

The use of stable nitrogen and carbon isotopes to examine the sources, sinks and cycling of nitrogen in a small, artificially eutrophic Boreal lake

by

Nicholas Alexander Paquette Flinn

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## **Author's declaration**

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## Abstract

This research examines components of nitrogen cycling in the artificially eutrophic Lake 227 at the Experimental Lakes Area. The components are: 1) the atmospheric sources of nitrogen to the lake through precipitation and dry deposition; 2) the sediment record of past nitrogen sources and primary productivity; and 3) the internal processes of nitrogen cycling. These topics are examined using stable nitrogen and carbon isotopes.

The  $\delta^{15}\text{N}$  of atmospheric nitrogen deposition in Canada is not well studied. The results of this work are the first  $\delta^{15}\text{N-NH}_4^+$  values for Canada, while the  $\delta^{15}\text{N-NO}_3^-$  values add to a very limited data base for Canada. The collection of  $\delta^{15}\text{N}$  of precipitation samples was accomplished through the deployment of bulk precipitation collectors over the summer of 2010. Samples were collected at approximately two week intervals, although the length varies depending on precipitation amounts. The  $\delta^{15}\text{N-NH}_4^+$  of precipitation was found to peak in midsummer with a mass weighted  $\delta^{15}\text{N}$  of -6.1‰, had a significant positive correlation with ammonium concentration. The  $\delta^{15}\text{N-NO}_3^-$  also peaked in midsummer with a mass weighted  $\delta^{15}\text{N}$  of -3.1‰. There was no relationship between  $\delta^{15}\text{N-NO}_3^-$  and nitrate concentration. There was a significant positive correlation between  $\delta^{15}\text{N-NO}_3^-$  and total solar radiation, suggesting that atmospheric photochemistry has a strong effect on the  $\delta^{15}\text{N-NO}_3^-$  of nitrate.

The detailed record of Lake 227 nutrient concentration, phytoplankton assemblages, and trophic state changes allows for a detailed look at the standard interpretations of sediment core isotopes and the information that can be gained from their study. The comparison between the standard interpretations of sediment core  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  and what was observed in the lake was accomplished through the collection, and isotopic analysis, of sections of a sediment core from the hypolimnion of Lake 227. The sediment core record of primary production in Lake 227, as inferred from the standard  $\delta^{13}\text{C}$  interpretation of sediment core  $\delta^{13}\text{C}$ , is at odds with what was observed in the lake over the past 15 years. The decline in  $\delta^{13}\text{C}$  over this period is likely the result of a decrease in alkalinity in Lake 227 following the cessation of experimental  $\text{NaNO}_3$  additions and not a decrease in primary production. The standard interpretation of sedimentary record  $\delta^{15}\text{N}$  accurately reflected actual changes in nitrogen sources as the lake progressed from an oligotrophic system; undergoing slight anthropogenic eutrophication, to being severely eutrophied with high levels of nitrogen and phosphorus, to the dominance of nitrogen fixating cyanobacteria following the end of nitrogen loading.

In order to synthesize the previous two chapters, the in lake nitrogen cycling was investigated in three ways; 1) sampling of multiple lakes over a one week period in the summer of 2010 for nitrogen isotopes of POM, zooplankton and DOM; 2) monthly sampling of nitrogen isotopes in Lakes 227 and 442;

and 3) the completion of a multiyear whole lake isotope mass budget for Lake 227. It was found that nitrogen fixation in Lake 227 does not cause the  $\delta^{15}\text{N}$  of epilimnetic POM to be significantly different than other ELA lakes, most likely due to the small range in the  $\delta^{15}\text{N}$  of the inputs that the lakes share such as precipitation and runoff. The mass weighted  $\delta^{15}\text{N}$  values for 2010 were 3.3‰ for precipitation, -1.5‰ for runoff, and 0‰ for nitrogen fixation. The hypolimnetic  $\delta^{15}\text{N}$  POM was significantly different in Lake 227 compared to other ELA lakes as a result of large reserves of ammonium in the hypolimnion due to increased sedimentation and mineralization, following eutrophication, which allows for large isotopic fractionations to occur. During the open water season the  $\delta^{15}\text{N}$  of epilimnetic POM in Lake 227 is fairly consistent, occupying a range of +2‰ to -2‰, while Lake 442 epilimnetic POM fluctuates more often and to greater extremes in  $\delta^{15}\text{N}$ , from +2‰ to -5.7‰. This larger range in  $\delta^{15}\text{N}$  PON in Lake 442 may be due to either, 1) changing nitrogen demands resulting in assorted levels of isotopic fractionation or, 2) the relative importance of isotopically distinct nitrogen sources changes during the open water season. Limited data suggests that nitrification and denitrification may be important processes that occur in ELA lakes under ice if there has been significant ammonium build up during the stratified season. Isotope mass budgets for 9 years indicate that the mass budget for Lake 227 is not well constrained because of possible issues in the calculation of inputs from terrestrial sources in the spring, uncertain nitrogen fixation inputs, and outflow chemistry from November to May or June in most years is not available. It was determined that any differences in Lake 227  $\delta^{15}\text{N}$  are likely the result of increased primary productivity and biomass following eutrophication and not the direct result of nitrogen fixation.

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Finally, this thesis would be filled with many grammatical errors and run-on sentences were it not for the diligent proofreading and editing of Suzanne Arner.

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# 1 Chapter 1 Introduction

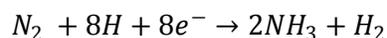
## 1.1 Overview

Nitrogen deposition to landscapes has been increasing since the rise of the internal combustion engine and has accelerated with the invention the Haber-Bosch process (Elser, 2011; Holtgrieve et al, 2011).

Traditionally, research on nitrogen deposition has focused on highly impacted locations in Europe, the North East United States, and increasingly China. Recent research has begun to examine the deposition of nitrogen to remote locations, long thought to be isolated from the sources of, and impacts caused by, increased nitrogen loading (Holtgrieve et al, 2011). Increasingly, nitrogen isotopes have been used to understand the impacts of nitrogen deposition in both terrestrial and aquatic ecosystems (Elliot et al, 2007; Spoelstra et al, 2001; Finlay et al, 2007; Burns & Kendall, 2007). An important aspect of the use of nitrogen isotopes is to understand the range of  $\delta^{15}\text{N}$  that may be encountered in order to constrain the isotopic composition of atmospheric inputs of nitrogen to the area of study. Techniques for isotopic analysis of low concentration nitrogen species are becoming increasingly common and allow for easier and more widespread application of nitrogen isotopes in precipitation (Mcilvin & Altabet, 2005; Zhang et al, 2007). However, there is limited  $\delta^{15}\text{N}\text{-NO}_3^-$  data for Canada (Spoelstra, 2004) and no  $\delta^{15}\text{N}\text{-NH}_4^+$  data.

One physiographic area of concern is the Boreal forests of Canada, long thought to be nitrogen limited with respect to ecosystem productivity, is now being subjected to increased loading of nitrogen (Parker et al, 2009). Increased nitrogen deposition can lead to increased forest productivity but in extreme conditions to forest decline (Aber et al, 1989). In some locations, the boreal forest is reaching nitrogen saturation and is becoming leaky with regards to nitrogen. Leaky forests result in increased nitrogen loss from forests to lakes and streams, which in some instances can amplify the effects of direct nitrogen loading onto the lake surface, leading to an increase in eutrophication of some lakes. Lakes where this is more likely to occur tend to have cold water, little stable stratification, short residence times or low available trace minerals that exclude nitrogen fixing cyanobacteria (Paerl, 2001).

Most aquatic systems are ultimately limited by phosphorus, as nitrogen limitation is overcome through species composition shifts to organisms that are able to fix atmospheric nitrogen into reactive forms of nitrogen (Schindler et al, 2008). Nitrogen fixation is an energetically demanding process so is only advantageous under conditions of severe nitrogen limitation (Howard & Rees, 1996). The conversion of  $\text{N}_2$  gas to ammonia during biological nitrogen fixation follows the chemical equation:



Nitrogen fixation can occur in terrestrial, marine and freshwater environments through either free living organisms or symbiotic partnerships between species (Howard & Rees, 1996). Understanding the controls on biological nitrogen fixation is of paramount importance to effectively manage eutrophic or at risk aquatic ecosystems.

Some of our understanding of nitrogen and phosphorus dynamics has come from work completed at the Experimental Lakes Area (ELA), Ontario, Canada. The original mandate of the ELA was to study biological productivity and provide a greater understanding of eutrophication (Hecky et al, 1994). This mandate has led to a series of whole ecosystem experiments to determine whether, carbon, nitrogen or phosphorus, was ultimately limiting as small scale bottle experiments had yielded conflicting and contradictory results (Schindler & Fee, 1974). The findings of these whole ecosystem experiments clearly point to phosphorus as the ultimately limiting nutrient due to the large reservoirs of nitrogen and carbon available from the atmosphere (Schindler & Fee, 1974). This research has helped direct public policy on nutrient reduction and has exerted pressure on the detergent industry to reduce phosphorus concentration in its products (Schindler, 1974). However, there are still some individuals that support nitrogen as the ultimate limiting nutrient (Scott & McCarthy, 2010). This discrepancy highlights the value of long term experiments that allow experimental manipulation of inputs in a manner similar to the efforts of ecosystem managers. Lake 227 is the longest running eutrophication experiment at the ELA, currently under experimentation for 40 years. During this time, the amount of nitrogen added to the lake has been changed three times, while the additions of phosphorus have remained constantly elevated (Findlay et al, 1994). Nitrogen was added at a high loading (315kg/yr), low loading (110kg/yr) and no loading (0kg/yr) rate, without observed changes in the levels of primary production over any of the experimental conditions (Paterson et al, 2011). The decreases in experimental nitrogen loading were replaced by atmospheric nitrogen fixation by cyanobacteria. The findings support the hypothesis that reducing nitrogen loading will not control eutrophication because nitrogen fixation can overcome nitrogen limitation causing phosphorus to be the ultimately limiting nutrient.

While the Lake 227 experiment has been ongoing for 40 years there are still many unknowns with regards to the mechanisms controlling nitrogen and phosphorus in lakes such as sediment sequestration and return of nutrients. Without a more detailed understanding of these, and other, mechanisms in Lake 227 the results from this long term experiment are not as transferable to other systems. The inputs and outputs of nitrogen and phosphorus can be calculated using a mass balance but the internal mechanisms controlling these nutrients is more difficult to study. To address the internal cycling of nutrients the use of natural abundance stable isotopes is common. However, phosphorus only has one stable isotope so is therefore not an available tool to address internal cycling. Nitrogen has two stable isotopes,  $^{15}\text{N}$  and  $^{14}\text{N}$ ,

which have been used extensively in aquatic studies for nitrogen source identification (Finlay et al, 2007; Holtgrieve et al, 2011; Minagawa & Wada, 1986), paleolimnological reconstructions (Galman et al, 2009; Lu et al, 2010), food web studies (Kidd et al, 1999), and quantifying individual processes such as nitrification, denitrification (Chan & Campbell, 1980), and assimilation (Waser et al, 1998) among other uses. By quantifying the isotopic composition of the sources and pools of nitrogen the different rates and amounts of nitrogen transformations can be more accurately estimated than by using a purely mass based approach. Alternatively, by studying the isotopic composition of sediment cores the historical in lake conditions and nitrogen utilization can be deduced, a powerful tool in situations where changes have occurred previous to monitoring efforts. Studying the stable nitrogen isotopes of the eutrophic Lake 227, to increase the understanding of the processes controlling nitrogen cycling, will aid in the application of the results from Lake 227 to other eutrophic systems.

## **1.2 Thesis Objectives**

The overall objectives of this thesis are to investigate the nitrogen sources, sinks and cycling in Lake 227 at the Experimental Lakes Area through the use of stable isotopes.

Chapter 2 is an overview of the research site, the sampling regime and analytical methods that are common to further chapters of this thesis.

Chapter 3 contains the changes in nitrogen deposition to the ELA over the past 40 years and the characterization of the  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and total nitrogen in atmospheric deposition to the ELA between June and October 2010. Values found at the ELA are compared to samples collected in other parts of the world.

Chapter 4 contains the results of a sediment core from Lake 227. In this chapter the sediment core carbon and nitrogen isotopes are compared to historical changes in lake nutrient additions and trophic status, the mass of nitrogen lost to net sedimentation is estimated in pre and post eutrophication periods.

Chapter 5 contains the results of a nitrogen isotope mass budget used to elucidate internal nitrogen cycling in Lake 227 employing isotopic compositions for precipitation from Chapter 3 and  $\delta^{15}\text{N}$  of particulate matter and sediments from Chapter 4. The question of whether nitrogen species in Lake 227 are isotopically different than other ELA lakes because of nitrogen fixation and eutrophication is also explored.

Chapter 6 contains an overall summary of conclusions and implications from chapters 3, 4, and 5.

## 2 Chapter 2: Site Description and Methodology

### 2.1 Site Description

This study was conducted on lakes at the Experimental Lakes Area (ELA) in Northern Ontario, Canada (93° 30'-94°00'W and 49°30'-49°45'N). The ELA was started in 1969 to examine the issue of eutrophication on the ecosystem scale by allowing whole lake and watershed manipulation experiments. Building on the original purpose to explore eutrophication, subsequent experiments have studied the effects of acidification, clear cut logging, heavy metal pollution, and forest fires for example (Schindler, 1980). ELA consists of 58 small lakes, ranging in size from 1 to 84 hectares, as well as watersheds designated for research. The surrounding watersheds are composed primarily of jack pine, black spruce, trembling aspen and white birch. The ELA is situated on primarily Precambrian acid granites, which are covered in places by a thin layer of glacial drift, mainly composed of sand and gravel. In low lying places, there are variable depths of brunisols covering the granites (Brunskill & Schindler, 1971).

The main study lake of this project was Lake 227, an artificially eutrophic, 5 hectare, headwater lake with a mean depth of 4 metres and a maximum depth of 10 metres (figure 2.1). Lake 227 has a watershed area measuring 34.4 hectares with no permanent stream section draining into the lake. Lake 227 undergoes occasional incomplete mixing in the fall and spring and is stratified for much of the open water season (Brunskill & Schindler, 1971). The epilimnion extends down to approximately 4 metres in July.

Lake 227 is the first, and longest lasting, eutrophication experiment at the ELA. Beginning in June of 1969, Lake 227 was fertilized with nitrogen and phosphorus and has continually been fertilized with a combination of these elements since that time. The artificial loading has changed Lake 227 from an unproductive oligotrophic lake to a highly productive eutrophic lake that experiences yearly summer algal blooms composed of N<sub>2</sub> fixing cyanobacteria (Findlay et al, 1994).

The ratio of nitrogen to phosphorous added to Lake 227 has been adjusted three times to explore the effects of changing N:P ratio on eutrophication (table 2.1). It has been found that, even with no fertilizer nitrogen added to Lake 227 (1990 to present), the lake has remained eutrophic with increased dominance of nitrogen fixing cyanobacteria in midsummer. In 1993-1996, pike were added to Lake 227 as part of a food manipulation experiment and removed in 1996, leaving Lake 227 fishless ever since.

A reference lake was also sampled as part of this research to allow for a direct comparison of a natural, oligotrophic lake under the same climatic conditions. Lake 442 was used as the comparison lake. Lake 442 is a 16 hectare second order lake, situated in a 145 hectare terrestrial watershed, with a mean depth of 9 metres and a maximum depth of 17.8 metres (figure 2.2). The total volume for Lake 442 is  $14.40 \times 10^6 \text{ m}^3$ . Lake 442 is situated downstream of Lake 438, and the connecting stream is bisected by Pine Road (McCullough and Campbell, 1993). Lake 442 has not been experimentally manipulated previously; a portion of the catchment (65%) was logged though between 1975 and 1979 (Paterson et al, 1998). There is a 25 year data set for Lake 442 resulting from its use as a reference lake in lake trout population studies as well as lake variability and climate change studies (Sellers et al, 1998; McCullough and Campbell, 1993).

### **2.1.1 Climate**

A meteorological station was installed at the ELA in June of 1969, within the L239 catchment, providing over 40 years of meteorological data. The annual average temperature, from 1969 to 2005, is  $2.8^\circ \text{ Celsius}$ , with a December to February mean temperature of  $-14.2^\circ \text{C}$ , and a June to August mean temperature of  $17.9^\circ \text{C}$  (Parker et al, 2009). The annual average water year precipitation, from 1969 to 2005, is 702mm per year. More precipitation falls in the period of April to October, at an average of 543 mm, than from November to March, which sees an average of 158mm of precipitation (Parker et al, 2009).

Temperature has been recorded at the ELA meteorological site for 40 years. The year 2010 was generally close to the median temperature, but there are occasions where the temperature varies from the mean, most notably in May through to September, where the daily mean temperature is lower than average (figure 2.3).

In 2010, there were a number of months that saw higher precipitation on average compared to the previous 40 year record of precipitation at the ELA meteorological site (figure 2.4). July of 2010 was the wettest July on record at the ELA, with a total of 252mm of precipitation. The open water season of 2010, May to September, was the wettest recorded at the ELA while the full calendar year was the third wettest year on record.

## **2.2 Methods**

### **2.2.1 Stable isotopes**

Stable isotope results presented in this thesis are expressed in delta ( $\delta$ ) permil (‰) notation for all samples analyzed. Delta per mil is the ratio of two isotopes of the sample (heavy/light) compared to the ratio of a known standard as shown in the expression:

$$\delta_{Sample} = \left( \frac{R_{Sample}}{R_{Standard}} - 1 \right) \times 1000$$

Where  $R_{sample}$  is the ratio of heavy isotope to light isotope in the sample and  $R_{standard}$  is the same ratio for a known standard. The standards used in this study are Vienna Pee Dee Belemite (VPDB) for  $\delta^{13}\text{C}$  and atmospheric nitrogen, which has been shown to be isotopically homogenous, for  $\delta^{15}\text{N}$  measurements. Many reactions show preference for one isotope over the other; in biological reactions this is predominantly the light isotope. Analyzing for stable isotopes allows for source or process identification in many natural systems with favourable conditions (ie. distinct source isotopic composition, or strongly fractionating processes).

### 2.2.2 Sampling regimen

To characterize the stable isotope abundance of the various species of nitrogen and carbon, Lakes 227 and 442 were sampled on a regular schedule throughout the 2010 open water season and once under ice cover by personnel at the ELA using established protocols. Lake 227 was sampled at discrete depths of 1, 4, 6, 8, and 10 metres while Lake 442 was sampled at 1, 5, 9, 13, and 17 metres. Discrete sampling was performed monthly, with samples collected for dissolved oxygen, general chemistry, as well as samples for isotopic analysis. General chemistry (including but not limited to: nitrate, ammonium, nitrite, suspended nitrogen, suspended carbon, anions, and cations) on lake samples was completed by the Experimental Lakes Area onsite laboratory and the Freshwater Institute at the University of Manitoba in Winnipeg, Manitoba using methods by Stainton et al, (1977). Two weeks after the discrete sampling was completed, an integrated epilimnion and metalimnion sample was collected for chemistry, but only POM samples were collected for isotopic analysis. The metalimnion is defined as the section of lake depth that has the greatest rate of change in temperature with respect to depth. Above the metalimnion is the epilimnion which is in direct contact with the atmosphere. Dependent on the depth and time of year, samples were collected for the isotopic analysis of  $\delta^{13}\text{C}$ -Dissolved Organic Carbon (DOC),  $\delta^{13}\text{C}$ -Particulate Organic Carbon (POC),  $\delta^{13}\text{C}$ -Sediments,  $\delta^{13}\text{C}$ -Zooplankton,  $\delta^{15}\text{N}$ - $\text{NO}_3^-$ ,  $\delta^{15}\text{N}$ - $\text{NH}_4^+$ ,  $\delta^{15}\text{N}$ -Dissolved Organic Nitrogen (DON),  $\delta^{15}\text{N}$ -Particulate Organic Nitrogen (PON),  $\delta^{15}\text{N}$ -Sediments, and  $\delta^{15}\text{N}$ -Zooplankton. Collection and analysis methods are presented in section 2.2.3.

A survey of lakes and streams at the ELA was performed, in the period of July 19<sup>th</sup> to July 30<sup>th</sup> 2010, to characterize the lake to lake variability in nitrogen and carbon stable isotope composition. The lakes included in the survey were Lake 239, Lake 626, Lake 373, Lake 375, Lake 303, Lake 304, Lake 979, Lake 114, Lake 442 and Lake 658. Streams included in the survey were Lake 979 east inflow, a catchment inflow from the cliff on the east side of Lake 114, inflow to Lake 240 from Lake 470, and the

channeled flow over Lake 302 uplands. At each lake, a sample was collected for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic analysis of DOM, POM, DIC, methane, sulphate, and bulk zooplankton. The streams were sampled for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes of POM and DOM. In this time period, lake sediments were collected in a transect using an Ekman dredge, at six points under different depths of water, in both Lake 227 and Lake 442 for isotopic analysis.

To collect samples for the analysis of stable nitrogen isotopes in precipitation a bucket collector, consisting of a 40 liter bucket lined with a new plastic bag for each sample, was deployed at the ELA meteorological site from the beginning of June to the end of September. Samples were collected approximately every two weeks, if there had been sufficient rain fall in that period. The samples were passed through a  $150\mu\text{m}$  screen to remove large particles and frozen in high density polyethylene (HDPE) bottles until processing could be completed at the Environmental Geochemistry Lab at the University of Waterloo.

### **2.2.3 Collection and Analysis Protocols**

#### **2.2.3.1 $\delta^{15}\text{N}$ , $\delta^{13}\text{C}$ , C:N POM**

Samples were collected in 1 litre HDPE bottles from discrete depths using a peristaltic pump and brought back to the ELA chemistry lab on site. Samples were kept cold until filtering could take place, typically within one day. To collect particulate samples, the water was prescreened through a  $150\mu\text{m}$  mesh to remove large zooplankton and collected on pre-combusted Quartz filters with a  $2.2\mu\text{m}$  pore size. Filters were packed until water no longer passed freely. Filters were placed in snap lid petri dishes and stored in a frozen state.

Filters were dried under vacuum then analysed at the Environmental Isotope lab at the University of Waterloo with a Carlo Erba 1108 (Italy) elemental analyzer, coupled to a Thermo Finnigan Delta plus continuous flow mass spectrometer. The precision on these measurements is  $\pm 0.3\%$  and  $\pm 0.2\%$  for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  respectively.

#### **2.2.3.2 $\delta^{15}\text{N}$ , $\delta^{13}\text{C}$ , C:N DOM**

The sample water collected and filtered for POM was used to obtain a sample for DOM. After POM filtration the sample was filtered through a  $0.45\mu\text{m}$  membrane filter, acidified with HCl to pH 3 for preservation and stored cold at  $4^\circ\text{C}$ .

DOM samples were frozen and freeze-dried to dryness. Samples with inorganic nitrogen concentrations that comprised greater than 5% of total nitrogen in the sample were dialysed in a 100 dalton Floatalyzer tube to remove the inorganic nitrogen before being re-freeze dried. Samples were analysed on the same mass spectrometer as  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  POM with the same analytical precision.

### **2.2.3.3 $\delta^{15}\text{N}$ , $\delta^{13}\text{C}$ , C:N Sediments and Zooplankton**

Zooplankton were collected using a 153 $\mu\text{m}$  net hauled vertically over the whole depth of the lake multiple times, in order to collect enough material for stable isotope analysis. Zooplankton samples were stored in Whirlpack bags and frozen. To prepare samples for analysis, samples were freeze-dried, packed into tin cups and analysed on the same mass spectrometer as  $\delta^{13}\text{C}/\delta^{15}\text{N}$ -DOM and  $\delta^{13}\text{C}/\delta^{15}\text{N}$ -POM.

Sediment samples were collected two different ways. In order to collect surface sediment samples, an Eckman Dredge was used. Transects were completed to gather sediment under different depths of water. The top 0-1.5 cm from the dredge was collected in the field in centrifuge tubes. The other method of sample collection was the use of a freeze-corer filled with dry ice and methanol to obtain a sediment core. Cores were taken at 3 different depths of overlying water in Lake 227 in winter. Sediment cores were maintained in a frozen state and section with a bandsaw in a freezer by depth at 0.5cm thickness from 0 to 11cm and 1cm thick sections from 11cm onward. Sample preparation for stable isotopes involved freeze-drying samples to dryness before the samples were packed into tin cups and analysed on the mass spectrometer.

### **2.2.3.4 $\delta^{15}\text{N}$ - $\text{NH}_4^+$**

Two methods were used for the analysis of  $\delta^{15}\text{N}$ - $\text{NH}_4^+$ , depending on sample type and concentration. For lake samples ( $\text{NH}_4^+$  concentration  $>0.6\text{mgN/L}$ ), the diffusion method was used, while the oxidation method ( $\text{NH}_4^+$  concentration  $0.14\text{mgN/l}$  to  $0.6\text{mgN/L}$ ) was used for precipitation samples. Ammonium isotope samples from the lakes were collected in 125ml HDPE bottles using a peristaltic pump. Samples were acidified soon after collection with HCl to a pH of around 3 and then frozen until analyses.

The  $\delta^{15}\text{N}$  of ammonium in precipitation was analysed using the ammonium oxidation method that converts ammonium to  $\text{N}_2\text{O}$ . The method used was after Zhang et al (2007). In brief, ammonium was oxidized to nitrite with  $\text{BrO}^-$  and subsequently reduced to  $\text{N}_2\text{O}$  with sodium azide. The resulting  $\text{N}_2\text{O}$  was analysed using a Micromass continuous flow mass spectrometer attached to a Micromass Trace Gas Analyzer. The precision on this measurement is  $\pm 0.3\%$ .

The second  $\delta^{15}\text{N-NH}_4^+$  determination method was a modified version of the ammonium diffusion procedure (Brooks et al, 1989; Holmes et al, 1998) after Spoelstra et al (2006). This method was performed with samples in duplicate, by diluting the sample to 0.6ml/L in 20ml with 4M potassium chloride solution. The sample and solution was made basic by the addition of 0.2M sodium hydroxide, buffering with sodium tetraborate at a pH of 9.3. After samples were made basic, a sealed polytetrafluoroethylene (PTFE) packet, containing an acidified (with  $\text{H}_2\text{SO}_4$ ) quartz disk, was added to the bottle and the bottle is sealed tightly. Samples were shaken for 14 days, during which time, ammonia volatilizes out of solution, through the PTFE packet where the ammonia reacts with the  $\text{H}_2\text{SO}_4$  to form ammonium sulphate. After 14 days the quartz disks were removed from the packets and freeze dried, before being analysed on a Carlo Erba 1108 (Italy) elemental analyzer, coupled to a Thermo Finnigan Delta plus continuous flow mass spectrometer as  $\text{N}_2$  gas. The precision on this measurement is  $\pm 0.3\%$

#### **2.2.3.5 $\delta^{15}\text{N-NO}_3^-$**

Water for nitrogen isotope analysis of nitrate in precipitation was sub sampled and filtered to  $0.45\mu\text{m}$  and freeze dried to obtain 0.6mg-N from the frozen bulk precipitation sample. Nitrate was converted by chemical denitrification to  $\text{N}_2\text{O}$  for isotopic analyses of both  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$ . The method used was after McIlvin and Altabet, 2005. In this method, the nitrate was reduced to nitrite through the use of cadmium subsequently nitrate is reduced again to  $\text{N}_2\text{O}$  with sodium azide. The  $\text{N}_2\text{O}$  was analysed using a Micromass continuous flow mass spectrometer attached to a Micromass Trace Gas Analyzer. The precision on measurements using this method are  $\pm 0.2\%$  for  $\delta^{15}\text{N-NO}_3^-$  and  $\pm 0.5\%$  for  $\delta^{18}\text{O-NO}_3^-$ .

#### **2.2.3.6 Concentration of Ammonium**

Ammonia concentration of precipitation samples was analysed colourmetrically using a manual spectrophotometer at the University of Waterloo. The ammonia was reacted with alkaline phenol and then hypochlorite forming indophenol blue, which is intensified through the addition of sodium nitroprusside before analysis at 600nm. Precision on ammonia concentration using this method is  $\pm 0.08\text{mgN/l}$ .

#### **2.2.3.7 Concentration of Nitrate**

For precipitation samples analysed at the University of Waterloo, nitrate concentration was determined using the Westco SmartChem 200 with a method based on USEPA 353.2 Revision 2.0 (1993). The nitrate was reduced to nitrite by passage of the sample through a tubular copperized cadmium reductor from which the nitrite was treated with sulphanilamide and N-(naphthyl)-ethylenediamine dihydrochloride to form a dye measured colourmetrically at 550nm. Precision on the measurement of nitrate concentration is  $\pm 0.02\text{mgN/l}$ .

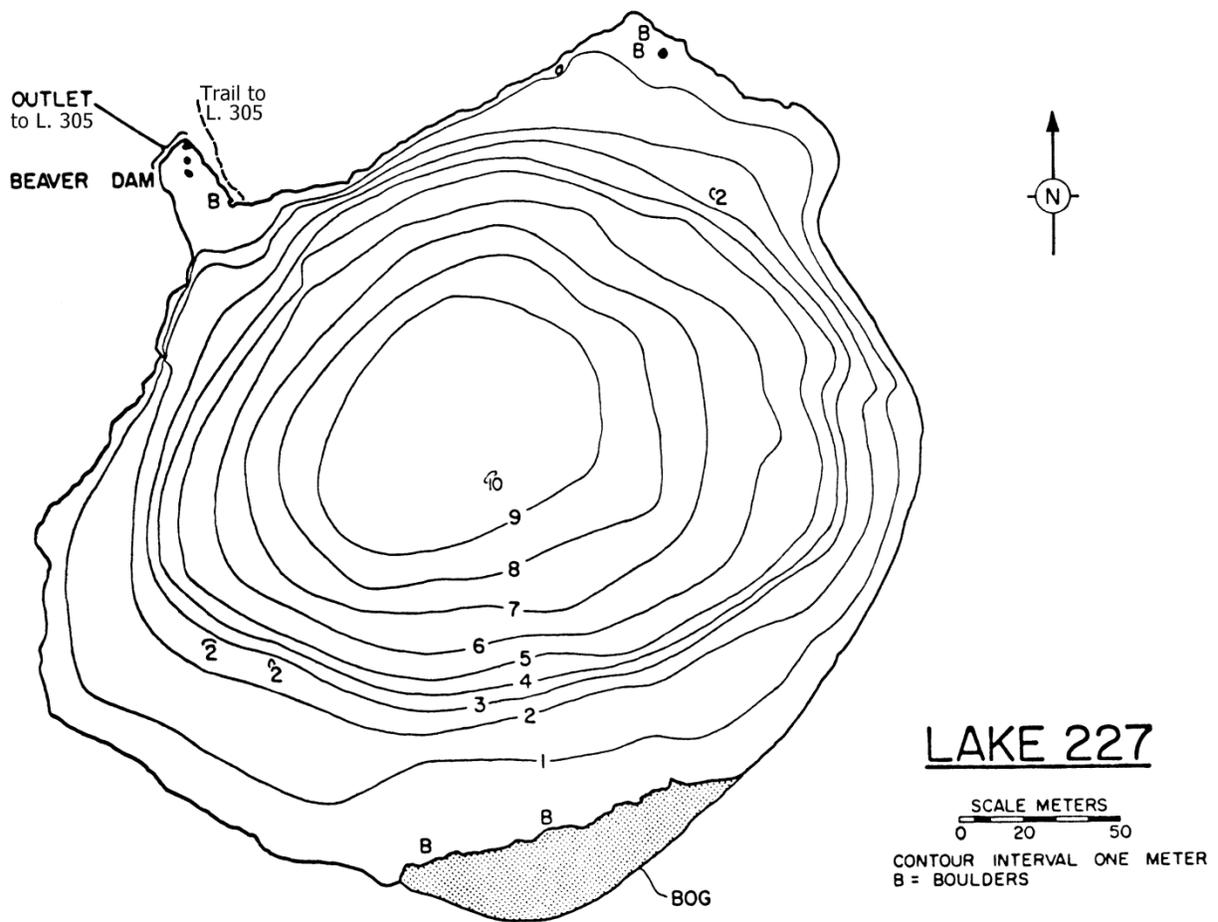


Figure 2:1: Bathymetric map of Lake 227, location of centre buoy is at deepest point (10m)

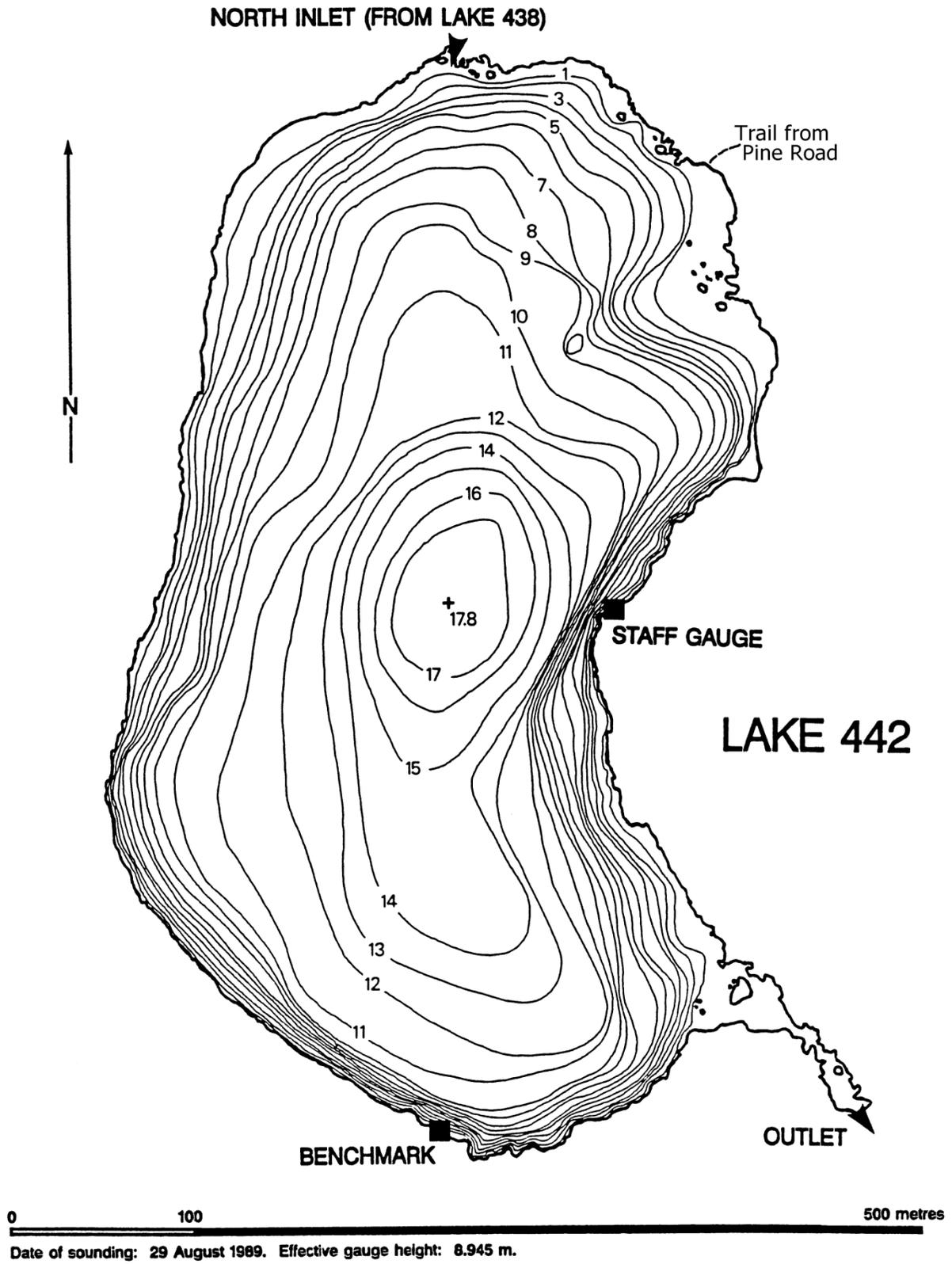


Figure 2:2: Bathymetric map of Lake 442, location of centre buoy is at deepest point (17.8m)

**Table 2-1 Artificial loading of nitrogen and phosphorus to Lake 227. Nitrogen was added as sodium nitrate ( $\text{NaNO}_3$ ) while phosphorus was added as phosphoric acid ( $\text{H}_3\text{PO}_4$ ). Nutrients were added from May to October each year**

<b>Year</b>	<b>Nitrogen mass (kg)</b>	<b>Phosphorus Mass (kg)</b>	<b>N:P ratio</b>
<b>1970-1974</b>	315	24	13:1
<b>1975-1989</b>	112	23	5:1
<b>1990-present</b>	0	23	0:1

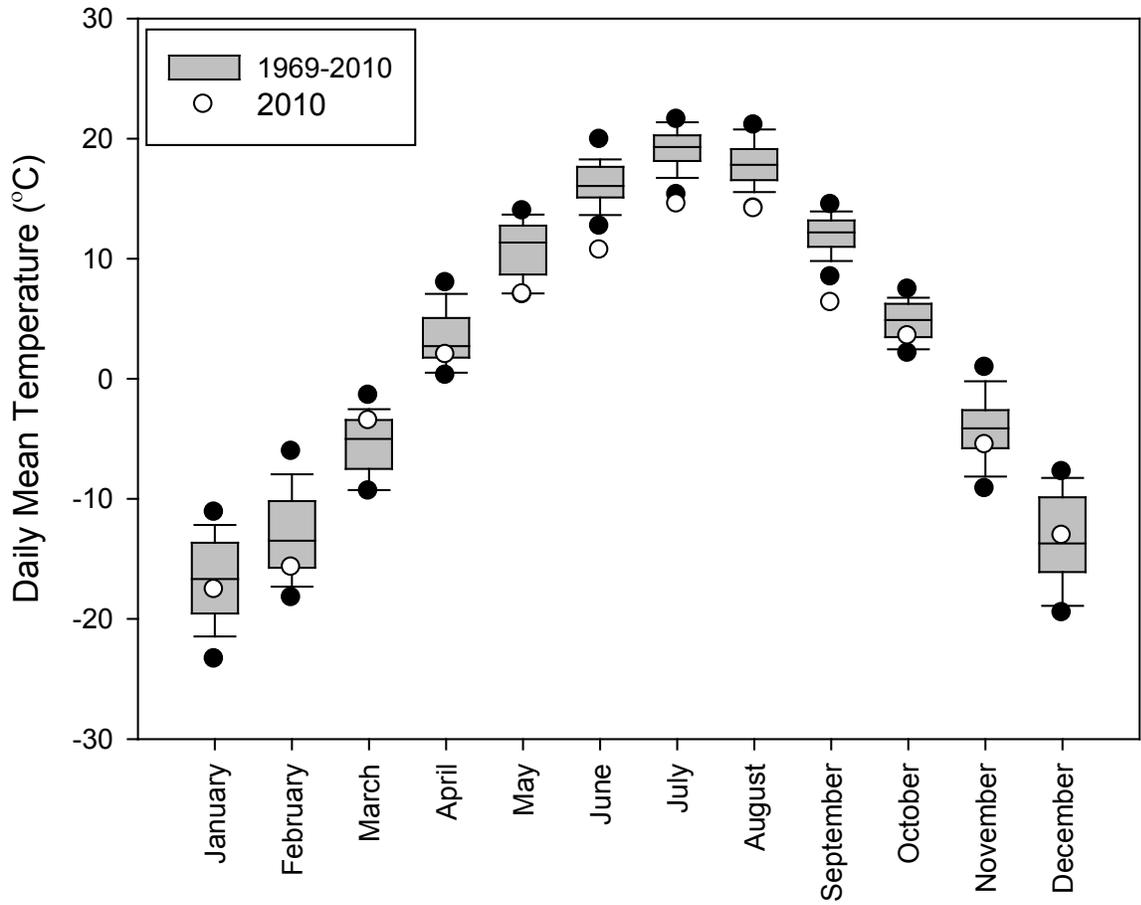


Figure 2:3 Monthly mean temperatures measured at the ELA meteorological site over 40 years (boxes with 5th and 95th percentiles) with 2010 monthly mean temperatures (open circles)

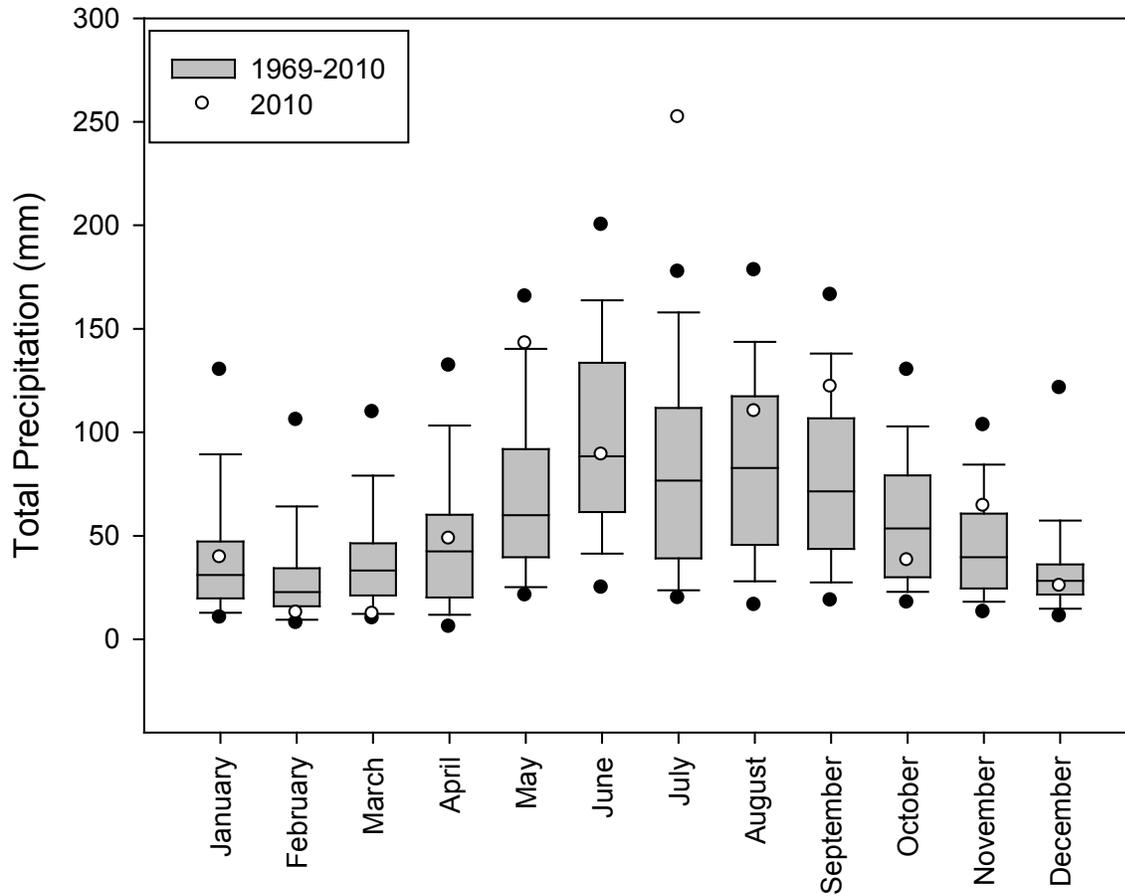


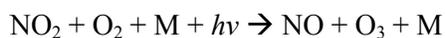
Figure 2:4 Monthly total precipitation depth measured at the ELA meteorological site over 40 years (boxes with 5th and 95th percentiles) with 2010 total monthly precipitation (open circles)

## 3 Chapter 3: Nitrogen Isotopes in Precipitation

### 3.1 Introduction

Inputs of nitrogen to the atmosphere have been increasing globally due to increased emissions of nitrogen from human activities (Vitousek et al, 1997). These emissions result in higher concentrations of nitrogen that react to form different species of nitrogen which are then deposited back to the surface onto ecosystems at distances of hundreds of kilometres from the source (Asman et al, 1998). In some non-impacted headwater lake systems atmospheric nitrogen deposition can account for more than 50% of the total nitrogen input, strictly from direct deposition on the lake surface (Findlay et al, 1994). Increases in nitrogen loading can have large impacts on oligotrophic environments that were previously nitrogen limited, increasing production and aiding eutrophication of these ecosystems (Bergstrom & Jansson, 2006). Increased nitrogen loading is a large issue for the Boreal forest, which is generally considered nitrogen limited. At high levels of nitrogen saturation forest productivity and biomass decrease while emissions of the potent green house gas nitrous oxide increase (Aber et al, 1989).

