculation of Source $\delta^{15}N$

In order to identify potential source contributions of NO_3^- to these agricultural streams, the $\delta^{15}N$ must be adjusted for the isotopic enrichment due to denitrification. In order to do this, the $\delta^{18}O$ of the source NO_3^- has to be estimated. The lowest $\delta^{18}O$ value for Boomer, Cox, Swan and Carroll Creek was -0.81‰, -1.70‰, -1.92‰ and -1.01‰ respectively (Fig 3.11).

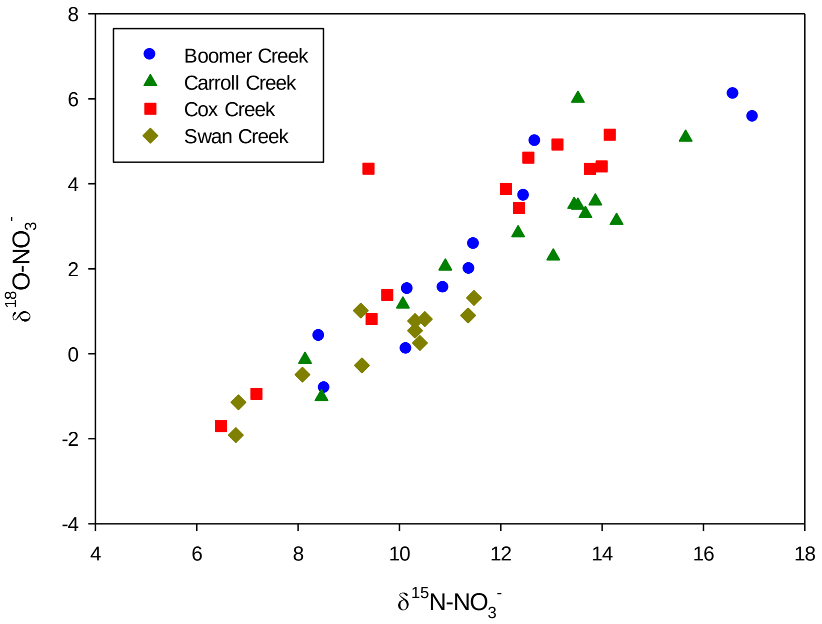


Figure 3.11: $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, samples collected at various times of year throughout the study period.

By rearranging equation 3.1 it is possible to calculate the $\delta^{15}N$ of the NO_3^- source. The $\Delta^{18}O$ value is calculated by subtracting the NO_3^- - $\delta^{18}O$ of the sample by the estimated NO_3^- - $\delta^{18}O$ of the source. If denitrification is the only process controlling isotopic ratios then the calculation of the $\delta^{15}N$ of the source should lead to a common value. A type II regression performed on $\delta\,^{15}N$ and $\delta\,^{18}O$ data from all the creeks was used to find the denitrification slope (DS).

$$DS = \frac{\Delta\delta^{18}O}{\Delta\delta^{15}N} \qquad \textbf{Equation 3.1}$$

$$\frac{\Delta\delta^{18}O}{DS} = \Delta\delta^{15}N$$

$$\frac{\Delta\delta^{18}O}{DS} = \delta^{15}N_2 - \delta^{15}N_1$$

$$\delta^{15}N_1 = \delta^{15}N_2 - \frac{\Delta\delta^{18}O}{DS}$$

This simple equation makes several critical assumptions:

1. All fractionation in O or N occurs as a result of denitrification
2. Single NO_3^- source.

The results of correcting the individual $\delta^{15}N$ values for denitrification can be seen in (Fig 3.12). All of the agricultural creeks corrected to a similar $\delta\,^{15}N$ source range (+ 6 to + 8 ‰). This gives strong evidence that these watersheds share similar sources of NO_3^- . The majority of data points were within the + 6‰ to + 8‰ range, which is slightly below the expected $\delta^{15}N$ values of manure.

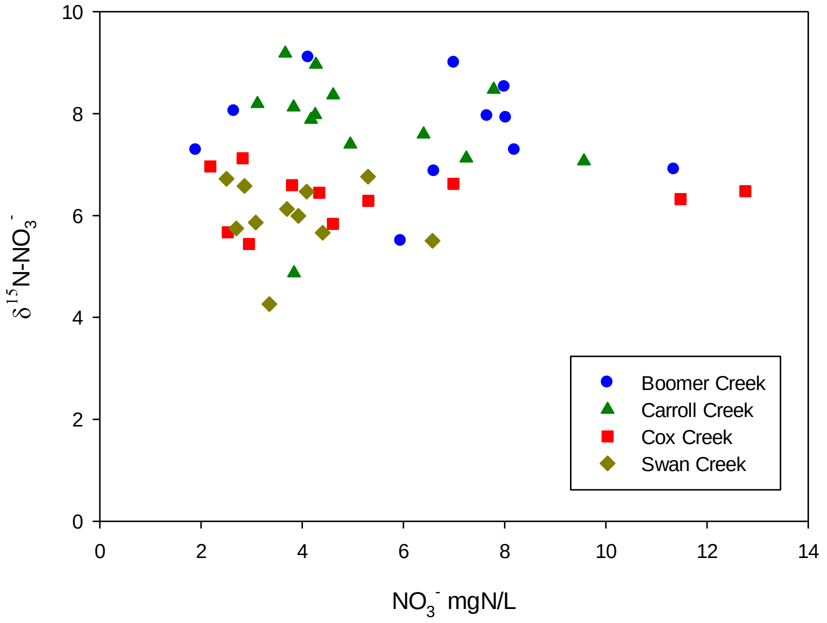


Figure 3.12: Corrected δ^{15}N-NO$_3^-$ values and NO$_3^-$ concentrations from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, samples collected at various times of year throughout the study period. δ^{15}N-NO$_3^-$ values have been corrected for denitrification.

3.4.3 NO$_3^-$ Sources to Streams

After correcting for denitrification a more accurate estimation of the source of NO$_3^-$ can be made. Stable isotopic analysis reveals (Fig 3.13) δ^{15}N values (+6 to +8‰) that are slightly below values associated with manure (> + 8‰). Despite being close to the δ^{15}N which has been empirically found for manure, several studies also describe similar δ^{15}N values to belong to other sources. A study conducted by Chang et al. (2002) on small streams in the upper Mideast US described δ^{15}N values of +5.5‰ as characteristic of inorganic fertilizer and soil nitrate. Gormly & Spalding (1979) stated that differentiating the NO$_3^-$ sources between inorganic and organic fertilizers solely on δ^{15}N values in groundwater between 3.5‰ to 9.5‰ was not possible. In addition there is also strong evidence that ammonia volatilization of inorganic fertilizers can cause an enrichment of +6‰ (Flipse and Bonner 59-67). Therefore although the δ^{15}N values for the creeks are within which has been found for manure, it is not possible at this time to definitively say that NO_3^- in these creeks is derived from manure.

