Nitrate sources and cycling at the Turkey Lakes Watershed: A stable isotope approach

by

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Abstract

Increased atmospheric deposition of nitrate and ammonium is a consequence of the anthropogenic creation of reactive nitrogen. The impacts of elevated nitrogen deposition to Earth's temperate forests are of particular interest due to their economic importance and the disproportionately large role they play in the global carbon cycle. Stable isotopic analysis of nitrate ($^{15}N/^{14}N$ and $^{18}O/^{16}O$) can be used to trace the fate of atmospheric nitrate deposition. Widespread application of the dual nitrate isotope technique in forested watersheds has been limited by the low nitrate concentrations generally found in the groundwater and surface waters and high concentrations of dissolved organic matter (DOM) that interfere with the isotopic analysis of nitrate.

The overall goal of the research presented in this thesis was to employ the dual nitrate isotope technique to trace nitrate sources and cycling in a temperate hardwood forest under undisturbed conditions and following harvest. This research was done at the Turkey Lakes Watershed (TLW), an old-growth sugar maple basin located near Sault Ste. Marie, Ontario, Canada. Although the TLW receives a moderate atmospheric loading of nitrogen, averaging 8.7 kg $N \cdot ha^{-1} \cdot yr^{-1}$, it has an unusually low ability to retain inorganic nitrogen (45 to 86% of deposition) and exhibits relatively high concentrations of nitrate in streams and groundwater. The use of stable isotope techniques in this study provide new insights into forest nitrogen cycling that are not possible to attain using more conventional methods. The knowledge gained through the research presented in this thesis builds on what has already been learned from over 20 years of nitrogen cycling research at the TLW.

Bulk precipitation collected biweekly at the TLW from 1995 to 2000 had nitrate isotope values that ranged from +42.4 to +80.4‰ for $\delta^{18}O$ and -6.3 to +2.8‰ for $\delta^{15}N$, with

the highest values for both generally found during the winter months. Mass-weighted mean atmospheric nitrate $\delta^{18}O$ and $\delta^{15}N$ values were +58.1‰ and -1.7‰, respectively. An incubation experiment indicated that the isotopic composition of atmospheric nitrate was not compromised by collection methods whereby unfiltered bulk precipitation samples can remain in the collector for up to two weeks. The large separation between the $\delta^{18}O$ values of atmospheric nitrate and those predicted for nitrate produced by chemolithoautotrophic nitrification (microbial nitrate) in TLW soils ($\delta^{18}O$ = -5.7 to +4.7‰) provided the basis for determining the proportion of nitrate from these two sources in streams and groundwater.

Prediction of the $\delta^{18}O$ of microbial nitrate is based on the results of laboratory experiments that indicated one of the oxygens on nitrate comes from soil O_2 and two from water during chemolithoautotrophic nitrification. The first direct measurement of the isotopic composition of microbial nitrate produced *in situ* was obtained by eliminating precipitation inputs to three TLW forest floor lysimeters and subsequently watering the area with a nitrate-free solution over a two-week period. Microbial nitrate had $\delta^{18}O$ values that ranged from +3.1 to +10.1‰ with a mean value of +5.2‰, only slightly higher than values predicted based on the $\delta^{18}O$-H_2O of the watering solution used. $\delta^{18}O$ values of soil O_2 (+23.2 to +24.1‰) down to a depth of 55cm were not significantly different from atmospheric O_2 (+23.5‰) and therefore respiratory enrichment of soil O_2 did not affect the $\delta^{18}O$ values of microbial nitrate produced at the TLW. As expected because of the large discrimination against $^{15}NH_4^+$ during nitrification, microbial nitrate was depleted in ^{15}N ($\delta^{15}N$ = -10.4 to -7.3‰) relative to organic nitrogen in the forest floor ($\delta^{15}N$ = -2.7 to +0.1‰).

Nitrate export from two undisturbed first-order stream basins was dominated by microbial nitrate, with the contribution of atmospheric nitrate peaking at about 30% during snowmelt. Clear-cutting of catchment 31 in 1997 resulted in elevated nitrate concentrations, reaching levels that exceeded the drinking water limit of 10 mg N/L. Isotopic analysis indicated that the source of this nitrate was predominantly chemolithoautotrophic nitrification. The use of nitrate isotope ratios to quantify the relatively small contribution of atmospheric nitrate to the stream was complicated by the very high concentrations of microbial nitrate and the fact that microbial nitrate $\delta^{18}O$ values progressively increased during the post-harvest period. Therefore the variability in the $\delta^{18}O$ values of stream nitrate collected at the catchment outlet was not solely due to a change in the proportion of nitrate from the two sources. The higher $\delta^{18}O$ values of the microbial end-member were thought to have resulted from an increase in the proportion of nitrification that occurred in the summer months, when $\delta^{18}O$-H_2O values of meteoric water are greater. Despite drastic alteration of nitrogen cycling in the catchment by the harvest, $\delta^{15}N$-nitrate values in shallow groundwater did not change from the pre-harvest. The concentration and isotopic composition of nitrate exported in the stream from the clear-cut catchment were affected by nitrate attenuation in a small wetland located near the start of the stream. Denitrification and plant uptake of nitrate in the forested swamp attenuated 65 to 100% of surface water nitrate inputs, thereby reducing catchment-scale nitrate export by 35 to 80% for the post-harvest periods studied.

The dual nitrate isotope technique worked well for tracing nitrate sources and cycling at the TLW. The use of new ultrafiltration and dialysis techniques to remove DOM from samples prior to nitrate collection by ion exchange methods was critical for the accurate determination of nitrate $\delta^{18}O$ values. Future use of the dual nitrate isotope technique will

yield additional insights into forest nitrogen cycling processes, especially when combined with $\delta^{15}N$ analysis of ammonium, soil organic matter, and vegetation.

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Chapter 1. Introduction.

1.1 Anthropogenic impacts on the forest nitrogen cycle

Human activities such as the burning of fossil fuels and the excessive use of inorganic nitrogen (N) fertilizers in agriculture have resulted in a doubling of the global terrestrial pool of reactive nitrogen since pre-industrial times, and as a result, many parts of the world are experiencing elevated levels of atmospheric nitrogen deposition (e.g. Galloway et al., 1995). Much of the recent forest nitrogen research is driven by the need to better understand how forests will respond to elevated nitrogen deposition and to the anticipated effects of climate change. While pristine areas experience nitrogen deposition levels of less than 5 $kg \cdot ha^{-1} \cdot yr^{-1}$, the Eastern USA receives about 28 $kg \cdot ha^{-1} \cdot yr^{-1}$ (Van Miegroet et al., 1992). Heavily polluted areas in Europe have nitrogen deposition in excess of 75 $kg \cdot ha^{-1} \cdot yr^{-1}$ (Dise and Wright, 1995). One of the consequences of these deposition levels is that forested ecosystems, which have historically been thought of as nitrogen-limited (Tamm 1991), are becoming nitrogen-saturated (Agren and Bosatta, 1988; Aber et al., 1989). Although forest productivity may initially increase due to higher deposition levels, nitrogen saturation can decrease forest health through increased water stress, reduced frost tolerance, soil acidification, the leaching of nutrients, and decreased fine root biomass (Aber et al., 1989).

To sustain annual forest productivity, trees require more nitrogen than the inputs received through atmospheric deposition. Therefore nitrogen-limited forests have efficient mechanisms of retaining and recycling available nitrogen (Figure 1.1). These mechanisms include processes such as the translocation of nitrogen from senescing foliage, which increases the C:N ratio of the litter and thus promotes the immobilization of inorganic nitrogen in the soil. Nitrogen-saturated catchments become leaky with respect to inorganic

nitrogen and often experience elevated levels of nitrate in streams and groundwater (e.g. Dise and Wright, 1995; Peterjohn et al., 1996). High nitrate export has detrimental effects on terrestrial and aquatic systems. In order to maintain charge balance, nitrate losses must be accompanied by increased cation leaching from soils. Therefore nitrate export can decrease forest health by concomitant loss of nutrient cations such as calcium and potassium as well as lead to acidification through the export of hydrogen ions (Aber et al., 1989). High nitrate concentrations can also threaten potable water supplies for humans (e.g. Bouchard et al., 1992; Wolfe and Patz, 2002), have detrimental effects on some amphibians, even at relatively low concentrations (Baker and Waights, 1993, 1994; Hecnar, 1995; Marco et al., 1999), and cause the eutrophication of downstream aquatic ecosystems (e.g. Paerl and Fogel, 1994).

Nitrogen deposition can have significantly different environmental impacts depending on its fate in forested watersheds. Although denitrification of excess atmospheric nitrate may benefit the forest through the reduction of nitrate leaching, denitrification is also a source of nitrous oxide to the atmosphere. Nitrous oxide is a powerful greenhouse gas, having 296 times the global warming potential of carbon dioxide (IPCC, 2001). The nitrification of atmospheric ammonium to nitrate in forest soils is an acid producing process that also contributes to nitrous oxide emissions from forests. Nitrate generated by nitrification could be leached from the soil and therefore potentially contribute to the problems associated with nitrate export. Alternatively, atmospheric nitrogen inputs could be assimilated into forest biomass as organic nitrogen, thereby also sequestering atmospheric carbon dioxide in the process. Forest organic matter reservoirs have C:N ratios that range from less than 15 in soils to greater than 500 in wood (Nadelhoffer et al., 1999). Therefore

the magnitude of carbon sequestration depends on where the nitrogen is stored (Rastetter et al., 1992; Houghton et al., 1998). Therefore, forest nitrogen cycling is intimately linked to the global carbon cycle, and the fate of anthropogenic nitrogen inputs to forests partially controls their net sink strength with respect to the global greenhouse gas budget.

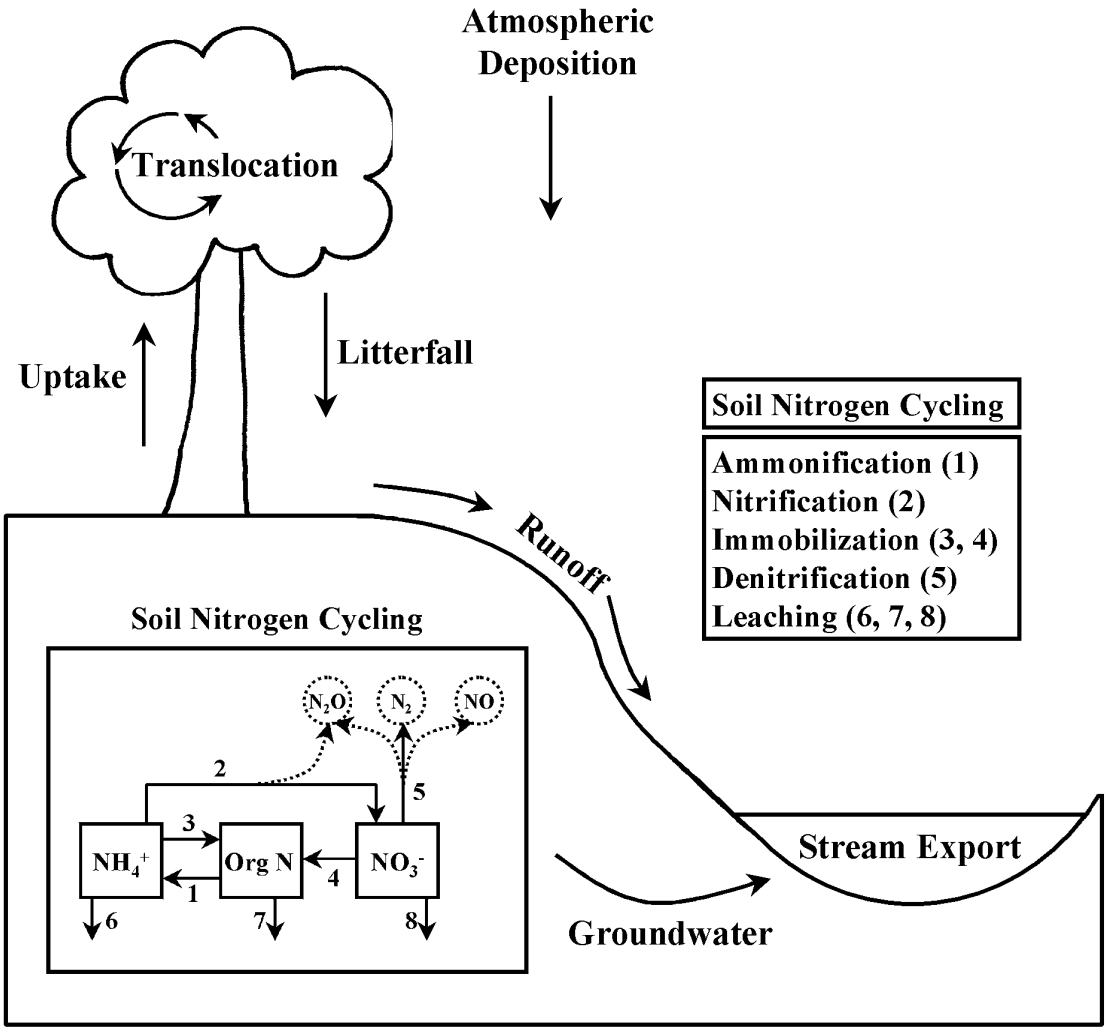

Figure 1.1 Diagrammatic representation of the major components of the forest nitrogen cycle.

1.2 Stable isotope techniques in forest nitrogen cycling research

The need for a more comprehensive understanding of nitrogen cycling in forests has led to an increase in the number of studies using stable isotopic analysis of nitrogen in forest ecosystems. These studies can be grouped into two categories: 1) ^{15}N tracer additions, and 2) natural abundance studies. The addition of inorganic nitrogen tracers enriched in ^{15}N has been particularly useful in forested catchments for tracing the long and short-term cycling of ammonium and nitrate deposition into microbial, vegetation, and soil nitrogen pools (e.g. Zak et al., 1990; Nadelhoffer et al., 1995; Lamontagne et al., 2000). These studies also provide information on the potential for carbon sequestration related to atmospheric nitrogen deposition (e.g. Nadelhoffer et al., 1999). Nitrogen isotope tracers have also been used to measure the gross rates of processes such as ammonification, nitrification, and immobilization of nitrate and ammonium in forest soils (e.g. Davidson et al., 1992; Pedersen et al., 1999). Natural abundance studies in forested ecosystems have been used to quantify nitrogen fixation (Shearer and Kohl, 1986), examine sources of nitrogen to plants (Nadelhoffer et al., 1996), differentiate sources of nitrous oxide emissions from soils (Perez et al., 2000), detect denitrification (Koba et al., 1997), and as an indicator of catchment nitrogen status (Högberg, 1990).

The fate of atmospheric nitrate is of particular interest because it is a form of acidic deposition (acid rain) and because of its mobility in soils. The nitrate exported from forested watersheds is typically a mixture of nitrate from two sources: 1) atmospheric deposition (atmospheric nitrate) and 2) nitrification in soils (microbial nitrate). Previous studies have shown that these two nitrate sources have widely separated oxygen isotope ratios and therefore $\delta^{18}O$-nitrate can be used to evaluate the importance of atmospheric versus

microbial nitrate in streams and groundwater (e.g. Durka et al., 1994). Although nitrogen isotope ratios ($^{15}N/^{14}N$) cannot be directly used for source separations due to overlapping ranges (Kendall, 1998), $\delta^{15}N$ analysis is required to distinguish ^{18}O enrichment due to denitrification from ^{18}O enrichment due to a change in nitrate source contributions. The dual nitrate isotope technique can be applied at the plot scale to quantify heterogeneity in soil nitrogen cycling, and at the catchment scale for a spatially integrated assessment of nitrate sources and cycling.

Application of the dual nitrate isotope technique to forested watersheds has largely been limited by low nitrate concentrations and high levels of dissolved organic matter (DOM) in these systems (Kendall, 1998). DOM interferes with $\delta^{18}O$-nitrate determination because it contains about 40% oxygen by weight (Thurman, 1985) and is retained by anion exchange resins used to isolate nitrate from water samples (Chang et al., 1999; Silva et al., 2000). Durka et al. (1994) examined nitrate sources in forest springs in Germany and found that atmospheric nitrate was less predominant in the springs of healthy forests compared to those that showed more severe signs of decline. Several investigations of nitrate isotope ratios in forested watersheds have been published since the German study (Kendall et al., 1996; Spoelstra et al., 2001; Williard et al., 2001; Burns and Kendall, 2002; Schiff et al., 2002; Sickman et al., 2003) although the number of papers is relatively low considering the decadal time span.

1.3 Thesis outline

The overall goal of the research presented in this thesis was to employ the dual nitrate isotope technique to trace nitrate sources and cycling at the TLW under undisturbed

conditions and following forest harvest. To accomplish this, new methods for the separation of DOM from nitrate were developed (ultrafiltration and dialysis) and some of the underlying assumptions with the application of this isotopic technique were tested. This research was done in conjunction with a forest harvest experiment initiated at the TLW in 1997 to investigate the impacts of forest management practices on a tolerant hardwood ecosystem.

Chapter 2 describes the TLW research site and the nitrate isotope methods used throughout the thesis. Other chapter-specific information on sample collection, catchment instrumentation, and experimental design information is described in individual chapters.

Chapter 3 presents an evaluation of the common assumption that atmospheric nitrate isotope ratios are not affected by bulk precipitation collection techniques. This study is the first to evaluate the isotopic stability of nitrate in precipitation and has been accepted for publication in *Environmental Science & Technology* pending minor revision. Chapter 3 includes these revisions.

Chapter 4 examines nitrate sources in streams and groundwaters of two undisturbed, first-order basins, catchment 31 (c31) and c47, from 1995 to 1996. The results, which are the first nitrate isotope ratios reported for a Canadian forest, were published in *Ecosystems* (Spoelstra et al., 2001) as part of a special TLW issue. This paper also describes the first use of ultrafiltration for the removal of DOM prior to collection of nitrate by ion exchange methods. Spoelstra et al. (2001) provided the first analysis of the temporal dynamics of nitrate sources in headwater streams at the TLW.

In May 1999, it was discovered that a wetland in c31 was attenuating high concentrations of nitrate that resulted from clear-cutting of the catchment uplands in 1997. Chapter 5 describes a two-year study of nitrate attenuation in this small forested swamp,

which included the isotopic analysis of nitrate and vegetation. This study demonstrates the disproportionately large influence that small wetlands can have on catchment nitrate export and their potential importance in future forest management practices.

Use of the dual nitrate isotope technique for determining nitrate source contributions requires knowledge of the $\delta^{18}O$ of microbial nitrate. Chapter 6 describes the first *in situ* experiment designed to test the assumption that microbial nitrate $\delta^{18}O$ values can be calculated for forest soils based on the range of $\delta^{18}O$-H_2O values of local meteoric water. This study is also the first to use a dialysis method for the removal of DOM prior to collection of nitrate by ion exchange methods. Previous studies of the isotopic composition of microbial nitrate from forest soils (Mayer et al., 2001; Burns and Kendall, 2002) have used soil incubation techniques rather than *in situ* methods. Chapter 6 is also the first study to examine the $\delta^{18}O$ of soil O_2 in conjunction with the isotopic analysis of nitrate. Previous studies using the dual nitrate isotope technique have assumed that the $\delta^{18}O$ of soil O_2 available to nitrifiers is identical to atmospheric O_2 and that soil $\delta^{18}O$-O_2 values are not altered by soil respiration.

Chapter 7 discusses results of the first stable isotope study of a nitrate pulse resulting from forest harvest. In addition, a five-year record of the $\delta^{15}N$ and $\delta^{18}O$ values of bulk atmospheric nitrate deposition to the TLW is presented.

Finally, the major conclusions of the thesis are summarized in Chapter 8. A conceptual model of the processes controlling the isotopic composition of nitrate exported from headwater catchments at the TLW is also presented along with recommendations for future research.

Chapter 2. Site description and methods.

2.1 The Turkey Lakes Watershed

Biogeochemical research at the TLW, now in its third decade, was initiated in 1980 to investigate the effects of acidic deposition on an acid-sensitive Canadian Shield ecosystem (Jeffries et al., 1988; www.tlws.ca). The TLW (47°03'N, 84°24'W) encompasses a 10.5 km^2 area located approximately 50 km north of Sault Ste. Marie, Ontario, Canada and contains a series of five lakes that ultimately drain into Lake Superior via Norberg Creek and the Batchawana River (Figure 2.1). Hydrology and stream chemistry has been monitored since the early 1980s in thirteen first-order stream catchments within the TLW that range in area from 4.06 to 66.54 ha (Beall et al., 2001). The TLW is jointly operated by Environment Canada, the Canadian Forest Service, and the Department of Fisheries and Oceans, in cooperation with the Ontario Ministry of Natural Resources.

Climate of the area is affected by it leeward position with respect to Lake Superior. The basin receives 1200-1300mm of precipitation annually (1981-1997 mean, Sirois et al., 2001) with approximately 35% deposited as snow (Semkin et al., 2002). Seasonal average temperatures range from +17°C in summer (July) to -13°C in winter (January) (Foster et al., 1992a). Soils are Ferro-Humic and Humo-Ferric podzols (Canada Soil Survey Committee, Subcommittee on Soil Classification, 1978) developed from a shallow, two-component glacial till (Elliot, 1985) that is underlain predominantly by Precambrian Shield, granodiorite bedrock with minor occurrences of granite (Semkin and Jeffries, 1983). The topography of the basin is quite varied, ranging in elevation from 630m at the top of Batchawana Mountain to 340m where Norberg Creek exits the watershed.

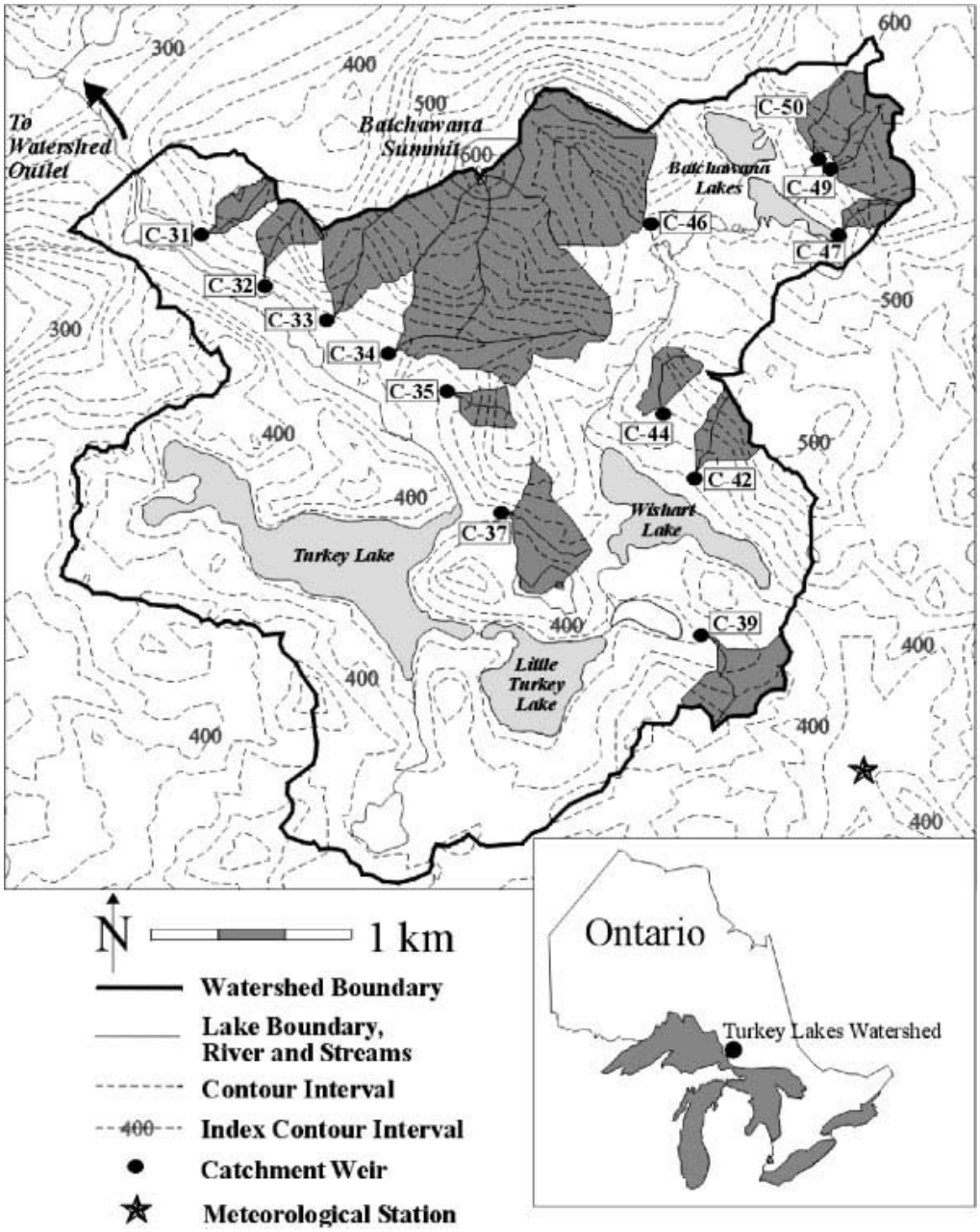

Figure 2.1 Location and topographic map of the Turkey Lakes Watershed (from Creed et al., 2003).

A detailed description of the physical, biological, and chemical characteristics of the TLW is given by Jeffries et al. (1988). In summary, the TLW is located at the northern margin of the Great Lakes – St. Lawrence Forest Region (Rowe, 1972) and can be described

as an uneven-aged, mature to over-mature hardwood forest dominated by sugar maple (90% of total phytomass), with most of the remaining trees consisting of other hardwoods (9% of total phytomass) such as red maple (*A. rubrum*), ironwood (*Ostrya virginiana* (Mill.) K. Koch), yellow birch (*Betula alleghaniensis* Britton), and red oak (*Quercus rubra* L.). Conifers such as white spruce (*Picea glauca* (Moench) Voss), white pine (*Pinus strobus* L.), balsam fir (*Abies balsamea* (L.) Mill.), eastern white cedar (*Thuja occidentalis* L.), and tamarack (*Larix laricina* (Du Roi) K. Koch) contribute about 1% of the total phytomass. The basin has no known history of forest fire and only minimal previous disturbance from logging in the mid-1950s when about 3% of the total standing volume was removed.

Although the TLW receives a moderate atmospheric loading of nitrate and ammonium, averaging 8.7 kg $N \cdot ha^{-1} \cdot yr^{-1}$, it has an unusually low ability to retain inorganic nitrogen (45 to 86% of deposition) (Nicolson, 1988). Mitchell et al (1992) suggested that the relatively high export of nitrogen by the TLW is a result of elevated atmospheric nitrogen deposition, stand maturity, and high mineralization and nitrification rates. The high mineralization and nitrification rates have been attributed to several factors which include the low C:N ratio (17:1) of the mineral soil (Foster, 1985; Mitchell et al., 1992), the high degradability of sugar maple litter (Mitchell et al., 1992), and the high organic nitrogen reserve in the soil (10500 kg $N \cdot ha^{-1}$, Foster et al., 1989a). Intensive plot-scale studies have shown that the majority of the nitrate that leaches past the effective rooting zone in TLW soils is produced by nitrification (72%-83%, Foster et al., 1989a; 60%-82%, Foster et al., 1992).

2.2 Analysis of nitrate isotope ratios

Methods used for the collection and isotopic analysis of nitrate were dependent on sample volume and chemistry and have changed over the course of the project due to advances in the techniques. In summary, water samples collected from the TLW (precipitation, groundwater, surface water, lysimeter samples) were first ultrafiltered or dialyzed to remove dissolved organic matter (DOM) if levels were high enough to interfere with the determination of nitrate isotope ratios. Nitrate was isolated by collection on ion exchange resin and was subsequently eluted and converted to a silver nitrate salt. Nitrogen and oxygen isotopic ratios ($^{15}N/^{14}N$ and $^{18}O/^{16}O$) of silver nitrate were initially determined using breakseal combustion techniques and were later replaced by continuous flow methods. All isotopic ratios were determined at the Environmental Isotope Lab (EIL), Department of Earth Sciences, University of Waterloo, and are reported in delta (δ) notation in units of per mil (‰) relative to atmospheric N_2 (Air) and Vienna Standard Mean Ocean Water (VSMOW) standards for $\delta^{15}N$ and $\delta^{18}O$, respectively.

2.2.1 Removal of DOM

Nitrate isotope studies of forested watersheds, especially those using $\delta^{18}O$-nitrate, are complicated by the combination of low nitrate concentrations and high levels of DOM. Natural DOM is composed of about 35-40% oxygen and about 1% nitrogen by weight (Thurman, 1985), and therefore DOM can interfere with the isotopic analysis of nitrate. DOM also takes up exchange sites on the anion resin used to collect nitrate from water samples and can clog resin columns when large samples are processed. Forest waters are generally problematic because of the high ratio of DOM-oxygen to nitrate-oxygen. $\delta^{18}O$-nitrate of waters with especially high DOM concentrations, such as soil extracts, wetland

waters, and lysimeters samples, generally cannot be accurately determined using current ion exchange techniques (e.g. Chang et al., 1999; Silva et al., 2000) without prior removal of the DOM.

Activated carbon has been used in several studies to reduce DOM oxygen contamination for $\delta^{18}O$ analysis of nitrate (Amberger and Schmidt, 1987; Durka et al., 1994; Silva et al., 2000). Chang et al. (1999) tested the effectiveness of activated charcoal, pre-processing through cation resins, and the type of anion resin used, for the separation of DOM from nitrate. Results of their study showed that the effectiveness of the techniques varied for samples from different sites, presumably due to differences in the physical and chemical characteristics of DOM. Considering that activated carbon also absorbs nitrate (Haberhauer and Blochberger, 1999), Chang et al. (1999) concluded that activated carbon did not absorb sufficient DOM to warrant its use. Instead, they recommended processing samples through a cation resin prior to nitrate collection by anion exchange. This method protonates the DOM, decreasing its ability to bind to the anion resin and therefore reducing the amount of DOM contamination of the nitrate sample. Other techniques to remove DOM prior to nitrate isotope analysis include the use of organic binding resins (Mayer et al., 2001) and polymers (Haberhauer and Blochberger, 1999).

Two techniques were employed to remove DOM from water samples prior to nitrate collection on anion exchange resin. Ultrafiltration was used for small volume samples (less than 4L) with dissolved organic carbon (DOC) concentrations from 2 to 10 mg C/L and a dialysis technique modified from Feuerstein et al. (1997) was used when more than 4L of water was required or if DOC exceeded 10 mg C/L. Additional DOM removal occurred

during collection of the nitrate by ion exchange and its subsequent conversion to silver nitrate.

2.2.1.1 Ultrafiltration

Water samples, previously filtered to 0.45μm, were ultrafiltered using a Pall, Ultrasette™ ultrafiltration cassette with a 1000 Dalton molecular weight cut-off membrane. Samples were placed in a beaker surrounded by ice during the ultrafiltration process, which took up to five hours for a 4L sample. Recirculation of the sample through the filter cartridge at a flow rate of about 1L/min and a pressure of 85 KPa resulted in a filtrate flow rate of about 15-30 mL/min. Nitrate and other small molecules passed through the polyethersulfone filter membrane and were collected as filtrate while the larger DOM molecules became concentrated in the recirculating sample (retentate) as the volume was reduced. Although increasing the pressure did increase the filtrate flow rate, it was also found to reduce the efficiency of DOM removal. Ultrafilters were cleaned between samples using a 0.1 N NaOH solution containing 200 ppm NaOCl until DOC concentrations in the retentate and filtrate were non-detectable. Chemical analysis of sub-samples taken of the filtrate, retentate, and original sample, along with their respective volumes, permitted determination of the proportion of DOM (as DOC) removed from each sample.

2.2.1.2 Dialysis

Samples to be dialyzed were first filtered to 0.45μm and then evaporated at 90°C to a total volume of about 500mL. Concentrated samples were then frozen and freeze-dried. The solids were redissolved in 50mL of ultrapure deionized water (DI) and put into 20cm long, 2cm diameter, Spectrum-Spectra/Por® cellulose ester dialysis tubes having a 100 Dalton molecular weight cut-off. Dialysis tubes were suspended in a 2L glass cylinder containing

1.8L of DI and allowed to dialyze while the DI was slowly mixed with a magnetic stirrer. After 24 hours, the water surrounding the dialysis bag was collected and replaced with another aliquot of DI. This step was repeated three times for a total dialysis time of 72 hours, which was found to be sufficient for removal of at least 98% of the nitrate. The three 1.8L aliquots containing the nitrate were pooled together prior to nitrate isolation by ion exchange techniques (Section 2.2.2). Chemical and volumetric determinations on the original, post-evaporation, DI extractants, and retentates for each sample dialyzed were used to calculate the proportion of DOC removed.

2.2.1.3 DOM removal by anion resin

Removal of DOM was also accomplished by the BioRad AG 1-X8 anion resin used to collect nitrate from water samples (Section 2.2.2). DOM contains negatively charged functional groups that give natural DOM a weakly anionic character, causing it to bind to exchange sites on the anion exchange resin. Anion resins can also retain DOM by non-exchange mechanisms such as hydrophobic interactions with the resin matrix (Silva et al., 2000) and/or becoming trapped in the pores of the resin beads.

In order for the resin to function as a DOM removal mechanism, the DOM must remain on the resin when the nitrate is eluted. The behavior of DOM on anion exchange resins depends on the characteristics of the DOM molecules and is therefore expected to vary with sample type (Chang et al, 1999). Once collected, nitrate is eluted from the anion resin using hydrochloric acid, which replaces all bound ions with chloride. Therefore, DOM bound to the resin by non-exchange mechanisms is more likely to remaining on the resin during the elution process.

DOM in TLW samples was strongly retained by the AG 1-X8 anion resin. A visual comparison of the columns before and after elution indicated a consistently large proportion of the DOM remained on the columns during the elution of nitrate. Although its magnitude was not measured, DOM removal by flocculation was also observed during neutralization of the elutant with silver oxide. Silva et al. (2000) suggested that additional DOM removal occurs through volatilization and adsorption following elution of the nitrate. Measurements done to determine the mass of DOC in samples prior to processing through the anion resin and again immediately prior to freeze-drying of the silver nitrate solution indicated that approximately 85% of the DOC was removed during this part of the procedure for TLW samples.

While the recommended methods of Chang et al. (1999) are designed to minimize DOM accumulation on the anion resin, methods used for TLW samples utilize the strong permanent retention of DOM by the AG 1-X8 resin as a DOM removal mechanism. These findings reinforce the conclusions of Chang et al. (1999) that the behavior of natural DOM on anion and cation exchange resins depends on the characteristics of the DOM itself. Therefore the effectiveness of DOM removal and nitrate collection techniques are likely to vary at different research sites.

2.2.2 Nitrate collection by ion exchange resin

The methods used to isolate nitrate and subsequently convert it to silver nitrate were adapted from Chang et al. (1999) and Silva et al. (2000). Once thawed, sample volumes were reduced to less than 500mL by evaporating at 90°C so they could be processed through the anion resin within one working day. Nitrate was isolated by dripping samples through columns containing 2mL of Bio-Rad, AG 1-X8, 100-200 mesh, anion exchange resin in the

chloride form at a rate of approximately 2mL/min. Columns containing nitrate were refrigerated until further processing, which ranged from hours to months later. Silva et al. (2000) showed that nitrate-loaded columns could be stored for at least two years without affecting the isotopic results. Nitrate was subsequently eluted using six 3mL additions of 3M HCl and the resulting solution neutralized with silver oxide to a pH of 5.3 to 5.9. Following filtration to remove the resulting silver chloride solid, an excess of barium chloride was added to precipitate oxygen-bearing anions such as sulfate and phosphate. The elutant was then refrigerated overnight to allow the precipitate to develop and filtered to 0.45μm the following day. The filtrate was passed through a column containing 2mL of Bio-Rad, AG 50W-X8, strong cation exchange resin (H^+ form) at a rate of approximately 2mL/min to remove excess barium ions. The resulting solution was re-neutralized with about one gram of silver oxide and filtered to 0.2μm to remove the silver chloride precipitate and excess silver oxide. The final solution was freeze-dried to yield a silver nitrate salt, which was stored in amber vials to prevent photo-degradation.

2.2.3 Measurement of δ^{15}N-nitrate

Nitrogen isotope ratios of silver nitrate were determined using slightly modified versions of both breakseal combustion and elemental analysis - isotope ratio mass spectrometry (EA-IRMS) methods described by Silva et al. (2000). The EA-IRMS technique requires significantly less sample preparation compared to the breakseal method. The smaller amount of silver nitrate required also enables the analysis of water samples with lower nitrate concentrations. Samples run by both δ^{15}N methods demonstrated that the two techniques produced the same results within the stated precision of the techniques.

2.2.3.1 Breakseal method

For $\delta^{15}N$ determination by breakseal combustion, 8mg of silver nitrate sample was combined with calcium oxide, cupric oxide, and copper metal granules, in a 6mm quartz breakseal. Loaded breakseals were evacuated to less than 10^{-4} atm for one hour prior to flame-sealing while under vacuum. Nitrogen gas was produced by baking the evacuated breakseals at 850°C for three hours. $\delta^{15}N$ of the resulting N_2 was determined relative to an atmospheric N_2 standard using a VG Isogas Prism Series II mass spectrometer. Depending on the expected range in $\delta^{15}N$ values, two to four internal silver nitrate standards ($\delta^{15}N$ = +1.0, +10.3, +13.8, and +18.4‰), which were previously calibrated against international $\delta^{15}N$ standards, were processed and analyzed with each batch of samples. Repeat analysis of standards and selected samples yielded a precision of ±0.3‰.

2.2.3.2 EA-IRMS method

One milligram of silver nitrate sample was combined with 2mg of sucrose in tin weighing cups and loaded into the autosampler of a Carlo Erba elemental analyzer coupled to a Micromass Isochrom continuous flow stable isotope mass spectrometer for determination of $^{15}N/^{14}N$ isotope ratios. Within each run, samples were bracketed by sets of three internal silver nitrate standards ($\delta^{15}N$ = +1.0, +13.8, and +18.4‰). Repeat analysis of standards and selected samples yielded a precision of ±0.3‰.

2.2.4 Measurement of $\delta^{18}O$-nitrate

Oxygen isotope ratios were determined using a modified version of the breakseal combustion method described in detail by Silva et al. (2000) and more recently using an EA-pyrolysis method modified from Mengis et al. (2001). For samples run using both $\delta^{18}O$-

nitrate methods, pyrolysis values were generally within ±0.5‰ of values obtained by breakseal combustion.

2.2.4.1 Breakseal method

δ^{18}O-nitrate analysis by the breakseal method involved combining 8mg of silver nitrate sample with 10mg of previously baked carbon powder in a 6mm Vycor breakseal. Samples were then evacuated to less than 10^{-4} atm overnight and flame sealed under vacuum. Breakseals were baked in a muffle furnace until the temperature reached 825°C and then the oven was cooled at a rate of 100°C per hour. The resulting CO_2 was analyzed on a VG Isogas Prism Series II mass spectrometer and values reported relative to Vienna Standard Mean Ocean Water (VMSOW). Repeat analysis of standards and selected samples yielded a precision of ±0.8‰. Depending on the expected range in δ^{18}O values, three to five internal silver nitrate standards (δ^{18}O = -0.2‰, +11.0‰, +20.5‰, +28.0‰, and +45.2‰), which had been previously calibrated to IAEA-N3 using a value of +23.0‰, were processed and analyzed with each batch of samples.

2.2.4.2 EA-pyrolysis method

In contrast to breakseal combustion, the pyrolysis method does not require the off-line conversion of nitrate oxygen to CO_2. As a result, much less silver nitrate salt is required for this method and therefore samples with lower amounts of nitrate (due to either low concentration or small volume) can be analyzed. 0.2mg of desiccated silver nitrate was loaded into tin cups for analysis using a Eurovector EA coupled to a Micromass Isoprime mass spectrometer. Samples were pyrolyzed at 1290°C in a ceramic pyrolysis tube containing glassy carbon and the resulting CO was analyzed for $^{18}O/^{16}O$ isotope ratios. Repeat analysis of standards and samples gave a precision of ±0.8‰ or better. As with the

breakseal method, multiple sets of three to five internal silver nitrate standards were run with each batch of samples.

Chapter 3. The effect of storage on the isotopic composition of nitrate in bulk precipitation.