Nitrogen isotopes are used in precipitation studies to estimate the source of atmospheric nitrogen to a system, partition the amount of atmospheric nitrogen in the terrestrial nitrogen pool, and generate isotope mass balances in which precipitation plays a part (Heaten, 1986). Sources of nitrogen can have characteristic isotopic signatures. Biogenic sources of NO<sub>x</sub> and ammonium are thought to have lower δ<sup>15</sup>N values than anthropogenic emissions (Freyer, 1978; Heaton, 1990). The use of nitrogen isotopes is complicated by different reactions the atmospheric precursors undergo before being deposited to the surface. The basic reaction mechanism of atmospheric nitrate nitrogen precursors in the daytime is:



The M in this case is an inert molecule that absorbs excess energies and  $h\nu$  is solar radiation. The NO<sub>2</sub> in this case is produced from NO<sub>x</sub> that is released from various biogenic and anthropogenic sources. The HNO<sub>3</sub> in this reaction is the species of nitrogen that is deposited to the landscape through dissolution in precipitation (Wallace & Hobbs, 2006). At night when UV light is unavailable, a separate reaction mechanism dominates:





These different reactions are suspected to have different isotopic fractionation factors due to the nature of the reaction series, kinetic versus equilibrium in the day and night respectively (Freyer et al, 1993). The release of ammonia from anthropogenic and biological sources results in a different reaction mechanism. The released ammonia is quickly deposited back to the surface as dry deposition, while some of the ammonia is transformed to ammonium by acidic atmospheric molecules such as  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$  or  $\text{HCl}$  (Asman et al, 1998). The ammonium can then be transported long distances from the source before it is deposited in rain events, during which the  $\delta^{15}\text{N-NH}_4^+$  can be altered by atmospheric interactions (Heaton, 1987). Even with these complications, important information can still be garnered from studies of atmospheric nitrogen isotopes. However, the literature presents relatively few studies on atmospheric nitrogen isotopes in Canada. Most work on this subject has been done in heavily impacted systems in Europe, North Eastern United States and China.

The Experimental Lakes Area (ELA) is located in north-western Ontario, in the boreal forest. The long term data available from the ELA allows for study of changing depositional patterns at a location remote from most point sources of anthropogenic pollution. In the early 1970's nitrogen deposition at the ELA was among the lowest in North America, this is no longer the case. However deposition amounts at the ELA are currently less than one third of those at the most impacted sites in Eastern North America and Europe. For a more detailed examination of deposition at the ELA relative to other forested locations refer to Watmough et al (2005).

The goals of this study are to investigate changing nitrogen deposition at the Experimental Lakes Area (ELA), characterize the  $\delta^{15}\text{N-NO}_3^-$ ,  $\delta^{18}\text{O-NO}_3^-$ ,  $\delta^{15}\text{N-NH}_4^+$ ,  $\delta^{15}\text{N-Total Nitrogen (TN)}$  of atmospheric bulk deposition at the ELA and compare the results from the ELA to the values found in literature.

### **3.2 Methods**

The historical and current work for this study was conducted at the Experimental Lakes Area in northern Ontario, Canada (93° 41'W 49° 41'N). Historical samples were collected at three locations, L239 Island, L240 Island, and the meteorological site (figure 3.1). The samples for this work were collected at the meteorological site over the summer period, June to October, of 2010.

The historical data of precipitation chemistry and depth of precipitation was retrieved from the Experimental Lakes Area database. These data provided concentrations of major nitrogen species to allow analysis of possible changes in nitrogen deposition at the ELA. Due to the large skew in the data the

median was used to provide the best description of a “normal” precipitation event. Concentration data were right skewed, when plotted as a histogram, with a small number of high concentration events. The samples collected for this study were only from the summer months (June through to the end of September) as such the data from the historical ELA database were treated to only show data collected in this same time period. The data were restricted to the summer months to understand the input of nitrogen to lakes during the open water season when lakes are most productive. The data were plotted over the whole period of available records at the ELA, June 1969 to the end of 2008. More recent data was not available on the ELA data retriever. The data were also separated to show the depositional trends at the two sites where measurements were taken. One sampling location was on the island in Lake 239 from 1969 to 1983 when the location of the sampler was moved to the island closest to the outflow in Lake 240. Precipitation has been collected continuously at one of these two locations from 1969 to present. The other sampling location is at the ELA Meteorological site (Met site). This location has been used from 1969 until present as a site for winter sample collection. In 1986 samples were taken during the summer months at the Met site, as well as the island site, over the same period which allows for a comparison of the two locations. In 1996 the Met site switched from a wet collector to a bulk collector similar to the one deployed at the island site.

The samples for this study were collected in 2010 at the ELA Meteorological site at an open canopy location. Samples were collected as bulk deposition in plastic bags, over approximately two week intervals. After sample collection, the bag was sealed and placed in a fridge until it could be transferred to Nalgene bottles for freezer storage, which usually occurred on the same day. Samples were passed through a 150 $\mu$ m screen prior to freezing to remove large particles such as leaves. Samples were kept frozen until analysis.

Samples collected over the summer of 2010 were analysed for concentration of nitrate, ammonium, and total nitrogen as well as  $\delta^{15}\text{N-NO}_3^-$ ,  $\delta^{18}\text{O-NO}_3^-$ ,  $\delta^{15}\text{N-NH}_4^+$  and  $\delta^{15}\text{N-TN}$ . A brief description of the methods used for these analyses is presented below.

Nitrate concentration was determined on filtered precipitation samples using the Westco SmartChem 200 with a method based on USEPA 353.2 Revision 2.0 (1993). The nitrate was reduced to nitrite by passage of the sample through a tubular copperized cadmium reactor from which the nitrite was treated with sulphanilamide and N-(naphthyl)-ethylenediamine dihydrochloride to form a dye measured colourmetrically at 550nm. Precision on the measurement of nitrate concentration was  $\pm 0.02\text{mg-N/l}$ .

Ammonia concentration was also done colourmetrically but was analysed manually using spectrophotometer on unfiltered samples. The ammonia was reacted with alkaline phenol and then

hypochlorite forming indophenol blue which was intensified through the addition of sodium nitroprusside before analysis at 600nm. Precision on ammonia concentration using this method was  $\pm 0.08\text{mg-N/l}$ .

Nitrate was converted by chemical denitrification to  $\text{N}_2\text{O}$  for isotopic analyses of both  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  in duplicate. The method used was from McIlvin and Altabet, 2005. Briefly the nitrate was reduced to nitrite through the use of cadmium and subsequently nitrate was reduced again to  $\text{N}_2\text{O}$  with sodium azide. The  $\text{N}_2\text{O}$  was analysed using a continuous flow mass spectrometer attached to a trace gas unit used to concentrate the sample before analyses. The precision on measurements using this method are  $\pm 0.2\text{‰}$  for  $\delta^{15}\text{N-NO}_3^-$  and  $\pm 0.5\text{‰}$  for  $\delta^{18}\text{O-NO}_3^-$ .

The  $\delta^{15}\text{N}$  of ammonium was analysed using the ammonium oxidation method that converts ammonium to  $\text{N}_2\text{O}$  in duplicate. The method used was from Zhang et al 2007. In brief ammonium is oxidized to nitrite with  $\text{BrO}^-$  and subsequently reduced to  $\text{N}_2\text{O}$ . The resulting  $\text{N}_2\text{O}$  was again analysed using the continuous flow mass spec attached to a trace gas unit. The precision on this measurement is reported at  $\pm 0.3\text{‰}$ .

To analyse total nitrogen for  $\delta^{15}\text{N}$ , the sample water was evaporated at below boiling to reduce the sample volume from generally two litres down to approximately 100ml. The sample was then frozen and freeze-dried to dryness. Freeze-dried samples were packed into tin cups and analysed on a Carlo Erba 1108 (Italy) elemental analyser coupled to a Thermo Finnigan Delta plus continuous flow mass spectrometer. The precision on this measurement is  $\pm 0.3\text{‰}$ .

### 3.3 Results

#### 3.3.1 Historical Nitrogen Deposition at the ELA

The historical record of summer precipitation nitrogen deposition from ELA is shows that there has been no appreciable change in the nitrate deposition while the ammonium has increased to a level higher than what was observed in the early years of the record (figure 3.2). The majority of the increase has occurred since 1980. The years 1982 to approximately 1990, show a large increase in total dissolved nitrogen (TDN), from generally less than  $200\text{mg/m}^2/\text{summer}$  to greater than  $300\text{mg/m}^2/\text{summer}$ , with a significant proportion of that being dissolved organic nitrogen. In 1980 there was a forest fire that burned large parts of the catchments around Lakes 239 and 240 that may have contributed to this increase. It is noted that the large increase in TDN in the 1980s is not seen at the meteorological station (figure 3.3). The two sites are in agreement on the increase in ammonium concentration from the mid 1980s to the present. The  $\text{NH}_4^+:\text{NO}_3^-$  ratio has increased over the 40 year record as more ammonium is deposited relative to the stable inputs of nitrate. The average ratio of  $\text{NH}_4^+:\text{NO}_3^-$  in the first 10 years of the record is 0.8, while in the last ten years the ratio is 1.6. The island site DON made up a significant portion (up to 40%) of the

total nitrogen deposition in the 1980s (figure 3.4). In more recent times, 1989 onward, the DON has decreased to less than 20 percent of total nitrogen deposition. The meteorological site shows an increasing proportion of suspended nitrogen that is not as evident at the island site. Nitrite was never a significant nitrogen species in precipitation.

Both sites have similar total nitrogen deposition amounts in summer over the period when both records exist. The chemistry at both sites follows similar general trends over time with regards to changes in nitrogen loading to the area, except in the mid-80s when the island had higher DON deposition.

### **3.3.2 Concentration of $\text{NO}_3^-$ and $\text{NH}_4^+$ in Deposition in 2010**

Samples taken over the summer of 2010 were analysed at the University of Waterloo for nitrate, ammonium, and total nitrogen concentrations (figure 3.5). There are some gaps in the data where samples were not available for concentration analysis for the period of June 10<sup>th</sup> to 16<sup>th</sup> and also August 7<sup>th</sup> to 22<sup>nd</sup>. The samples that were analysed for concentration represent 90% of the total precipitation over the summer of 2010. There were some large precipitation events in the summer of 2010, most notably on July 3<sup>rd</sup> and July 22<sup>nd</sup>, when 86mm and 60.8mm of rain fell respectively, in 24 hour periods. Overall, the summer of 2010 was one of the wettest on record at the ELA. The average summer rainfall at the ELA, from 1970 to 2010, is 377mm, 572mm was received in 2010.

Ammonium concentration in the collected precipitation peaked in midsummer, with lows at the beginning and end of the summer. The volume weighted mean ammonium concentration over this period was 238ugN/L, with a range of 90ugN/L to 430ugN/L. Nitrate concentration followed a more cyclical trend, peaking at the beginning of July and again at the beginning of September. The range in nitrate concentration was 80ugN/L to 250ugN/L, with an average volume weighted concentration of nitrate in summer of 2010 was 157ugN/L. The longer than usual sampling interval between August 23<sup>rd</sup> and September 13<sup>th</sup> resulted because of insufficient precipitation for analysis, semi dry conditions persisted until the 10<sup>th</sup> of September when 29mm of rain fell.

The two largest precipitation events, July 3<sup>rd</sup> and 22<sup>nd</sup>, represent a significant proportion, greater than 40%, of the ammonium and more than 30% of the total nitrate deposition over the whole sampling period (figure 3.6). The two precipitation events represent 28% of total precipitation in the sampling period.

### **3.3.3 Nitrogen Isotopes in Deposition**

The mean for the published  $\delta^{15}\text{N-NO}_3^-$  data is -3‰ with a standard deviation of 3.5‰, the mean of published  $\delta^{18}\text{O-NO}_3^-$  data is 65‰ with a standard deviation of 15.2‰ (figure 3.7). The range in published precipitation nitrate isotopes is large, for  $\delta^{15}\text{N}$  the range is 5‰ to -13‰ while the range in  $\delta^{18}\text{O}$  extends from 12‰ to 86‰. Published data comes from sites worldwide from urban, rural, and forested sites. The Turkey Lakes Watershed and Southern shore of Lake Superior are close to the ELA and show a similar range in  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  although the Turkey Lakes Watershed has lower  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  than the ELA or Lake Superior.

For the 7 precipitation samples from the summer of 2010 the  $\delta^{15}\text{N-NO}_3^-$  increases from the beginning of June until the end of June and early part of July after which the  $\delta^{15}\text{N-NO}_3^-$  decreases (figure 3.8). The range for  $\delta^{15}\text{N-NO}_3^-$  extends from a low of -6.1‰ to a high of -0.9‰. The  $\delta^{18}\text{O-NO}_3^-$  shows the opposite trend, reaching the lowest  $\delta^{18}\text{O}$  at the beginning of August then increasing over the rest of the summer. The range in  $\delta^{18}\text{O-NO}_3^-$  spans from 63.5‰ to 74.4‰. Other locations in North America have seen similarly large ranges in  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  from one site over the summer months (Barnes et al, 2008; Burns & Kendall, 2002). The mass weighted mean for  $\delta^{18}\text{O-NO}_3^-$  deposition over the period of study was 66.2‰ while the mass weighted mean  $\delta^{15}\text{N}$  of the nitrate is -3.1‰ for the same period. There was no relationship between nitrate concentration and  $\delta^{15}\text{N-NO}_3^-$  or  $\delta^{18}\text{O-NO}_3^-$  (figure 3.9).

The mean  $\delta^{15}\text{N-NH}_4^+$  presented in the literature is -6.4‰ with a standard deviation of 5.5‰ and a total range of -21.8‰ to 5.7‰ (figure 3.10). There are no published  $\delta^{15}\text{N-NH}_4^+$  samples from Canada to this author's best knowledge.

The nitrogen isotope ratio of ammonium in precipitation was also analysed on samples collected over the summer of 2010 at the ELA (figure 3.11). The range of the data is large, ranging from -20.8‰ to -1.9‰ with a mass weighted mean  $\delta^{15}\text{N-NH}_4^+$  of -6.1‰. The  $\delta^{15}\text{N-NH}_4^+$  follows a similar trend to the  $\delta^{15}\text{N-NO}_3^-$ , with a peak in  $\delta^{15}\text{N}$  in midsummer. The  $\delta^{15}\text{N-NH}_4^+$  shows a positive relationship with ammonium concentration in the precipitation, with higher  $\delta^{15}\text{N}$  in samples coming with higher concentrations of ammonium (figure 3.12).

The range of the  $\delta^{15}\text{N-TN}$  data extends from -2.0‰ to 9.0‰ with a mass weighted mean of 3.3‰ (figure 3.13). The  $\delta^{15}\text{N-TN}$  follows the same patterns as the  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{15}\text{N-NH}_4^+$ , with the highest  $\delta^{15}\text{N}$  values in the middle of summer, 26 July to 6<sup>th</sup> of August. There have not been many studies on strictly  $\delta^{15}\text{N-TN}$  but a study in Bermuda that measured  $\delta^{15}\text{N-TN}$  found lower  $\delta^{15}\text{N-TN}$  with an average annual value of -2.3‰ (Knapp et al, 2010). The warm season had higher  $\delta^{15}\text{N}$  than the cool season (-4.6‰ in cool and 0.0‰ in warm) in that study, precipitation from cool periods were not measured in this study of  $\delta^{15}\text{N}$  of precipitation.

## 3.4 Discussion

### 3.4.1 Historical Deposition

The relatively long record of measured nitrogen deposition in precipitation at the ELA allows for analyses of the changing chemical composition of rain in this region, previously one of the sites with the lowest dissolved inorganic nitrogen (DIN) deposition rates in North America (Parker et al, 2009). The local effects from sampling at two separate locations and with different sampler set-ups at the two locations are evident in the different DON loading in the 1980s. The 1980 forest fire appears to have had an impact on the nitrogen deposition at the ELA, with a greater effect on the island site collector. This is seen in the long term data through the increase in DON deposition following the year of the fire and lasting for much of the decade. The increase in DON in precipitation at the island site could be related to the lower vegetative height following the forest fire which would result in increased local wind speed (Parker et al, 2009; Proe et al, 2001). Increased wind speed can lead to greater wind disturbance and distribution of soil organic matter, the results of which could be expected to be more pronounced at the island site because of the type of sample collector utilized. The island site uses a bulk collector which captures the dry deposition of dissolvable or suspended particles, while the Met site uses a wet only collector. The difference in the samplers is highlighted by the lack of agreement between the Met site and the island site data in the early part of the overlapping record, when the samplers were different. Following the change in sampling equipment from wet only to bulk collector at the Met site (in 1996) the TDN data from both locations becomes more harmonious.

The changing levels of ammonium in precipitation at the ELA are noted at both sites and are not related to the type of sampler used to collect samples in the early years of overlapping records. This could indicate that most ammonium deposited at the ELA is due to rain events and not from dry deposition, otherwise a difference in deposition in the period of different sampling equipment would be expected. This would signify that the source of ammonium is distant from the ELA as most dry deposition of ammonia occurs over a short distance, 1 to 2km, from the source (Asman et al, 1998). The residence time for ammonium, which is deposited primarily in rain events, is approximately ten days (Asman et al, 1998). A ten day residence time allows for distant transport from sources hundreds of kilometres away. As most ammonium deposited at the ELA is suspected to be the result of distant transport, the increasing trend more likely results from regional increase and not from a local increase in ammonia emissions. The prevailing wind conditions at the ELA result in a West to East flow of air masses. While the ELA is situated in a relatively pristine northern forest, the land to the west is heavily impacted. The land just over 200 hundred kilometres away is mainly agricultural, extending westward through Manitoba and Saskatchewan to Alberta. Heavily farmed land leads to increased ammonia emissions through the

application of ammonium fertilizer and the excrement from farm animals, such as cattle and swine (Asman et al, 1998). These two land uses, crop and animal, result in the release of gaseous ammonia through volatilization (Lee et al, 2011). In Manitoba the application of fertilizer increased by an order of magnitude from 1970 to 2011 (figure 3.14). In recent years, 2006 to 2010, the number of hogs and cattle has declined. However, the overall number of hogs has more than quadrupled (figure 3.14) and the total number of cattle has increased by 25% (figure 3.14) since 1970 (Honey, 2010). This recent decline in livestock numbers occurs in a period for which the historical deposition chemistry data is not available so the impact of decreases in livestock production on nitrogen deposition at the ELA is not known.

### 3.4.2 Nitrogen Isotopes in Deposition

The isotopic nitrogen ratios of precipitation from the ELA for  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of nitrate and  $\delta^{15}\text{N}$  ammonium are very close to the mean from published literature. The mass weighted  $\delta^{15}\text{N}-\text{NO}_3^-$  in literature and at the ELA is 3.1‰, while the literature mean and ELA mean for  $\delta^{18}\text{O}-\text{NO}_3^-$  are only different by 1.6‰ (66.2‰ at the ELA and 64.6‰ in literature). The  $\delta^{15}\text{N}-\text{NH}_4^+$  at the ELA is also very similar to the mean found in the literature, differing by 0.3‰ (-6.1‰ at the ELA and -6.4‰ in literature). There are few studies of  $\delta^{15}\text{N}-\text{NO}_3^-$  in Canada and none that looked at the  $\delta^{15}\text{N}-\text{NH}_4^+$ , this makes comparisons of locations with similar climates difficult but at the two locations near to the ELA (TLW and Lake Superior) there is available published data. These locations have a large range in isotopic data, but the results are fairly similar to values found at the ELA in 2010. The ELA is close to Lake Superior and the TLW but local differences in airsheds such as climate and nitrogen sources, could impact the isotopes of nitrate. Samples collected at these three locations were collected over a span of 10 years, 2000 to 2002 at TLW, 2004 to 2006 for Lake Superior, and 2010 at the ELA. The difference in  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  could therefore be due to inter annual variability or regional climate influences on nitrate deposition and isotopic composition.

The effect of spring snowmelt is suspected to be of major importance to the total  $\delta^{15}\text{N}$  nitrogen that is deposited to a lake. During spring, when snow melts, any nitrogen species contained in the snow flushes quickly into lakes and wetlands (Burns and Kendall, 2002). From other research conducted in this area (Spoelstra, 2004; Finlay et al, 2007) the  $\delta^{15}\text{N}-\text{NO}_3^-$  of winter deposition has higher  $\delta^{15}\text{N}$  than in the summer months, moving the annual mass weighted values closer to 0‰. This is important when considering using nitrogen isotopes to study lake nitrogen cycling, if the majority of the inputs are around 0‰ than there are not distinct end members for studies of cycling and sources.

The nitrogen isotope sampling conducted at the ELA took place strictly in the warm months of the year which may provide a less accurate depiction of the seasonal isotopic trends. The literature describes the seasonal trend, when one is observed, as nitrate being higher in  $\delta^{15}\text{N}$  in the winter months

and lower  $\delta^{15}\text{N}$  during the summer months (Freyer, 1978). This paradigm is difficult to interpret when the samples in this study were collected only within the months typically associated with summer and early autumn. The  $\delta^{15}\text{N-NO}_3^-$  is in fact higher at the peak of the summer in this study. When compared to work completed in areas near to the ELA (Spoelstra, 2004; Finlay et al, 2007) it is found that these may be short term increases and the truly high  $\delta^{15}\text{N}$  values, greater than 0‰, occur outside of the temporal scale of this study, October to May. The seasonal trend for  $\delta^{18}\text{O-NO}_3^-$ , documented in the literature, is a  $\delta^{18}\text{O}$  decrease occurring in summer followed by increasing  $\delta^{18}\text{O}$  values in winter (Williard et al, 2001). The results from ELA support this trend as values in midsummer all have lower  $\delta^{18}\text{O}$  relative to the June and September samples from 2010 (approximately 6‰ lower in midsummer). The short time span, roughly 120 days, as well as the season of those days, predominately the growing season, allows for the assumption that the  $\text{NO}_x$  source that supplies the nitrate is relatively constant. Outside of the growing season the inputs of atmospheric nitrogen are suspected to be of different origin than during summer when sources are predominantly natural and anthropogenically influenced biogenic emissions (Freyer, 1978). The differences in  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  could possibly be related to changing atmospheric photochemistry over the course of the summer. The  $\delta^{15}\text{N-NO}_3^-$  shows a strong positive relationship ( $r=0.84$ ,  $P=0.0095$ ) with the mean daily total radiation where the higher the level of total radiation, the higher the  $\delta^{15}\text{N-NO}_3^-$ . The  $\delta^{18}\text{O-NO}_3^-$  shows a strong negative relationship with the total radiation, with the lowest  $\delta^{18}\text{O}$  values when the mean radiation is highest. There was no strong relationship ( $P>0.05$ ) found for  $\delta^{15}\text{N-NO}_3^-$  and nitrate concentration (figure 3.9), precipitation depth, precipitation intensity, or average daily precipitation. There are many factors that have been shown to influence the isotopic composition of nitrate including, but not limited to; temperature, UV radiation (Freyer et al, 1993),  $\text{NO}_x$  source, storm track (Buda & DeWalle, 2009), duration of rain event (Buda & DeWalle, 2009), temporal separation from previous rain events (Heaton, 1987), and atmospheric chemical reactions of  $\text{NO}_3$  precursors (Freyer et al, 1993). The nitrate deposited at the ELA is likely to be of biogenic origin owing to the relatively more negative  $\delta^{15}\text{N-NO}_3^-$  values measured in this study and the distance to any significant anthropogenic source of  $\text{NO}_x$  such as a power station or large scale industry.

The measurements of  $\delta^{15}\text{N-NH}_4^+$  in this work are the first known  $\delta^{15}\text{N-NH}_4^+$  values from Canada, as such the year to year variability and seasonal trends in Canada are unknown. The values for the  $\delta^{15}\text{N-NH}_4^+$  follow a trend similar to the one present for  $\delta^{15}\text{N-NO}_3^-$ , with highest  $\delta^{15}\text{N}$  in midsummer. However, the range of the  $\delta^{15}\text{N-NH}_4^+$  data is larger. The large range in the  $\delta^{15}\text{N-NH}_4^+$  must be considered in any source partitioning study that uses isotopes to characterize precipitation end members because if any precipitation events are missed the assigned atmospheric  $\delta^{15}\text{N}$  could be very different from what is actually deposited. The ammonium in precipitation at the ELA is suspected to be composed of ammonium

of agricultural origin because of the correlation between increased agricultural activity in Manitoba and the increase in ammonium deposition at the ELA, noted in the historical record. Samples collected in the winter months would help to identify possible sources of ammonium in the summer if a noted difference appeared when the agricultural industry was not as active. The ammonium fertilizer that is spread over the fields in Manitoba as well as livestock excrement can volatilize as ammonia to become an atmospheric pollutant allowing transport to distant locations, such as the ELA (Lee et al, 2011). The rate of ammonia volatilization, and therefore possible atmospheric pollutant available for transport, is controlled by a combination of water content, pH, temperature and wind speed (Sommer et al, 1991). How differences in volatilization rates over seasonal timescales would influence the isotopic composition of ammonium deposited many kilometres away is not known, although it could be assumed that greater rates of volatilization would result in higher  $\delta^{15}\text{N-NH}_4^+$  in the atmosphere as the source ammonium  $\delta^{15}\text{N}$  would increase over time because of isotopic fractionation. The  $\delta^{15}\text{N-NH}_4^+$  was found to be significantly related ( $r= 0.778$   $P=0.023$ ) to the concentration of ammonium in the precipitation in this work (figure 3.12) and others (Knapp et al, 2008; Xiao and Liu, 2002). The reason for this relationship is not known at the ELA but some possible explanations suggested in the literature are; the wash out of high  $\delta^{15}\text{N}$  ammonium by previous storms (Heaton, 1987), the changing availability of atmospheric acid species (such as  $\text{H}_2\text{SO}_4$ ,  $\text{HCl}$ , or  $\text{HNO}_3$ ) for the formation of ammonium aerosols (Asman, 1998), or some influence on the  $\delta^{15}\text{N-NH}_4^+$  by atmospheric photochemistry, but no relationships were found with the parameters available in this study. The combination of multiple factors impacting ammonium volatilization, changes in source  $\delta^{15}\text{N}$ , and ammonium transport are likely the cause of the large range in the  $\delta^{15}\text{N-NH}_4$  over the summer.

The trend in  $\delta^{15}\text{N-TN}$  follows that of the DIN which is expected because  $\text{NO}_3^-$  and  $\text{NH}_4^+$  comprise a large (80% in 2010) proportion of total nitrogen in precipitation; the remainder is a combination of dissolved organic and suspended nitrogen. The very high  $\delta^{15}\text{N}$  values (8.1‰ and 9‰) are difficult to explain as it would require an even higher  $\delta^{15}\text{N}$  material to offset the lower  $\delta^{15}\text{N}$  DIN to this degree. Some possible explanations are alteration of  $\delta^{15}\text{N}$  during sample preparation, reworking of sample fractions before freezing, or unknown sources of high  $\delta^{15}\text{N}$  suspended or dissolved organic nitrogen. Further samples need to be analyzed to determine if this is an artefact of sampling/processing error or a true  $\delta^{15}\text{N}$  value.

### 3.5 Summary and Recommendations

The historical record of precipitation chemistry at the ELA shows that the deposition of ammonium has increased almost 2 fold in the 40 years of recorded chemistry while nitrate inputs have remained relatively constant. This increase in ammonium deposition is most likely due, in large part, to an increase in the agricultural intensity in Manitoba and the prairies. The increase in inorganic nitrogen deposition has

resulted in the ELA no longer being among the sites of lowest deposition, being with higher nitrogen deposition than locations in Quebec (Lac la Flamme and Lac Tirasse). At the ELA total inorganic nitrogen ( $\text{NO}_3^- + \text{NH}_4^+$ ) deposition during the 1990s was 4.27kg/ha/yr compared to 3.72 kg/ha/yr at Lac Laflamme and 2.83kg/ha/yr at Lac Tirasse (Wamouth et al, 2005). Deposition is lower at the ELA than that at the Turkey Lakes Watershed (total inorganic nitrogen deposition of 8.31kg/ha/yr) in Northern Ontario (47°02'N 84°22' W), approximately 700km to the east southeast of the ELA.

Importantly this research shows the open water input of nitrogen and nitrogen isotopes from the atmosphere to remote locations, like the ELA. The  $\delta^{15}\text{N-NH}_4^+$  measurements are the first known in Canada, and the  $\delta^{15}\text{N-NO}_3^-$  adds to a very limited collection of Canadian values. The mass weighted  $\delta^{15}\text{N-NO}_3^-$  over the open water season was -3.1‰, the  $\delta^{18}\text{O-NO}_3^-$  mean was 66.2‰, the mean  $\delta^{15}\text{N-NH}_4^+$  was -6.2‰ and the mean  $\delta^{15}\text{N-TN}$  was 3.3‰. This work shows that nitrogen isotopes are highly variable over a short period of time therefore it is important when planning studies of nitrogen sources to measure nitrogen isotopes in atmospheric deposition over all time periods because the assumption of an unchanging isotopic source is not valid. If a large, isotopically unique, precipitation event was missed the assumed  $\delta^{15}\text{N}$  of atmospheric nitrogen could be vastly different than what was actually deposited. To improve upon this work it is recommended that samples be collected from the rest of the calendar year, that the time period covered for each sample is shortened to improve the resolution of the data and the spatial scale of this work be increased by collecting samples from multiple locations to understand the variability of atmospheric nitrogen deposition to remote locations across Canada.

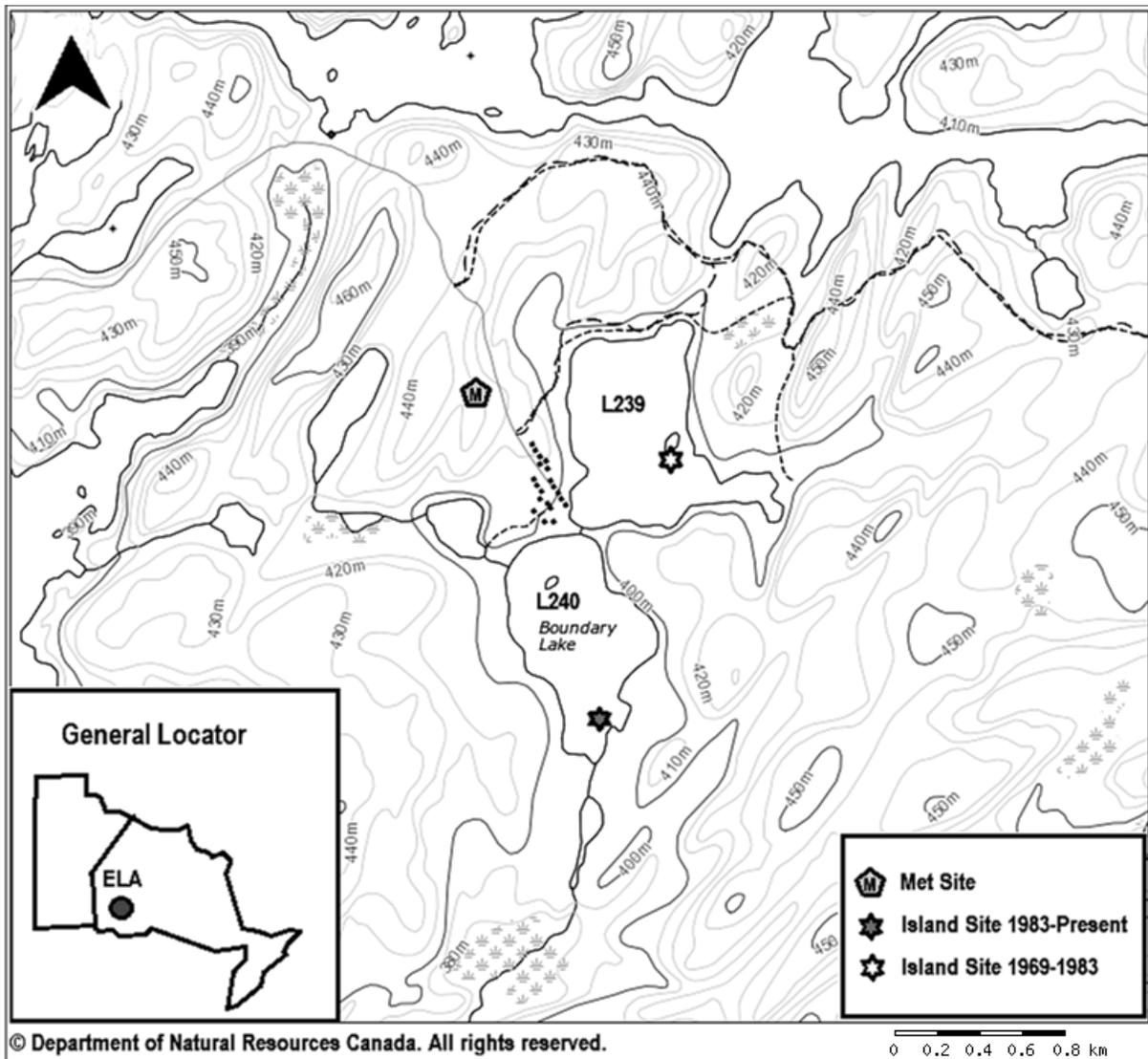
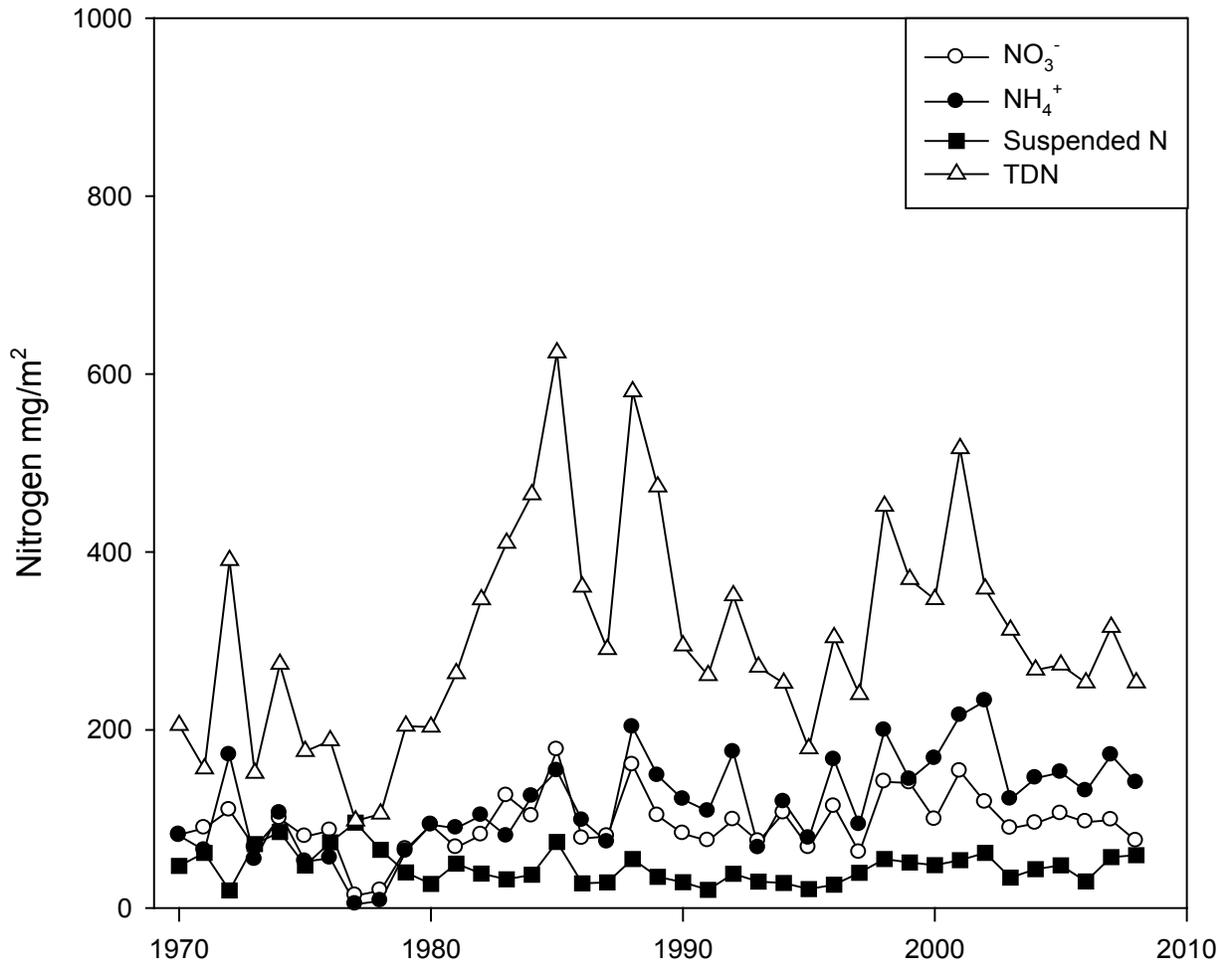


Figure 3:1 Topographic map of selected portion of the ELA with 10m contour intervals; in the bottom left corner the location of the ELA in Ontario. Black dots between L239 and L240 represent the location of ELA base camp buildings. Map shows the locations and years for precipitation sample collection. The L239 island site (open star) was active from 1969 to 1983 when it was moved to the L240 island site (filled star) where samples have been collected since. Summer precipitation collection started at the meteorological site (pentagon with the letter “M”) in 1986 and has continued to the present. Map modified from Department of Natural Resources Canada



**Figure 3:2** Historical record of yearly nitrogen deposition collected at the island sites (Lake 239 until 1983, Lake 240 thereafter) during the open water season from 1970 to 2008

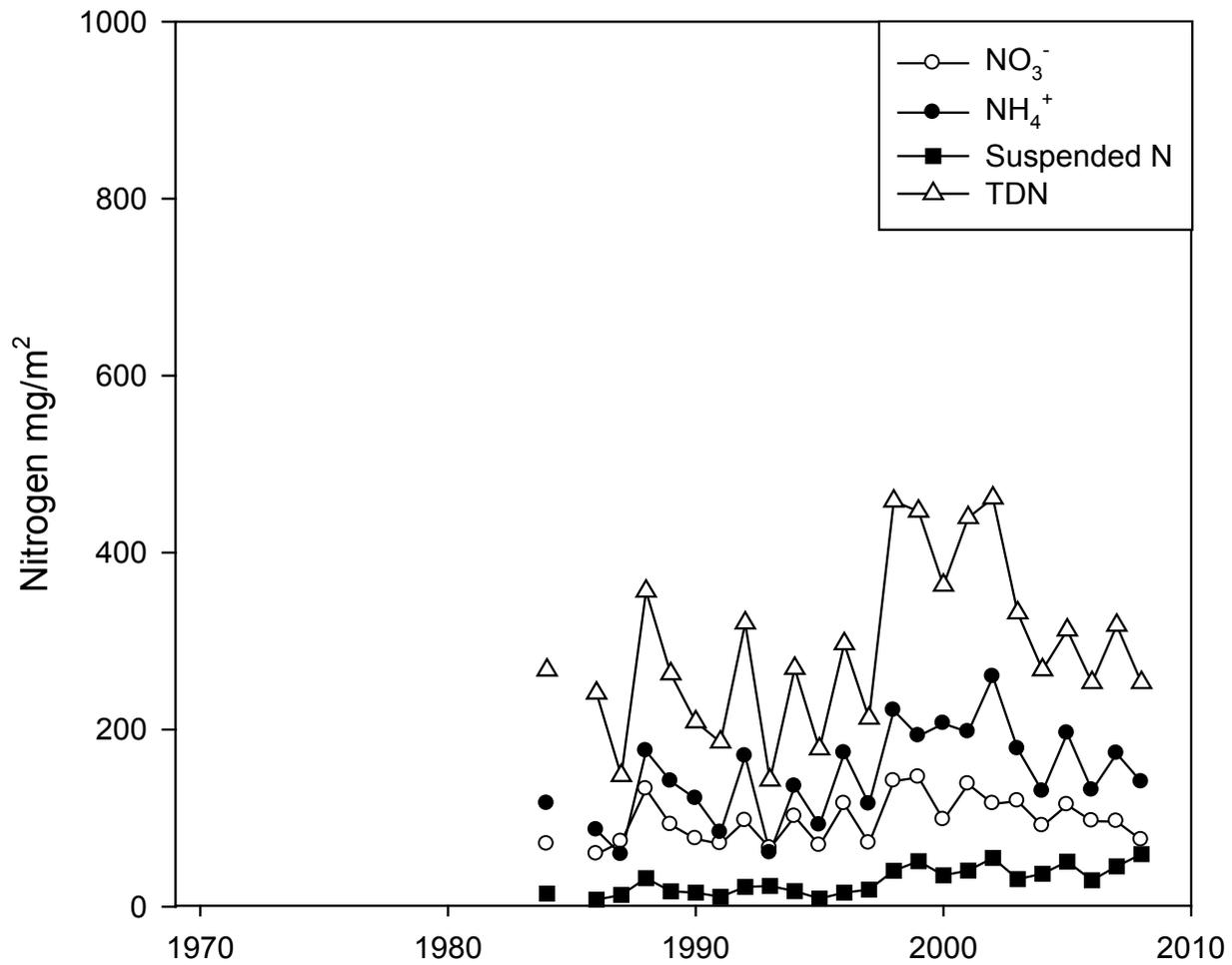
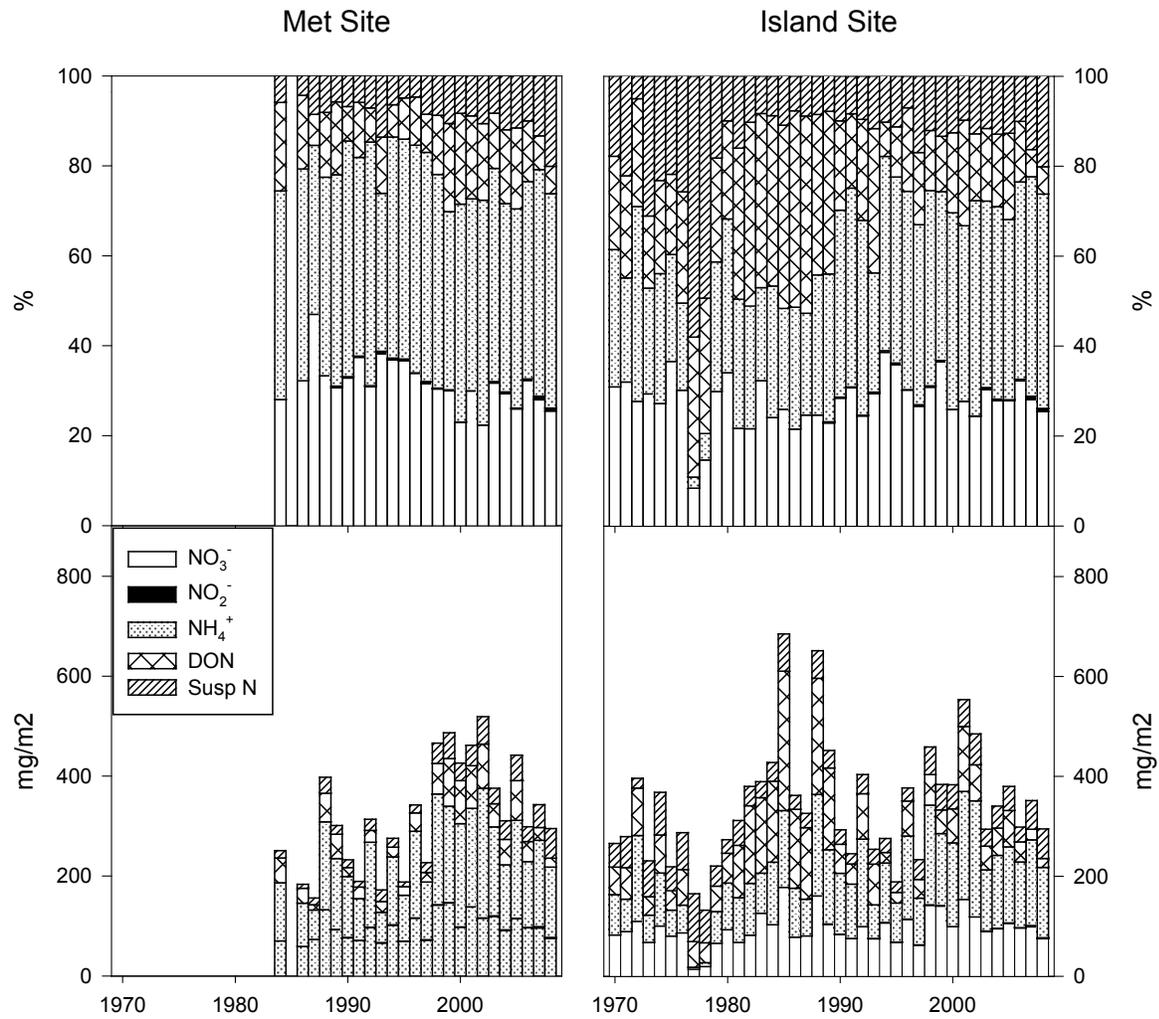


Figure 3:3 Historical record of yearly nitrogen deposition collected at the meteorological site during the open water season from 1986 to 2008



**Figure 3:4 Historical record of yearly nitrogen deposition at the meteorological site (left figures) and the island sites (right figures). The top two figures are the relative percent contribution of nitrate, nitrite, ammonium, DON and Suspended nitrogen to total nitrogen deposition at the meteorological and island sites. The bottom two figures are the median yearly inputs of nitrogen fractions at both the meteorological island sites**