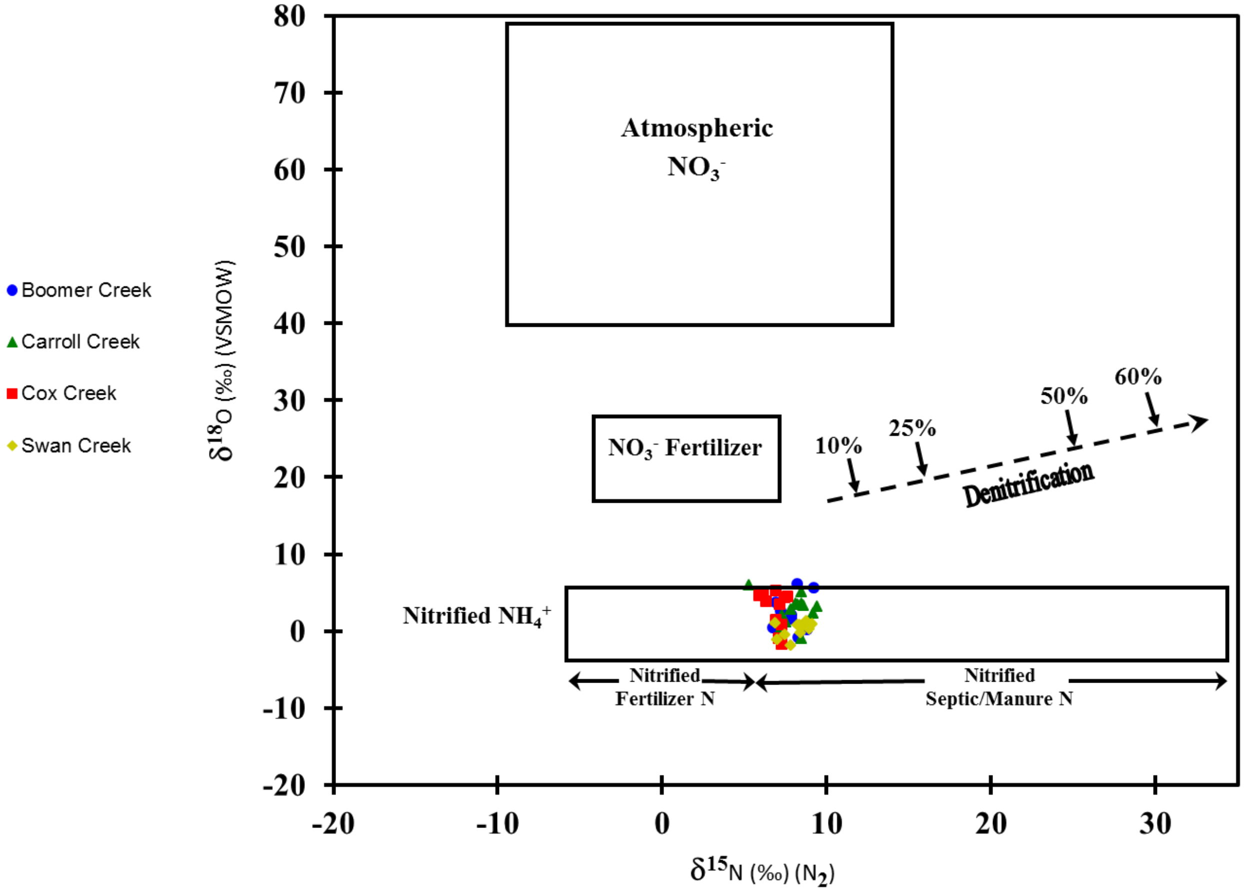


Figure 3.13: $\delta^{15}N-NO_3^-$ and $\delta^{18}O-NO_3^-$ values from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, samples collected at various times of year throughout the study period. $\delta^{15}N-NO_3^-$ has been corrected for denitrification. Boxes indicate empirical $\delta^{15}N$ and $\delta^{18}O$ of different NO_3^- sources. The denitrification line (dashed line) represents the relationship between $\delta^{15}N$ and $\delta^{18}O$ values of the residual NO_3^- that results from denitrification and has been observed in many groundwater studies to typically be regarded as a 2:1 ratio (Kendall and McDonnell). The percentages on the denitrification line correlate to the fraction of NO_3^- that has been denitrified. The location of the percentages on the denitrification line is dependent on the enrichment factor.

3.4.4 The Effects of Land-Use on δ^{15}N-NO$_3^-$

Despite differences in land-use, there was very little difference in the δ^{15}N-NO$_3^-$ values (Fig 3.14). The percent of total nitrogen attributed to manure varied between creeks with Boomer Creek having the most (68%) and the other creeks falling having between (45-53%) (Ontario Ministry of the Environment). Percent agriculture (The Ontario Ministry of Natural Resources) is similar in all creeks (77-86%) yet there is a large difference in % tile drains, total nitrogen density and manure density between creeks data (Ministry of Agriculture, Food and Rural Affairs). Despite these differences all creeks had similar δ^{15}N values (+ 6‰ to + 8).

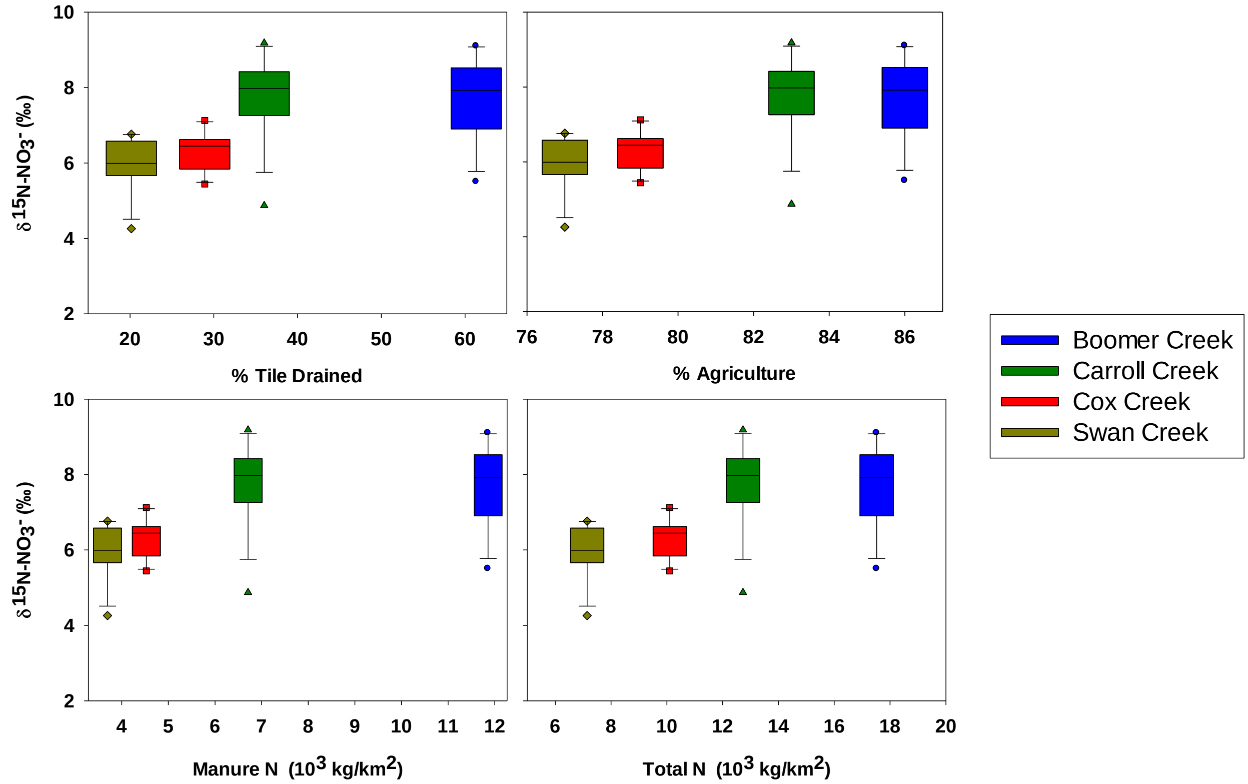


Figure 3.14: Box plot of corrected δ^{15}N-NO$_3^-$ values as well as land-use data from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek, samples collected at various times of year throughout the study period.

3.4.5 Effect of Tile Drains on δ^{15}N-NO_3^- and δ^{18}O-NO_3^-

In order to better understand the sources and processes controlling NO_3^- concentrations on agricultural creeks, stable isotopic analysis was conducted on water originating from the tile drains as well as water from adjacent shallow groundwater along Boomer Creek. Unfortunately the majority of groundwater samples had NO_3^- concentrations very close to the detection limit or had NO_2^- concentrations that were too high to be run for isotopic analysis. The tile drains showed a large range in both δ^{15}N-NO_3^- and δ^{18}O-NO_3^- (Fig 3.15).

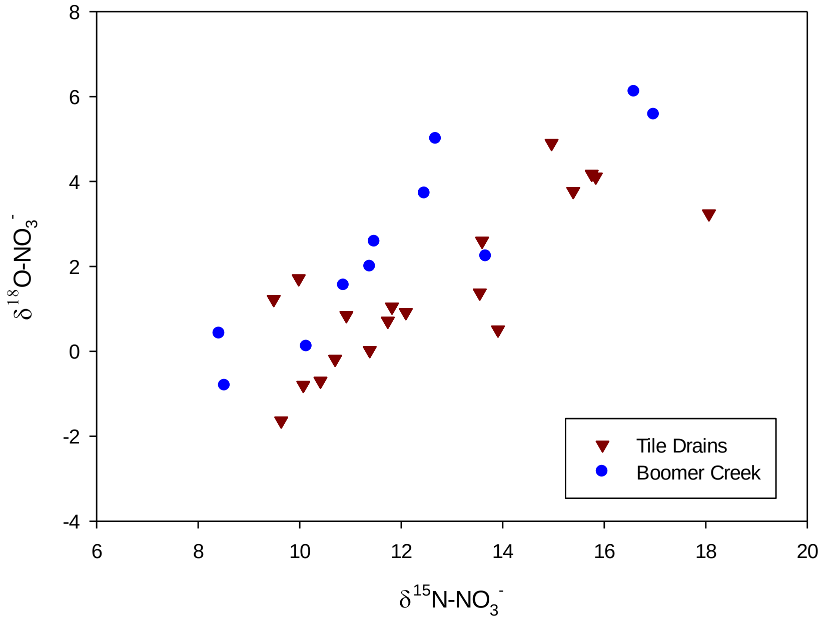


Figure 3.15: δ^{15}N-NO_3^- and δ^{18}O-NO_3^- values from Boomer Creek and tile drains located along Boomer Creek. Samples for Boomer Creek were collected at various times of year throughout the study period. Samples for tile drains were collected in November 2013.

Tile drains along Boomer Creek had similar δ^{15}N and δ^{18}O-NO_3^- values when compared to Boomer Creek. Minimum δ^{15}N values in the tile drains were approximately + 9.5‰ whereas Boomer Creek minimum δ^{15}N values occurred close to + 8‰. Although there was a large range (10 mg/L) in NO_3^- values in both the tile drains and Boomer Creek δ^{15}N values were similar at similar concentrations. This gives further evidence that tile drains strongly influence NO_3^- concentrations within Boomer Creek. In addition δ^{15}N and δ^{18}O values at higher concentrations-from tile drains are very close to that of Boomer Creek at high concentrations (+ 8 to + 10‰ and 0‰) (Fig 16a & b).