3.1 Introduction

Human activities such as the burning of fossil fuels and the excessive use of inorganic nitrogen (N) fertilizers by the agriculture sector have been implicated for recent increases in atmospheric nitrogen deposition across the globe (eg. Galloway et al., 1995). While pristine areas experience nitrogen deposition levels of less than 5 $kg \cdot ha^{-1} \cdot yr^{-1}$, the Eastern USA receives about 28 $kg \cdot ha^{-1} \cdot yr^{-1}$ (Van Miegroet et al., 1992). Heavily polluted areas in Europe have nitrogen deposition in excess of 75 $kg \cdot ha^{-1} \cdot yr^{-1}$ (Dise and Wright, 1995). One of the consequences of these deposition levels is that forested ecosystems, which have historically been thought of as nitrogen-limited, are becoming nitrogen-saturated (Agren and Bosatta, 1988; Aber et al., 1989). Nitrogen saturation can decrease forest health through increased water stress, reduced frost tolerance, soil acidification, the leaching of nutrients, and decreased fine root biomass (Aber et al., 1989).

Concerns over the long-term effects of elevated nitrogen deposition have led to an increase in the number of studies using stable isotope analysis to trace the fate of atmospheric nitrate deposition in forest ecosystems. The two potential sources of nitrate in most forested watersheds are: 1) nitrate from atmospheric deposition and 2) nitrate produced by nitrification in soils (microbial nitrate). Atmospheric and microbial nitrate are isotopically distinct and therefore isotopic ratios, particularly $^{18}O/^{16}O$, can be used to study nitrate sources and cycling in forested catchments (Durka et al., 1994; Kendall et al., 1996; Spoelstra et al., 2001; Williard et al., 2001; Burns and Kendall, 2002; Schiff et al., 2002).

Even at sites receiving elevated nitrate deposition, several liters of water are often required for isotopic analysis of atmospheric nitrate using the methods of Chang et al. (1999) and Silva et al. (2000). In pristine areas, 10L or more of precipitation might be required to determine both $\delta^{15}N$ and $\delta^{18}O$ values of nitrate. Typical bulk precipitation collectors designed to sample water for chemical analyses might not accumulate sufficient water from individual rain events to determine nitrate isotope ratios and therefore multiple collectors are often required. However, recently developed nitrate isotope methods, which use denitrifying bacteria to convert nitrate to nitrous oxide (N_2O) for determination of $\delta^{15}N$ and $\delta^{18}O$ values, reduce the required sample size by two orders of magnitude (Sigman et al., 2001; Casciotti et al, 2002). Therefore, utilization of these new techniques will significantly decrease the duration of the precipitation collection period necessary for accumulation of sufficient sample volume.

For many long-term catchment studies it would be impractical to analyze nitrate isotope ratios for each precipitation event. A significantly less expensive and less labor-intensive method of determining the isotopic signature of annual nitrate deposition is to combine individual precipitation samples acquired over an extended time interval. Thus, a mass-weighted mean $\delta^{15}N$ and $\delta^{18}O$ value is determined for the period of collection.

At remote sites, or where resources are limiting, bulk collectors might accumulate precipitation for several days or weeks before filtering and preservation occurs. With this approach, microbial reactions that consume or produce nitrate might alter the concentration and isotopic signature of nitrate in unfiltered samples. Maximum alteration of nitrate would be expected during summer months when elevated temperatures promote greater microbial activity. Microbial alteration would lead to the determination of an erroneous isotopic

composition for atmospheric nitrate, thus affecting subsequent source contribution calculations.

Several studies have investigated the temporal stability of ion concentrations in precipitation with varying conclusions with respect to nitrate. Although wet-only precipitation samples generally show more stable ionic compositions because of the decreased effects of particulate deposition (Galloway and Likens, 1976, 1978; Peden and Skowron, 1978), nitrate stability in wet-only samples ranges from days to months (Galloway and Likens, 1976, 1978; Peden and Skowron, 1978; Madsen, 1982; Keene and Galloway, 1984; de Pena et al., 1985; Ridder et al., 1985; Sisterson et al., 1985; Slanina et al., 1987).

A wide range of nitrate stability has also been measured for unfiltered bulk precipitation. Galloway and Likens (1976, 1978) found no change in the ionic composition of unfiltered bulk precipitation samples stored at 25ºC for seven months and attributed the results to the preservation effect of low pH (<4.5). Karlsson et al. (2000) did not see a change in nitrate concentration over a seven-week period for samples kept at 4ºC. In contrast, other studies have shown significant changes in nitrate levels for precipitation stored under a similar range of conditions (Peden and Skowron, 1978; Mahendrappa, 1985; Michalzik et al., 1997).

Since 1995, bulk precipitation samples have been collected and processed for nitrate isotope ratios as part of a study using stable isotope techniques to investigate nitrate sources and cycling at the TLW (Spoelstra et al., 2001). After a two-week collection period, samples are retrieved, filtered, and frozen until further processing at the University of Waterloo to determine $\delta^{15}N$ and $\delta^{18}O$ values of nitrate. Although precipitation is in the dark while in the

collector, the sample remains unfiltered at daytime temperatures that often exceed +25°C during the summer.

To date, the isotopic stability of nitrate in precipitation samples has not been assessed. The goal of this chapter was to determine if the nitrate isotope composition of unfiltered bulk precipitation samples is altered due to a two-week storage period at ambient temperatures.

3.2 Methods

For the longer term study of nitrate stable isotope ratios at the TLW (Spoelstra et al., 2001), precipitation has been collected using a Teflon® coated, stainless steel funnel (2500 cm^2) that drains into a 20L bottle that is kept in a dark chamber below the collector. Minimal evaporation can occur because the 20L bottle is closed to the atmosphere except through a small tube that connects it to the funnel. In this study, a custom-built bulk collector was used to acquire large volume precipitation samples from discrete rain events between May 2000 and June 2002. The collector consisted of a 110 x 90 cm plastic sheet that was suspended 70 cm above the ground using a frame constructed of ½" PVC pipe. A 20L jug collected water through a 3cm-diameter drain in the center of the plastic sheet. The rain collector was erected in an open area prior to forecasted rain events.

Immediately following sample collection, a sub-sample was filtered to 0.45µm and retained for chemical analysis. The remaining water was divided into three aliquots, providing a control sample and two samples for incubation experiments. Control samples were immediately filtered to 0.45µm to remove particulate matter and bacteria and then frozen until processing to determine unaltered atmospheric nitrate isotope ratios. Filtered

(0.45μm) and unfiltered aliquots were incubated to evaluate the effect of microbial reactions on the nitrate concentration and isotopic composition of the precipitation samples. Incubated samples were kept at 25-28°C for two weeks to mimic storage conditions in bulk collectors during the summer. The bottles were covered in a manner that prevented contamination by dust while allowing gaseous exchange with the atmosphere. Following incubation, samples were filtered to 0.45μm, sub-sampled for chemical analysis, and frozen until processing to determine nitrate isotope ratios.

The methods used for the isolation of nitrate from water samples and its subsequent conversion to silver nitrate were adapted from Chang et al. (1999) and Silva et al. (2000) and are described in Section 2.2.2. $\delta^{15}N$ and $\delta^{18}O$ values of silver nitrate were determined by continuous flow isotope ratio mass spectrometry methods as outlined in Sections 2.2.3.2 and 2.2.4.2, respectively.

3.3 Results and Discussion

Following the two-week incubation, none of the treatments, filtered or unfiltered, showed a significant difference in nitrate concentrations compared to initial values for each sample (Table 3.1). However, the isotopic composition of samples could have shifted without a change in nitrate concentration if nitrate production and consumption occurred at the same rate.

Bacteria could produce nitrate in unfiltered precipitation samples by nitrification of atmospheric ammonium (NH_4^+) present in the sample or ammonium produced from dissolved organic matter (DOM) decomposed during the incubation period. Nitrifying bacteria strongly

Table 3.1 Nitrate concentrations and nitrate isotope results for control and incubated bulk precipitation samples. The analytical precision for nitrate concentration is ±0.02mg N/L.

Sample	Treatment	Nitrate (mg N/L)		Isotope Ratios	
		Initial	Final	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)
BP-1-C	Filtered, frozen	0.80		-4.6	60.7
BP-1-F	Filtered, incubated	0.80	0.76	-4.9	60.4
BP-1-U	Unfiltered, incubated	0.80	0.80	-4.8	61.6
BP-2-C	Filtered, frozen	0.27		-5.7	52.0
BP-2-F	Filtered, incubated	0.27	0.28	-5.5	56.5
BP-2-U	Unfiltered, incubated	0.27	0.27	-5.4	55.8
BP-3-C	Filtered, frozen	0.23		-7.3	51.1
BP-3-F	Filtered, incubated	0.23	0.22	-7.3	49.0
BP-3-U	Unfiltered, incubated	0.23	0.24	-7.4	52.6
BP-4-C	Filtered, frozen	0.34		-7.6	51.1
BP-4-F	Filtered, incubated	0.34	0.35	-7.8	54.8
BP-4-U	Unfiltered, incubated	0.34	0.33	-7.4	54.8
BP-5-C	Filtered, frozen	0.26		-5.8	53.9
BP-5-F	Filtered, incubated	0.26	0.26	-5.9	55.4
BP-5-U	Unfiltered, incubated	0.26	0.26	-5.7	53.9

fractionate against $^{15}NH_4^+$ (ε = -12 to -29‰, Shearer and Kohl, 1986) and therefore the initial nitrate produced is markedly depleted in ^{15}N compared to the ammonium source. However, the overall $\delta^{15}N$ of nitrate produced by nitrification depends on several factors including the $\delta^{15}N$ of the ammonium source and the fraction of ammonium nitrified. As a larger proportion is nitrified the $\delta^{15}N$ of that nitrate approaches the $\delta^{15}N$ of the original ammonium pool. Atmospheric ammonium is generally depleted in ^{15}N compared to coexisting nitrate in precipitation (Freyer, 1978; Garten, 1992). However, without nitrogen isotope data for ammonium or DON in TLW precipitation, it is not known whether nitrification nitrate will be significantly depleted compared to the original atmospheric nitrate.

Interpretation of $\delta^{18}O$ values, in conjunction with $\delta^{15}N$, more reliably assesses the effects of nitrification. Nitrate produced by nitrification is significantly depleted in ^{18}O

relative to atmospheric nitrate. During the nitrification of ammonium, oxygen is added to the nitrogen molecule from O_2 (+23.5‰) and from water (Aleem et al., 1965; Kumar et al., 1983; Andersson and Hooper, 1983; Hollocher, 1984), the $\delta^{18}O$ of which largely depends on the $\delta^{18}O$-H_2O of local precipitation. Depending on the ratio of oxygen from O_2 to oxygen from water the $\delta^{18}O$ of nitrate produced is expected to be -5 to +15‰ (Kendall, 1998). Therefore the overall result of gross nitrification occurring in unfiltered precipitation samples would be: 1) an increase in nitrate concentration and 2) a shift to lower $\delta^{18}O$ (and likely $\delta^{15}N$) values. Since nitrate concentrations did not change and none of the treatments had ^{18}O-depleted nitrate isotope ratios compared to the controls (Figure 3.1), it appears that nitrate production by nitrification did not occur as a result of the incubation.

Microbes that utilize nitrate as a nitrogen source have to first reduce it to ammonium. Assimilative nitrate reduction requires energy and thus ammonium is generally preferred by microorganisms as an initial nitrogen source (Rosswall, 1981). Ammonium and nitrate concentrations in the precipitation samples were similar (Table 3.2). Therefore microbial ammonium assimilation could occur in samples, however, this process would not affect nitrate concentrations or isotope ratios. Compared to nitrate, the isotopic composition of atmospheric ammonium is probably more susceptible to microbial alteration during sample storage.

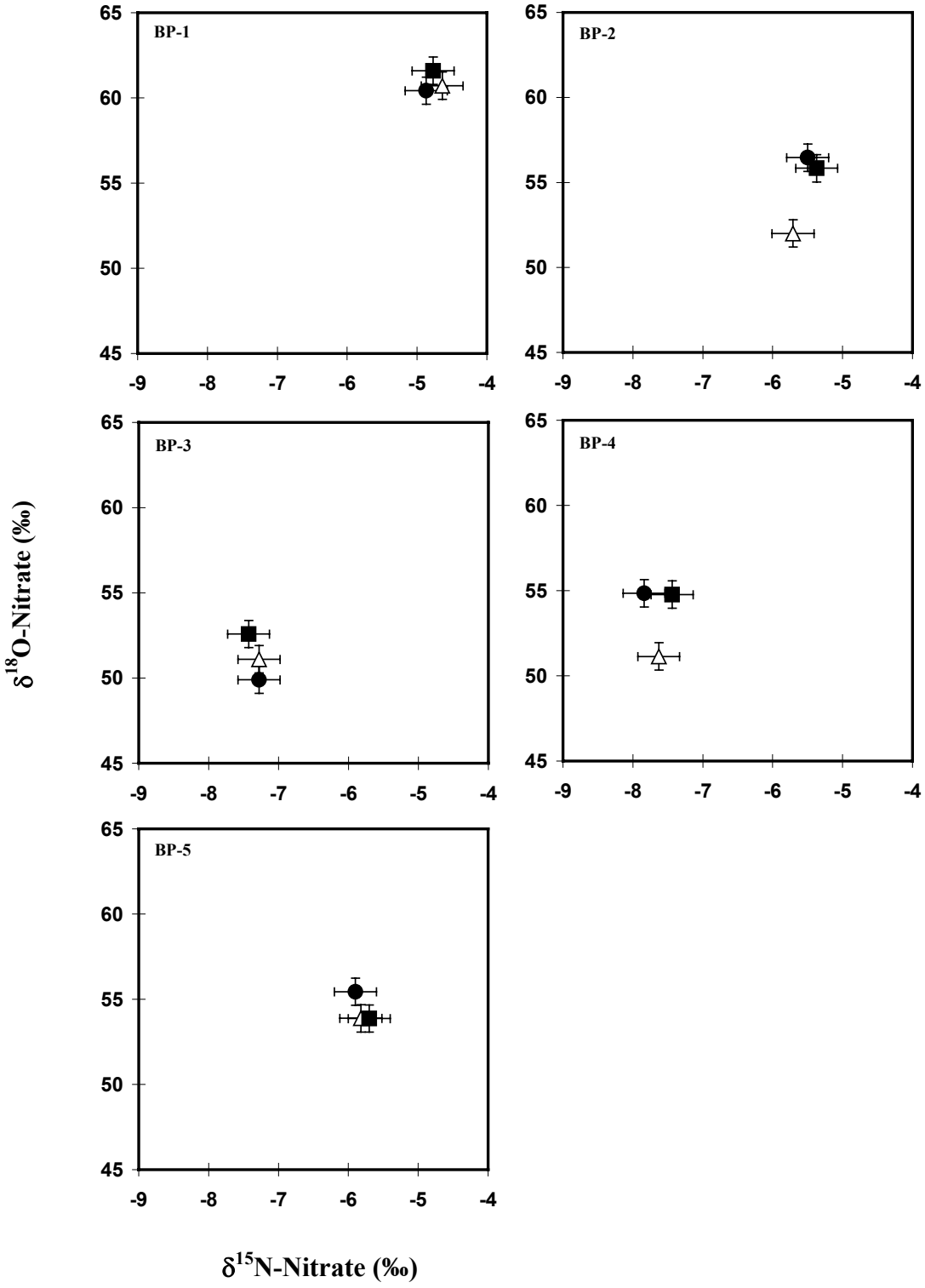

Figure 3.1 Nitrogen and oxygen isotope ratios of control (△), filtered and incubated (●), and unfiltered and incubated (■) bulk precipitation samples for five separate rain events. Horizontal and vertical bars indicate the precision of $\delta^{15}N$ (±0.3‰) and $\delta^{18}O$-nitrate (±0.8‰) analyses.

Table 3.2 Selected chemical parameters for bulk precipitation samples at time of collection.

Sample	NH_4^+ (mg N/L)	NO_3^- (mg N/L)	SO_4^{2-} (mg/L)	DOC (mg C/L)
BP-1	0.74	0.80	4.12	1.40
BP-2	0.31	0.27	1.51	1.52
BP-3	0.27	0.23	1.76	0.60
BP-4	0.40	0.34	1.68	3.27
BP-5	0.29	0.26	1.51	2.24

Several types of microorganisms have been shown to fractionate against ^{15}N to varying degrees during nitrate assimilation (e.g. Hübner, 1986). Although little or no information exists on the associated isotopic discrimination for $^{18}O\text{-}NO_3^-$ during nitrate assimilation, biochemical reactions generally discriminate against heavy isotopes (Kendall, 1998). Therefore, microbial nitrate assimilation is expected to preferentially utilize isotopically light nitrate, progressively enriching the residual nitrate in ^{15}N and ^{18}O. The overall effect of this type of microbial activity in bulk precipitation samples would be 1) a decrease in nitrate concentration over time and 2) likely a concomitant increase in the $\delta^{15}N$ and $\delta^{18}O$ values of the residual nitrate.

Denitrification, a dissimilatory nitrate reduction pathway, converts nitrate to N_2O and N_2 under anaerobic conditions. This microbial reaction causes a decrease in nitrate concentration while increasing $\delta^{15}N$ and $\delta^{18}O$ values of the remaining nitrate in a characteristic ratio of approximately 2:1 (Amberger and Schmidt, 1987; Böttcher et al., 1990; Voerkelius and Schmidt, 1990; Aravena and Robertson, 1998; Cey et al., 1999; Mengis et al., 1999). However, denitrification was not expected to be a significant process affecting samples in bulk precipitation collectors because the likelihood of anaerobic conditions

developing was limited by low DOC concentrations and the fact that oxygen could freely diffuse into the collection vessels.

Isotopic analysis did not reveal a concomitant increase of $\delta^{15}N$ and $\delta^{18}O$ values for atmospheric nitrate following the two-week incubation (Figure 3.1). In fact, none of the incubated samples had $\delta^{15}N$ values that differed from the controls, which were filtered and frozen immediately after collection. The absence of a reduction in nitrate concentration, in conjunction with no increase in $\delta^{15}N$ values, strongly indicates that neither assimilative nitrate reduction nor denitrification occurred in any of the incubated samples. The approximately 4‰ difference between the $\delta^{18}O$ values of control and treatments for BP-2 and BP-4, is likely the result of slight DOM contamination of the silver nitrate produced from the controls of both these samples.

Natural DOM consists of approximately 40% oxygen by weight (Thurman, 1985) and is isotopically depleted in ^{18}O ($\delta^{18}O$ = +8.2 to +25.3‰; Humphries, 2003) relative to atmospheric nitrate. DOM also contains nitrogen, but at only about 1% by weight (Thurman, 1985). Small amounts of DOM contamination that interfere with $\delta^{18}O$-nitrate analysis would not necessarily contain enough nitrogen to shift $\delta^{15}N$-nitrate values. Other common oxygen bearing ions such as sulfate, phosphate and bicarbonate, which could cause a shift in the measured $\delta^{18}O$ without affecting $\delta^{15}N$ values, are quantitatively removed during nitrate collection by anion exchange and its subsequent conversion to silver nitrate (Silva et al., 2000).

The effectiveness of DOM removal from water samples prior to nitrate isotope analysis is dependent on the physical and chemical properties of the DOM and therefore can vary with sample type and research site (Chang et al., 1999). Approximately 85% of the

DOM in samples collected from the TLW was removed by the combined effects of permanently binding to the anion exchange resin and flocculation during conversion of aqueous nitrate to a silver nitrate salt. The incubation period might have affected the chemical and physical characteristics of the DOM in the treatment samples, resulting in a higher proportion of the DOM being removed and less or no contamination of the resulting silver nitrate in incubated samples compared to the controls. BP-4 and BP-2 had the highest and third-highest initial DOC concentrations of the five precipitation samples, 3.3 and 1.5 mg C/L, respectively (Table 3.2). The difference between $d^{18}O$ values of the treatment and control samples for BP-2 and BP-4, without a change in nitrate concentrations or $\delta^{15}N$ values, is not consistent with a microbial effect. As a result, it is suspected that the controls have been shifted slightly to lower $\delta^{18}O$ values relative to incubated samples. Our results further underscore the need for DOM removal prior to $\delta^{18}O$ analysis of atmospheric nitrate, even at relatively low DOC concentrations.

The length of time that unfiltered precipitation can remain in collectors without alteration of nitrate isotope ratios is likely a function of temperature and initial sample chemistry. Winds at the TLW are typically from the west to southwest, coming across Lake Superior and land dominated by forest cover. Therefore, low nutrient levels in TLW precipitation could be one reason why nitrate concentration and isotopic composition did not change during the two-week incubation. Bacterial growth in TLW bulk precipitation might also be limited by elements such as phosphorus and labile organic carbon and by the low pH (mean of 4.3; Morrison et al., 1992). Sites that are downwind of agricultural areas with disturbed soils likely receive higher concentrations of nutrients, especially in particulate form, and therefore could be more susceptible to microbial alteration (e.g. Peden and

Skowron, 1978). Windblown soil particles are also expected to have high concentrations of bacteria that could inoculate bulk precipitation samples and expedite nitrogen transformations. Precipitation collected as throughfall is also enriched in certain nutrients and labile organic carbon because trees, especially conifers, are efficient collectors of dry deposition (Kloeti et al., 1989). Michalzik et al. (1997) found that nitrate and ammonium concentrations in bulk precipitation were more stable than those of throughfall samples.

3.4 Conclusions

Bulk precipitation has been collected from the TLW for nitrate isotope analysis since 1995. These samples remain in the collector for up to two weeks prior to filtering and preservation. Results of the incubation experiment presented in this paper confirm that the isotope signature of atmospheric nitrate is not compromised by current precipitation collection methods employed at the TLW. The ability to collect precipitation over an extended time period without the alteration of nitrate $\delta^{15}N$ or $\delta^{18}O$ values reduces the total number of samples to be processed and is especially relevant for precipitation collection at remote sites where immediate sample retrieval is not always possible. We caution that nitrate isotope ratios might be more susceptible to alteration where higher nutrient concentrations in precipitation are less limiting to bacterial growth or where temperatures exceed those used in this study.

The effects of storage on the isotopic composition of other ions in bulk precipitation remains to be investigated. Isotopic data for nitrate presented in this paper, in conjunction with previous studies showing little or no change in sulfate concentrations (Galloway and Likens, 1976, 1978; Peden and Skowron, 1978; Mahendrappa, 1985; Ridder et al., 1985;

Karlsson et al., 2000), suggest that the isotopic ratios of less biologically active ions such as sulfate also remain unmodified after extended storage times. Conversely, the results of previous concentration-based studies (Ridder et al., 1985; Sisterson et al., 1985; Michalzik et al., 1997; Karlsson et al., 2000) indicate that nutrient compounds such as ammonium likely require prompt preservation to avoid isotopic alteration by microorganisms.

To dr. John Spoelstra

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Chapter 4. Tracing the sources of exported nitrate in the Turkey Lakes Watershed using $^{15}N/^{14}N$ and $^{18}O/^{16}O$ isotopic ratios.

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4.1 Introduction

Although the TLW receives a moderate atmospheric loading of nitrate and ammonium, averaging 8.7 kg $N \cdot ha^{-1} \cdot yr^{-1}$, it has an unusually low ability to retain inorganic nitrogen. The nitrate exported from forested watersheds is typically a mixture of two sources: 1) atmospheric deposition (atmospheric nitrate) and 2) nitrification in soils (microbial nitrate). Previous studies have shown that these two nitrate sources have widely separated oxygen isotope ratios and therefore $\delta^{18}O$-nitrate can be used to evaluate the importance of atmospheric and microbial nitrate in streams and groundwater (e.g. Durka et al., 1994). The highest nitrate concentrations in TLW headwater streams generally occur during snowmelt (Creed et al. 1996), resulting in stream acidification through a concomitant increase in the export of hydrogen ions. If the nitrate exported during snowmelt is largely derived from the snowpack then future increases in acidic nitrogen deposition are likely to have a significant effect of stream pH during the melt period. The purpose of this study is to test the applicability of the dual nitrate isotope technique at the TLW and to determine the relative contributions of the two nitrate sources to the nitrate exported from two undisturbed headwater catchments at this site.

4.1.1 Microbial nitrate

The use of isotopes to distinguish between nitrate from different sources requires a measurable difference in the isotope ratios of nitrate from these sources. Intensive plot-scale studies indicate that the nitrification of ammonium (NH_4^+) in forest soils is a major source of the nitrate in TLW groundwater on an annual basis (Foster et al., 1989a; Foster et al., 1992). The $\delta^{15}N$ of the microbial nitrate available for leaching depends on the rates of several soil processes including mineralization, nitrification, and assimilation of inorganic nitrogen that control the $\delta^{15}N$ of both the ammonium available to be nitrified and the resulting nitrate. Since the rates of these processes vary spatially and temporally, it is expected that the $\delta^{15}N$ of nitrate produced by nitrification would also vary. Isotopic fractionation occurs with the soil nitrogen processes, making it difficult to accurately estimate the $\delta^{15}N$ of the microbial nitrate in the absence of direct measurements. Enzymatically controlled processes such as soil nitrogen cycling tend to fractionate against the heavy isotope by preferential utilization of the lighter isotope.

Based on the results of laboratory incubations (Kumar et al., 1983; Andersson and Hooper, 1983; Hollocher, 1984), the $\delta^{18}O$ value of nitrate produced from nitrification of ammonium can be calculated using Equation 4.1. In these experiments, one third of the oxygen was derived from atmospheric O_2 and two thirds from soil water. The $\delta^{18}O$ of atmospheric O_2 is about +23.5‰ (Kroopnick and Craig, 1972) whereas the $\delta^{18}O$ of shallow soil water is expected to vary as the $\delta^{18}O$-H_2O of precipitation changes during the year. Bottomley et al. (1986) found that the $\delta^{18}O$ of precipitation at the TLW ranged from -20.1‰ for the snowpack to -4.5‰ for summer rain. The corresponding $\delta^{18}O$ of microbial nitrate produced from soil water with these values would be -5.7‰ and +4.7‰ respectively.

$$\delta^{18}O\text{-}NO_3^- \text{ (microbial)} = {}^1/_3(\delta^{18}O\text{-}O_2) + {}^2/_3(\delta^{18}O\text{-}H_2O) \quad 4.1$$

Due to mixing in the groundwater flow system, the $\delta^{18}O$ of microbial nitrate that enters headwater streams via groundwater is likely to have a range of values intermediate between the two seasonal extremes. Mixing also narrows the range of observed groundwater $\delta^{18}O\text{-}H_2O$ values. The effect of groundwater mixing on the $\delta^{18}O$ value of microbial nitrate in groundwater would depend on the timing and the amount of nitrate entering the groundwater flow system. Nitrification rates are highest during the growing season, however, this is also a time of high nitrogen utilization by vegetation and microbes (Foster et al., 1989b) coupled with low groundwater recharge. A lack of water flux through the soil often prevents the leaching of nitrate below the rooting zone during the summer (Foster et al., 1989a). Nitrification rates are lower during the dormant season but a larger proportion of this nitrate may enter the groundwater because of lower rates of nitrate assimilation and higher recharge rates (Foster et al., 1989b). Thus, the expected $\delta^{18}O$ of nitrate leached to the water table likely follows temporal dynamics similar to the pattern of groundwater recharge. The $\delta^{18}O\text{-}H_2O$ of groundwater at the TLW ranges from about -10.5 to -13‰ (Bottomley et al., 1986) and the $\delta^{18}O$ of atmospheric O_2 is +23.5‰. Therefore, according to Equation 4.1, the average $\delta^{18}O$ value of nitrate produced by nitrification at the TLW is expected to fall within a range of -0.8 to +0.8‰.

4.1.2 Atmospheric nitrate

The stable isotopic ratios for nitrate in precipitation are controlled by complex atmospheric processes that are not well understood. These ratios show large spatial and

temporal variability, even within a single storm event (Heaton, 1986). Previous studies have shown that the $\delta^{18}O$ of atmospheric nitrate is generally enriched in ^{18}O relative to atmospheric O_2 (+23.5‰), having values that are generally between +25 and +75‰ (Durka et al., 1994; Kendall et al., 1996; Schiff et al., 2002). The oxygen isotope ratios for atmospheric nitrate are also very enriched compared to the $\delta^{18}O$ values for nitrate produced by nitrification. Even though atmospheric nitrate displays a wide range of $\delta^{18}O$ values there is a large isotopic separation between atmospheric and microbial nitrate. Therefore, $\delta^{18}O$-nitrate provides information on the importance of nitrate from these two sources in surface and ground waters.

The $\delta^{15}N$ values that have been measured for atmospherically derived nitrate are centered around the value of atmospheric dinitrogen gas (0‰), most within a range of -4 to +6‰ (Kendall, 1998). The isotopic separation between the two nitrate sources is significantly less with $\delta^{15}N$ compared to $\delta^{18}O$, however, concomitant $\delta^{15}N$-nitrate analysis is important because it permits the detection of isotopic enrichment due to denitrification. As nitrate is denitrified, the residual nitrate becomes increasingly enriched in ^{18}O and ^{15}N in an approximate ratio of 1:2 (Amberger and Schmidt, 1987; Böttcher et al., 1990; Voerkelius and Schmidt, 1990; Aravena and Robertson, 1998; Cey et al., 1999; Mengis et al., 1999). The dual nitrate isotope approach enables the distinction between an enriched $\delta^{18}O$ value from atmospheric nitrate, which would have a $\delta^{15}N$ value around zero, and a similar $\delta^{18}O$ value from nitrate that has been enriched due to denitrification, which would also have an enriched $\delta^{15}N$ value.

4.2 Methods

4.2.1 Sample collection

Two-liter water samples for nitrate isotope analysis were taken from piezometers during high water table periods, biweekly from bulk precipitation collectors, and monthly from streams, from April 1995 to December 1996. Both catchments contain an upper and lower transect of piezometers that intersect the stream. Each transect consists of 6 pairs of drive-point piezometers with a screened interval of 15cm, one shallow (approximately 40cm) and one deep (approximately 105cm) per pair (Hazlett et al., 2001). Some piezometers could not be sampled because they were dry or recharge was insufficient. Prior to November 1995, precipitation samples were taken at a site in catchment 47 and, since that time, have been collected at a Canadian Air and Precipitation Monitoring Network (CAPMoN) site located within 1.5 km of the TLW. Stream samples were collected at V-notch weirs located near the base of the catchments. Samples were frozen in polyethylene bottles until processing for isotopic analysis.

4.2.2 DOM removal

For samples that contained sufficient dissolved organic matter (DOM) (dissolved organic carbon (DOC) greater than 3.0 mg $C \cdot L^{-1}$), DOM was removed by ultrafiltration for isotopic analysis (results not included in this thesis). Ultrafiltration using a Pall Filtron Ultrasette with a 1000 dalton molecular weight cut-off removed approximately 80% of the DOM (Section 2.2.1.1). Further DOM removal occurred on the ion exchange resin where a significant amount of the DOM was retained during elution of the nitrate (Section 2.2.1.3).

4.2.3 Nitrate isotope analysis

Ion exchange methods for the isolation of aqueous nitrate and its subsequent conversion to silver nitrate were adapted from Chang et al. (1999) and Silva et al. (2000) and are described in Section 2.2.2. $\delta^{15}N$ and $\delta^{18}O$ values of silver nitrate were determined by breakseal combustion techniques outlined in Sections 2.2.3.1 and 2.2.4.1.

4.3 Results and Discussion

4.3.1 Isotopic composition of nitrate

The isotope values for atmospheric nitrate at the TLW fall within the range of values seen in other studies (Durka et al., 1994, Kendall et al., 1996, Kendall, 1998). Due to low nitrate concentrations, only one isotopic ratio ($\delta^{15}N$ or $\delta^{18}O$) could be measured for most precipitation samples. The 16 precipitation samples analyzed for $\delta^{18}O$-nitrate had a range of values between +35 and +59‰ with a mass weighted mean of +50.2‰ (Figure 4.1). The range of $\delta^{15}N$-nitrate values for 10 precipitation samples was -4.0 to +0.8‰ with a mass weighted mean of -2.1‰. No clear seasonal trend in either $\delta^{18}O$ or $\delta^{15}N$ was observed for nitrate in bulk precipitation samples and there did not appear to be a relationship between nitrate isotope values and nitrate concentration. The $\delta^{18}O$ of atmospheric nitrate at the TLW is significantly enriched compared to the calculated $\delta^{18}O$ of microbial nitrate, and therefore, excellent isotopic separation exists between the two nitrate sources at this site.

Hydrograph separations show that, even during spring melt, the source of water to the headwater streams at the TLW is largely groundwater (Bottomley et al., 1984; Bottomley et

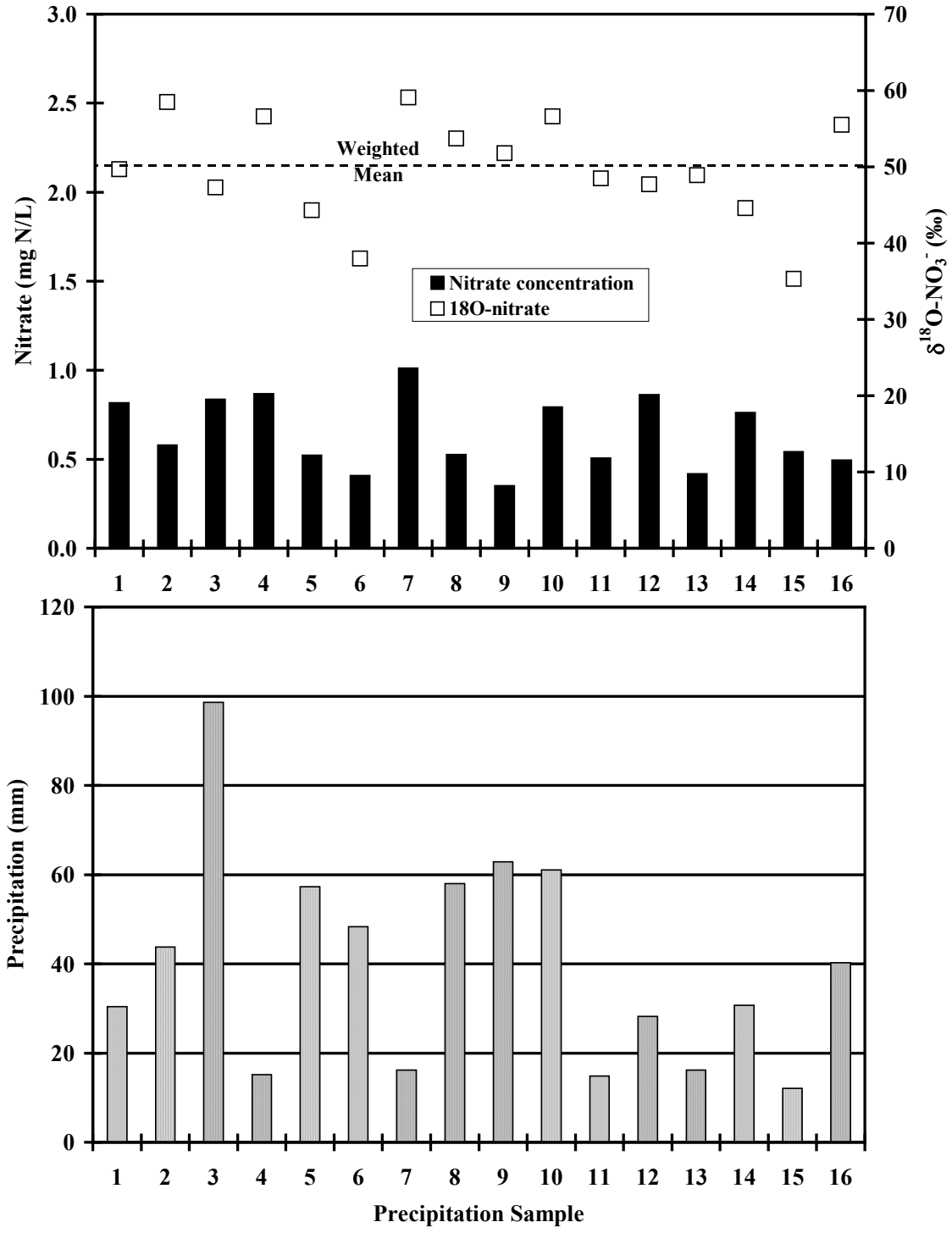


Figure 4.1 $\delta^{18}O\text{-}NO_3^-$, nitrate concentration, and precipitation amount for 16 precipitation samples collected at the TLW from 1995 to 1997. The analytical precisions for $\delta^{18}O\text{-}NO_3^-$ and nitrate concentration are ±0.8‰ and ±10% respectively. The precipitation amounts shown for rain and snow samples are water equivalents for the two-week collection period for each sample.

al., 1986). Therefore, the range in $\delta^{18}O$-H_2O of the streamwater is a good proxy for the $\delta^{18}O$-H_2O of groundwater which was only measured during the spring melt and fall recharge periods. The $\delta^{18}O$-H_2O for streams 31 and 47 ranged from -11.03 to -15.34‰ for samples taken throughout 1995 and 1996. Using the assumptions outlined earlier, the corresponding expected range of microbial $\delta^{18}O$-nitrate values would be -2.4 to +0.5‰, with an average value of -1.0‰.

Measured $\delta^{18}O$-NO_3^- values ranged from +3.3 to +14.5‰ in the streams and from +5.5 to +21.2‰ in groundwater (Figure 4.2). These relatively depleted values indicate that nitrification is the dominant source of the nitrate exported by streams 31 and 47. Nitrate concentrations in the streams exceeded the volume weighted average nitrate concentration of wet plus dry deposition (0.57 mg $N \cdot L^{-1}$ - derived from Sirois et al. (2001)) when evapo-concentration is low (Figure 4.3). Based on this observation alone, microbial nitrate must be entering the streams at these times. Throughout the summer and fall the concentration of nitrate in the streams decreases due to increased inorganic nitrogen demands by vegetation and microbes. During this time period, the concentration of nitrate in precipitation is often greater than the stream nitrate concentration. The isotopes however, show that microbial nitrate is still the major source of exported nitrate at this time and throughout the entire year.

Amberger and Schmidt (1987) and Böhlke et al. (1997) suggested that evaporative enrichment could lead to even more enriched $\delta^{18}O$ values for soil water and thus for microbial nitrate. Higher than expected microbial nitrate $\delta^{18}O$ values could also occur if the O_2 available for nitrification was enriched by microbial respiration (Kendall, 1998). These two oxygen enrichment effects would be greatest during the warm summer months. Several

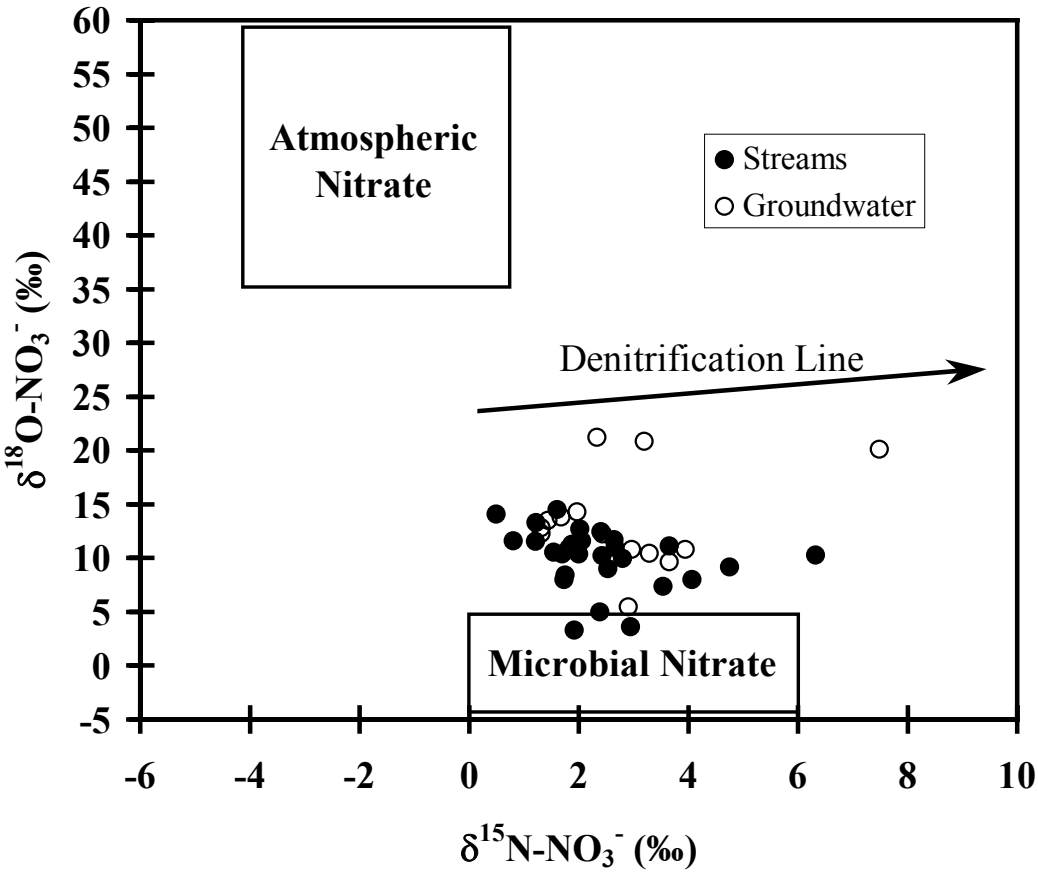


Figure 4.2 Isotopic ratios of nitrate in streamwater and groundwater for catchments 31 and 47 with boxes indicating measured ranges for nitrate in TLW precipitation and estimated ranges for microbial nitrate produced at the TLW. The analytical precisions for $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NO_3^-$ are ±0.8‰ and ±0.3‰ respectively. The denitrification line shows the slope of the expected isotopic shift for nitrate that has been enriched by denitrification.

samples from catchments 31 and 47, including samples taken during summer and early fall, had $\delta^{18}O$-nitrate values less than +10‰ (Figure 4.2). The low $\delta^{18}O$ values for these samples suggest that an enriched oxygen source for nitrification may not be a significant factor in these catchments.