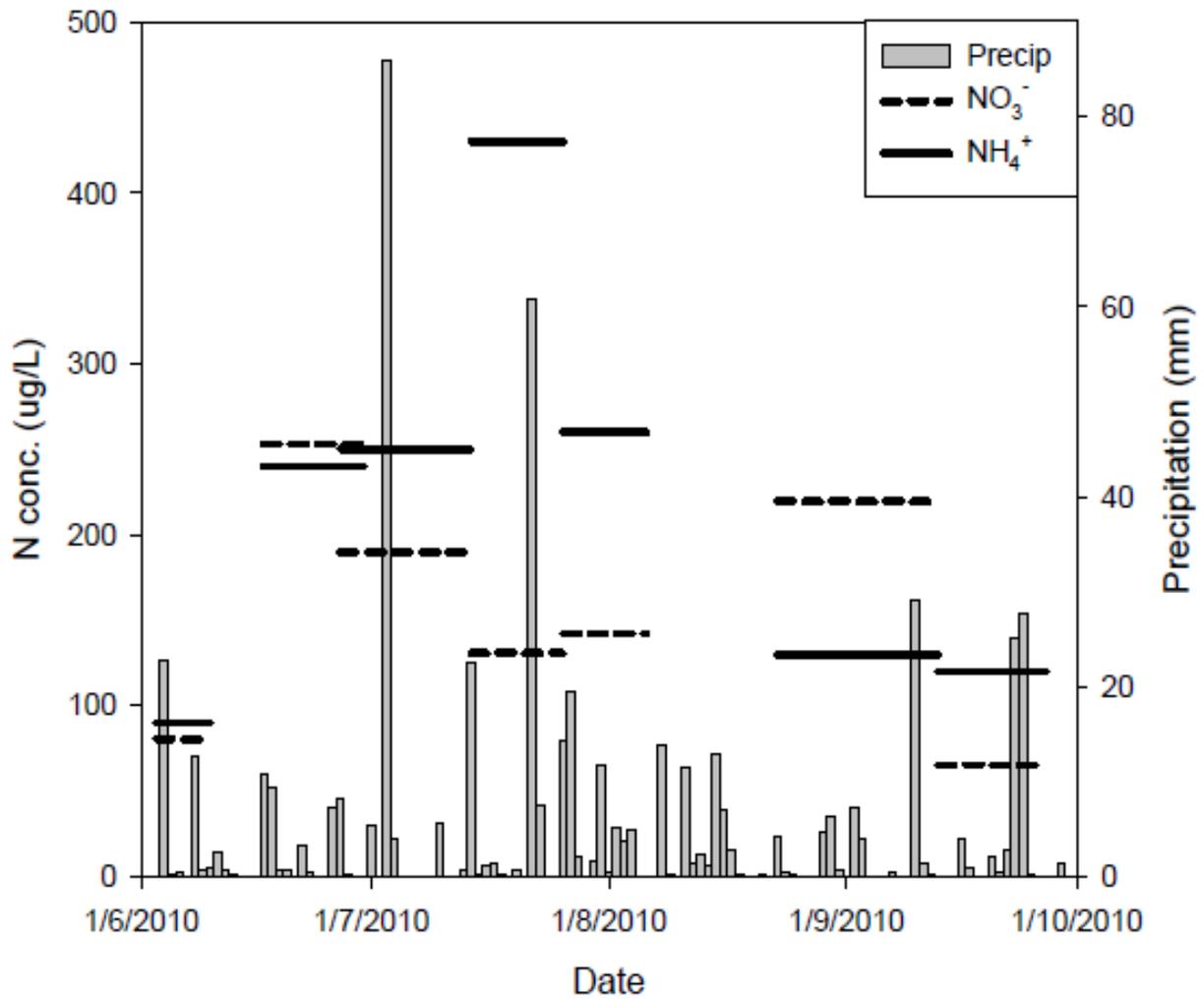
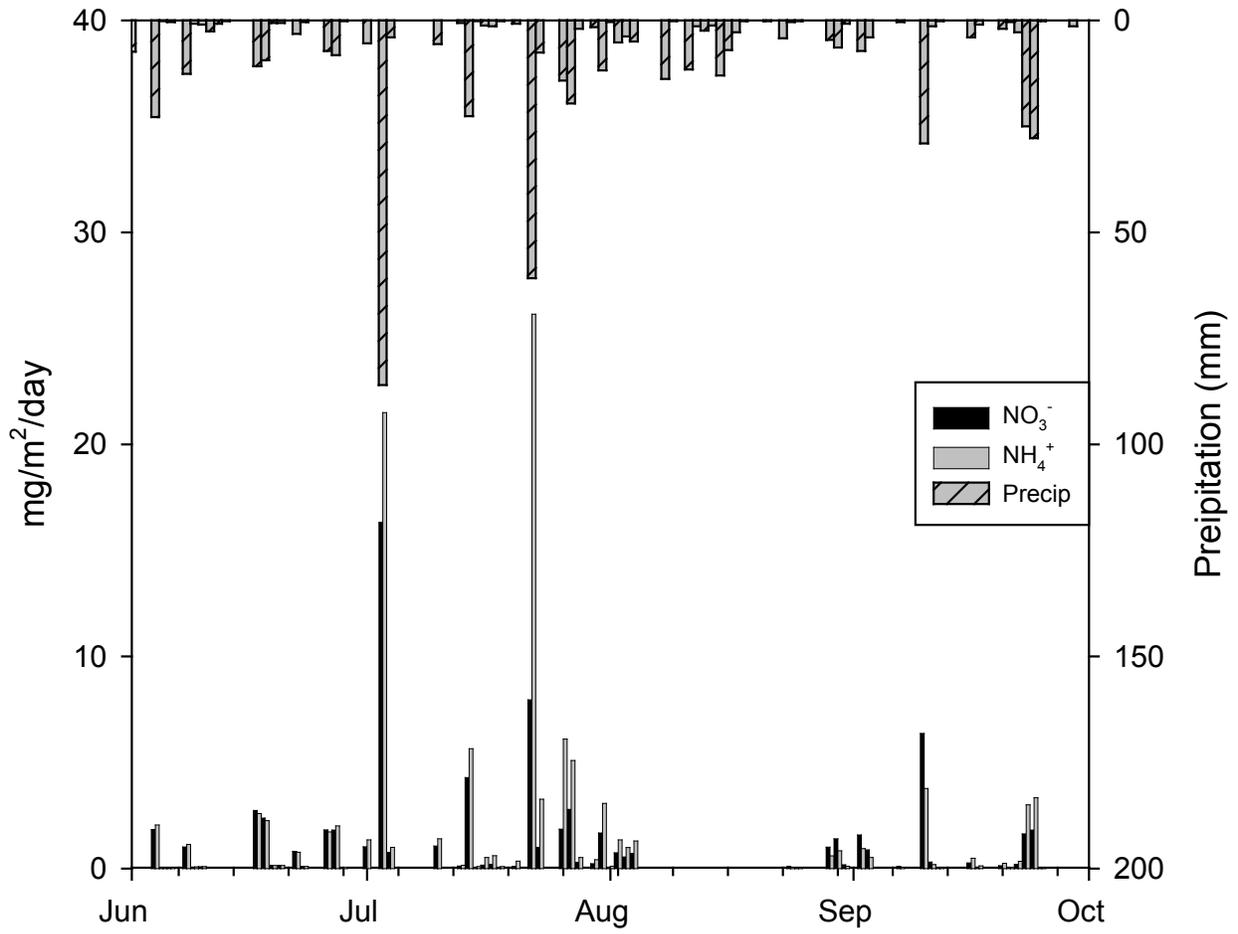


Figure 3:5 Measured nitrate (dashed line) and ammonium (solid line) concentration in atmospheric deposition in 7 periods between June and October of 2010 with the daily total precipitation depth (gray bars, right axis). Samples were bulk collected so breadth of dashed and solid lines signifies the duration of sample collection



**Figure 3:6 Total deposition of nitrate (black bar) and ammonium (gray bar) per day based on average concentration per period and rainfall amount on each day (descending hatched bar). The gap in nitrate and ammonium deposition amounts, between the 6th and 23rd of August, results from missing sample concentration data. The large rain events in July account for 34% of nitrate and 44% of ammonium open water season deposition**

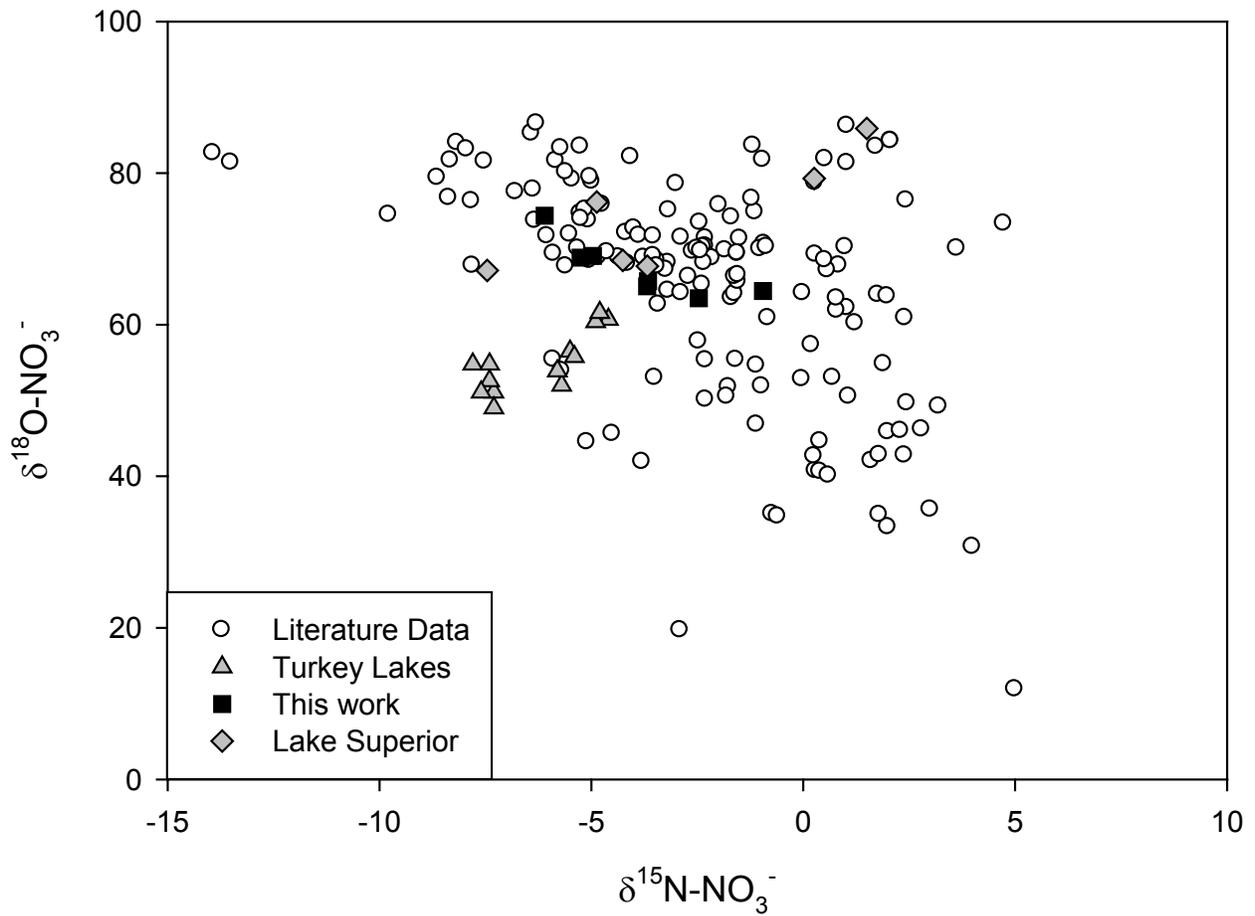


Figure 3:7 Comparison of published (open circles)  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  versus results from this work (filled squares). The Turkey Lakes Watershed (TLW) is located in Ontario, Canada, east of the ELA, while samples from Lake Superior were collected from the south shore of the Lake. These are the geographically closest isotopic values in the published literature

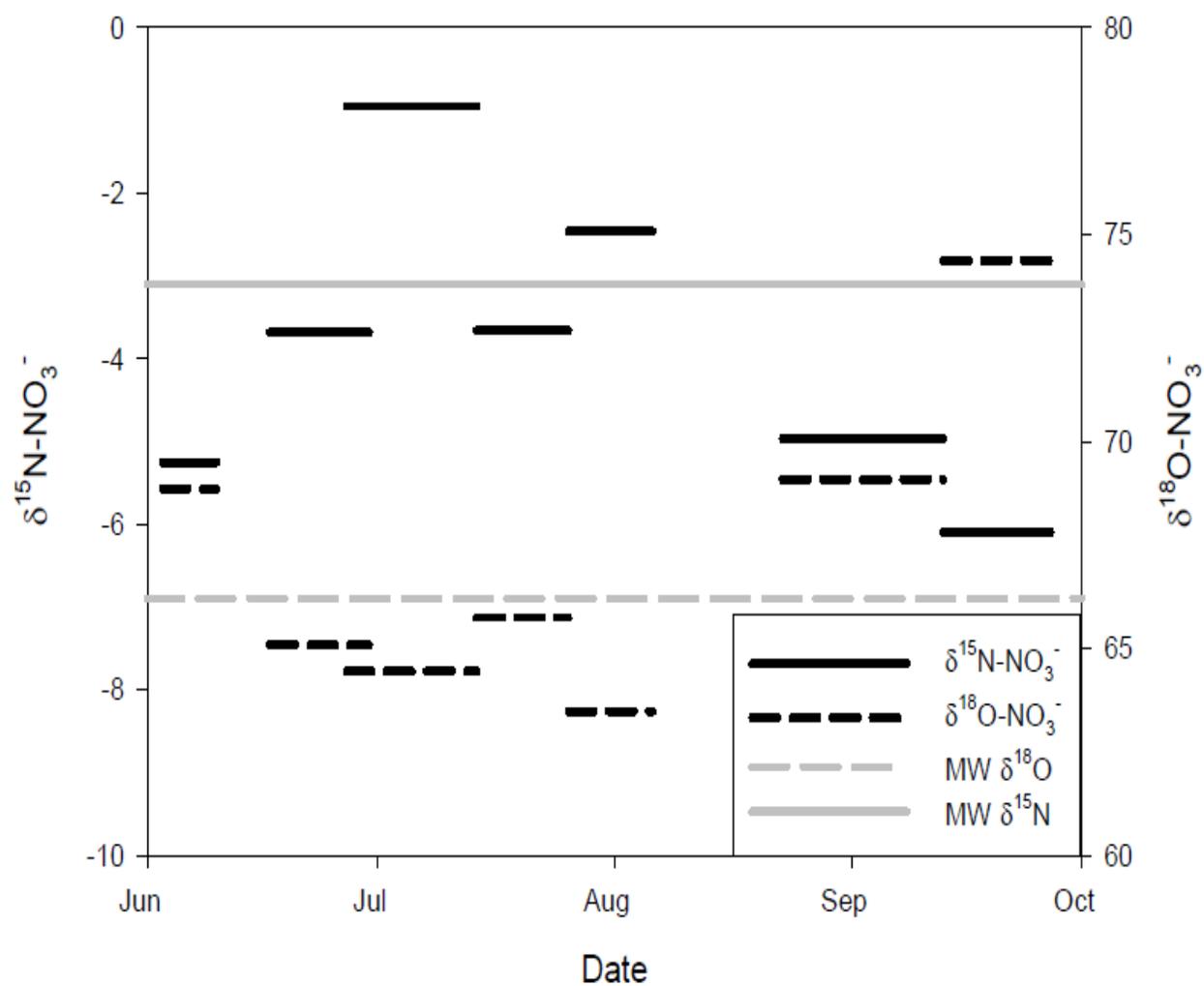


Figure 3:8 Seasonal plot of  $\delta^{15}\text{N-NO}_3^-$  (solid line) and  $\delta^{18}\text{O-NO}_3^-$  (dashed line) values from 7 periods in 2010. Mass weighted average for  $\delta^{15}\text{N-NO}_3^-$  is -3.1‰ while the mass weighted average for  $\delta^{18}\text{O-NO}_3^-$  is 66.2‰. Samples were bulk collected so breadth of dashed and solid lines signifies the duration of sample collection

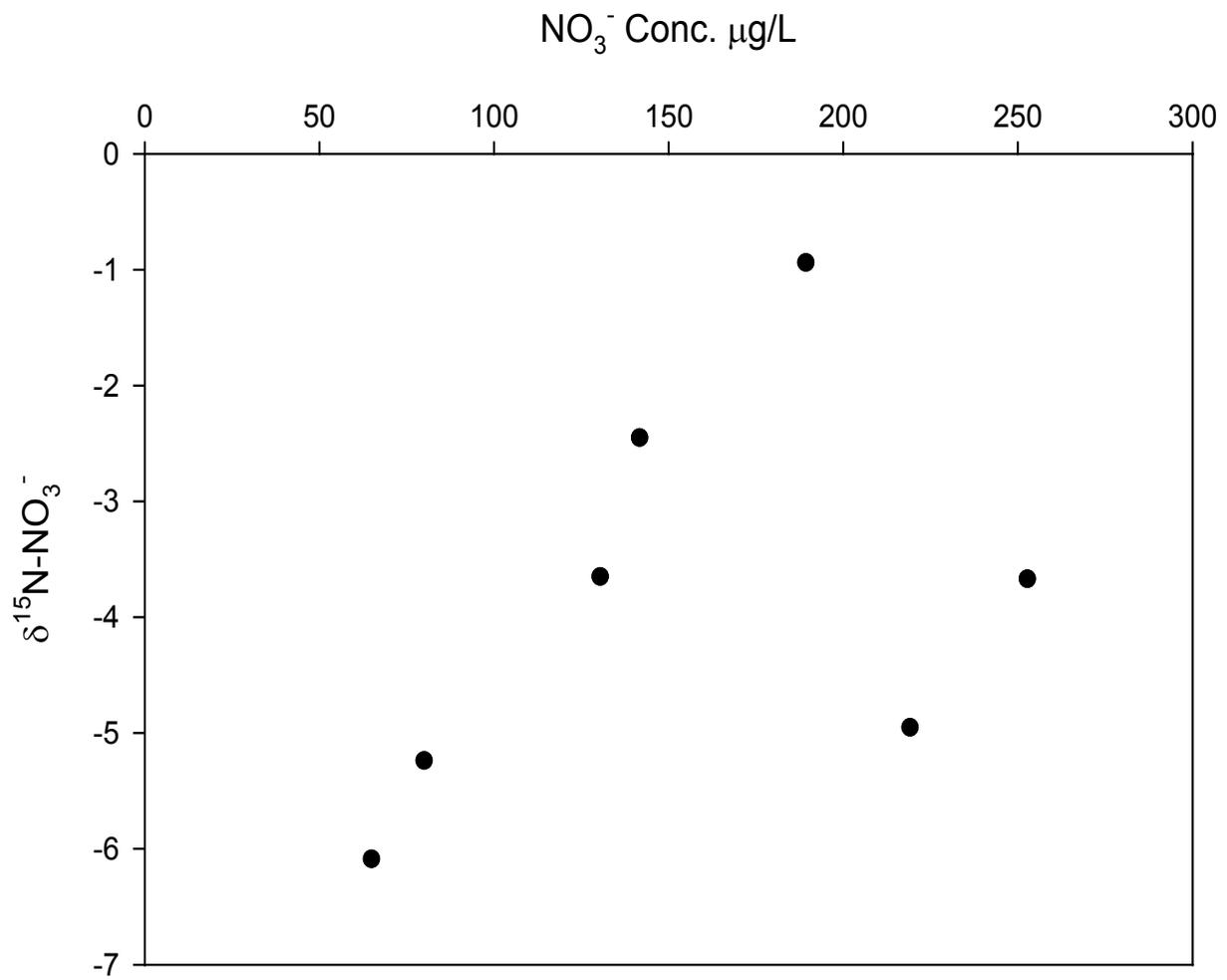


Figure 3:9 The non significant ( $r=0.44$ ,  $p=0.269$ ) relationship between  $\delta^{15}\text{N-NO}_3^-$  and  $\text{NO}_3^-$  concentration in atmospheric deposition in 7 periods in 2010

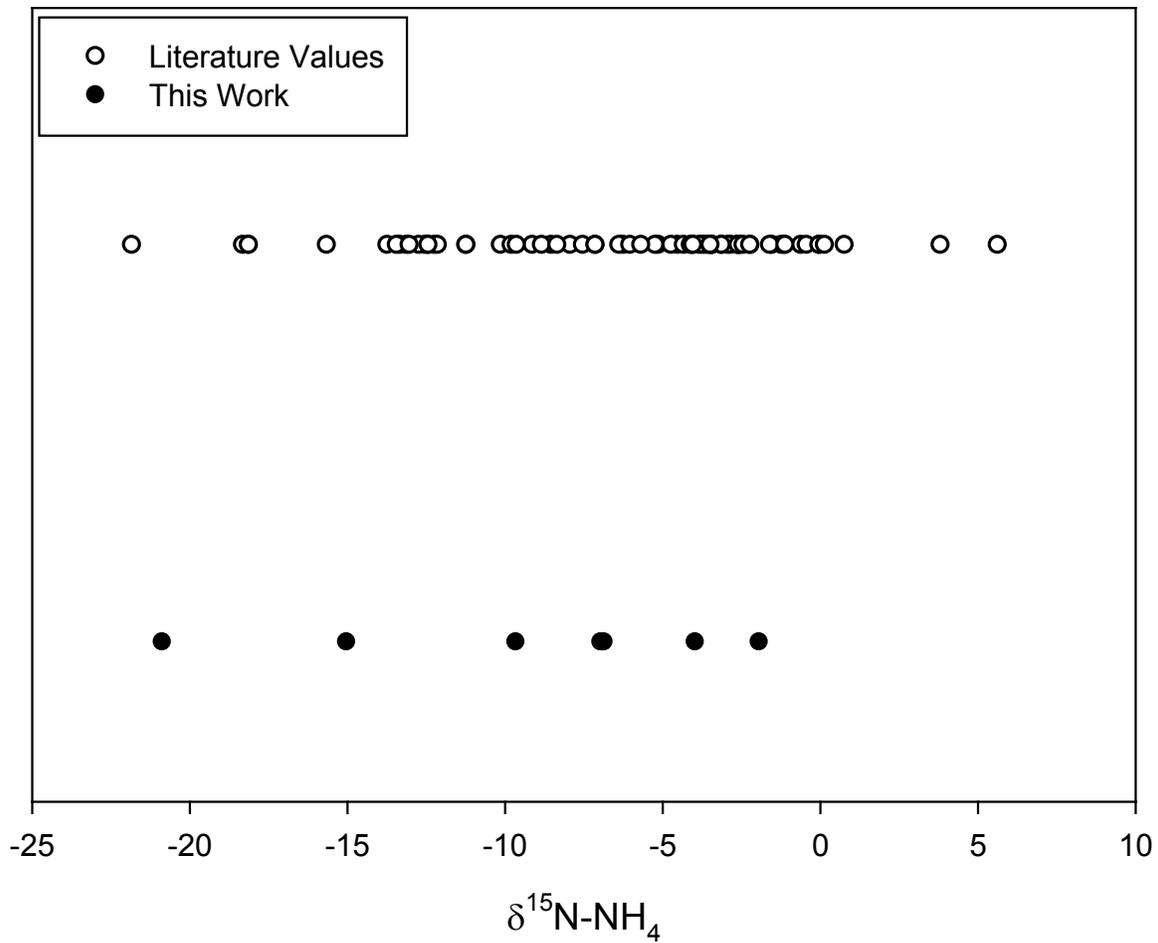


Figure 3:10 Comparison of published (open circles)  $\delta^{15}\text{N-NH}_4^+$  versus results from this work (filled circles). The  $\delta^{15}\text{N-NH}_4^+$  results from this work are the first known in Canada

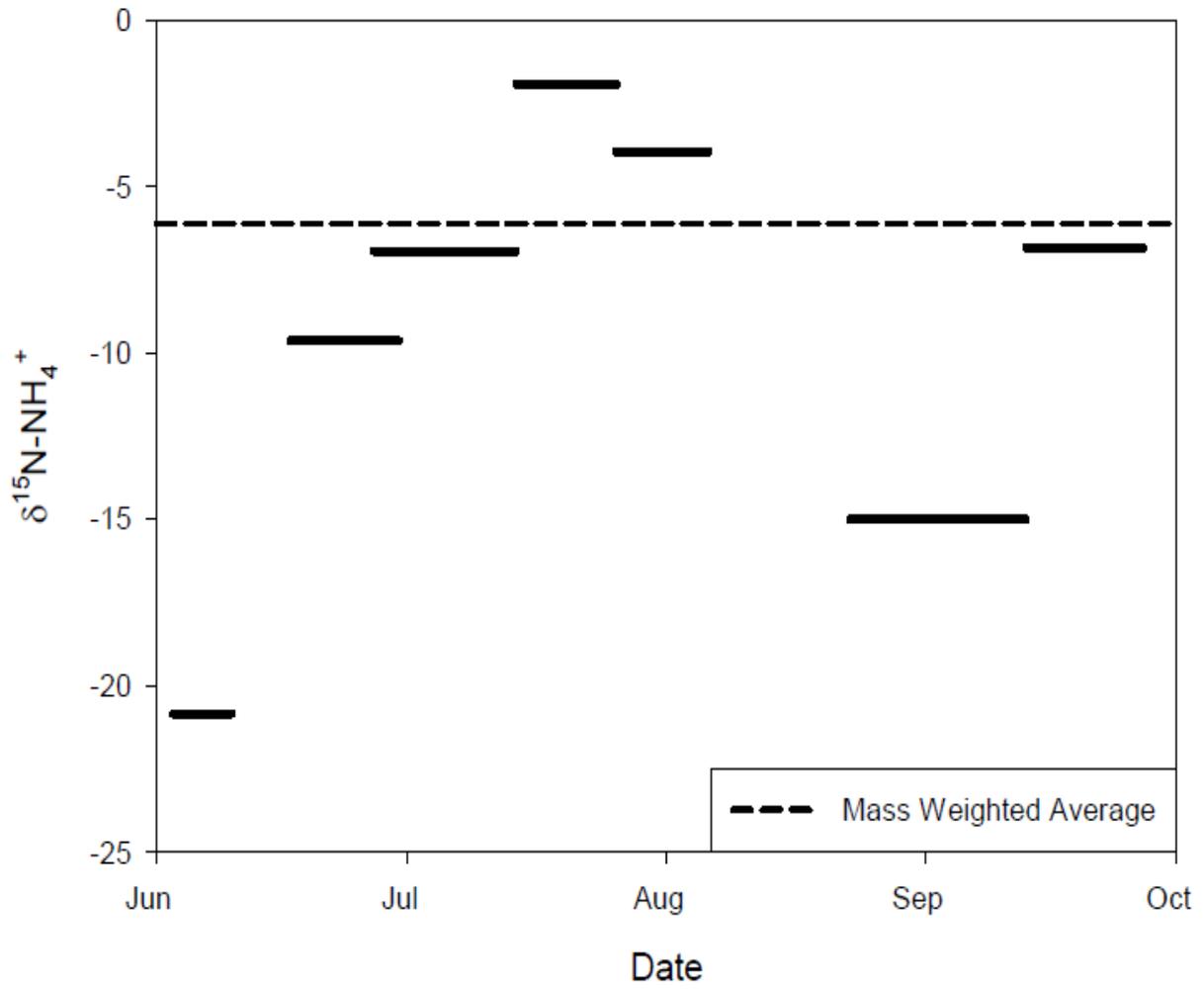


Figure 3:11 Seasonal plot of  $\delta^{15}\text{N-NH}_4^+$  values from 7 periods in 2010. Mass weighted average of  $\delta^{15}\text{N-NH}_4^+$  is -6.1‰. Samples were bulk collected so breadth of dashed and solid lines signifies the duration of sample collection

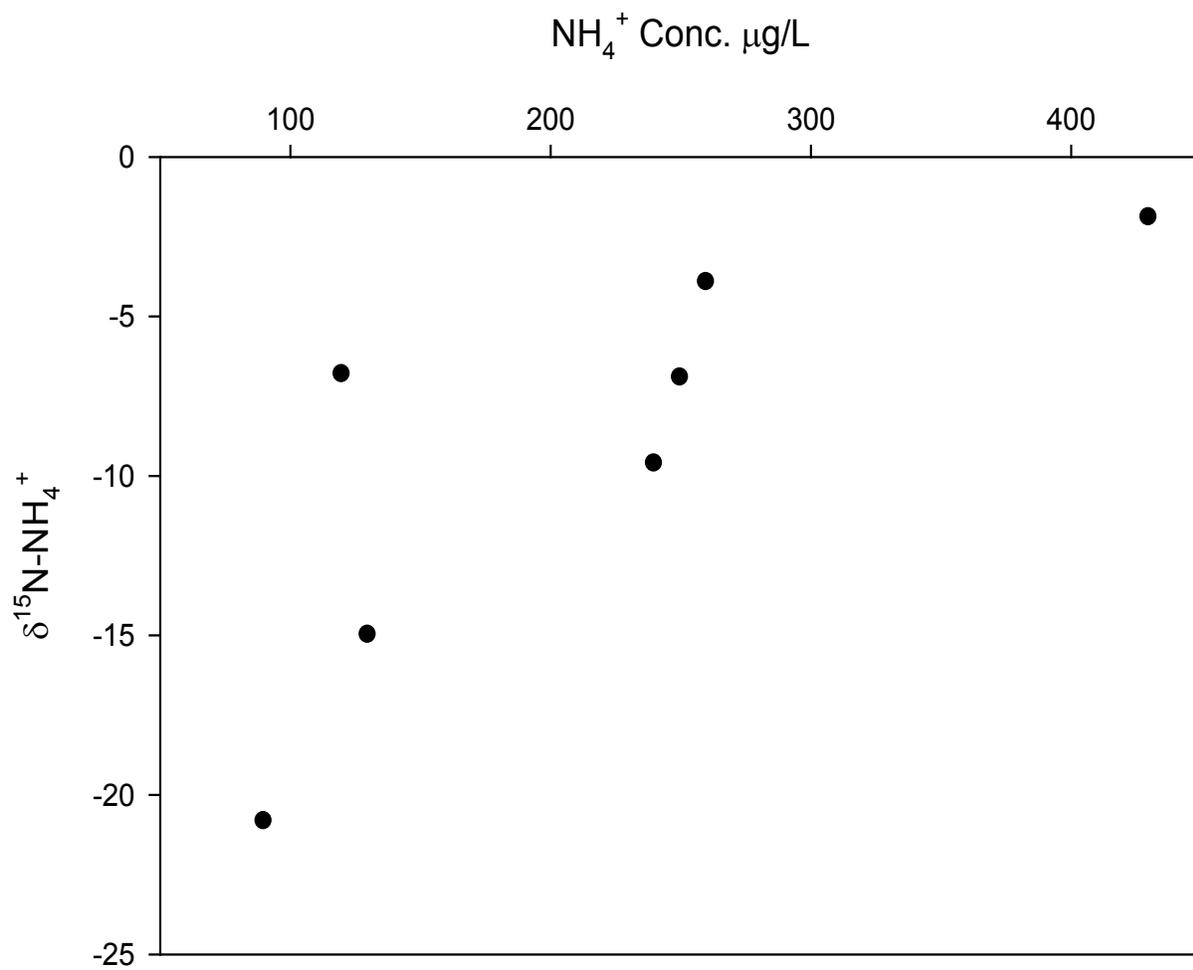


Figure 3:12 The significant ( $r=0.78$ ,  $p=0.02$ ) correlation between  $\delta^{15}\text{N-NH}_4^+$  and  $\text{NH}_4^+$  concentration in atmospheric deposition in 7 periods in 2010

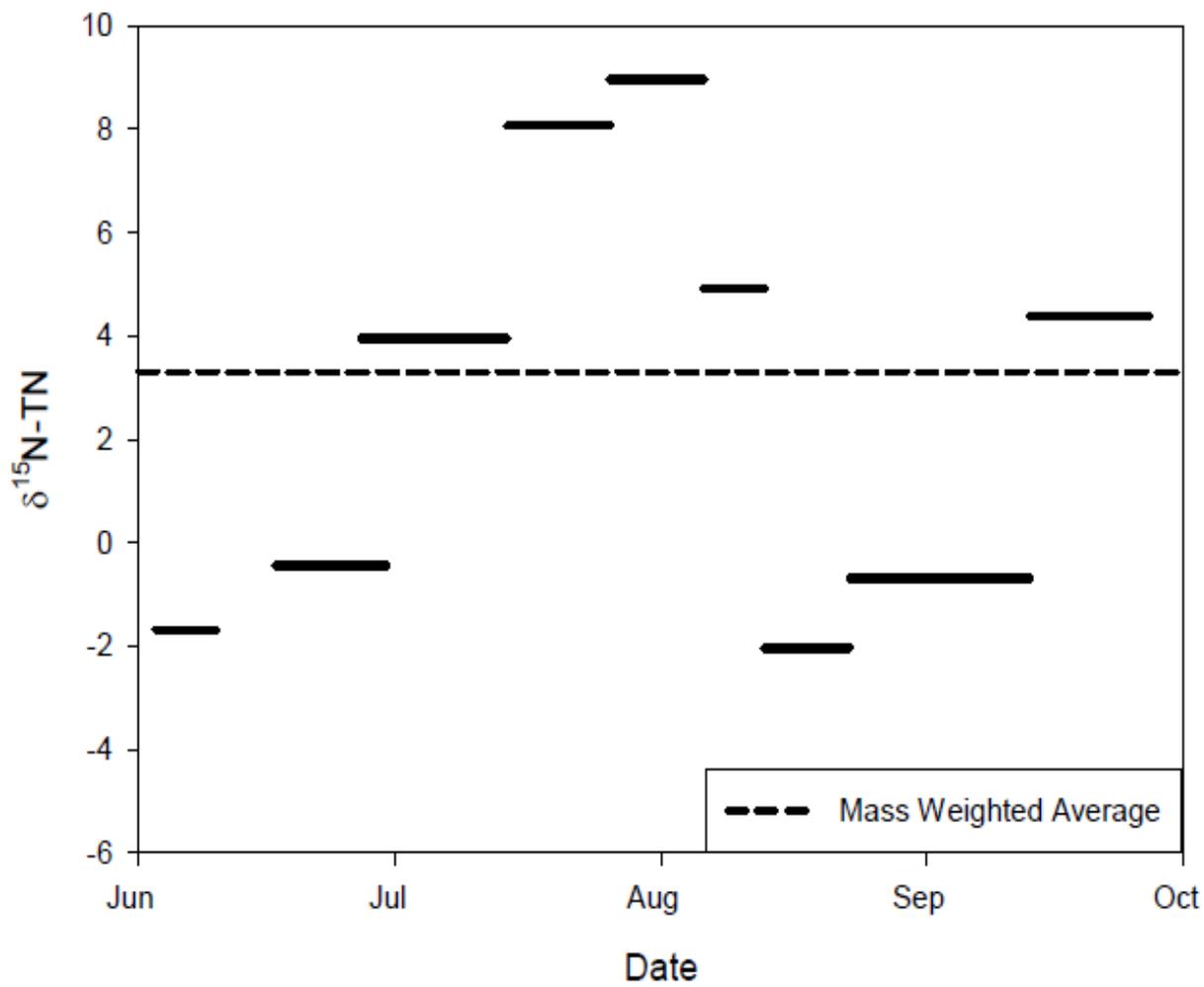


Figure 3:13 Seasonal plot of  $\delta^{15}\text{N-TN}$  values from 7 periods in 2010. Mass weighted average of  $\delta^{15}\text{N-TN}$  is 3.3‰. Samples were bulk collected so breadth of dashed and solid lines signifies the duration of sample collection

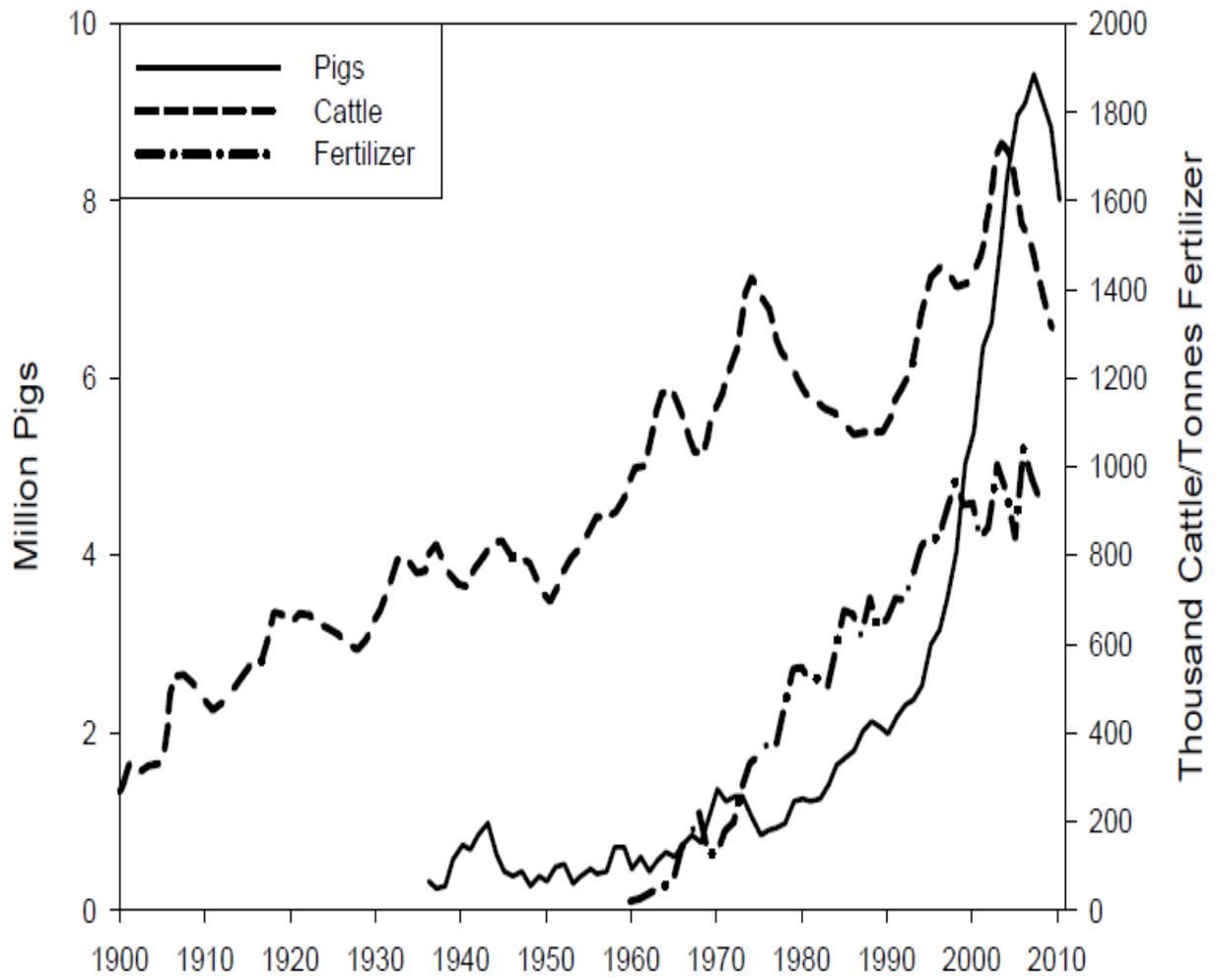


Figure 3:14 Historical heads of cattle, pigs on farms and tonnes of fertilizer purchased in Manitoba, Canada

## 4 Chapter 4: Sediment Isotopes of Carbon and Nitrogen

### 4.1 Introduction

Understanding the past record of nitrogen utilisation, as well as the sources, are important considerations in determining how lakes have changed over time either due to natural succession or anthropogenic disturbance. Nitrogen is utilised by algae and aquatic vegetation for growth, and when nitrogen becomes limiting there are species that can compensate through the fixation of atmospheric nitrogen gas (Schindler, 1977). Many nitrogen fixing species are responsible for the nuisance blooms that occur in lakes and rivers, which can impact the aesthetic qualities of the water as well as pose a potential drinking water hazard due to the release of toxins (Stewart et al, 2006). Management practices strive to control the size and duration of these blooms by enacting measures to reduce nutrient inputs to water bodies (Paerl et al, 2001). Targeting the correct nutrient source is an important management decision based on cost, impact, and feasibility (Paerl et al, 2001). The culmination of 37 years of experimentation on artificially eutrophic Lake 227 at the Experimental Lakes Area has found controlling nitrogen alone to be an ineffective strategy for managing algal blooms, due to the ability of cyanobacteria to fix atmospheric nitrogen (Schindler et al, 2008). The reduction of phosphorus is thought to be the best way to manage nuisance algal blooms (Schindler et al, 2008).

According to the literature, increases in lake primary production from increases in phosphorus loading alter algal carbon and nitrogen isotopes in predictable ways that can be preserved in the sedimented organic matter (Hollander & Smith, 2001; Lu et al, 2010; Meyers & Teranes, 2001). Increases in primary production can cause higher algal  $\delta^{15}\text{N}$  as the available pool of dissolved nitrogen is depleted and the algae biomass  $\delta^{15}\text{N}$  more closely resembles the algae's source of nitrogen (Talbot, 2001). The increase in  $\delta^{15}\text{N}$  of algal biomass continues until the reactive nitrogen concentration becomes low enough to offer nitrogen fixing cyanobacteria a competitive advantage over other algal species (Talbot, 2001). The species composition shift results in the introduction of atmospherically fixed nitrogen from cyanobacteria with a  $\delta^{15}\text{N}$  of 0‰, lowering the  $\delta^{15}\text{N}$  of sediments preserved from this period (Talbot, 2001). However, there are exceptions to this paradigm such as in severely phosphorus limited systems where the increase in  $\delta^{15}\text{N}$  with increased production may not be evident if the amount of nitrogen assimilated by algae is not a significant proportion of the total dissolved nitrogen pool, or when dissolved nitrogen is isotopically similar to atmospheric nitrogen (Talbot, 2001). The  $\delta^{13}\text{C}$  of algal biomass also increases with increased production because of the more complete utilization of available dissolved inorganic carbon, which has progressively higher  $\delta^{13}\text{C}$  because of preferential uptake of  $^{12}\text{C}$  by algae (Meyers & Teranes, 2001). Under times of carbon stress algae can also have a decreased fractionation

factor during uptake of CO<sub>2</sub>. Sedimentation of epilimnetic algae can sometimes deposit the organic matter into conditions where the isotopic composition of the algae can be preserved (Meyers & Ishiwatari, 1993). The isotopic composition of carbon and nitrogen can reflect the growing conditions at that time (Meyers & Teranes, 2001). Long term and continuous deposition of epilimnetic algae builds up a record in sediment that can be collected through coring, to allow for historical reconstructions of in lake conditions. Diagenesis and reworking of the sediments can negatively impact the degree to which the sediments accurately reflect the isotopic composition of the algae (Meyers & Teranes, 2001).

The interpretation of past lake processes from a sediment core relies on methods to assign accurate dates to depths in the core. There are a number of methods to do this, each with advantages and disadvantages. Isotopic methods include the use of <sup>210</sup>Pb, <sup>137</sup>Cs, and <sup>14</sup>C, while physical methods include varve counting and stratigraphy (Refer to Last & Smol, 2001, for a review of chronological techniques). Sinking particles in lakes with little sediment disturbance can form annual bands in the sediment called varves, or varve couplets if distinct summer and winter bands are present. Varve couplets generally consist of a dark and light band signifying winter and summer deposition, respectively (Wolfe et al, 1994). Unless otherwise stated, the use of the word varve in this work refers to a full year of deposition, including both the light and dark bands. By counting annual varves an estimate of the date of sediment deposition in a core can be made (Lamoureux, 2001). Coupling the results from isotopic analysis of sediment core materials with accurate dating allows for the interpretation of past changes in lake productivity and nutrient cycling.

Trends in sediment isotopic composition have been used to interpret sediment cores from many lakes. Lake 227, the site of a long running eutrophication experiment, offers a unique opportunity to test the validity of these interpretations because of the 40 years of detailed historical records of algal species and biomass as well as nutrient concentrations. The goals of this work are to: 1) compare epilimnion  $\delta^{15}\text{N}/\delta^{13}\text{C-POM}$  and  $\delta^{15}\text{N}/\delta^{13}\text{C}$  of surficial sediments to determine if the epilimnetic algae's carbon and nitrogen isotopic signatures are preserved in the sediments of Lake 227 and how this is influenced by diagenesis; 2) assess the validity of standard paleolimnology literature interpretations of changing  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and C:N utilizing a sediment core from the hypolimnion of Lake 227; 3) examine changes in nitrogen cycling of Lake 227 using  $\delta^{15}\text{N}$  in sediment record; 4) examine changes in primary productivity in Lake 227 using  $\delta^{13}\text{C}$  in sediment record; and 5) determine the total masses of nitrogen and carbon stored in the sediments of Lake 227 from pre-eutrophication and post-eutrophication periods .