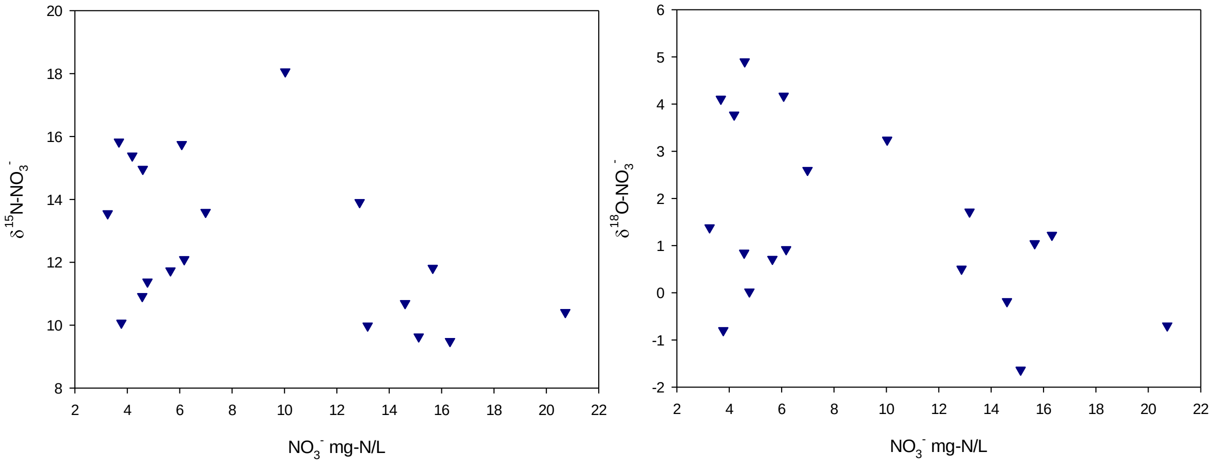


Figure 3.16a & 3.16b: Figure 3.16a: δ^{15}N-NO_3^- values and NO_3^- concentrations from tile drains located along Boomer Creek. Figure 3.16b: δ^{18}O-NO_3^- and NO_3^- concentrations from tile drains located along Boomer Creek. Samples for tile drains were collected in November 2013.

3.4.6 δ^{15}N and δ^{18}O at High flows

Times of high discharge often occurred during time of increased tile drain flow such as spring melt and large precipitation events. During times of increased discharge tile drains were observed to have lower values in both δ^{15}N and δ^{18}O (Fig 3.17 a & b). Times of increased discharge were also related to lower ranges in both δ^{15}N and δ^{18}O in all creeks. During times of lower flows, the δ^{18}O and δ^{15}N values of the creeks show a larger range (Fig 3.18 a & b). This is likely a result of several factors, reduced input from tile drains and increased denitrification. During the summer there is a reduced amount of water being discharged from the tile drains as a result of the water table being lower, causing some tile drains to cease to flow. Although water from flowing tile drains was observed to have NO_3^- concentrations > 4mg/L, their water contribution to the creeks is likely very small during this time. Also during the summer, increased temperatures will cause denitrification to be favourable both within the soil and in stream increasing both the $\delta^{18}O$ and $\delta^{15}N$ within the remaining water.

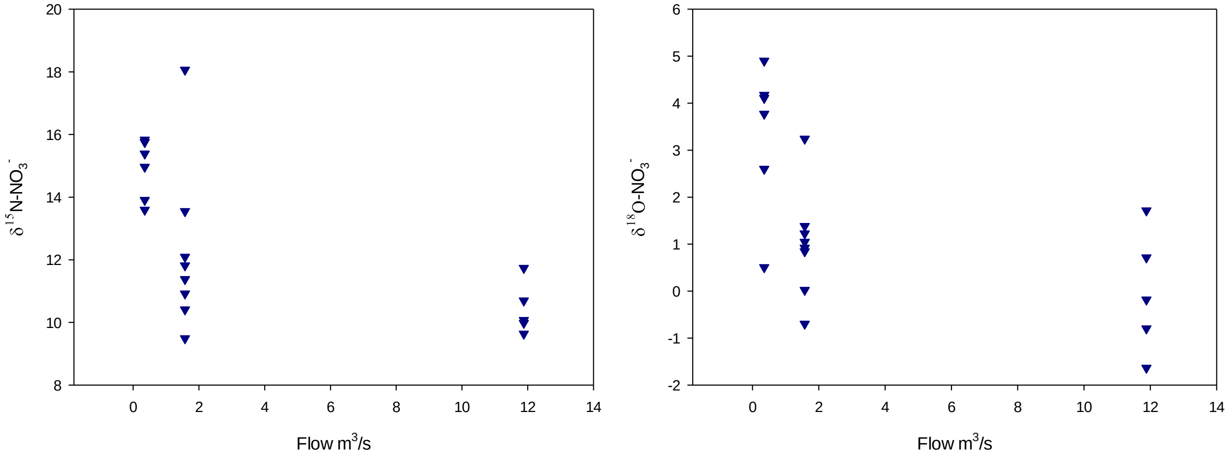


Figure 3.17a & 3.17b: Figure 3.17a: $\delta^{15}N\text{-}NO_3^-$ values from tile drains located along Boomer Creek and estimated Boomer Creek discharge. Figure 3.17a: $\delta^{18}O\text{-}NO_3^-$ values from tile drains located along Boomer Creek and estimated Boomer Creek discharge. Samples for tile drains were collected in November 2013.

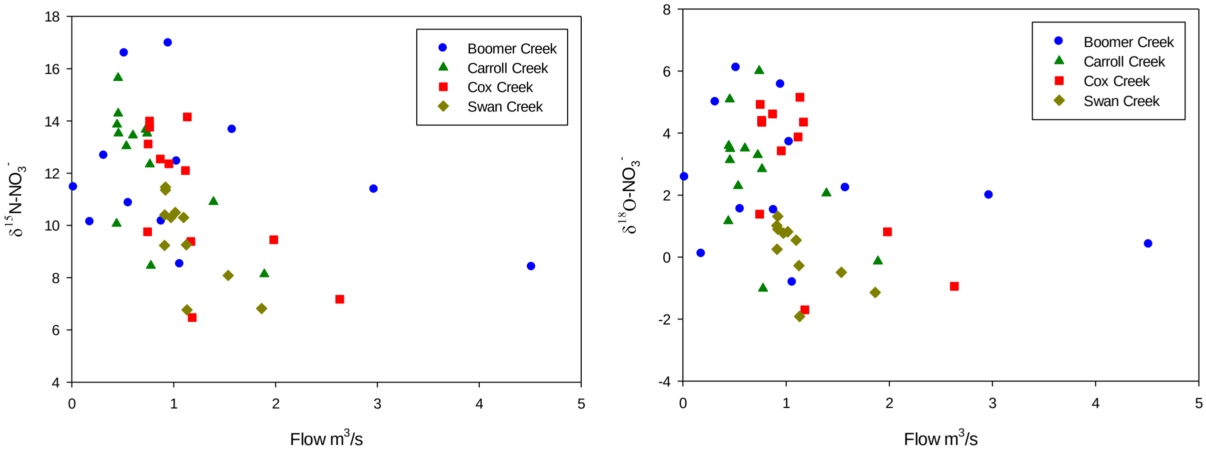


Figure 3.18a & 3.18b: Figure 3.18a: δ^{15}N-NO$_3^-$ values from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek and respective estimated creek discharge. Figure 3.18b: δ^{18}O-NO$_3^-$ values from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek and estimated creek discharge. Samples collected at various times of year throughout the study period.

3.4.7 Systematics of NO_3^- stable isotopes in Small Agricultural Catchments

In order to better explain variations in annual NO_3^- isotopic a simplified conceptual model was created. In this model NO_3^- that has undergone denitrification will have elevated δ^{15}N -NO_3^- and δ^{18}O -NO_3^- values, while NO_3^- that has not undergone denitrification will have isotopic values close to that of the source. If a denitrification line can be modelled and plotted on a δ^{15}N -NO_3^- vs. NO_3^- graph, then a theoretical area under which all possible data should fall can be calculated (Fig 3.19). How close data matches do this denitrification line will be dependent on the fraction of NO_3^- remaining and the isotopic fractionation factor.