All stream and groundwater samples had $\delta^{15}N$-nitrate values greater than +0.5‰. Since the stream nitrate is largely microbial in origin as indicated by $\delta^{18}O$ and the average

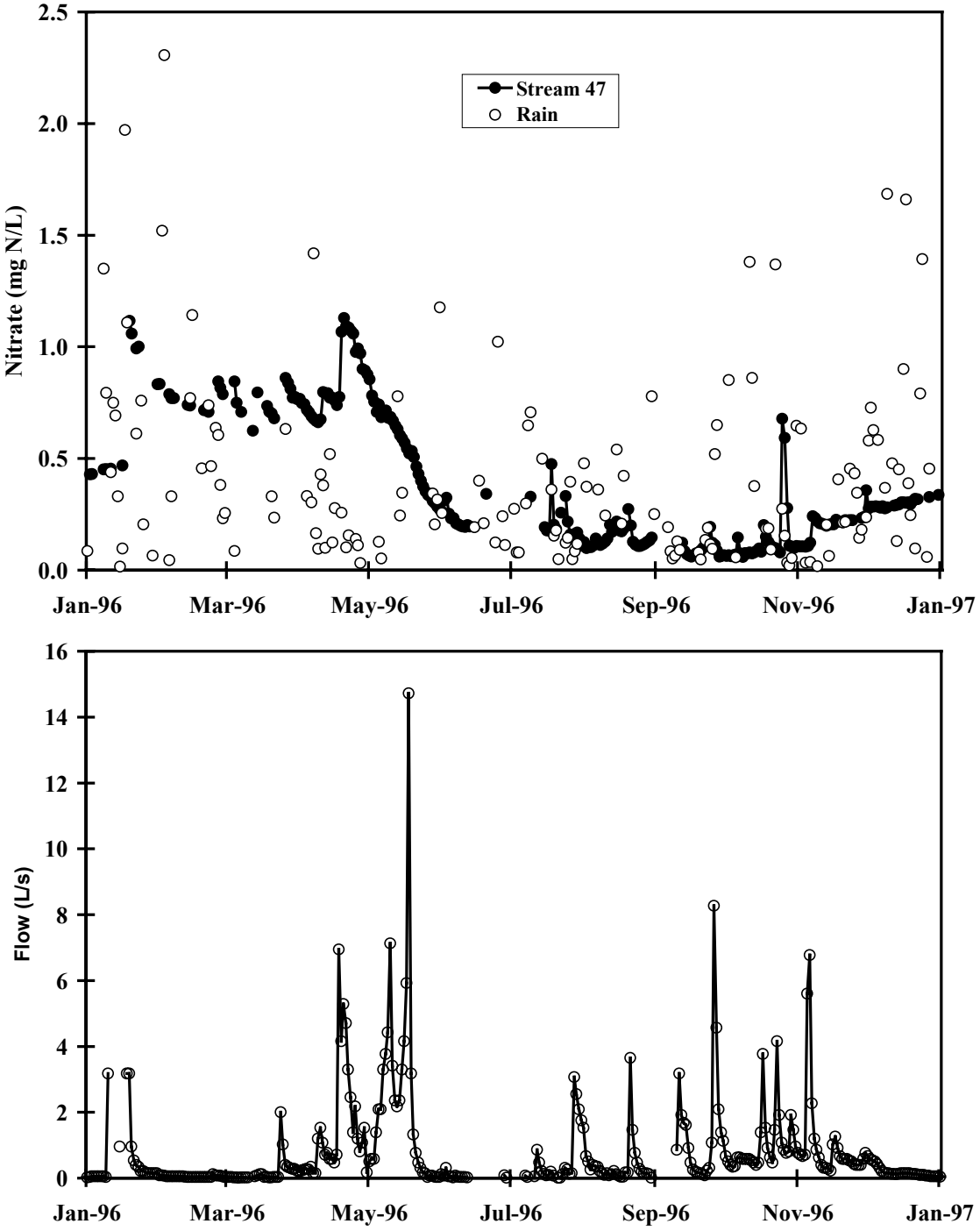


Figure 4.3 Nitrate concentration and discharge for stream 47 and nitrate concentration in wet-only precipitation (events 1.0mm or greater) for 1996. The volume weighted average nitrate concentration is 0.33 mg $N{\cdot}L^{-1}$ for wet deposition and 0.57 mg $N{\cdot}L^{-1}$ for wet plus dry deposition (derived from Sirois et al., 2001). Daily precipitation chemistry data provided by Environment Canada, Atmospheric Environment Service.

atmospheric nitrate $\delta^{15}N$ is -2.1‰, the $\delta^{15}N$ of microbial nitrate must be greater than 0‰. Based on stream and groundwater samples, it is estimated that this value was within the range of 0 to +6‰. Although the isotopic separation of the two sources is not as extreme for $\delta^{15}N$-nitrate as it is for $\delta^{18}O$-nitrate, $\delta^{15}N$ remains a useful indicator of nitrate source and cycling at the TLW.

4.3.2 Nitrate source contributions

One of the goals of this study was to determine the relative contributions from each nitrate source to the nitrate in streams. Large ranges in the nitrate isotopic ratios of the sources, especially those for atmospherically derived nitrate, render these calculations only approximate. The $\delta^{18}O$-nitrate is highly variable between the precipitation samples that were collected only two weeks apart. Due to this variability over short time periods and the mixing of atmospherically derived nitrate once it enters the groundwater flow system, the mass weighted average of the $\delta^{18}O$-nitrate in precipitation was used to calculate percent source contributions.

The calculated average $\delta^{18}O$-nitrate values in precipitation (+50.2‰) and of microbial nitrate (-1.0‰) were used along with the measured $\delta^{18}O$-nitrate of stream samples to calculate the percent microbial nitrate in streams 31 and 47 for 1995 and 1996 (Figure 4.4). The proportion of microbial nitrate in the streams ranged from 70 to 92% with both streams showing similar trends. These results agree with the results of plot-scale (1.0 ha) studies of catchment 31 (Foster et al., 1989a; Foster et al., 1992) which showed that 60 to 83% of the nitrate leaching past the effective rooting zone annually was produced by nitrification. If the $\delta^{18}O$ values of microbial nitrate were more enriched due to an enriched oxygen source (O_2 or H_2O), the microbial contribution would be even higher than calculated.

Streams 31 and 47 showed the same temporal pattern in nitrate source contributions (Figure 4.4). Atmospherically derived nitrate was most evident in the streams during spring melt when it reached a maximum value of about 30%. Despite the higher contribution from precipitation nitrate during spring melt, microbial nitrate was still the dominant source of nitrate in the streams. The isotopic signature of stream nitrate showed that the episodic acidification of the streams resulting from the high nitrate concentrations was caused more by microbial nitrate than by nitrate from atmospheric deposition. Microbial nitrate diluting the atmospheric nitrate signature from the melting snow could be produced by nitrification under the snowpack (Hazlett et al., 1992) or derived from groundwater discharge where nitrate was largely microbial in origin.

The fraction of microbial and atmospheric nitrate exported from forested catchments depends on the seasonality and rates of several processes that can vary significantly between study sites. These factors include nitrate deposition, biological nitrate assimilation, groundwater recharge and nitrogen mineralization/nitrification (Durka et al., 1994; Kendall et al., 1996). Kendall et al. (1996) used the dual isotope approach to show the importance of atmospheric nitrate as a source of nitrate to streams during spring melt in three watersheds in the United States. Unlike this study, the authors concluded that atmospherically derived nitrate stored in the shallow groundwater was a major source of the nitrate pulse that was seen during early melt in these catchments. Durka et al. (1994) suggested that the fraction of atmospheric nitrate in spring water for eight Norway spruce (*Picea abies* [L.] Karst.) forests in Germany was related to forest health. In the German study the fraction of atmospheric nitrate in the spring water ranged from 14 to 46 percent with the declining forests showing

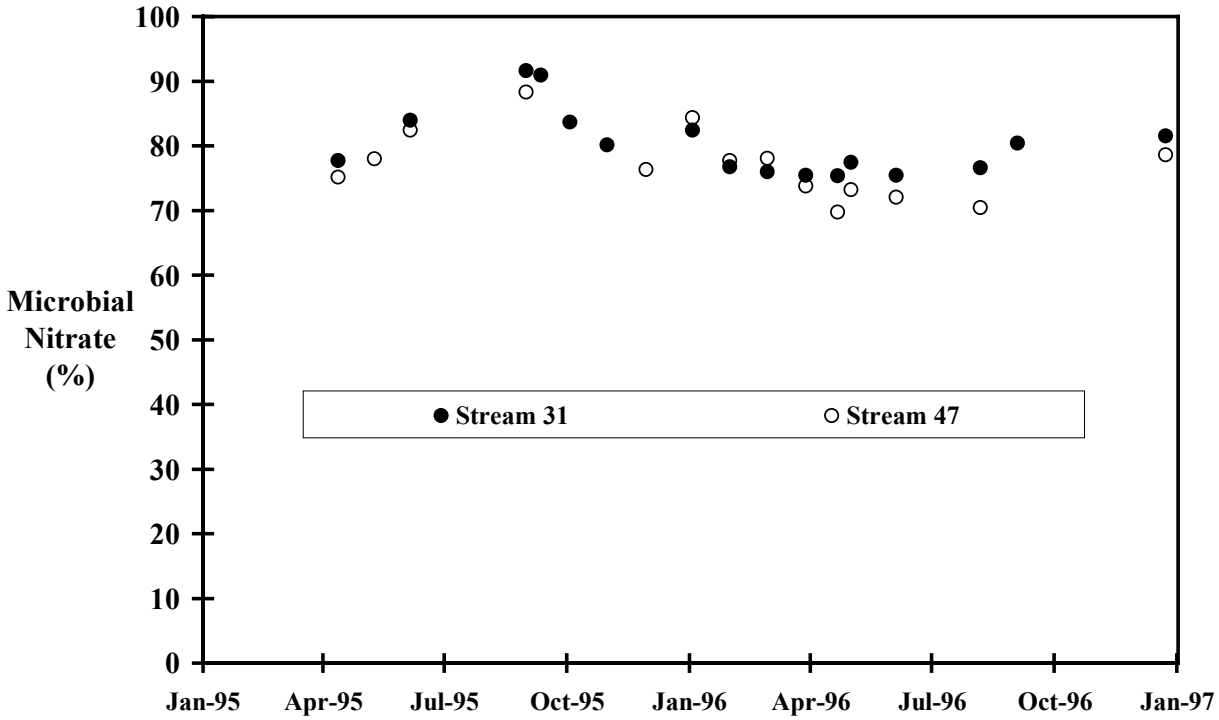


Figure 4.4 Temporal trends in the proportion of microbial nitrate in streams 31 and 47 for 1995 and 1996 as calculated using the $\delta^{18}O$ of streamwater nitrate and the mass weighted average $\delta^{18}O\text{-}NO_3^-$ for precipitation (+50.2‰) and microbial (-1.0‰) nitrate sources.

the highest contribution from atmospheric nitrate. Results of the current study suggest that the fraction of atmospheric nitrate in nitrate exported from catchments 31 and 47 is similar to the fraction leached from the healthy German forests.

4.3.3 Importance of denitrification

Previous research in catchment 31 has shown that there is a large difference between the amount of nitrate that leaches past the rooting zone (16.2 kg $N \cdot ha^{-1} \cdot yr^{-1}$, Foster et al., 1992) and the amount exported in stream discharge (~3 kg $N \cdot ha^{-1} \cdot yr^{-1}$, Foster et al., 1986; Nicolson, 1988; Foster et al., 1989b). One possible explanation for this difference is that most of the nitrate is denitrified and the nitrate that reaches the stream is residual nitrate from

this process. The nitrate isotope ratios of almost all of the stream and groundwater samples fell within the range of values that would be produced by a simple mixing of the two nitrate sources (Figure 4.2). The slightly enriched nitrate isotope ratios (+8‰ > $\delta^{15}N$ > +6‰) for one stream and one groundwater sample may suggest denitrification. Both samples were taken during spring melt when soil saturation could produce favorable conditions for denitrification. Other groundwater samples taken at the same time from different piezometers did not show evidence of denitrification.

The net export of nitrate by these catchments is the result of nitrogen processes with rates that vary spatially and temporally. Although denitrification was not detected isotopically in stream and groundwater samples it does not necessarily mean that the process was not occurring in certain areas within catchments 31 and 47. The results, however, show that the nitrate in the stream was not only residual nitrate from incomplete denitrification. Detection of denitrification via isotopic enrichment requires that residual nitrate remains to be measured. If denitrification goes to completion and all the nitrate is denitrified, denitrification could not be detected by an isotopic shift. Therefore, it is unlikely that incomplete denitrification along the groundwater flow path or in the streambed is the cause of the large difference between the amount of nitrate that leaches past the rooting zone and the amount that is exported by the stream. Another possibility is that the enriched signature from the small amount of residual nitrate is masked by a much larger amount of nitrate that has not been enriched by denitrification. Further study is required to assess the importance of denitrification at the TLW.

4.4 Conclusions

The excellent isotopic separation between the two nitrate sources in comparison to the analytical precision confirmed the usefulness of this technique for determining nitrate source contributions to streams and groundwater at the TLW. Plot-scale studies previously conducted at the TLW have shown that nitrate produced by nitrification is the major source of the nitrate that leaches past the rooting zone on an annual basis. The results of this study show that microbial nitrate is also the dominant source of the nitrate exported by headwater streams. In addition, the nitrate isotope technique determines source contributions at discrete points in time, giving information on the temporal dynamics of nitrate source contributions in surface and ground waters at much shorter time scales than otherwise possible.

The largest contribution of atmospheric nitrate to the streams occurred during spring melt, however, microbial nitrate still remained the dominant source at this time and throughout the rest of the year. The headwater streams in catchments 31 and 47 had similar nitrate source contributions and displayed similar temporal trends. Denitrification does not appear to have significantly affected the isotopic composition of nitrate in the streams or groundwater of the two catchments (with the possible exception of two values). The isotopic effects of denitrification would not be noticed if complete denitrification of nitrate was occurring within certain areas of these catchments.

Compared to plot-scale studies, there are several advantages of using the nitrate isotope technique for determining nitrate source contributions. The determination of nitrogen mineralization, nitrification, and assimilation rates at the plot scale is very labor intensive. The quantification of nitrogen fluxes between the various soil horizons requires the installation of lysimeters to determine water chemistry and the use of water-balance

equations to estimate the amount of soil leachate. Rates of nitrogen cycling processes and water flux are difficult to determine under the snowpack, during mid-winter thaws, and during rain-on-snow events. In addition, the plots are usually very small compared to the total catchment area. Plots are also not usually located in or near streams and might miss important processes in riparian zones. Thus, scaling up of plot-scale results will not always accurately predict what is happening at the catchment scale. The nitrate isotope technique, in comparison, is less time consuming and requires less field instrumentation. Measurements in groundwater integrate upland processes. Stream measurements include any processes occurring in riparian zones and discharge areas. In this study, upland nitrogen processes controlled the isotopic signature of nitrate in headwater streams. Thus, the oxygen and nitrogen isotopic ratios of streamwater nitrate at the catchment outflow provide information about nitrogen cycling that was integrated over the whole catchment.

Chapter 5. Nitrate Attenuation In A Small Temperate Wetland Following Forest Harvest.

5.1 Introduction

Under natural conditions, nitrogen (N) is the most growth-limiting element in terrestrial ecosystems (Tamm, 1991). Annual inputs of new nitrogen (deposition, fixation, mineral weathering) are often insufficient to meet annual vegetative requirements and therefore forests have evolved efficient methods of recycling available nitrogen (Rosswall, 1976). Forest harvest and other disturbances generally result in the loss of nitrogen from catchments by decoupling the mechanisms responsible for the tight internal cycling (Vitousek, 1981). These losses can affect forest regeneration time and species composition as well as impacting downstream aquatic systems (Vitousek et al., 1979). Consequently, nitrogen losses are particularly important when considering the biogeochemical consequences of forest management practices.

Elevated export of dissolved inorganic nitrogen, particularly in its most mobile form of nitrate (NO_3^-), has been documented following forest harvest in both hardwood (e.g. Likens et al., 1970) and conifer forests (e.g. Tamm et al., 1974). Nitrate export following harvest results from increased ammonification (organic N to ammonium (NH_4^+)) and nitrification (NH_4^+ to NO_3^-) in conjunction with drastically reduced vegetative nitrogen uptake (Vitousek, 1981). Atmospheric nitrate could also contribute to increased nitrate export following forest harvest. Decreased interception of precipitation by vegetation, combined with lower infiltration rates in disturbed soils, can result in a greater potential for overland flow in harvested catchments (Elliot et al., 1998).

High nitrate export has detrimental effects on terrestrial and aquatic systems. In order to maintain charge balance, nitrate losses must be accompanied by increased cation leaching from soils. Therefore nitrate export can decrease forest health by concomitant loss of nutrient cations such as calcium and potassium as well as lead to acidification through the export of hydrogen ions (Aber et al., 1989). Even at relatively low concentrations, nitrate can be harmful to the aquatic life stages of amphibians (Baker and Waights, 1993, 1994; Hecnar, 1995; Marco et al., 1999). Nitrate concentrations from forest harvest can exceed drinking water standards and threaten potable water supplies (Likens et al., 1970).

In 1997, a forest harvest experiment was initiated at the Turkey Lakes Watershed (TLW), located near Sault Ste. Marie, Ontario, Canada (Figure 2.1), to study the effects of current forest management practices on a tolerant hardwood ecosystem. Although not traditionally employed in Canadian hardwood forests, a clear-cut treatment was used to provoke a maximum disturbance effect to contrast with conventional management approaches (selection and shelterwood cuts). As part of the TLW harvest experiment stable isotope techniques were used to trace nitrate sources and cycling in undisturbed and harvested catchments.

For the long-term study, stream samples for nitrate isotope analysis were collected regularly at weirs at the catchment outlets. In May 1999, sequential stream sampling in the clear-cut catchment revealed a decrease in stream nitrate concentrations through a small wetland located in the upper part of the catchment. Studies examining wetland responses to harvest have focused on the effects of harvesting of the wetland itself (e.g. Shepard, 1994). A large body of literature also deals with the effects of draining peatlands to enhance forest growth (e.g. Paavilainen and Päivänen, 1995). No previous studies have investigated

wetland attenuation of elevated nitrate concentrations caused by harvesting of the adjacent uplands.

Nitrate removal in the wetlands of clear-cut and undisturbed catchments could affect the interpretation of nitrate isotope ratios in stream water collected at the catchment outlets. Nitrate attenuation processes such as denitrification, which discriminate against isotopically heavy nitrate, leave the residual nitrate enriched in ^{15}N and ^{18}O (Kendall, 1998). If nitrate exiting the wetland results from incomplete denitrification, the isotopic enrichment could be detectable downstream at the weir and would complicate the calculation of nitrate source contributions that are based on $^{18}O/^{16}O$ of nitrate (e.g. Spoelstra et al., 2001). Alternatively, if denitrification of nitrate goes to completion in localized areas of the wetland and this water subsequently dilutes incoming surface water, no isotopic enrichment would be detected downstream of the wetland. Plant uptake of nitrate or dilution from low nitrate groundwater discharging into the wetland, which has been unaffected by denitrification, would also not affect nitrate isotope ratios at the weir.

The goals of this study were to: 1) document the ability of a small wetland to buffer downstream areas from the full effects of forest harvest with respect to nitrate export, 2) identify the main mechanisms responsible for declining nitrate concentrations through the wetland, and 3) determine if nitrate attenuation in the wetland affects nitrate isotope ratios measured at the catchment outlet.

5.2 Methods

5.2.1 Study Site

The focus of this study is a wetland located in TLW catchment 31 (c31) (Figure 5.1).

Catchment 31 is a 4.6 hectare first order stream basin located near the outlet of the TLW (Figure 2.1) at an elevation ranging from 359 to 418m (Beall et al., 2001). In the summer of 1997, c31 was clear-cut as part of the TLW forest harvest experiment. Trees with a diameter at breast height (dbh) greater than 20cm were felled, delimbed, and the stems removed by skidder. In addition, all trees with a dbh of 10 to 20cm were cut and left to decompose where they fell. All branches removed during delimbing were also left in the catchment at their stumps.

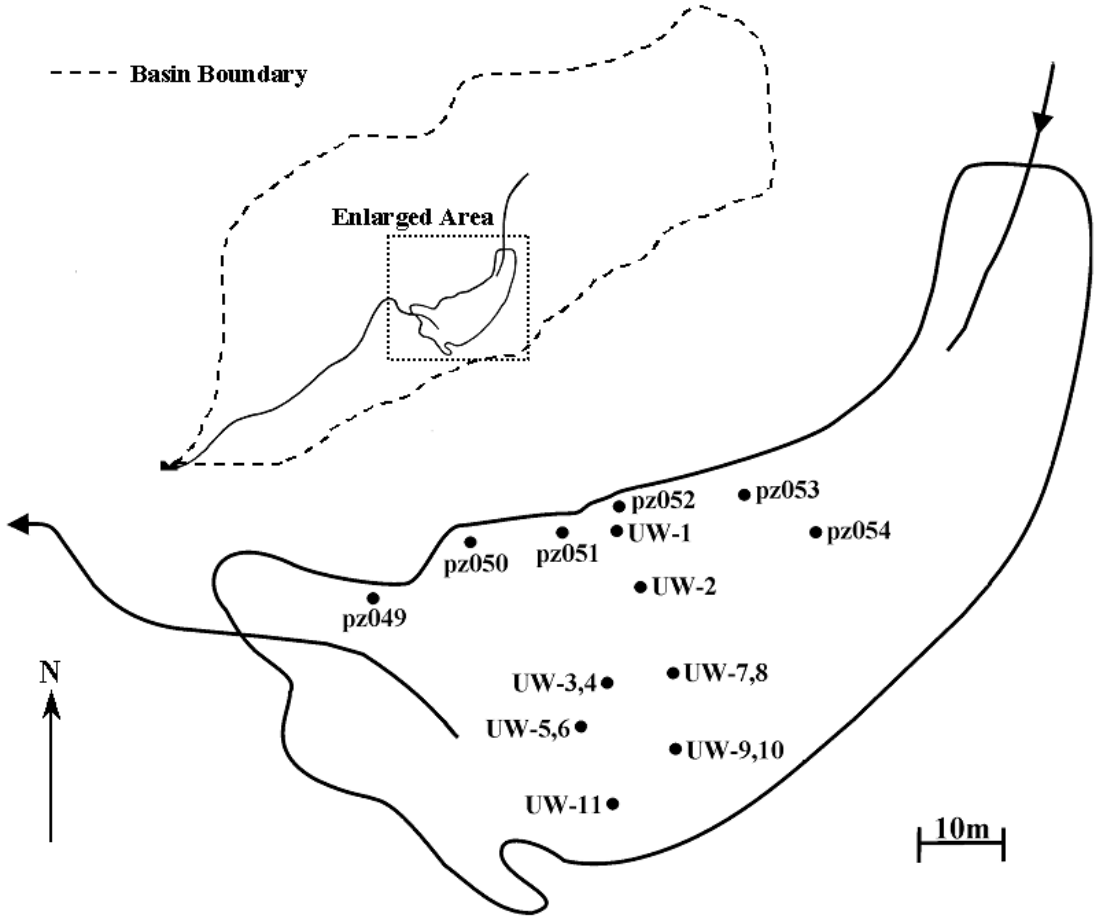


Figure 5.1 Location and instrumentation of the catchment 31 wetland.

The c31 wetland is a forested swamp (0.2 hectares) located in a topographic depression with slopes defining its northern and eastern boundaries. Since the harvest, a dense community of herbaceous vegetation has developed on the organic deposits that are 40 to 65cm in depth. Although a defined stream channel is present at the inlet and outlet of the swamp, none exists in the wetland interior. Water moves slowly along diffuse pathways, in intimate contact with rich organic horizons and herbaceous wetland vegetation. Prior to this study, six 2" diameter piezometers (pz 049 - pz 054) with a screened interval of 30cm existed near the northern margin of the wetland. Additional ¾" PVC drive-point piezometers with a screened interval of 15cm were installed in May 2000 (UW# 1-6) and in September 2000 (UW# 7-11) (Figure 5.1). The center of the screened intake for c31 wetland piezometers ranged from 8 to 50cm below ground surface.

Catchment 50 (c50), also a first-order stream basin, was not part of the TLW harvest experiment and is located in the headwaters (Figure 2.1) at an elevation ranging from 507 to 590m (Beall et al., 2001). Catchment 50 (11.6 ha) contains three wetlands in series and has very low stream water nitrate concentrations during the growing season (Creed and Band, 1998). The downstream wetland in c50 was used as a reference for this study to determine the $\delta^{15}N$ of wetland vegetation with low ambient available nitrate.

5.2.2 Sampling

Surface and groundwater samples were collected during late spring (May/June) and fall (September/October) periods from 2000 to 2002. Depending on nitrate concentrations, one to four liters of water were collected for isotopic analysis of nitrate. Prior to freezing, samples were filtered to 0.45μm and sub-samples sent to the Great Lakes Forestry Centre for chemical analysis using standard methods (Nicolson, 1988). Sub-samples taken to monitor

sample processing for nitrate isotope ratios (e.g. efficiency of DOM removal) were analyzed by the Environmental Geochemistry Lab at the University of Waterloo (Spoelstra et al., 2001).

5.2.3 DOM removal

Water samples with dissolved organic carbon (DOC) concentrations greater than 2.5 mg C/L were ultrafiltered as described in Section 2.2.1.1. DOC removal by ultrafiltration ranged from 30 to 93% for wetland samples with a mean value of 64%. DOM removal was also accomplished by the BioRad AG 1-X8 anion resin used to isolate nitrate from water samples. Even for samples that had not been ultrafiltered, nitrate collection on the resin and its subsequent conversion to silver nitrate eliminated about 85% of the DOM from the final silver nitrate solution (Section 2.2.1.3). Taking both mechanisms into account, overall DOM removal for ultrafiltered samples ranged from 90 to 99%.

5.2.4 Nitrate isotope analysis

The methods used for the isolation of nitrate from water samples and its subsequent conversion to silver nitrate were adapted from Chang et al. (1999) and Silva et al. (2000) and are described in Section 2.2.2. Breakseal combustion and continuous flow methods used for the isotopic analysis of silver nitrate were modified from Silva et al. (2000) and Mengis et al. (2001). Details regarding the modifications to the breakseal and continuous flow methods are described in Sections 2.2.3 and 2.2.4.

5.2.5 Vegetation isotopes and sampling

Vegetation from c31 and c50 wetlands was sampled in September 2000 and May 2001. Five leaves from deciduous trees (sugar maple (*Acer saccharum*), yellow birch (*Betula alleghaniensis*)) or needles from five different branches of conifer species (eastern

white cedar (*Thuja occidentalis*), white spruce (*Picea glauca*)) were collected and pooled for each tree sampled. Therefore, each $\delta^{15}N$ analysis corresponded to an individual tree. The above ground portion of wetland herbaceous vegetation (jewel weed (*Impatiens capensis*), wood horsetail (*Equisetum sylvaticum*), sensitive fern (*Onoclea sensibilis*), marsh marigold (*Caltha palustris*), cattails (*Typha latifolia*), trout lily (*Erythronium americanum*), peat moss (*Sphagnum* spp.), sedge (*Carex* spp.), marsh blue violet (*Viola cucullata*)) was sampled from three individual plants of a particular species collected within $0.25m^2$ and pooled as one sample.

Prior to freeze-drying, vegetation was rinsed with deionized water to remove dust. Dried plants were ground in a Wiley mill and then pulverized using a ball mill. Two to five milligrams of powdered plant material was weighed into tin capsules and run on a Carlo Erba elemental analyzer coupled to a Micromass Isochrom continuous flow stable isotope mass spectrometer to determine $\delta^{15}N$ and %N. International Atomic Energy Association (IAEA) nitrogen standards, IAEA-N1 ($\delta^{15}N = 0.36‰$) and IAEA-N2 ($\delta^{15}N = 20.3‰$), were run with each batch of samples. Samples and standards run in duplicate throughout each run yielded a reproducibility of ±0.3‰.

5.2.6 Hydrologic measurements

Quantification of surface and groundwater fluxes in the wetland was not possible for this study. Weirs could not be constructed above or below the wetland because of the potential effects on stream chemistry and sediment loading, which were being monitored in response to forest harvest. The addition of a chemical tracer above the wetland to calculate flows and hydrological residence time was also rejected because of possible interference with the interpretation of stream chemistry changes due to harvest. Estimates of wetland stream

flows were obtained by measuring stream velocity and cross-sectional area. Flows above and below the wetland were approximately equal for each sampling period and ranged from about 0.25 to 1.0 L/s.

Instrumentation was insufficient to permit the calculation of groundwater flux. Individual piezometers exhibited a range in recovery rates following pumping, suggesting a large spatial variability in hydraulic conductivity. Since stream flows above and below the wetland were similar, the groundwater flux must be relatively small and therefore difficult to quantify by mass balance. In addition, some of the groundwater in the wetland might discharge into the stream down-slope of the wetland outlet. Water loss by evapotranspiration was unquantified but was also expected to be small since tree cover was sparse.

5.3 Results

5.3.1 Nitrate concentrations

Nitrate concentrations at the wetland outflow were significantly lower than stream water entering the wetland (Figure 5.2). Non-detectable nitrate concentrations at the wetland outflow indicated complete attenuation of incoming nitrate in September 2001 and subsequent sampling periods. Although stream 31 nitrate concentrations progressively increased downstream of the wetland, concentrations at the weir did not reach levels measured above the wetland (Figure 5.2). Nitrate concentrations entering the wetland also decreased over the duration of the study, presumably due to increased nitrogen demands of regrowing vegetation in the catchment uplands.

The maximum groundwater nitrate concentration also decreased over time following

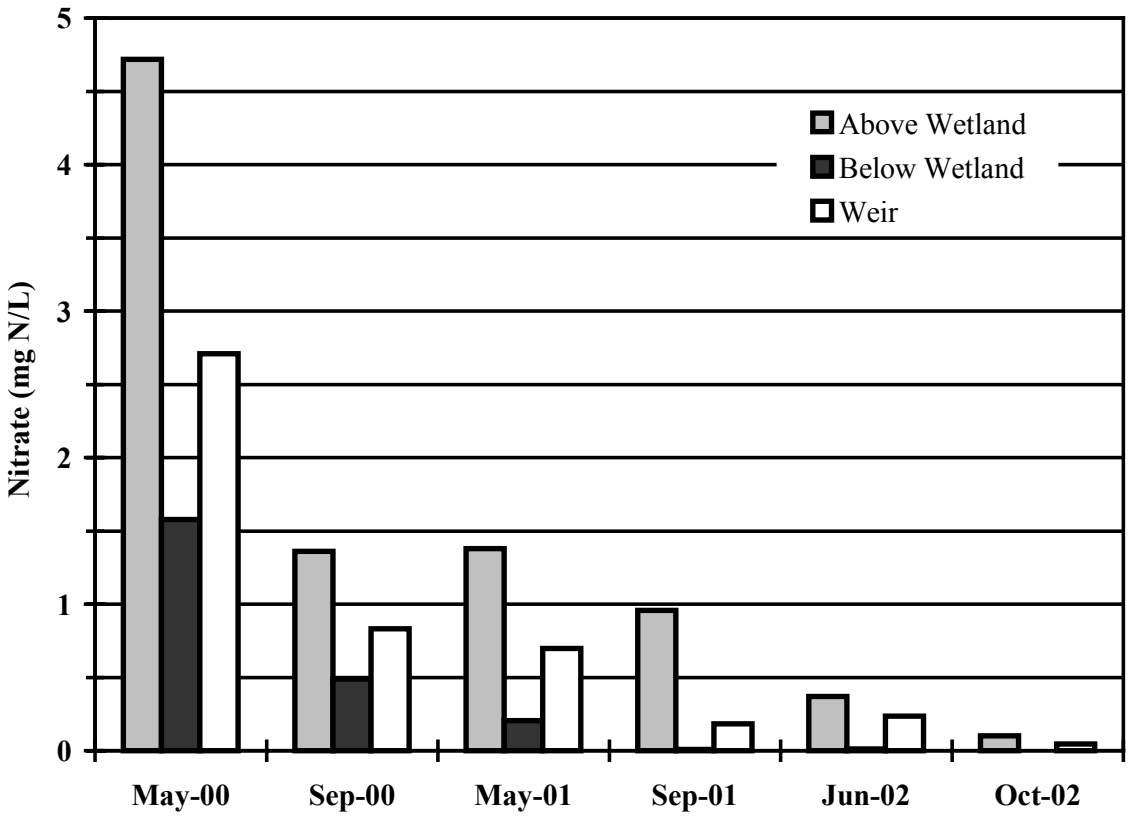


Figure 5.2 Nitrate concentrations measured in stream water above and below the wetland and at the c31 outlet.

harvest. However, piezometers with low nitrate were detected for each sampling period (Figure 5.3). If discharged into the wetland, low nitrate groundwater could dilute high nitrate stream water inputs. However, hydraulic heads indicate that groundwater flow in the wetland was predominantly horizontal or weakly downward. Limited measurements near the northern margin of the wetland suggested groundwater discharged into the wetland at the toe of this slope. Regardless of the direction of the hydraulic gradients, water flux through the wetland was likely dominated by the lateral movement of incoming stream water. The magnitude of groundwater recharge or discharge was constrained by the low hydraulic conductivity of wetland sediments. Typically in wetlands, hydraulic conductivities are

highest in the near-surface layers (0-20cm) and decrease quickly by up to four orders of magnitude with depth (e.g. Mewhinney, 1996). Localized zones of higher groundwater discharge or recharge are possible in areas where the thin wetland soils have been disturbed. These areas included several holes created by the root systems of windfall trees. The movement of standing trees by the wind was also observed to move saturated wetland soils in the c31 wetland. Therefore, a detailed understanding of the groundwater movement in this small wetland was complicated by the extreme heterogeneity, low ambient hydraulic conductivity, and unquantified loss by evapotranspiration.

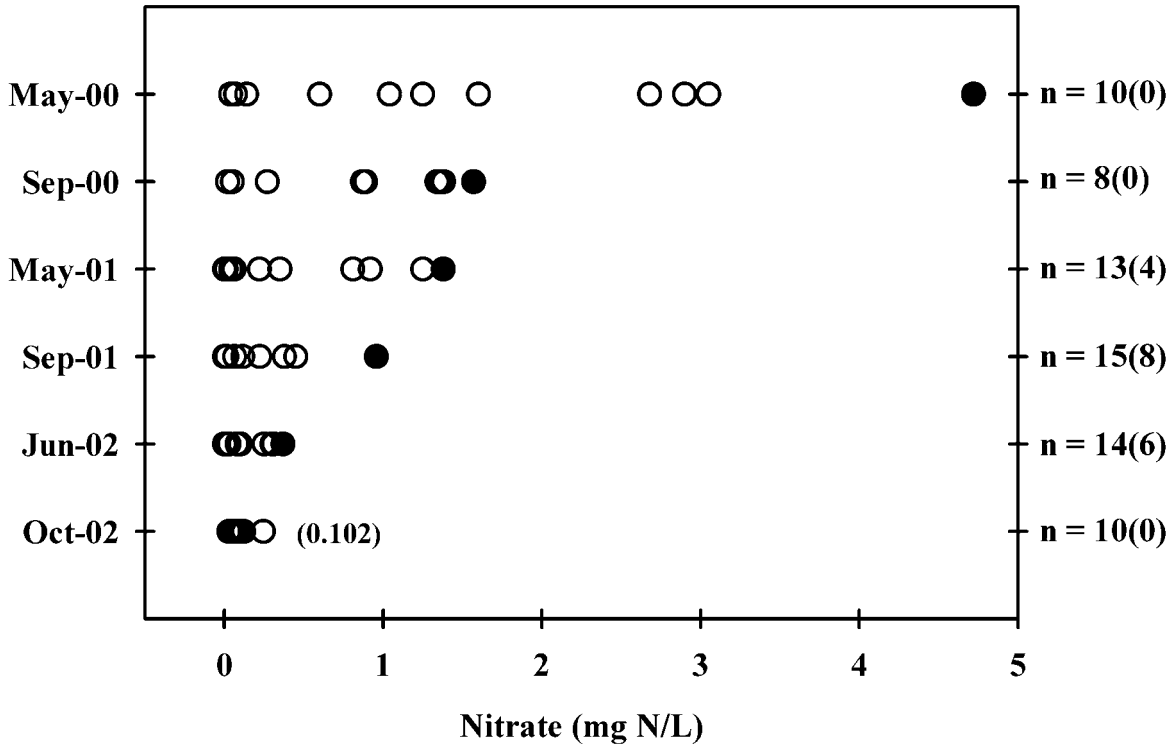


Figure 5.3 Nitrate concentrations in c31 wetland groundwater (○) and stream water entering the wetland (●, indicated in brackets for October 2002) for each sampling period. The total number of piezometers sampled (n) and the number with nitrate concentrations less than or equal to 0.01 mg N/L (in brackets) are indicated on the right-hand axis.

5.3.2 Nitrate isotope ratios

Isotopic analysis of nitrate in wetland surface water (Figure 5.4) and groundwater (Figure 5.5) revealed a trend of increasing $\delta^{15}N$ values with decreasing nitrate concentration for each sampling period. This observation is consistent with a nitrate attenuation mechanism that preferentially utilized isotopically light nitrate, enriching the residual nitrate in ^{15}N. Nitrate attenuation in groundwater was accompanied by greater discrimination against ^{15}N (i.e. more negative enrichment factors) compared to those observed in surface waters.