## 4.2 Methods

Lake 227 is the longest running eutrophication study at the Experimental Lakes Area (ELA) located east of Kenora Ontario, Canada. Lake 227 is a five hectare headwater lake, with a maximum depth of 10m and an average depth of 4m (Brunskill & Schindler, 1971). Lake 227 is generally meromictic, with deeper mixing occurring at fall overturn compared to the spring (Brunskill & Schindler, 1971). Anoxia is present in the hypolimnion of Lake 227 for much of the year (Schindler et al, 1971). Lake 227 has been artificially fertilized with nitrogen and phosphorus, at three different loading rates, for over 40 years (Hendzel et al, 1994). During this time, Lake 227 has been intensely studied, resulting in a large historical data set. Sampling for this research occurred over the period of March to the end of October in 2010, and in March of 2011 for sediment core collection.

Surficial lake sediment samples (0-1.5cm) were collected from Lake 227 under different depths of overlying water using an Eckman dredge. The samples were collected from 1m, 2.5m, 4.4m, 6.5m, 8.4m, and 10.6m of water column depth. Samples were stored in Whirlpack<sup>®</sup> bags and kept frozen until being freeze-dried to dryness. Sample analysis was done using a Carlo Erba 1108 (Italy) elemental analyser coupled to a Thermo Finnigan Delta Plus continuous flow mass spectrometer. Precision for  $\delta^{15}\text{N}$  was  $\pm 0.3\text{‰}$  and for  $\delta^{13}\text{C}$  was  $\pm 0.2\text{‰}$ .

Sediment cores were collected under ice on March 2<sup>nd</sup>, 2011, at Lake 227 by freeze coring, following a method modified from Huttunen and Merilainen (1978). Cores were taken from a number of depths, and a 50cm core from 7m was analysed for the purpose of this study. The 7m core was sectioned using a band saw in a freezer, at 0.5cm intervals to a depth of 11cm, and at 1.0cm intervals to the 50cm mark of the core. The same pre-analysis treatment was performed on sediment core samples as was performed on surficial sediment samples with analysis completed on the same instrument.

In order to determine the isotopic composition of particulate matter in the epilimnion, water samples were collected every two weeks during the open water season, and once under ice in the late winter of 2010. Samples were filtered onto pre-combusted QMA filters and frozen until analyses at the Environmental Isotope Lab (EIL) at the University of Waterloo. These filters were packaged into tin cups before being analysed on the same instrument as sediment isotope samples.

Oxygen and nutrient concentrations of lake water were retrieved from the ELA database. Samples were processed according to Stainton et al (1977).

## **4.3 Results**

### **4.3.1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of Particulate Matter in the Epilimnion and Surficial Sediments**

In order to find the degree to which the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of the epilimnetic particulate matter in Lake 227 is recorded in the hypolimnetic sediments, the  $\delta^{15}\text{N}/\delta^{13}\text{C}$ -POM of the epilimnion was compared to the  $\delta^{15}\text{N}/\delta^{13}\text{C}$  of surficial lake sediments. Over the course of the 2010 open water season the  $\delta^{15}\text{N}$  of particulate matter in Lake 227 was slightly variable (figure 4.1.). The means of  $\delta^{15}\text{N}$ -PON from 1m and 4m over the open water season were not significantly different (T-test,  $p=0.1$ ). The non-weighted mean of epilimnion (1m and 4m)  $\delta^{15}\text{N}$ -PON samples was  $0.4\pm 0.9\text{‰}$ , ranging from  $-1.2\text{‰}$  to  $2.3\text{‰}$ . The lowest  $\delta^{15}\text{N}$ -PON was during the cyanobacteria bloom in June 2010. The open water and under ice  $\delta^{15}\text{N}$ -PON were significantly different (T-test,  $p<0.01$ ), with under ice  $\delta^{15}\text{N}$ -PON being higher.

Particulate carbon mean  $\delta^{13}\text{C}$  in the epilimnion at 1m and 4m (figure 4.2) were significantly different (T-test,  $p<0.05$ ), the mean at 1m was  $-27.8\text{‰}$  and  $-31.2\text{‰}$  at 4m.  $\delta^{13}\text{C}$  POM reached a maximum of  $-22.9\text{‰}$  at 1m during the cyanobacteria bloom in June 2010. The under ice  $\delta^{13}\text{C}$ -POM of the epilimnion was significantly different (T-test,  $p<0.05$ ) than the open water  $\delta^{13}\text{C}$ -POM.

The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of the open water particulate had similar isotopic composition to the hypolimnetic sediments (figure 4.3 and 4.4). The range for  $\delta^{15}\text{N}$  of particulate from the epilimnion was  $-1.2\text{‰}$  to  $2.3\text{‰}$  (mean  $0.4\pm 0.9\text{‰}$ ), while the sediment below the oxycline was on average  $0.2\text{‰}$ . The  $\delta^{13}\text{C}$  of the sediments below the oxycline also fell within the  $\delta^{13}\text{C}$  range of open water epilimnetic POM, with the sediments having an average of  $-29.9\text{‰}$ . To determine whether the epilimnion particulate matter was conservatively isotopically labeling the sediment, a mass weighted mean  $\delta^{15}\text{N}/\delta^{13}\text{C}$ -POM from 1m and 4m was calculated using suspended particulate matter masses (figure 4.5) from the ELA database. The concentrations of suspended nitrogen were converted to masses by multiplying the concentration on each date at 1m and 4m by the volume of water in those strata. The 1m and 2m water volumes were assigned to the 1m concentration while 3m and 4m volumes were applied to the 4m concentration. The resulting calculated values of  $0.5\text{‰}$  and  $-29.8\text{‰}$  for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  respectively are very close, within  $0.3\text{‰}$  for both carbon and nitrogen, to the anoxic sediment isotopic composition. This comparison relies on the assumption that there is no preferential incorporation of algal mass of certain periods (all dates are retained in sediments dependent only on relative percentage of total epilimnion mass). The hypolimnetic  $\delta^{15}\text{N}$ -PON and  $\delta^{13}\text{C}$ -POC are significantly different than the epilimnetic  $\delta^{15}\text{N}$ -PON (T-test,  $p<0.05$ ) and  $\delta^{13}\text{C}$ -POC (T-test,  $p<0.05$ ), with a mean  $\delta^{15}\text{N}$  of  $-7.3\text{‰}$  and  $\delta^{13}\text{C}$  of  $-38.2\text{‰}$ . The impact of this much lower  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  particulate from the hypolimnion was not evident in the surficial sediments, possibly because there was not enough mass produced in the hypolimnion to alter the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of sediments. It is also possible that the slow shifting of higher  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  sediments from the oxic epilimnion to the hypolimnion, due to wave action, was offsetting the contribution from low  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  hypolimnetic POM in the sediment record. The C:N of the surficial sediments does not reflect the C:N of the POM in

Lake 227 (figure 4.6). The mass weighted average C:N of epilimnetic POM in Lake 227 was 9.8, while the hypolimnetic sediments averaged 11.8. The hypolimnetic sediments likely had a higher C:N than particulate because of the influence of high C:N allochthonous material that is not retained on filters due to material size or the episodic nature of allochthonous additions to Lake 227. No relationship exists between depth of water or oxygen concentration and C:N of surficial sediments.

The surficial sediment  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  was sampled in July 2010, at six different water depths in Lake 227. Above 4m, where there was oxygen present, the  $\delta^{15}\text{N}$  of the sediments was higher by approximately 2‰, than the sediments below 4m, where oxygen was not present (figure 4.7). This trend is also true for the  $\delta^{13}\text{C}$  of the sediment with lower  $\delta^{13}\text{C}$  values under water that was low in dissolved oxygen, mean of -29.9, in comparison to oxygen rich water where the  $\delta^{13}\text{C}$  has a mean of -27.1‰. Below 6m of water column depth the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of surficial sediments do not change greatly, so core taken at any depth of water greater than 6m should have the same isotopic signature recorded, although the sedimentation rate could be different due to focusing of sediment to deeper depths.

#### **4.3.2 Lake 227 Sediment Core Characterization and Sedimentation Rates**

The core collected from Lake 227 at 7m of water column depth showed visible varves down to a depth of 11cm of the core, this core was not dated by radiological methods. However, the transition from unvarved to varved is consistent with an increase in hypolimnetic anoxia arising from artificially induced eutrophication. Oxygen concentrations from 6m and 8m depth at that time are at the detection limit on the low end so anoxia was present at 7m during the stratified season in the earliest available samples. Thus this horizon likely corresponds to the beginning of the eutrophication experiment in 1970. An estimation of the depositional rate was made. Taking 11cm to be the start of the eutrophication experiment, based on the appearance of visible varves at this depth, this depth of core represents approximately 40 years of deposition. Accumulation of 11cm of sediments over 40 years is equal to an average depositional rate of 0.28cm/yr for the eutrophic period. Estimating the sedimentation rate in this way comes with caveats because in older sediments the compound effects of compression and sediment diagenesis will result in lower sedimentation rates relative to new, uncompressed sediments. Using methods similar to Anderson et al (1987) an estimation of mass accumulation was also made based on estimated porosity and water content of Lake 227 sediments. The calculated mass accumulation rate was 0.009g/cm<sup>2</sup>/yr. This sedimentation rate estimation using the varve horizon is in agreement with the literature values for the post-eutrophication period of Lake 227 (Table 4.1).

#### **4.3.3 Inventory of Lake 227 Sediment Nitrogen and Carbon Masses**

The average annual net mass of nitrogen lost to the sediment in Lake 227 was calculated based on the percent nitrogen from the Delta EA and estimated sedimentation rates from pre and post eutrophication from Leavitt and Findlay (1994) (table 4.2). The sedimentation rates from other literature were used to provide a consistent source for Pre and Post eutrophication estimates as in this work an accurate pre-fertilization estimate of sedimentation rate could not be made. The mass of nitrogen lost per year previous to eutrophication, accounting for sediment focusing, was 47kg-N. The loss of nitrogen to sedimentation post eutrophication, for all experimental conditions, was 148kg-N/year. Sedimentation amounts were calculated for each experimental condition, the high nitrogen loading sedimentation mass was 121kg-N/year, the low nitrogen mass was 141kg-N/year, and the no nitrogen loading mass was 169kg-N/year. The different sedimentation mass from the high, low and no nitrogen loading conditions is likely a product of the loss of nitrogen mass over time in the sediments due to diagenesis.

The mass of carbon in the sediments was calculated using the same methods as nitrogen (table 4.2). Pre-eutrophication mass loss to sedimentation was 600kg-C/yr while post-eutrophication average was 1555kg-C/yr. In the three experimental conditions the mass lost was 1369kg-C/yr, 1505kg-C/yr, and 1683kg-C/yr for high nitrogen, low nitrogen, and no nitrogen loading respectively.

#### **4.3.4 Lake 227 Sediment Core Record of $\delta^{15}\text{N}$ , $\delta^{13}\text{C}$ and C:N During Three Experimental Conditions**

Using the estimated post-eutrophication sedimentation rate of 0.28cm/yr the core was divided up into four time periods: Pre-1970, 1970-1975, 1976-1990, and 1990 until present, corresponding to changes in the experimental loading to Lake 227. The loading changes are: No fertilizer (Pre-1970), High Nitrogen (1970-1975), Low Nitrogen (1976-1989) and No Nitrogen (1990-present). The no fertilizer time period reaches from the bottom of the core to 11 cm and using estimates of pre-fertilization sedimentation rates from the literature could extend back as far as 1855. The three experimental treatments resulted in the deposition of sediments with significantly different  $\delta^{15}\text{N}$  ( $p < 0.01$ ) according to a one-Way ANOVA (figure 4.8 and figure 4.9). The highest  $\delta^{15}\text{N}$  occurred at the highest artificial nitrogen loading level, while the lowest  $\delta^{15}\text{N}$  occurred most recently with no fertilizer nitrogen additions. The low nitrogen addition time period was at an intermediate  $\delta^{15}\text{N}$ . The pre-fertilization time period most closely resembled the low nitrogen loading conditions with regards to  $\delta^{15}\text{N}$  of the sediment recorded. For  $\delta^{13}\text{C}$ , the pre-fertilization time period had a mean  $\delta^{13}\text{C}$  of  $-30.4 \pm 0.4\text{‰}$ , while both the low nitrogen and high nitrogen loading conditions resulted in significantly different mean  $\delta^{13}\text{C}$  values (one-way ANOVA,  $p < 0.01$ ),  $-28.1 \pm 0.2\text{‰}$  and  $-28.4 \pm 0.3\text{‰}$  respectively (figure 4.9). The  $\delta^{13}\text{C}$  has been decreasing since reaching a maximum near

the high nitrogen to low nitrogen transition, but the rate of decrease in  $\delta^{13}\text{C}$  was highest beginning at 4.0cm, approximately 1996, until present (figure 4.11).

The C:N was lower and in the 40 years post eutrophication, than the 100 years previous to eutrophication (figure 4.7 and figure 4.9). The range post eutrophication was 9.8 to 13.3 while, previous to eutrophication, the C:N ranged from 13.7 to 15.4. The C:N with depth (figure 4.12) shows the sudden decrease in C:N that occurred at the time of eutrophication (from 14.8 to 13.1).

The section of core with the lowest  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was at the surface of the sediment core. This sample was lower in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  than the surficial sediment sample collected in July of 2010. Recent  $\delta^{15}\text{N}$  POM data from January 2011 (Schiff et al, unpublished data) suggest that incorporation of recent algal remains from the under ice period into the sediment core could be a possible cause of the very low  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  section at the very top of the core. However it makes up only a small percentage of the total mass compared to the large input of organic matter from the open water season epilimnetic growth.

## **4.4 Discussion**

### **4.4.1 The Effect of Diagenesis on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$**

Preservation of the isotopic composition of autochthonous material in the sediments provides a resource to study past lake conditions, but only if what is preserved in the sediment has not been greatly altered from the source (Meyers & Teranes, 2001). The distance of overlying water travelled during sedimentation has an impact on the degree of isotopic reworking of particles, as breakdown of organic matter can increase the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the residual organic matter (Meyers & Iswhatari, 1993). The longer the path to the sediment the more time available to decompose the organic matter in the water column and therefore the greater the expected change in the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (Teranes & Bernasconi, 2000). The similarity between the open water season  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  POM and the hypolimnetic surficial sediments indicates that the isotopic composition of particulate is not greatly altered on the path to the sediments through the water column in this small lake. While time of travel is an important consideration for organic matter reworking, oxygen concentration in the path of the particulate can dominate the degree of microbial break down of organic matter (Lehmann et al, 2002). Under oxic conditions the proportion of organic matter that is resistant to degradation is lower than under anoxic conditions (Lehmann et al, 2002). This can lead to greater increases in  $\delta^{15}\text{N}$  under oxic conditions as the hydrolysis of organic matter preferentially removes  $^{14}\text{N}$  of organic matter, but would require large mass loss because of the small fractionation associated with mineralization (Lehmann et al, 2002). In a study of multiple sediment cores retrieved from an anoxic varved lake over a period of 27 years, Galman et al (2009) found that  $\delta^{13}\text{C}$  increased between 0.4- 1.5‰ in the first five years following deposition, which corresponded with a 20%

loss of initial carbon over that time period. Following this initial 5 year period of high carbon loss, the  $\delta^{13}\text{C}$  became relatively stable with only minor variability, possibly related to differences in individual cores (Galman et al, 2009). The amount of change in the  $\delta^{15}\text{N}$  of sediments in the same study was more variable and not dependent on the time since deposition. The  $\delta^{15}\text{N}$  decreased by 0.3-0.7‰ over the entire 27 year period, with most of the change in  $\delta^{15}\text{N}$  occurring during early depositional loss of 30% of initial nitrogen. The changes in the Lake 227 sediment core  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  are greater than the amount of change expected to be caused by diagenesis, so they should still retain information relevant to in-lake nitrogen cycling and productivity following diagenesis.

#### **4.4.2 Allochthonous versus Autochthonous Material in Lake 227 Sediments**

Lake sediments are a mixture of allochthonous and autochthonous material. Eutrophic lakes, such as Lake 227, have high levels of autochthonous production and as such, the sediments in these lakes have a higher proportion of autochthonous to allochthonous material. In Lake 227 the sedimentation rate doubled at the start of eutrophication because of increases in autochthonous production (Wolfe et al, 1994). The decrease in C:N in the sediment core at the start of the eutrophication experiment indicates that the sediment contains more autochthonous material post-eutrophication than pre-eutrophication. Based on estimated C:N values for autochthonous production (5-8) and allochthonous material (20-100) the proportion of autochthonous material in the sediments is at least 60%, using the most conservative estimate, although it is likely higher. Therefore changes in the sediments in Lake 227 should represent in-lake processes.

#### **4.4.3 Isotopic Labeling of Sediments by Epilimnetic Particulate Matter**

The particulate matter from the photic zone (1m and 4m) during the open water season in Lake 227 has a similar  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  to the hypolimnetic sediments. The impact of under ice particulate on the isotopic composition of Lake 227 sediments is suspected to be small. Wolfe et al (1994) concluded that the amount of total yearly deposition was determined predominantly by the summer sedimentation based on dark and light band thickness in varve couplets from a Lake 227 sediment core. This is true deeper in the core, but the very top of the core may be heavily influenced by the under ice particulate because it has not been compressed or decomposed. Under-ice particulate likely comprises the whole top core section (0-0.5cm), even though the total material is small because of very high porosity.

The  $\delta^{15}\text{N}$  values in the oxic surficial sediments of Lake 227 are higher than anoxic sediments, possibly because of the higher total loss of  $^{14}\text{N}$  reactive organic matter or denitrification of remineralized nitrogen in the epilimnetic sediments. In Lake 227, the  $\delta^{13}\text{C}$  in the oxic zone sediments is increased relative to the source particulate. The anoxia and cold temperatures in the hypolimnion of Lake 227 probably aid in the preservation of the original isotopic composition of deposited particulate by reducing

the breakdown of the organic matter. The anoxic hypolimnion also acts to preserve the isotopic signature of the sediment by not providing a suitable habit for benthic invertebrates requiring oxygen, eliminating bioturbation of sediments (Meyers & Ishwatari, 1993). Resuspension and transport of shallow sediments to the hypolimnion (focusing) is a confounding factor for paleo-reconstructions as it has been shown in this work that shallow surficial sediments are isotopically different than hypolimnetic sediments. Crusius and Anderson (1995) calculated the focusing of shallow sediments to deep sediments in Lake 227 to occur at a rate of 5-10% a year. The contribution of epilimnetic sediments, with isotopic compositions shown in this work, would not greatly change the isotopic composition of hypolimnetic sediments, increasing the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  by 0.3‰ and 0.2‰ respectively, at the higher level of focusing. Thus, the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of the sediment core is likely a good indicator of in lake algal biomass isotopic composition and therefore nitrogen and carbon dynamics.

#### **4.4.4 $\delta^{15}\text{N}$ as a Proxy for Historical Lake 227 Nitrogen Cycling**

The past 40 years of nitrogen loading to Lake 227 has been extensively studied and the changes in dominant sources of nitrogen and lake phytoplankton assemblages are well known. Previous to the experimental additions, a short period of study was conducted to create an oligotrophic baseline for the eutrophication experiment (Schindler et al, 1971). It was later discovered through paleolimnology by Leavitt et al (1994) that Lake 227 had experienced some natural eutrophication in the ten years preceding the artificial fertilizer additions. This was based on the increased abundance of mesotrophic diatom microfossils and increased sedimentary phosphorus content. When the report of pre-experimental eutrophication is compared to the  $\delta^{15}\text{N}$  from the sediment core, the increase in eutrophication is not evident under the standard interpretation of increased eutrophication leading to increased  $\delta^{15}\text{N}$  in sediments (Talbot, 2001). However, Holtgrieve et al (2011) have found decreases in sediment core  $\delta^{15}\text{N}$  in remote lakes beginning in the early 1900's and accelerating after 1950 due to increased deposition of anthropogenic nitrogen with a low  $\delta^{15}\text{N}$ . The decreasing trend in the Lake 227 sediment core begins at approximately the correct time to be the result of similarly increased anthropogenic nitrogen deposition. The majority of the decrease in  $\delta^{15}\text{N}$  in lake sediments found by Holtgrieve et al (2011) occurred in the period after the beginning of the eutrophication experiment and was masked by the artificial nitrogen additions in Lake 227.

The start of the eutrophication experiment is marked by a large increase in  $\delta^{15}\text{N}$  and also the C:N. The increase in  $\delta^{15}\text{N}$  can be attributed to either: 1) uptake of  $\delta^{15}\text{N}$  nitrate added to the lake as  $\text{NaNO}_3$  that has  $\delta^{15}\text{N}$  greater than or equal to the maximum  $\delta^{15}\text{N}$  seen in the sediments or 2) denitrification increasing the remaining  $\delta^{15}\text{N}$  pool of nitrogen (Talbot, 2001). The production of  $\text{NaNO}_3$  fertilizer (the fertilizer added to Lake 227) has been found to produce  $\text{NO}_3^-$  with a  $\delta^{15}\text{N}$  greater than atmospheric nitrogen (0‰),

due to equilibrium exchange reactions in the formation process (Freyer & Aly, 1974). The analysis of two samples of  $\text{NaNO}_3$  (J.T. Baker<sup>®</sup>  $\text{NaNO}_3$  laboratory reagent and USGS35 from the Environmental Geochemistry Lab at the University of Waterloo) resulted in a mean  $\delta^{15}\text{N}$  of  $\text{NaNO}_3$  of 3.1‰, which supports  $\delta^{15}\text{N}$  greater than 0‰ for  $\text{NaNO}_3$ . However, these samples are not from the same companies that provided the  $\text{NaNO}_3$  for the Lake 227 experiment. If the  $\delta^{15}\text{N}$  of the fertilizer added to Lake 227 was similar to these samples this would provide an explanation for the increase of  $\delta^{15}\text{N}$  in the lake sediments. Alternatively, denitrification could increase the  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  in the water column before assimilation by algae. The sudden loading of high amounts of nitrate to Lake 227, where the fertilizer was 5 to 9 times the natural nitrogen loading to the system, should have provided suitable conditions (anoxic sediments, high nitrate, high organic carbon) for high levels of denitrification (Seitzinger et al, 2006). Measurements of denitrification during summer stratification were made in the summers of 1973, 1974 and 1975 when nitrogen loading was highest (Chan & Campbell, 1980). It was found that the epilimnetic sediment-water interface was a more significant site for denitrification than the thermocline and the anoxic hypolimnion. The added nitrate was not observed to diffuse below 5m two days after each addition, and therefore the hypolimnion was not considered an important location for denitrification. Chan and Campbell (1980) estimated that 1.4% of total fertilizer nitrate added was denitrified per year while the remaining nitrate was consumed by phytoplankton. As the amount of denitrification is small relative to the uptake of nitrogen by phytoplankton, it is likely that the increase in the sediment  $\delta^{15}\text{N}$  seen in the high nitrogen condition is the result of the near complete consumption of added nitrate with little fractionation, as is expected under the standard interpretation of  $\delta^{15}\text{N}$  values in eutrophic lakes. In the second experimental condition the nitrogen inputs were decreased to 35% of the high nitrogen loading period. The decrease in fertilizer loading led to a shift in algal species composition with blooms of nitrogen fixing cyanobacteria appearing every year since the decrease (Findlay et al, 1994; Schindler, 2008). The growth of nitrogen fixing cyanobacteria provided a new pathway for nitrogen to enter the lake. This pathway, plus the natural loading from runoff and precipitation, accounted for no less than 30% of total nitrogen entering the lake over the whole period of low artificial nitrogen loading (Findlay et al, 1994). The shift in species community is seen in the  $\delta^{15}\text{N}$  of the sediments, as the  $\delta^{15}\text{N}$  of the sediment decreases to a mean of 0.9‰ because of the incorporation of atmospheric nitrogen and the lessening of artificial fertilizer dominance in the nitrogen budget. In 1990, the nitrogen additions were stopped while phosphorus additions continued (Findlay et al, 1994). The response in the lake was an increase in the amount of atmospheric nitrogen fixed from an average of 32kg-N fixed per year previous to the cessation of nitrogen loading, to an average of 61kg-N fixed after nitrogen loading stopped (Paterson et al, 2011). The increase in fixation led to greater incorporation of 0‰ nitrogen into biomass and in turn the sediments. The impact of stopping the fertilizer additions on the  $\delta^{15}\text{N}$  was strengthened by the natural external loading (precipitation and

runoff) which in 2010 had a mass weighted mean  $\delta^{15}\text{N}$  of 0.2‰ over the open water season. The combination of nitrogen fixation, precipitation and runoff decrease the  $\delta^{15}\text{N}$  of in lake biomass and in turn the sediments after deposition, as is expected under the standard interpretation of sediment nitrogen isotopes in lakes with large amounts of nitrogen fixation. In other lakes the changes in  $\delta^{15}\text{N}$  would not be as clear, as it is unlikely that the complete removal of anthropogenic nitrogen, while maintaining phosphorus concentrations, would be achieved as it was in Lake 227. Therefore in other lakes there would be a more subtle change in  $\delta^{15}\text{N}$  as the lake progressed through different trophic states if nitrogen remained in excess of biological requirements.

#### **4.4.5 $\delta^{13}\text{C}$ as a Proxy for Historical Primary Production in Lake 227**

Lake 227 offers an ideal location to examine the standard interpretations of sediment core carbon isotopic signatures. At the beginning of eutrophication the increase in  $\delta^{13}\text{C}$  coincides with the increase in primary production. The increase in  $\delta^{13}\text{C}$  may, however, be a product of carbon limitation and changing alkalinity due to  $\text{NaNO}_3$  additions. The relatively high alkalinity (between 150-300 $\mu\text{eq/L}$ ) in Lake 227 during  $\text{NaNO}_3$  loading resulted in higher pH during the 1970-1990 period which in turn shifted the speciation of carbon from  $\text{CO}_2$  to  $\text{HCO}_3^-$ . Less  $\text{CO}_2$ , because of a shift towards  $\text{HCO}_3^-$ , results in quicker depletion of available  $\text{CO}_2$  by algae which will increase the  $\delta^{13}\text{C}$  of both the residual  $\text{CO}_2$  and algae (Meyers & Teranes, 2001). The recent ( $\approx 15$  years) changes in  $\delta^{13}\text{C}$  of the sediments could result from: 1) diagenesis; which in this case would be the progressive preferential loss of  $^{12}\text{C}$  that increases the bulk isotopic composition; 2) a change in original  $\delta^{13}\text{C}$  of the deposited particulate in the upper portion of the core due to a change in primary production and DIC utilization, increased invasion of atmospheric  $\text{CO}_2$ , or changing fractionation factors; 3) increased incorporation of low  $\delta^{13}\text{C}$  particulate from the hypolimnion (Hollander & Smith, 2001); and 4) changes in lake pH and carbon speciation (Hassan et al, 1997). The degree of  $\delta^{13}\text{C}$  change during diagenesis found by Galman et al (2009) was an increase in  $\delta^{13}\text{C}$  between 0.4-1.5‰ during the first 5 years after deposition. The degree of diagenesis found in that work would not explain the longer time period (15 years) or the greater amount of change in the  $\delta^{13}\text{C}$  (2‰) of the Lake 227 sediment core. To address possible changes in primary productivity Chl *a* concentrations in Lake 227 from Paterson et al (2011) were compared to the  $\delta^{13}\text{C}$  of the sediment core (figure 4.13). Coinciding with the start of the experiment, the  $\delta^{13}\text{C}$  increased with the increase in primary production until approximately 1978. Post 1978 there has been no significant change ( $p=0.16$ ) in the Chl *a* concentration, indicating that primary productivity has not declined in Lake 227, while the  $\delta^{13}\text{C}$  of the sediments has decreased significantly ( $p<0.01$ ). Supporting evidence for increased and sustained production following the start of eutrophication is the C:N ratio of the sediments and the increased sedimentation rate. The third scenario that could serve to lower the  $\delta^{13}\text{C}$  of the sediment comes from a higher percentage of input to the

sediment from the more negative  $\delta^{13}\text{C}$  hypolimnetic organisms, although this would require a very large increase in sedimentation from the hypolimnion. There is no evidence of increased biomass in the hypolimnion of Lake 227 over the past 15 years (ELA database, unpublished data), however this does not eliminate the possibility of increased sedimentation and preservation of low  $\delta^{13}\text{C}$  hypolimnetic particulate organic matter. The fourth possibility for the decline in the  $\delta^{13}\text{C}$  is changing carbon speciation under times of carbon limitation. The cessation of  $\text{NaNO}_3$  additions to Lake 227 caused the alkalinity to decrease from 150-300  $\mu\text{eq/L}$  to 50-150  $\mu\text{eq/L}$  (figure 4.14). Decreases in pH, because of decreased alkalinity in Lake 227, can result in lower  $\delta^{13}\text{C}$  of freshly produced organic matter. At a lower pH the available carbon source is predominantly  $\text{CO}_2$  leading to more negative  $\delta^{13}\text{C}$  of organic matter relative to periods with high pH, such as the phase of  $\text{NaNO}_3$  loading to Lake 227, because of the increased availability of  $\text{CO}_2$ . The lack of evidence to suggest that there has been a change in primary production, an increase in hypolimnetic biomass, or significant diagenesis would indicate that the change in  $\delta^{13}\text{C}$  from the top of the core to 5cm is likely the result of changes in pH, caused by decreased alkalinity, after the additions of  $\text{NaNO}_3$  ceased. Therefore, In Lake 227  $\delta^{13}\text{C}$  cannot be used as a proxy for past productivity changes.

#### **4.4.6 Sediment Storage of Carbon and Nitrogen**

The eutrophication experiment led to a 160% and 215% increase in the mass of carbon and nitrogen, respectively, stored in the sediments of Lake 227 per year. The greater increase in nitrogen relative to carbon stored is caused by the increased deposition of high nitrogen content autochthonous material, such as algae. There is a 28% difference in the mass of nitrogen and a 19% difference of carbon stored in the most recent experimental condition compared to high nitrogen loading. The difference in nitrogen mass could be caused by remineralization of nitrogen into the sediment pore waters and eventually diffusion to the water column. Sedimentation nitrogen mass calculated by Findlay et al (1994) is 86 to 200kg and approximately the same as the estimates made here for the post eutrophication period 148kg-N/year.

#### **4.5 Summary and Conclusions**

The sediment in Lake 227 is a combination of allochthonous and autochthonous material, a conservative estimate places at least 60% (likely higher) of sediment as autochthonous in origin. The similarity between the surficial lake sediments and the photic zone particulate indicates that the majority of the in-lake sediments are derived from the top 4m of Lake 227. Diagenesis alters the  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and C:N ratio of the sediment soon after deposition, although the impact has been shown to be small by other authors (Galman et al, 2009). In Lake 227 eutrophication resulted in increases in sedimentation rate, a decreased C:N ratio of the sediments, and large changes in both the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . The  $\delta^{15}\text{N}$  in the sediment core increased to greater than 2‰ during high nitrogen loading, decreased to approximately 0.7‰ during low

nitrogen loading and decreased again to approximately 0‰ after the cessation of nitrogen loading and the increased dominance of nitrogen fixing cyanobacteria. The  $\delta^{13}\text{C}$  increased following the start of the eutrophication period, from less than -30‰ to approximately -28‰, and stayed high until the end of nitrogen loading, when the  $\delta^{13}\text{C}$  decreased to an average of approximately -30‰. The decrease in  $\delta^{13}\text{C}$  over the top 4cm of the core, implying a decrease in primary production, was not substantiated by Chl *a* concentrations over the same period, indicating that the  $\delta^{13}\text{C}$  is not a useful proxy for historic primary production in Lake 227. The changes in  $\delta^{13}\text{C}$  at the beginning of the eutrophication experiment and following the end of nitrogen loading are thought to be the result of changes in alkalinity and carbon speciation caused by the alteration of artificial  $\text{NaNO}_3$  loading rate. The  $\delta^{15}\text{N}$  and C:N ratio retain information relevant to nitrogen sources in Lake 227 such as the addition of artificial nitrogen fertilizer, and the increased dominance of nitrogen fixing cyanobacteria.

The interpretation of the core through the isotopes of carbon and nitrogen benefited greatly from the body of work already amassed for Lake 227, including records of primary production (Paterson et al, 2011), artificial nitrogen loading (Findlay et al, 1994), nitrogen fixation (Schindler et al, 2008; Paterson et al, 2011), previous interpretation of sediments cores for dating and pre-experimental conditions (Wolfe et al, 1994; Leavitt and Findlay, 1994; Anderson et al, 1987; Kipphut et al, 1978; Jeremiason et al, 1999; Crusius & Anderson, 1995), measurements of denitrification (Chan & Campbell, 1980) and sediment focusing (Crusius & Anderson, 1995) among other unpublished information. Even with this extensive supporting information the interpretation of sediment core isotope data was not straightforward. Therefore, in lakes without supporting data on historical conditions, sediment core isotopes have the potential to be misinterpreted.

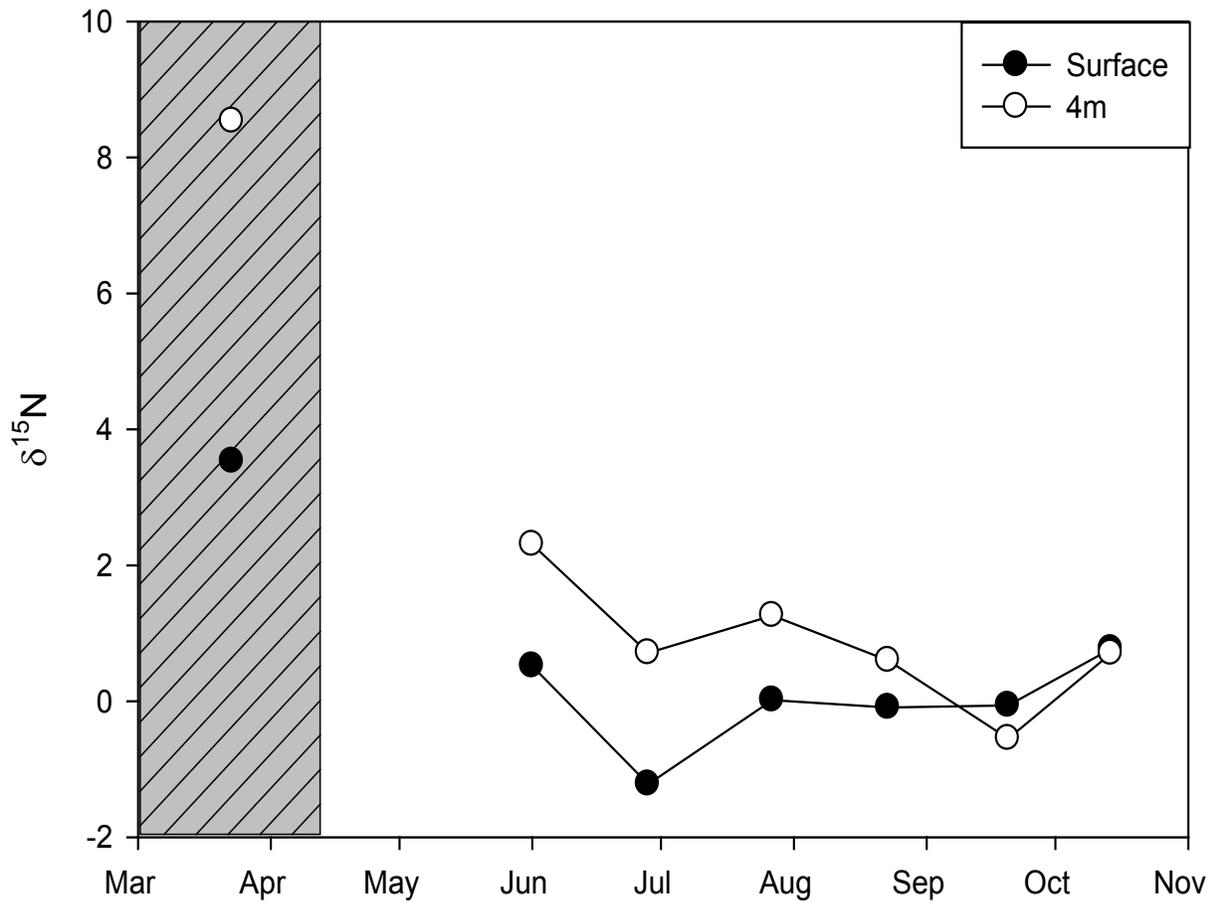


Figure 4:1 Seasonal  $\delta^{15}\text{N}$ -POM from surface (filled circle) and 4m (open circle) from March to October 2010 in Lake 227. Diagonal hatched box represents the ice covered period of early 2010. The June 28th sample is from the peak of the cyanobacteria bloom

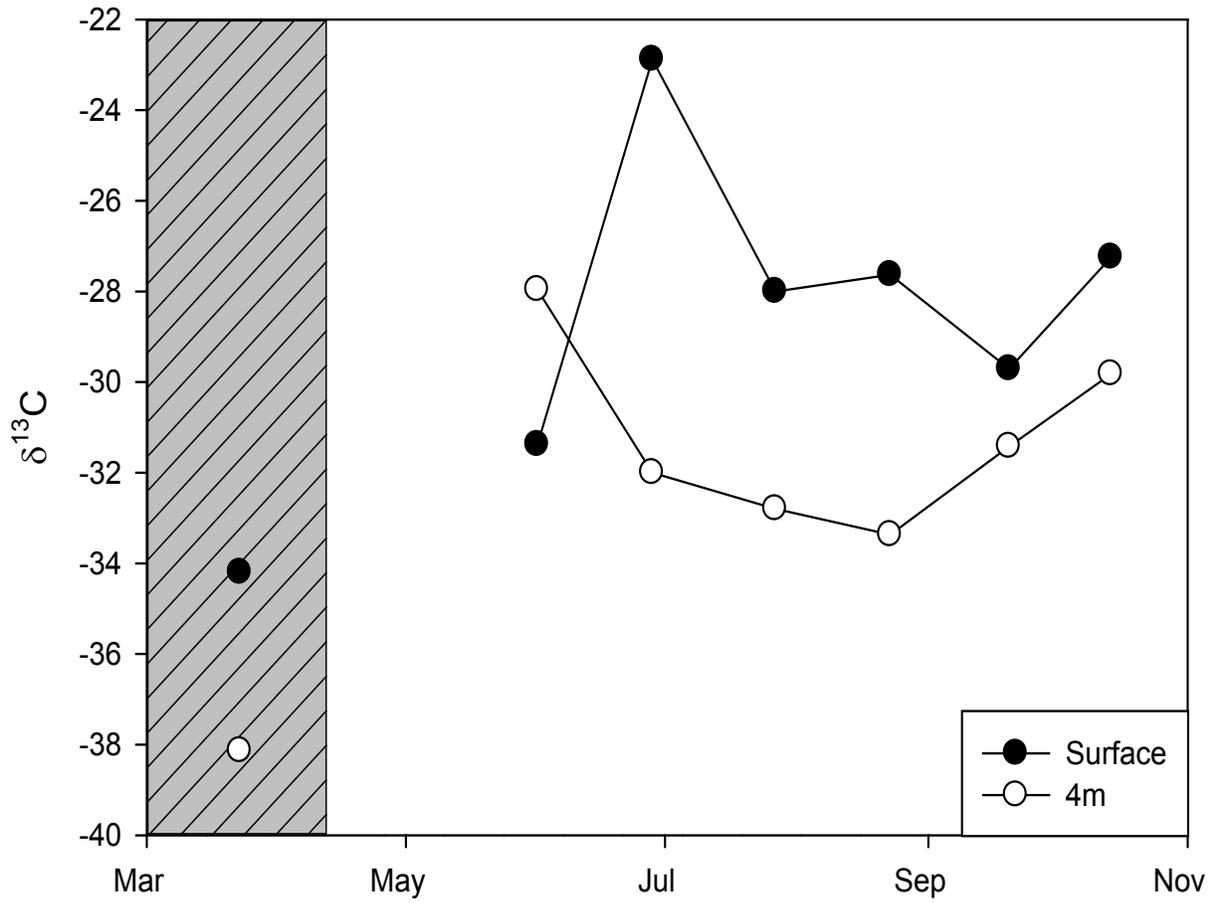


Figure 4:2 Seasonal  $\delta^{13}\text{C}$ -POM from surface (filled circle) and 4m (open circle) from March to October 2010 in Lake 227. Diagonal hatched box represents the ice covered period of early 2010. The June 28th sample is from the peak of the cyanobacteria bloom

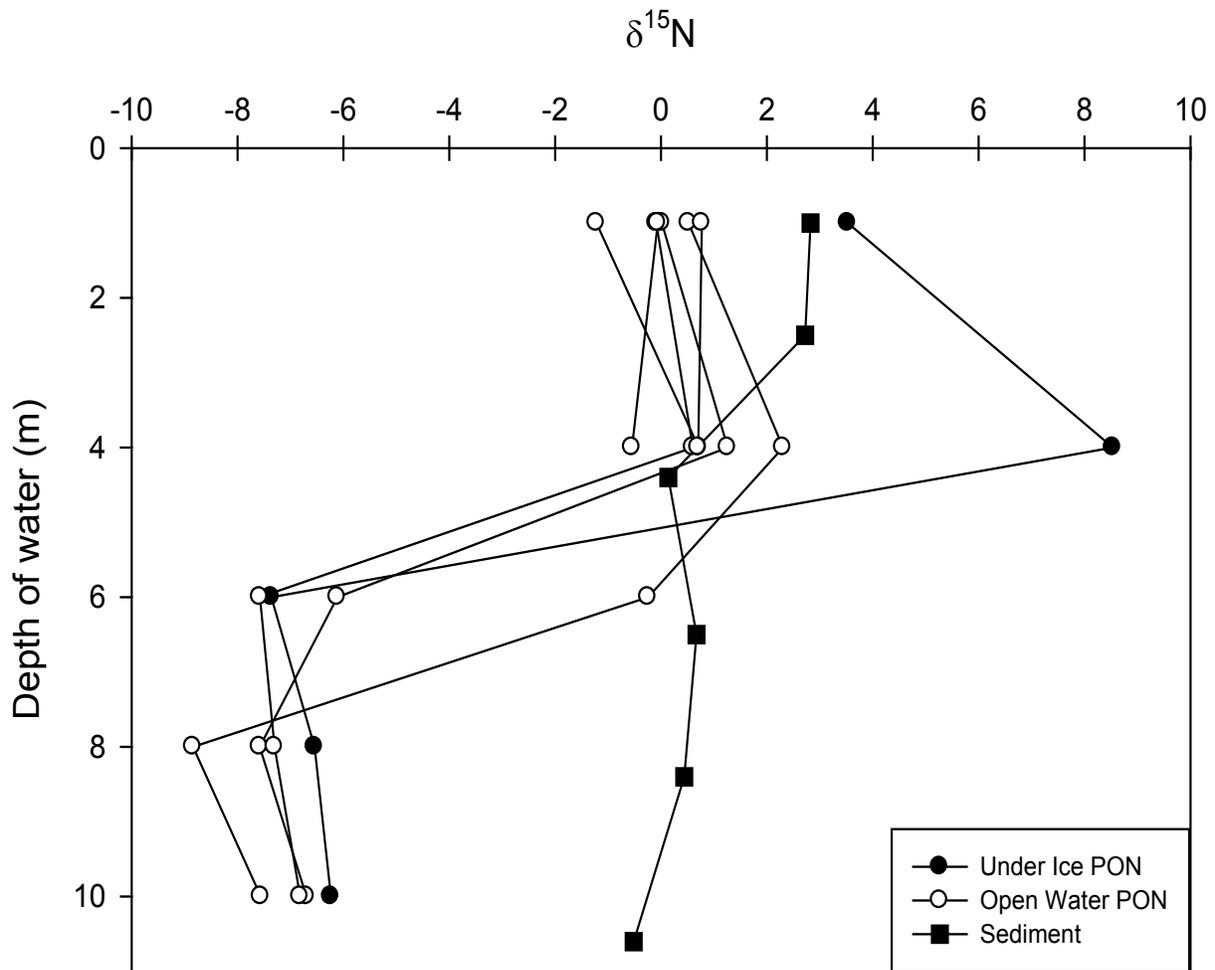


Figure 4:3 Depth of water profile of  $\delta^{15}\text{N}$ -POM from the open water season (June to October, open circles), under ice (March, closed circles) and  $\delta^{15}\text{N}$  of surficial sediments (filled in boxes) in Lake 227 in 2010