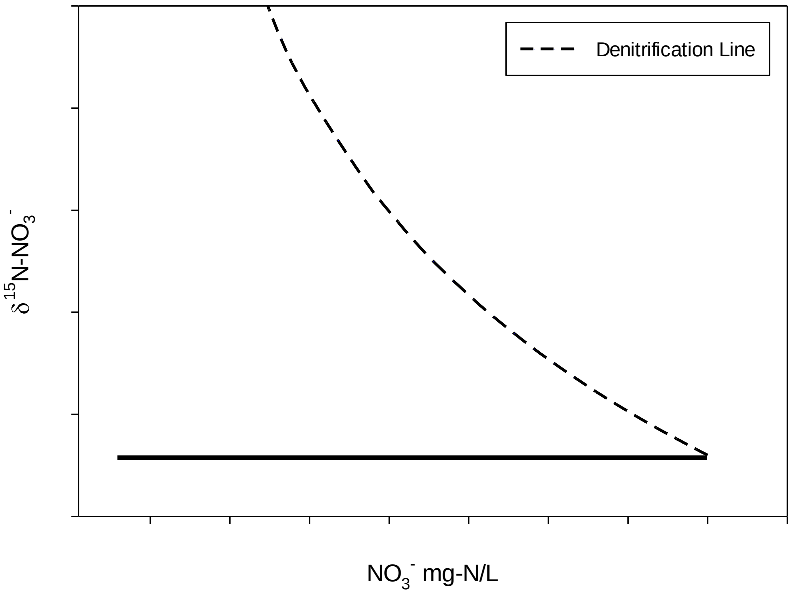


Figure 3.19: Model of possible $\delta^{15}N\text{-}NO_3^-$ values and associated NO_3^-. The dashed black line represents the enrichment in $\delta^{15}N\text{-}NO_3^-$ as a result of denitrification causing NO_3^- concentrations to decrease. This was calculated using the Rayleigh fraction equation with a ε of -27.6‰. The start of this line is based on an estimation of source values. The solid black line represents dilution, decreasing the NO_3^- concentration without any change in $\delta^{15}N\text{-}NO_3^-$.

The Rayleigh distillation equation (Equation 3.2) allows for the calculation of fractionation effects on $^{15}N$of the remaining NO_3^- .

$$\delta \approx \delta_0 + \varepsilon_{N_2-NO_3} \ln(f) \qquad \textbf{Equation 3.2}$$

Where δ is the composition of the remaining NO_3^- , δ_0 is the initial composition of the NO_3^-, *f* is the remaining fraction of substrate and ε the enrichment factor. The application of the Rayleigh distillation equation in this study is based on the following assumptions; closed system, finite source of NO_3^- and the enrichment factor does not change throughout the reaction (Kendall and McDonnell). These assumptions are not valid in this system as this is an open system where NO_3^- is being contently added through the addition of fertilizer or the nitrification of NH_4^+, also it is very unlikely that the enrichment factor is constant throughout as this will be dictated by the environmental conditions.

An ^{15}N enrichment factor of -27.6‰ will be used for calculations, as this was found to be the enrichment factor in a similar catchment (Strawberry Creek) within the Grand River Watershed (Mengis et al. 448-457). Once the δ^{15}N values were calculated based on the fraction of NO_3^- remaining, this curve was then plotted on a δ^{15}N vs NO_3^- and compared to δ^{15}N and NO_3^- concentrations from the creeks (Fig 3.20).

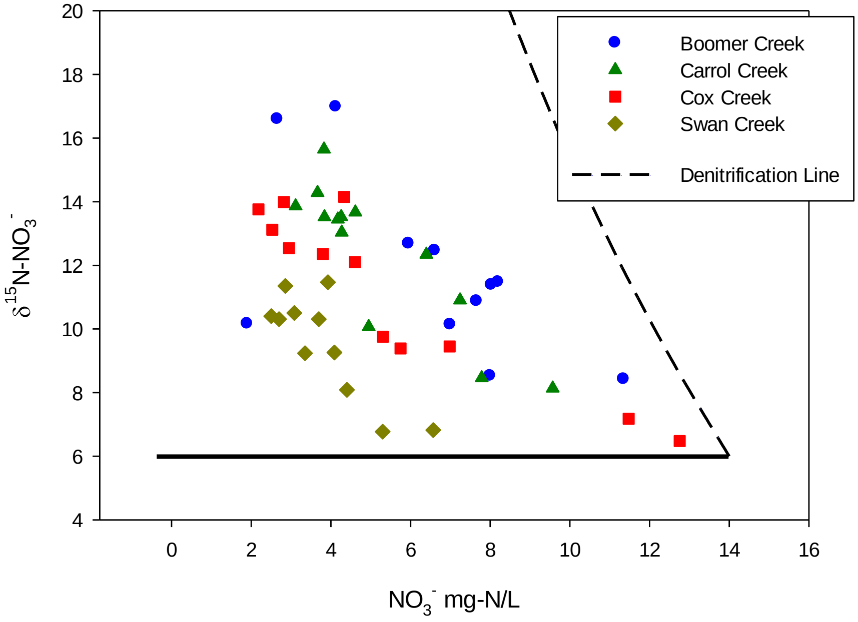


Figure 3.20: $\delta^{15}N-NO_3^-$ values and associated NO_3^- from Boomer Creek, Carroll Creek, Cox Creek and Swan Creek. The dashed black line represents the enrichment in $\delta^{15}N-NO_3^-$ as a result of denitrification causing NO_3^- concentrations to decrease. This was calculated using the Rayleigh fraction equation with a ε of -27.6‰. The start of this line at 14 mg N/L and 6‰ is based on an estimation of the average source NO_3^- values in each creek. The solid black line represents dilution, decreasing the NO_3^- concentration without any change in $\delta^{15}N-NO_3^-$. Samples collected at various times of year throughout the study period.

All creeks show an increase in the range of both δ^{15}N and δ^{18}O at low concentrations. Higher concentrations tend to have lower δ^{15}N and δ^{18}O values. Depending on the initial NO_3^- concentration chosen, in a system where denitrification was the lone factor affecting NO_3^- concentrations and isotope fractionation, the $\delta^{15}N$ values should fall along the same slope as the denitrification line calculated with the Rayleigh equation. If there was little denitrification occurring then the $\delta^{15}N$ and $\delta^{18}O$ would not show a marked change with a decrease in NO_3^- concentration. The creeks appear to be strongly influenced by denitrification as there is a clear increasing trend in $\delta^{15}N$ as the NO_3^- concentrations decreases. However there is also some dilution occurring as the data does not fall along the slope for denitrification. This is unsurprising as the creeks receive inputs from groundwater which has strong evidence of denitrification and from the tile drains which has little evidence of denitrification. In addition the amount of input from tile drains as well as the conditions for denitrification changes throughout the year leading to the input from the different sources changing throughout the year.

This denitrification model was also applied when interpreting tile drain isotopic values. The tile drains located in Boomer Creek displayed a large range in $\delta^{15}N\text{-}NO_3^-$ in comparison to NO_3^- concentration (Fig 3.21).

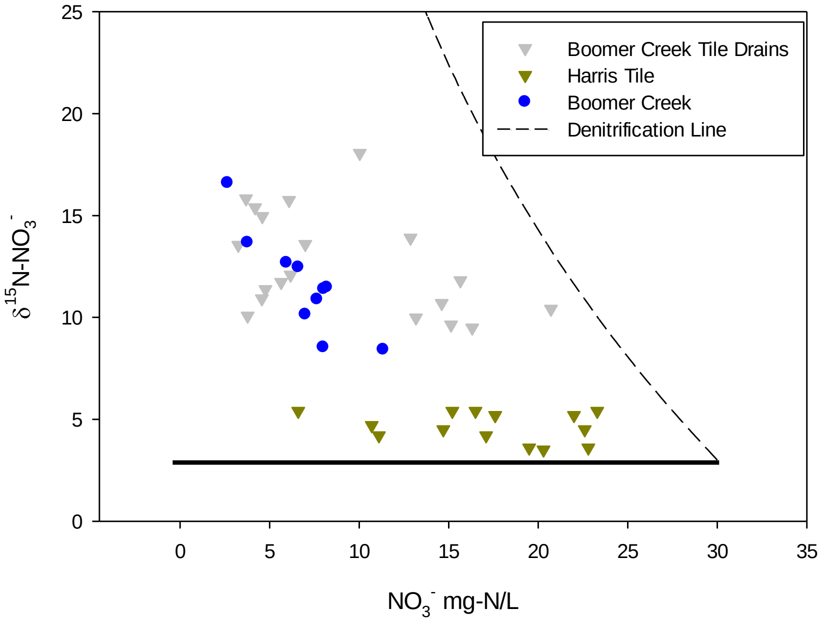


Figure 3.21: δ^{15}N-NO$_3^-$ values and associated NO$_3^-$ from Boomer Creek, tile drains along Boomer Creek and from a tile drain (Harris Tile) from the Strawberry Creek watershed (Rempel, 2008). The dashed black line represents the enrichment in δ^{15}N-NO$_3^-$ as a result of denitrification causing NO$_3^-$ concentrations to decrease. This was calculated using the Rayleigh fraction equation with a ε of -27.6‰. The start of this line at 30 mg N/L and 3‰ is chosen on an estimation of source values. The solid black line represents dilution, decreasing the NO$_3^-$ concentration without any change in δ^{15}N-NO$_3^-$. Boomer Creek samples were collected at various times of year throughout the study period. Tile drain samples were taken November 2013 and samples from the Harris tile were taken between November 1998 and July 2000.