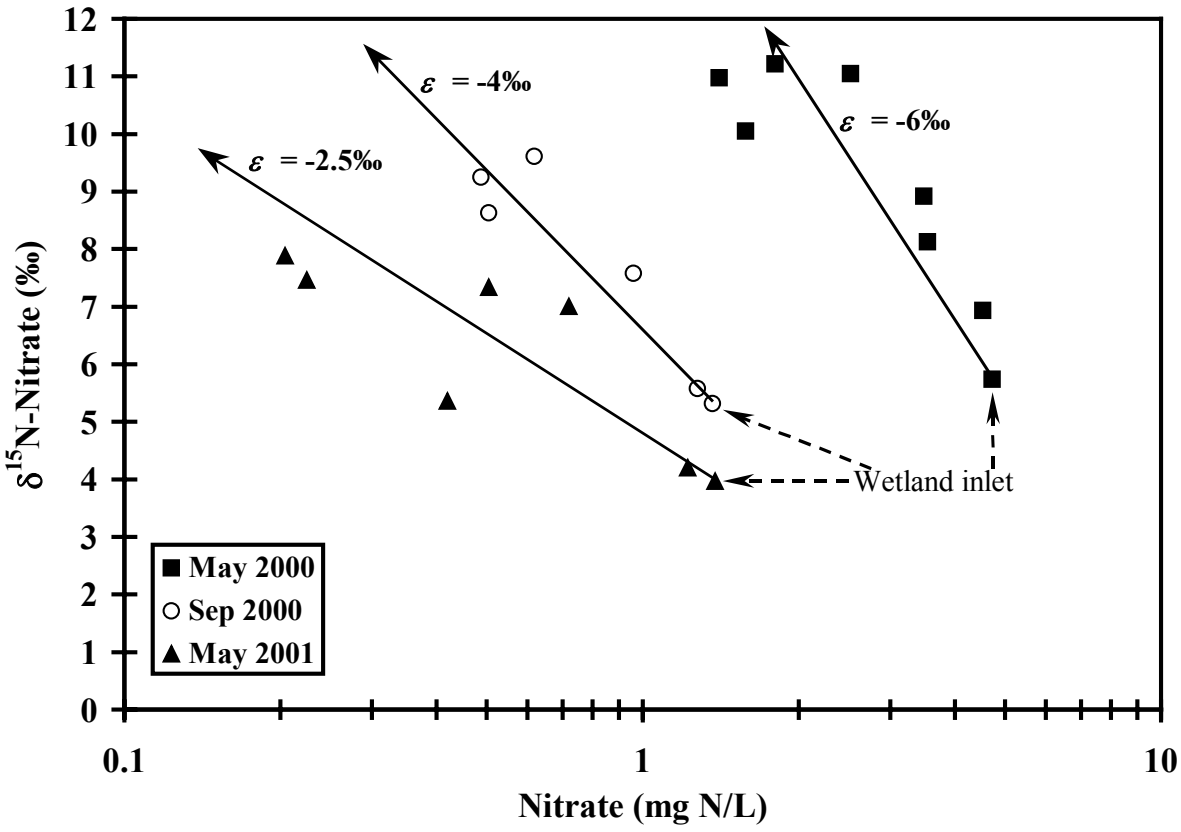


Figure 5.4 Relationship between nitrogen isotope values and nitrate concentration in wetland surface water. Lines representing the trajectories for several denitrification enrichment factors (ε) are shown.

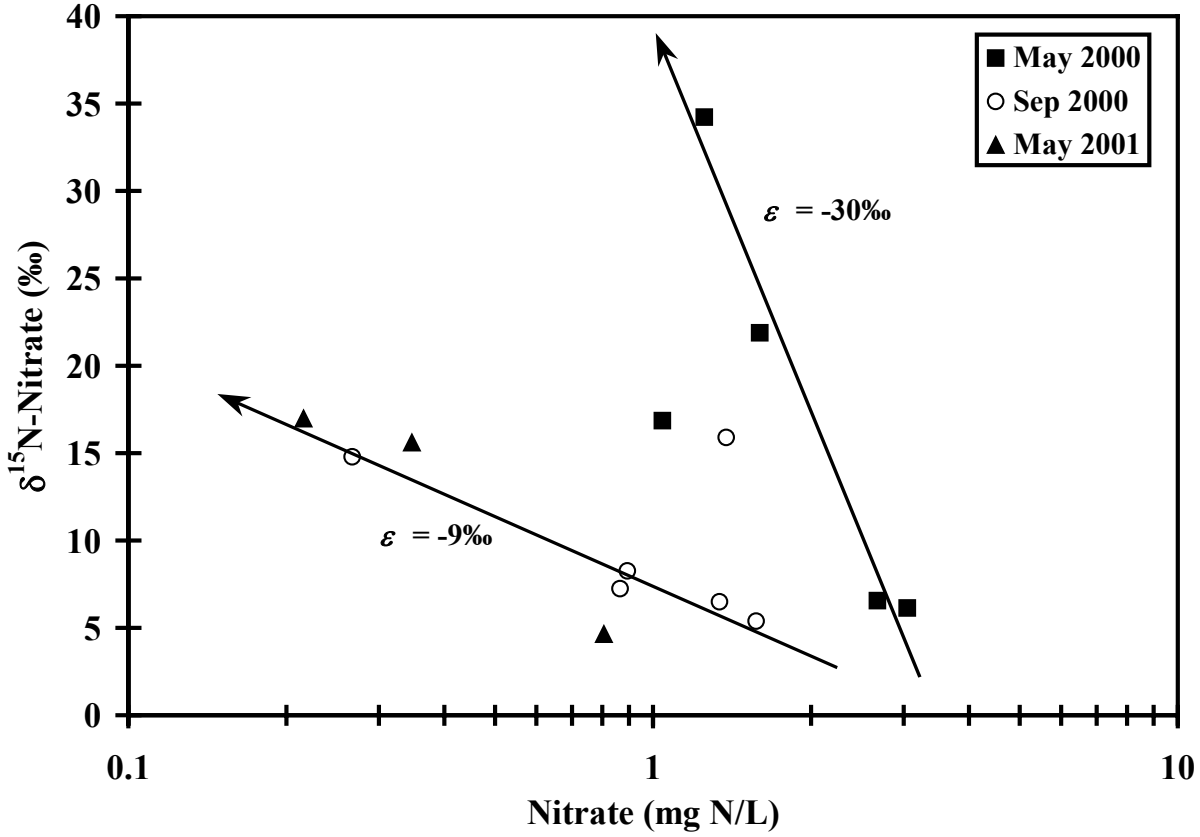
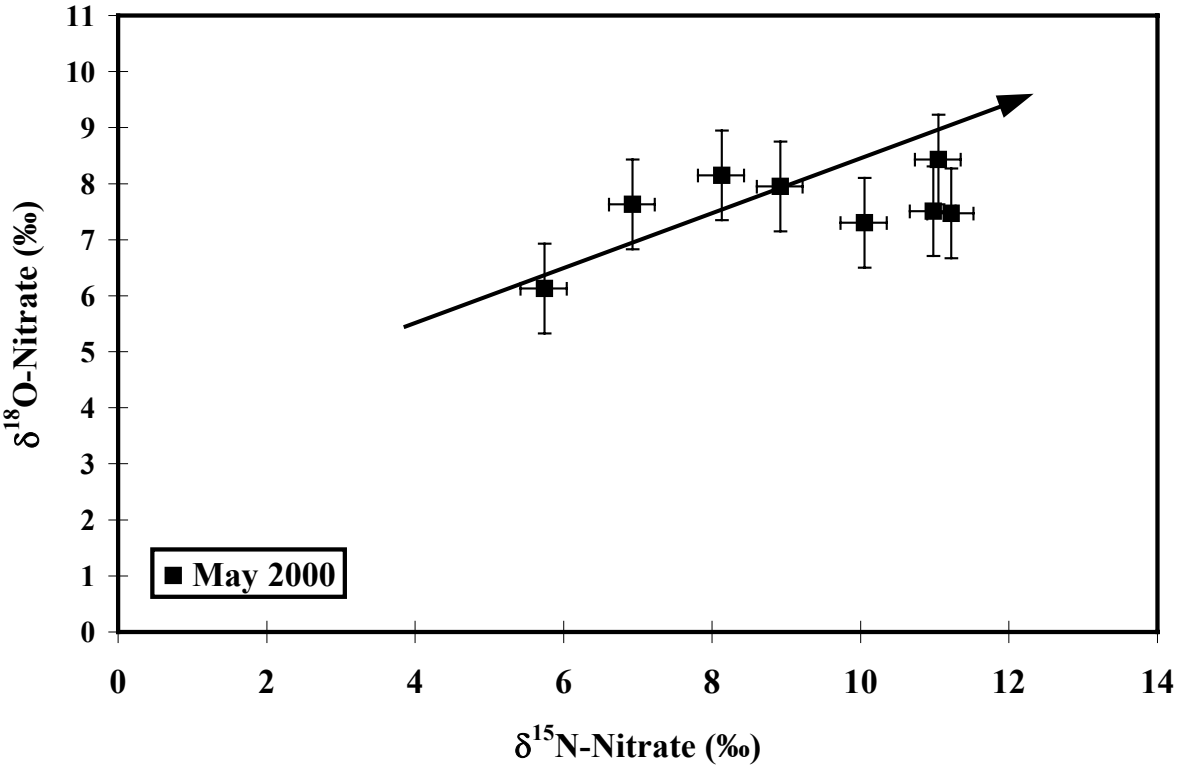
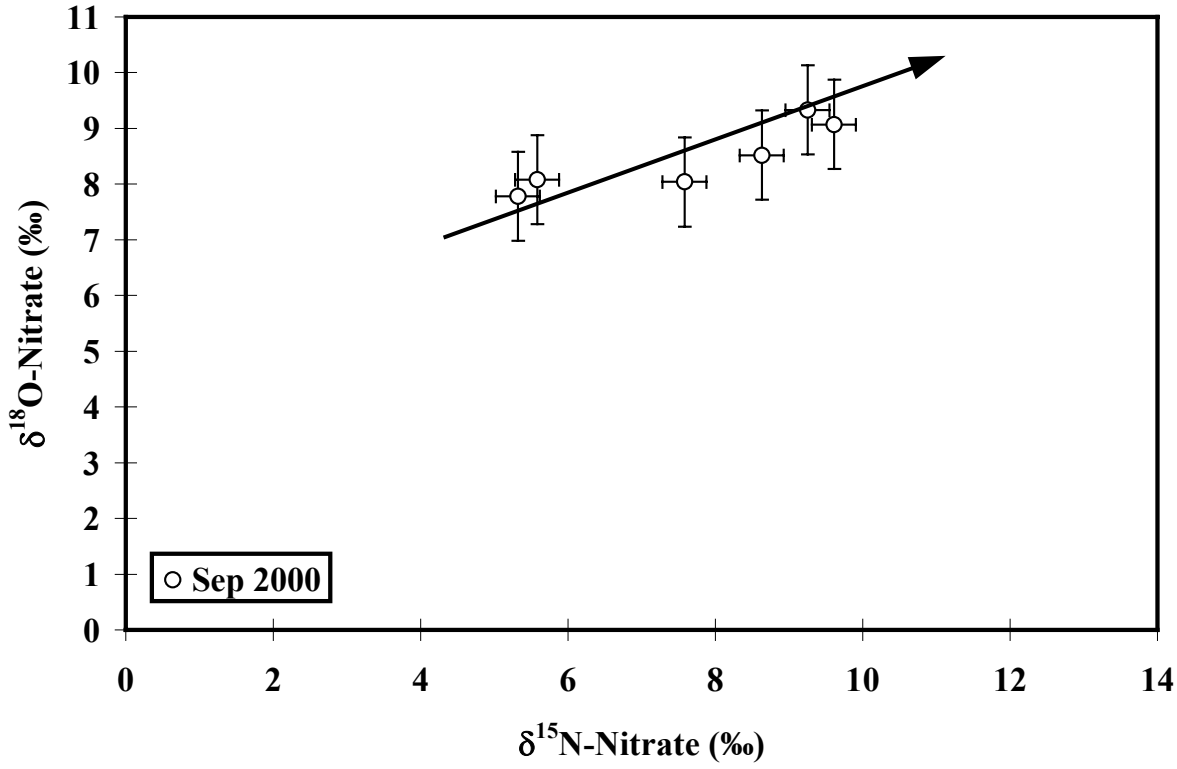


Figure 5.5 Relationship between nitrogen isotope values and nitrate concentration in wetland groundwater. Lines representing the trajectories for denitrification enrichment factors (ε) of -9 and -30‰ are shown.

Laboratory and field studies have shown that denitrification results in an approximately 2:1 increase in the $\delta^{15}N$:$\delta^{18}O$ values of the residual nitrate pool (Amberger and Schmidt, 1987; Böttcher et al., 1990; Voerkelius and Schmidt, 1990; Aravena and Robertson, 1998; Cey et al., 1999; Mengis et al., 1999; Devito et al., 2000). Even if other mechanisms of nitrate removal are partially responsible for the decreasing nitrate concentrations, the enrichment relationship between the two nitrate isotope ratios during denitrification will remain provided the other mechanisms do not fractionate. Although

wetland surface water nitrate generally fits the 2:1 enrichment trend (Figure 5.6), the results are inconclusive on their own because of the relatively small range in $\delta^{15}N$ and $\delta^{18}O$ values.





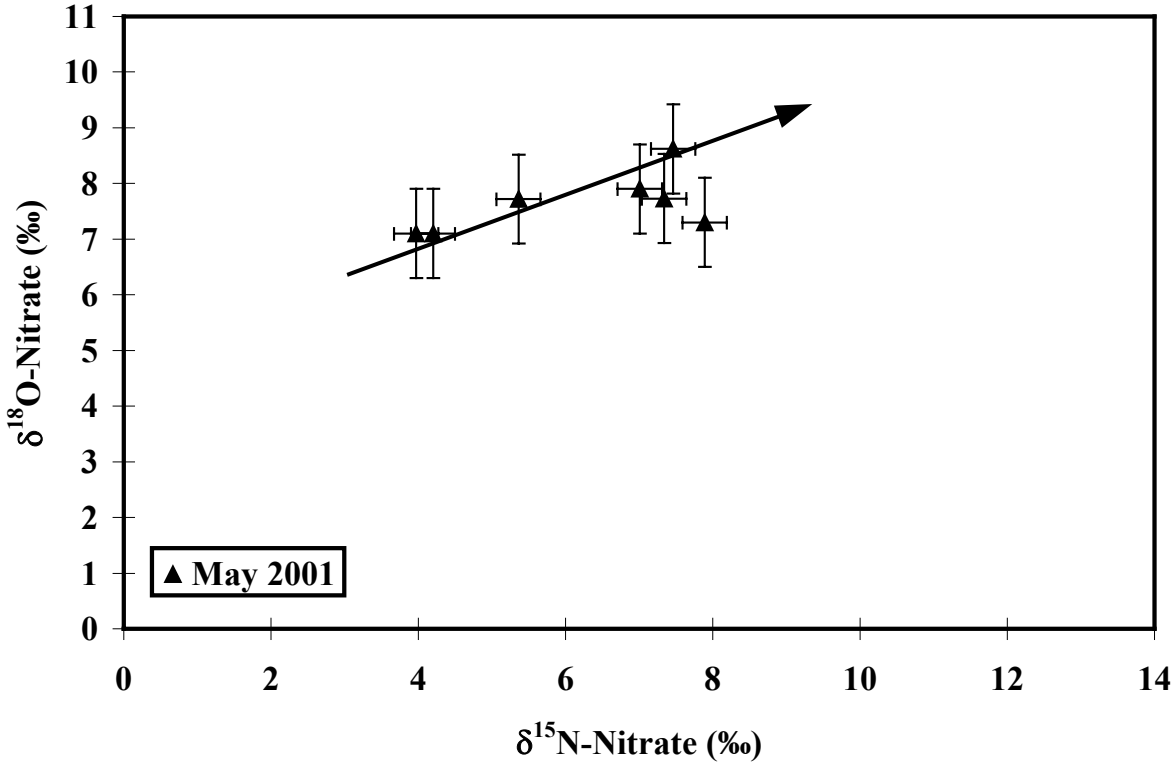


Figure 5.6 Dual nitrate isotope plot of wetland surface water samples. The line on each diagram indicates the 2:1 enrichment slope for $\delta^{15}N$:$\delta^{18}O$ expected for samples affected by denitrification. Error bars show the analytical precisions of ±0.8‰ and ±0.3‰ for $\delta^{18}O$ and $\delta^{15}N$ of nitrate, respectively.

Wetland groundwater shows the expected relationship between $\delta^{18}O$ and $\delta^{15}N$ for nitrate affected by denitrification (Figure 5.7) and confirms that denitrification was occurring in the wetland. The nitrate isotope signature of the source water entering the denitrification zone determines the starting point of the denitrification line. As a result of harvest, the oxygen isotope ratios of nitrate entering the c31 wetland through groundwater and the stream changed over time (Section 7.3.4) and therefore the groundwater nitrate data, which were collected over the entire study period, should not necessarily plot on a single line with a 2:1 slope. The arrows on Figure 5.7 indicate that the one outlier could have resulted from the denitrification of high concentrations of ^{18}O-depleted nitrate produced by the nitrification of ammonium (microbial nitrate) shortly after harvest.

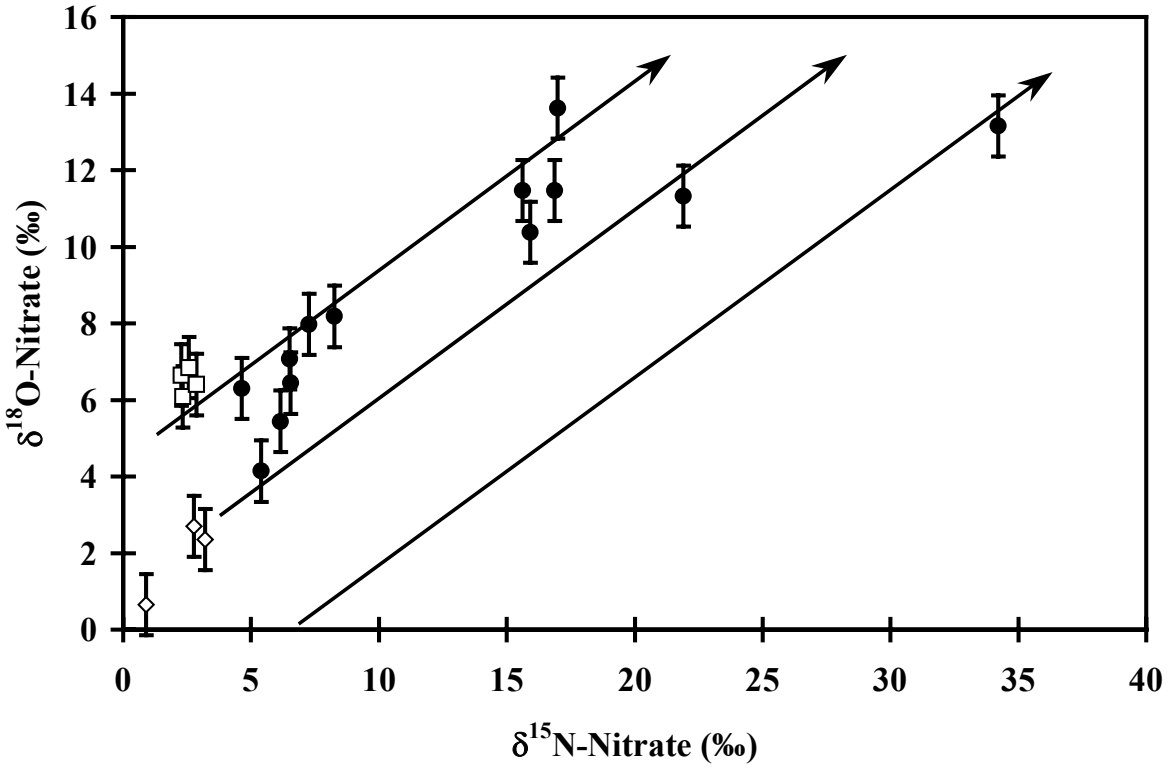


Figure 5.7 Dual nitrate isotope plot of groundwater samples collected from the wetland (●) and from c31 upland piezometers (38 to 75cm depth) in November 1997 (◊) and April 1999 (□). Lines on the diagram indicate the 2:1 slope for isotopic enrichment by denitrification. Error bars indicate the analytical precision of ±0.8‰ for $\delta^{18}O\text{-}NO_3^-$ analysis and the width of the points is equal to the ±0.3‰ precision for $\delta^{15}N\text{-}NO_3^-$.

5.3.3 Vegetation $\delta^{15}N$ values

Wetland vegetation in the c50 wetland was similar in species composition to c31 however the species abundances differ significantly between the two catchments. The higher density of trees in the c50 wetland and the presence of trees in the surrounding uplands lead to significantly less light reaching the surface of the wetland compared to c31. Less penetration of light through the forest canopy, together with lower nutrient concentrations in c50 wetland surface water, were likely responsible for the lower density of herbaceous

vegetation. Sphagnum species dominated the wetland ground cover in c50 but were of minor importance in the c31 wetland.

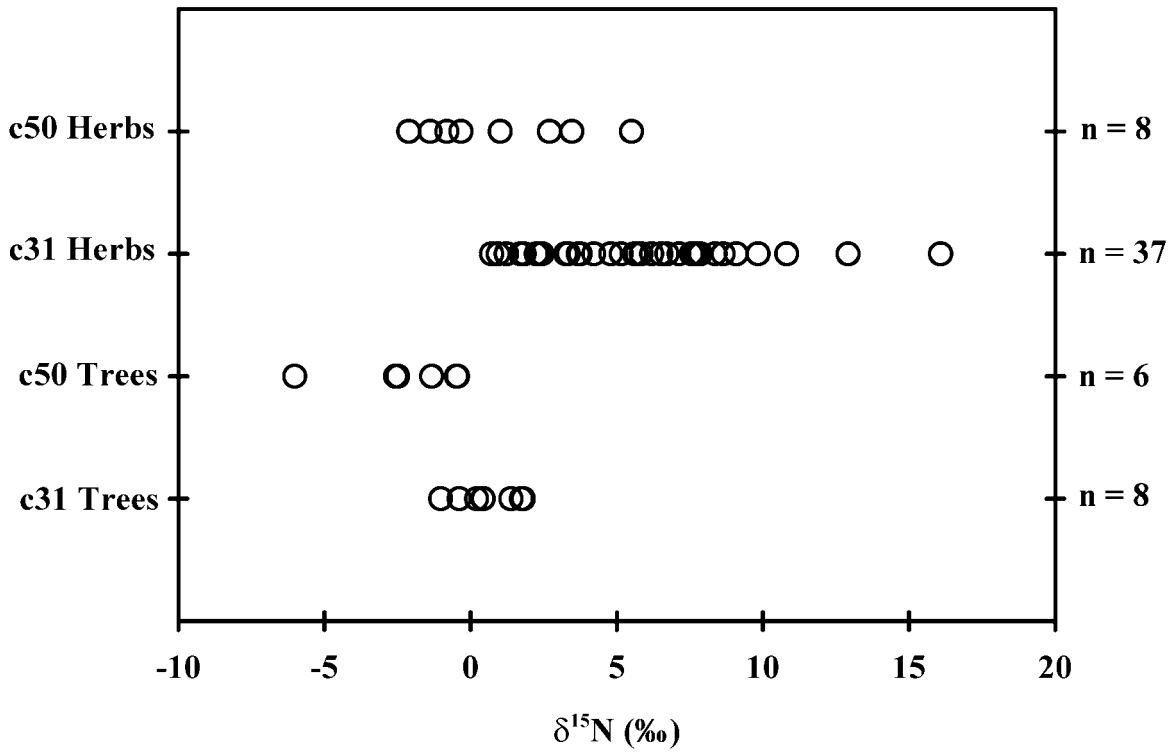


Figure 5.8 $\delta^{15}N$ of herbaceous plant species and tree leaves collected from wetlands in harvested (c31) and reference (c50) catchments in May and September of 2000. The number of samples in each category (n) is indicated on the right-hand axis.

Wetland vegetation from the clear-cut catchment was enriched in ^{15}N compared to that collected from the c50 wetland (Figure 5.8), which does not have high surface water nitrate available for uptake. Nitrate was probably the most available nitrogen source to plants in the c31 wetland since ammonium concentrations were less than 0.01 mg N/L in groundwater and surface water throughout the study. The enrichment in ^{15}N was greater in the herbaceous vegetation compared to wetland trees.

5.4 Discussion

5.4.1 Nitrate attenuation in the wetland

Nitrate attenuation in the wetland had a dramatic effect on stream 31 nitrate concentrations following the harvest. In May 2000-2001, the wetland was able to remove 65 to 85% of incoming stream water nitrate, which ranged in concentration from 1.4 to 4.7 mg N/L (Figure 5.2). Nitrate concentrations less than 1 mg N/L (September 2001 to Oct 2002) were completely attenuated, resulting in no nitrate export from the wetland during these periods.

Denitrification, which converts nitrate to nitrous oxide (N_2O) and dinitrogen gas (N_2) under anaerobic conditions, is often the dominant mechanism for nitrate attenuation in wetlands experiencing high nitrate inputs (e.g. Reilly et al., 2000). Wetland vegetation can also be an important nitrate sink, either directly by assimilative nitrate uptake or indirectly by contributing to favorable conditions for denitrification (Bachand and Horne, 2000). Other processes that could contribute to nitrate removal include dissimilatory nitrate reduction to ammonium and algal nitrate uptake. Enrichment in ^{15}N with decreasing nitrate concentration (Figure 5.4 and 5.5) is consistent with denitrification as a mechanism for nitrate removal in the c31 wetland.

No evidence was found to suggest that dissimilatory nitrate reduction to ammonium was a significant process in this wetland. This reaction would lead to increased ammonium concentrations, which were always less than 0.01 mg N/L in wetland surface and ground waters. Isotopically light ammonium would be produced by preferential reduction of $^{14}NO_3^-$. Therefore, if plants were fortuitously taking up ammonium as it was produced, it would lead to a depletion of plant tissue $\delta^{15}N$ rather than the observed enrichment.

Algae fractionate against $^{15}NO_3^-$ when exposed to high nitrate concentrations (Fogel and Cifuentes, 1993), leading to an enrichment of the residual nitrate pool. However, there were few standing pools of water in the wetland and algal biomass was observed to be insignificant relative to herbaceous vegetation. Therefore, algae did not likely contribute significantly to nitrate removal.

5.4.2 Plant nitrate assimilation

In most natural nitrogen-limited forested ecosystems, plants are not expected to significantly fractionate nitrate during uptake (Högberg, 1997) and therefore the $\delta^{15}N$ of plant tissue should be similar to available nitrate if nitrate is the dominant nitrogen source of the plant. Fractionation might occur through plant-mycorrhizal fungi interactions or when inorganic nitrogen concentrations are high relative to plant nitrogen demand (Högberg, 1997). However, both effects result in the incorporation of isotopically light nitrate into plant tissue and therefore do not explain the high plant $\delta^{15}N$ values.

Trees were less enriched in ^{15}N than herbaceous vegetation on the wetland surface. Wetland trees in c31 were generally growing on raised hummocks and had shallow root systems. As a result, wetland trees derive some of their nitrogen from localized areas where denitrification activity does not influence the $\delta^{15}N$ values of available nitrogen in the soil. Also, TLW trees acquire about 56% of their annual nitrogen requirements through internal translocation of previously assimilated nitrogen (Mitchell et al., 1992). Nitrogen assimilated before the harvest would dilute the $\delta^{15}N$ shift expected from the uptake of ^{15}N-enriched nitrate following the harvest. In contrast, the herbaceous vegetation grew on the wetland surface and was largely rooted in the saturated zone. Therefore herbs had access to nitrate in both surface water and shallow groundwater. The extreme ^{15}N enrichment of some plants,

well beyond the $\delta^{15}N$ values of surface water nitrate exiting the wetland, indicates that these plants were assimilating residual nitrate with highly enriched ^{15}N in a zone of higher denitrification rates.

The c31 wetland vegetation community was well established by the third post-harvest growing season, which coincides with the start of this research. Vegetation species composition and distribution was not observed to change significantly from May 2000 to May 2001. Therefore, it is suspected that the magnitude of annual nitrate removal by plant uptake was also similar during these sampling periods. As a result, the difference in wetland nitrate attenuation between these two dates was likely caused more by changes in denitrification activity than plant uptake.

Although $\delta^{15}N$ data indicated that plant assimilation of nitrate occurred, it was not the dominant nitrate attenuation mechanism. Nitrate removal in late September was still significant (Figure 5.2) even though plant growth and nutrient uptake were minimal at that time since much of the wetland vegetation was senescing. A comparison of nitrate concentration data from September 2000 and May 2001 could be used to approximate the amount of nitrate removed by plant uptake. Although nitrate concentrations coming into the wetland were almost identical, nitrate attenuation was greater in May 2001 when the plants were active (Figure 5.2). Shallow soil temperatures, which affect rates of microbially mediated attenuation processes, are similar in May and September at the TLW (Semkin, unpublished data). Surface water flow through the wetland, however, appeared greater in September and therefore part of the difference in nitrate attenuation between the two dates might be due to a shorter hydrologic residence time in September. The difference in nitrate concentrations in wetland outflow from these two periods (~0.3 mg N/L) might represent an

estimate of the maximum contribution of plant nitrate uptake. Thus, a combination of vegetative uptake and a slower transit time increased nitrate retention in the wetland by 20%.

5.4.3 Denitrification enrichment factor

Large nitrogen isotope enrichment factors (ε) have been measured for denitrification (ε_d = -10 to -30‰) under laboratory (Wellman et al., 1968; Delwiche and Steyn, 1970; Blackmer and Bremner, 1977; Mariotti et al., 1981; Sebilo et al., 2003) and field conditions (Vogel et al., 1981; Heaton et al., 1983; Böttcher et al., 1990; Smith et al., 1991; Spalding and Parrott, 1994; Aravena and Robertson, 1998; Bates and Spalding, 1998; Bates et al., 1998; Mengis et al., 1999; Sidle et al., 2000). Low (less negative) $\delta^{15}N$ enrichment factors (ε_d = -3.6 to -6.0‰) have been associated with systems where denitrification rates are limited by diffusive transport of nitrate to the denitrification site (Mariotti et al., 1988; Fustec et al., 1990; Koba et al., 1997; Sebilo et al., 2003).

The observed $\delta^{15}N$ enrichment factor (ε_{obs}) depends on several factors including: 1) the denitrification enrichment factor (ε_d), 2) the proportion of denitrification that goes to completion (i.e. dilution by nitrate-free water), and 3) the proportional contribution of plant nitrate uptake. ε_{obs} declined over the course of this study for both wetland surface water (Figure 5.4) and groundwater (Figure 5.5). The measured enrichment in surface water samples was less than expected if partial denitrification was solely responsible for decreasing nitrate concentrations in the wetland.

ε_{obs} for the c31 wetland (Figure 5.4) was also affected by dilution with nitrate-free waters. When denitrification goes to completion, isotopic enrichment is not detectable because there is no residual nitrate to measure. Addition of nitrate-free water to water undergoing partial denitrification lowers the magnitude of ε_{obs}. If nitrate availability is high,

denitrification activity might not be sufficient to reduce all the nitrate. Therefore the enrichment associated with denitrification is more likely to be detected at higher initial nitrate concentrations. As nitrate concentrations declined over time, complete denitrification became responsible for an increasingly larger proportion of nitrate attenuation in the wetland and thus ε_{obs} decreased.

Plant uptake of nitrate also contributed to lowering the ^{15}N enrichment associated with nitrate attenuation. Because plants do not significantly fractionate nitrate on uptake, vegetative assimilation removes nitrate without changing the isotopic signature of the remaining pool. The maximum potential nitrate removal by plant uptake is fixed by the plant's nitrogen demand and therefore denitrification was expected to be the dominant attenuation mechanism at high nitrate concentrations. The magnitude of nitrate attenuation by plant uptake (20%) was smaller than that by denitrification (65%) in May 2001 when the stream nitrate concentration coming into the wetland was 1.4 mg N/L. Plant uptake of nitrate has been implicated in other wetland studies for causing low ε_{obs} (-2.5‰; Lund et al., 2000) and likely contributed to less observed isotopic discrimination against $^{15}NO_3^-$ in surface water compared to the wetland groundwater (Figure 5.4 and 5.5).

Denitrification of surface water nitrate apparently occurred as water moved horizontally through the near surface environment of the wetland. Nitrogen isotope ratios in the herbaceous vegetation that are significantly more enriched than surface water nitrate indicate that some plants were rooted in or near the zones of higher denitrification. Flow was not visible in the wetland outside of the stream channels at either end of the wetland. Even though oxygen can diffuse into the surface waters from the atmosphere, anaerobic conditions can develop in shallow water systems due to retarded water movement, high organic matter

content, and dense vegetation at the surface of the wetland. In addition, the presence of aerobic surface waters does not preclude anaerobic conditions from developing at the water/sediment interface (Bachand and Horne, 2000).

5.4.4 Isotopic effects at c31 weir

The use of nitrate oxygen isotope ratios to separate microbial and atmospheric nitrate sources at the weir was not significantly affected by isotopic enrichment from attenuation in the wetland from May 2000 to October 2002. The enrichment in ^{18}O caused by wetland denitrification (max. of 1.5‰, Table 5.1) was further reduced through additional groundwater inputs to the stream prior to reaching the weir. Assuming that the nitrate concentration of groundwater entering the stream between the wetland and the weir was similar to that of groundwater above the wetland, and that evapotranspiration in the wetland was small, calculations indicate that about 60% of stream discharge at the catchment weir was water from the wetland outlet. Therefore the additional groundwater inputs upstream of the weir, but downstream of the wetland, reduce the observed increase in $\delta^{18}O$ due to denitrification in the wetland (0.1 to 0.9‰). This enrichment is generally within the analytical uncertainty for $\delta^{18}O$-nitrate (±0.8‰) and would not affect source contribution calculations. $\delta^{18}O$-nitrate values measured at the weir, which were lower than in nitrate upstream of the wetland, indicated that the additional groundwater nitrate inputs had a greater proportion of microbial nitrate than groundwaters above the wetland.

The effect of wetland denitrification on nitrate isotope ratios was greater for $\delta^{15}N$ than for $\delta^{18}O$ (Table 5.1). For the three periods examined, the increase in $\delta^{15}N$-nitrate values below the wetland ranged from 3.9 to 4.3‰, which translates to an approximately 2.5‰

enrichment of nitrate collected at the weir. However, δ^{15}N-nitrate is not directly used to calculate nitrate source contributions at the TLW (Spoelstra et al., 2001).

Table 5.1 Isotopic ratios of stream 31 nitrate collected at the wetland inlet, outlet, and weir.

Isotope Ratio	Location	May 2000	Sept. 2000	May 2001
Nitrate	Wetland inlet	4.719	1.362	1.379
(mg N/L)	Wetland outlet	1.579	0.488	0.204
	c31 weir	2.711	0.831	0.697
δ^{18}O (‰)	Wetland inlet	6.1	7.8	7.1
	Wetland outlet	7.3	9.3	7.3
	c31 weir	4.8	5.4	5.8
δ^{15}N (‰)	Wetland inlet	5.7	5.3	4.0
	Wetland outlet	10.0	9.3	7.9
	c31 weir	8.1	5.6	3.4

5.4.5 Catchment nitrate export

Despite being too small to appear on standard topographic maps (Creed et al., 2003) and having a midpoint location in the catchment, the c31 forested wetland significantly reduced catchment-scale nitrate export following harvest. Nitrate attenuation in the wetland was responsible for lower nitrate concentrations measured at the weir. Therefore per-hectare catchment nitrate export would be underestimated when calculated from discharge and concentration data collected at the weir. Assuming that stream 31 nitrate concentrations at the weir would be similar to those measured upstream of the wetland if attenuation had not occurred, the effect of the wetland on catchment-scale nitrate export for each of the study periods was calculated. According to these calculations, wetland nitrate attenuation reduced

catchment nitrate export by 40 to 50% for the May 2000, September 2000, and May 2001 periods. For the later post-harvest periods (September 2001, Jun 2002, October 2002) nitrate export was reduced by 35 to 80%.

Increasing $\delta^{15}N$ values with time were observed for nitrate collected at the c31 weir in the early post-harvest period (1997-1999) and these results were attributed to denitrification activity in the wetland (Chapter 7). However, no corresponding stream samples were collected directly above or below the wetland during this period, so the wetland's effect on nitrate concentrations was not quantified. Nitrate concentrations at the c31 weir ranged from about 2 to greater than 10 mg N/L during the early post-harvest period (Section 7.3.2). In the first growing season following harvest, plant uptake of nitrate was probably lower than subsequent years since the vegetation community would take some time to establish. Changes in the vegetation community could have also affected denitrification activity since the submerged portions of wetland plants can act as an attachment substrate for bacteria as well as increasing surface hydrologic residence time by flow restriction (Hammer, 1992). Also, high nitrate or labile organic carbon concentrations might have promoted higher denitrification rates during the first post-harvest growing season.

Seasonal variability in the magnitude of wetland nitrate attenuation and its effect on catchment nitrate export were expected due to temperature effects on microbial activity, the seasonality of plant nitrogen demand, and changes in the hydrologic residence time of the wetland. For TLW headwater catchments, 30-60% of the annual discharge occurs during the snowmelt period (Nicolson, 1988). Therefore, on an annual basis, the effect of the wetland on nitrate export would be somewhat reduced because high flows would limit the wetland's ability to lower nitrate concentrations.

Samples collected at various locations along stream 31 in 1985 (W. Johns, unpublished data) indicate that nitrate attenuation was also occurring in the wetland prior to harvest. Therefore, the large discrepancy between the amount of nitrate leached below the rooting zone (16.2 kg/ha/yr, Foster et al., 1992b) and the catchment-scale export of nitrate by stream 31 (approximately 3 kg/ha/yr, Foster et al., 1986; Nicolson, 1988; Foster et al., 1989) is partially caused by wetland nitrate attenuation. Isotopic evidence for denitrification in the c31 wetland for two years prior to harvest (1995-1996) was limited, with the most enriched weir sample having a δ^{15}N-nitrate of +4.8‰ (Spoelstra et al., 2001). However, at pre-harvest nitrate concentrations (mean of 0.5 mg N/L for May and September; Creed and Band, 1998), nitrate removal by the wetland may have been complete and therefore not detectable using stable isotope analysis of nitrate collected at the weir.

5.5 Conclusions

This study demonstrates the ability of a small wetland to attenuate high nitrate concentrations in a headwater stream caused by harvesting of the associated forested uplands. For fall and spring sampling periods from 2000 to 2002, the c31 wetland was responsible for retaining 65 to 100% of incoming surface water nitrate inputs, thereby decreasing nitrate export from the entire catchment by 35 to 80%. Stable isotope analysis of nitrate (δ^{15}N, δ^{18}O) and vegetation (δ^{15}N) were used to examine the mechanisms responsible for wetland nitrate attenuation. The inverse relationship between δ^{15}N and nitrate concentration in wetland surface and ground water, and the 2:1 concomitant increase in nitrate δ^{15}N:δ^{18}O values, indicated that attenuation by denitrification was occurring. Elevated vegetation δ^{15}N values revealed that plants were assimilating nitrate enriched in ^{15}N

by partial denitrification in the rooting zone or from the overlying surface water. The nitrogen isotope signature of herbaceous plants might be useful to qualitatively map denitrification activity within the rooting zone of shallow sediments and riparian areas.

Isotopic enrichment by nitrate attenuation in the c31 wetland does not appear to interfere with the use of $\delta^{18}O\text{-}NO_3^-$ to identify nitrate sources in headwater streams at the TLW. However, wetland effects on stream nitrate concentration need to be recognized and accounted for when determining catchment nitrogen budgets. Forest management practices designed to preserve or enhance wetland nitrate attenuation could potentially be used to reduce the detrimental aquatic effects of nitrate export caused by harvest operations.

Chapter 6. The isotopic composition of nitrate produced from nitrification in a hardwood forest floor.

6.1 Introduction

Analysis of nitrate stable isotope ratios ($^{18}O/^{16}O$ and $^{15}N/^{14}N$) have proved to be a powerful tool for investigating nitrate sources and cycling in forested watersheds (Durka et al., 1994; Kendall et al., 1996; Spoelstra et al., 2001; Williard et al., 2001; Burns and Kendall, 2002; Schiff et al., 2002; Sickman et al., 2003). $\delta^{15}N$ and $\delta^{18}O$ values of both nitrate end-members are needed to calculate the fraction of nitrate from atmospheric deposition and nitrification in streams and groundwater. The isotopic signature of atmospheric nitrate can be measured directly by analysis of precipitation. However, the microbial end-member is not as easily determined from field samples, which usually contain a mixture of nitrate from both sources. Laboratory studies have shown that two oxygens come from water and one from O_2 during the conversion of ammonium to nitrate by chemolithoautotrophic bacteria (Aleem et al., 1965; Kumar et al., 1983; Andersson and Hooper, 1983; Hollocher, 1984). Therefore, Equation 6.1 can be used to calculate microbial nitrate $\delta^{18}O$ from the $\delta^{18}O$-H_2O of available water and the $\delta^{18}O$ of atmospheric O_2.

$$\delta^{18}O\text{-}NO_3^- \text{ (microbial)} = {}^1/_3(\delta^{18}O\text{-}O_2) + {}^2/_3(\delta^{18}O\text{-}H_2O) \qquad 6.1$$

$\delta^{18}O$-nitrate values in the range of the predicted microbial end-member have been measured in groundwaters in agricultural areas (Böttcher et al., 1990; Wassenaar, 1995; Cey et al., 1999; Mengis et al., 1999, 2001) and septic plumes (Aravena et al., 1993; Aravena and Robertson, 1998). Although some studies in forested watersheds have observed $\delta^{18}O$ values

in streams and groundwater depleted enough to be 100% microbial nitrate (Spoelstra et al., 2001, Schiff et al., 2002, Williard et al., 2002), others only measure values well above the calculated microbial nitrate end-member (Durka et al., 1994; Kendall et al., 1996; Burns and Kendall, 2002; Sickman et al., 2003). The elusiveness of the microbial nitrate signature in some forested systems lead to speculation that Equation 6.1 may not predict the $\delta^{18}O$ signature of nitrate produced by nitrification in all forest soils.