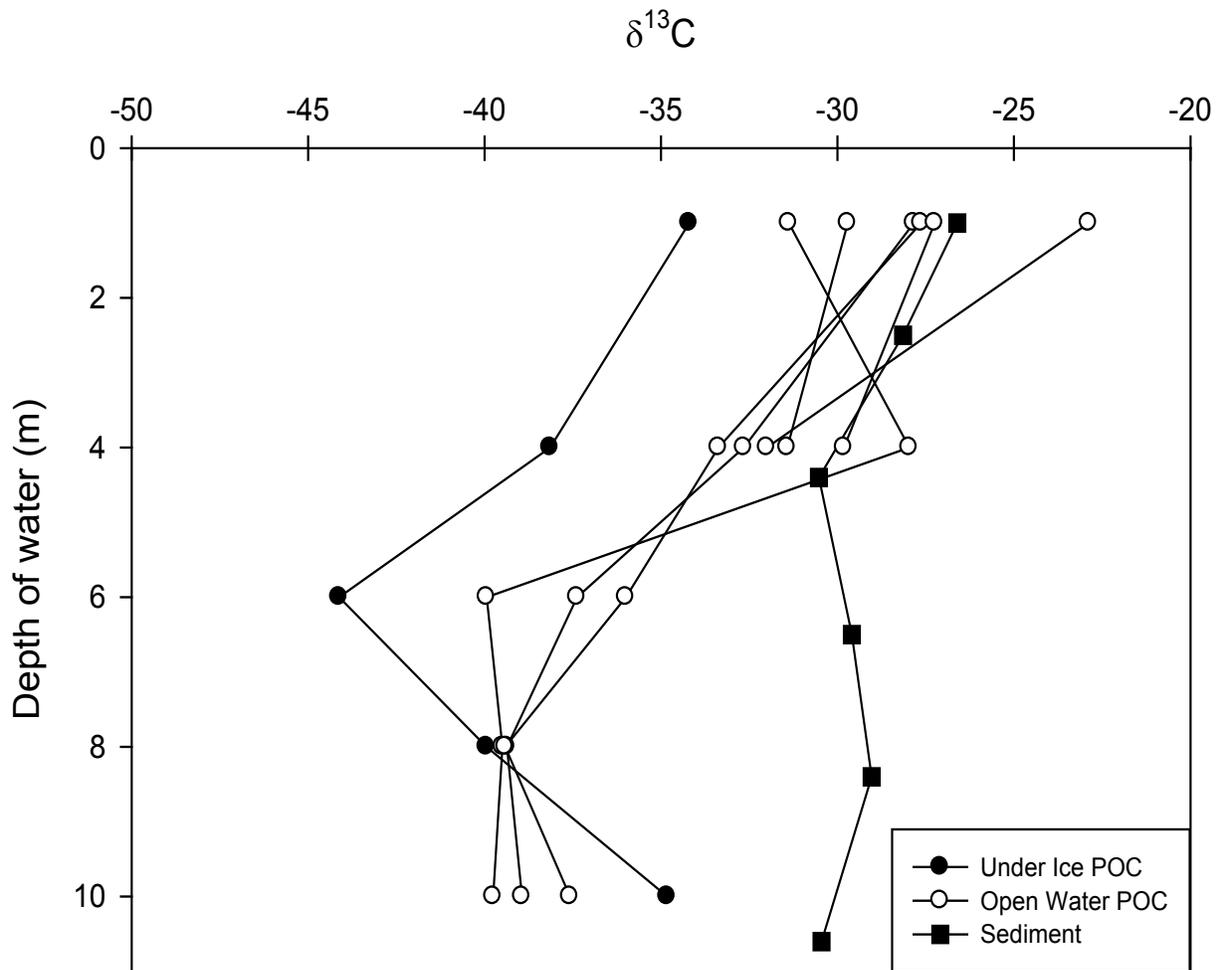


Figure 4:4 Depth of water profile of  $\delta^{13}\text{C}$ -POM from the open water season (June to October, open circles), under ice (March, closed circles) and  $\delta^{13}\text{C}$  of surficial sediments (filled in boxes) in Lake 227 in 2010

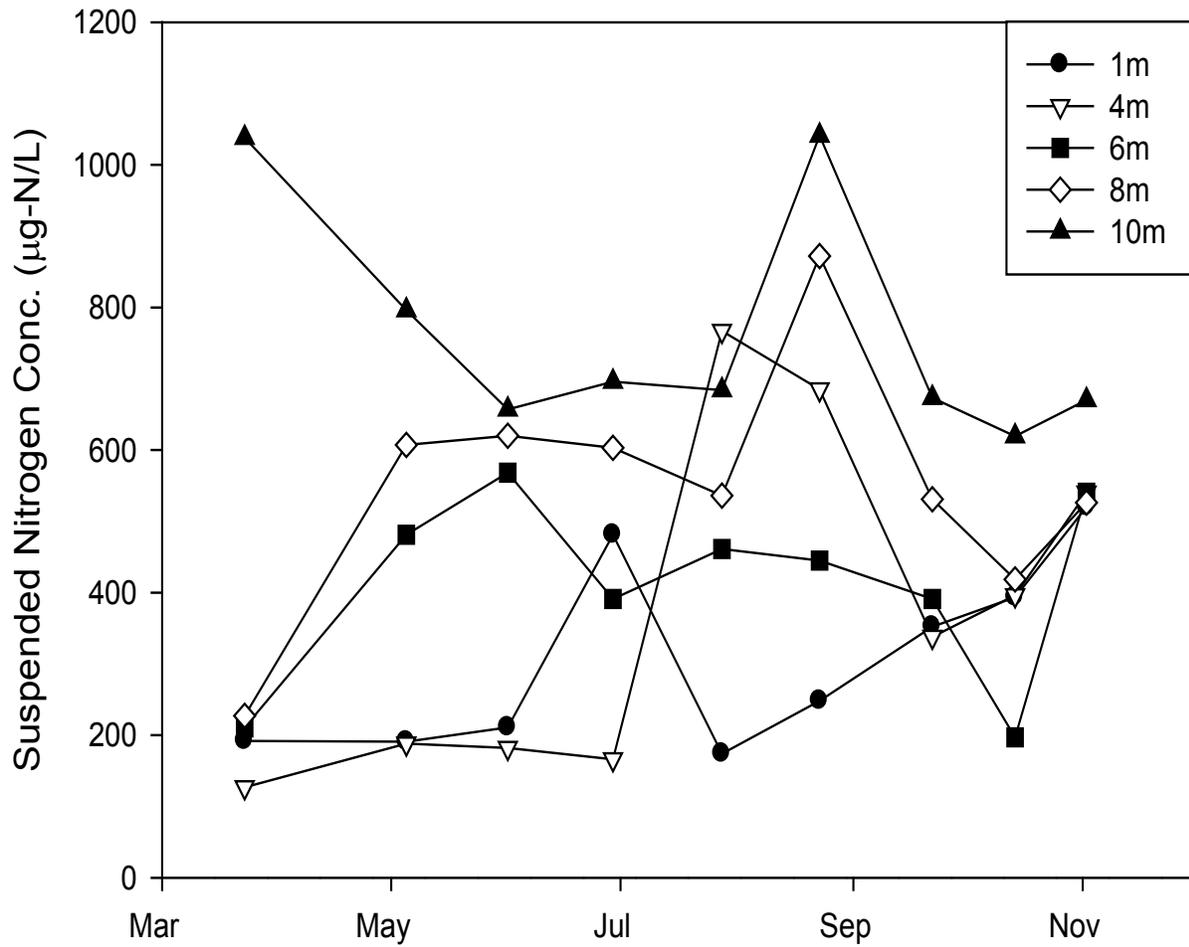


Figure 4:5 Masses of suspended nitrogen in Lake 227 used for the calculation of mass weighted averages for  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ , and C:N

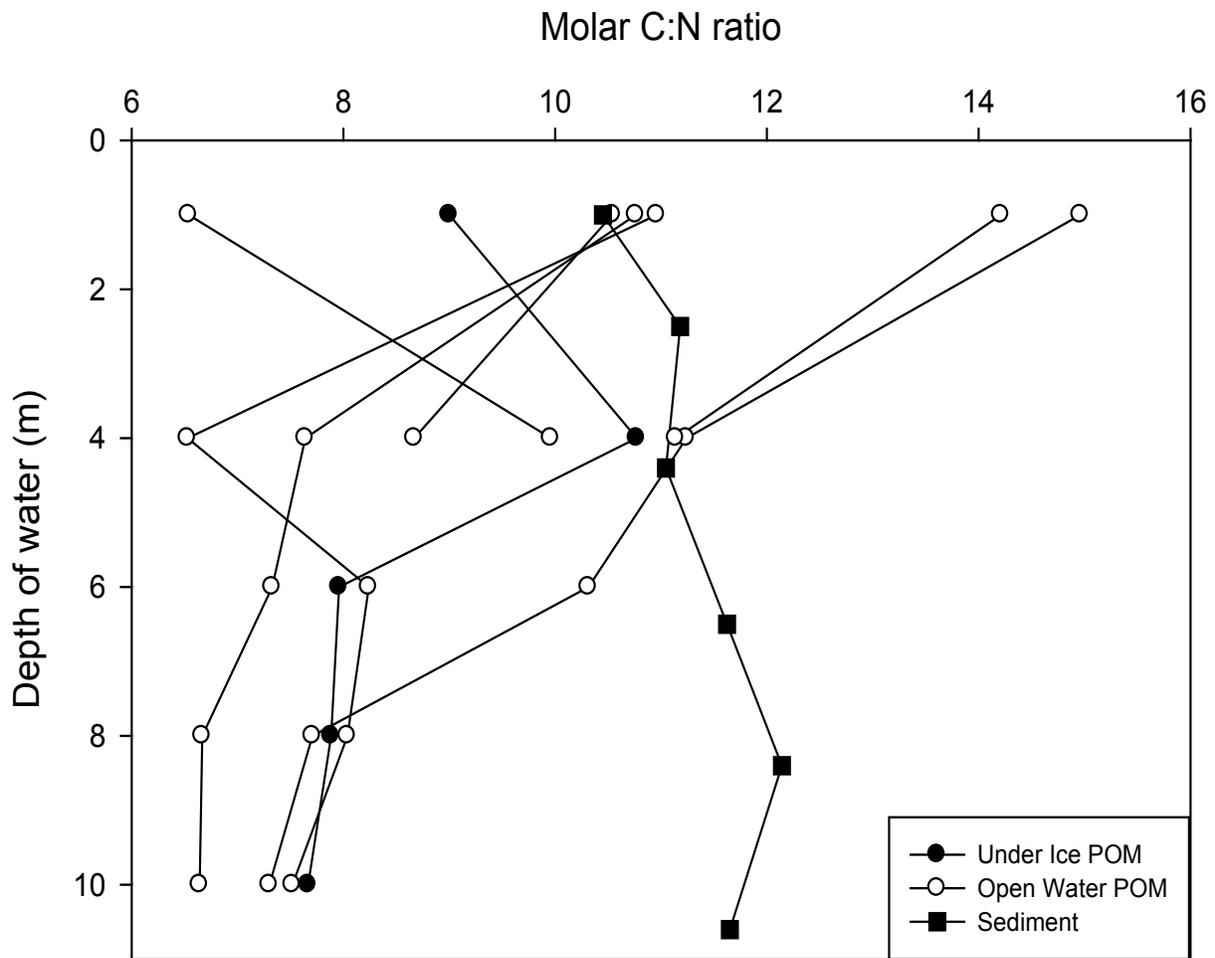


Figure 4:6 Depth of water profile of molar C:N ratio of POM from the open water season (June to October, open circles), under ice (March, closed circles) and molar C:N ratio of surficial sediments (filled in boxes) in Lake 227 in 2010

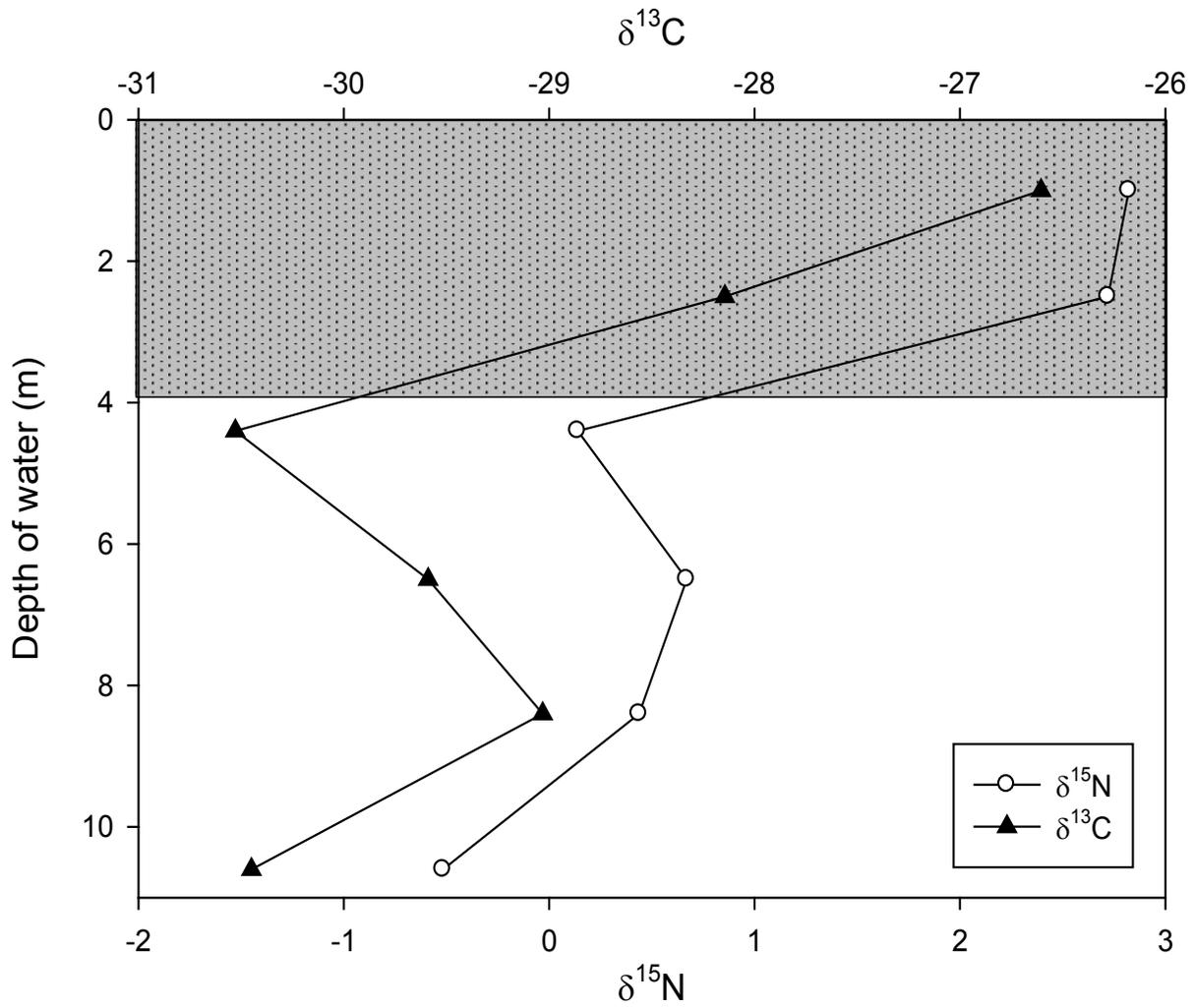


Figure 4:7 Depth of water profile of surficial sediment  $\delta^{15}\text{N}$  (open circle, bottom axis) and  $\delta^{13}\text{C}$  (closed circle, top axis) in Lake 227, sampled on July 24th 2010. Dotted box represents approximate depth of oxygen penetration in the water column on date of sampling

**Table 4-1 Literature estimations of Lake 227 sedimentation rates. The table includes the date the core was sampled, sedimentation rate estimation method and the period covered by the estimate. Full record estimations did not separate pre and post eutrophication period. Pre-eutrophication time period is defined as earlier than 1969 while post eutrophication is from 1969 to present**

Sedimentation Rate	Unit	Method	Core Date	Time Period	Author
0.09	cm/yr	210Pb Curve	1973	Full Record	Kipphut, 1978
0.04+/- 0.03	cm/yr	14C curve	1973	Full Record	Kipphut, 1978
0.43	cm/yr	210Pb log Linear	1980	Full Record	Anderson et al, 1987
0.021	g/cm <sup>2</sup> /yr	210Pb log Linear	1980	Full Record	Anderson et al, 1987
0.005 to 0.02	g/cm <sup>2</sup> /yr	137Cs	1987	Full Record	Wolfe et al, 1994
0.0084	g/cm <sup>2</sup> /yr	137Cs	1987	Pre-Eutrophication	Wolfe et al, 1994
0.0062	g/cm <sup>2</sup> /yr	210Pb	1987	Pre-Eutrophication	Wolfe et al, 1994
0.0087	g/cm <sup>2</sup> /yr	Varve	1987	Full Record	Wolfe et al, 1994
0.18	cm/yr	210Pb Curve	1988	Full Record	Crusius & Anderson, 1995
0.009	g/cm <sup>2</sup> /yr	Laminae	1988	Full Record	Crusius & Anderson, 1995
0.008	g/cm <sup>2</sup> /yr	210Pb	1988	Full Record	Crusius & Anderson, 1995
0.009	g/cm <sup>2</sup> /yr	137Cs, 239+240Pu	1988	Full Record	Crusius & Anderson, 1995
0.006	g/cm <sup>2</sup> /yr	Varve	1990	Pre-Eutrophication	Leavitt & Findlay, 1994
0.011	g/cm <sup>2</sup> /yr	Varve	1990	Post-Eutrophication	Leavitt & Findlay, 1994
0.0059 +/- 0.0011	g/cm <sup>2</sup> /yr	Varve	1994	Pre-Eutrophication	Jeremiason et al, 1999
0.0083 +/- 0.0023	g/cm <sup>2</sup> /yr	Varve	1994	Post-Eutrophication	Jeremiason et al, 1999
0.28	cm/yr		2010	Post-Eutrophication	This work
0.009	g/cm <sup>2</sup> /yr		2010	Post-Eutrophication	This work

**Table 4-2 Lake 227 sediment accumulation mass from 7m water depth sediment core using sedimentation rates from Leavitt & Findlay (1994)**

Period		Sedimentation rate (g/cm <sup>2</sup> /yr)	Kg-N/yr	Kg-C/yr
Total	Pre-Fert	0.006	47	600
Total	Post-Fert	0.011	148	1555
High N	Post-Fert	0.011	121	1369
Low N	Post-Fert	0.011	141	1505
No N	Post-Fert	0.011	169	1683

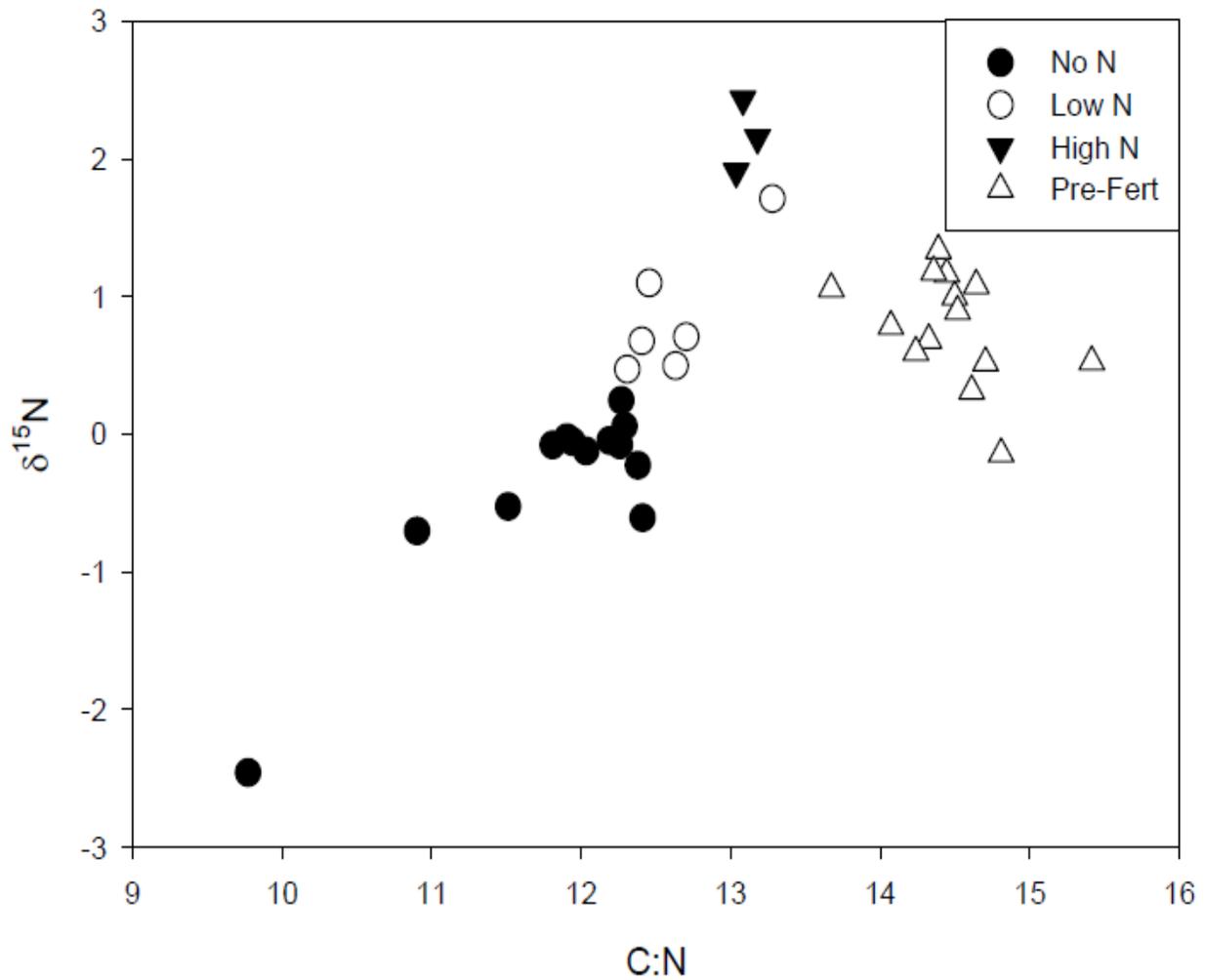


Figure 4:8  $\delta^{15}\text{N}$  and molar C:N of sediment core sections separated into approximate historic experimental conditions. Pre-fert (open triangles) is before 1969, high N (filled downward triangles) experimental nitrogen loading was 315kg/year (1970-1975), Low N (open circles) experimental nitrogen loading was 112kg (1976-1990), and No N (closed circles) experimental nitrogen loading was ceased (1990 to present). Nitrogen fixing cyanobacteria blooms have occurred every summer since 1975, excluding 1996 at the end of a food web manipulation experiment

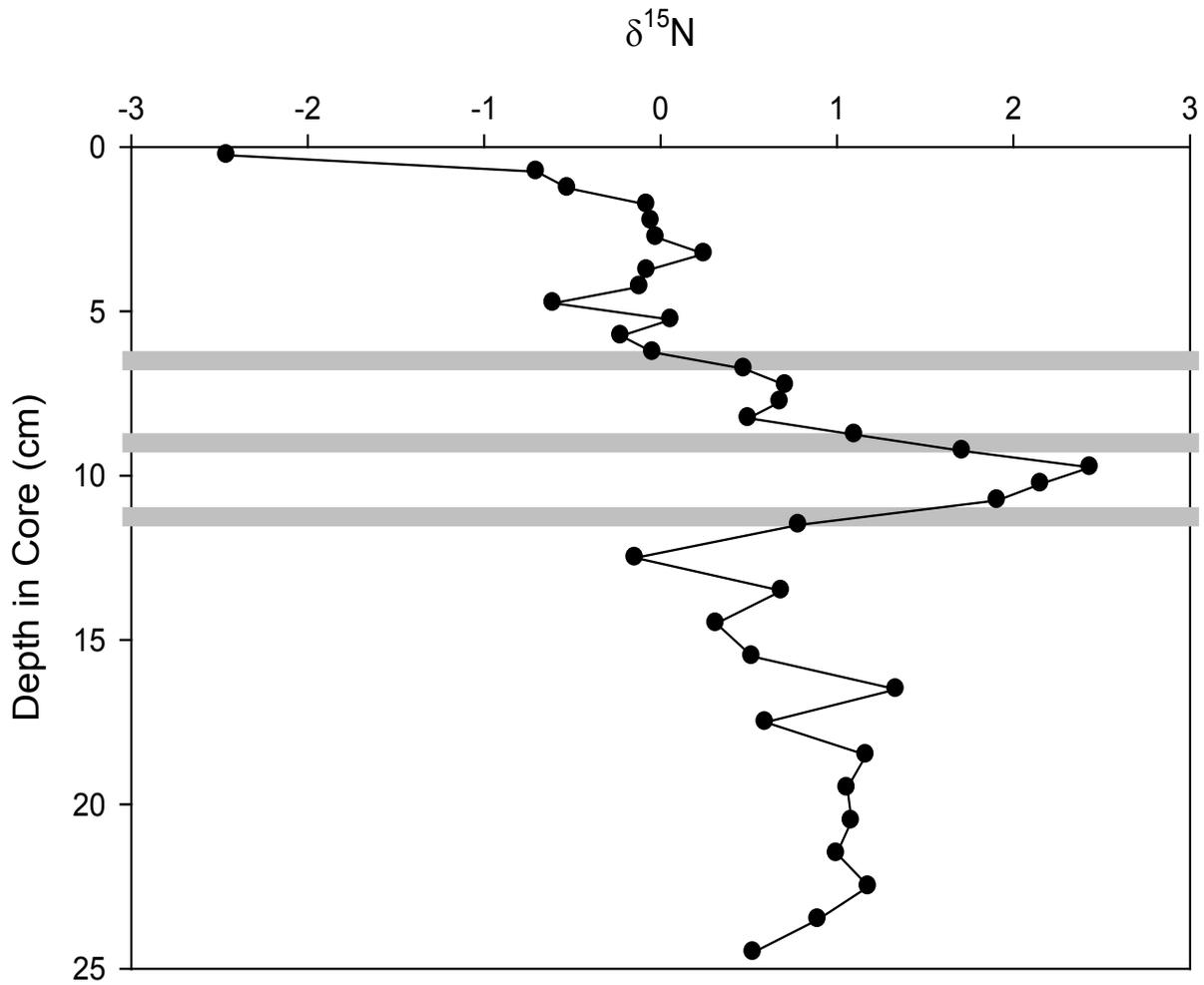
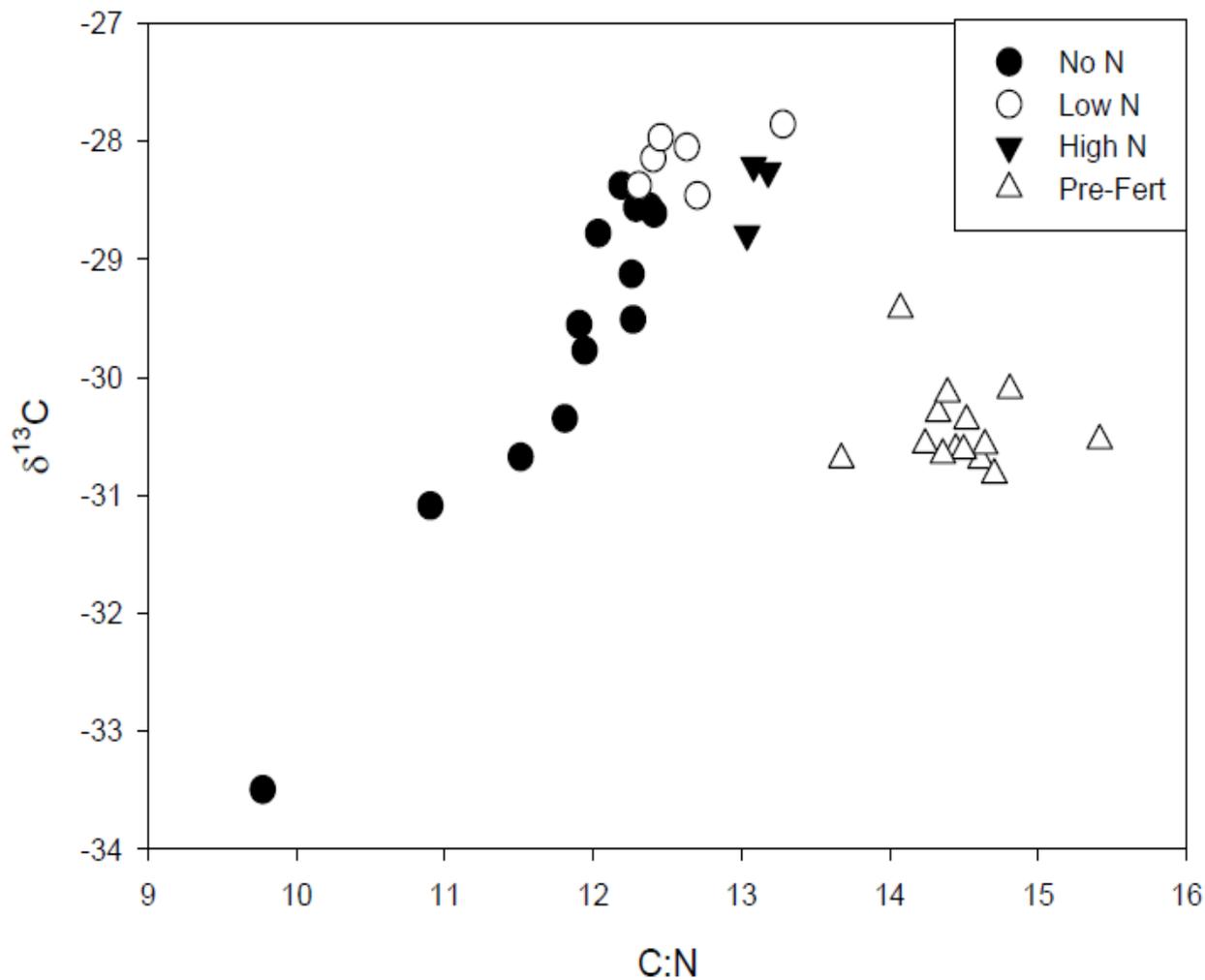


Figure 4:9 Sediment core profile of  $\delta^{15}\text{N}$ . Core was retrieved from under 7m of water in March of 2010 using a freeze corer. Marked with gray bands are the beginning of eutrophication experiment recorded at 11.5cm, change to Low nitrogen dosing at 9cm, and no nitrogen dosing at 6cm



**Figure 4:10**  $\delta^{13}\text{C}$  and molar C:N of sediment core sections separated into approximate historic experimental conditions. Pre-fert (open triangles) is before 1969, high N (filled downward triangles) is when experimental nitrogen loading was 315kg/year (1970-1975), Low N (open circles) is when experimental nitrogen loading was 112kg (1976-1990), and No N (closed circles) is when experimental nitrogen loading was ceased (1990 to present). Nitrogen fixing cyanobacteria blooms have occurred every summer since 1975, excluding 1996 at the end of a food web manipulation experiment

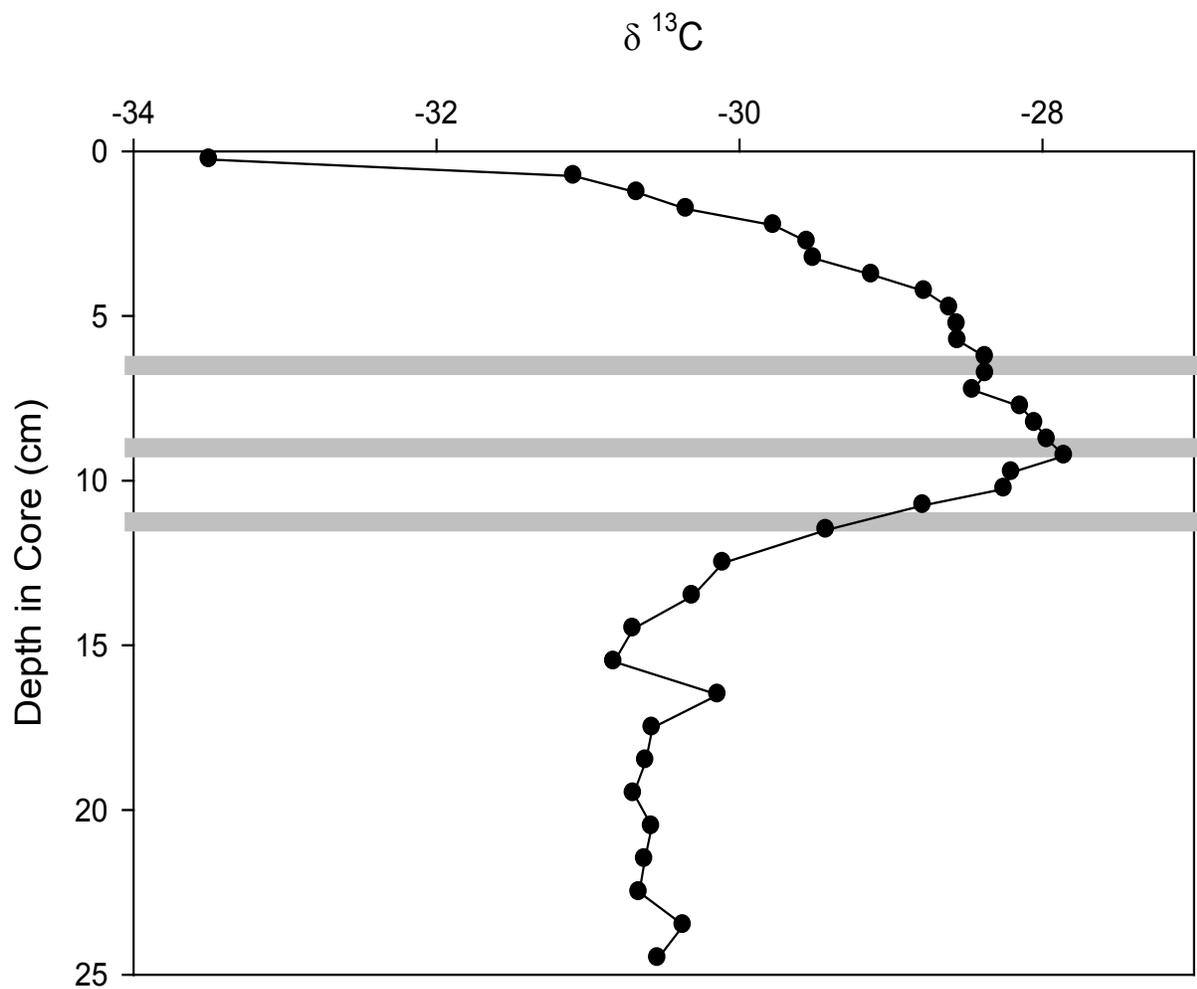


Figure 4:11 Sediment core profile of  $\delta^{13}\text{C}$ . Core was retrieved from under 7m of water in March of 2010 using a freeze corer. Marked with gray bands are the beginning of eutrophication experiment recorded at 11.5cm, change to Low nitrogen dosing at 9cm, and no nitrogen dosing at 6cm

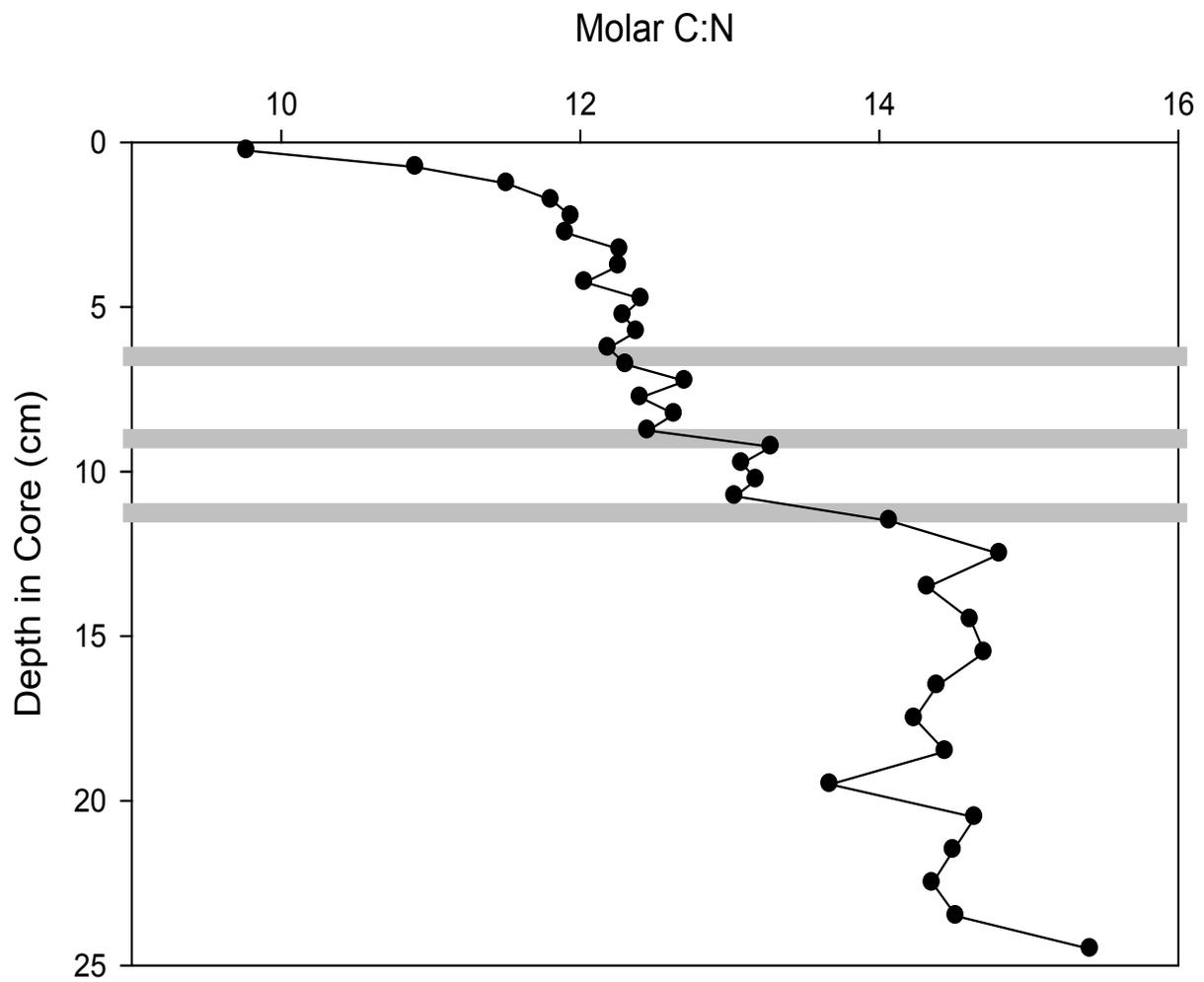


Figure 4:12 Sediment core profile of C:N. Core was retrieved from under 7m of water in March of 2010 using a freeze corer. Marked with gray bands are the beginning of eutrophication experiment recorded at 11.5cm, change to Low nitrogen dosing at 9cm, and no nitrogen dosing at 6cm

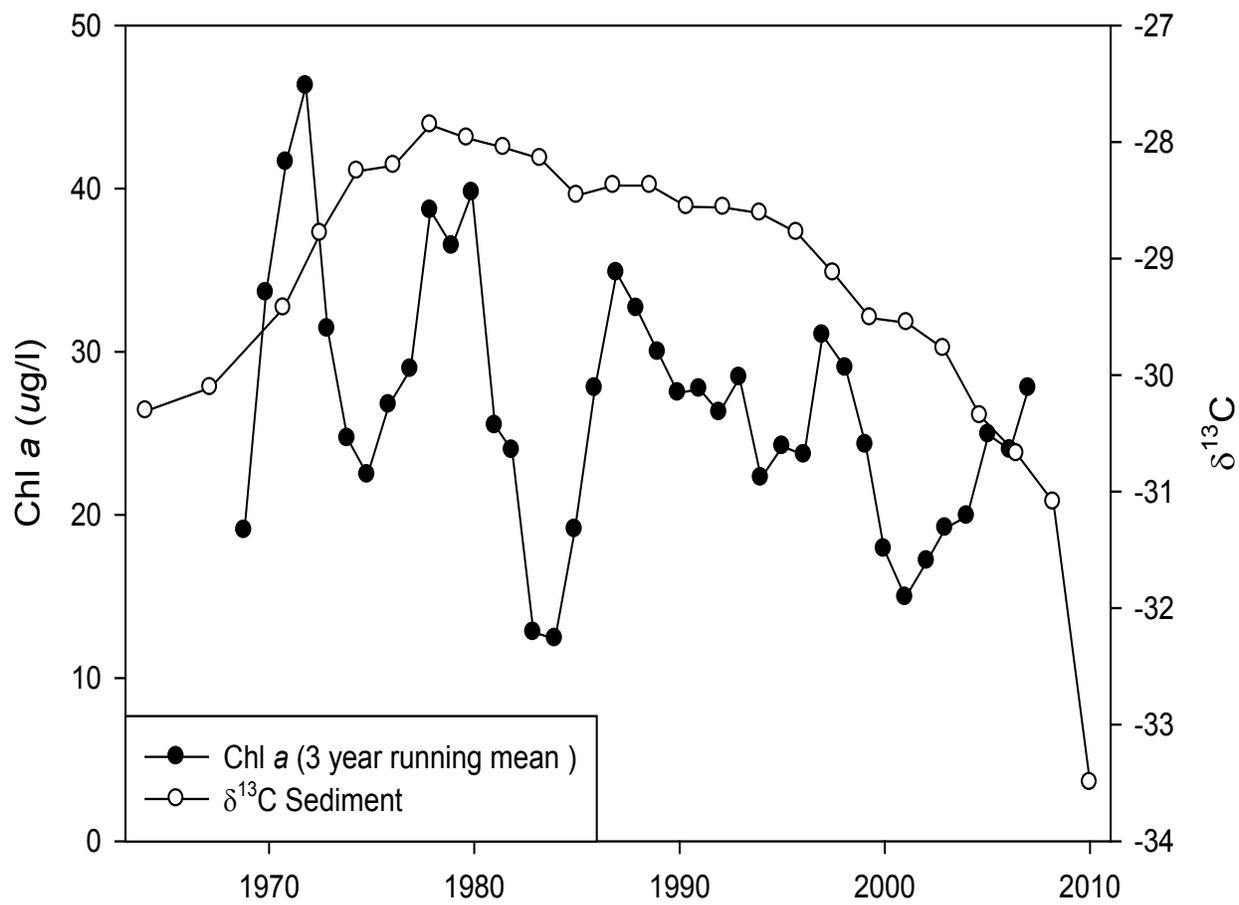


Figure 4:13 Historical comparison of 3-year running mean of Chl *a* (filled circles) from Patterson et al (2011), as a proxy for primary production, and  $\delta^{13}\text{C}$  of the sediment core (open circles). Core date estimates are based on sedimentation rate estimates of 0.28cm/year

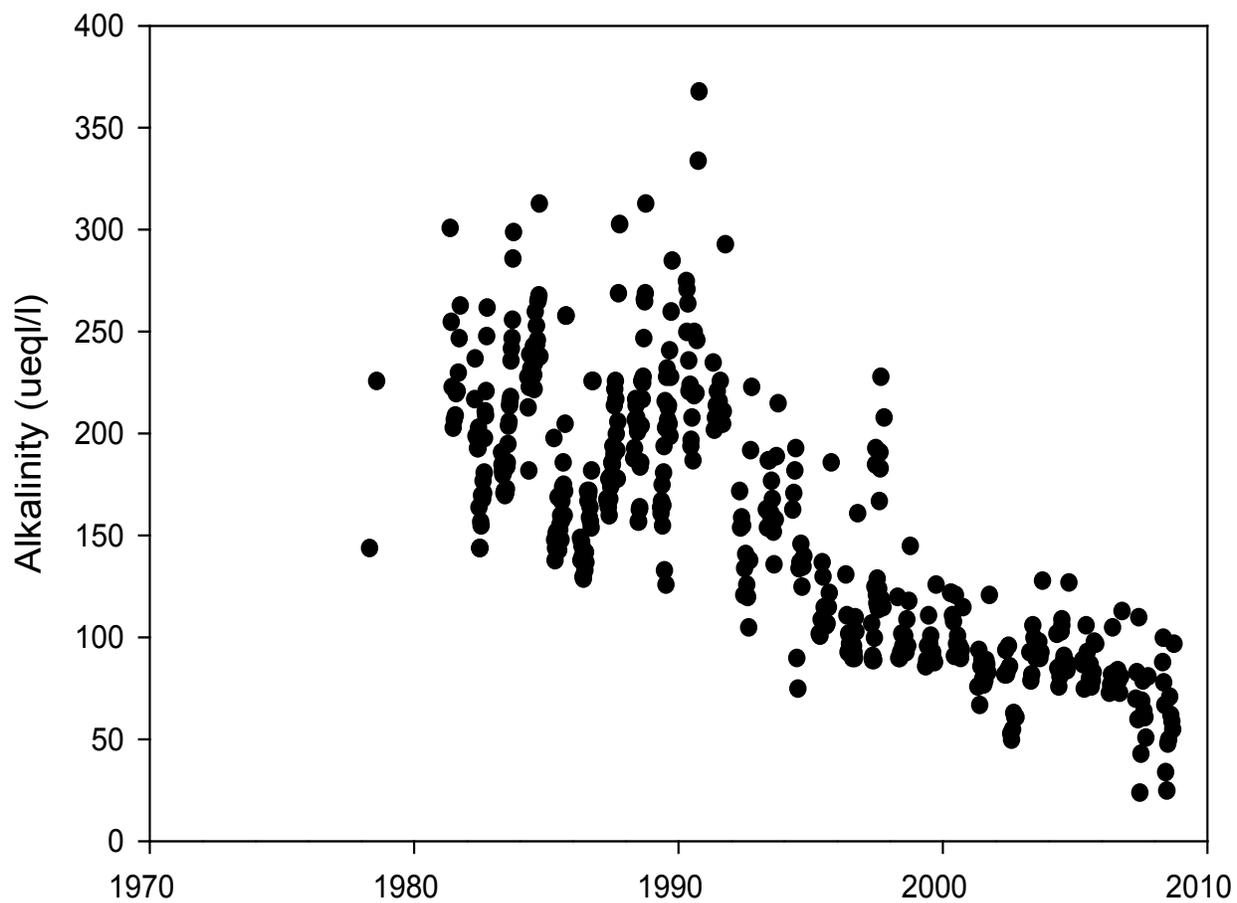


Figure 4:14 Historical alkalinity in the epilimnion of Lake 227 between May 1st and October 31<sup>st</sup>, no alkalinity data exists for the period of high nitrogen loading

## 5 Chapter 5: Nitrogen Isotopes in Lakes at the Experimental Lakes Area

### 5.1 Introduction

The eutrophication of aquatic ecosystems is a global problem that has major impacts on biodiversity, drinking water, and human recreation (Vitousek et al, 1997). Inputs of nitrogen from human activities, such as fertilizer production and combustion of fossil fuels, have doubled the rate of nitrogen input to the terrestrial nitrogen cycle. Phosphorus is generally accepted as the main element controlling eutrophication in aquatic ecosystems, with nitrogen playing a secondary role, as some ecosystems are able to compensate for nitrogen deficiencies through atmospheric nitrogen fixation (Schindler, 2008). Blooms of nitrogen fixing algae can have a more detrimental impact on water quality than some other nonfixing algae species because of their ability to overcome nitrogen limitation, allowing for the formation of nuisance blooms that can release toxins, deplete nutrients, increase turbidity and decrease inorganic carbon supply (Paerl et al, 2001). Blooms can crash suddenly because of the exhaustion of resources, resulting in oxygen depletion and adverse odours from decay (Paerl et al, 2001).