The tile drains along Boomer Creek show little variation in δ^{15}N values at elevated NO$_3^-$ concentrations (> 14 mg N/L) with most of the variability occurring at lower concentrations. As seen with δ^{15}N-NO$_3^-$ in the creeks, water from the tile drains is also appear to be affected by both denitrification and dilution. A previous study conducted by Rempel (2008) on tile drains in the Strawberry Creek watershed found that the tile drain known as the Harris Tile which drained an agricultural field fertilized with inorganic fertilizer, experienced little change in the δ^{15}N values regardless of NO$_3^-$ concentration. This data may suggest that that field conditions were not ideal for denitrification.

At high concentrations the isotopic abundances of the tile drains along Boomer Creek likely reflect the source of NO_3^- as there has likely been little denitrification. The $\delta^{15}N$ value of approximately +10 ‰ found in the tiles at high NO_3^- concentrations closely matched $\delta^{15}N$ values found in Boomer Creek (+8 to +10 ‰) at high NO_3^- concentrations. Evidence presented in this study would indicate that tile drains dictate the isotopic abundances of $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- within Boomer Creek. As mentioned in the previous chapter, there is evidence that tile drains play a strong role in the annual NO_3^- concentration patterns, tile drains also have a strong influence on $\delta^{15}N$ and $\delta^{18}O$ values. Data suggests that during times of decreased tile discharge water coming from tile drains likely undergoes denitrification. An increase in the $\delta^{15}N$ and $\delta^{18}O$ values as well as the range was observed during times with reduced tile drain flow (summer), possibly reflecting shallow groundwater discharging into the creek or in stream processing. This shallow groundwater based on observed NO_3^- concentrations has likely undergone denitrification as it passed through favourable denitrification conditions (low O_2, high organic matter) in riparian zones. This is thought to occur in all the creeks studied as elevated $\delta^{15}N$ and $\delta^{18}O$ values were observed in all creeks during the summer when compared to times of tile flow (fall-spring). During times of elevated water tables (fall through spring) tile drains flow, and contribute large amounts of water and NO_3^- to Boomer Creek. During times when tile drains were flowing Boomer Creek had similar $\delta^{15}N$ and $\delta^{18}O$ values to that of water from tile drains. During times of tile drain flow isotopic signatures from samples taken from Boomer Creek resemble those found in tile drains, indicating that tile drains may control the isotopic signatures of the of $\delta^{15}N$ -NO_3^- and $\delta^{18}O$- NO_3^- found within the creek.

3.5 Conclusions

Isotopes alone cannot in this instance differentiate between sources of NO_3^- to these catchments, it can however give insight into processes such as denitrification, which strongly influence NO_3^- concentrations within these creeks. The stable isotopic ratios of tile drain samples at high NO_3^- concentrations match those of Boomer Creek at high NO_3^- concentrations. During times when the tile drains are not flowing both $\delta^{15}N$ and $\delta^{18}O$ increase, indicating that the shallow groundwater discharging into the creek has likely undergone denitrification in the riparian zone, which is supported by supplementary data (N_2O, CH_4). This leads to the conclusion that tile drains influence the isotopic abundances of NO_3^- throughout the year.

Chapter 4 − Conclusions and Recommendations

4.1 Conclusions

Several research objectives were achieved during this study. The first objective was to characterize annual fluctuations in NO_3^- concentration within four small (44-87 km^2) agricultural catchments, as agricultural catchments represent a large non-point source of nitrogen to the environment. It was found that the range in annual NO_3^- concentrations differed between catchments but the annual pattern of elevated concentrations during the fall through to spring and decreased concentrations during the summer (June-September) existed in all catchments. The pattern found in this study was also seen in historical concentration data collected by the PWQMN over the past 10 years. Historical data and data collected during this study indicated that this pattern exists independent of scale as it also occurred in the Conestogo River watershed (819km^2) and the Grand River watershed (2253 km^2).

Annual NO_3^- concentrations observed during this study indicated that samples taken by the PWQMN did not adequately capture the annual NO_3^- concentration fluctuations. As PWQMN's aim is to simply track general changes in stream chemistry throughout time and not try to accurately estimate annual NO_3^- concentration fluctuations, samples are typically taken on a monthly grab-sample basis, and only typically from March to November. As a result concentration information at key times of discharge is not collected. This is particularly true during melt and storm events where it was found that NO_3^- concentrations rapidly increase by up to two to threefold for a short duration of time. Year-round and storm/melt event sampling is also crucial in estimating annual NO_3^- export as increased concentrations occurred with increased discharge. Therefore if the goal is to accurately estimate export a more rigorous approach to sampling must be taken, as any export calculations based on monthly grab samples may underestimate NO_3^- export.

Historical concentration data on both Boomer Creek and the Conestogo River allowed for the comparison between years classified as wet and dry based on monthly precipitation data. Boomer Creek was found to have small NO_3^- concentration differences between wet and dry years. However there was a notable difference in summer NO_3^- concentrations in the Conestogo River between wet and dry years. Dry years were found to have lower summer NO_3^- concentrations when compared to wet years. This may be due to longer water residence time and/or denitrification occurring within the reservoir during dry years. Subsequently any water discharged from the reservoir for the remainder of the year will likely have a lower NO_3^- concentration. Wet years had higher summer NO_3^- concentrations when compared to dry years, this may result from a large influx of NO_3^- latent water during the spring, which is slowly discharged the remainder of the year. Regardless of the weather conditions of a given year (wet or dry) the reservoir appears to influence NO_3^- concentrations downstream.

Investigation into NO_3^- concentrations in Boomer Creek, tile drains along Boomer Creek and shallow groundwater within the riparian zone adjacent to tile drains revealed insight into important processes governing NO_3^- processes. Tile drains had consistently elevated NO_3^- concentrations when compared to shallow groundwater at the same location. Groundwater from the adjacent riparian zone had consistently low NO_3^- and was low in all samples collected. Boomer Creek had consistently higher NO_3^- concentrations than that of shallow riparian groundwater. Elevated NO_3^- concentrations within Boomer Creek compared to groundwater demonstrates the influence that tile drains have.

During times of significant tile drain flow (fall to spring) Boomer Creek had elevated NO_3^- concentrations. Boomer Creek experienced decreased NO_3^- concentrations during the summer when tile drain flow was minimal and Boomer Creek was predominantly fed by groundwater discharge. This led to the hypothesis that tile drains have a significant influence on NO_3^- concentrations within Boomer Creek and that increased concentrations in the creek will occur when tile drains are flowing, with decreased concentrations occurring when tile drain flow is minimal.

Increased NO_3^- concentrations during the fall and spring are believed to partially result from a “wet up” that occurs (a rapid increase in water table elevation).This increase in water table elevation allows groundwater to gain access to a greater contributing NO_3^- source. Calculations on export found that spring melt represents a large amount of the annual NO_3^- export. During the 2013 spring melt >20% of the annual NO_3^- export occurred within 45 days. In contrast, export during the summer (125 days) only accounts for 7-22% of annual export as water tables and NO_3^- concentrations within the creeks are low. Fall (70 days) made up 9-18% of annual discharge as increased water tables flush nutrients from agricultural soils. Whereas winter and spring made up between 15-26 % of export again most likely as a result of elevated water tables and reduced plant uptake.

Despite differences in various land-use categories there existed little difference in NO_3^- concentration and isotope ranges. In Boomer Creek, there was nearly triple the amount of manure density and total nitrogen in comparison to Swan Creek yet the median NO_3^- concentration differed by only 1.5 mg/L and the difference in $\delta^{15}N$ was less then 2‰. Based on current data Boomer Creek was observed to have the greatest amount of annual NO_3^- export (27.3 x 10^3kg/ha) while Swan Creek which had the lowest manure and total nitrogen density of all the studied creeks had the second highest annual NO_3^- export (26.1 x 10^3kg/ha). There is little evidence that land-use has an effect on NO_3^- concentrations, isotopes or export in agricultural creeks.

The second objective of this study was to evaluate the applicability of stable isotopic ratios as a tool to analyze the sources and processes governing annual NO_3^- concentrations within these agricultural catchments. Elevated $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values occurred during late spring and summer months (May to September), with lower values occurring during the fall-spring (October to April). Isotopic abundances followed an opposite pattern to that observed in NO_3^-. Elevated $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values occurred when NO_3^- concentrations were low, whereas low$\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values were associated with times of high NO_3^- concentrations. This indicated that denitrification was likely affecting NO_3^- concentrations. Slopes from $\delta^{15}N\text{-}NO_3^-$ vs $\delta^{18}O\text{-}NO_3^-$ regression lines also gave evidence that denitrification was occurring. Data from all creeks displayed slopes between 0.5-0.9, with three of the four creeks having slopes greater than 0.8 which is higher than the range expected for denitrification in groundwater and closer to that observed in marine environments.

N_2O and CH_4 concentration data was also used in an effort to confirm the presence and appropriate redox conditions for denitrification. Boomer Creek N_2O concentrations were greatest in early spring. The N_2O concentration pattern closely matched that of NO_3^-. Although isotopes and NO_3^- concentrations may not indicate denitrification during the fall and spring, it is likely occurring and being masked by the relatively high NO_3^- low $\delta^{15}N$ and low $\delta^{18}O$ water coming from tile drains. CH_4 concentration data indicated a change in redox conditions during late spring, and early summer. Increasingly reduced conditions demonstrated by the presence of CH_4 would indicate that denitrification is likely going to completion, which would cause a decrease in the N_2O:N_2, one possible explanation as to why N_2O concentrations during summer months are so low. In addition ongoing nitrification of NH_4^+ from fertilizers throughout the year may also generate N_2O within the soil, yet the evidence of denitrification in this environment would indicate that likely denitrification generates the majority of N_2O.