Several hypotheses have been suggested to explain why microbial nitrate might be more enriched than predicted by Equation 6.1 (summarized in Kendall, 1998). Water used as an oxygen source for nitrification in shallow soils might be enriched in ^{18}O by evaporation. Therefore $\delta^{18}O$-H_2O values of precipitation or groundwater used in the calculation would not accurately reflect the $\delta^{18}O$ of water available to nitrifiers. By measuring the isotopic composition of twig water ($\delta^{18}O$ and δD), Tang and Feng (2001) found that the water taken up by a sugar maple tree had been enriched by evaporation, by as much as 7‰ for $\delta^{18}O$. The enrichment effects of evaporation decrease rapidly with depth in the soil profile (e.g. Allison, 1983). Since trees can take up water from both shallow and deep sources, the shallow soil water taken up by the tree in the Tang and Feng (2001) study must have been enriched by more than 7‰. Therefore it also seems likely that the water available to nitrifiers could periodically be enriched in ^{18}O, especially in the upper most soil layers.

Heterotrophic nitrification, which derives less than two oxygens from water, has also been identified as a possible mechanism for producing microbial nitrate with enriched $\delta^{18}O$ values relative to those predicted using Equation 6.1 (Mayer et al., 2001). Heterotrophic nitrifiers, which consist largely of fungi and some bacteria species, predominantly convert organic nitrogen to nitrite and nitrate through biochemical pathways that do not provide

energy to the microorganisms (Killham, 1986; Bock et al., 1991). Relatively little is known about the conditions that favor heterotrophic nitrification in soils (Killham, 1986; Pedersen et al., 1999). Heterotrophic nitrifiers only produce small amounts of inorganic nitrogen and therefore chemolithoautotrophs are generally the dominant nitrifiers in environments with high nitrate production (Focht and Verstraete, 1977; Killham, 1986; Bock et al., 1991).

Finally, the $\delta^{18}O$ of oxygen gas, which is constant in the atmosphere (+23.5‰ relative to VSMOW; Kroopnick and Craig, 1972), might be modified in the subsurface. Laboratory experiments have shown that respiratory oxygen consumption by plant roots and microbes can cause isotopic enrichment of soil O_2 (Lane and Dole, 1956; Forstel and Schleser, 1976; Schleser, 1979; Guy et al., 1989, 1993; Robinson et al., 1992; Angert and Luz, 2001a; Lee et al., 2003). In environments where diffusion controls soil gas oxygen isotope ratios, lower $\delta^{18}O$ values are expected with depth since $^{16}O^{16}O$ diffuses faster than $^{18}O^{16}O$ (e.g. Severinghaus et al., 1996). Lower soil $\delta^{18}O$-O_2 values with decreasing O_2 concentration can also occur when slow diffusion rates through soil aggregates or root tissues limit O_2 concentration at the site of respiration, resulting in minimal isotopic discrimination (Angert and Luz, 2001b). Factors such as respiration rate, microbial community, soil moisture, temperature, and soil structure, control the balance between the fractionation effects of respiration and those of diffusion and therefore determine the magnitude and direction of any isotopic shift in soil $\delta^{18}O$-O_2 values.

A limited number of field studies in non-forest systems have measured $\delta^{18}O$ values of soil O_2 and these studies have shown both directions of isotopic shift with depth. Wassenaar and Koehler (1999) and Lee et al. (2003) found significant ^{18}O enrichment of soil O_2 with decreasing O_2 concentration in a mining waste rock deposit. Aggarwal and Dillon (1998)

observed the same effect at a site in Nebraska consisting of unsaturated silty clay and sand. A minor ^{18}O depletion of O_2 with depth was measured in sand dunes (Severinghaus, et al., 1996) and in orchard soils (Angert and Luz, 2001b) where fractionation resulting from diffusion controlled soil O_2 isotope ratios.

To date, only one study has reported $\delta^{18}O$-O_2 data for forest soils (Angert et al., 2003). In this study, tropical, temperate, and boreal forest sites were examined and variations in soil $\delta^{18}O$-O_2 values relative to atmospheric O_2 ranged from -0.64 to +3.53‰ (+22.9 to +27‰ relative to VSMOW) at depths between 40 to 155cm. Even using the most enriched values, the effect on the oxygen isotope composition of nitrate produced by chemolithoautotrophic (Equation 6.1) for these sites would be less than 1.2‰.

Equation 6.1 assumes that chemolithoautotrophic bacteria do not isotopically fractionate O_2 or water during nitrification. Even if these fractionations exist, there is evidence that they are relatively minor (Mayer et al., 2001). Since biological processes typically discriminate against heavy isotopes, the nitrate produced would be more depleted than predicted by Equation 6.1 and therefore this effect does not explain enriched nitrate $\delta^{18}O$ values.

Previous experiments to determine the $\delta^{18}O$ of microbial nitrate had widely varying results, with $\delta^{18}O$-nitrate ranging from less than 0 to +16‰ (Amberger and Schmidt, 1987; Voerkelius, 1990; Williard et al., 2001; Burns and Kendall, 2002). In the most detailed study of the isotopic composition of microbial nitrate produced in forest soils to date, Mayer et al. (2001) demonstrated that, under conditions of high ammonium availability and high nitrification rates, $\delta^{18}O$ values were consistent with those predicted by Equation 6.1. However, when nitrification rates and ammonium concentrations were low, microbial nitrate

$\delta^{18}O$ values were considerably enriched in ^{18}O because less than two oxygens were derived from water, presumably as a result of heterotrophic nitrification.

The $\delta^{15}N$ of microbial nitrate is difficult to predict because it depends on the $\delta^{15}N$ of available ammonium and kinetic isotope fractionation effects associated with nitrification. The nitrogen isotope signature of ammonium is expected to be similar to soil organic nitrogen since little isotopic fractionation occurs during ammonification (Kendall, 1998). However, the $\delta^{15}N$ of ammonium available for nitrification can subsequently be altered by assimilative ammonium uptake. A large fractionation factor exists for nitrification (-12‰ to -29‰, Shearer and Kohl, 1986) and therefore $\delta^{15}N$-nitrate values should be significantly lower than those of soil ammonium and organic nitrogen. Temperature and substrate availability effects on isotopic fractionation affect the magnitude of this depletion. Two previous studies measuring microbial nitrate $\delta^{15}N$ from forest soils have shown a wide range of values (-22 to +16‰) (Mayer et al., 2001; Burns and Kendall, 2002)

The goals of this study were to: 1) use a field study approach to determine the $^{18}O/^{16}O$ and $^{15}N/^{14}N$ isotopic ratios of nitrate produced by nitrification in a hardwood forest floor, 2) measure the $\delta^{18}O$ of soil gas to determine if respiratory enrichment of O_2 affects the $\delta^{18}O$ of microbial nitrate, and 3) confirm that calculations of microbial nitrate $\delta^{18}O$ values using Equation 6.1 are appropriate for nitrification at the TLW.

6.2 Methods

6.2.1 *Experimental design*

In order to collect nitrate solely from nitrification, precipitation inputs were eliminated to three zero-tension lysimeters installed on a well-drained slope. A nitrate and

ammonium free solution, that otherwise resembled the chemical composition of TLW precipitation, was used to simulate rainfall on the forest floor. The microbial nitrate collected in the lysimeters was then analyzed for oxygen and nitrogen isotope ratios.

The setup used for this experiment is shown in plan view in Figure 6.1. In May 2001, three zero-tension lysimeters were installed below the F-horizon (Oe) in TLW catchment 32. The lysimeters drained by gravity into bottles kept in a lined pit down slope. A 1m x 1.5m area around the lysimeters was marked with flagging tape to delineate the boundary for watering. The area was given four months to recover from the installation process before the first run of the experiment in September 2001.

At the beginning of each collection period (September 20 to October 4, 2001 and May 30 to June 11, 2002) a 2.4 x 3.0m waterproof tarp was erected over the area (Figure 6.1) and remained for the two-week duration of each experiment. The tarp was held 1m above the ground with a slight dip to allow water to drain to a second tarp that carried intercepted precipitation an additional 1.8m down slope.

The chemistry of the watering solution (Table 6.1) was designed to simulate TLW precipitation without contributing nitrate or ammonium to the lysimeters. The watering solution was prepared by pipetting several stock solutions of the reagents into ultrapure deionized water (DI) at the Environmental Geochemistry Lab (EGL), University of Waterloo. Twenty liters of the solution, which is equivalent to a 13.3mm rain event, was applied to the watering area over a 45-minute period every 48 hours using a plastic watering jug. The $\delta^{18}O\text{-}H_2O$ of the solutions used for the September 2001 and June 2002 periods were -10.15‰ and -10.47‰, respectively.

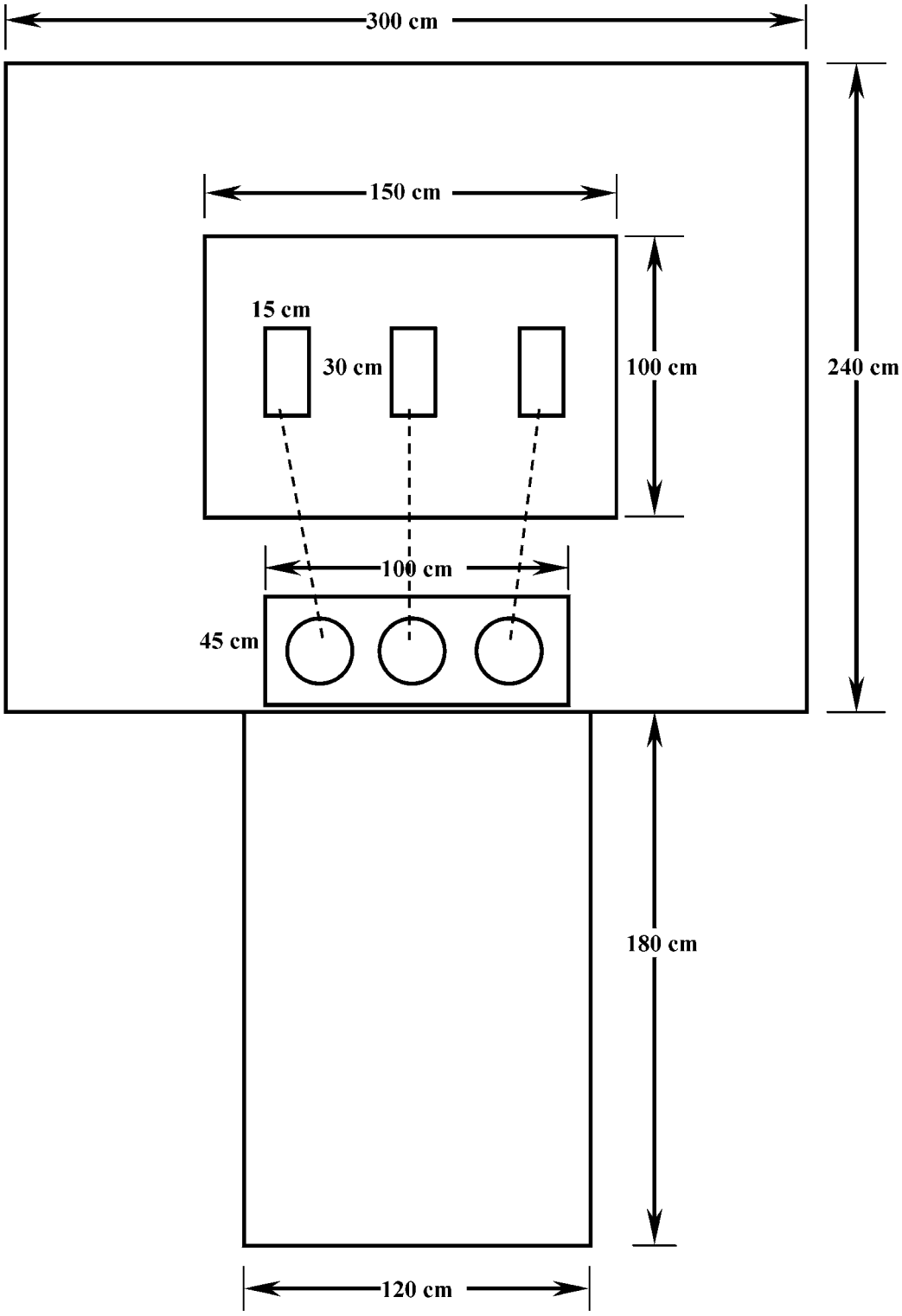


Figure 6.1 Plan view of the lysimeter installation and precipitation exclusion apparatus.

Table 6.1 Chemistry of the watering solution and bulk precipitation at the TLW. *Ion concentrations are volume-weighted means for 1981-1985 (Foster and Nicolson, 1988).

	Watering Solution (μmol/L)	Bulk Precipitation* (μmol/L)
H^+	35	38
Ca^{2+}	10	8
Mg^{2+}	5	3
K^+	5	2
Na^+	10	6
NH_4^+	0	18
SO_4^{2-}	30	25
NO_3^-	0	28
Cl^-	20	6
HCO_3^-	0	6
pH	4.46	4.42

Forest floor leachate was retrieved 24 hours after each watering to allow sufficient time for soil and lysimeters to completely drain. Water from the three individual lysimeters was pooled, filtered to 0.45μm, and sub-sampled for chemical and $\delta^{18}O$-H_2O analyses. The remaining sample was frozen until further processing for nitrate isotope ratios ($^{18}O/^{16}O$ and $^{15}N/^{14}N$) at the EGL.

6.2.2 DOM removal

A dialysis technique modified from Feuerstein et al. (1997) was used to separate nitrate from DOM in forest floor leachate (Section 2.2.1.2). Chemical and volumetric determinations on sub-samples collected from the dialysis process were used to calculate that 79 to 95% of the dissolved organic carbon (DOC) was removed by this procedure. The anion resin used to isolate nitrate from water samples also strongly retained DOM during elution of

the nitrate (2.2.1.3). Taking both mechanisms into account, the overall DOM removal for lysimeter samples was greater than 99%.

6.2.3 Nitrate isotope analysis

Ion exchange methods for the isolation of aqueous nitrate and its subsequent conversion to silver nitrate were adapted from Chang et al. (1999) and Silva et al. (2000) and are described in Section 2.2.2. $\delta^{15}N$ and $\delta^{18}O$ values of silver nitrate were determined by continuous flow isotope ratio mass spectrometry methods as outlined in Sections 2.2.3.2 and 2.2.4.2, respectively.

6.2.4 Soil gas O_2

Soil gas was collected in late May/June 2000-2002 and late September 2001 from several locations in catchment 32, as well as other catchments within the TLW, using a hollow stainless steel tube (length = 1500mm, outer diameter = 6mm) inserted into the soil. Once at the desired depth, an inner rod, which prevented the tube from filling with soil, was removed and the tube raised about 1cm to create an area for gas flow from the soil. A cap with silicon septa was attached to the top of the gas sampler to allow gas collection by syringe. A 60mL syringe and 3-way valve assembly was used to purge 60cc of gas from the sampling apparatus (two times the tube volume) at about 1cc/s prior to the collection of 20cc of soil gas. The syringe was removed from the septa while slowly expelling sample at a rate of 1cc/s to prevent atmospheric contamination. The last 10cc was injected into a 10mL bottle that was previously evacuated and filled to atmospheric pressure with helium and sealed with a butyl blue stopper and aluminum crimp. Where soil conditions permitted, the inner rod was reinserted and the gas sampler pushed to a second or third depth and subsequent samples collected.

Techniques used for isotopic analysis of soil gas O_2 were adapted from Wassenaar and Koehler (1999) and are described by Venkiteswaran (2002). Briefly, the reference gas line of a Gilson autosampler connected to a Micromass Isochrom mass spectrometer was modified to allow direct introduction of gas samples via an inline, splitless injection port. Following carbon dioxide and water removal, a 2m column containing a 5Å molecular sieve at 80ºC separated oxygen and nitrogen gas. Results of $\delta^{18}O$-O_2 analysis are reported relative to VSMOW with a precision of ±0.3‰.

6.2.5 Other isotopic analyses

Oxygen isotope ratios of water were determined by carbon dioxide equilibration using a VG Micromass 903. Results are reported as per mil deviations from VSMOW with an analytical precision of ±0.1‰.

Nitrogen isotope ratios of soils were determined using a Carlo Erba elemental analyzer coupled to a Micromass Isochrom mass spectrometer. Results were calibrated against IAEA-N1 ($\delta^{15}N$ = +0.36‰) and IAEA-N2 ($\delta^{15}N$ = +20.3‰) and reported relative to atmospheric N_2 with a precision of ±0.3‰.

6.3 Results and Discussion

6.3.1 Water and nitrogen yields

At the watering rate used for this study, the theoretical combined yield of the three lysimeters (amount of watering solution applied (cm) x lysimeter area (cm^2)) was 1800mL. Actual water volumes collected ranged from 950 to 1860mL (Table 6.2). Variability in lysimeter yield was expected as a result of imperfections in the evenness of watering and from preferential flow paths in the forest floor that can direct water to or away from the

lysimeters. Water yields lower than 1800mL would also be caused by evaporation and/or vegetative uptake of the residual soil moisture. Evapotranspiration was likely more important during the June sampling since TLW trees are senescing in September. Therefore, it is suspected that the lower average sample volumes in September 2001 (1313mL) compared to June 2002 (1548mL) are a residual effect of soil structure disturbance caused by lysimeter installation.

Although similar in magnitude, concentrations of ammonium were higher than nitrate and the yield of both species was greater during the June sampling (Table 6.2). Increased inorganic nitrogen collection in June (75% greater) resulted from higher average concentrations of both nitrogen species and was not solely due to the 18% increase in water yield for the June period (Table 6.2). Greater microbial activity, including ammonification and nitrification, was favored by higher average temperatures in June (Table 6.3). The F-horizon at the TLW contains three to four times as much nitrogen as the L-horizon (Morrison and Foster, 2001) and has a lower C/N ratio (Figure 6.2a,b). Lower C/N ratios favor net nitrogen mineralization whereas higher ratios generally resulted in net nitrogen immobilization (Alexander, 1977; Rosswall, 1981; Janssen, 1996). Therefore, differences in the amount of nitrogen available for leaching during each period predominantly result from nitrogen cycling changes in the F-horizon. In addition, higher C/N ratios of the litter, especially during September, might have resulted in the immobilization of nitrogen produced by mineralization in the F-horizon, thereby also contributing to lower inorganic nitrogen concentrations.

Table 6.2 Results of chemical and isotopic analysis of forest floor leachate collected from three, zero-tension lysimeters watered with a nitrogen free solution.

Collection	Day	Volume	NO_3^--N	NH_4^+-N	NO_3^- + NH_4^+	DOC-C	$\delta^{15}N$-NO_3	$\delta^{18}O$-NO_3	$\delta^{18}O$-H_2O
Date		mL	mg/L	mg/L	mg N	mg/L	‰	‰	‰
21-Sep-01	2	1710	0.17	0.37	0.92	8.8	-5.8	13.1	-10.42
23-Sep-01	4	1585	0.26	0.57	1.31	14.8	-7.7	4.5	-10.31
25-Sep-01	6	1125	0.27	0.42	0.77	14.5	-7.3	3.7	-9.98
27-Sep-01	8	1345	0.22	0.56	1.05	10.8	-8.5	3.6	-9.61
29-Sep-01	10	950	0.27	0.27	0.51	9.7	-7.6	4.0	-9.86
1-Oct-01	12	1250	0.28	0.59	1.08	11.9	-8.9	4.3	-9.87
3-Oct-01	14	1485	0.29	0.92	1.80	14.9	-10.4	4.1	-9.55
4-Oct-01	15	1050	0.30	0.49	0.82	13.6	-9.6	3.1	-9.36
31-May-02	2	1275	0.34	0.94	1.64	20.9	-7.6	14.5	-10.51
2-Jun-02	4	1495	0.54	0.94	2.21	28.1	-9.8	8.3	
4-Jun-02	6	1460	0.41	0.53	1.37	15.7	-9.1	7.4	-10.21
6-Jun-02	8	1860	0.56	0.48	1.93	14.3	-8.7	5.4	
8-Jun-02	10	1365	0.26	0.47	1.00	15.4	-10.2	10.1	-10.35
10-Jun-02	12	1710	0.65	0.80	2.48	18.0	-8.5	5.0	
11-Jun-02	13	1670	0.58	0.63	2.02	18.3	-9.1	4.1	-10.24

Table 6.3 Selected meteorological parameters averaged for the two periods of study. Data was measured at 10-minute intervals at the Canadian Air and Precipitation Monitoring Network (CAPMoN) site located about 1.5km from the TLW (Figure 2.1).

	Air Temperature (ºC)			Relative	Wind Speed
Period	Minimum	Maximum	Mean	Humidity (%)	(m/s)
20-Sep to 4-Oct-2001	2	20	10.4	85	2.2
31-May to 11-Jun-2002	5	26	14.2	67	2.0

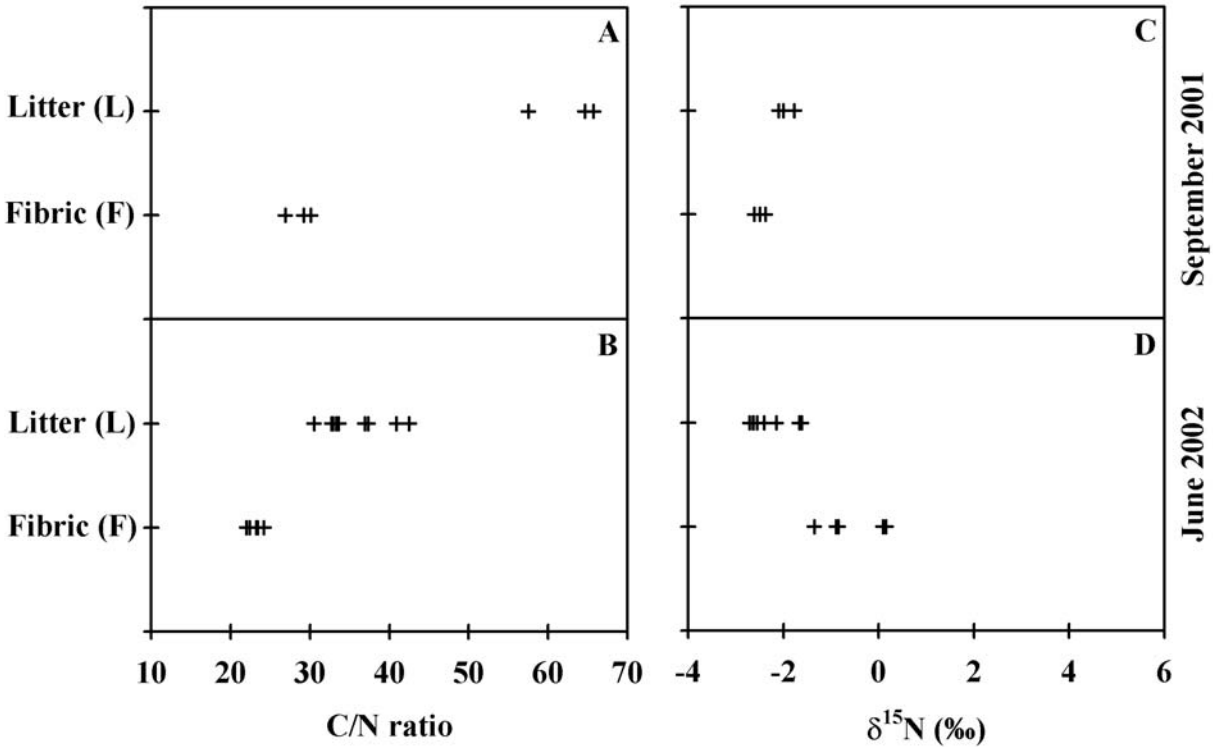


Figure 6.2 Molar C/N and nitrogen isotope ratios for L and F soil horizons collected at the TLW during each run of the experiment.

6.3.2 Isotope composition of soil O_2

The isotopic composition of O_2 in TLW soils was not significantly different from atmospheric O_2 (+23.5‰; Kroopnick and Craig, 1972) (Figure 6.3). Substituting the most enriched soil O_2 value (+24.1‰) into Equation 6.1 instead of +23.5‰ only increases the calculated microbial nitrate $\delta^{18}O$ by 0.2‰, well within the analytical uncertainty of the $\delta^{18}O$-

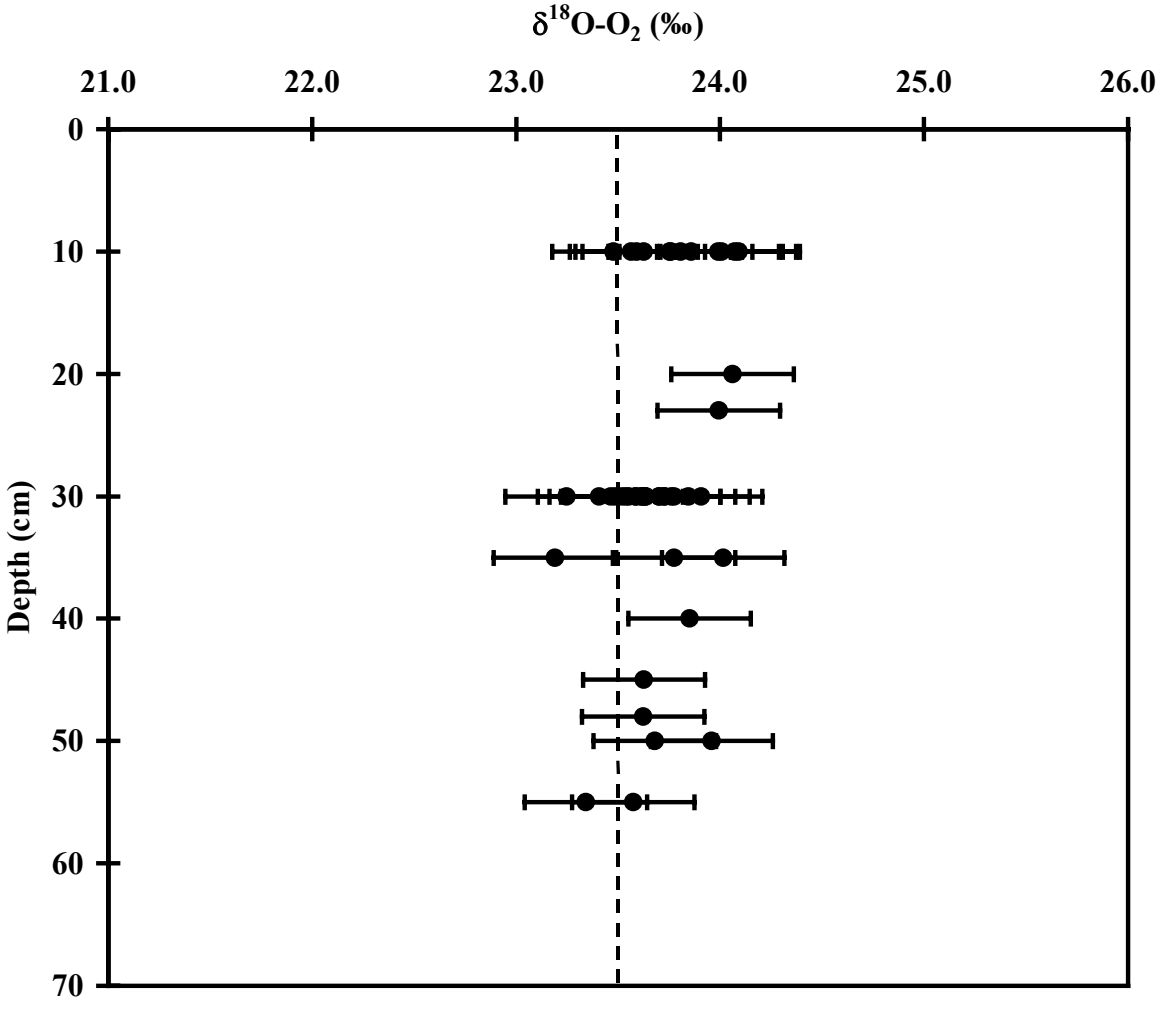


Figure 6.3 Isotopic composition of soil gas O_2 collected from various depths below the top of the litter layer in May/June 2000-2002 and September 2001. The isotopic composition of atmospheric O_2 (+23.5‰; Kroopnick and Craig, 1972) is marked by the dashed line and error bars (±0.3‰) indicate the analytical uncertainty of $\delta^{18}O$-O_2 analysis.

nitrate technique (±0.8‰). Soil incubation experiments revealed that approximately 95% of nitrification occurs within the top 15cm of TLW soils (Foster et al., 1986). Therefore, the lack of an isotopic shift in $\delta^{18}O\text{-}O_2$ to a depth of 55cm indicates that a value of +23.5‰ can be used in Equation 6.1 for calculating microbial nitrate $\delta^{18}O$ values at the TLW.

The soil O_2 isotope data in this study are consistent with those of Angert et al. (2003) who found a maximum $\delta^{18}O$ enrichment of +3.53‰ for soil O_2 collected at depths ranging from 40 to 155cm in tropical, temperate, and boreal forests. Results for the temperate forest sites showed enrichments ranging from +0.08 to +0.47‰ above the $\delta^{18}O$ value of atmospheric O_2. Since many of the published studies using the dual nitrate isotope technique in forested catchments are situated in the temperate zone (including the TLW), results from this study and those of Angert et al. (2003) suggest that a value of +23.5‰ can be used in Equation 6.1 as a reasonable proxy for the $\delta^{18}O$ value of O_2 available to nitrifiers in forest soils.

6.3.3 Oxygen isotope composition of microbial nitrate

Precipitation $\delta^{18}O\text{-}H_2O$ values ranged from -18.2 to -4.5‰ and -11.2 to -7.9‰ for the September and June periods, respectively (Figure 6.4). However, the $\delta^{18}O$ of water collected by the lysimeters was very similar to the watering solution (Figure 6.4), confirming that the exclusion apparatus was effective at eliminating precipitation to the lysimeters. Most of the solution added during each watering period rapidly drained through the forest floor and was collected by the underlying lysimeters. A fraction of the added water was retained by the soil and this residual moisture was then available for plant uptake, microbial processes (including nitrification), and evaporation. The small $\delta^{18}O\text{-}H_2O$ enrichment (~0.2‰) of some

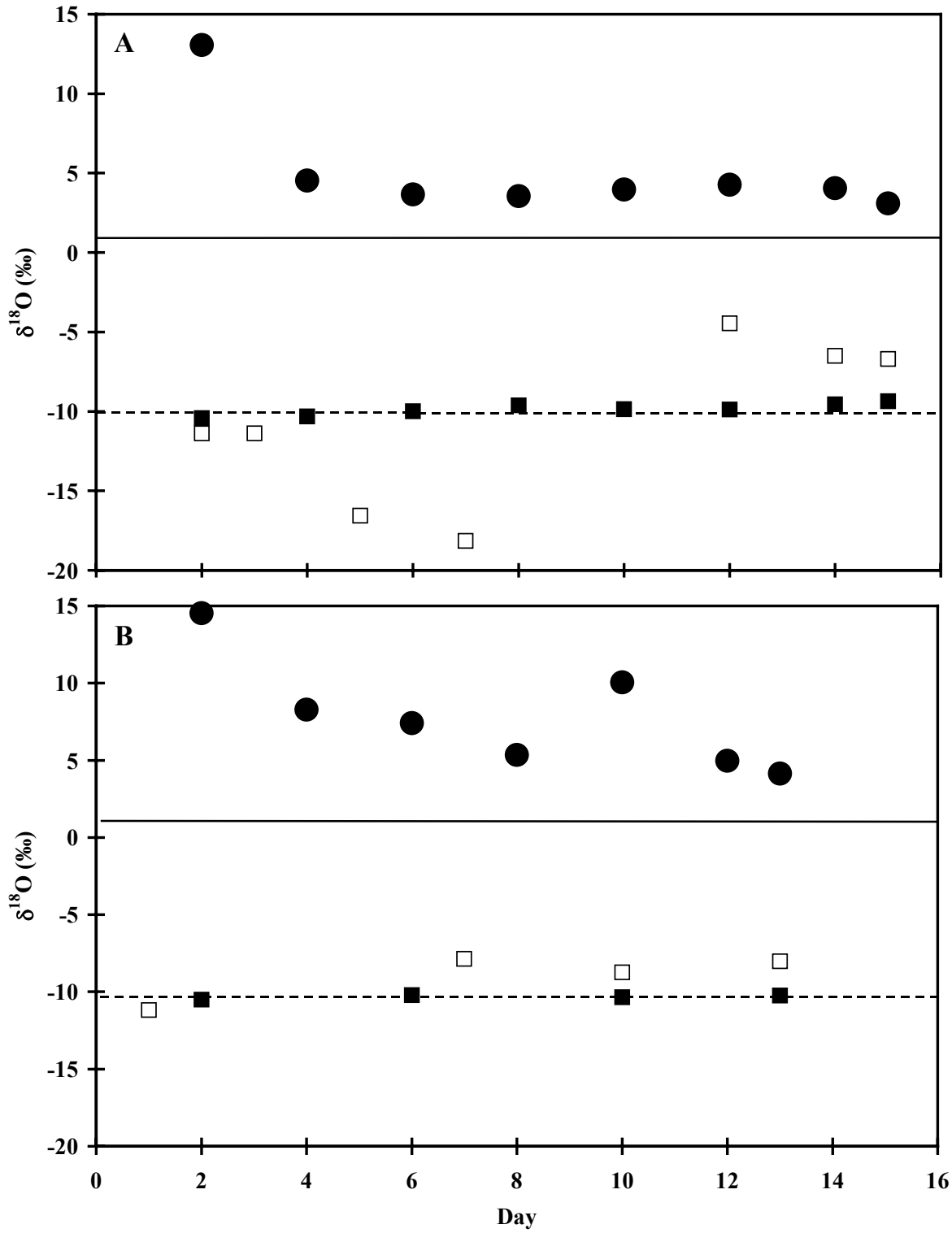


Figure 6.4 Oxygen isotope ratios ($^{18}O/^{16}O$) of forest floor leachate water (■) and nitrate (●) and precipitation water (□) for the September 2001 (A) and June 2002 (B) periods. Solid and dashed lines indicate the expected $\delta^{18}O$ of microbial nitrate (from Equation 6.1) and the $\delta^{18}O$-H_2O of the watering solution, respectively. The heights of the symbols for $\delta^{18}O$-nitrate are equivalent to the analytical uncertainty (±0.8‰).

lysimeter samples might be caused by evaporation, which would enrich the residual soil water in ^{18}O by preferential evaporation of $H_2{}^{16}O$. The magnitude of this enrichment would be masked by the much larger volume of water flushed directly to the lysimeters during each watering, without the chance for evaporation effects on isotopic composition.

Immediately following each watering, the $\delta^{18}O$ of water available to nitrifiers was expected to be very similar to the $\delta^{18}O$ of the watering solutions, which were -10.15 and -10.47‰ for the September and June periods, respectively. Therefore, the nitrate produced by chemolithoautotrophic nitrification, which uses one oxygen from O_2 and two from the watering solution, would have $\delta^{18}O$ values of approximately 1‰ according to Equation 6.1. For both runs of the experiment, $\delta^{18}O$-nitrate values of lysimeter samples approached the calculated microbial nitrate $\delta^{18}O$ values. In September, $\delta^{18}O$-nitrate values remained relatively steady (+3.1 to +4.5‰) (Figure 6.4a) after the first watering flushed out the mixture of microbial and atmospheric nitrate initially present in the soil. In June, $\delta^{18}O$ values of microbial nitrate produced in the forest floor (+4.1 to +10.1‰) declined more gradually following the initial flushing, with $\delta^{18}O$-nitrate decreasing to +4.1‰ by the end of the experiment (Figure 6.4b).

Evaporative enrichment of soil water could be responsible for the slight ^{18}O-enrichment of forest floor microbial nitrate compared to values calculated using the $\delta^{18}O$-H_2O of the watering solutions and Equation 6.1. The greater ^{18}O enrichment of microbial nitrate in June (3-9‰) compared to September (2-3.5‰) supports this hypothesis since higher temperatures and lower relative humidity in June (Table 6.3) favored greater evaporation from surface soils. The magnitude of soil water ^{18}O enrichment depends on the

fraction of soil water evaporated, which can be calculated using a simplified Rayleigh equation (Equation 6.2).

$$\delta_f - \delta_i = \varepsilon \ln(f) \qquad 6.2$$

where: δ_f = $\delta^{18}O$ value of the water after enrichment

δ_i = initial water $\delta^{18}O$ value

ε = enrichment factor between water vapor and liquid water

f = fraction of soil water remaining

In order to produce microbial nitrate enriched by 2 to 9‰, the average $\delta^{18}O$ values of soil water used by the nitrifiers would have to be enriched by 3 to 13.5‰ since only two of the three oxygens come from soil water. Using a value of -9.7‰ for ε (calculated at 20ºC from Majoube, (1971)) in Equation 6.2, about 17 to 75% of the watering solution initially retained by the forest floor would need to be evaporated to enrich the remaining soil water by 3 to 13.5‰, respectively.

By replenishing forest floor soil water with watering solution every 48 hours, the period in which evaporation could occur was limited. Soil moisture content was not monitored during this experiment so the actual reduction in soil moisture between watering periods is not known. Under natural conditions, the interval between rain events can be substantially larger than those simulated in this experiment, and thus, the isotopic enrichment effects on forest floor water could also be much greater. However, nitrifiers are very moisture sensitive and nitrification activity in TLW soils is significantly reduced at moisture levels less than 50% of field capacity (Foster et al., 1992b; Foster et al., in prep). Therefore,

most nitrification probably occurs while soil moisture is high and evaporative enrichment of water is low, producing nitrate with $\delta^{18}O$ values relatively close to that predicted using the $\delta^{18}O$ value of added water.

A minor contribution of nitrate from heterotrophic nitrification could also have produced the small increase of nitrate $\delta^{18}O$ values. During heterotrophic nitrification, soil organic matter oxygen might be incorporated into the newly formed nitrate molecule (Mayer et al., 2001). The oxygen isotopic composition of soil organic matter in the upper horizons should be similar to that of the plant matter from which it is derived. The $\delta^{18}O$ of organic oxygen in fresh leaf litter (predominantly sugar maple and birch) and leaf leachate were similar and ranged from +23.4 to +25.3‰ (VSMOW) at a site in central Ontario (Humphries, 2003). Therefore, nitrate produced by heterotrophic nitrification would be considerably more enriched in ^{18}O than nitrate produced by the chemolithoautotrophic pathway. The close agreement between measured forest floor $\delta^{18}O$-nitrate values and those predicted from the $\delta^{18}O$ of the watering solution indicate that heterotrophic nitrification was not at significant source of microbial nitrate at the TLW.

6.3.4 Nitrogen isotope composition of microbial nitrate

Higher $\delta^{15}N$-nitrate values on day two of each run were due to a mixture of atmospheric and microbial nitrate initially present in the soil and are consistent with $\delta^{18}O$-nitrate results (Figure 6.5 and 6.6). Microbial nitrate $\delta^{15}N$ values from subsequent water samples were similar for both periods, ranging from -10.4 to -7.3‰ and -10.2 to -8.5‰ for September and June, respectively. The constancy of the $\delta^{18}O$-nitrate values indicated that the general decline in $\delta^{15}N$ values for the September period was not due to progressive removal of precipitation nitrate from the soil.