A mass budget is one way to assess the flux of nutrients into and out of an aquatic system. A mass budget accounts for inputs to the lake from direct precipitation, terrestrial runoff, inflow streams, nitrogen fixation (in some systems), and regeneration of nitrogen from organic lake sediments. In addition to inputs, a mass budget needs to quantify losses of nitrogen from the water column through the outflow, sedimentation of organic matter, and denitrification. Some processes are difficult to measure accurately, specifically denitrification and regeneration, so they are estimated by calculating them from other portions of the mass budget. A major issue with mass budgets is that they do not elucidate internal cycling of nitrogen, only external sources and sinks of nitrogen.

Another tool used to study nitrogen cycling in many aquatic studies is nitrogen isotopes. Measuring the isotopic ratio of different pools of nitrogen (suspended, dissolved organic, and dissolved inorganic) can give information on sources of nitrogen being utilized by algae or bacteria through known fractionation factors associated with different processes such as assimilation, nitrification, denitrification and mineralization. Assimilation is the uptake of nitrogen by algae and has been shown to fractionate nitrogen under conditions of high nitrogen availability (Fogel & Cifuentes, 1994). Nitrification is the conversion of ammonium to nitrate, this reaction mostly occurs under oxic conditions, and also strongly fractionates nitrogen, leaving the residual ammonium with higher  $\delta^{15}\text{N}$  (Mariotti et al, 1981). The process of denitrification occurs in anoxic environments where it can strongly fractionate nitrogen isotopes during a reaction that converts nitrate to  $\text{N}_2$  gas if it goes to completion, otherwise some intermediaries are formed such as nitrous oxide (Mariotti et al, 1981). The mineralization of organic matter produces

ammonium and has been shown to have a small fractionation factor, so the ammonium produced will have a  $\delta^{15}\text{N}$  similar to that of the source organic matter (Kendal, 1998).

An exclusively isotopic approach may not be the most effective study method due to the similarity of the source nitrogen inputs from precipitation and terrestrials sources to many lakes. The low concentrations of some dissolved species of interest, such as nitrate and ammonium, can make analysis difficult or not possible, limiting the scope of an isotopic study. This study uses a combination of the mass budget and stable isotope approach to understand the sources and sinks of nitrogen in an artificially eutrophic lake at the Experimental Lakes Area, Ontario, Canada, by comparison with a naturally oligotrophic lake.

The differences in nitrogen isotopes in eutrophic and non-eutrophic lakes at the Experimental Lakes Area have been explored tangentially in the past during food web studies. In one such study by Kidd et al (1999), stable isotopes of sediment trap material were analyzed as part of a food web manipulation experiment in Lakes 227 and 110 at the Experimental Lakes Area (ELA). Lake 227 has been the site of a long running eutrophication experiment that has turned it from a low productivity lake typical of the area to a highly eutrophic lake that experiences annual nitrogen fixing cyanobacteria blooms. The comparison of the food webs for the artificially eutrophic Lake 227 and the oligotrophic Lake 110 provided results that showed that the particulate organic matter (POM) from Lake 227 had lower  $\delta^{15}\text{N}$  than the POM from Lake 110 (figure 5.1). The reason for this was not examined in this paper but the fixation of atmospheric nitrogen could be a potential explanation for the lower  $\delta^{15}\text{N}$  in POM in Lake 227. The fixation of atmospheric nitrogen could force the  $\delta^{15}\text{N}$  of particulate material in Lake 227 closer to 0‰, the  $\delta^{15}\text{N}$  of atmospheric nitrogen, while Lake 110 had higher  $\delta^{15}\text{N}$  because it lacked inputs of relatively low  $\delta^{15}\text{N}$  nitrogen. The lower  $\delta^{15}\text{N}$  in biomass that was fixing atmospheric nitrogen has been demonstrated in mesocosm experiments by Vrede et al (2009) as well as in natural terrestrial and aquatic environments (Delwiche & Steyn, 1970; Minagawa & Wada, 1986). Ideally the nitrogen from fixation is isotopically different than other nitrogen sources allowing for identification of pathways for nitrogen in Lake 227. However, if the  $\delta^{15}\text{N}$  of other sources and sinks of nitrogen are similar to that of nitrogen fixation then the impact of fixation on lake nitrogen cycling would be masked by the other sources.

Nitrogen inputs to lakes, aside from nitrogen fixation, can include direct precipitation and runoff. Nitrogen inputs from precipitation at the ELA have been shown to be an increasingly important part of the nitrogen budget because of increased inorganic nitrogen concentrations in precipitation (Chapter 3). The  $\delta^{15}\text{N}$  of precipitation is not well studied in Canada. At ELA, runoff is predominantly comprised of

dissolved organic nitrogen as the catchments retain a high percentage of inorganic nitrogen (Parker et al, 2009). The  $\delta^{15}\text{N}$  of runoff is controlled by the source nitrogen in the soil and litter fall.

Physical losses of nitrogen can occur in lakes either through the outflow or to the sediments. The amount of nitrogen lost out of the outflow depends on the in lake nitrogen concentration and the flow rate, both of which can change seasonally. The  $\delta^{15}\text{N}$  of nitrogen lost via the outflow is dependent on the  $\delta^{15}\text{N}$  of inputs to the lake as well as in lake nitrogen cycling that can fractionate the nitrogen before it is lost. Losses to the sediment are also controlled by nitrogen concentrations in the lake, as lakes with higher biomass have higher sedimentation rates than less productive lakes. The  $\delta^{15}\text{N}$  of sedimented organic matter is controlled by the inputs of nitrogen, much like the outflow, because the source of material to the sediment is from the epilimnion or allochthonous material.

The goals of this work are to: 1) characterize the  $\delta^{15}\text{N}$  of inflows and outflows in ELA lakes and specifically in Lake 227; 2) construct yearly isotope mass budgets for Lake 227, between 1999-2007, to determine if the mass budgets are accurately representing nitrogen inflows, outflows and cycling; 3) determine whether nitrogen isotopes aid in the understanding of internal nitrogen processes in Lake 227; and 4) determine if differences in nitrogen cycling in Lake 227 impart a distinct nitrogen isotope signal to Lake 227 in comparison to other ELA Lakes.

## **5.2 Site description and methods**

### **5.2.1 Site Description**

This study was conducted at the Experimental Lakes Area, in North Western Ontario, Canada. All sampling occurred in 2010, the majority of which was focused on the open water season (May to November). The artificially eutrophied Lake 227 and oligotrophic Lake 442 were the foci for this study. Lake 227 is a 5 hectare headwater lake with a maximum depth of 10m, surrounded by a 34.4 hectare watershed with no permanent inflow stream (Brunskill & Schindler, 1971). Lake 227 has been extensively studied for the impacts of anthropogenic eutrophication for 40 years. Two previous mass budgets were completed for Lake 227; one by Findlay et al (1994), and the other by Schindler et al (1987) which can be referred to for past nitrogen budgets and changes with regards to nutrient loading.

Lake 442 is a second order lake, situated downstream of Lake 438, that has a surface area of 16 hectares with a maximum depth of 17.8m. Lake 442 is situated in a 145 hectare watershed of which 65% was disturbed due to logging activities in the winters between 1975 and 1979. The logging in the Lake 442 watershed left only a narrow buffer strip of undisturbed land around lakes 442 and 438 (Paterson et al, 1998). Since this time, the watershed has not been impacted by the fires that burned other portions of

the ELA. Lake 442 has not experienced active experimentation, unlike Lake 227, but has been used as a reference lake in previous studies on lake trout, climate change, and watershed disturbance (Sellers et al, 1998; McCullough & Campbell, 1993; Paterson et al, 1998).

### **5.2.2 Sampling and Analysis**

Sampling for the isotopic values included in the isotope mass budget was conducted during the open water season of 2010 for the precipitation and terrestrial inputs, and in March of 2011 for the sediment core  $\delta^{15}\text{N}$ .

The detailed methodology for the sampling and analysis of precipitation nitrogen isotopes can be found in Chapter 3. In brief, total deposition was collected using an open bucket collector at the ELA meteorological site on a roughly two week sampling interval. Samples were screened and frozen until analysis for  $\delta^{15}\text{N}$  - $\text{NO}_3^-$ ,  $\delta^{15}\text{N}$  - $\text{NH}_4^+$  and  $\delta^{15}\text{N}$  -total nitrogen.

Physical description, sampling method, and analysis protocols for the sediment core can be found in Chapter 4. In brief the sediment core was collected using a freeze corer in March of 2011, sectioned with a band saw while frozen into 0.5cm sections from 0cm-11cm deep in the core and 1cm sections for the rest of the core. Core sections were freeze dried and analyzed on a Carlo Erba 1108 (Italy) elemental analyzer, coupled to a Thermo Finnigan Delta plus continuous flow mass spectrometer. The precision on these measurements is  $\pm 0.3\%$  and  $\pm 0.2\%$  for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  respectively.

The sampling of terrestrial inputs was conducted in July of 2010 at four locations at the ELA that were used as proxies for the  $\delta^{15}\text{N}$  of the inflow to Lake 227. The locations were the 979 East inflow, Lake 240 inflow from Lake 470, Lake 302 uplands, and the overland flow entering the east side of Lake 114. Samples were collected for terrestrial input in one litre HDPE bottles, filtered to  $0.45\mu\text{m}$  on the same day, acidified with HCl to pH of 4, and kept cold until analysis. Analysis consisted of evaporating samples to roughly 100ml before freeze drying and dialyzing samples to remove inorganic nitrogen. The material was freeze dried post dialysis before being analyzed with a Carlo Erba 1108 (Italy) elemental analyzer, coupled to a Thermo Finnigan Delta plus continuous flow mass spectrometer. The precision is the same as for the sediment core samples.

### **5.2.3 Construction of the N Mass and N isotope Budgets**

Nitrogen mass budgets were provided by Michael Paterson (personal communication, 2011). Data for the mass balance is from the ELA data retriever system which contains hydrological, chemical and biological data collected during routine sampling. They were constructed using similar methods to Findlay et al

(1994). Direct precipitation was calculated using water volumes and total dissolved nitrogen concentrations from the ELA meteorological site. The input from terrestrial runoff required a proxy catchment to estimate flow volume and nitrogen concentrations. The Lake 239 East Inflow (EIF) was used for both flow and total dissolved nitrogen concentrations, the nitrogen concentrations used were the flow weighted mean from each calendar year. The east inflow to Lake 239 flow volume was measured throughout the entire year. Nitrogen fixation was calculated from heterocyst counts taken twice monthly during the open water season which were converted to a mass of nitrogen fixed using equations from Findlay et al (1994). The outflow from Lake 227 is not gauged so a regression with the Lake 239 outflow, lagged one day, was used. The regression was  $L227 \text{ (m}^3\text{/s)} = 0.00029 + (1.007 \times L239\text{OF} - 1 \text{ Day})$ . Outflow nitrogen masses were calculated from the average yearly nitrogen concentration in the epilimnion using available chemistry data in the ELA database. In recent winters the instances of winter sampling have been reduced so under ice nitrogen concentrations are not available for the outflow. The average nitrogen mass in the lake was calculated by using profile chemistry data and strata volumes to determine a nitrogen mass. The masses used were the average over the open water season to reduce the impacts of anomalous data points. The change in lake nitrogen mass was calculated by calculating the average nitrogen mass in the lake for each year over the time span of May to October and finding the difference between two consecutive years for the period of the mass balance. The loss to the sediments and denitrification was calculated as the residual using the equation:

$$\text{Residual} = \text{Change in lake mass} - (\text{Runoff} + \text{Precipitation} + N_2\text{Fixation} - \text{Outflow}).$$

Negative residual means nitrogen was lost to a combination of sedimentation and denitrification.

#### 5.2.4 Isotope Mass Budget

Nitrogen isotope  $\delta^{15}\text{N}$  values were assigned to the precipitation, runoff, nitrogen fixation, outflow and change in lake nitrogen mass. The N mass budget was then used to construct the N isotope budget. The  $\delta^{15}\text{N}$  of precipitation and runoff were assigned based on measurements made in 2010, and the assumption was made that these input  $\delta^{15}\text{N}$  values have not changed significantly over the past 10 years. Nitrogen fixation is known to have an inconsistent isotopic fractionation on nitrogen (Minagawa & Wada, 1986). However, there is a small range in published values, and as a direct measurement in Lake 227 was not performed the input from nitrogen fixation was assumed to have the  $\delta^{15}\text{N}$  of atmospheric  $\text{N}_2$ ; 0‰. The outflow  $\delta^{15}\text{N}$  was assigned the value of the yearly mass weighted  $\delta^{15}\text{N}$  of the inputs from nitrogen fixation, runoff and direct precipitation. This was calculated using the equation:

$$\delta^{15}\text{N}_{\text{Outflow}} = \frac{(\delta_{\text{Precip}} \times M_{\text{Precip}} + \delta_{\text{Runoff}} \times M_{\text{Runoff}} + \delta_{\text{N}_2\text{Fix}} \times M_{\text{N}_2\text{Fix}})}{(M_{\text{Precip}} + M_{\text{Runoff}} + M_{\text{N}_2\text{Fix}})}$$

To assign the outflow  $\delta^{15}\text{N}$  this way required the assumptions that nitrogen stress in Lake 227 limits isotopic fractionation and that all nitrogen entering the lake is bioavailable. An alternative way to define the outflow is to use the measured isotopic values of DON and PON from 2010 and assume that the average from that year is applicable to the other years in the mass budget. This method however does not take into account changing input conditions resulting from differences in precipitation and differences in values from the late fall and winter that are not available.

The loss to the sediments  $\delta^{15}\text{N}$  was calculated using equation:

$$\delta^{15}\text{N} = \frac{(\delta_{Lake\ M} \times M_{Lake\ M} - (\delta_P \times M_P + \delta_R \times M_R + \delta_{N_2\text{Fix}} \times M_{N_2\text{Fix}} - \delta_O \times M_O))}{(M_{Lake\ M} - (M_P + M_R + M_{N_2\text{Fix}} - M_O))}$$

The equation contains parameters for change in lake mass (Lake M), precipitation (P), runoff (R), outflow (O), and nitrogen fixation ( $\text{N}_2$  Fix). The calculated  $\delta^{15}\text{N}$  of the sediments was compared to measured  $\delta^{15}\text{N}$  of sediments from a sediment core taken from the hypolimnion of Lake 227 (Chapter 4).

## 5.2.5 Lake Survey

In July of 2010, between the 19<sup>th</sup> and 29<sup>th</sup>, a lake survey was completed to determine the isotopic signature of organic matter from several lakes at the ELA. Specifically the  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and C:N ratio of POM, DON, and zooplankton were sampled in several lakes. The lakes included in the survey were 442, 375, 658, 373, 304, 626, 979, 303, 239, and 114. For more information on these lakes consult Brunskill and Schindler (1971).

## 5.3 Results

### 5.3.1 Isotopic Values of Nitrogen Sources and Sinks

The isotopic values used in the mass balance are compiled from a combination of field work in 2010 and literature values. Field work in 2010 provided isotopic values for deposition [e.g. both wet and dry deposition], runoff, and an estimate for what the  $\delta^{15}\text{N}$  of the outflow would be in other years of the mass balance. Nitrogen fixation  $\delta^{15}\text{N}$  was from the literature and was based on the assumption that this process does not isotopically fractionate nitrogen.

Nitrogen in deposition is a combination of nitrate, nitrite, ammonium, dissolved organic nitrogen and particulate nitrogen. The total dissolved nitrogen in precipitation was comprised of roughly 25% nitrate plus nitrite, 55% ammonium, and 20% dissolved organic nitrogen in 2010 (Chapter 3, this work).

The isotopic composition of nitrogen in precipitation in Canada's boreal forest is not well studied so the range in  $\delta^{15}\text{N}$  used for the mass balance is based on work presented in Chapter 3. The  $\delta^{15}\text{N}$  of total nitrogen from atmospheric sources ( $\delta^{15}\text{N}-\text{P}_d$ ) was measured in 9 periods collected between June 1<sup>st</sup> and September 27<sup>th</sup> 2010 (Chapter 3). The mass weighted  $\delta^{15}\text{N}$  -TN value of 3.3‰ was used to represent precipitation input for all years in this isotope mass budget (table 5.1).

The runoff component of the mass budget required the use of proxy watersheds to obtain a  $\delta^{15}\text{N}$  value. The watersheds that were used were Lake 979 east inflow, Lake 114, and Lake 302 (U8) upland catchment; the latter two both require rainfall to have inflow to their lakes, similar to Lake 227. The  $\delta^{15}\text{N}$  of the proxy watersheds runoff was measured once, in July 2010, and extrapolated historically for all years in this mass budget. The average  $\delta^{15}\text{N}$  -DON of runoff from these proxy catchments was -1.5‰, which was used in the isotope mass budget. Data collected from the Lake 114 inflow in 2001-2003 suggests that the  $\delta^{15}\text{N}$  -DON does not follow a seasonal pattern (figure 5.2) nor is it different than samples collected in 2010 (Venkiteswaran & Schiff, unpublished data). The average  $\delta^{15}\text{N}$  is similar to the value used in this work.

The final external input parameter is biological nitrogen fixation. This process has been shown to have no significant fractionation (Hoering & Ford, 1960; Minagawa & Wada, 1986). As such, any nitrogen entering Lake 227 via  $\text{N}_2$  fixation would have an isotopic value reflecting the atmospheric source. Atmospheric nitrogen is used as the benchmark for isotopic analyzes and has an assigned value of 0‰ (Mariotti, 1983).

The  $\delta^{15}\text{N}$  of the outflow is a composite of the nitrogen sources entering the lake. Therefore the  $\delta^{15}\text{N}$  of the outflow should equal the  $\delta^{15}\text{N}$  of the inputs when the nitrogen is limiting in the lake, since there is not expected to be any fractionation during nitrogen assimilation at low nitrogen concentration. Nitrogen return from the sediment likely plays a role in supplying nitrogen to the epilimnion however the amount is difficult to quantify, so for this isotope mass budget it will be assumed that the sediments are not a significantly large or isotopically distinct source of nitrogen in the epilimnion. The  $\delta^{15}\text{N}$  loss from the outflow during each year was estimated by taking the mass weighted  $\delta^{15}\text{N}$  value of the inputs for each specific year. This is a significant assumption but without actually measuring the  $\delta^{15}\text{N}$  of the outflow, it is likely the best way to account for year to year variability in the relative importance that different inputs have in setting the  $\delta^{15}\text{N}$  of outflow.

The sediment of Lake 227 is a large reservoir of nitrogen because of high sedimentation rates post eutrophication (Wolfe et al, 1994). The sediment core at 7 metres, following diagenesis and compaction, had a mean  $\delta^{15}\text{N}$  of -0.06‰ and displayed little change over the section of core representing 1999-2007

(Chapter 4). The measured sediment core  $\delta^{15}\text{N}$  is being used to compare the result of the isotope mass budget. However, half of the lake sediments are above 4 metres and therefore subject to oxic conditions for the majority of the year. As was shown in Chapter 4 this leads to higher  $\delta^{15}\text{N}$  values in the surficial sediments above 4m depth. No sediment core was analyzed from this depth so the change in  $\delta^{15}\text{N}$  of sediment at this depth over time is not known and therefore the loss of nitrogen historically in the isotopic mass budget has this as a major source of error. The amount of sediment accumulation in the shallower portions of the lake is less than deeper parts because of focusing of sediments to deeper parts of the lake due to the sediment slope plus wind and wave action (Crusius & Anderson, 1995)

The year to year change in lake nitrogen mass  $\delta^{15}\text{N}$  was assumed to be 0‰ as there is no evidence from the sediment core in Chapter 4 that there was a considerable change in the  $\delta^{15}\text{N}$  in the past 10 years.

### **5.3.2 Isotope Mass budget**

In the years 1999 to 2007, the input from nitrogen fixation was the dominant form of nitrogen entering Lake 227 at 41% of the total, followed by runoff at 33% and precipitation at 26% (table 5.2). The outflow and the change in lake mass nitrogen were highly variable across the years. Outflow losses ranged from 28kg to 131kg, while the change in nitrogen mass ranged from a loss of 145kg to a gain of 63kg. In every year included in this mass budget, nitrogen was lost from the lake. Some years had a much greater loss than others, with a range of 18kg to 186kg and an average of 91kg.

The  $\delta^{15}\text{N}$  of the material added to the sediment that was calculated from the lake mass budget does not match what has been recorded in the sediment core in any of the years tested (table 5.2). The calculated  $\delta^{15}\text{N}$  of the sediment input was higher than the  $\delta^{15}\text{N}$  measured in the sediment core in all years except 2004. The difference between the calculated  $\delta^{15}\text{N}$  of the sediment deposition and what was recorded in the sediment core varies in the amount of difference, ranging from 0.1-1.1‰, with an average difference of 0.4‰.

### **5.3.3 $\delta^{15}\text{N}$ in POM and Sediments of Lake 227 and Lake 442**

In the epilimnion of Lake 227, the  $\delta^{15}\text{N}$  of particulate nitrogen remained relatively constant, following ice off, for the whole of the open water season (figure 5.3). PON collected at 0.5 m had  $\delta^{15}\text{N}$  values that were within the range of the  $\delta^{15}\text{N}$  of external nitrogen inputs (precipitation, runoff and  $\text{N}_2$  fixation). The range in the external inputs was -1.5‰ to 3.3‰, while the range in particulate was -1.2‰ to 2.3‰ (figure 5.4). The mass weighted  $\delta^{15}\text{N}$  of epilimnetic particulate matter was 0.5‰ (Chapter 4). This is close to the mass weighted mean  $\delta^{15}\text{N}$  of input nitrogen for the open water season of 2010 which was 0.0‰. Under ice, there are only three POM samples from different years, but they have very different  $\delta^{15}\text{N}$  values (figure 5.5). There was a very low  $\delta^{15}\text{N}$  PON at the beginning of February 2010 and a very high  $\delta^{15}\text{N}$  PON near

the end of March 2011 at both 1m and 4m. The  $\delta^{15}\text{N}$  of PON deeper than 5m remained constant over the year, except in early June at 6m when the  $\delta^{15}\text{N}$  at 6m was near 0‰. Particulate matter from the hypolimnion of Lake 227 had an average  $\delta^{15}\text{N}$  of -7.3‰, ranging from -8.8‰ to -6.1‰ (figure 5.6). Hypolimnetic particulate was significantly different ( $p < 0.05$ ) than epilimnetic particulate matter. There was no relationship between  $\delta^{15}\text{N}$  and depth in the hypolimnion.

Ammonium in the hypolimnion averaged -0.3‰ at 8m where it was measured monthly, having a seasonal range of -2.0‰ to 0.3‰ (figure 5.7). One sample from both 6m and 9m showed that ammonium had higher  $\delta^{15}\text{N}$  as the distance from the sediments increased, and the concentration decreased. The surficial sediments in the hypolimnion had an average  $\delta^{15}\text{N}$  of 0.2‰, and there was no association between  $\delta^{15}\text{N}$  and depth below 4m (figure 5.6).

The  $\delta^{15}\text{N}$  of epilimnetic particulate matter in Lake 442 had a wider seasonal range than particulate matter in Lake 227 (figure 5.8). Lake 442 epilimnion particulate matter  $\delta^{15}\text{N}$  was low at the beginning of June, for both 1m and 5m, before increasing to a higher  $\delta^{15}\text{N}$  that was more constant, except for one large decrease in  $\delta^{15}\text{N}$  in August at 5m and in September at 1m. The particulate matter at 9m in Lake 442 remained around 2.5‰, which was higher than any other depth in Lake 442 during the open water season, except in October. The  $\delta^{15}\text{N}$  of particulate matter in Lake 442 increased with depth to 9m on most days, except for one sample day where the  $\delta^{15}\text{N}$  decreased at 9m to resemble particulate found deeper in the lake (figure 5.9). Under ice particulate in Lake 442 had higher  $\delta^{15}\text{N}$  than open water particulate at 5m and 9m but not at 1m where the  $\delta^{15}\text{N}$  was within the same range as open water samples. Hypolimnion  $\delta^{15}\text{N}$  - POM was lower than 9m particulate but was not different from the particulate in the epilimnion; however hypolimnion  $\delta^{15}\text{N}$  - POM was only available from one date in the open water season. Surficial sediment in Lake 442 had lower  $\delta^{15}\text{N}$  below 10m than above, a similar trend in surficial sediment  $\delta^{15}\text{N}$  was seen in Lake 227 (figure 5.9). The higher  $\delta^{15}\text{N}$  of the sediments in the epilimnion of Lake 227 corresponded to oxic conditions (Chapter 4); in a similar fashion to the higher sediment  $\delta^{15}\text{N}$  in the upper part of Lake 442.

#### 5.3.4 Lake Survey

The range in  $\delta^{15}\text{N}$  of PON in Lake 227 spanned -8.8‰ to 8.5‰, (figure 5.10). All samples from the other lakes in the lake survey conducted in July of 2010 fell within this range, with the majority between -6‰ and 2‰. Epilimnion PON, zooplankton and DON samples from Lake 227 during July 2010 only are within the same  $\delta^{15}\text{N}$  range as similar samples from the epilimnion of the other lakes in the survey.

The C:N separates the DON from other species of nitrogen with all DON having C:N ratios greater than 20 and less than 40. Lake 227 POM had a significantly ( $p < 0.01$ ) different C:N in POM compared to

POM from other ELA lakes. The mean C:N of Lake 227 POM was 9.1 while the C:N of POM from other lakes was 15.2. The  $\delta^{15}\text{N}$  of the hypolimnion POM were significantly different ( $p < 0.01$ ) but the  $\delta^{15}\text{N}$  of epilimnion POM was not significantly different ( $p > 0.05$ ) between Lake 227 and the other ELA lakes. Lake 227 POM had a non-mass weighted mean of  $0.4 \pm 0.9\text{‰}$  while the other lakes had a mean of  $-0.3 \pm 2.5\text{‰}$ .

## 5.4 Discussion

### 5.4.1 Reasons for Discrepancy Between Isotope Mass Budget and Sediment $\delta^{15}\text{N}$ values

The calculated  $\delta^{15}\text{N}$  values of sediments from the isotope mass budget do not equal the  $\delta^{15}\text{N}$  values that are observed in the sediment core. This could happen because; 1) the errors in the nitrogen mass input and loss calculations are significant; 2) the  $\delta^{15}\text{N}$  assigned to the inflows and outflow are not reflective of the actual  $\delta^{15}\text{N}$  of inputs and losses; or 3) there is an unaccounted for source/sink of nitrogen in Lake 227 supplying/removing nitrogen in the sediments.

The mass budget calculation is subject to several assumptions. For the inflows, there is no defined inflow stream in the Lake 227 catchment so the flow cannot be gauged or sampled for chemical and isotopic analysis. A proxy catchment is thus required. The inputs from nitrogen fixation are estimated by heterocyst counts and related to the amount of nitrogen fixed by an equation in Findlay et al, 1994. This estimation does not account for heterocysts that are not actively fixing nitrogen or are fixing nitrogen at a different rate compared to when the regression of heterocysts to nitrogen fixed was calculated (Storch et al, 1990). In addition, it is assumed that the equation for 1994 is applicable to the nine years of the mass budget calculation.

The outflow from Lake 227 is another potential source of error because the flow rate is not gauged. The Lake 239 outflow is used as a proxy to estimate the outflow from Lake 227 but the lake and watershed area are much larger than in Lake 227, so it may not be an accurate proxy for outflow volume. A comparison of the Lake 227 outflow when gauges were present, 1970-1998, to the back calculated outflow using the Lake 239 regression show that the outflow volume from Lake 227 may be overestimated by the regression in wet years (Michael Patterson, Personal communications 2011). Without a gauge at the outflow of Lake 227 in the years used in this mass budget any error introduced by the Lake 239 regression is unknown in magnitude and unavoidable because there is not a better known option.

The cumulative impact of the input output mass errors is manifested in the residual or sedimentation number. The amount of sedimentation varies over an order of magnitude between the years

1999 and 2007. One way to check the mass budget is to ground truth it using a measured value which, in this case, is the actual average sediment accumulation mass from a sediment core with known chronology. The average mass accumulation between 1999 and 2007, according to the sediment core in Chapter 4, is 170kg-N/yr. In only three of the years (1999, 2003, and 2006) in this mass budget does the amount of sedimentation come close to the average accumulation rate from the core. The average sedimentation from the mass budget is 91kg/year, which is almost half of the average yearly sedimentation from the sediment core. Although it is likely that there is some winnowing of epilimnetic sediments and re-deposition in the hypolimnion, the sediments in the epilimnion are organic rich with observable accumulation with time so not all sediment is transported to the hypolimnion. Thus re-deposition is unlikely to account for the offset between observed and calculated deposition. The large variation in the sedimenting mass from the mass budget indicates that there are large errors in the inputs and outputs of nitrogen.

The yearly change in the  $\delta^{15}\text{N}$  of the inputs to Lake 227 is not known. Nitrogen fixation is assumed to have a constant  $\delta^{15}\text{N}$  input because it is assumed that fixation does not fractionate nitrogen isotopes and will therefore always have a value of 0‰ (Hoering & Ford, 1960). However the fractionation factor in Lake 227 for cyanobacteria has not been directly quantified and studies have shown that there can be a range in the  $\delta^{15}\text{N}$  of nitrogen fixed from the atmosphere under different growth and aeration rates (Minagawa & Wada, 1986). The  $\delta^{15}\text{N}$  of DON in runoff should not vary significantly year to year, barring some form of major disruption to the catchment, such as a forest fire of which there have been none in the Lake 227 catchment (Schindler et al, 1980). However, the impact of inorganic nitrogen pulses in spring runoff are not accounted for when estimating the  $\delta^{15}\text{N}$  of terrestrial input so the yearly  $\delta^{15}\text{N}$  of runoff is not completely characterized. This could be significant as the total dissolved nitrogen in the North West, East, and North East inflows to the reference Lake 239 are composed of 13%, 35% and 6% inorganic nitrogen respectively on an annual timescale (Parker et al, 2009).

The year to year variability and overall trend of the  $\delta^{15}\text{N}$  of precipitation is also uncertain as these measurements are among the first in Canada. The precipitation inputs also do not cover winter deposition, so the average mass weighted  $\delta^{15}\text{N}$  could be different depending on the mass of nitrogen and the  $\delta^{15}\text{N}$  of winter precipitation. A study conducted at the Turkey lakes watershed in Northern Ontario found higher  $\delta^{15}\text{N}$  nitrate by approximately 2.6‰ (-3.4‰ between April and October and -0.8‰ between November and March) in winter deposition (Spoelstra, 2004). Nitrate would be available mostly during the spring runoff period where it could either be rapidly flushed from the lake or utilized by aquatic organisms depending on the year and degree of mixing, so the impact on in-lake  $\delta^{15}\text{N}$  is unknown and likely variable from year to year. Throughout the open water season lakes only receive nitrate by direct precipitation on

the lake surface or during extreme precipitation events that result in overland flow (Lamontagne & Schiff, 1999).

Known losses of nitrogen in Lake 227 are through the outflow and to the sediment. The loss out the outflow has been estimated by taking the mean  $\delta^{15}\text{N}$  of the inputs for each year. This assumption was made because Lake 227 is under severe nitrogen stress, which has led to nitrogen fixation during the summer months. In low available nitrogen conditions there should be little isotopic fractionation during assimilation of nitrogen (Fogel & Cifuentes, 1994). One problem with this approach is that the nitrogen of the inputs may not all be bioavailable, such as the suspended matter in deposition or the DON from the catchment. In the deposition, the  $\delta^{15}\text{N}$  of suspended and dissolved nitrogen must be very high compared to the  $\delta^{15}\text{N}$  of nitrate and ammonium, which have mass weighted values of -3.1‰ and -6.2‰ respectively, to bring the total average nitrogen  $\delta^{15}\text{N}$  of precipitation to 3.3‰. Nitrate and ammonia are readily bioavailable and if only these two nitrogen species are used in the budget, the  $\delta^{15}\text{N}$  of the outflow would be lower than what was used. However, if the measured  $\delta^{15}\text{N}$  of the outflow from 2010 is compared to calculated outflows from 1999 to 2007 (using the mass weighted  $\delta^{15}\text{N}$  of inputs for each year) the values are not extremely different. In 2010 the mass weighted  $\delta^{15}\text{N}$  of PON and DON in the epilimnion during the open water season was 0.6‰, compared to calculated outflow values the greatest difference historically is within 1‰ of the 2010 value. So therefore the calculated  $\delta^{15}\text{N}$  outflow values in the mass budget are within a reasonable range.

Other possibilities for the discrepancy between isotope mass budget and observed sediment  $\delta^{15}\text{N}$  is an unaccounted loss of nitrogen from the sediments that has a positive  $\delta^{15}\text{N}$ , an unaccounted gain of negative  $\delta^{15}\text{N}$  nitrogen to the sediments, or a combination of both. Considering potential loss mechanisms the loss of positive  $\delta^{15}\text{N}$  material from the sediments is difficult based on standard isotope fractionation during most kinetic processes. The possible processes responsible for losses of nitrogen from the sediment are denitrification and mineralization.

Denitrification has been shown to exert a strong preference for the light nitrogen isotope, making the substrate progressively higher in  $\delta^{15}\text{N}$  and the product lower than the source  $\delta^{15}\text{N}$  (Marriotti et al, 1981). This makes denitrification not feasible for removing positive  $\delta^{15}\text{N}$  nitrogen from the sediments as the sediments have  $\delta^{15}\text{N}$  values less than 0‰ in the period of study, so any nitrogen lost through denitrification will be much less than 0‰. Mineralization is a process that is quite active in Lake 227, as significant amounts of ammonium build up in the hypolimnion over the stratified season. This process however has been found to only slightly fractionate nitrogen which would again leave the sediments more positive in  $\delta^{15}\text{N}$  (Kendal, 1998). A small apparent fractionation factor for the production of ammonium

from sediment organic matter was observed in Lake 227. The average  $\delta^{15}\text{N}$  - $\text{NH}_4^+$  in Lake 227 was -0.3‰, while the  $\delta^{15}\text{N}$  of the surficial sediments was 0.2‰, a difference of only 0.5‰. This would mean that any nitrogen lost from the sediments as ammonium would be equal to or less than the sediment organic matter  $\delta^{15}\text{N}$ . Neither denitrification or mineralization are capable of releasing positive  $\delta^{15}\text{N}$  nitrogen from the sediment, so it is unlikely that the sediments are acting as a source of positive  $\delta^{15}\text{N}$  nitrogen to the lake.

A negative  $\delta^{15}\text{N}$  source of particulate matter to the sediments could come from the hypolimnion or from the epilimnion following turnover and under ice when there can be significant ammonium in the epilimnion. The surficial sediment sample retrieved in the summer of 2010 had a  $\delta^{15}\text{N}$  of 0.2‰, which was very close to the  $\delta^{15}\text{N}$  of epilimnetic particulate matter from the open water season (Chapter 4). If there was a large input of low  $\delta^{15}\text{N}$  particulate from the hypolimnion over the course of the stratified season it would have been expected to be evident in that sample. Another source of negative  $\delta^{15}\text{N}$  particulate is under ice when there can be low  $\delta^{15}\text{N}$  particulate in the lake because of large stores of ammonium available for assimilation and therefore the possibility of isotopic fractionation. Evidence for this is from the sediment core retrieved from Lake 227 in March of 2011 which had low  $\delta^{15}\text{N}$  measured at the very top of the core (Chapter 4, this work). This was suspected to be recently deposited, under ice particulate, which had not been compressed by, and blended with, higher  $\delta^{15}\text{N}$  summer or very later winter deposition (Chapter 4, this work). For either of these options to work there must be export of higher  $\delta^{15}\text{N}$  nitrogen from the lake because if all nitrogen that was regenerated was again deposited to the sediment the net effect on the  $\delta^{15}\text{N}$  would be negligible. The outflow following turnover would be the most likely path for the loss of high  $\delta^{15}\text{N}$  nitrogen because it does not actively discriminate against the heavier nitrogen isotope like biological processes can.

Discrepancies show that the mass budget of nitrogen is likely not well enough constrained given mismatch of sediment inventory and calculated deposition. The inputs of nitrogen, most likely the terrestrial and fixation sources, are not known well enough because of the use of proxy catchments and assumptions on the amount and rate of nitrogen fixation. Also the  $\delta^{15}\text{N}$  of inputs and losses are not well enough constrained to provide information on the cycling of nitrogen in Lake 227 due to a data set that does not include multiple years and the changing hydrologic conditions or winter and early spring samples of precipitation and runoff  $\delta^{15}\text{N}$ .

#### **5.4.2 Nitrogen Cycling in Lake 227: Evidence from Nitrogen isotopes**

Nitrogen isotopes in Lake 227 particulate can provide information on the sources of nitrogen for algae and whether these sources change. In 2010, the  $\delta^{15}\text{N}$  of epilimnetic POM in Lake 227, during the open

water season, does not occupy a large range, staying within roughly 2‰ to -2‰. The consistent  $\delta^{15}\text{N}$  of POM indicates that the source of nitrogen to the algae in Lake 227 during the summer stratified period has a relatively stable  $\delta^{15}\text{N}$  (a combination of nitrogen fixation, precipitation and runoff) and/or that the lake is under high nitrogen stress which limits the apparent isotopic fractionation. As nitrogen fixation now comprises a large portion of the open water nitrogen budget in Lake 227 the constant input of nitrogen could dampen the effect of possible changes in precipitation and runoff input  $\delta^{15}\text{N}$ .

The  $\delta^{15}\text{N}$  of the POM in the hypolimnion indicates that there is at least a different nitrogen source for hypolimnetic particulate than the epilimnion, if not an entirely different biological community. The source of nitrogen in the hypolimnion is likely ammonium, from the mineralization of sedimented organic matter, as it is available in high concentrations and is the dominant form of inorganic nitrogen in the hypolimnion. The surficial sediments in Lake 227 have similar nitrogen isotopic signatures as the mass weighted epilimnetic POM, both of which are approximately 0.2‰ (Chapter 4). The mineralization of organic matter has been shown to exhibit little fractionation (Kendall, 1998). In Lake 227 the ammonium produced through mineralization had similar  $\delta^{15}\text{N}$  (-0.3‰) relative to the  $\delta^{15}\text{N}$  of the surficial sediments indicating that in the hypolimnion of Lake 227 mineralization is not a strongly fractionating process. The POM in the hypolimnion of Lake 227 is on average -7.3‰, which is 7.0‰ lower than the  $\delta^{15}\text{N}$  of available ammonium. A fractionation of this magnitude is large relative to the other processes in Lake 227 such as assimilation in the epilimnion and nitrogen fixation. The average ammonium concentration in the hypolimnion of Lake 227 is 165  $\mu\text{mols/L}$ , which has been found to be approximately the concentration with the greatest isotopic fractionation during utilization by heterotrophic bacteria while diatoms fractionate most strongly at around 50  $\mu\text{mols/L}$  of ammonium (Fogel & Cifuentes, 1993). Different fractionation factors occur depending on what is the rate limiting step in the utilization of nitrogen. The highest fractionations occur when the reaction is diffusion limited, followed by enzyme limited and lastly, with the least fractionation, nitrogen limited. In the epilimnion the organisms are nitrogen limited so little fractionation occurs, while in the hypolimnion they may be either diffusion, enzyme, or perhaps some other process limited thus resulting in a distinct  $\delta^{15}\text{N}$  POM isotopic signal.

The two available POM samples from under-ice show that there is an increase in the  $\delta^{15}\text{N}$  of the nitrogen source over the course of the under-ice period. A possible explanation for the higher  $\delta^{15}\text{N}$  of POM under ice is the assimilation of overturned hypolimnetic ammonium by algae following the coupled processes of nitrification and denitrification acting on the ammonium. Algae have been shown to preferentially take up ammonium when presented with both ammonium and nitrate so the  $\delta^{15}\text{N}$  of POM should initially reflect the  $\delta^{15}\text{N}$  of ammonium until it is depleted and a switch to nitrate occurs (Waser et al, 1998). At turnover some hypolimnetic ammonium is mixed into the rest of the water with a  $\delta^{15}\text{N}$  of

approximately 0‰, the amount of ammonium depends on the degree of turnover which varies year to year. However, fall turnover is generally complete to at least 8 metres. Mixing in the water column to 8 metres in 2010 resulted in an ammonium concentration of 300µg-N/l and a nitrate concentration of 25µg-N/l in the top 6 metres of Lake 227 on the 2<sup>nd</sup> of November. At this time there has likely already been some uptake of ammonium because there was a 50% increase in the amount of suspended nitrogen following turnover. The increase in suspended nitrogen does not appear to be the direct result of hypolimnetic suspended nitrogen being mixed into the water column because the concentration of suspended nitrogen in the hypolimnion is not high enough to increase the lake suspended nitrogen to such a large degree. The earliest POM sample available after fall turnover (February 1<sup>st</sup>) has a  $\delta^{15}\text{N}$  of -5.1‰; this is higher than the  $\delta^{15}\text{N}$  of hypolimnetic POM during the stratified season. Assuming the same fractionation on uptake, the source ammonium would be around 2.1‰. This more positive  $\delta^{15}\text{N-NH}_4^+$  value could arise from a combination of uptake, although productivity should be low at this time, and nitrification. The next POM sample is from the end of March and has a  $\delta^{15}\text{N}$  of +8.5‰, which is much higher than the expected starting  $\delta^{15}\text{N}$  for the ammonium following turnover of roughly 0‰. This suggests that a great deal of the initial ammonium has been consumed by assimilation and nitrification, which is supported by chemistry data on that date showing ammonium concentrations to be less than 15µg-N/L. At this time nitrate concentrations are high (greater than 500µg-N/L) and the algae may also been using nitrate as a nitrogen source. This is important because if they have switched to nitrate then the nitrate must have very high  $\delta^{15}\text{N}$  values, indicating that a significant amount of denitrification has occurred (Delwiche & Steyn, 1970). However, if the algae are still utilizing the ammonium it only shows that there has been a great deal of nitrification occurring to increase the  $\delta^{15}\text{N}$  of residual ammonium, and not necessarily denitrification. If the presence and amount of denitrification can be confirmed by further research, specifically the measurement of  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{15}\text{N-NH}_4^+$  under ice, then it could represent a significant portion of the nitrogen budget of Lake 227 that is not currently accounted for (Schindler et al, 1987; Findlay et al, 1994).

#### **5.4.3 Differences in Nitrogen Cycling Between Lakes 442 and 227**

The  $\delta^{15}\text{N}$  of epilimnetic particulate matter in Lake 227 has a smaller range than in Lake 442 even though both lakes have similar input. Differences in input nitrogen between the lakes could be caused by the input of atmospherically fixed nitrogen to Lake 227 or the upstream lake input of Lake 438 to Lake 227. The larger range in the  $\delta^{15}\text{N}$  in Lake 442 is suspected to be related to changing nitrogen availability during the summer stratified season and missing input of nitrogen fixation which stabilizes the  $\delta^{15}\text{N}$  of POM in Lake 227. Higher nitrogen concentrations can occur in the epilimnion of Lake 442 during the spring due to turnover and because of increased terrestrial inputs during spring snowmelt (figure 5.11) allowing for greater isotopic fractionation (Waser et al, 1998). At times of greater nitrogen stress or after

exhaustion of the nitrogen source, the  $\delta^{15}\text{N}$  of the particulate should increase to be closer to the source nitrogen  $\delta^{15}\text{N}$  (figure 5.12) (Waser et al, 1998). The relationship between nitrogen concentration and  $\delta^{15}\text{N}$  will not always be straightforward as the source of nitrogen being utilized can have distinct initial isotopic ratios. Therefore, changing nitrogen demand and availability in Lake 442 is likely responsible for the differences between Lake 442 and Lake 227 epilimnion nitrogen isotopes of POM.