Due to the kinetic fractionation associated with denitrification, $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- values must be corrected for denitrification before source determination analysis can be performed. Correction for denitrification was done by finding the $\delta^{15}N$-NO_3^- vs $\delta^{18}O$-NO_3^- slope from all creeks and finding the lowest $\delta^{18}O$ value for each creeks. The majority of corrected $\delta^{15}N$-NO_3^- values from all creeks fell within + 6 to + 8‰, which is within the expected range of manure and outside the range expected for synthetic fertilizers. Plotting $\delta^{15}N$-NO_3^- vs $\delta^{18}O$-NO_3^- values on a NO_3^- source plot found that despite the apparent match in $\delta^{15}N$ to that which has been empirically found for manure, several studies also describe similar $\delta^{15}N$ values to belong to other sources such as inorganic fertilizer or soil nitrate.

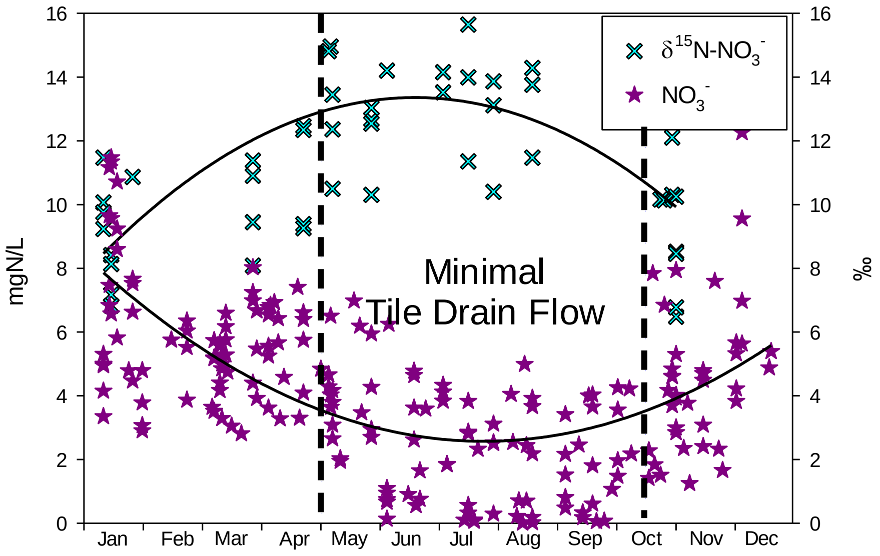
Despite large ranges between catchments in land-use parameters (manure density, nitrogen application density and tile drains) all creeks had similar $\delta^{15}N$ values (+ 6 to + 8).

Isotopic analysis was also conducted on samples from tile drains along Boomer Creek. Tile drain $\delta^{15}N-NO_3^-$ and $\delta^{18}O-NO_3^-$ values were very close to those samples from Boomer Creek taken during increased tile drain flow (fall-spring). The highest NO_3^- concentrations in both the tile drains and Boomer Creek had similar $\delta^{15}N-NO_3^-$ values. Furthermore during times of increased flow both the tile drains and Boomer Creek had decreased $\delta^{15}N-NO_3^-$ and low $\delta^{18}O-NO_3^-$ values. These values are thought to reflect source $\delta^{15}N-NO_3^-$ which has not undergone significant denitrification as a result of rapid transport from agricultural fields via tile drains.

These results were used to develop a model to explain $\delta^{15}N-NO_3^-$ values found within these creeks. Water that has undergone denitrification should follow the denitrification line on a $\delta^{15}N-NO_3^-$ vs. NO_3^- graph. Groundwater discharging through the riparian zone into the stream will have likely undergone denitrification, which is supported by the low (< 2 mg N/L) NO_3^- concentrations found in this study regardless of season or flow conditions. Alternatively as NO_3^- concentration decreases, water which does not undergo denitrification should have very little change in the $\delta^{15}N$ value. Times of elevated NO_3^- concentrations and lower $\delta^{15}N$ values in both tile drain and Boomer Creek occurred during times of increased flow. During times of increased flow there would be a lower possibility of denitrification when compared to times of low flow, as water is quickly transported from crop fields to creeks through tile drains. Therefore water in the creeks should theoretically fall between lines representing denitrification and dilution as creek water will be a mix of groundwater discharge and water discharging from tile drains. During times of tile drain flow Boomer Creek had NO_3^- concentrations above groundwater and $\delta^{15}N$ and $\delta^{18}O$ that were close to that in the tile drains. At low NO_3^- concentrations when tile drains are contributing minimal amounts of water to the creeks the $\delta^{15}N-NO_3^-$ will have a slope closer to that of denitrification with lower NO_3^- concentrations. This indicates the influence of tile drains on creek NO_3^- concentrations.

The overall objective of this thesis was to try to identify the sources and processes governing NO_3^- concentration and export in small agricultural catchments within the Grand River Watershed. The use of NO_3^- isotope for source identification as not applicable in this situation as isotopic values obtained for NO were too similar to that of other sources. Isotopic ratios of $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- did however give strong evidence that denitrification was occurring. Isotopic ratios also provided evidence that tile drains are the likely source of high NO_3^- concentrations observed in agricultural creeks.

In summary during times of reduced tile drain flow (summer), agricultural creeks will have lower NO_3^- concentrations and high $\delta^{15}N$ and $\delta^{18}O$, in contrast during times of tile drain flow (fall-spring) agricultural creeks will have elevated NO_3^- concentrations and low $\delta^{15}N$ and $\delta^{18}O$ (Fig 4.1).



4.2 Recommendations

In order to protect groundwater and surface water resources nutrient dynamic from small agricultural catchments must be understood. Based on the results found in this study in order to mitigate high NO_3^- concentrations in streams and to minimize export the following recommendations were made: monitoring of the Conestogo reservoir nutrient levels, maintaining and protecting riparian zones, the use of in-line bioreactors and fertilizer best management practices.

Monitoring of the Conestogo reservoir nutrient concentrations should be performed on a regular basis to better understand concentrations downstream, as NO_3^- concentrations from spring melt were perceived to affect NO_3^- concentrations in the Conestogo River throughout the rest of the year. With knowledge of reservoir concentration, reservoir management practices could be altered to allow for incoming high NO_3^-concentration water during spring melt to be discharged. Assuming there is no flood risk, water that has high NO_3^- concentrations like that in spring melt could be discharged as soon as possible. This could help to avoid discharging high NO_3^- concentrations for the remainder of the year. This is based on the assumption that after the initial pulse of high NO_3^- during spring melt, the remainder of the year the reservoir will fill up with low NO_3^- concentration water. This scenario also does not change to amount of annual NO_3^- export.

Protecting and enhancing riparian zones is also important in maintaining good water quality. Riparian zones can work as zones of concentrated denitrification and can effectively decrease the amount of NO_3^- being discharged into nearby creeks by groundwater. In this study it was found that groundwater within the riparian zone had consistently low (< 1 mg N/L) concentrations. Yet tile drains are the major contributors of NO_3^- to these agricultural catchments and circumvent the riparian zone. Bioreactors could be installed in-line of tile drains to effectively remove NO_3^-. These bioreactors work as denitrification hotspots by providing the ideal environment for denitrifying bacteria. Blowes et al. (1994) found that an in-line bioreactor filled with sand and organic matter was effective at decreasing NO_3^- concentrations. This is a relatively simple and cost effective way to reduce NO_3^- concentrations in stream.

Best management practices by farmers would also help to reduce the amount of nitrogen remaining in the soil, eventually this may lead to a decrease in the NO_3^- in the groundwater and surface water systems. Best management practices include ensuring that the right fertilizer is applied to the right crop, at the right rate, at the right time and the right place known as the “Four R’s”. Fertilizer timing is critical to minimizing NO_3^- pollution. If precipitation occurs close to the time of fertilization there is a high probably much of the fertilizer will run-off through tile drains into a nearby creek.

The use manure as a fertilizer can also be troublesome as the nitrogen in manure must first be mineralized in order to be taken up by plants. This process can be preferable as plants are given more time to sequester nitrogen, but the amount of manure needed each year must be accurately estimated in order to reduce the chance over fertilizing, as the process of mineralization of organic nitrogen can take years and can create a legacy of excess NO_3^- in the soil as more manure will be applied in the following years. Since manure must be disposed of when it can no longer be safely stored in a farmer's storage tank, it is often applied to agricultural fields throughout the year when crops are no longer growing. During these times there is little to no plant uptake, which allows for much of the nutrients in the manure to directly flow into the groundwater or off fields via tile drains. If laws instead allowed for the amount of manure stored to be correlated to the number of animals allowed on a property then this may prevent over fertilization as well as large nutrient runoff as a result of spreading manure during non-ideal conditions.