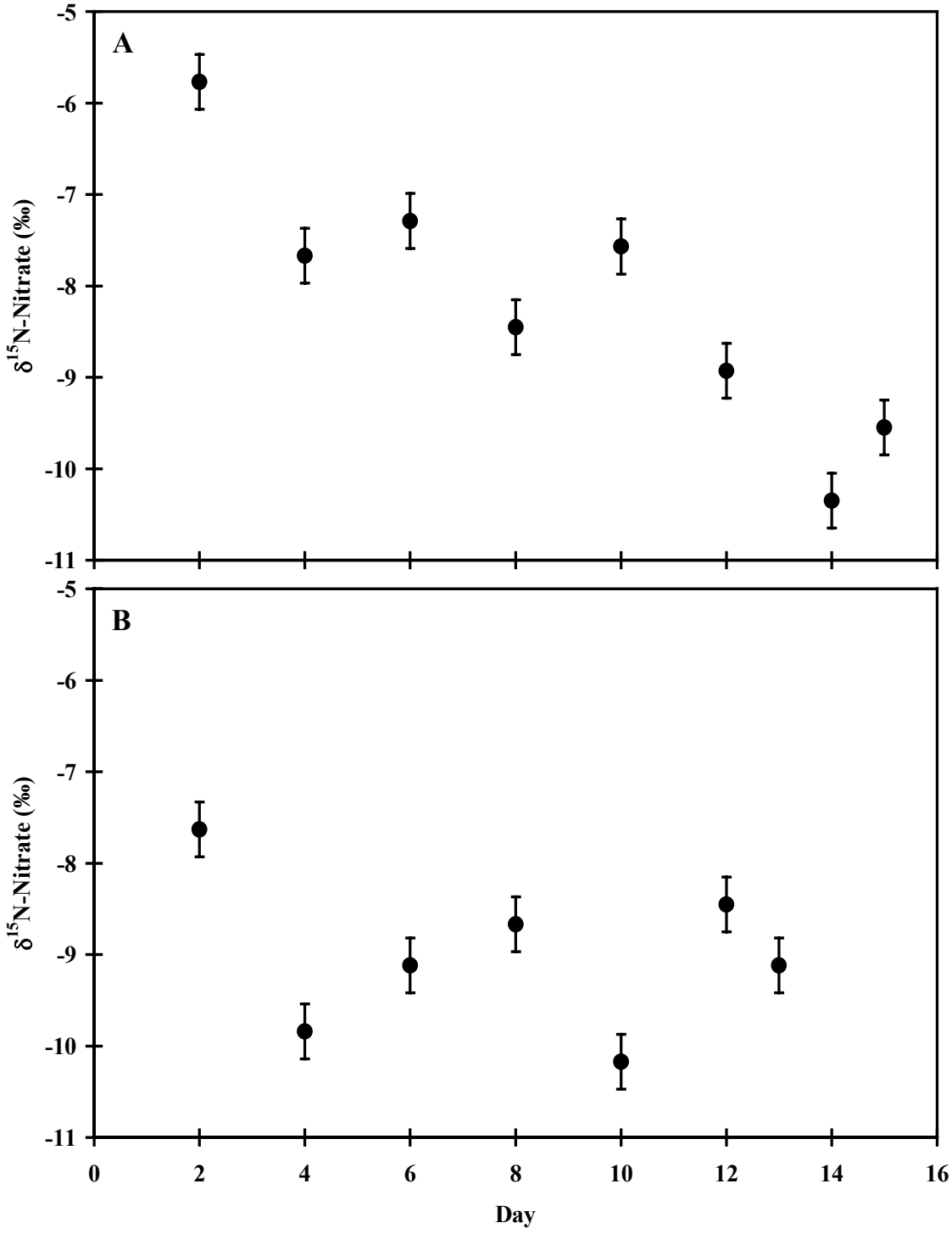


Figure 6.5 Nitrogen isotope ratios ($^{15}N/^{14}N$) of nitrate in forest floor leachate collected for the September 2001 (A) and June 2002 (B) periods. The analytical uncertainty for δ^{15}N-nitrate analysis (±0.3‰) is indicated by the error bars.

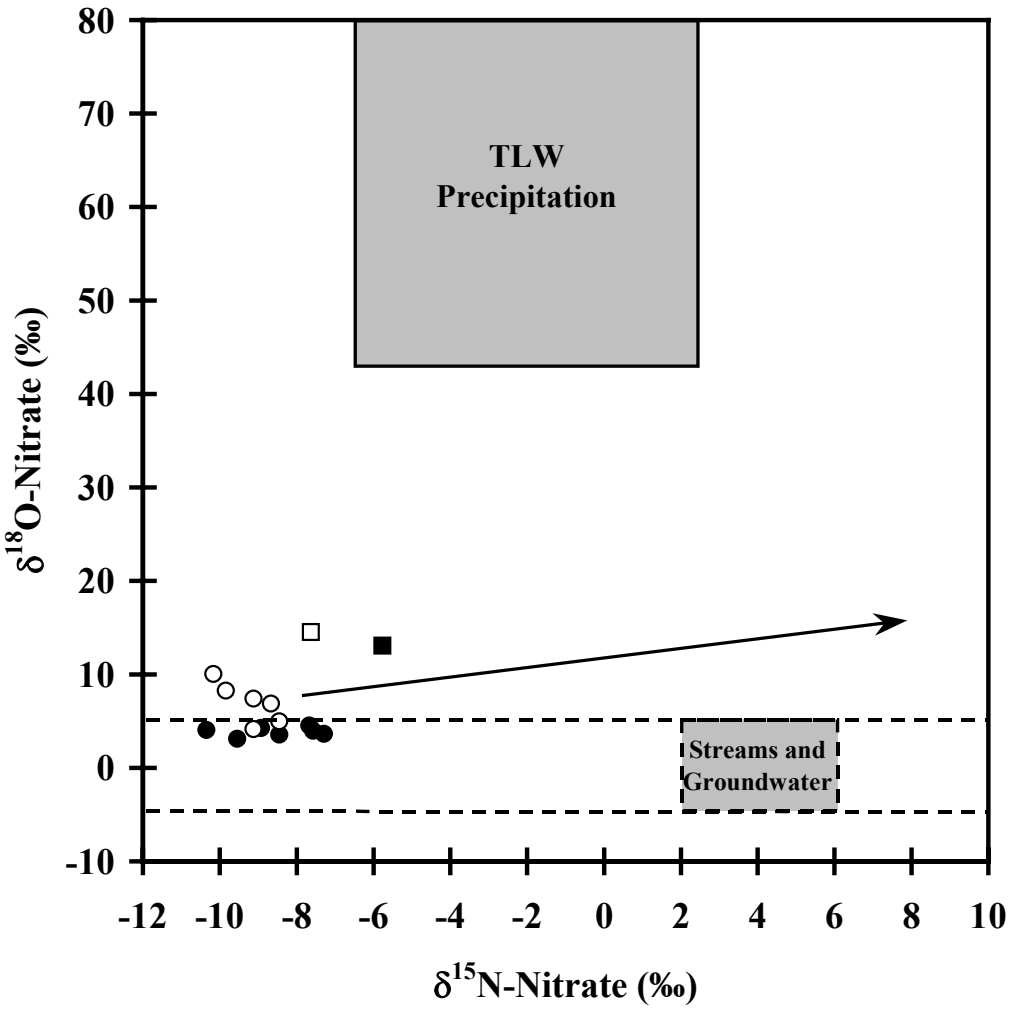


Figure 6.6 Isotopic composition of forest floor lysimeter nitrate collected in September 2001 (●) and June 2002 (○) in relation to nitrate in bulk precipitation (rain and snow) collected bi-weekly at the TLW (1995-1999). The first samples collected from the lysimeters are shown for September 2001 (■) and June 2002 (□). The horizontal dashed lines indicate the calculated range of microbial nitrate $\delta^{18}O$ (Equation 6.1) using atmospheric O_2 (+23.5‰) and the range of $\delta^{18}O$-H_2O for TLW meteoric water (-20.1 to -4.5‰, Bottomley et al., 1986). The $\delta^{15}N$ of microbial nitrate found in TLW streams and groundwater (Spoelstra et al., 2001; Chapter 7) is also indicated. The arrow shows the expected slope for progressive denitrification of the forest floor nitrate.

Many processes occurring simultaneously in the soil control the nitrogen isotope signature of microbial nitrate. The first step in the overall conversion of organic nitrogen to nitrate is the ammonification of soil organic nitrogen. Ammonification does not significantly

fractionate against $\delta^{15}N$ (Kendall, 1998) and therefore the ammonium produced by this reaction is expected to have $\delta^{15}N$ values close to soil organic nitrogen. The $\delta^{15}N$ of total soil nitrogen, which consists predominantly of organic nitrogen, ranged from -2.7 to +0.1‰ for L and F horizons collected at the TLW (Figure 6.2c,d).

In contrast, the nitrification of ammonium results in a large isotopic discrimination against $^{15}NH_4^+$ (ε = -12 to -29‰; Shearer and Kohl, 1986). In a closed system, the nitrate produced initially would be very depleted, becoming more enriched as preferential conversion of $^{14}NH_4^+$ decreased the size of the residual ammonium pool and enriched it in ^{15}N. If 100% of the available ammonium was nitrified, mass balance principles dictate that the resulting nitrate would have the same $\delta^{15}N$ as the original ammonium pool. In forest soils, prediction of the $\delta^{15}N$ of ammonium available for nitrification is complicated by the simultaneous production and nitrification of ammonium, microbial and vegetative ammonium assimilation, and temperature effects on fractionation factors.

As expected, the microbial nitrate produced in this study was depleted relative to soil organic nitrogen. The proportion of ammonium nitrified might explain some of the variability in microbial nitrate $\delta^{15}N$ values. Nitrate and ammonium concentrations in lysimeter samples could not be compared as a proxy for the net mineralization to net nitrification ratio and its effect on microbial nitrate $\delta^{15}N$ because it was found that the ratio of nitrate to ammonium collected changed with water yield. For example, the highest NO_3^--N: NH_4^+-N in September occurred when the lowest volume was collected (950mL - Day 10) as a result of one lysimeter only yielding 20mL.

The range of microbial nitrate $\delta^{15}N$ values produced by the TLW forest floor (-10.4 to -7.3‰) was not as depleted as that found by Mayer et al. (2001) for soil incubations of

deciduous mor humus (-22 to -12‰) but similar to their results for raw humus of coniferous origin (-12 to -7‰). Mayer et al. (2001) provided evidence that the variability in $\delta^{15}N$ values of microbial nitrate in their study was due to ammonium availability, with low ammonium leading to less fractionation during nitrification and resulting in microbial nitrate $\delta^{15}N$ values closer to those of the bulk soil. Relatively high concentrations of ammonium in TLW forest floor leachates (Table 6.2) suggest that microbial nitrate was not enriched in ^{15}N by ammonium limitation in this study. A possible explanation given for the highly variable $\delta^{15}N$ values of microbial nitrate (+1.5 to +16.1‰) from soil incubations by Burns and Kendall (2002) was repeated mineralization, nitrification, and immobilization cycles progressively enriching the residual pool. Given the complex controls on microbial nitrate $\delta^{15}N$, a large range in the values measured in different studies is not surprising.

Nitrogen and oxygen isotope ratios of microbial nitrate might vary between lab incubation studies and those done *in situ*. Soil processing for incubation experiments is likely to affect microbial nitrate $\delta^{15}N$ values by changing ammonification, nitrification, and immobilization rates and the associated fractionation factors. Microbial nitrate $\delta^{18}O$ values are sensitive to changes in the rates of processes controlling the $\delta^{18}O$ of H_2O (i.e. evaporation) and O_2 (i.e. respiration and diffusion) available to nitrifiers. Despite these possible complications, field samples collected from forest floor lysimeters by Mayer et al. (2001) were consistent with the findings of their soil incubation experiments.

In this study, the presence of live tree roots could have indirectly affected microbial nitrate $\delta^{15}N$ values by modifying ammonium concentrations in the forest floor, thereby influencing the net mineralization to net nitrification ratio. However, nitrogen substrate availability effects do not affect the $^{18}O/^{16}O$ ratios of nitrate produced by nitrification.

Nitrate assimilation by trees was not expected to affect observed nitrate isotope ratios since most plants do not significantly fractionate nitrate during uptake (Högberg, 1997). The use of an intact soil structure, including live roots, means that the nitrate collected by TLW lysimeters in this study reflects the $\delta^{15}N$ and $\delta^{18}O$ values of microbial nitrate available for leaching to lower soil horizons.

6.3.5 Effect of the mineral soil on the isotopic ratios of exported nitrate

The very ^{15}N-depleted nitrate produced in the forest floor has not been detected in TLW streams or shallow (~40cm) or deep groundwater (~105cm), not even during the period of high nitrate leaching following forest harvest in catchment 31 (Spoelstra et al., 2001; Chapter 7). Previous studies using the dual nitrate isotope approach have shown that nitrate in TLW streams and groundwater was predominantly microbial in origin and that this microbial nitrate had $\delta^{15}N$ values of approximately +2 to +6‰ (Figure 6.6) (Spoelstra et al., 2001; Chapter 7), significantly enriched compared to microbial nitrate generated in the forest floor in this study (-7.3 to -10.4‰). Forest floor microbial nitrate might not be detectable at depth for several reasons including: 1) the production of ^{15}N-enriched microbial nitrate in the mineral soil, 2) the immobilization of forest floor nitrate, and 3) the enrichment of forest floor nitrate by denitrification.

Nitrification rates are generally highest in surface organic horizons where high organic nitrogen contents promote net ammonification and net nitrification (e.g. Foster et al., 1986). However, the mineral soil can also be a significant source of microbial nitrate since lower organic nitrogen contents are often compensated for by the larger mass of the mineral soil. At the TLW, the forest floor has a higher nitrogen content (3.17%) but contains far less nitrogen (888 kg $N \cdot ha^{-1}$) than the top 60cm of mineral soil (0.24% N, 9710 kg $N \cdot ha^{-1}$)

(Mitchell et al., 1992). The importance of mineral soil nitrification at the TLW has been demonstrated by soil incubations (Foster et al., 1986) and plot-scale studies that showed 8.7 kg $N \cdot ha^{-1} \cdot yr^{-1}$ is leached from the forest floor and 18.6 kg $N \cdot ha^{-1} \cdot yr^{-1}$ is exported below the rooting zone in the mineral soil (Mitchell et al., 1992).

The isotopic signature of microbial nitrate generated in the mineral soil is expected to differ somewhat from that produced in the forest floor, particularly with respect to $\delta^{15}N$. Soil organic matter $\delta^{15}N$ values generally increase with depth in the soil profile (e.g. Shearer et al., 1978) and therefore the ammonium and nitrate produced by nitrogen mineralization might also be more enriched with depth. TLW soil horizons range in $\delta^{15}N$ from about -2.5‰ in the LF-horizon (Figure 6.2) to approximately +8‰ at the top of the mineral soil (Spoelstra, unpublished data). The enrichment effects of evaporation decrease rapidly with depth in the soil profile (e.g. Allison, 1983) and therefore the $\delta^{18}O$ of microbial nitrate produced in the mineral soil is more likely to match values predicted using Equation 6.1.

If it is assumed, for calculation purposes, that the 8.7 kg $N \cdot ha^{-1} \cdot yr^{-1}$ of nitrate exported from the forest floor (Mitchell et al., 1992) behaves conservatively, and that this nitrate mixes with nitrate produced in the mineral soil, the $\delta^{15}N$ of the mineral soil microbial nitrate can be calculated. Using the measured $\delta^{15}N$ range for forest floor nitrate (-7.3 to -10.4‰) and the observed $\delta^{15}N$ range of microbial nitrate exported in streams and groundwater (+2 to +6‰), the $\delta^{15}N$ of nitrate produced by nitrification in the mineral soil at the TLW would have to be in the range of +10 to +20‰. However, fractionation during nitrification results in nitrate that is generally depleted in ^{15}N relative to soil organic nitrogen. Even if 100% of the ammonium produced by ammonification were nitrified so that no fractionation would be observed, the highest $\delta^{15}N$ values possible for nitrate produced in the mineral soil would be

about +8‰. Therefore, the previously observed $\delta^{15}N$ values for microbial nitrate in streams and groundwater at the TLW cannot be explained solely through a simple mixing of microbial nitrate produced in the forest floor with that produced in the mineral soil.

Denitrifying bacteria preferentially convert isotopically light nitrate to N_2O and N_2, increasing $\delta^{15}N$ and $\delta^{18}O$ values of the residual, unreacted nitrate in a characteristic ratio of approximately 2:1, respectively (Amberger and Schmidt, 1987; Böttcher et al., 1990; Voerkelius and Schmidt, 1990; Aravena and Robertson, 1998; Cey et al., 1999; Mengis et al., 1999; Devito et al., 2000). Therefore, partially denitrification of forest floor nitrate would cause it to lose its very depleted $\delta^{15}N$ signature.

Significant denitrification in TLW upland soils has not been detected. Variation in the $\delta^{15}N$ values of microbial nitrate measured in this study was not due to denitrification effects. The September 2001 samples did not show a concomitant increase in $\delta^{18}O$ that would result if the increase in $\delta^{15}N$ were due to denitrification and, in June 2002, $\delta^{18}O$ values decreased with increasing $\delta^{15}N$ (Figure 6.6). In addition, Foster et al. (in prep) did not find evidence for significant denitrification in shallow TLW soils using acetylene inhibition and stable isotope techniques. The isotopic composition of nitrate collected from TLW groundwater, with the exception of one sample, plotted on a mixing line between atmospheric nitrate and mineral soil microbial nitrate (Spoelstra et al., 2001; Chapter 7) and not on the denitrification line originating from the forest floor microbial nitrate (Figure 6.6) except where the two ranges intersect. Contrary to the effects of denitrification, even though $\delta^{15}N$ values were about 8 to 13‰ more enriched, some groundwater samples had $\delta^{18}O$ values similar to or lower than those of the forest floor microbial nitrate produced in this study, (Chapter 7).

More detailed studies are needed to assess denitrification activity in upland TLW soils and its possible effect on the isotope composition of soil nitrate, especially during spring melt and fall recharge periods when soil moisture content of is highest. However, evidence compiled to date suggests that denitrification is not the reason that microbial nitrate collected in groundwater and streams is enriched in ^{15}N relative to nitrate produced in the forest floor.

The immobilization of nitrate by microorganisms and through abiotic mechanisms can be an important nitrogen cycling process in forest soils (e.g. Davidson et al., 1992; Berntson and Aber, 2000; Dail et al., 2001) and therefore likely contributes to the disappearance of ^{15}N-depleted forest floor nitrate. A reduced concentration of forest floor nitrate in soil solution would make it possible to get the observed groundwater nitrate $\delta^{15}N$ values (+2 to +6‰) without requiring mineral soil microbial nitrate $\delta^{15}N$ values to be greater than +8‰, as previously calculated. Immobilization could also increase the residual forest floor nitrate $\delta^{15}N$ through isotopic discrimination although little is known about the magnitude of fractionation during assimilative nitrate uptake by soil microorganisms.

Evidence for the immobilization of isotopically light nitrate in the mineral soil is provided by $\delta^{15}N$ analysis of TLW soils. As previously discussed, TLW soils have $\delta^{15}N$ values of about -2.5‰ in the LF horizon that increase to about +8‰ in the top of the mineral soil, a pattern typical of forest soils. However, the profile becomes more depleted further down in the mineral soil, often reaching +6‰ at a depth of about 60 to 80cm (Spoelstra, unpublished data). It has been suggested that this reversal of the typical enrichment trend with depth in forest soils might be caused by the immobilization of isotopically light nitrate (Högberg, 1997).

6.4 Conclusions

Current methods used to calculate the oxygen isotope ratio of nitrate produced from chemolithoautotrophic nitrification (Equation 6.1) accurately predict the $\delta^{18}O$ values of microbial nitrate at the TLW. Nitrification in the forest floor produced mean nitrate $\delta^{18}O$ values only 4.2‰ enriched above calculated values. ^{18}O-enrichment of soil gas O_2, which has been suspected of increasing microbial nitrate $\delta^{18}O$ values in other studies, was not detected in TLW soils. It is suspected that evaporative enrichment of soil water was largely responsible for the slight enrichment of microbial nitrate $\delta^{18}O$ values. However, a small contribution of nitrate from heterotrophic nitrifiers could have also produced the same effect. Nitrate cycling in the mineral soil may reset the $\delta^{18}O$ of nitrate leached from the upper soil horizons, thus reducing or eliminating any evaporative enrichment effects before the nitrate reaches the saturated zone.

As expected, nitrification in the forest floor produced nitrate with $\delta^{15}N$ values depleted compared to soil organic nitrogen since nitrification strongly fractionates against $^{15}NH_4^+$. Concomitant analysis of soil nitrate and ammonium $\delta^{15}N$ are needed for a better understanding of factors affecting microbial nitrate $\delta^{15}N$ values and, if done using several soil horizons, would also provide useful information related to the development of soil nitrogen isotope profiles. The current understanding of the isotopic composition of nitrate produced by nitrification in forest soils would benefit from further studies, both *in situ* and soil incubations.

The wide range of $\delta^{15}N$ values for microbial nitrate (Mayer et al., 2001; Burns and Kendall, 2002; this study) which span the entire range measured for atmospheric nitrate (Kendall, 1998), prevent $\delta^{15}N$ analysis from being employed on its own for determining

nitrate sources in forested catchments. In contrast, $\delta^{18}O$ of nitrate provides excellent isotopic separation between atmospheric and microbial nitrate sources and can be used in conjunction with $\delta^{15}N$ analysis to identify denitrification effects. Work done at the TLW also indicates that the $\delta^{15}N$ of microbial nitrate in stream and groundwater samples, back-calculated along source mixing lines, might provide information about the relative importance of nitrification in organic versus mineral soil horizons.

Chapter 7. The isotopic composition of a nitrate pulse resulting from forest harvest.

7.1 Introduction

Natural forest ecosystems are typically nitrogen (N) limited (Tamm, 1991). Although these systems are surrounded by N_2, which comprises 78% of the atmosphere, relatively few organisms, mostly bacteria, have direct access to this vast nitrogen pool. Forests receive bioavailable nitrogen through wet and dry atmospheric inputs in the form of ammonium and nitrate. However, atmospheric nitrogen deposition is usually small compared to annual biological nitrogen demand (Rosswall, 1976). Therefore, forests largely rely on recycling of soil organic matter for their nitrogen requirements. Soil microorganisms convert organic nitrogen, which is largely unavailable to most plants, to inorganic nitrogen through the process of nitrogen mineralization.

The elimination or drastic reduction of vegetative nitrogen uptake resulting from forest harvest decouples the tight internal nitrogen cycle that normally prevents or minimizes inorganic nitrogen leaching from forests. Forest harvest can increase ammonium concentration in soils by eliminating vegetative ammonium assimilation and increasing nitrogen mineralization (Vitousek and Melillo, 1979). High ammonium availability increases the native population of nitrifiers in the soil (Duggin et al., 1991) and increases nitrification activity, potentially leading to greater leaching losses of nitrate. As a result, elevated losses of nitrate, the most mobile form of inorganic nitrogen in soils, are often observed following harvest. Increased nitrate export following harvest has been reported for both hardwood (e.g. Likens et al., 1970) and conifer dominated systems (e.g. Tamm et al., 1974) with stream concentrations varying from negligible (Vitousek et al., 1979) to well over

the drinking water limit for nitrate (e.g. Likens et al., 1970). Nitrate export can increase forest regeneration time through the concomitant leaching of nutrient cations and soil acidification, and can also negatively affect downstream water quality (e.g. Aber et al., 1989). High nitrate concentrations can also threaten potable water supplies for humans (e.g. Bouchard et al., 1992; Wolfe and Patz, 2002) and can have detrimental effects on some amphibians, even at relatively low concentrations (Baker and Waights, 1993, 1994; Hecnar, 1995; Marco et al., 1999).

Atmospheric nitrate could also contribute to the increase in nitrate export following forest harvest. Decreased interception of precipitation by vegetation, combined with lower infiltration rates in disturbed soils, can result in a greater potential for overland flow in harvested catchments (Elliot et al., 1998). Therefore biotic and abiotic mechanisms in the soil that can rapidly immobilize nitrate (Stark and Hart, 1997; Davidson et al., 1992; Berntson and Aber, 2000; Dail et al., 2001) could largely be bypassed during high flow periods. As a result, a greater proportion of atmospheric nitrate inputs might be exported by headwater streams compared in recently harvested catchments.

In 1997, a forest harvest experiment was initiated at the TLW (Figure 2.1) to study the effects of current forest management practices on a tolerant hardwood ecosystem. Although not traditionally employed in Canadian hardwood forests, a clear-cut treatment was used to provoke a maximum disturbance effect to contrast with conventional management approaches (selection and shelterwood cuts). As part of the TLW harvest experiment, stable isotope techniques were used to trace nitrate sources and cycling in undisturbed and harvested catchments.

The objectives of this study were to: 1) present and interpret a five year record of atmospheric nitrate isotope ratios from bulk precipitation collected at the TLW, and 2) analyze $^{15}N/^{14}N$ and $^{18}O/^{16}O$ isotopic ratios of nitrate in order to investigate changes in nitrate source contributions following clear-cutting of a hardwood forest.

7.2 Methods

7.2.1 Clear-cut study site

In the summer of 1997, c31 was clear-cut as part of the TLW forest harvest experiment. Trees with a diameter at breast height (dbh) greater than 20cm were felled, delimbed, and the stems removed by skidder. In addition, all trees with a dbh of 10cm or greater were cut and left to decompose where they fell. All branches removed during delimbing were also left in the catchment at their stumps.

7.2.2 Sampling

Bulk precipitation was retrieved biweekly at a site within the TLW prior to December 1995 and from a Canadian Air and Precipitation Monitoring Network (CAPMoN) site located about 1.5km outside the watershed boundary (Figure 2.1) since that time. Precipitation was collected using a Teflon®-lined, stainless steel funnel ($0.25m^2$) that drained into a 20L jug contained within a closed cabinet below the funnel. The precipitation collection methods employed, whereby unfiltered samples can remain in the collector for up to two weeks, did not affect the isotopic composition of atmospheric nitrate (Chapter 3).

Stream samples for nitrate isotope analysis were obtained weekly at the c31 weir located at the base of the catchment. Groundwater was collected from ½" diameter, drive-point piezometers when water levels permitted, typically during snowmelt and the fall

months. Catchment 31 has lower and upper piezometer transects that intersect the stream, each consisting of six pairs of piezometers, one shallow (~40cm) and one deep (~105cm) per pair (Hazlett et al., 2001). The specific piezometers sampled during each period depended on water levels and individual recharge rates, which determined if 2L-samples could be collected in a reasonable amount of time.

An aliquot of each sample collected was filtered to 0.45μm and subsequently analyzed for a suite of chemical parameters at the Water Chemistry Laboratory at the Great Lakes Forestry Centre, Sault Ste. Marie, Ontario. Samples for nitrate isotope analysis were filtered (0.45μm) and frozen until further processing at the Environmental Geochemistry Lab (EGL) at the University of Waterloo.

7.2.3 Nitrate isotope analysis

Prior to the collection of nitrate by ion exchange methods (Section 2.2.2), samples with DOM concentrations high enough to interfere with nitrate isotope analysis were ultrafiltered (Section 2.2.1.1) or dialyzed (Section 2.2.1.2) to separate DOM from nitrate. Even for samples that had not been ultrafiltered or dialyzed, nitrate collection on the anion resin and its subsequent conversion to silver nitrate eliminated about 85% of the DOM from the final silver nitrate solution (Section 2.2.1.3). Nitrate isotope ratios were determined using breakseal or, more recently, continuous flow techniques as detailed in Sections 2.2.3 and 2.2.4.

7.3 Results and Discussion

7.3.1 Isotopic composition of atmospheric nitrate

Bulk precipitation samples collected at the TLW from 1995 to 1999 had nitrate isotope ratios

that ranged from +42.4 to +80.4‰ for $\delta^{18}O$ and -6.3 to +2.8‰ for $\delta^{15}N$ (Figure 7.1). Mass-weighted mean atmospheric nitrate $\delta^{18}O$ and $\delta^{15}N$ values were +58.1‰ and -1.7‰, respectively. The isotopic signature of atmospheric nitrate deposition at the TLW was within the overall range found by other catchment studies (Voerkelius, 1990; Durka et al., 1994; Kendall et al., 1996, Kendall, 1998; Williard et al., 2001; Burns and Kendall, 2002; Campbell et al., 2002; Schiff et al., 2002; Sickman et al., 2003). The large separation between $\delta^{18}O$ values measured for atmospheric nitrate and those calculated for nitrate produced by chemolithoautotrophic nitrification in soils (-5 to +15‰; Kendall, 1998) make nitrate isotope analysis a powerful tool for tracing nitrate sources and cycling in forested catchments.

When reanalyzed by newer isotope methods (EA, pyrolysis), it was found that two precipitation samples with depleted nitrate $\delta^{18}O$ values (+35 and +38‰) previously reported by Spoelstra et al. (2001), were contaminated with non-nitrate oxygen. The source of the extraneous oxygen was not determined but incomplete removal DOM would produce artificially low $\delta^{18}O$-nitrate values. Low nitrate waters such as precipitation are particularly susceptible to $\delta^{18}O$ contamination. Kendall et al. (1996) also found that small samples sometimes had lower than expected $\delta^{18}O$ values and hypothesized that these questionable samples could be contaminated with oxygen from DOM (Kendall, 1998). The elimination of the two samples from the TLW database does not change the interpretation of pre-harvest stream data by Spoelstra et al. (2001), causing only a minor increase in the percentage of microbial nitrate determined from source contribution calculations.

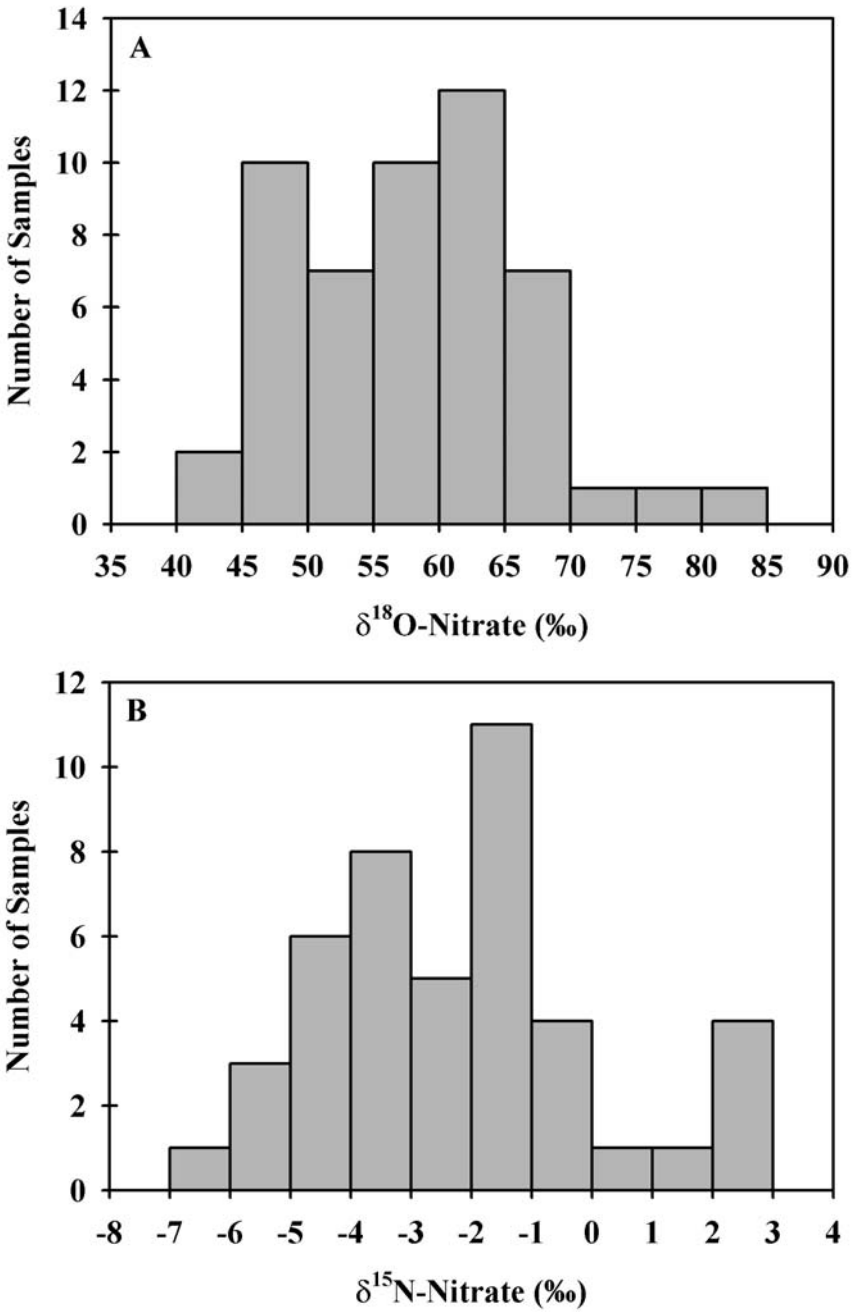


Figure 7.1 Frequency distribution diagrams for the oxygen (A) and nitrogen (B) isotopic compositions of bulk precipitation samples collected at the TLW from 1995 to 1999.

Precipitation samples for the five-year period were plotted on a generic year axis in order to elucidate seasonal trends in atmospheric nitrate isotope ratios (Figure 7.2).

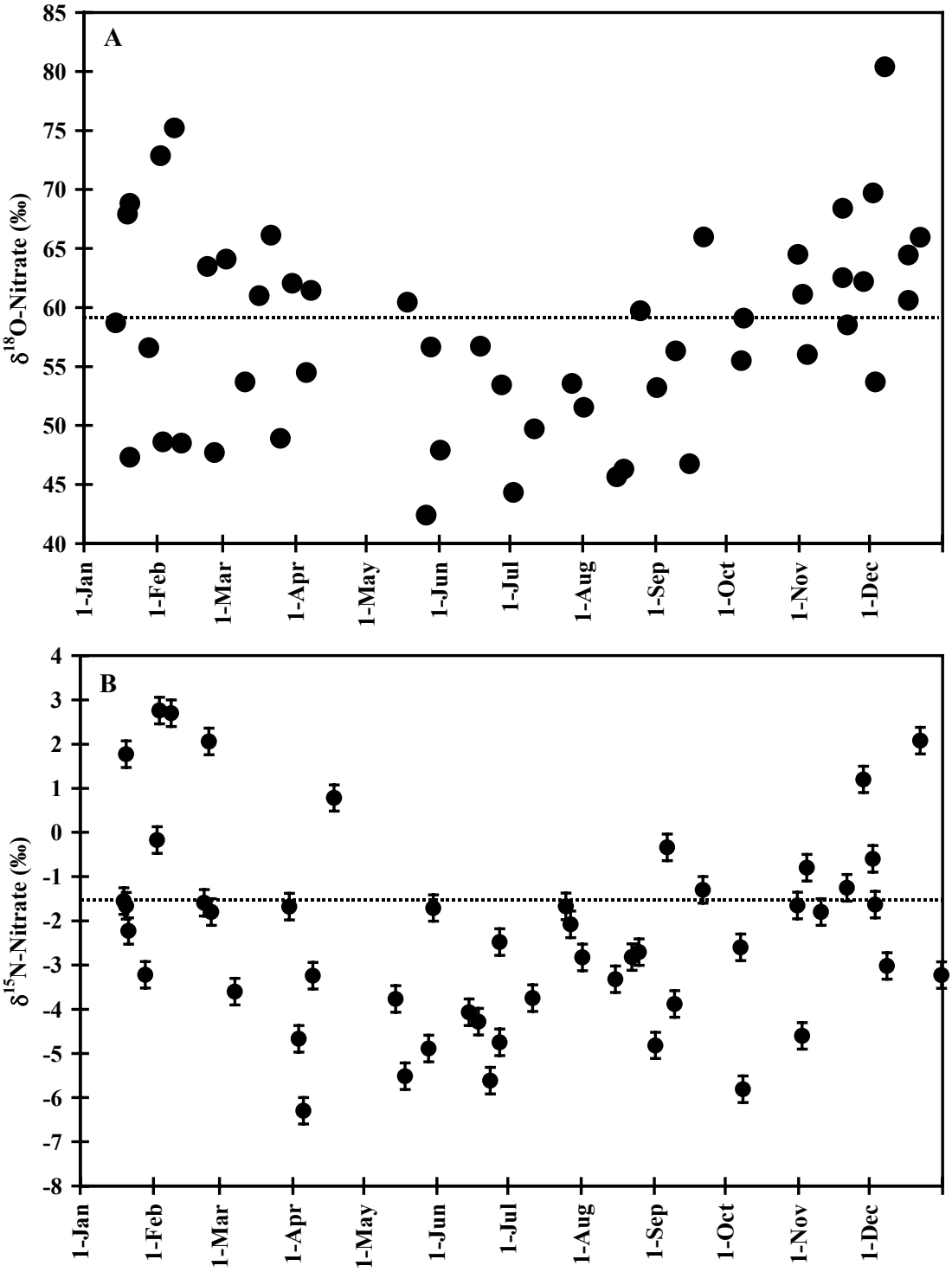


Figure 7.2 $\delta^{18}O$ (A) and $\delta^{15}N$ (B) values of atmospheric nitrate plotted on a generic year axis for bulk precipitation collected at the TLW from 1995 to 1999. A dashed line represents the mass-weighted mean value for each measured parameter. The height of the symbols in (A) and error bars in (B) indicate the analytical uncertainty for $\delta^{18}O$ (±0.8‰) and $\delta^{15}N$ (±0.3‰), respectively.

Although low nitrate $\delta^{18}O$ values occurred in all seasons, the highest values were observed in the late fall to winter period (November to February). A similar pattern was observed for $\delta^{15}N$, however, the most depleted values (<-4‰) were restricted to precipitation in the form of rain. Higher winter and lower summer trends in atmospheric nitrate $\delta^{18}O$ and $\delta^{15}N$ values have also been reported for other North American sites (Williard et al., 2001; Campbell et al., 2002).

Several other studies, using $\delta^{15}N$ only (Freyer, 1978, 1991; Heaton, 1986, 1987; Yeatman et al., 2001a), have also observed seasonal trends in atmospheric nitrate isotope ratios. Very little is known about the temporal variability in atmospheric nitrate $\delta^{18}O$ or what controls it (Kendall, 1998). Mechanisms responsible for variability in atmospheric nitrate $\delta^{15}N$ values may also be responsible for some of the variability and seasonal trends that have been recognized for $\delta^{18}O$ values. Factors known or suspected of causing $\delta^{15}N$ variation include: kinetic and equilibrium isotope effects of atmospheric nitrogen reactions, proximity to the ocean, NO_x precursor source (coal, petroleum, soil, industrial), and selective washout effects (Freyer, 1978, 1991; Heaton, 1986, 1987, 1990; Hübner, 1986; Freyer et al., 1993; Yeatman et al., 2001a,b; Russell et al., 1998).