The mineralization of organic matter in the anoxic portion of the hypolimnion of Lake 442 is expected to undergo a similarly slight degree of isotopic fractionation as the same process in Lake 227. The ammonium produced in the anoxic hypolimnion of Lake 442 should have a  $\delta^{15}\text{N}$  close to the sediments, which are around 2‰. The particulate matter in anoxic hypolimnion of Lake 442 is approximately -2‰, which is 5‰ more positive than in the hypolimnion of Lake 227. The difference in the  $\delta^{15}\text{N}$  of ammonium in Lake 227 and the estimated  $\delta^{15}\text{N}$  of ammonium in Lake 442 (-0.3‰ versus 2‰) is not enough to explain the difference in the  $\delta^{15}\text{N}$  of hypolimnetic POM between the two lakes. In Lake 442 the offset between the estimated  $\delta^{15}\text{N}$  of ammonium and the  $\delta^{15}\text{N}$  of hypolimnetic POM is only 4.1‰, compared to a 7‰ offset between ammonium and POM in Lake 227. The apparent isotopic fractionation is lower in Lake 442 than 227 so other factors must have an influence on the  $\delta^{15}\text{N}$  of POM in the hypolimnion. One such factor could be a higher percentage of ammonium utilization in Lake 442 versus Lake 227; therefore the product POM is more isotopically similar to the original  $\delta^{15}\text{N}$  of the ammonium (Waser et al, 1998). To confirm this possibility the  $\delta^{15}\text{N}$  of the ammonium would need to be known, as the  $\delta^{15}\text{N}$  would be expected to increase as the ammonium supply was depleted. Alternatively, knowing the rate that ammonium is produced at in the hypolimnion of Lake 442 would allow for the calculation of how much ammonium has been utilized and therefore an approximation of the expected increase in the  $\delta^{15}\text{N}$  of residual ammonium. Qualitatively it can be assumed that mineralization in Lake 227 is higher than in Lake 442 because of the increased sedimentation rate in Lake 227 following eutrophication (Wolfe et al, 1994). Increased sedimentation resulted in a greater reservoir of organic rich, labile, autochthonous material available for mineralization compared to the lower organic content, allochthonous sediments of Lake 442 (figure 5.13). There is a large difference in the ammonium concentrations in the anoxic hypolimnions of Lakes 227 and 442. Lake 227 had an average ammonium concentration of 2.6mg/L while Lake 442 had an average ammonium concentration of just 0.1mg/L. A second factor that decreases the apparent fractionation during ammonium assimilation could be the influence of sinking epilimnetic particulate offsetting the lower  $\delta^{15}\text{N}$  particulate in the hypolimnion, resulting in a mixed epilimnetic/hypolimnetic signal  $\delta^{15}\text{N}$  signal in Lake 442 although this would also be an issue in Lake 227 as well, possibly more so because of the high sedimentation rate. However, this material is not suspected to be comprised of entirely settled epilimnetic particulate matter based on POM

carbon isotopes, showing the Lake 442 hypolimnetic particulate has a similar  $\delta^{13}\text{C}$  as organisms growing in the hypolimnion of Lake 227 (Schiff, unpublished data). In Lake 227, the higher ammonium concentration might support a larger hypolimnetic community and the influence of sedimenting particulate would be less. The difference between the  $\delta^{15}\text{N}$  of POM in Lake 227 compared to Lake 442 is likely the indirect result of increased inputs of phosphorus in Lake 227 which led to increased sedimentation and subsequently higher ammonium concentrations in the hypolimnion in Lake 227, which is the product of eutrophication.

#### **5.4.4 Lake Survey**

The external inputs of nitrogen to Lake 227 occupy a 4.8‰ range, with runoff  $\delta^{15}\text{N}$  at the low end (-1.5‰) and precipitation at the high  $\delta^{15}\text{N}$  end (3.3‰) while nitrogen fixation is in between at 0‰. The impact of nitrogen fixation may not be seen in the  $\delta^{15}\text{N}$  of the particulate matter as the inputs from runoff and precipitation would result in a  $\delta^{15}\text{N}$  between the two values. Samples collected during the lake survey in July of 2010 in Lake 227 and the other lakes do not have different mean  $\delta^{15}\text{N}$  for epilimnetic particulate matter. The range in Lake 227 epilimnetic particulate over the whole open water season is smaller than Lake 442, and presumably because other lakes at ELA are more like Lake 442 than 227 they will also have a wider range in the  $\delta^{15}\text{N}$  of POM than Lake 227. The controls on nitrogen cycling in non-eutrophic lakes at the ELA could be better understood with a larger sampling of lakes throughout the open water season to understand if all of the lakes follow similar trends related to nitrogen availability and sources or if there is some other control mechanism.

In comparison with whole season data from Lakes 442 and 227 but only one date for all other lakes sampled at the ELA there is no difference in the mean  $\delta^{15}\text{N}$  of epilimnetic particulate in Lake 227 versus the other lakes included in the survey, likely the product of the other lakes having the same external inputs as Lake 227. However, the range in the  $\delta^{15}\text{N}$  of epilimnetic particulate from other lakes extends more negative than in Lake 227 because of a number of possible reasons, one is that the inputs of nitrogen fixation will keep Lake 227 closer to 0‰ (Minagawa & Wada, 1986), a second reason is that other lakes at the ELA are phosphorus limited so nitrogen is generally in excess and can be fractionated upon assimilation (Schindler et al, 2008; Fogel & Cifuentes, 1994) and the relative importance of runoff (more negative  $\delta^{15}\text{N}$ ) versus precipitation (more positive  $\delta^{15}\text{N}$ ) is unique to each lake depending on the catchment to lake surface area ratios and surrounding topography. Other factors that can impact the  $\delta^{15}\text{N}$  of particulate nitrogen are lake morphology (percent of the sediments that regenerate nitrogen to the epilimnion), lake order, and wetland area. These many factors play a part in setting the  $\delta^{15}\text{N}$  of particulate matter and the input of nitrogen through fixation is not significantly different enough to offset the particulate matter in Lake 227 from other ELA lakes.

Nitrogen fixation does not directly set Lake 227 apart isotopically, but the nitrogen fixation is the product of eutrophication which has likely changed internal nitrogen cycling in Lake 227 by increasing nitrogen demand in the epilimnion and increasing sedimentation of organic matter (Findlay et al, 1994; Wolfe et al, 1994). The increased sedimentation is responsible for the buildup of high levels of ammonium in the hypolimnion of Lake 227 which in turn leads to changes in the hypolimnetic and under ice nitrogen cycles.

## 5.5 Summary and conclusions

Based upon the mass budget it is likely that the mass inputs and outflow loss of nitrogen are not accurately reflecting true inputs of nitrogen to Lake 227. The evidence for this is the highly variable sedimentation mass that is not supported by sediment core measurements of sediment accumulation. However, if the errors introduced in the mass budget are less than the difference between the calculated sediment  $\delta^{15}\text{N}$  and the measured  $\delta^{15}\text{N}$  of sediments, then the isotope mass budget points to an unknown source of negative  $\delta^{15}\text{N}$  material to the sediments and concurrent loss of positive  $\delta^{15}\text{N}$  material from the lake. The suspected source of unaccounted material to the sediment is under ice POM, a period during which high positive  $\delta^{15}\text{N}$  nitrate and ammonium (because of assimilation nitrification and denitrification) may also be lost out of the outflow. Denitrification is suspected to be a loss mechanism for nitrogen during the ice covered period based on a small sample of  $\delta^{15}\text{N}$  -POM samples from this period, future work would need to measure the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of DIN available under ice to determine if it is changing in a way indicative of denitrification.

The range in input  $\delta^{15}\text{N}$  for lakes at the ELA (precipitation, runoff, and nitrogen fixation) is not very large, only 4.8‰. The mass weighted average  $\delta^{15}\text{N}$  of the inputs in 2010 were 3.3‰ for precipitation, -1.5‰ for runoff and 0‰ for nitrogen fixation. The small range in input  $\delta^{15}\text{N}$  has resulted in Lake 227 not being significantly different in the  $\delta^{15}\text{N}$  composition of epilimnetic particulate matter compared to the other lakes sampled at the ELA. However, it is not known how much the  $\delta^{15}\text{N}$  these inputs vary seasonally or year to year due to limited measurements at this location.

Some differences are apparent when a direct comparison between an oligotrophic (Lake 442) and eutrophic lake (Lake 227) are made. The epilimnion  $\delta^{15}\text{N}$  POM in Lake 442 extends more negative than the  $\delta^{15}\text{N}$  of Lake 227 POM likely because of greater fractionation in Lake 442 during nitrogen assimilation because of lower nitrogen demand or differences in nitrogen source  $\delta^{15}\text{N}$ . The hypolimnetic POM in the anoxic zone is more negative in Lake 227 compared to Lake 442 because of either; 1) greater fractionation due to more available ammonium in Lake 227 from the mineralization of organic matter

from higher sedimentation rates; or 2) a higher percentage of material sampled being hypolimnetic in origin as opposed to sinking epilimnetic matter.

Nitrification and denitrification are suspected in Lake 227 during ice coverage based on POM samples and changing nitrogen concentrations. The importance of this process in the Lake 227 nitrogen budget is currently unknown. Lake 442 may also have some nitrification and denitrification under ice but the amount is expected to be less than Lake 227 because of lower starting ammonium concentration at turnover. Further studies would require more regular sampling of under ice for ammonium and nitrate isotopes to elucidate changes in the nitrogen pool that concentration analyzes may not identify.

Differences in nitrogen isotopes between Lake 227 and other ELA lakes do not appear to be the direct result of nitrogen fixation but are likely the result of eutrophication. Lake 227 and other ELA lakes cycle nitrogen in similar ways, but Lake 227 has had an intensification of nitrogen cycling because of increased inputs and productivity.

Fig. 1. (a)  $\delta^{15}\text{N}$  and (b)  $\delta^{13}\text{C}$  of sediment trap material (circles), bulk zooplankton (squares), and *Chaoborus* spp. (triangles) collected from May to October 1993 from oligotrophic Lake 110 (solid symbols) and eutrophic Lake 227 (open symbols), ELA, northwestern Ontario.

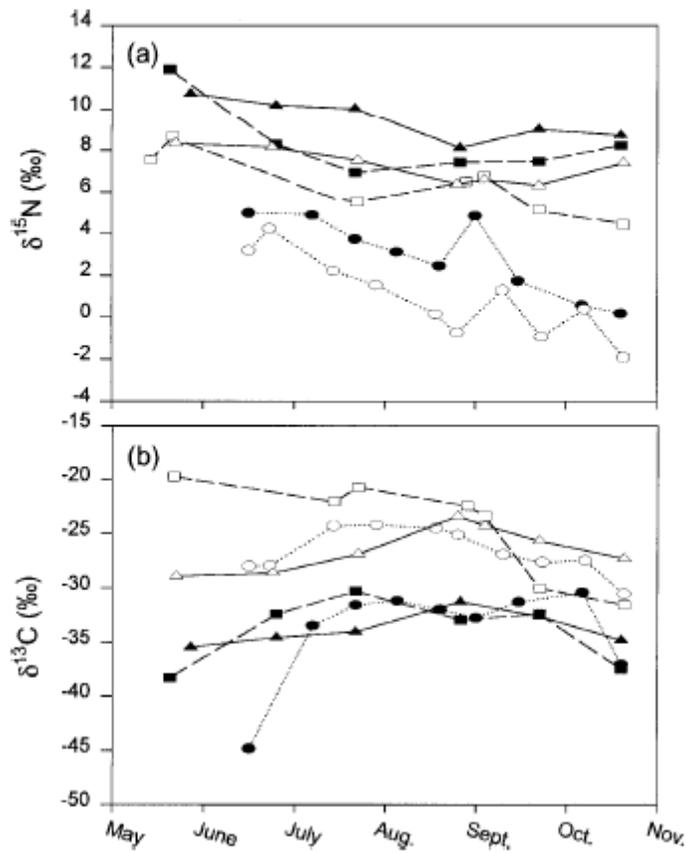


Figure 5:1 Lake 227 and 110  $\delta^{15}\text{N}$  of bulk zooplankton, *chaoborus* spp. and sediment trap material from Kidd et al, 1999

**Table 5-1 Isotope mass budget  $\delta^{15}\text{N}$  values used for outflow, precipitation, runoff and change in lake mass**

Year	$\delta^{15}\text{N}$ Outflow	$\delta^{15}\text{N}$ Precipitation	$\delta^{15}\text{N}$ Runoff	$\delta^{15}\text{N}$ change in lake mass
1999	0.5	3.3	-1.5	0.0
2000	0.2	3.3	-1.5	0.0
2001	0.7	3.3	-1.5	0.0
2002	0.9	3.3	-1.5	0.0
2003	0.6	3.3	-1.5	0.0
2004	-0.1	3.3	-1.5	0.0
2005	0.4	3.3	-1.5	0.0
2006	0.2	3.3	-1.5	0.0
2007	0.0	3.3	-1.5	0.0

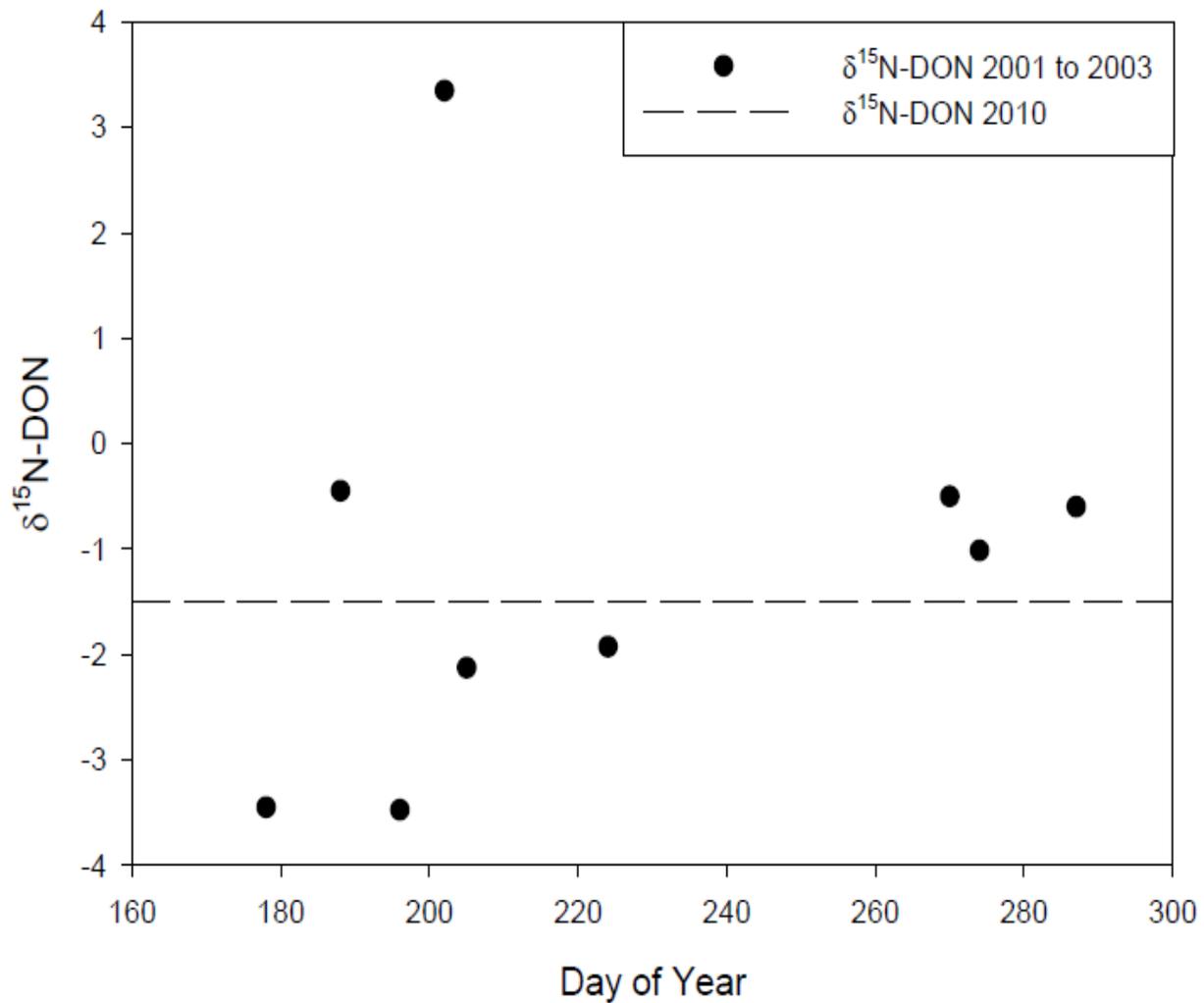


Figure 5:2 Historical  $\delta^{15}\text{N-DON}$  from the Lake 114 inflow (filled circles) from 2001 to 2003 plotted by day of year. Average for 2001 to 2003 is  $-1.1\text{‰}$ . Dashed line represents  $\delta^{15}\text{N-Runoff}$  value used in this mass balance ( $-1.5\text{‰}$ )

**Table 5-2 Inputs and losses of nitrogen for mass budget with sedimentation calculated as the residual to balance the masses. The calculated  $\delta^{15}\text{N}$  from the isotope mass budget is also presented. Measured sediment core  $\delta^{15}\text{N}$  is from three measured values from a Lake 227 sediment core linearly interpolated for points in between. The calculated  $\delta^{15}\text{N}$  of sediments is higher than actual measured  $\delta^{15}\text{N}$  in the sediments in all years except for 2004**

Year	Runoff (kg)	Precipitation (Kg)	N <sub>2</sub> fixation (kg)	Outflow (Kg)	Change in lake mass (kg)	Residual (sedimentation + denitrification) (kg)	Calculated $\delta^{15}\text{N}$ sediments	$\delta^{15}\text{N}$ Sediment Core
1999	59	49	48	116	-145	-184	0.1	-0.05
2000	65	40	43	131	-35	-52	0.1	-0.06
2001	53	59	44	101	16	-41	1.0	-0.06
2002	43	53	32	90	-39	-77	0.4	-0.07
2003	25	31	52	28	-86	-166	0.3	-0.08
2004	68	27	68	107	8	-49	-0.1	-0.08
2005	33	32	93	83	31	-44	0.6	-0.08
2006	33	27	106	43	-63	-186	0.2	-0.09
2007	70	32	75	97	63	-18	0.0	-0.09

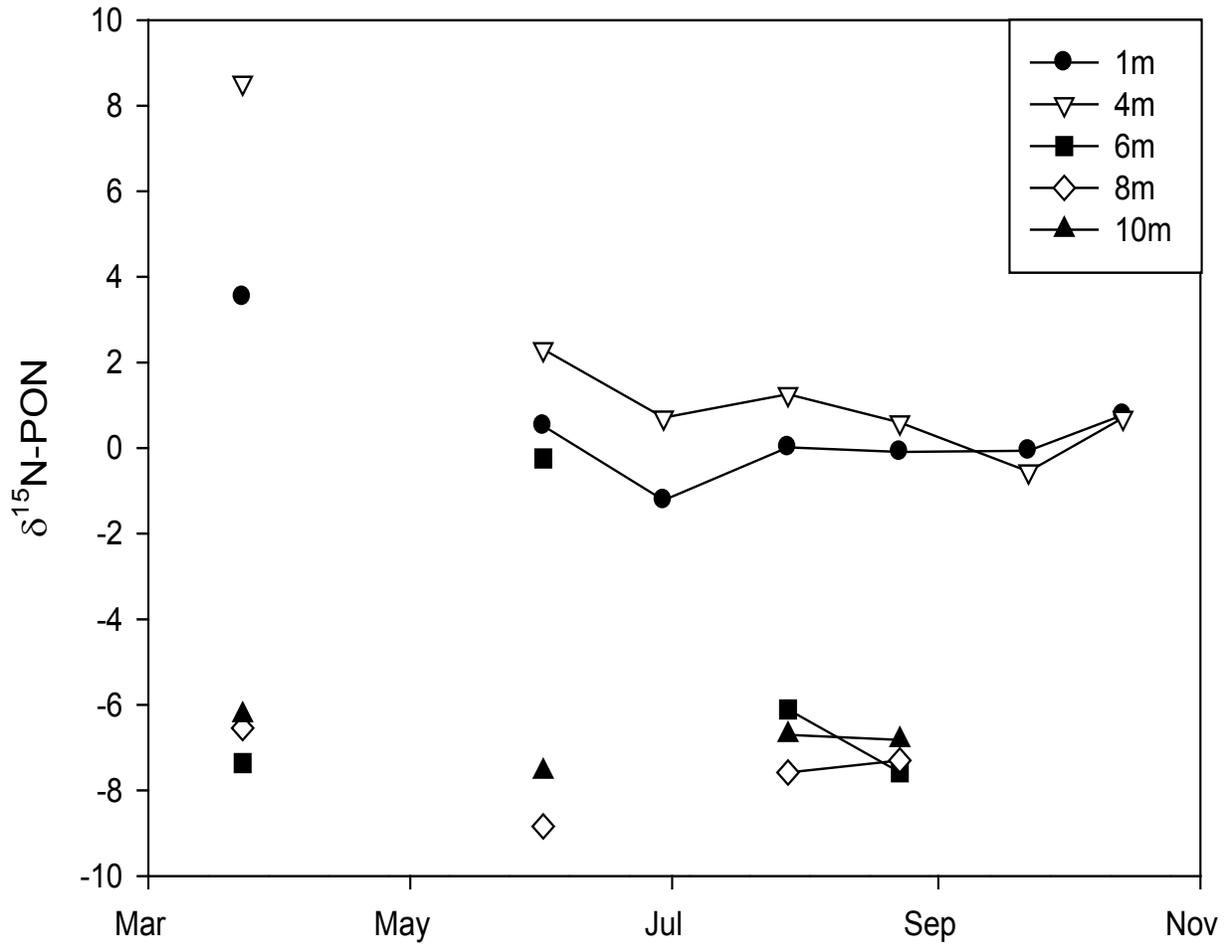


Figure 5:3  $\delta^{15}\text{N-POM}$  from 5 depths in Lake 227 during 2010. Points in March separated by the gap were taken under ice. The oxycline was around 4m for much of the open water season in 2010

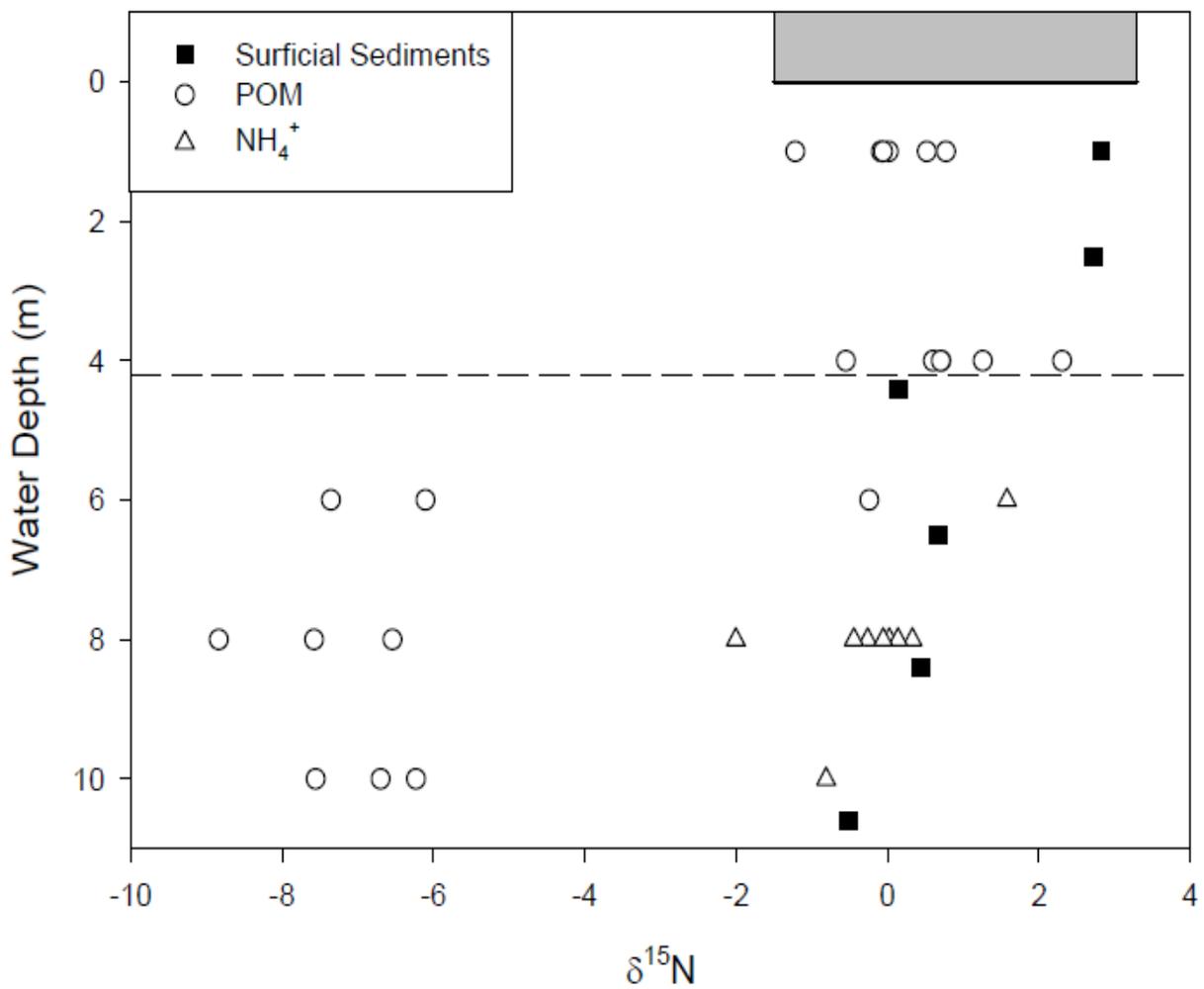


Figure 5:4 Lake 227 nitrogen species  $\delta^{15}\text{N}$  over the open water season in 2010. Gray square at top encompasses the average  $\delta^{15}\text{N}$  of inputs (including runoff, precipitation, and nitrogen fixation), while the dashed line just below 4m is the normal location of the oxycline in Lake 227

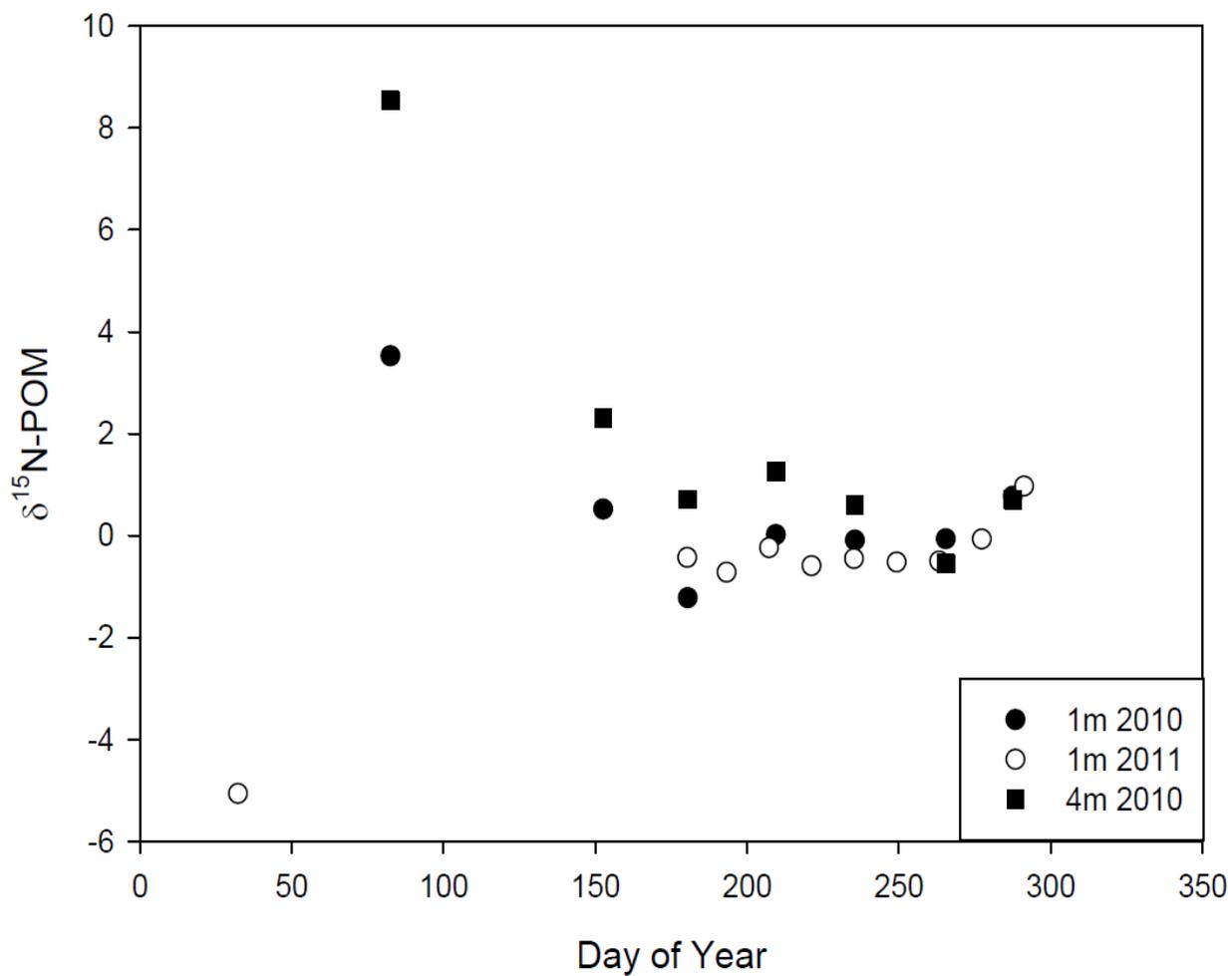


Figure 5:5  $\delta^{15}\text{N-POM}$  from Lake 227 at 1m and 4m in 2010 and 1m from 2011 by day of year. Turn over occurs soon after ice off in the spring, roughly day 115, and before ice on in the fall, roughly day 300

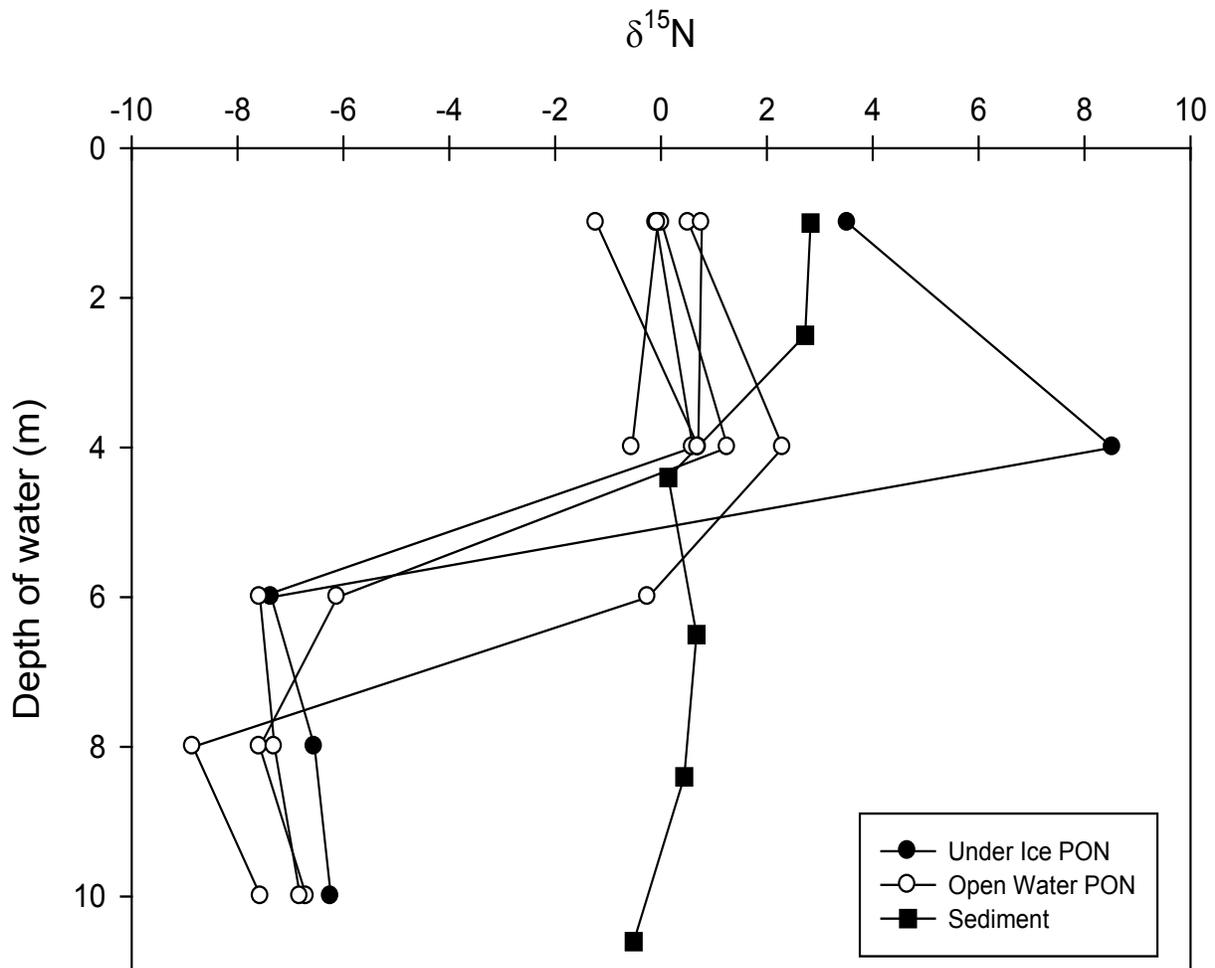


Figure 5:6 Depth of water profile of  $\delta^{15}\text{N}$ -PON from Lake 227 during the open water season (June to October, open circles), under ice (March, closed circles) and  $\delta^{15}\text{N}$  of surficial sediments (filled in boxes) in Lake 227 in 2010

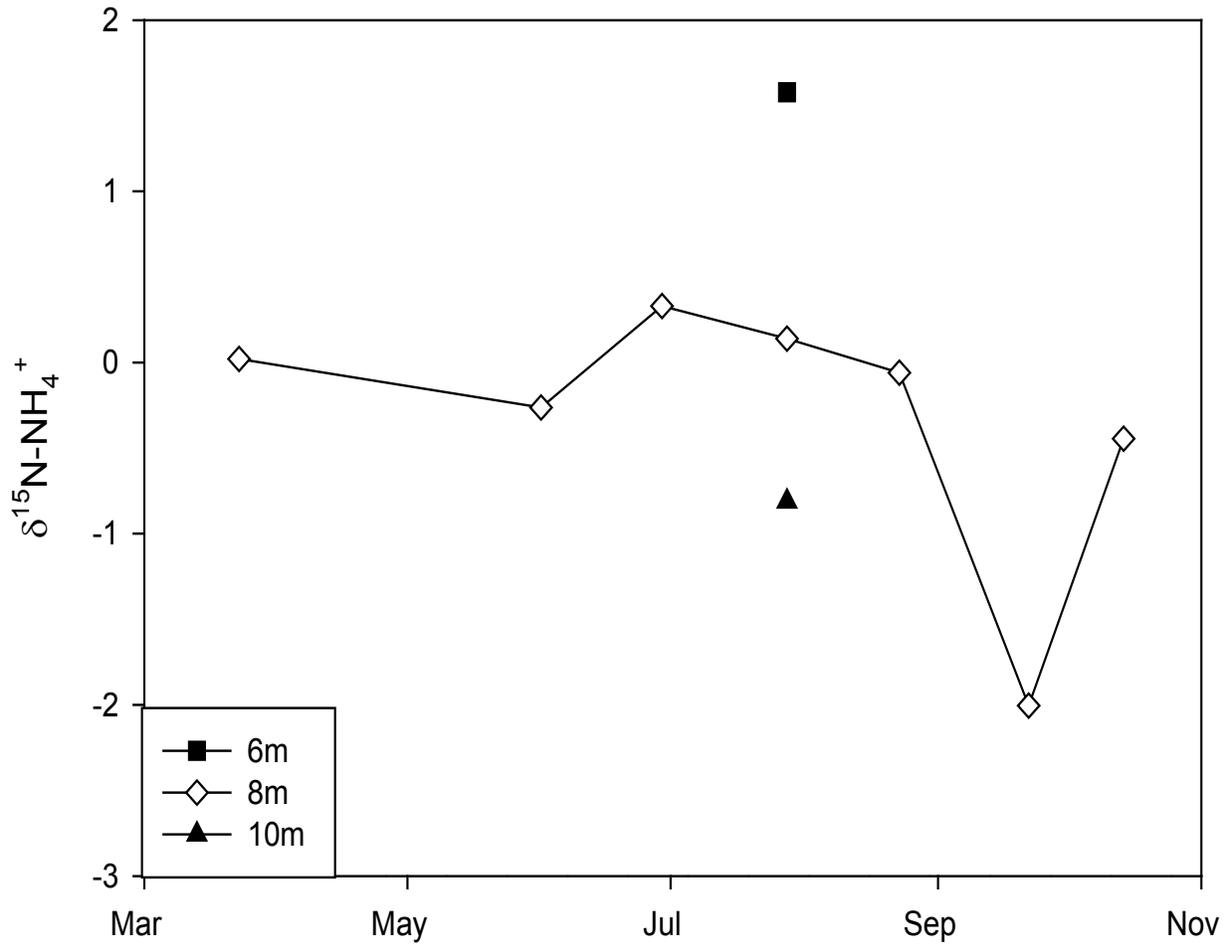


Figure 5:7 Seasonal  $\delta^{15}\text{N-NH}_4^+$  from 8m (open diamond) and one time measurements at 6m (filled square) and 10m (filled triangle). Mean  $\delta^{15}\text{N-NH}_4^+$  at 8m is -0.4‰, the source of the organic matter is the organic matter in the sediment

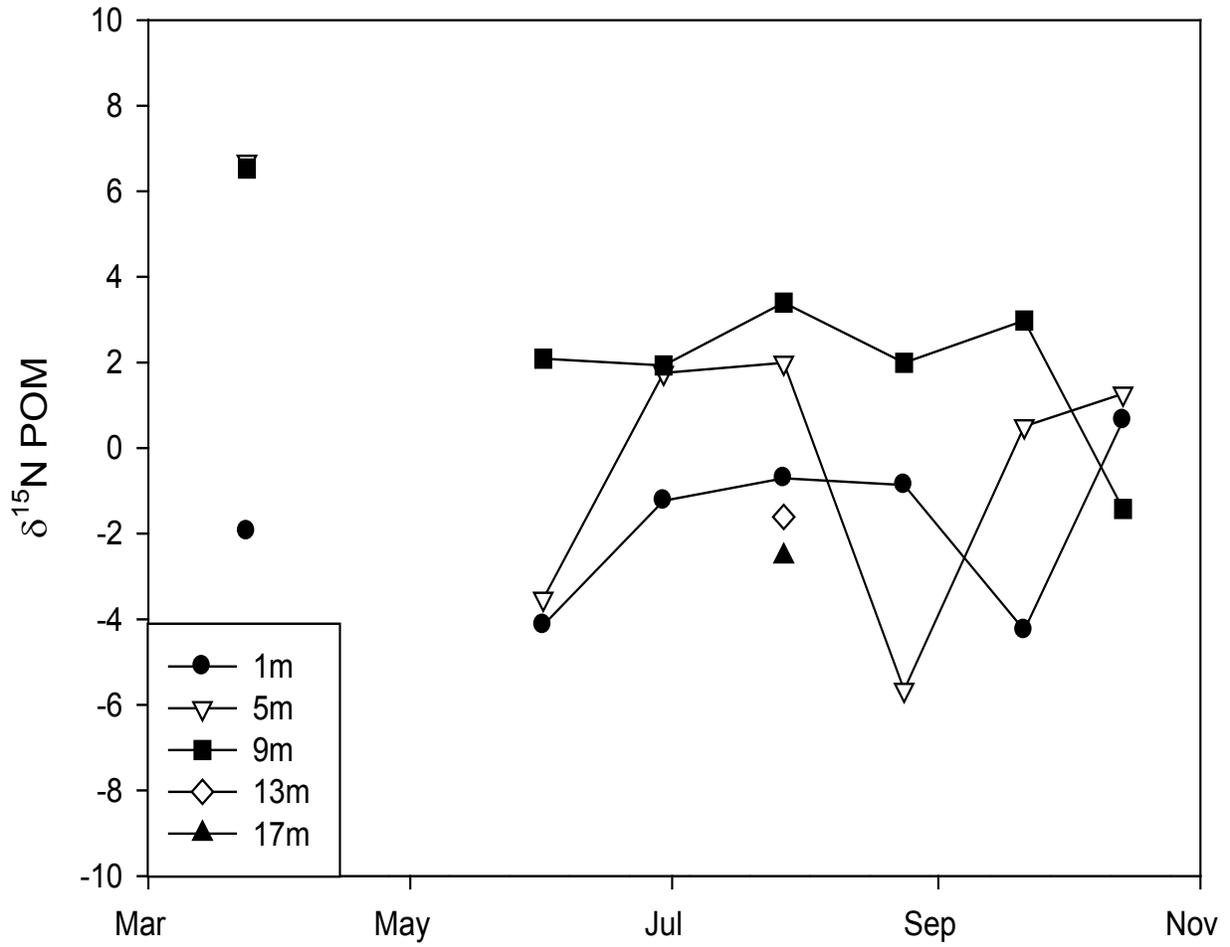


Figure 5:8 δ<sup>15</sup>N-POM from 5 depths in Lake 442 during 2010. Points in March separated by the gap were taken under ice

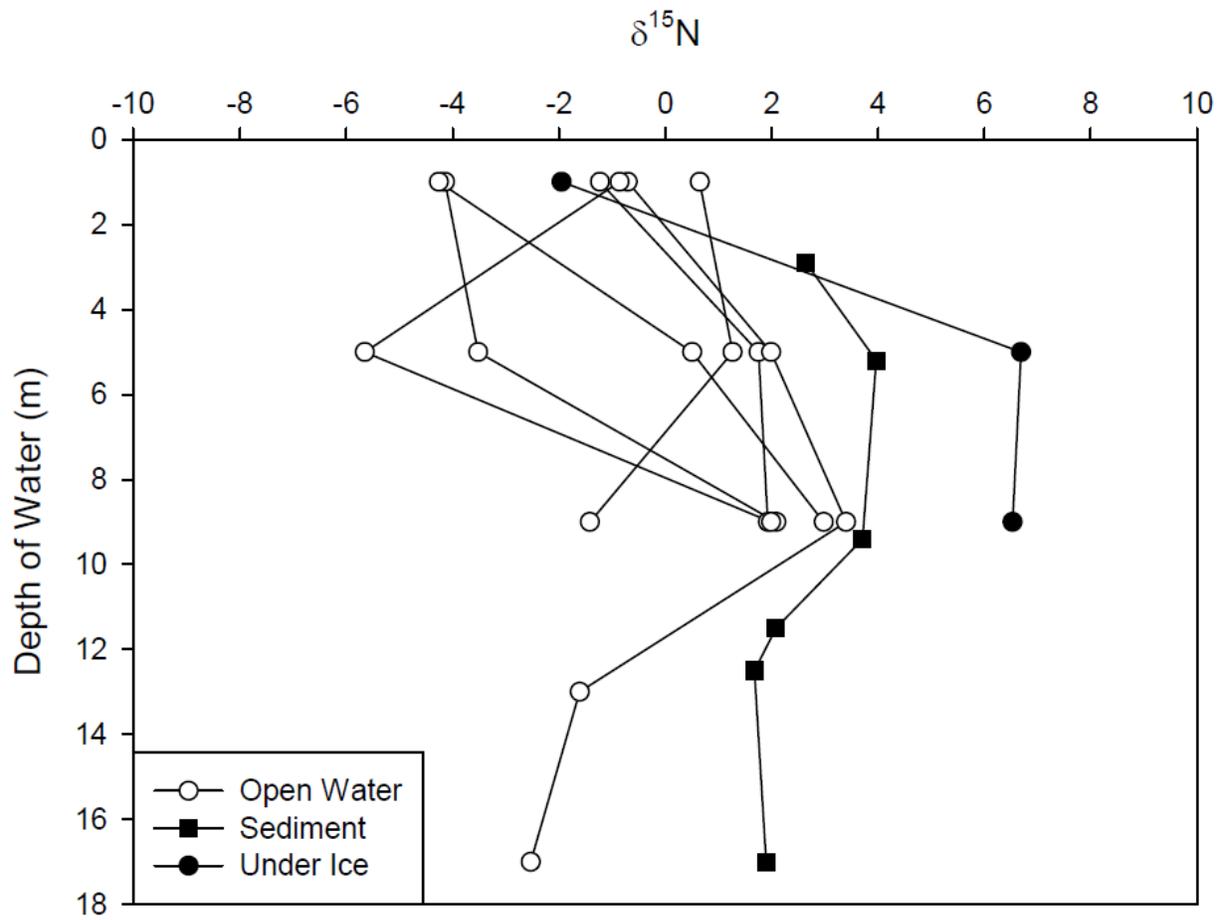


Figure 5:9 Depth of water profile of  $\delta^{15}\text{N}$ -POM from Lake 442 during the open water season (June to October, open circles), under ice (March, closed circles) and  $\delta^{15}\text{N}$  of surficial sediments (filled in boxes) in Lake 442 in 2010