References

Adam, JWH. "Health Aspects of Nitrate in Drinking-Water and Possible Means of Denitrification (Literature Review)." *Water SA* 6.2 (1980): 79. Print.

Allison, SM, and JI Prosser. "Ammonia Oxidation at Low pH by Attached Populations of Nitrifying Bacteria." *Soil Biology and Biochemistry* 25.7 (1993): 935-41. Print.

Angle, JS, et al. "Soil Nitrate Concentrations Under Corn as Affected by Tillage, Manure, and Fertilizer Applications." *Journal of environmental quality* 22.1 (1993): 141-7. Print.

Aravena, Ramon, and William D. Robertson. "Use of Multiple Isotope Tracers to Evaluate Denitrification in Ground Water: Study of Nitrate from a Large-flux Septic System Plume." *Groundwater* 36.6 (1998): 975-82. Print.

Birgand, Françoisx, et al. "Nitrogen Removal in Streams of Agricultural Catchments—a Literature Review." *Critical Reviews in Environmental Science and Technology* 37.5 (2007): 381-487. Print.

Blowes, DW, et al. "Removal of Agricultural Nitrate from Tile-Drainage Effluent Water using in-Line Bioreactors." *Journal of contaminant hydrology* 15.3 (1994): 207-21. Print.

Bremner, John M. "Sources of Nitrous Oxide in Soils." *Nutrient Cycling in Agroecosystems* 49.1-3 (1997): 7-16. Print.

Breuer, Lutz, Ralf Kiese, and Klaus Butterbach-Bahl. "Temperature and Moisture Effects on Nitrification Rates in Tropical Rain-Forest Soils." *Soil Science Society of America Journal* 66.3 (2002): 834-44. Print.

Canadian Council of Ministers of the Environment. *Canadian Water Quality Guidelines for the Protection of Aquatic Life: Nitrate. In: Canadian Environmental Quality Guidelines*. Winnipeg: Canadian Council of Ministers of the Environment, 2012. Print.

Cey, Edwin E., et al. "Role of the Riparian Zone in Controlling the Distribution and Fate of Agricultural Nitrogen Near a Small Stream in Southern Ontario." *Journal of contaminant hydrology* 37.1 (1999): 45-67. Print.

Cooke, G. Dennis, et al. *Restoration and Management of Lakes and Reservoirs*. CRC press, 2005. Print.

Creed, IF, et al. "Regulation of nitrate-N Release from Temperate Forests: A Test of the N Flushing Hypothesis." *Water Resources Research* 32.11 (1996): 3337-54. Print.

Earth Tech. *Region of Waterloo Wastewater Treatment Masterplan*., 2007. Print.

Ecoregions Working Group. "Ecoclimatic Regions of Canada, First Approximation." *Ecological land classification series* 23 (1989)Print.

Eilers, W., et al. "Environmental Sustainability of Canadian Agriculture: Agri-Environmental Indicator Report Series–Report# 3." (2010)Print.

Flipse, William J., and Francis T. Bonner. "Nitrogen-Isotope Ratios of Nitrate in Ground Water Under Fertilized Fields, Long Island, New York." *Groundwater* 23.1 (1985): 59-67. Print.

Fogg, Graham E., et al. "Spatial Variation in Nitrogen Isotope Values Beneath Nitrate Contamination Sources." *Groundwater* 36.3 (1998): 418-26. Print.

Friedl, Gabriela, and Alfred Wüest. "Disrupting Biogeochemical Cycles-Consequences of Damming." *Aquatic Sciences* 64.1 (2002): 55-65. Print.

Frink, Charles R., Paul E. Waggoner, and Jesse H. Ausubel. "Nitrogen Fertilizer: Retrospect and Prospect." *Proceedings of the National Academy of Sciences* 96.4 (1999): 1175-80. Print.

Galloway, James N., and Ellis B. Cowling. "Reactive Nitrogen and the World: 200 Years of Change." *AMBIO: A Journal of the Human Environment* 31.2 (2002): 64-71. Print.

Garcia-Ruiz, R., SN Pattinson, and BA Whitton. "Denitrification in River Sediments: Relationship between Process Rate and Properties of Water and Sediment." *Freshwater Biology* 39.3 (1998): 467-76. Print.

Gormly, JR, and RF Spalding. "Sources and Concentrations of Nitrate-Nitrogen in Ground Water of the Central Platte Region, Nebraskaa." *Ground Water* 17.3 (1979): 291-301. Print.

Grand River Conservation Authority, et al. "The Boomer Creek Experience: Changing Traditions." 2007.Web. <http://www.ontariopork.on.ca/Portals/0/Docs/Environment/BoomerCreek.pdf>.

Grand River Conservation Authority. "GRIN Geospatial Information." 2014.Web. <http://maps.grandriver.ca/data-gis.html>.

Granger, Julie, et al. "Nitrogen and Oxygen Isotope Fractionation during Dissimilatory Nitrate Reduction by Denitrifying Bacteria." *Limnology and Oceanography* 53.6 (2008): 2533. Print.

Hayatsu, Masahito, Kanako Tago, and Masanori Saito. "Various Players in the Nitrogen Cycle: Diversity and Functions of the Microorganisms Involved in Nitrification and Denitrification." *Soil Science and Plant Nutrition* 54.1 (2008): 33-45. Print.

Hill, AR. "Nitrate Distribution in the Ground Water of the Alliston Region of Ontario, Canada." *Ground Water* 20.6 (1982): 696-702. Print.

Hill, Alan R., and Kandiah Sanmugadas. "Denitrification Rates in Relation to Stream Sediment Characteristics." *Water research* 19.12 (1985): 1579-86. Print.

Howarth, Robert W., and Roxanne Marino. "Nitrogen as the Limiting Nutrient for Eutrophication in Coastal Marine Ecosystems: Evolving Views Over Three Decades." *Limnology and Oceanography* 51.1 (2006): 364-76. Print.

Janzen, HH, et al. "The Fate of Nitrogen in Agroecosystems: An Illustration using Canadian Estimates." *Nutrient Cycling in Agroecosystems* 67.1 (2003): 85-102. Print.

Jones Jr, Jeremy B., et al. "Methanogenesis in Arizona, USA Dryland Streams." *Biogeochemistry* 31.3 (1995): 155-73. Print.

Junk, Gregor, and Harry J. Svec. "The Absolute Abundance of the Nitrogen Isotopes in the Atmosphere and Compressed Gas from various Sources." *Geochimica et Cosmochimica Acta* 14.3 (1958): 234-43. Print.

Kellman, LM, and C. Hillaire-Marcel. "Evaluation of Nitrogen Isotopes as Indicators of Nitrate Contamination Sources in an Agricultural Watershed." *Agriculture, Ecosystems & Environment* 95.1 (2003): 87-102. Print.

Kellman, LM. "A Study of Tile Drain Nitrate-δ15N Values as a Tool for Assessing Nitrate Sources in an Agricultural Region." *Nutrient Cycling in Agroecosystems* 71.2 (2005): 131-7. Print.

Kendall, Carol, and Jeffrey J. McDonnell. *Isotope Tracers in Catchment Hydrology*. Elsevier, 1999. Print.

Kendall, Carol, and Ramon Aravena. "Nitrate Isotopes in Groundwater Systems." *Environmental Tracers in Subsurface Hydrology.*Springer, 2000. 261-297. Print.

Kendall, Carol, Emily M. Elliott, and Scott D. Wankel. "Tracing Anthropogenic Inputs of Nitrogen to Ecosystems." *Stable isotopes in ecology and environmental science* 2 (2007): 375-449. Print.

Khalil, K., B. Mary, and P. Renault. "Nitrous Oxide Production by Nitrification and Denitrification in Soil Aggregates as Affected by O 2 Concentration." *Soil Biology and Biochemistry* 36.4 (2004): 687-99. Print.

Knowles, Roger. "Denitrification." *Microbiological reviews* 46.1 (1982): 43. Print.

Kool, Dorien M., et al. "Oxygen Exchange with Water Alters the Oxygen Isotopic Signature of Nitrate in Soil Ecosystems." *Soil Biology and Biochemistry* 43.6 (2011): 1180-5. Print.

Kreitler, Charles W., and David C. Jones. "Natural Soil Nitrate: The Cause of the Nitrate Contamination of Ground Water in Runnels County, Texasa." *Ground Water* 13.1 (1975): 53-62. Print.

Maag, M., and Finn Pilgaard Vinther. "Nitrous Oxide Emission by Nitrification and Denitrification in Different Soil Types and at Different Soil Moisture Contents and Temperatures." *Applied Soil Ecology* 4.1 (1996): 5-14. Print.

McIlvin, Matthew R., and Mark A. Altabet. "Chemical Conversion of Nitrate and Nitrite to Nitrous Oxide for Nitrogen and Oxygen Isotopic Analysis in Freshwater and Seawater." *Analytical Chemistry* 77.17 (2005): 5589-95. Print.

Mengis, M., et al. "Multiple Geochemical and Isotopic Approaches for Assessing Ground Water NO3− Elimination in a Riparian Zone." *Groundwater* 37.3 (1999): 448-57. Print.