No relationship between nitrate concentration and isotopic signature was observed for most precipitation samples (Figure 7.3). However, three samples with the highest nitrate concentrations also had the highest $\delta^{15}N$ values. These samples also had ammonium-N concentrations similar to those of nitrate-N. It is suspected that the high nitrogen contents and ^{15}N enriched nitrate are related to a distinct source of nitrogen deposition to the TLW. Nitrogen isotopic analysis of both ammonium and nitrate, in conjunction with the modeling

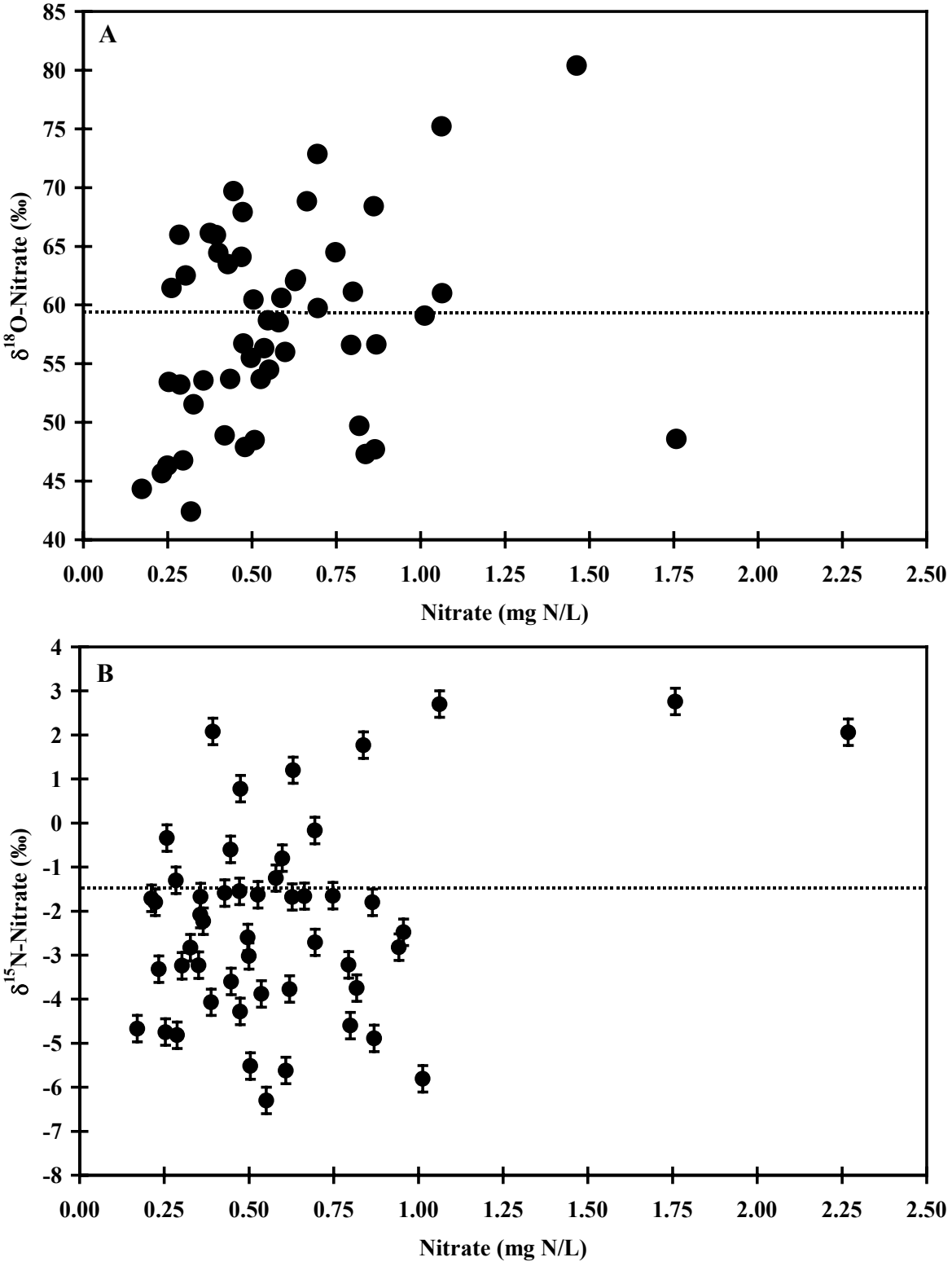


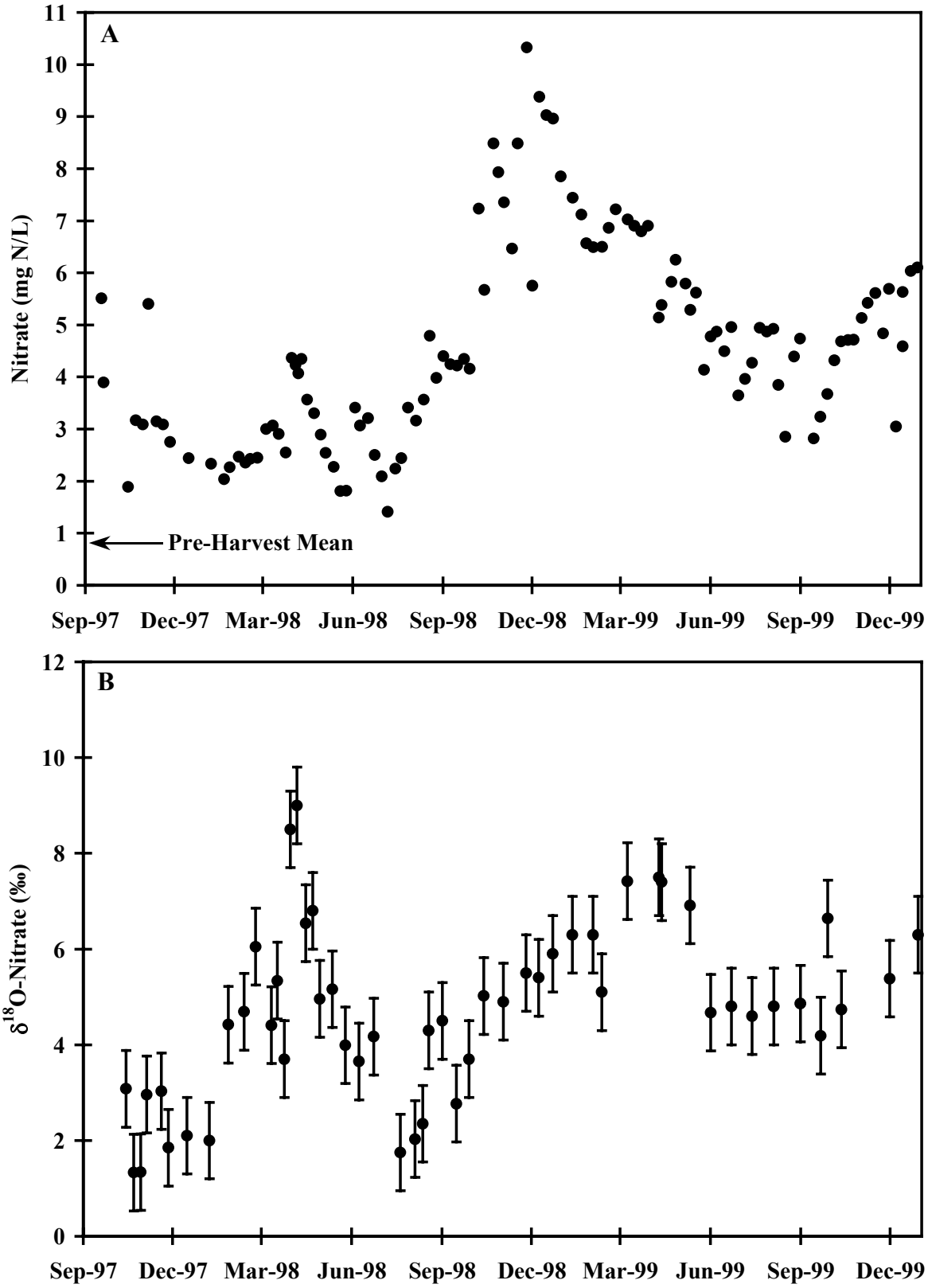
Figure 7.3 Relationship between the nitrate concentration and isotopic signature of atmospheric nitrate in bulk precipitation. A dashed line represents the mass-weighted mean value for each measured parameter. The height of the symbols in (A) and error bars in (B) indicate the analytical uncertainty for $\delta^{18}O$ (±0.8‰) and $\delta^{15}N$ (±0.3‰), respectively.

of air mass trajectories, are needed to determine if $\delta^{15}N$ analysis of precipitation can be used to trace sources of atmospheric nitrogen deposition to the TLW.

7.3.2 Nitrate concentrations in stream water

Nitrate export by stream 31 increased dramatically as a result of the clear-cut (Figure 7.4a). The mean nitrate concentration for monthly stream samples collected for nitrate isotope analysis prior to the harvest (April 1995 to July 1997) (Spoelstra et al., 2001) was 0.8 mg N/L. Post-harvest nitrate concentrations in stream 31 peaked in late November 1998, briefly reaching levels above the drinking water standard of 10 mg N/L. Nitrate concentrations subsequently decreased but remained elevated above pre-harvest levels throughout 1999. The initial pulse of nitrate in September 1997 likely resulted from the combined effects of the harvest and a drought that occurred at the TLW that summer. Elevated nitrate concentrations are often observed in TLW headwater streams for a couple of days immediately following a significant post-drought rain event, presumably caused by a sudden flushing of nitrate that was produced and stored in the soil (Creed et al., 1996). Tree removal in c31 increased the amount of nitrate available to be flushed following the drought, resulting in a stream nitrate concentration of 5.5 mg N/L on 17-Sep-1997 compared to 1.3 mg N/L in the stream draining a non-harvested reference catchment (c47).

Spikes in stream 31 nitrate concentration were observed during the snowmelt period in 1998 and, to a lesser extent, 1999. Elevated nitrate concentrations in streams during snowmelt are also found in undisturbed TLW catchments (Creed et al., 1996) and the pattern is typical of other forested basins in the temperate zone (e.g. Shepard et al., 1990; Martin et al., 2000). These episodic increases are due to the release of nitrate from the snowpack, the


A
Nitrate (mg N/L)
Pre-Harvest Mean
B
δ18O-Nitrate (‰)
Sep-97
Dec-97
Mar-98
Jun-98
Sep-98
Dec-98
Mar-99
Jun-99
Sep-99
Dec-99

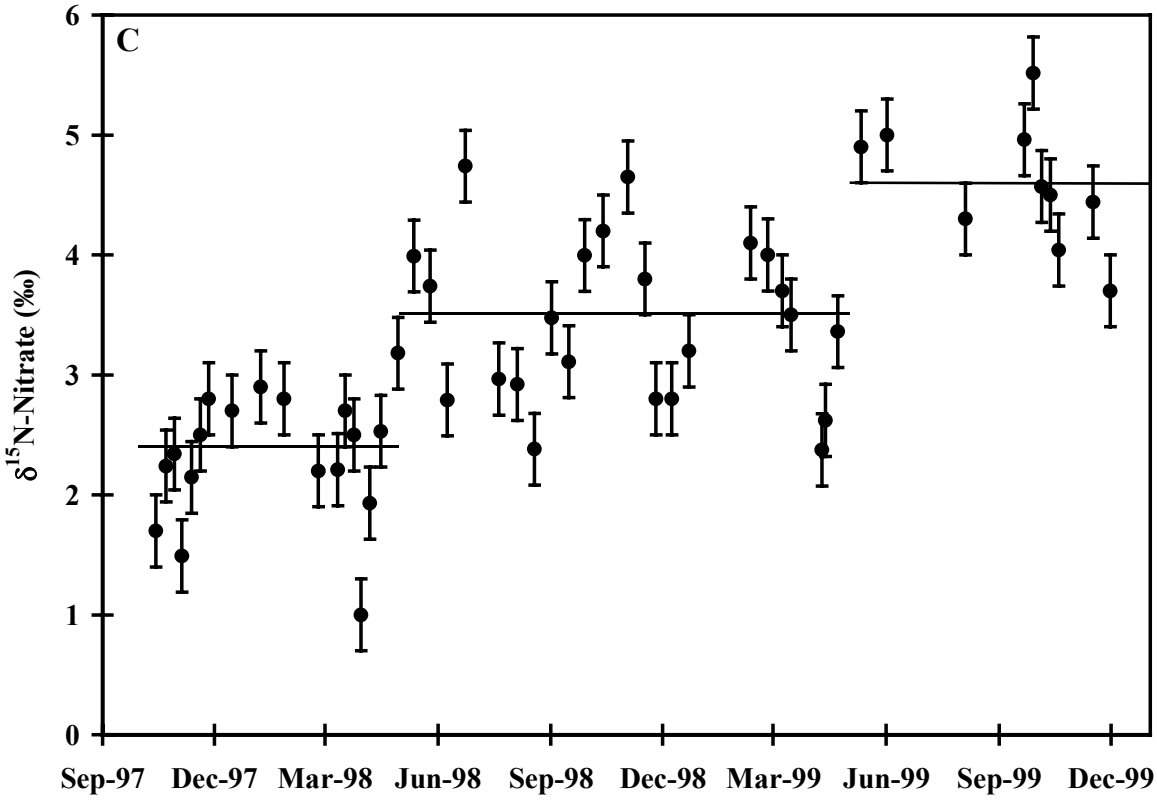


Figure 7.4 Nitrate concentration (A) and isotopic composition (B,C) for stream 31 samples collected at the catchment outlet. Error bars indicate the analytical uncertainty for $\delta^{18}O$ (±0.8‰) and $\delta^{15}N$ (±0.3‰), respectively. The mean $\delta^{15}N$ values for September 1997 to April 1998, May 1998 to April 1999, and May to December 1999, not including two snowmelt samples in both years, are indicated by horizontal lines for each period.

flushing of nitrate produced by nitrification under the snowpack, and the contribution of nitrate stored in groundwater.

7.3.3 Nitrogen isotopic ratios of the nitrate pulse

The $\delta^{15}N$ of nitrate exported by stream 31 after harvest ranged from +1.0 to +5.5‰, which was similar to values found in c31 prior to the clear-cut and in a reference catchment (+0.4 to +6.3‰, Spoelstra et al., 2001). The lowest $\delta^{15}N$ values for both post-harvest years corresponded to snowmelt (Figure 7.4c) and were due to a maximum proportional contribution of atmospheric nitrate at that time, as also indicated by the $\delta^{18}O$-nitrate data

(Figure 7.4b). Stream nitrate generally became progressively more enriched in ^{15}N following the harvest, increasing from a mean of +2.4‰ (Sep 1997 to Apr 1998) to +4.6‰ (May to Dec 1999) over the study period (Figure 7.4c).

Changes in soil nitrogen cycling that resulted from the harvest could be responsible for the ^{15}N enrichment trend for nitrate in stream water collected at the c31 weir. Several enriching mechanisms are possible and include increases in: 1) ammonia volatilization, 2) soil organic nitrogen $\delta^{15}N$ values over time, 3) the proportion of ammonium nitrified, and 4) the importance of denitrification. However, shallow (~40cm) and deep (~105cm) groundwater nitrate collected in c31 did not exhibit similarly elevated $\delta^{15}N$ values (Figure 7.5). These samples, collected between November 1997 and February 2000, indicate that microbial nitrate in groundwater had $\delta^{15}N$ values generally between 2 to 3.5‰, which was similar to c31 pre-harvest values (2 to 5‰ – derived from Spoelstra et al., 2001) and to those of a non-harvested reference catchment (c47) (Figure 7.5). Therefore the ^{15}N enrichment trend for nitrate in stream water collected at the c31 weir was not the result of processes occurring in the soils or groundwater of the harvested uplands.

In Chapter 5 it was shown that plant uptake and denitrification in a small wetland near the start of stream 31 were responsible for the attenuation of high nitrate concentrations following the harvest. Although trees do not typically fractionate nitrogen isotopes significantly during nitrate uptake (Högberg, 1997), denitrification preferentially converts isotopically light nitrate to N_2 and N_2O, thereby enriching the residual nitrate in ^{15}N and ^{18}O (e.g. Böttcher et al., 1990). Several studies have shown that denitrification progressively increases the $\delta^{15}N$ and $\delta^{18}O$ values of the remaining nitrate in a ratio of approximately 2:1 (Amberger and Schmidt, 1987; Böttcher et al., 1990; Voerkelius and Schmidt, 1990; Aravena

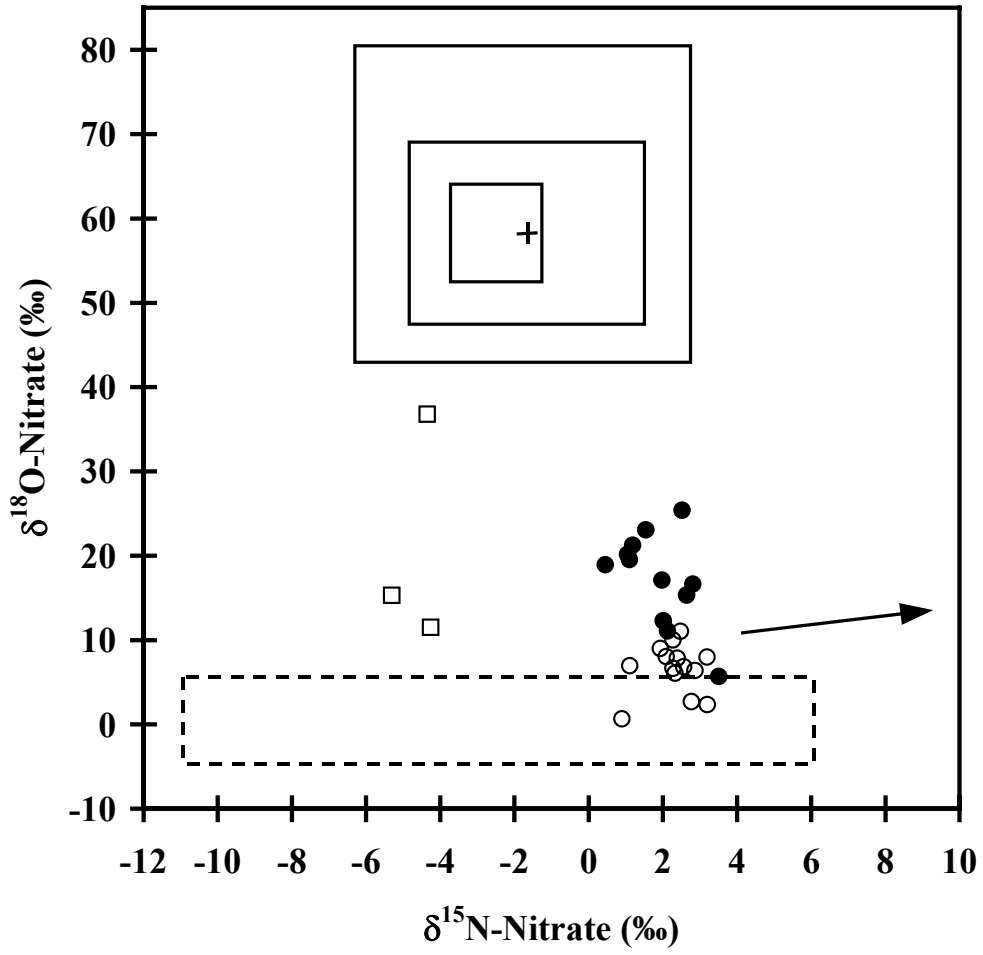


Figure 7.5 Dual isotopic ratio plot of nitrate in TLW bulk precipitation (solid-line boxes), groundwater from c31 (○) and an undisturbed reference catchment - c47 (●), and soil leachate (□) collected below the F-horizon in c31. The crosshair indicates the volume-weighted mean isotopic composition of TLW atmospheric nitrate. Solid-line boxes of increasing size delineate ranges that encompass 50, 80, and 100% of TLW precipitation samples, respectively. The dashed-line box marks the calculated $\delta^{18}O$ and observed $\delta^{15}N$ ranges for nitrate produced by nitrification in TLW soils. An arrow indicates the 2:1 enrichment slope for $\delta^{15}N$:$\delta^{18}O$ for samples affected by denitrification.

and Robertson, 1998; Cey et al., 1999; Mengis et al., 1999; Devito et al., 2000). Nitrate in stream water exiting the c31 wetland in 2000-2002 was isotopically enriched by denitrification and it was calculated that the ^{15}N enrichment effects would still be detectable

at the weir despite additional groundwater inputs of non-enriched nitrate downstream of the wetland (Section 5.4.4).

In the current study, the ^{15}N enrichment trend of nitrate collected at the c31 weir is interpreted as evidence for denitrification in the wetland starting in the first growing season following the harvest. Since the study described in Chapter 5 started in May 2000, wetland nitrate attenuation effects on stream nitrate concentration and isotopic composition in 1997-1999 were not measured. However, it was hypothesized that denitrification in the wetland could have been significant as early as the first summer post-harvest (1998) because of the high nitrate availability (Section 5.4.5). The concomitant increase in $\delta^{18}O$ values also implicates denitrification as the cause of the ^{15}N enrichment. However, the small range of $\delta^{18}O$ values (Figure 7.6) make the detection of denitrification by the 2:1 increase in $\delta^{15}N$:$\delta^{18}O$ inconclusive on its own.

Considering the drastic alteration of nitrogen cycling in c31 caused by the harvest, it was surprising that the $\delta^{15}N$ of microbial nitrate in groundwater did not change from pre-harvest values. The mass of the forest floor decreased rapidly following the harvest (N.W. Foster, per. comm.). Although erosion was undoubtedly responsible for some of this loss, much of the organic matter initially present must have been mineralized. Nitrification strongly fractionates against $^{15}NH_4^+$, producing nitrate that is isotopically depleted relative to soil organic nitrogen. Therefore, it was expected (e.g. Pardo, 1999) that ammonification and nitrification of the organic soil horizons would lead to a decrease in the $\delta^{15}N$ values of exported nitrate. In an undisturbed catchment, the TLW forest floor produced nitrate with a $\delta^{15}N$ values of -10.4 to -7.3‰ (Section 6.3.4). However, this depleted microbial nitrate was not detected in groundwater or headwater streams. It was hypothesized that production of

^{15}N-enriched nitrate in the mineral soil, and the immobilization of forest floor nitrate by the mineral soil, was responsible for the disappearance of the ^{15}N-depleted forest floor nitrate signal (Section 6.3.5).

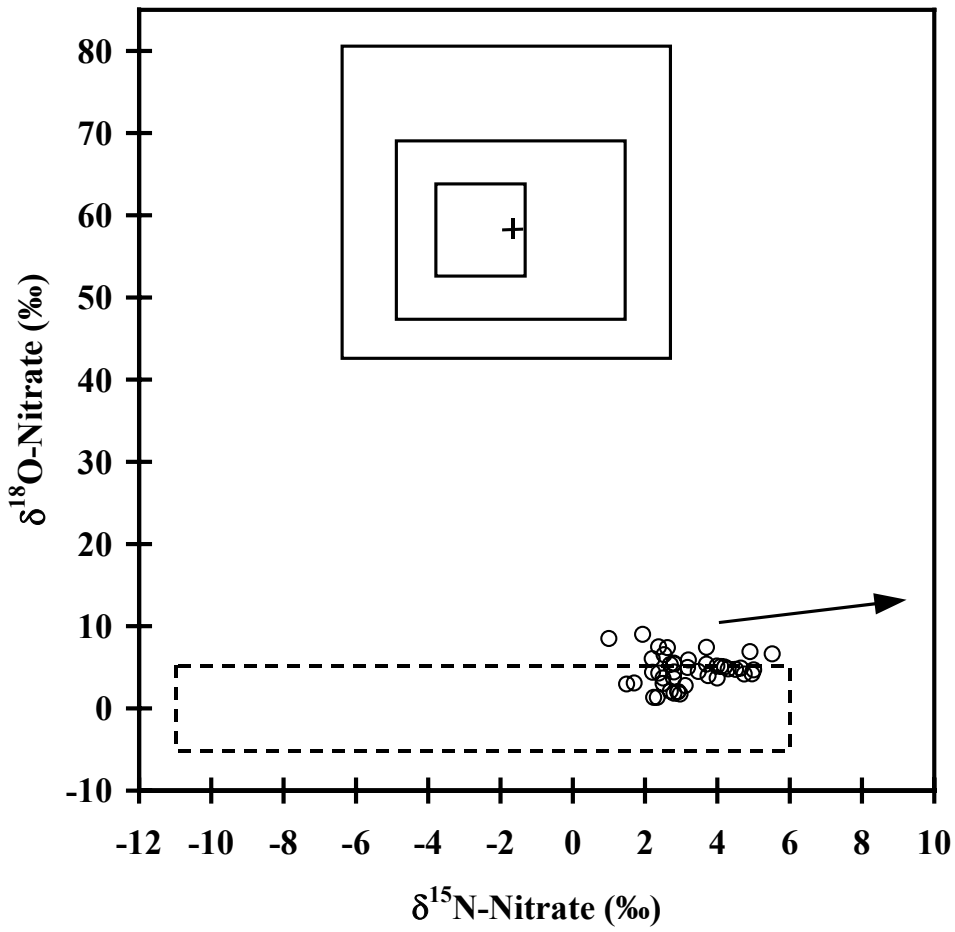


Figure 7.6 Dual isotopic ratio plot of nitrate in bulk precipitation collected at the TLW (solid-line boxes) and in stream-31 (○) during the 1997 to 1999 post-harvest period. The crosshair indicates the volume-weighted mean isotopic composition of TLW atmospheric nitrate. Solid-line boxes of increasing size delineate ranges that encompass 50, 80, and 100% of TLW precipitation samples, respectively. The dashed-line box marks the calculated $\delta^{18}O$ and observed $\delta^{15}N$ ranges for nitrate produced by nitrification in TLW soils. An arrow indicates the 2:1 enrichment slope for $\delta^{15}N$:$\delta^{18}O$ for samples affected by denitrification.

Forest floor lysimeters sampled shortly after the harvest (September and October 1997) indicated that nitrate produced in the forest floor had low $\delta^{15}N$ values (Figure 7.5). Therefore, the soil mechanisms responsible for preventing ^{15}N-depleted forest floor nitrate from being detected in groundwater and streams in undisturbed catchments were not overwhelmed, and possibly even enhanced, by high nitrate concentrations in the post-harvest period. More research on nitrate cycling in the mineral soil is needed to better understand what controls the $\delta^{15}N$ values of nitrate export at the TLW.

7.3.4 Oxygen isotopic ratios of the nitrate pulse

As expected based on concentration data alone, dual nitrate isotope analysis of stream samples indicated that the high concentrations of nitrate exported by the harvested catchment were due to nitrification. $\delta^{18}O$ values of nitrate collected at the c31 weir ranged from +1.3 to +9.0‰ with the majority of samples having values less than +5‰, within the calculated microbial nitrate end-member range (Figure 7.6). Nitrate in groundwater exhibited a similar range of $\delta^{18}O$ values (+0.7 to 11.0‰) (Figure 7.5).

The highest $\delta^{18}O$ values for stream nitrate occurred during snowmelt for each year (Figure 7.4b). This observation is consistent with trends found at the TLW during the pre-harvest period (Spoelstra et al., 2001) and indicates the highest proportional contribution of atmospheric nitrate in the stream at the time when atmospheric nitrate is eluted from the snowpack. Although not enough samples were obtained for a detailed analysis of seasonal trends in groundwater, the highest $\delta^{18}O$ values measured did correspond to snowmelt. The groundwater contribution to discharge, which is approximately 50 to 60% during peak melt (Bottomley et al., 1986), is largely responsible for diluting the isotopic signature of snowpack-derived nitrate at that time. Similar observations have been made in other

undisturbed forested basins (Kendall et al., 1996; Burns and Kendall, 2002; Schiff et al., 2002). Low stream nitrate $\delta^{18}O$ values were measured during summer baseflow conditions, indicating that groundwater was a significant source of microbial nitrate to the stream. $\delta^{18}O$-nitrate values progressively increased during the fall period, culminating in snowmelt.

If changes in the proportion of atmospheric and microbial nitrate were solely responsible for the observed isotopic variability of stream 31 nitrate then $\delta^{18}O$ values would be inversely related to nitrate concentration. However, nitrate $\delta^{18}O$ values did not exhibit a clear relationship with nitrate concentrations (Figure 7.7). In addition, given the very high nitrate concentrations that could only be supplied by large increases in microbial nitrate, the $\delta^{18}O$ values were expected to be lower, especially in the late-1998 to early-1999 period. Nitrate could have been enriched in ^{18}O by denitrification occurring in the small wetland, which was also suspected of increasing $\delta^{15}N$-nitrate values measured at the weir. In order to examine the temporal trend of microbial nitrate $\delta^{18}O$ values in the stream following harvest, the effect of denitrification and the contribution of atmospheric nitrate on the observed variability in $\delta^{18}O$-nitrate values measured at the weir must be estimated.

7.3.4.1 Isotopic effect of wetland nitrate attenuation.

$\delta^{15}N$ values of nitrate in groundwater did not exceed +3.5‰ during the post-harvest period (Figure 7.5). Without partial denitrification in wetlands, $\delta^{15}N$ values of stream nitrate are similar to groundwater nitrate (Spoelstra et al. 2001) since TLW headwater streams are largely groundwater fed. Therefore it was assumed that stream samples with $\delta^{15}N$-nitrate values higher than +3.5‰ were influenced by the effects of wetland denitrification. Based

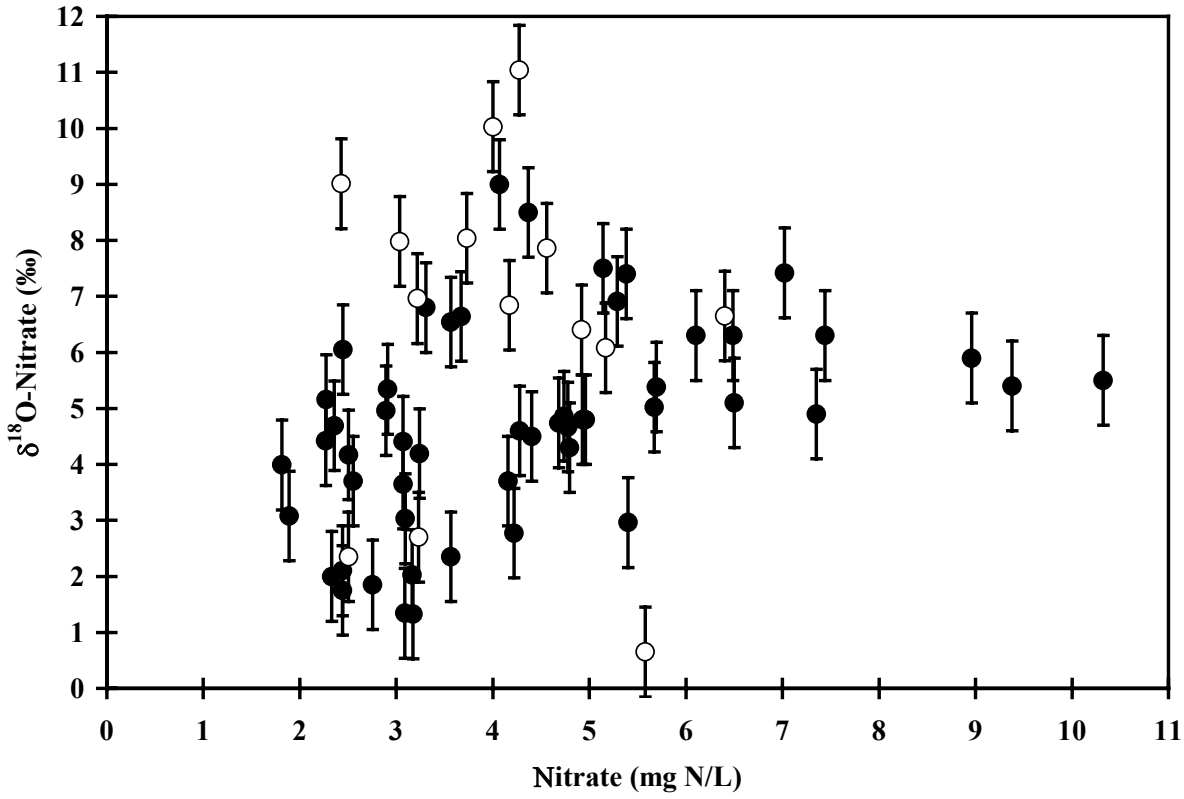


Figure 7.7 Post-harvest relationship between nitrate concentration and oxygen isotope composition for c31 stream samples collected at the weir (●) and c31 groundwater (○). Error bars indicate the analytical uncertainty for $\delta^{18}O$ (±0.8‰).

on the 2:1 increase in $\delta^{15}N$:$\delta^{18}O$ that occurs during denitrification (e.g. Böttcher et al., 1990), $\delta^{18}O$ values of the initial nitrate were estimated using Equation 7.1.

$$\delta^{18}O_i = \delta^{18}O_w - \left(\frac{\delta^{15}N_w - 3.5‰}{2} \right) \qquad 7.1$$

where:

$\delta^{18}O_i$ = $\delta^{18}O$ of stream nitrate without wetland denitrification.

$\delta^{18}O_w$ = $\delta^{18}O$ of stream nitrate collected at the weir.

$\delta^{15}N_w = \delta^{15}N$ of stream nitrate collected at the weir.

The original nitrate concentration of each sample was then estimated using a simplified Rayleigh equation (Equation 7.2). The enrichment factor used (ε_{obs} = -2‰) was based on isotopic fractionation found for c31 wetland nitrate attenuation in May 2000 (Chapter 5). The low ε_{obs} value, relative to those found for groundwater denitrification studies (e.g. -18.3‰; Mengis et al., 1999), was due to the combined effects of complete denitrification of nitrate in localized areas of the wetland and plant uptake of nitrate, both of which dilute the isotopic enrichment resulting from incomplete denitrification.

$$\delta^{18}O_w - \delta^{18}O_i = \varepsilon \ln\left(\frac{[NO_3^-]_w}{[NO_3^-]_i}\right) \qquad 7.2$$

where:

$[NO_3^-]_i$ = Stream nitrate concentration without wetland nitrate attenuation.

$[NO_3^-]_w$ = Stream nitrate concentration measured at the weir.

ε_{obs} = Observed $\delta^{18}O$ enrichment factor for c31 wetland nitrate attenuation.

7.3.4.2 Isotopic effect of atmospheric nitrate.

The contribution of atmospheric nitrate to stream nitrate export changes seasonally, reaching a maximum at springmelt (Spoelstra et al., 2001). An isotope mass balance approach (Equation 7.3 and 7.4) can be used to establish the pre-harvest pattern of atmospheric nitrate in stream 31.

$$\delta^{18}O_w = (1 - f_a)(\delta^{18}O_{mpre}) + f_a(\delta^{18}O_a) \quad 7.3$$

$$[NO_3^-]_a = f_a[NO_3^-]_w \quad 7.4$$

where:

f_a = Fraction of stream nitrate that is atmospheric

$[NO_3^-]_a$ = Concentration of atmospheric nitrate in the stream.

$\delta^{18}O_a$ = Mass-weighted mean annual $\delta^{18}O$ of atmospheric nitrate.

$\delta^{18}O_{mpre}$ = $\delta^{18}O$ of microbial nitrate in stream 31 prior to harvest.

Results calculated for 1996 (Figure 7.8) show a progressive increase in the stream concentration of atmospheric nitrate beginning in January and culminating in snowmelt at about 0.3 mg N/L. Mid-winter thaw activity is rare at the TLW. Therefore, the increased contribution of atmospheric nitrate during the dormant period is thought to result from the recharge of atmospheric nitrate to shallow groundwater during fall storms when water tables are high, which subsequently supplies baseflow to streams during the winter. Atmospheric nitrate concentrations decreased following melt, remaining steady at about 0.1 mg N/L during summer baseflow conditions.

To estimate post-harvest microbial nitrate $\delta^{18}O$ values, the 1996 seasonal trend of atmospheric nitrate in stream 31 was used with modified concentration values. Atmospheric nitrate concentrations in stream 31 could have increased as a result of the harvest since soil compaction and removal of vegetation can increase overland flow (Elliot et al., 1998).

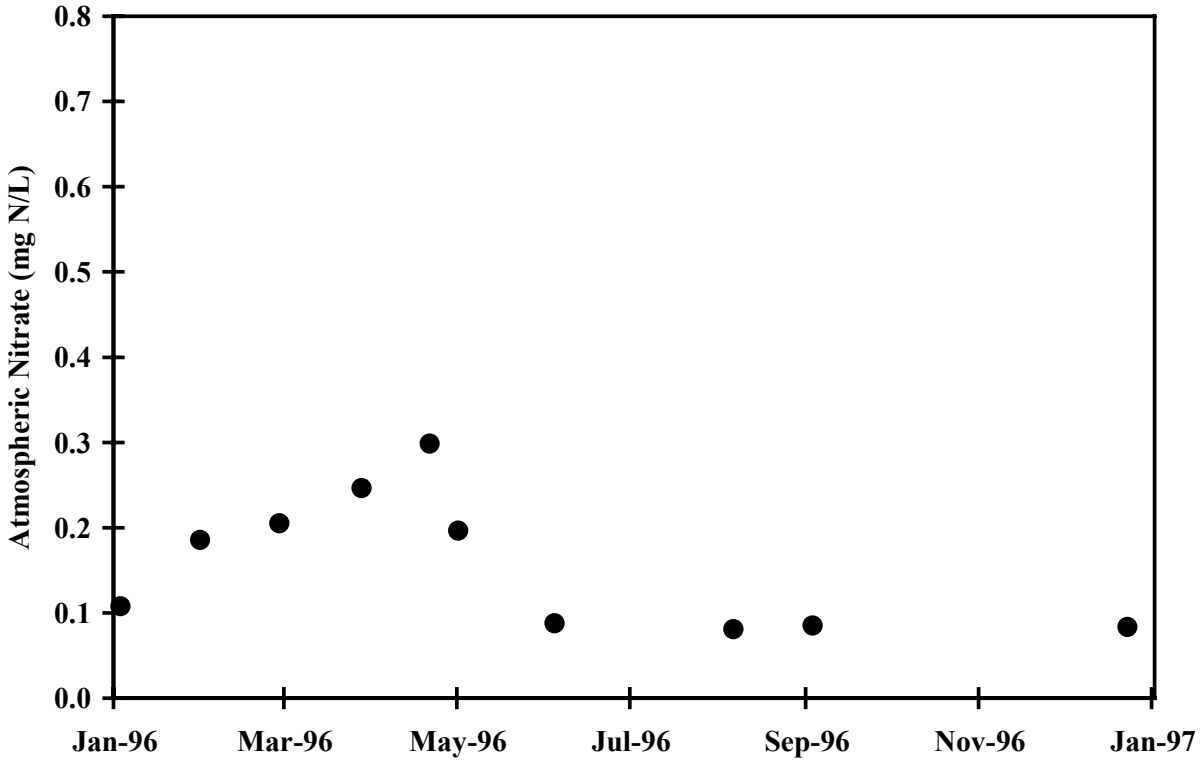


Figure 7.8 Calculated concentration of atmospheric nitrate at the outlet of TLW catchment 31 prior to harvest.

Experimenting with various atmospheric nitrate concentration scenarios demonstrated that the calculated microbial nitrate $\delta^{18}O$ values for the later post-harvest period (>September 1998) were relatively insensitive to changes in the assumed stream concentration of atmospheric nitrate. This is not surprising considering the concentration of microbial nitrate was up to two orders of magnitude greater than atmospheric nitrate in the stream. For example, in November 1998, doubling the assumed concentration of atmospheric nitrate in the stream from 0.1 to 0.2 mg N/L only changed the calculated microbial nitrate $\delta^{18}O$ by -0.6‰. Therefore, as a conservative estimate of microbial nitrate $\delta^{18}O$ values, it was assumed that the concentration of atmospheric nitrate in stream 31 doubled from pre-harvest

levels. However, this scenario produced $\delta^{18}O$ values that were as low as -13‰ prior to snowmelt 1998 (data not shown), values lower than the theoretically microbial nitrate range (Figure 7.5). This discrepancy indicated that the assumed atmospheric nitrate concentration was too high for the early post-harvest period. When pre-harvest atmospheric nitrate concentrations in stream 31 were assumed for the period prior to snowmelt 1998, reasonable microbial nitrate $\delta^{18}O$ values were calculated.

$$\left[NO_3^-\right]_i\left(\delta^{18}O_i\right) = \left(\left[NO_3^-\right]_i - \left[NO_3^-\right]_a\right)\left(\delta^{18}O_{mpost}\right) + \left[NO_3^-\right]_a\left(\delta^{18}O_a\right) \quad 7.5$$

where:

$(\delta^{18}O_{mpost})$ = $\delta^{18}O$ of microbial nitrate in stream 31 post-harvest.

$([NO_3^-]_i - [NO_3^-]_a)$ = Concentration of microbial nitrate in the stream.

The atmospheric nitrate concentration scenario used for the calculation of post-harvest microbial nitrate $\delta^{18}O$ values by mass balance (Equation 7.5) is shown in Figure 7.9. The high atmospheric nitrate concentration during peak melt (0.6 mg N/L) is not unrealistic considering that the volume-weighted mean concentration of nitrate in wet-plus-dry deposition is 0.57 mg N/L (derived from Sirois et al., 2001) and that nitrate stored in the snowpack can be concentrated by preferentially elution during the early melt period (Cadle et al., 1984; Semkin and Jeffries, 1986, 1988).

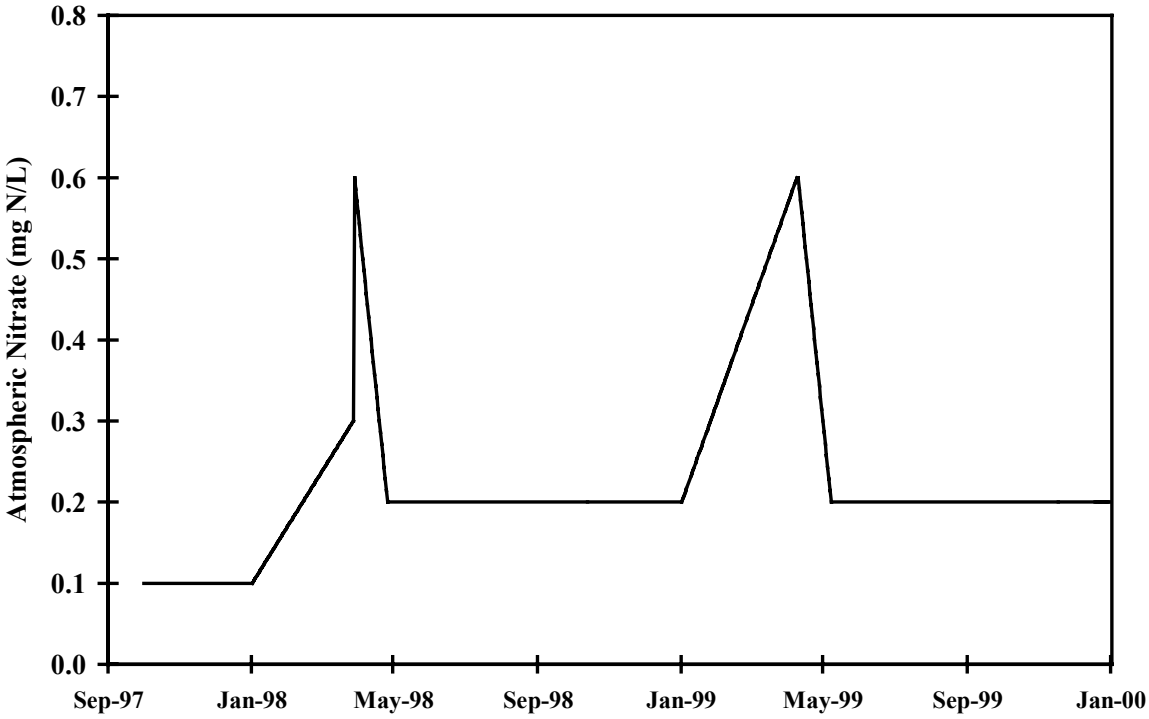


Figure 7.9 Hypothetical post-harvest concentration of atmospheric nitrate in stream 31 used to calculate the microbial nitrate $\delta^{18}O$ values shown in Figure 7.10 and 7.11.

7.3.5 $\delta^{18}O$ values of microbial nitrate in stream 31

Results of the calculations (Figure 7.10) indicate that $\delta^{18}O$ values of microbial nitrate in stream 31 were indeed increasing throughout the post-harvest period. Therefore, the trend in $\delta^{18}O$-nitrate values measured at the weir was not strictly due to changes in the proportion of nitrate from the two sources or from the effects of denitrification in the upstream wetland. Microbial nitrate could be enriched in ^{18}O by several mechanisms including: 1) an increase in the contribution of nitrification by heterotrophs, 2) evaporative enrichment of soil water, and 3) an increase in the proportion of summer nitrification.

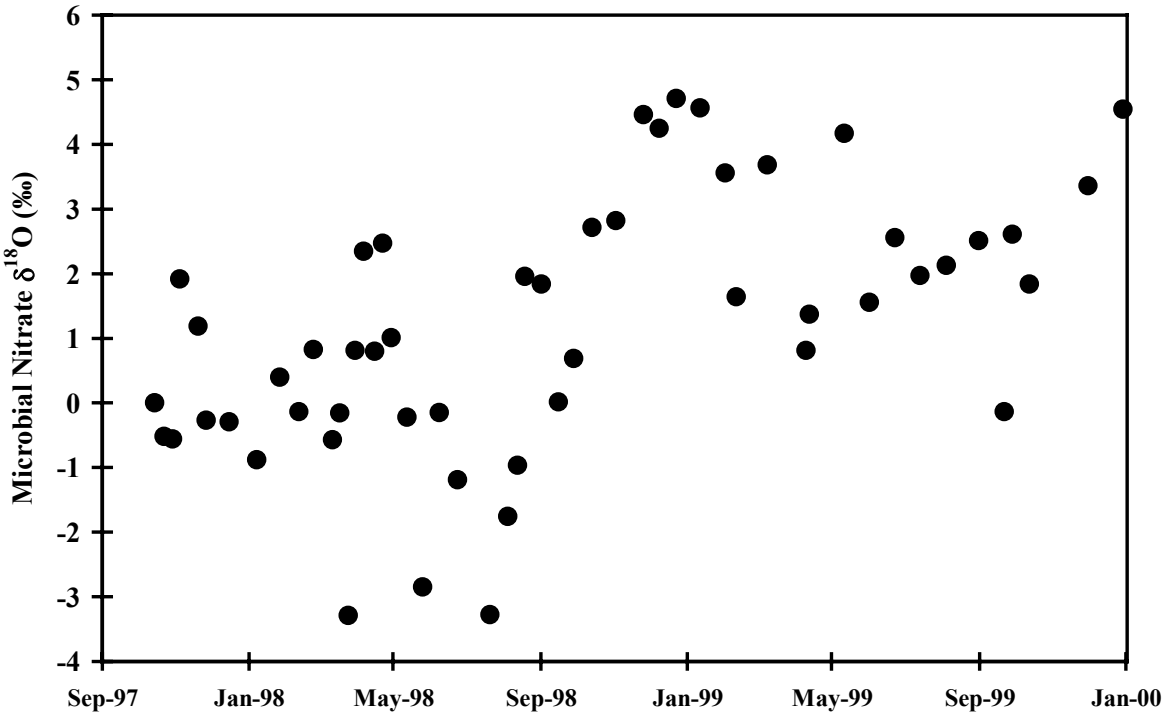


Figure 7.10 Calculated $\delta^{18}O$ values for microbial nitrate exported by stream 31 during the post-harvest period (1997-1999).

Although low $\delta^{18}O$ values for microbial nitrate indicate that nitrification was predominately by chemolithoautotrophs, a small but increasing contribution from heterotrophic nitrifiers cannot be ruled out (Chapter 6). Several studies have shown nitrate production by autotrophic and heterotrophic pathways can occur simultaneously in forest soils (Schimel et al., 1984; Duggin et al., 1991; Barraclough and Puri, 1995; Hart et al., 1997; Pedersen et al., 1999). The balance between the two types of nitrification can shift significantly as a result of forest harvest and both directions of change have been documented (Schimel et al., 1984; Duggin et al., 1991; Pedersen et al., 1999).

Heterotrophic nitrification derives two to three of the oxygens on the nitrate molecule from soil organic matter (Mayer et al., 2001). DOM in leaf leachate was found to have a

$\delta^{18}O$ ranging from +23.4 to +25.3‰ at a site in Ontario, Canada (Humphries, 2003). Therefore nitrate produced by heterotrophs is expected to be significantly enriched in ^{18}O relative to that produced by chemolithoautotrophs. As a result, an increase in the ratio of heterotrophic to autotrophic nitrification following harvest would increase microbial nitrate $\delta^{18}O$ values.

Duggin et al. (1991) found that heterotrophs were better at nitrifying older organic matter and that reduced inputs of fresh litter following harvest could increase the contribution of heterotrophic nitrification. The proportion of nitrification by heterotrophs would be highest in the later post-harvest period, once most of the relatively labile organic nitrogen had already been nitrified by chemolithoautotrophs. Therefore, if heterotrophic nitrification were responsible for the oxygen isotope trend of microbial nitrate, the highest $\delta^{18}O$ values would have occurred after the peak in stream nitrate concentration (November 1998). Contrary to this hypothesis, microbial nitrate $\delta^{18}O$ values exhibited a positive relationship with nitrate concentration (Figure 7.11). Although an increase in heterotrophic may have occurred as a result of the TLW harvest, it does not appear to be the principle mechanism responsible for the microbial nitrate $\delta^{18}O$ trend.

Increased evaporative enrichment of soil water in the upper soil layers is another process that could have increased $\delta^{18}O$ values of microbial nitrate produced following the harvest. Removal of the canopy results in higher soil temperatures, which could have increased evaporation and soil water $\delta^{18}O$ values. Evaporative enrichment of soil water would be limited mainly to the forest floor since the effect decreases rapidly with depth (e.g. Allison, 1983). Microbial nitrate generated in the forest floor is depleted in ^{15}N compared to microbial nitrate found in TLW groundwater and streams (Section 6.3.5). Therefore, if

microbial nitrate produced from evaporatively enriched water in the forest floor were responsible for the increase in microbial nitrate $\delta^{18}O$ values over time, it would be expected that microbial nitrate $\delta^{15}N$ values would also decrease. However, as previously discussed, the $\delta^{15}N$ of microbial nitrate in groundwater did not change as a result of the harvest. Therefore, although it may have contributed, evaporative enrichment of soil water does not appear to be the dominant mechanism responsible for increased microbial nitrate $\delta^{18}O$ values.

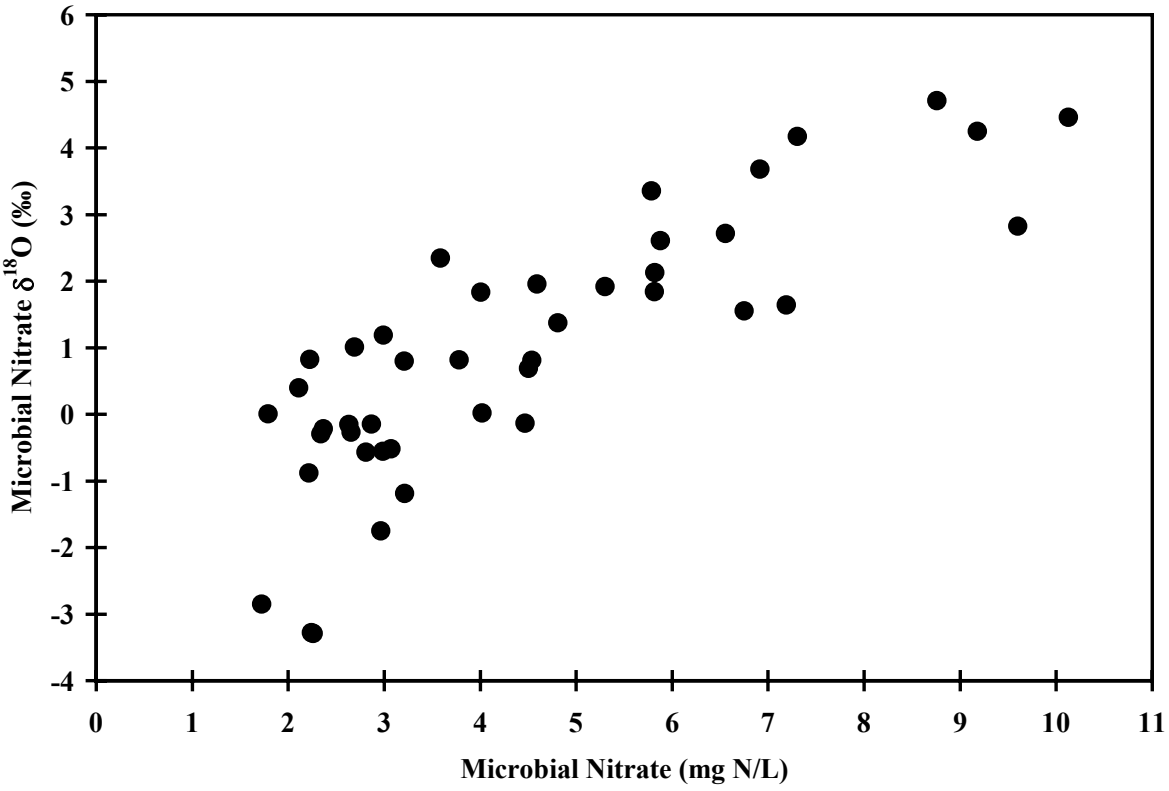


Figure 7.11 Relationship between calculated nitrate concentration and $\delta^{18}O$-nitrate values for microbial nitrate exported by stream 31 during the post-harvest period (1997-1999).

A third possible cause of the microbial nitrate $\delta^{18}O$ trend is an increase in the proportion of nitrate in the stream from nitrification occurring during the summer months (summer nitrate). The $\delta^{18}O$-H_2O of meteoric water at the TLW, which ultimately controls the $\delta^{18}O$-H_2O of soil water available to nitrifiers, reaches minimum values of about -20‰ as snow and is highest during the summer at about -4.5‰ (Bottomley et al., 1986). The range in precipitation $\delta^{18}O$-H_2O determines the expected range in $\delta^{18}O$ values of microbial nitrate produced at the TLW (-5.7 to +4.7‰, Figure 7.5), as calculated using Equation 6.1. Therefore, a greater fraction of summer nitrate in the stream would increase in the average $\delta^{18}O$ values of microbial nitrate leached to groundwater and exported by streams. The proportion of summer nitrate exported from the catchment could increase due to increased summer nitrification activity and/or an increase in the transport of summer nitrate to the stream.

Since the deciduous trees that dominate the TLW are inactive during winter, their absence due to the harvest does not likely affect nitrification rates significantly in the dormant period. Even if winter nitrification activity did increase, forest harvest would have a greater impact on soil microbial processes (e.g. nitrification) during the growing season when vegetative nitrogen uptake would have otherwise reduced inorganic nitrogen availability in the soil. Forest harvest has been shown to increase soil temperatures, increase ammonium availability, and reduce soil moisture limitations on nitrification activity (e.g. Likens et al., 1970), all of which could lead to greater summer nitrification. Stream discharge during the summers of 1998 and 1999 was also significantly greater than pre-harvest years (F.D. Beall, per. comm.), providing evidence that the transport of summer nitrate to the stream increased as a result of the harvest.

An increase in the ratio of summer to dormant season nitrate in stream 31 was likely the principal mechanism responsible for the greater $\delta^{18}O$ values of microbial nitrate exported from catchment 31 following the harvest. Microbial nitrate $\delta^{18}O$ values that plateau near the expected values for summer nitrification at the TLW (+4.7‰), and are positively correlated to microbial nitrate concentration (Figure 7.11), provide support for this hypothesis.

The delay between production of ^{18}O-enriched summer microbial nitrate and its appearance in the stream depends on several factors including hydrology and groundwater residence time. Peak values during the 1998/1999 winter (Figure 7.10) likely resulted from nitrate production during the summer, which was recharged to groundwater during fall rains and subsequently discharged to the stream as baseflow during the dormant period. Higher microbial nitrate $\delta^{18}O$ values in groundwater then sustained the elevated trend through the summer of 1999.

7.3.6 Implications of the microbial end-member $\delta^{18}O$ shift

The fact that the $\delta^{18}O$ of microbial nitrate in stream 31 shifted throughout the post-harvest period complicates nitrate source contribution calculations. The concentration of atmospheric nitrate exported by stream 31 was expected to increase in the post-harvest period because of increased surface runoff. However, quantification of the contribution of atmospheric nitrate using $\delta^{18}O$ analysis could not be accomplished because of the temporal variability in the $\delta^{18}O$ values of the microbial nitrate source. Calculations done to estimate microbial nitrate $\delta^{18}O$ values did reveal that atmospheric nitrate concentrations in stream 31 could not have increased significantly prior to the 1998 snowmelt. Future studies using the dual nitrate isotope approach to study nitrate sources in forested catchments will need to

consider how temporal variability in the $\delta^{18}O$ of the microbial nitrate end-member could affect interpretation of the $\delta^{18}O$ values of nitrate export.

7.4 Conclusions

Atmospheric nitrate deposition collected biweekly at the TLW from 1995 to 1999 had an isotopic composition within the range found in other studies (Kendall, 1998), ranging from +42.4 to +80.4‰ and -6.3 to +2.8‰ for $\delta^{18}O$ and $\delta^{15}N$, respectively. The most enriched isotopic ratios for both oxygen and nitrogen occurred for winter precipitation. Seasonal trends in atmospheric nitrate have been observed at other North American sites (Williard et al., 2001; Campbell et al., 2002) and these patterns are likely caused by seasonality in the sources of nitrate to the atmosphere or in atmospheric processes involved in nitrate deposition. Future research involving the modeling of air mass trajectories and the isotopic analysis of atmospheric nitrate and ammonium will provide insights into the processes responsible for seasonal variation in the isotopic composition of nitrogen deposition.

Clear-cutting of catchment 31 resulted in elevated nitrate concentrations in surface and ground waters, peaking at above 10 mg N/L in the stream at the catchment outlet. Low stream nitrate $\delta^{18}O$ values (+1.3 to +9.0‰) indicated that nitrate was predominantly produced by chemolithoautotrophic nitrification in forest soils. $\delta^{18}O$ values of microbial nitrate increased in stream 31 following the harvest. An increase in the fraction of nitrate in the stream that was produced by nitrification during the summer months, when the $\delta^{18}O$-H_2O of meteoric water is higher, was likely responsible for the oxygen isotope shift of the microbial nitrate end-member.

As also indicated by the isotopic study of microbial nitrate in Chapter 6 and other recent studies (e.g. Mayer et al., 2001; Burns and Kendall, 2002), more soil nitrogen cycling research is needed before the nitrogen isotope composition of nitrate exported from forest soils can be fully interpreted. It was expected that forest harvest would lead to a pulse of ^{15}N-depleted nitrate due to mineralization of the forest floor. The lack of a decrease in the $\delta^{15}N$ values of nitrate in groundwater and streams following harvest indicates that the rate of the soil processes responsible for masking the isotopic signal of forest floor nitrate in undisturbed catchments (Section 6.3.5) must have increased to compensate for the dramatic increase in nitrate production and leaching during the post-harvest. Future studies will need to identify these soil processes since they 1) control $\delta^{15}N$ values of the microbial endmember in streams and groundwater, 2) are quantitatively significant and, 3) likely are important for the development of soil $\delta^{15}N$ profiles.

The isotopic composition of nitrate exported from c31 will continue to be monitored in the latter post-harvest period (2000 +). As vegetative regrowth increases nitrogen demand in c31, growing season nitrification rates will decline due to lower ammonium availability. The $\delta^{18}O$ of microbial nitrate in stream 31 is also expected to decline as the ratio of growing season to dormant season nitrification decreases. However, the $\delta^{18}O$ of nitrate measured at the weir (mixture of both sources) could increase as nitrate concentrations return to pre-harvest levels and the relative proportion of atmospheric nitrate in stream water subsequently increases.

Chapter 8. Conclusions and recommendations

The overall goal of this thesis research was to investigate the use of oxygen and nitrogen isotopic ratios of nitrate for providing information on nitrate sources and cycling in forested watersheds. At the start of the TLW nitrate isotope study in 1995, previous application of the dual nitrate isotope technique to forested catchments was limited, largely because of the low nitrate and high DOM concentrations in these waters. This thesis presents results and interpretation of isotopic analyses, including nitrate ($\delta^{15}N$, $\delta^{18}O$), soil O_2 ($\delta^{18}O$), vegetation ($\delta^{15}N$), and soils ($\delta^{15}N$), for samples collected between April 1995 and June 2002.

8.1 Main conclusions and contributions to research.

1) Methods for the separation of DOM and nitrate.

Oxygen contamination from DOM is the most important factor limiting application of the dual nitrate isotope technique in forested watersheds. This thesis describes new ultrafiltration and dialysis methods used to separate DOM and nitrate in water samples. These techniques permitted the accurate determination of $\delta^{18}O$-nitrate values, even in samples such as forest floor lysimeters with DOC as high as 34.0 mg C/L. Nitrate retention by the anion resin used to collect the nitrate also removed about 85% of the residual DOM. Therefore, overall DOM removal ranged from about 85% to greater than 99%. Use of the ultrafiltration and dialysis methods for DOM removal will significantly extend the range of sites where $\delta^{18}O$-nitrate can be determined.

2) Assessment of the isotopic stability of nitrate in precipitation collectors.

A common assumption made when collecting precipitation for the analysis of nitrate isotope ratios is that the $\delta^{15}N$ and $\delta^{18}O$ values of atmospheric nitrate are not affected by microbial reactions during the collection period. An incubation experiment using bulk precipitation collected at the TLW is the first study to directly test this assumption. Results indicated that nitrate $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios in unfiltered bulk precipitation collected at the TLW were not altered by unfiltered storage in the collectors for up to two weeks at summer temperatures. The results of this study confirm the validity of current precipitation collection methods for the analysis of atmospheric nitrate isotope ratios at the TLW and are also relevant to past and future studies using stable isotopes to trace nitrate sources in forested watersheds.

3) Sources of nitrate export in an undisturbed, N-saturated, old-growth forest.

$\delta^{18}O$ analysis provided excellent isotopic separation between atmospheric and nitrification sources of nitrate at the TLW. Although $\delta^{15}N$ values of the two nitrate sources overlap ($\delta^{15}N_{atm}$ = -6.3 to +2.8‰, $\delta^{15}N_{microbial}$ = -10.4 to +6‰), atmospheric nitrate was highly enriched in ^{18}O ($\delta^{18}O_{atm}$ = +42.4 to +80.4‰) compared to the range calculated for microbial nitrate at the TLW ($\delta^{18}O_{microbial}$ = -5.7 to +4.7‰). The isotopic composition of nitrate accumulated by bulk atmospheric deposition collectors at the TLW was within the range found in other studies ($\delta^{15}N$ = -6 to +10‰, $\delta^{18}O$ = +14 to +76‰; Kendall, 1998). Nitrate export from undisturbed first-order stream basins was dominated by nitrate produced by nitrification in forest soils, even during the snowmelt period when the contribution of atmospheric nitrate in streams peaked at about 30%. Therefore, the detrimental effects of

nitrate export during snowmelt, such as episodic acidification, cannot be solely attributed to atmospheric nitrate accumulated in the snowpack. Other nitrate isotope studies have also documented the importance of microbial nitrate during snowmelt in forested watersheds (e.g. Kendall et al., 1996). Spoelstra et al. (2001) was the first study of nitrate isotope ratios in a forested watershed in Canada. Previous studies have scaled-up plot-scale measurements to determine that 60 to 83% of nitrate exported from the TLW annually is generated by nitrification in soils (Foster et al., 1989a; Foster et al., 1992). Analysis of nitrate isotope ratios in streams and groundwaters provided new information on the temporal dynamics of nitrate sources at the TLW.

4) $\delta^{18}O$ values of forest soil O_2.

The equation used to calculate the $\delta^{18}O$ of nitrate produced by chemolithoautotrophic nitrification requires the $\delta^{18}O$ value of the O_2 available to nitrifiers in the soil. Although shallow soil gas is assumed to have $\delta^{18}O$-O_2 values similar to atmospheric O_2, respiration could increase soil $\delta^{18}O$-O_2 values by preferentially utilizing $^{16}O^{16}O$. Isotopic analysis of soil gas O_2 collected from 10 to 70cm below the surface of the forest floor indicated that respiratory enrichment of ^{18}O was negligible and did not affect the $\delta^{18}O$ values of nitrate produced by nitrification in TLW soils. Therefore, an atmospheric $\delta^{18}O$-O_2 value of +23.5‰ can be assumed when calculating the $\delta^{18}O$ values of nitrate produced by chemolithoautotrophic nitrification. This is the first study to examine soil $\delta^{18}O$-O_2 values in conjunction with $\delta^{18}O$ analysis of nitrate produced by nitrification.

5) An in situ measurement of the isotopic composition of microbial nitrate.

The $\delta^{18}O$ values the microbial nitrate end-member is calculated based on laboratory experiments that indicate one of the oxygens comes from O_2 and two from H_2O. Chapter 6 is the first *in situ* study to directly measure the isotopic composition of microbial nitrate produced in forest soils. A precipitation exclusion apparatus and soil lysimeters were used to collect nitrate produced by nitrification in the TLW forest floor. Oxygen isotopic ratios of this microbial nitrate were generally only slightly enriched compared to values expected for chemolithoautotrophic nitrification. Therefore, these results provide direct evidence that the lab-based calculation of microbial nitrate $\delta^{18}O$ values is also applicable for determining the $\delta^{18}O$ values of nitrate produced by chemolithoautotrophic nitrification in forest soils. Forest floor microbial nitrate was depleted in ^{15}N ($\delta^{15}N$ = -10.4 to -7.3‰) relative to soil organic nitrogen ($\delta^{15}N$ = -2.7 to +0.1‰) due to a strong isotopic fractionation against $^{15}NH_4^+$ that occurs during nitrification. The $\delta^{18}O$ of nitrate in streams and groundwater also reached values calculated for the microbial end-member.

6) The isotopic composition of a nitrate pulse resulting from forest harvest.

Although many previous studies have examined the effects of forest harvest on nitrate export, this is the first study to combine a forest harvest experiment with isotopic analysis of the resulting nitrate pulse to determine nitrate source contributions. Following clear-cutting, low $\delta^{18}O$-nitrate values (+1.3 to +9.0‰) revealed that stream 31 was dominated by nitrate produced by chemolithoautotrophic nitrification. A maximum contribution of atmospheric nitrate was observed during snowmelt, however, the associated increase in stream nitrate $\delta^{18}O$ values were dampened by high concentrations of microbial nitrate also exported during

post-harvest snowmelt periods. The $\delta^{18}O$ of microbial nitrate increased in the stream following harvest, likely due to an increase in the ratio of growing season to dormant season nitrification. Drastic alteration of nitrogen cycling caused by clear-cutting of c31 did not change the $\delta^{15}N$ values of groundwater nitrate.

7) Disappearance of ^{15}N-depleted forest floor nitrate.

Nitrate produced in the forest floor was very depleted in ^{15}N (-10.4 to -7.3‰) compared to nitrate in groundwater and streams (+0.4 to +7.5‰). It is hypothesized that immobilization and assimilation of forest floor nitrate in the mineral soil were responsible for the disappearance of the depleted forest floor nitrate signature. Since the $\delta^{15}N$ values of microbial nitrate in groundwater did not change as a result of harvest, the rates of the mechanisms responsible for the change in nitrate $\delta^{15}N$ must have also increased to compensate for the increased leaching of ^{15}N-depleted nitrate from the forest floor. Therefore, the study of nitrate isotope ratios provided evidence for a previously unrecognized mechanism that strongly influences the $\delta^{15}N$ values of nitrate export at the TLW.

8) Wetland nitrate attenuation following clear-cutting of a hardwood catchment.

The isotopic composition, particularly $\delta^{15}N$, of nitrate exported from catchment 31 was affected by nitrate attenuation in a small wetland following the clear-cut. The c31 wetland is a forested swamp (0.2ha) that is too small to show up on maps that are based on aerial photography or satellite imagery. Despite only accounting for 4% of the catchment area, denitrification and plant nitrate uptake in the wetland reduced catchment nitrate export during the study periods by 35 to 80%. If the wetland were located closer to the catchment

outlet, rather than near the start of the stream, the nitrate attenuation effects would have been even more dramatic. Therefore, computer models that simulate nitrogen cycling in forested catchments must be able to recognize these small wetlands and account for their effects. Future forest management practices that recognize and preserve these small wetlands could significantly reduce the negative impacts of nitrate export on aquatic systems following forest harvest.

8.2 Implications for modeling nitrogen cycling

A conceptual model (Figure 8.1 and 8.2) was constructed in order to summarize the current understanding of the mechanisms controlling the isotopic composition of nitrate in TLW catchments, and to facilitate interpretation of results from more detailed soil nitrogen studies already underway at the TLW. The basic unit of the model (Figure 8.1) was not meant to represent all soil nitrogen cycling reactions, only those believed to exert significant control of nitrate levels and isotopic composition in a given compartment. Boxes represent the total pools for ammonium, organic nitrogen, and nitrate in the compartment. The size of each box is not proportional to the size of the pool. In addition to internal nitrogen cycling within the compartment and plant nitrogen uptake, the model unit has ammonium and nitrate inputs from above compartments (1, 2), leaching losses (3, 4), and lateral transport to surface waters (12, 13).

The soil compartment model in Figure 8.1 was expanded to a conceptual model of the processes controlling the isotopic composition of nitrate export from TLW first-order streams (Figure 8.2). Many assumptions were made that simplified the structure of the model, including:

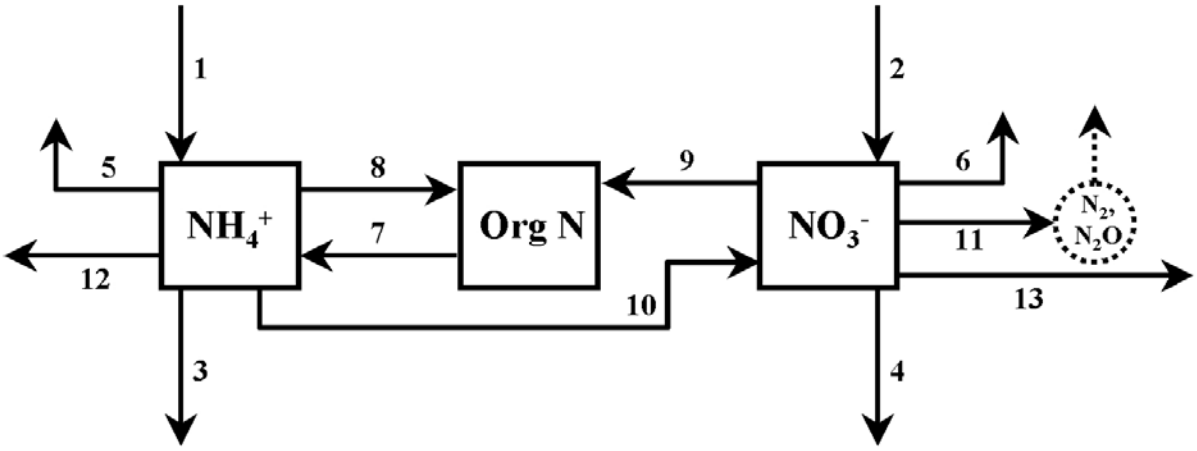


Figure 8.1 Conceptual model of processes influencing the isotopic composition of nitrate generated in forest soils. The nitrogen cycling reactions and fluxes represented are: ammonium and nitrate inputs from above soil layers (1, 2), leaching of inorganic nitrogen (3, 4), plant assimilation (5, 6), ammonification (7), immobilization (8, 9), nitrification (10), denitrification (11), and lateral transport of N to streams (12, 13).

1) Direct input of ammonium and nitrate to headwater streams is negligible in relation to inputs that transit through at least one soil layer first. Even during snowmelt, it is assumed that most of the melt-water has some contact with the forest floor, even if just traveling through it with minimal geochemical alteration. Therefore, atmospheric nitrogen inputs enter the model through the forest floor compartment.

2) Ammonium inputs to the stream are insignificant compared to nitrate inputs and can therefore be ignored for each compartment since they do not influence the isotopic composition of nitrate export.

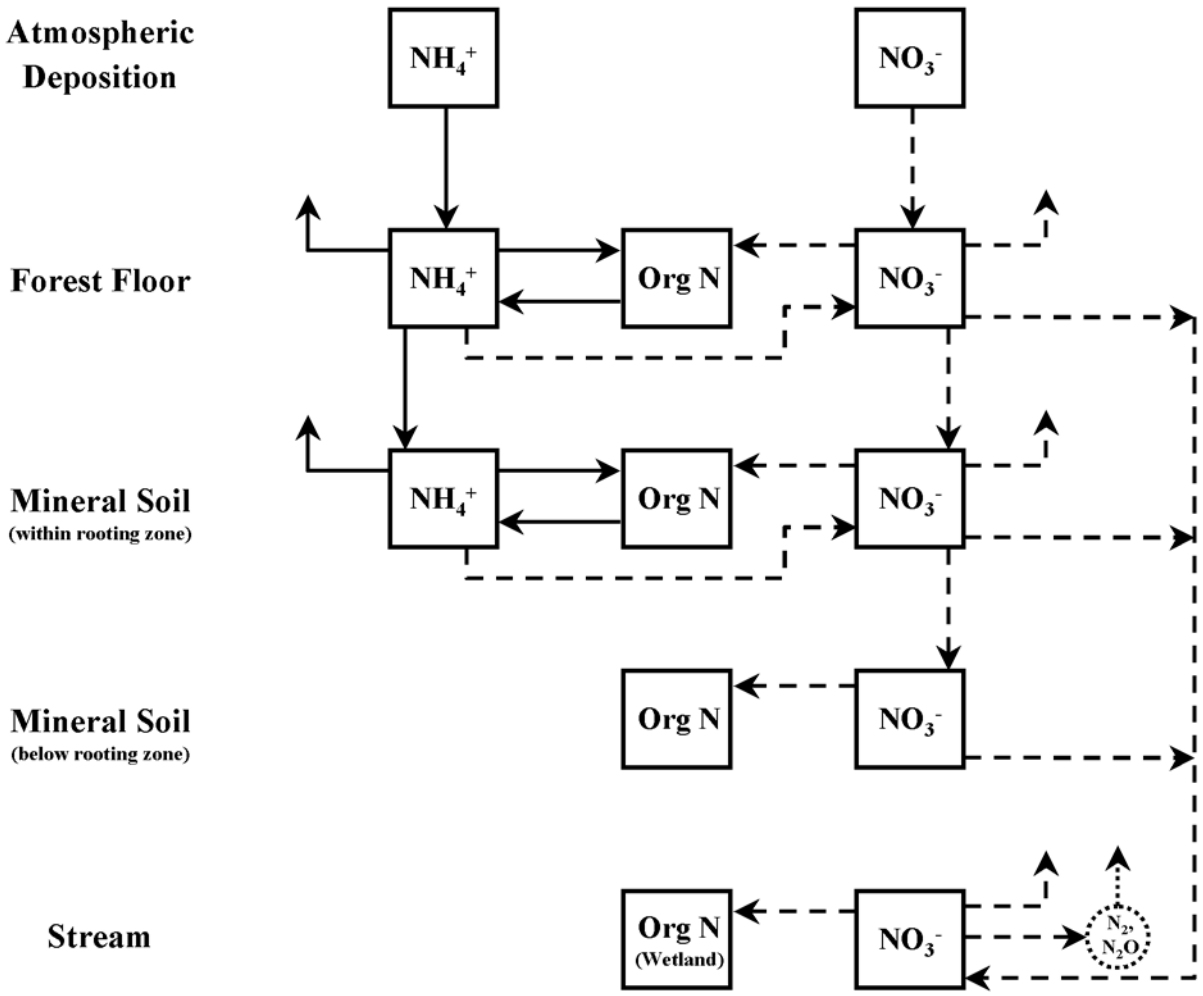


Figure 8.2 Conceptual model of soil and stream processes controlling the isotopic composition of nitrate exported from first-order stream basins at the TLW. Dashed arrows indicate processes and fluxes that can influence the $\delta^{18}O$ and $\delta^{15}N$ of nitrate exported by streams. Solid arrows indicate processes and fluxes that only affect the $\delta^{15}N$ of nitrate.

3) Ammonium export below the rooting zone (60cm) in the mineral soil is negligible (e.g. Foster et al., 1986).

4) Ammonification and nitrification below the rooting zone are small compared to rates in above compartments (Foster et al., 1986) and are therefore ignored. However, nitrate immobilization may be important with respect to nitrogen supply for microbes and therefore this flux remains in the model.

5) Studies to date suggest that denitrification is not significant in TLW soils and groundwater (see Section 6.3.5) but is important for nitrate concentration and isotopic composition in streams that intersect wetlands (Chapter 5).

6) In-stream nitrogen mineralization is insignificant in first-order streams with rapid transit times. A multiple-catchment sampling in late May 2000 showed little difference in nitrate concentration at the start of streams compared to catchment outlets in basins without wetlands (Spoelstra, unpublished data). Therefore, processes indicated for the stream compartment represent plant uptake, immobilization, and denitrification occurring in wetlands that are intersected by the stream and subsequently increase hydrologic residence time.

The forest floor and mineral soil are each shown as one compartment for simplicity but a working computer model should have a separate compartment for each soil horizon in the forest floor (L, F, H) and mineral soil (Ah, Ae, Bfh1, etc). This requirement is largely due to changing organic nitrogen contents and $\delta^{15}N$ values with depth in the soil profile. Subsequently, the nitrogen isotope signature of nitrification in each layer may also differ and therefore would need to be modeled separately for each horizon. A computer model based

on Figure 8.2 would require information on the size and $\delta^{15}N$ of the organic nitrogen pool, the rates of nitrogen cycling processes, and the associated fractionation factors, in order to predict the isotopic composition of nitrate export.

The nitrogen isotope composition of nitrate export is difficult to predict because of the combined effects of many processes that influence the $\delta^{15}N$ of nitrate produced by nitrification, either by affecting substrate concentrations, through fractionation effects, or both. In contrast, only the processes indicated with dashed arrows in Figure 8.2 control the $\delta^{18}O$ values of nitrate production and export. Therefore the $\delta^{18}O$ data can be used independently from $\delta^{15}N$ to constrain the model with respect to the magnitude of these fluxes and processes, subsequently also constraining the $\delta^{15}N$ portion of the model.

Extensive research has been done on nitrogen cycling at the TLW (see publication list at www.tlws.ca) and therefore many of the pools and fluxes in Figure 8.2 have already been quantified and much of the isotopic information required for the nitrate portion of the model is presented in this thesis. For example, results in Chapter 4 and 7 indicate that the $\delta^{18}O$ of nitrate in TLW headwater streams was predominantly controlled by a mixing of atmospheric and microbial nitrate sources without significant isotopic modification by other processes. The forest harvest experiment provides a significantly different set of nitrogen cycling conditions and flux rates that could eventually be used, in conjunction with the post-harvest nitrate isotope data, as an additional test of the conceptual model.

8.3 Recommendations for future research

Stream 31 nitrate isotope ratios may reveal a change in the nitrate source contribution patterns in the latter post-harvest period. As increased nitrogen demands from regrowing

vegetation lead to decreased nitrification and leaching of microbial nitrate, it is expected that the proportion of atmospheric nitrate found in the stream during snowmelt will increase. Therefore, monitoring of nitrate isotope ratios in c31 should continue as stream nitrate concentrations decrease below pre-harvest levels.

Isotopic analysis of atmospheric nitrate at the TLW (Section 7.3.1), as well as other North American sites (Williard et al., 2001; Campbell et al., 2002), suggest that nitrate isotope ratios could be used to trace sources of nitrate in precipitation (e.g. Freyer, 1978) or provide information on atmospheric processes that lead to increased nitrate deposition to forested watersheds. $\delta^{18}O$ and $\delta^{15}N$ analysis of atmospheric nitrate, in conjunction with $\delta^{15}N$ analysis of ammonium deposition and the modeling of air mass trajectories, should be employed to investigate causes of isotopic variability in atmospheric nitrogen deposition.

Although $\delta^{18}O$-nitrate analysis indicated that nitrification was predominantly performed by chemolithoautotrophs in TLW soils, a minor contribution from heterotrophic nitrifiers could not be conclusively rule out. Future nitrate isotope work at the TLW would benefit from a quantitative assessment of heterotrophic nitrification. A precipitation exclusion mechanism, similar to the one used in Chapter 6, in conjunction with acetylene inhibition of chemolithoautotrophic nitrification, could be used to measure *in situ* net heterotrophic nitrification and the isotopic composition of any resulting nitrate.

The ability of a very small wetland to dramatically reduce stream nitrate concentrations following forest harvest warrants consideration in forest management strategies. Harvesting procedures designed to recognize and protect these wetlands, which can be too small to appear on standard topographic maps, could reduce nutrient loading to rivers and lakes following forest harvest.

More research is required to determine what controls the $\delta^{15}N$ values of nitrate produced in forest soils and subsequent reactions that affect its isotopic composition prior to export in groundwater and streams. The current understanding of nitrogen cycling in forested catchments would benefit from further *in situ* and lab incubation studies of nitrogen cycling in soils, including concomitant isotopic analysis of ammonium, nitrate, and organic nitrogen. Information on the rates of nitrogen cycling processes and fluxes, along with associated isotopic fractionation factors, could then be incorporated into a model to formulate hypotheses and help answer questions such as:

1) Why is ^{15}N-depleted forest floor nitrate not detected in groundwater and streams at the TLW?
2) How do foliar $\delta^{15}N$ values relate to the $\delta^{15}N$ of nitrate and ammonium in the soil profile?
3) How do soil $\delta^{15}N$ profiles develop and what do they indicate about catchment nitrogen status?

Interpretation of nitrate isotope ratios and nitrogen cycling research would benefit greatly from the determination of nitrate oxygen isotope fractionation factors associated with processes that remove nitrate from solution (e.g. plant and microbial assimilation, abiotic immobilization). Although nitrogen fractionation factors have been determined for nitrate reactions by many species of bacteria and higher plants (e.g. Hübner, 1986; Shearer and Kohl, 1986; Handley and Raven, 1992), relatively little or nothing is known about the accompanying isotopic discriminations for nitrate oxygen. A notable exception to the previous statement is denitrification, for which an approximate 2:1 enrichment trend in

$\delta^{15}N$:$\delta^{18}O$ values has been documented in several studies (e.g. Böttcher et al., 1990). Future research employing a dual nitrate isotope approach may reveal diagnostic enrichment ratios for other nitrate-attenuating processes. In fact, the 2:1 enrichment ratio observed for denitrification will not be truly diagnostic on its own until it can be shown that other nitrate attenuating processes do not exhibit the same enrichment ratio. New analytical methods for nitrate isotope ratios (Sigman et al., 2001; Casciotti et al., 2002), which require significantly less sample volume and preparation time, are likely to facilitate more research that includes analysis of nitrate $^{18}O/^{16}O$ ratios.

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