Michalak, Anna M., et al. "Record-Setting Algal Bloom in Lake Erie Caused by Agricultural and Meteorological Trends Consistent with Expected Future Conditions." *Proceedings of the National Academy of Sciences* 110.16 (2013): 6448-52. Print.

Ministry of Agriculture, Food and Rural Affairs. "Census." 2014.Web. <http://www.omafra.gov.on.ca/english/stats/census/index.html>.

Ministry of Natural Resources and Forestry. "Ontario Flow Assesment Tool III." 2013.Web. <http://www.giscoeapp.lrc.gov.on.ca/web/mnr/wrip/ofat/Viewer/viewer.html>.

Nier, Alfred O. "A Redetermination of the Relative Abundances of the Isotopes of Carbon, Nitrogen, Oxygen, Argon, and Potassium." *Physical Review* 77.6 (1950): 789. Print.

Novotny, Vladimir. "Diffuse Pollution from Agriculture—a Worldwide Outlook." *Water Science and Technology* 39.3 (1999): 1-13. Print.

Surficial Geology of Southern Ontario Ontario Geological Survey, 2010.

Ontario Ministry of Agriculture. *OMAFRA: Land use. Ontario Ministry of Agriculture, Food, and Rural Affairs*, ., 2006. Print.

Ontario Ministry of the Environment. *Water Quality of 15 Streams in Agricultural Watersheds of Southwestern Ontario 2004-2009: Seasonal Patterns, Regional Comparisons, and the Influence of Land Use*., 2012. Print.

Paerl, Hans W. "Coastal Eutrophication and Harmful Algal Blooms: Importance of Atmospheric Deposition and Groundwater as" New" Nitrogen and Other Nutrient Sources." *Limnology and Oceanography* 42.5 (1997): 1154-65. Print.

Pearce, Ralph. "**Drainage statistics reveal some interesting facts about Ontario**." 2011.Web. <http://www.drainagecontractor.com/business/drainage-statistics-reveal-some-interesting-facts-about-ontario>.

Poor, Cara J., and Jeffrey J. McDonnell. "The Effects of Land use on Stream Nitrate Dynamics." *Journal of Hydrology* 332.1 (2007): 54-68. Print.

Ryabenko, Evgeniya, Mark A. Altabet, and Douglas WR Wallace. "Effect of Chloride on the Chemical Conversion of Nitrate to Nitrous Oxide for δ15N Analysis." *Limnology and Oceanography: Methods* 7 (2009): 545-52. Print.

Saad, Omar ALO, and Ralf Conrad. "Temperature Dependence of Nitrification, Denitrification, and Turnover of Nitric Oxide in Different Soils." *Biology and Fertility of Soils* 15.1 (1993): 21-7. Print.

Savard, Martine M., et al. "Winter Nitrification Contributes to Excess NO_3^- in Groundwater of an Agricultural Region: A Dual-isotope Study." *Water Resources Research* 43.6 (2007)Print.

Schepers, James Stuart, and William Raun. *Nitrogen in Agricultural Systems*. 49 Vol. ASA-CSSA-SSSA, 2008. Print.

Schindler, DW. "Evolution of Phosphorus Limitation in Lakes." *Science* 195.4275 (1977): 260-2. Print.

Schindler, David W., et al. "Eutrophication of Lakes Cannot be Controlled by Reducing Nitrogen Input: Results of a 37-Year Whole-Ecosystem Experiment." *Proceedings of the National Academy of Sciences* 105.32 (2008): 11254-8. Print.

Seglenieks, Frank. "University of Waterloo Weather Station Annual Summary - 2005." 2005.Web. <http://www.weather.uwaterloo.ca/download/historical/2005/2005-annual.pdf>.

Seglenieks, Frank. "UW Weather Station: Data Archives." 2014.Web. <http://weather.uwaterloo.ca/data.html>.

Sigman, DM, et al. "A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater." *Analytical Chemistry* 73.17 (2001): 4145-53. Print.

Smil, Vaclav. "Population Growth and Nitrogen: An Exploration of a Critical Existential Link." *Population and Development Review* 17.4 (1991): 569-601. Print.

Smith, Val H. "Cultural Eutrophication of Inland, Estuarine, and Coastal Waters." *Successes, Limitations, and Frontiers in Ecosystem Science.*Springer, 1998. 7-49. Print.

Smith, Val H., G. David Tilman, and Jeffery C. Nekola. "Eutrophication: Impacts of Excess Nutrient Inputs on Freshwater, Marine, and Terrestrial Ecosystems." *Environmental pollution* 100.1 (1999): 179-96. Print.

Smolders, Alfons JP, et al. "How Nitrate Leaching from Agricultural Lands Provokes Phosphate Eutrophication in Groundwater Fed Wetlands: The Sulphur Bridge." *Biogeochemistry* 98.1-3 (2010): 1-7. Print.

Snider, David M., Sherry L. Schiff, and John Spoelstra. "15N/14N and 18O/16O Stable Isotope Ratios of Nitrous Oxide Produced during Denitrification in Temperate Forest Soils." *Geochimica et Cosmochimica Acta* 73.4 (2009): 877-88. Print.

Snider, David M., et al. "Stable Oxygen Isotope Ratios of Nitrate Produced from Nitrification: 18O-Labeled Water Incubations of Agricultural and Temperate Forest Soils." *Environmental science & technology* 44.14 (2010): 5358-64. Print.

Socolow, Robert H. "Nitrogen Management and the Future of Food: Lessons from the Management of Energy and Carbon." *Proceedings of the National Academy of Sciences* 96.11 (1999): 6001-8. Print.

Spoelstra, J., B. J. Kralt, and R. J. Elgood. *A Chemical Method for the Conversion of Nitrate to Nitrous Oxide for Isotopic Analysis*. 14- 088 Vol. National Water Research Institute Environment Canada, 2014. Print.

Statistics Canada. "*Focus on Geography Series, 2011 Census*. Statistics Canada Catalogue no. 98-310-XWE2011004. Ottawa, Ontario. Analytical products, 2011 Census." 2012a.Web. <http://www12.statcan.gc.ca/census-recensement/2011/as-sa/fogs-spg/Facts-cma-eng.cfm?LANG=Eng&GK=CMA&GC=543>.

Statistics Canada. "*St. Jacobs, Ontario (Code 1245) and Ontario (Code 35)* (table). *Census Profile*. 2011 Census. Statistics Canada Catalogue no. 98-316-XWE. Ottawa." 2012b.Web. <http://www12.statcan.gc.ca/census-recensement/2011/dp-pd/prof/index.cfm?Lang=E>.

Tesoriero, Anthony J., Hugh Liebscher, and Stephen E. Cox. "Mechanism and Rate of Denitrification in an Agricultural Watershed: Electron and Mass Balance Along Groundwater Flow Paths." *Water Resources Research* 36.6 (2000): 1545-59. Print.

The Ontario Ministry of Natural Resources. *Southern Ontario Land Resource Information System (SOLRIS) Land use Data*. Toronto, Ontario: The Ontario Ministry of Natural Resources, 2008. Print.

Tilsner, Jens, et al. "Emission of Gaseous Nitrogen Oxides from an Extensively Managed Grassland in NE Bavaria, Germany." *Biogeochemistry* 63.3 (2003): 249-67. Print.

Tomer, MD, and MR Burkart. "Long-Term Effects of Nitrogen Fertilizer use on Ground Water Nitrate in Two Small Watersheds." *Journal of environmental quality* 32.6 (2003): 2158-71. Print.

Van Cleemput, Oswald. "Subsoils: Chemo-and Biological Denitrification, N2O and N2 Emissions." *Nutrient Cycling in Agroecosystems* 52.2-3 (1998): 187-94. Print.

Vitousek, Peter M., et al. "Human Alteration of the Global Nitrogen Cycle: Sources and Consequences." *Ecological Applications* 7.3 (1997): 737-50. Print.

Wassenaar, Leonard I., M. Jim Hendry, and Nikki Harrington. "Decadal Geochemical and Isotopic Trends for Nitrate in a Transboundary Aquifer and Implications for Agricultural Beneficial Management Practices." *Environmental science & technology* 40.15 (2006): 4626-32. Print.

Wassenaar, Leonard I. "Evaluation of the Origin and Fate of Nitrate in the Abbotsford Aquifer using the Isotopes of 15 N and 18 O in NO 3−." *Applied Geochemistry* 10.4 (1995): 391-405. Print.

Weiss, RF, and BA Price. "Nitrous Oxide Solubility in Water and Seawater." *Marine Chemistry* 8.4 (1980): 347-59. Print.

Wunderlich, Anja, Rainer U. Meckenstock, and Florian Einsiedl. "A Mixture of Nitrite-Oxidizing and Denitrifying Microorganisms Affects the Δ 18 O of Dissolved Nitrate during Anaerobic Microbial Denitrification Depending on the Δ 18 O of Ambient Water." *Geochimica et Cosmochimica Acta* 119 (2013): 31-45. Print.

Yokoyama, Kazuhira, and Tohru Ohama. "Effect of Inorganic N Composition of Fertilizers on Nitrous Oxide Emission Associated with Nitrification and Denitrification." *Soil Science & Plant Nutrition* 51.7 (2005): 967-72. Print.