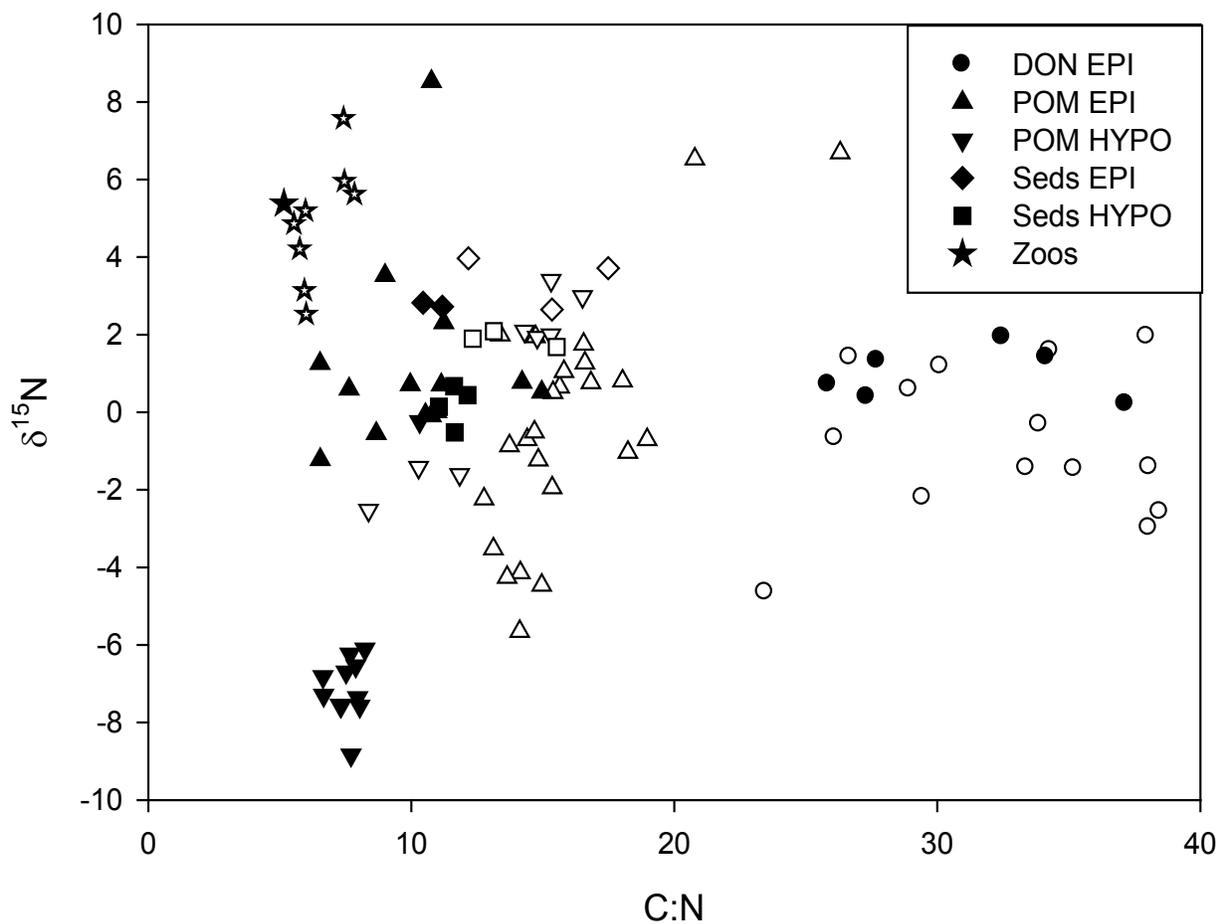


Figure 5:10 Cross plot of  $\delta^{15}\text{N}$  and molar C:N of Lake 227 (filled symbols) and lake survey samples (open symbols). Lakes included in the lake survey are 442, 375, 658, 373, 304, 626, 979, 303, 239, and 114. C:N of POM is significantly different ( $p < 0.05$ ) in Lake 227 compared to other ELA lakes

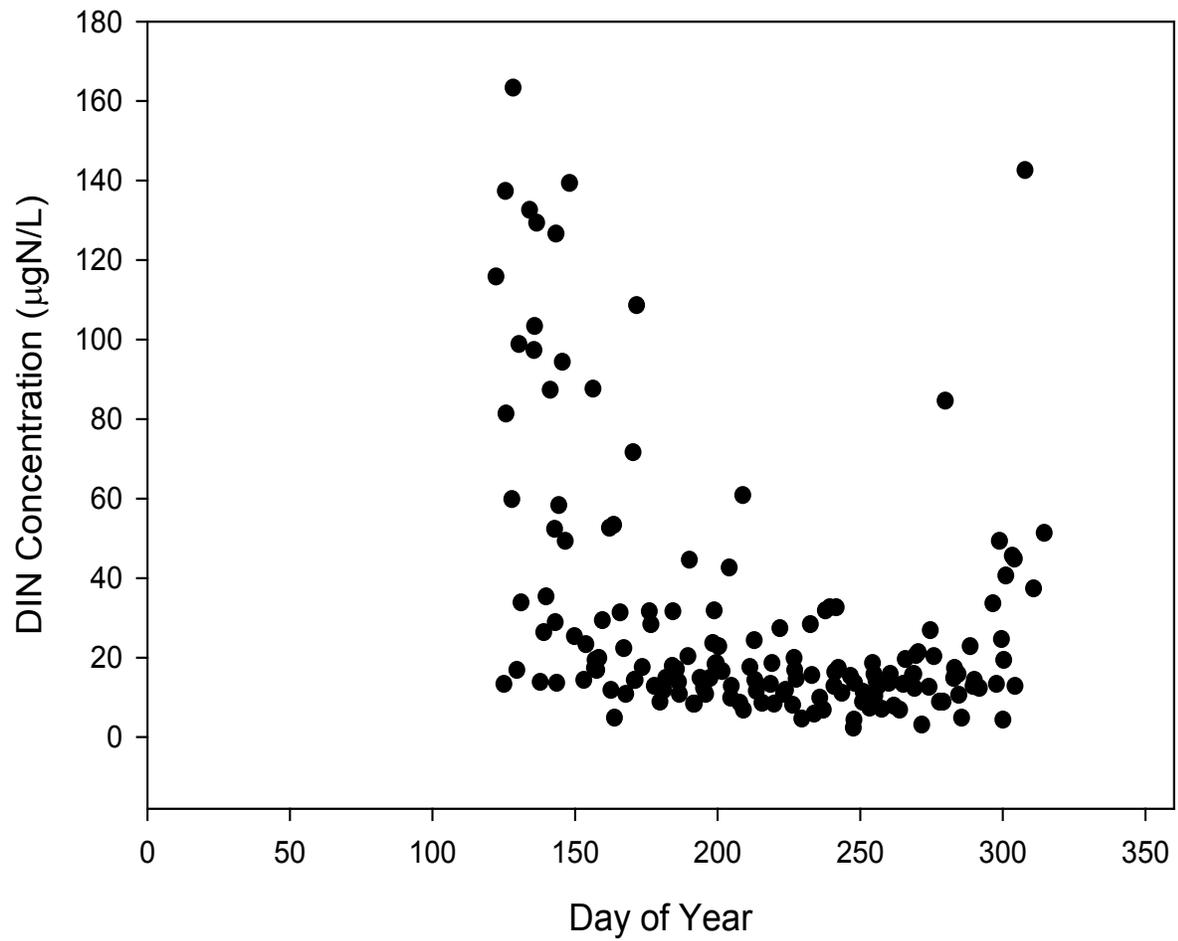


Figure 5:11 Epilimnion DIN ( $\text{NO}_3^- + \text{NH}_4^+$ ) concentration in Lake 442 from 1994 to 2008 demonstrating higher nitrogen concentrations in spring following turnover and spring melt

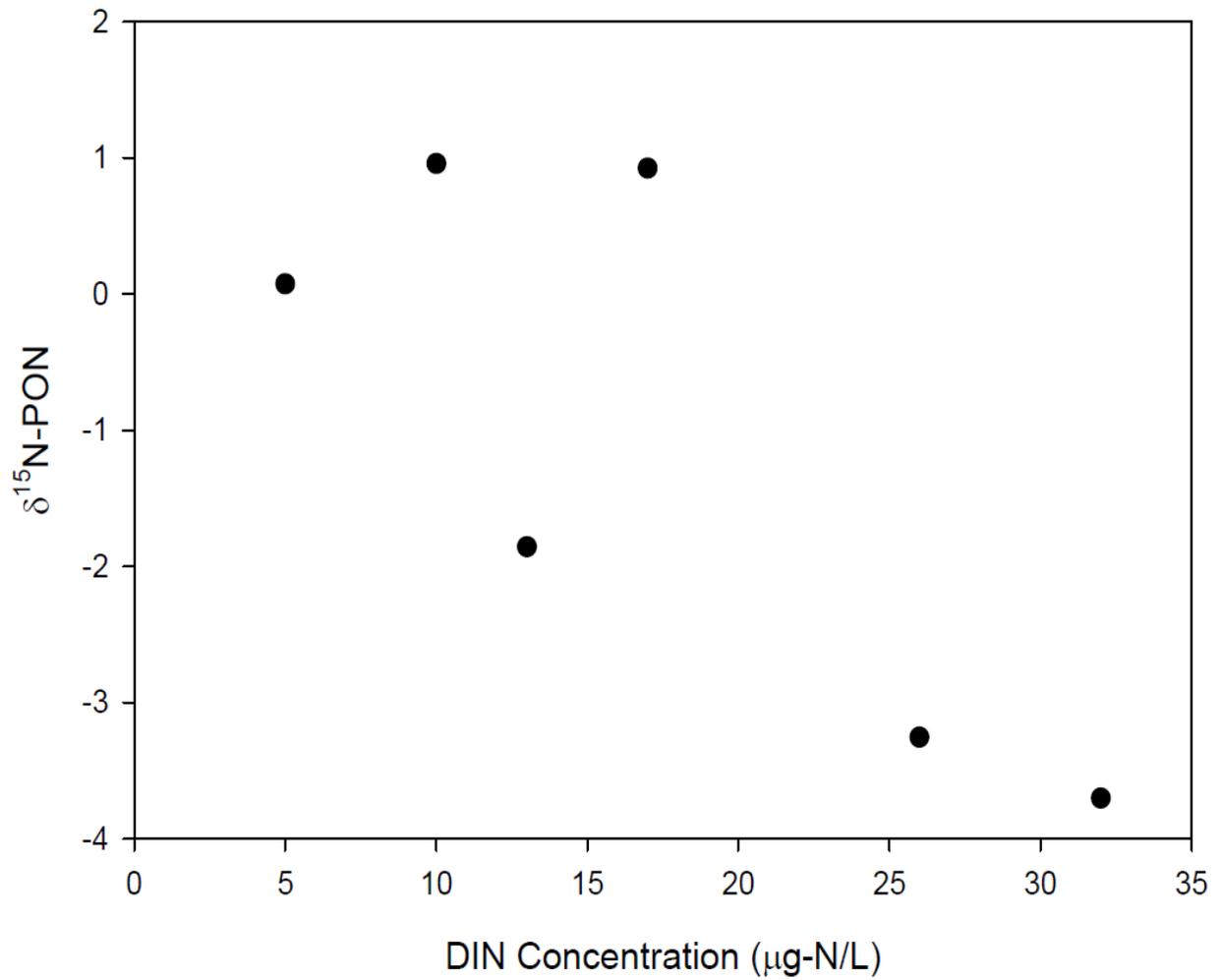


Figure 5:12 Lake 442 DIN ( $\text{NO}_3^- + \text{NH}_4^+$ ) concentration plotted with  $\delta^{15}\text{N-PON}$  from the epilimnion of Lake 442 in the open water season of 2010, a general trend of more negative  $\delta^{15}\text{N-PON}$  values at higher DIN concentration is seen

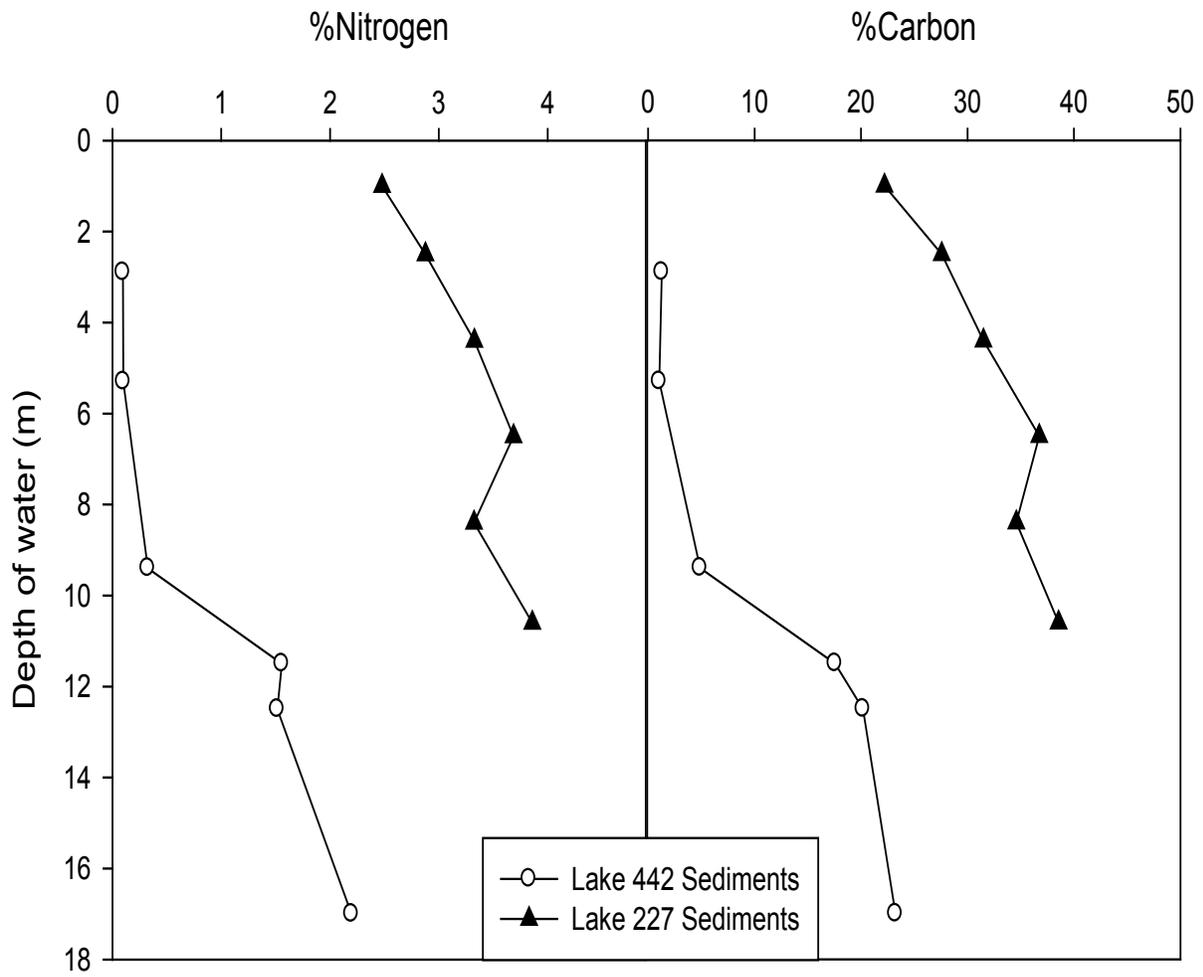


Figure 5:13 Lakes 442 and 227 surficial sediment percent nitrogen and carbon from July 2010, Lake 227 sediments have a much higher percent composition of nitrogen and carbon in both the oxic and anoxic portions of the lakes. Anoxia is present below 4m in Lake 227 and below 10m in Lake 442

## 6 Chapter 6: Conclusions

### 6.1 Nitrogen Isotopes in Precipitation

The historical look at nitrogen deposition at the Experimental Lakes Area (ELA) provides evidence of increased ammonium deposition, approximately doubling in the past 40 years. The large increase in ammonium deposition occurred over a period of intensification in farming practices in Manitoba, located west of the ELA, with a 16% increase in heads of cattle, a 700% increase in swine and a 640% increase in fertilizer application over the same 40 year period. Increased agriculture is likely responsible for higher levels of atmospheric nitrogen, which can be transported long distances to be deposited in pristine areas such as the ELA. Recent research has shown the ELA is no longer among the North American sites with the lowest inorganic nitrogen deposition (Fernandez et al, 2005).

Sampling of precipitation at the meteorological site at the ELA was conducted from June to October of 2011 to characterize the nitrogen isotopic values of deposition in this region of Canada, an understudied component of nitrogen sources in this country. The  $\delta^{15}\text{N-NH}_4^+$  measurements are the first in Canada while the  $\delta^{15}\text{N-NO}_3^-$  from this work add to a very limited body of literature values for Canada. Improvements on this research would include sampling of October to June precipitation, an entire year record, to fully characterize the  $\delta^{15}\text{N}$  of precipitation inputs.

The range observed in the results was large a 19‰ range in ammonium isotopes, an almost 5‰ range in nitrate and an 11‰ range in total nitrogen. The highest  $\delta^{15}\text{N}$  values occurred in July for nitrate and ammonium. Total nitrogen, composed primarily of inorganic nitrogen, followed the  $\delta^{15}\text{N}$  increases of nitrate and ammonium peaking in late July. The  $\delta^{15}\text{N}$  of nitrate was not related to the nitrate concentration. The  $\delta^{15}\text{N}$  of total nitrogen was higher than expected and the reason for this is not known. The influence of high  $\delta^{15}\text{N}$  suspended nitrogen is suspected to be the cause and requires further investigation. Mass weighted values for  $\delta^{15}\text{N}$  of ammonium, nitrate and total nitrogen were -6.2‰, -3.1‰ and 3.3‰ respectively. The mass weighted value of  $\delta^{18}\text{O-NO}_3^-$  was 66.2‰.

The range in  $\delta^{15}\text{N}$  of precipitation nitrogen necessitates that studies wishing to characterize precipitation isotopes as a nitrogen source require a temporally comprehensive sampling regime, measuring  $\delta^{15}\text{N}$  from all seasons to truly characterize the  $\delta^{15}\text{N}$  of precipitation.

### 6.2 Sediment Core Carbon and Nitrogen Isotopes

The use of sediment cores to interpret past lake productivity and nitrogen utilization is well documented in the literature. This study was undertaken to attempt to test the standard interpretations of changes in

sediment carbon and nitrogen isotopes through the comparison of a sediment core to known experimental changes in Lake 227.

Initial work was done to confirm that carbon and nitrogen isotopic composition of epilimnetic algae was being preserved in the sediment. This was completed through open water sampling of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  POM and a onetime sampling of surficial samples from Lake 227 in July of 2010. The results demonstrate that the epilimnetic POM was similar to the isotopic composition of the hypolimnion surficial sediments with mass weighted  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of epilimnetic POM within 0.3‰ of surficial sediment  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . Sediments in the epilimnion showed higher  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  than the epilimnetic POM, likely because of higher degradation of organic matter in the oxic epilimnion.

Analysis of the Lake 227 sediment core showed large changes in the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  over the past 40 years of experimental additions in Lake 227. Additions of  $\text{NaNO}_3$  and phosphorus increased the level of primary productivity as well as alkalinity in Lake 227. These two factors led to increased  $\delta^{13}\text{C}$  of particulate organic matter which was preserved in the sediment. Following the cessation of  $\text{NaNO}_3$  loading alkalinity in Lake 227 has decreased by 50%, which has resulted in lower pH levels and changing carbon speciation. This led to a decline in  $\delta^{13}\text{C}$  of sediment post-1990 because of different availability of carbon sources and source carbon  $\delta^{13}\text{C}$ . Under the traditional interpretation of sediment isotopes carbon isotopes would be interpreted as a decline in primary production that is not substantiated by in lake measures of primary productivity over that time period. The nitrogen isotopes in the sediment core appear to reflect changes in nitrogen loading and the source of algae nitrogen accurately with some diagenesis occurring in the top 1.5cm of the core. The increase in  $\delta^{15}\text{N}$  of the sediments coincided with the addition of large amounts of nitrogen fertilizer, while a decrease in  $\delta^{15}\text{N}$  occurred after the reduction in fertilizer and the increased prominence of nitrogen fixing cyanobacteria in the algal community.

The relationships between increases in  $\delta^{15}\text{N}$  and higher levels of primary production, as well as increased nitrogen fixation causing lower  $\delta^{15}\text{N}$  in the sediments appear reliable in Lake 227. The  $\delta^{13}\text{C}$  sediment record of Lake 227 productivity is clouded by the changes in alkalinity brought about by the different  $\text{NaNO}_3$  dosing levels. The discrepancy is most evident in recent times where the sediment  $\delta^{13}\text{C}$  deviates away from high  $\delta^{13}\text{C}$  values that are supposed to be indicative of high primary productivity. It should be noted that changes in nitrogen loading to Lake 227 happened rapidly, and the sediments are varved so the  $\delta^{15}\text{N}$  horizons between different loading rates are very sharp. In lakes with more gradual changes in nutrient loading and trophic states or non-varved sediments, the shift in the  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  of the sediments will not be as clear.

### 6.3 Nitrogen Isotope Mass Balance

A whole lake nitrogen isotope mass balance was completed for Lake 227 for the years 1999-2007. The range in the  $\delta^{15}\text{N}$  of nitrogen inputs is narrow at the ELA. Precipitation has a mass weighted  $\delta^{15}\text{N}$  of 3.3‰, runoff has a  $\delta^{15}\text{N}$  of -1.5‰ and nitrogen added from atmospheric fixation has a  $\delta^{15}\text{N}$  of 0‰. Nitrogen fixation inputs nitrogen at a  $\delta^{15}\text{N}$  between precipitation and runoff and does not result in a significant difference in  $\delta^{15}\text{N}$  of epilimnetic POM between Lake 227 and other ELA lakes. The range in  $\delta^{15}\text{N}$  of epilimnetic POM extends more negative in other lakes than Lake 227 because of a combination of no nitrogen fixation but more likely a combination of greater fractionation during assimilation and different nitrogen sources to the lakes due to unique watershed area to lake surface area ratios, catchment characteristics and lake order. Eutrophication in Lake 227 has caused an increase in biomass and sedimentation of organic matter which has led to greater build up of ammonium in the hypolimnion. The build up of ammonium in the anoxic hypolimnion of Lake 227 results in very negative  $\delta^{15}\text{N}$  POM because of a large isotopic fractionation (7‰) during nitrogen assimilation. In Lake 442 there is a smaller isotopic fractionation during nitrogen uptake in the anoxic hypolimnion than in Lake 227, likely due to the lower availability of ammonium and greater depletion of the existing ammonium pool. The larger build up of ammonium in L227 could also lead to more nitrification and denitrification under ice in Lake 227 following fall turnover compared to Lake 442, however more work must be done to quantify how significant a portion of the nitrogen budget this may account for.

The results from the mass and isotope budgets indicate that the mass budget does not accurately quantify inputs and losses of nitrogen for Lake 227. There are a number of unknowns in the isotope mass budget including ; 1) early spring terrestrial nitrogen input for concentration and isotopic composition not quantified; 2) outflow concentration and isotopic concentration outside of June to October also not quantified; 3) Precipitation isotopic composition between October and June is not known; 4) the assumed relationship between heterocyst counts and the rate of nitrogen fixation has not been retested in 18 years a period during which in-lake changes of contributing factors for nitrogen fixation may have occurred and; 5) the variation in isotopic fractionation during nitrogen fixation is not known in Lake 227. Completing some of the missing portions of the mass budget outline above would serve to increase the usefulness of the mass and isotope budgets to understand nitrogen processes in Lake 227.

The comparison of Lake 227 to other lakes at the ELA could be improved by a more widespread sampling of other lakes at multiple times throughout the course of the year to better understand the range and variation of nitrogen isotopes in non manipulated lakes. The widespread temporal study would increase the understanding of how early spring summer nitrogen availability influences nitrogen isotopes in particulate matter at the ELA, if the isotopic composition of the sources of nitrogen at this time can be

quantified. A more in depth analysis in to how the differences in lake morphometry and catchment characteristics influence  $\delta^{15}\text{N}$  in lakes at the ELA would benefit from a wider sample size as well.

## References

- Aber, J.D., Nadelhoffer, K.J., Steudler, P., Melillo, J.M. (1989). Nitrogen saturation in northern forest ecosystems. *Bioscience*, 39(6), 378-386
- Asman, W.A.H., Sutton, M.A., Schjorring, J.K. (1998). Ammonia: emission, atmospheric transport and deposition. *New Phyto.*, 139, 27-48
- Barnes, R.T., Raymond, P.A., Casiotti, K.L. (2008). Dual isotope analyses indicate efficient processing of atmospheric nitrate by forested watersheds in the North-eastern U.S.. *Biogeochemistry*, 90, 15-27
- Bergstrom, A.K., Jansson, M. (2006). Atmospheric nitrogen deposition has caused nitrogen enrichment and eutrophication of lakes in the northern hemisphere. *Global Change Biology*, 12, 635-643
- Brooks, P.D., Stark, J.M., McInter, B.B., Preston, T. (1989). Diffusion method to prepare soil extracts for automated nitrogen 15 analysis. *Soil Sci. Am. J.*, 53, 1707
- Brunskill, G.J., Schindler, D.W. (1971). Geography and bathymetry of selected lake basins, Experimental Lakes Area, Northwestern Ontario. *J. Fish. Res. Bd. Canada*, 28, 139-155
- Buda, A.R., DeWalle, D.R. (2009). Using atmospheric chemistry and storm track information to explain the variation of nitrate stable isotopes in precipitation at a site in central Pennsylvania, USA. *Atmospheric Environment*, 43, 4453-4464
- Burns, D.A., Kendall, C. (2002). Analysis of  $^{15}\text{N}$  and  $^{18}\text{O}$  to differentiate  $\text{NO}_3$  sources in runoff at two watersheds in the Catskill Mountains of New York. *Water Resources Research*, 38(5), 1051-1061
- Chan, Y.K., Campbell, N.E.R. (1980). Denitrification in Lake 227 during summer stratification. *Can. J. Fish. Aquat. Sci.*, 37, 506-512
- Cleveland, C.C., Townsend, A.R., Schimel, D.S., Fisher, H., Howarth, R.W., Hedin, L.O., Perakis, S.S., Latty, E.F., Von Fischer, J.C., Elseroad, A., Wasson, M.F. (1999). Global patterns of terrestrial biological nitrogen ( $\text{N}_2$ ) fixation in natural ecosystems. *Global Biogeochemical Cycles*, 13(2), 623-645
- Crusius, J., Anderson, R.F. (1995). Evaluating the mobility of  $^{137}\text{Cs}$ ,  $^{239+240}\text{Pu}$  and  $^{210}\text{Pb}$  from their distributions in laminated lake sediments. *Journal of Paleolimnology*, 13, 119-141
- Delwiche, C.C., Steyn, P.L. (1970). Nitrogen isotope fractionation in soils and microbial reactions. *Environmental Science & Technology*, 4(11), 929-935
- Elliot, E.M., Kendall, C., Wankel, S.D., Burns, D.A., Boyer, E.W., Harlin, K., Bain, D.J., Butler, T.J. (2007). Nitrogen isotopes as indicators of  $\text{NO}_x$  source contributions to atmospheric nitrate deposition across the Midwestern and Northeastern United States. *Environ. Sci. Technol.*, 41, 7661-7667
- Elser, J.J. (2011). A world awash with nitrogen. *Science*, 334, 1504-1505
- Findlay, D.L., Hecky, R.E., Hendzel, L.L., Stainton, M.P., Regehr, G.W. (1994). Relationship between  $\text{N}_2$ -fixation and heterocyst abundance and its relevance to the nitrogen budget of Lake 227. *Can. J. Fish. Aquat. Sci.*, 51, 2254-2266

- Finlay, J.C., Sterner, R.W., Kumar, S. (2007). Isotopic evidence for in-lake production of accumulated nitrate in Lake Superior. *Ecological Applications*, 17(8), 2323-2332
- Fogel, M.L., Cifuentes, L.A. (1993). Isotope fractionation during primary production. In *Organic Geochemistry*, ed M.H. Engel and S.A. Macko, 73-98, New York: Plenum
- Freyer, H.D. (1978). Seasonal trends of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  nitrogen isotope composition in rain collected at Julich, Germany. *Tellus*, 30, 83-92
- Freyer, H.D., Kley, D., Volz-Thomas, A., Kobel, K. (1993) On the interaction of isotopic exchange processes with photochemical reactions in atmospheric oxides of Nitrogen. *Geophysical Research*, 98 (8), 14791-14796
- Galman, V., Rydberg, J., Sjostedt de-Luna, S., Bindler, R., Renberg, I. (2008). Carbon and nitrogen loss rates during aging of lake sediment: Changes over 27 years studied in varved sediment. *Limnol. Oceanogr.*, 53(3), 1076-1082
- Galman, V., Rydberg, J., Bigler, C. (2009). Decadal diagenetic effects on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  studied in varved lake sediment. *Limnol. Oceanogr.*, 54(3), 917-924
- Hassan, K.M., Swinehart, J.B., Spalding, R.F. (1997). Evidence for Holocene environmental change from C/N ratios, and  $^{13}\text{C}$  and  $^{15}\text{N}$  values in Swan Lake sediments, western Sand Hills, Nebraska. *Journal of Paleolimnology*, 18, 121-130
- Heaton, T.H.E. (1987).  $^{15}\text{N}/^{14}\text{N}$  ratios of nitrate and ammonium in rain at Pretoria, South Africa. *Atmospheric Environment*, 21(4), 843-852
- Heaton, T.H.E. (1990).  $^{15}\text{N}/^{14}\text{N}$  ratios of  $\text{NO}_x$  from vehicle engines and coal-fired power stations. *Tellus*, 42B, 304-307
- Hecky, R.E., Rosenberg, D.M., Campbell, P. (1994). The 25<sup>th</sup> anniversary of the Experimental Lakes Area and the history of Lake 227. *Can. J. Fish. Aquat. Sci.*, 51, 2243-2246
- Hendzel, L.L., Hecky, R.E., Findlay, D.L. (1994). Recent changes of  $\text{N}_2$ -fixation in Lake 227 in response to reduction of the N:P loading ratio. *Can. J. Fish. Aquat. Sci.*, 51, 2247-2253
- Hoering, T.C., Ford, H.T. (1960). The isotope effect in the fixation of nitrogen by *Azotobacter*. *Journal of the American Chemistry Society*, 82(2), 376-378
- Hollander, D.J., Smith, M.A. (2001). Microbially mediated carbon cycling as a control on the  $^{13}\text{C}$  of sedimentary carbon in eutrophic Lake Mendota (USA): New models for interpreting isotopic excursions in the sedimentary record. *Geochimica et Cosmochimica Acta*, 65(23), 4321-4337
- Holmes, R.M., McClelland, J.W., Sigman, D.M., Fry, B., Peterson, B.J. (1998). Measuring  $^{15}\text{N}\text{-NH}_4^+$  in marine, estuarine and fresh waters: an adaptation of the ammonia diffusion method for samples with low ammonium concentration. *Mar. Chem.*, 60, 235-243
- Holtgrieve, G.W., Schindler, D.E., Hobbs, W.O., Leavitt, P.R., Ward, E.J., Bunting, L., Chen, G., Finney, B.P., Gregory-Eaves, I., Holmgren, S., Liscac, M.J., Lisi, P.J., Nydick, K., Rogers, L.A., Saros, J.E.,

- Selbie, D.T., Shapley, M.D., Walsh, P.B., Wolfe, A.P. (2011). Nitrogen deposition to remote watersheds of the northern hemisphere. *Science*, 334, 1545-1548
- Honey, J. (2011). Manitoba cattle and beef industry 2010. Department of Agribusiness and Agricultural Economics, University of Manitoba
- Honey, J. (2011). Manitoba pig and pork industry 2010. Department of Agribusiness and Agricultural Economics, University of Manitoba
- Honey, J. (2010). Crops in Manitoba 2009-2010. Department of Agribusiness and Agricultural Economics, University of Manitoba
- Howard, J.B., Rees, D.C. (1996). Structural basis of biological nitrogen fixation. *Chem. Rev.*, 96, 2965-2982
- Hubner, H. (1986). Isotope effects of nitrogen in the soil and biosphere. *In Handbook of environmental Isotope Geochemistry*, vol. 2B. Amsterdam. Elsevier, 361-425
- Huttunen, P., Merilainen, J. (1978) New freezing device providing large unmixed sediment samples from lakes. *Ann. Bot. Fennici IS*, 128-130
- Jeremiason, J.D., Eisenreich, S.J., Paterson, M.J. (1999). Accumulation and recycling of PCBs and PAHs in artificially eutrophied Lake 227. *Can. J. Fish. Aquat. Sci.*, 56, 650-660
- Kendall, C. (1998). Tracing nitrogen sources and cycling in catchments. *In Isotope tracers in catchment hydrology*, ed. C. Kendall and J.J. McDonnell, 519–576. Amsterdam: Elsevier
- Kidd, K.A., Paterson, M.J., Hesslein, R.H., Muir, D.C.G., Hecky, R.E. (1999). Effects of northern pike (*Esox lucius*) additions on pollutant accumulation and food web structure, as determined by  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , in a eutrophic and an oligotrophic lake. *Can. J. Fish. Aquat. Sci.*, 56, 2193-2202
- Kipphut, G.W. (1978). An investigation of sedimentary processes in lakes. Ph.D. Dissertation, Columbia University. pg 102
- Knapp, A.N., Hastings, M.G., Sigman, D.M., Lipschultz, F., Galloway, J.N. (2010), The flux and isotopic composition of reduced and total nitrogen in Bermuda Rain. *Marine Chemistry*, 120, 83-89
- Korel, M. (2002). Canadian fertilizer consumption, shipments and trade 2001/2002. Agriculture and Agri-Food Canada
- Lamontagne, S., Schiff, S. (1999). The response of a heterogeneous upland boreal shield catchment to a short term  $\text{NO}_3^-$  addition. *Ecosystems*, 2(5), 460-473
- Lamoureux, S. (2001) Varve Chronology Techniques. In “*Tracking environmental change using lake Sediments Vol 1 Basin Analysis, Coring, and Chronological Techniques*” Eds W.M. Last, and J.P. Smol, pp 247-260. Kluwer Academic Publishers, Boston
- Last, W.M., Smol, J.P. (2001). Tracking environmental change using lake sediments Volume 1: Basin analysis, coring and chronological techniques. Kluwer Academic Publishers, Boston

- Leavitt, P.R., Findlay, D.L. (1994). Comparison of fossil pigments with 20 years of phytoplankton data from eutrophic Lake 227, Experimental Lakes Area, Ontario. *Can. J. Fish. Aquat. Sci.*, 51, 2286-2299
- Leavitt, P.R., Hann, B.J., Smol, J.P., Zeeb, B.A., Christi, C.E., Wolfe, B., Kling, H.J. (1994). Paleolimnological analysis of whole lake experiments: an overview of results from Experimental lakes Area Lake 227. *Can. J. Fish. Aquat. Sci.*, 51, 2322-2332
- Lee, C., Hristov, A.N., Cassidy T., Heyler, K. (2011). Nitrogen isotope fractionation and origin of ammonia nitrogen volatilized from cattle manure in simulated storage. *Atmosphere*, 2, 256-270
- Lehmann, M.F., Bernasconi, S.M., Barbieri, A., McKenzie, J.A. (2002). Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early diagenesis. *Geochimica et Cosmochimica Acta*, 66(20), 3573-3584
- Lu, Y., Meyers, P.A., Johengen, T.H., Eadie, B.J., Robbins, J.A., Han, H. (2010).  $^{15}\text{N}$  values in Lake Erie sediments as indicators of nitrogen biogeochemical dynamics during cultural eutrophication. *Chemical Geology*, 273, 1-7
- Mariotti, A., Germon, J.C., Huber, P., Kaiser, P., Letolle, R., Tardieux, A., Tardieux, P. (1981). Experimental determination of nitrogen kinetic isotope fractionation: Some principles; illustration for the denitrification and nitrification process. *Plant and Soil*, 62, 413-430
- McCullough, G.K., Campbell, P. (1993). Lake variation and climate change study: ELA Lakes, 1986-1990. II. Watershed geography and lake morphology. *Canadian Technical Report of Fisheries and Aquatic Sciences*, 52(1898)
- McIlvin, M.R., Altabet, M.A. (2005). Chemical conversion of nitrate and nitrite to nitrous oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater. *Anal. Chem.*, 77, 5589-5595
- Meyers, P.A., Teranes, J.L. (2001) Sediment organic matter. In “*Tracking environmental change using lake Sediments Vol 2 Physical and Geochemical Methods*” Eds W.M. Last, and J.P. Smol, pp 247-260. Kluwer Academic Publishers, Boston
- Minagawa, M., Wada, E. (1986). Nitrogen isotope ratios of Red Tide organisms in the East China Sea: A characterization of biological nitrogen fixation. *Marine Chemistry*, 19, 245-259
- Paerl, H.W., Fulton, R.S., Moisander, P.H. (2001). Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *The Scientific World*, 1, 76-113
- Parker, B.R., Schindler, D.W., Beaty, K.G., Stainton, M.P., Kasian, S.E.M. (2009). Long-term changes in climate, streamflow, and nutrient budgets for first-order catchments at the Experimental Lakes Area (Ontario, Canada). *Can. J. Aquat. Sci.*, 66, 1848-1863
- Paterson, A.M., Cumming, B.F., Smol, J.P., Blais, J.M., France, R.L. (1998). Assessment of the effects of logging, forest fires and drought on lakes in north western Ontario: A 30 year paleolimnological perspective. *Can. J. For. Res.*, 28, 1546-1556

- Paterson, M.J., Schindler, D.W., Hecky, R.E., Findlay, D.L., Rondeau, K.J. (2011). Comment: Lake 227 shows clearly that controlling inputs of nitrogen will reduce or prevent eutrophication of lakes. *Limnol. Oceanogr.*, 56(4), 1545-1547
- Proe, M.F., Griffiths, J.H., McKay, H.M. (2001). Effect of whole-tree harvesting on microclimate during establishment of second rotation forestry. *Agricultural and forest Meteorology*, 110, 141-154
- Rudd, J.W., Furutani, A., Flett, R.J., Hamilton, R.D. (1976). Factors controlling methane oxidation in shield lakes: The role of nitrogen fixation and oxygen concentration. *Limnology and Oceanography*, 21(3), 357-364
- Schindler, D.W. (1974). Eutrophication and recovery in experimental lakes: Implications for lake management. *Science*, 184, 897-899
- Schindler, D.W. (1977). Evolution of phosphorus limitation in lakes. *Science*, 195, 260-262
- Schindler, D.W. (1980). Evolution of the experimental lakes project. *Can. J. Fish. Aquat. Sci.*, 37, 313-319
- Schindler, D.W., Armstrong, F.A.J., Holmgren, S.K., Brunskill, G.J. (1971). Eutrophication of Lake 227, Experimental Lakes Area (ELA), northwestern Ontario, by addition of phosphate and nitrate. *J. Fish. Res. Board Can.*, 28, 1763-1782
- Schindler, D.W., Fee, E.J. (1974). Experimental lakes area: Whole-lake experiments in eutrophication. *Journal of the Fisheries Research Board of Canada*, 31(5), 937-953
- Schindler, D.W., Hecky, R.E., Findlay, D.L., Stainton, M.P., Parker, B.R., Paterson, M.J., Beaty, K.G., Lyng, M., Kasian, S.E.M. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of 37-year whole-ecosystem experiment. *PNAS*, 105(32), 11254-11258
- Schindler, D.W., Hesslein, R.H., Turner, M.A. (1987). Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. *Can. J. Fish. Aquat. Sci.*, 44(Supp 1), 26-33
- Schindler, D.W., Newbury, R.W., Beaty, K.G., Prokopowich, J., Ruscynski, T., Dalton, J.A. (1980). Effects of a windstorm and forest fire on chemical losses from forested watersheds and on the quality of receiving streams. *Can. J. Fish. Aquat. Sci.*, 37, 328-334
- Scott, J.T., McCarthy, M.J. (2010). Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. *Limnol. Oceanogr.*, 53(3), 1265-1270
- Seitzinger, S., Harrison, J.A., Bohlke, J.K., bouwman, A.F., Lowrance, R., Peterson, B., Tobias, C., Van Drecht, G. (2006). Denitrification across landscapes and waterscapes: A synthesis. *Ecological Applications*, 16, 2064-2090
- Sellers, T.J., Parker, B.R., Schindler, D.W., Tonn, W.M. (1998). Pelagic distribution of lake trout (*Salvelinus namaycush*) in small Canadian Shield lakes with respect to temperature, dissolved oxygen, and light. *Can. J. Fish. Aquat. Sci.*, 55, 170-179

- Shapley, M.D., Walsh, P.B., Wolfe, A.P. (2011). Nitrogen deposition to remote watersheds of the northern hemisphere. *Science*, 334, 1545-1548
- Sommer, S.G., Olesen, J.E., Christensen, B.T. (1991). Effects of temperature, wind speed and air humidity on ammonia volatilization from surface applied cattle slurry. *The Journal of Agricultural Science*, 117, 91-100
- Spoelstra, J. (2004). The isotopic composition of a nitrate pulse resulting from forest harvest. In *Nitrate sources and cycling at the Turkey Lakes Watershed: A Stable isotope approach*. Department of Earth and Environmental Science, University of Waterloo, Chapt. 7, 107-136
- Spoelstra, J., Schiff, S.L., Elgood, R.J., Semkin, R.G., Jeffries, D.J. (2001). Tracing the sources of exported nitrate in the Turkey Lakes Watershed Using  $^{15}\text{N}/^{14}\text{N}$  and  $^{18}\text{O}/^{16}\text{O}$  isotopic ratios. *Ecosystems*, 4, 536-544
- Spoelstra, J., Murry, M., Elgood, R.J. (2006). A simplified diffusion method for delta  $^{15}\text{N}$  analysis of  $\text{NH}_4^+$ . In *Environmental geochemistry lake technical procedure 20*. Department of Earth and Environmental Science, University of Waterloo, pg 10
- Stainton, M.P., Capel, M.J., Armstrong, F.A.J. (1977). The chemical analysis of freshwater. 2<sup>nd</sup> ed. *Can. Fish. Mar. Serv. Misc. Spec. Publ.* 25:180
- Stewart, I., Webb, P.M., Schluter, P.J., Shaw, G.R. (2006). Recreational and occupational field exposure to freshwater cyanobacteria- a review of anecdotal and case reports, epidemiological studies and the challenges for epidemiologic assessment. *Environmental Health: A global Access Science Source*, 5(6)
- Storch, T.A., Saunders, G.W., Ostrofsky, M.L. (1990). Diel nitrogen fixation by cyanobacterial surface blooms in sanctuary lake, Pennsylvania. *Applied and Environmental microbiology*, 56(2), 466-471
- Talbot, M. (2001). Nitrogen isotopes in palaeolimnology. In "Tracking environmental change using lake Sediments Vol 2 Physical and Geochemical Methods" Eds W.M. Last, and J.P. Smol, pp 401-439. Kluwer Academic Publishers, Boston
- Teranes, J.L., Bernasconi, S.M. (2000). The record of nitrate utilization and productivity limitation provided by the  $^{15}\text{N}$  values in lake organic matter- A study of sediment trap and core sediments from Baldeggersee, Switzerland. *Limnology and Oceanography*, 45(4), 801-813
- Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G. (1997). Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, 7(3), 737-750
- Vrede, T., Ballantyne, A., Mille-Lindblom, C., Algersten, G., Gudasz, C., Lindahl, S., Brunberg, A.K. (2009). Effects of N:P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. *Freshwater Biology*, 54, 331-344
- Wallace, J.M., Hobbs, P.V. (2006). Atmospheric chemistry. In *Atmospheric Science: an introductory survey*. Ed Jennifer Hele, pp 162-164 Academic Press, Burlington, MA

Waser, N.A., Yin, K., Yu, Z., Tada, K., Harrison, P.J., Turpin, D.H., Calvert, S.E. (1998). Nitrogen isotope fractionation during nitrate, ammonium and urea uptake by marine diatoms and coccolithophores under various conditions of N availability. *Mar. Ecol. Prog. Ser.*, 169, 29-41

Waser, N.A.D., Harrison, P.J., Nielson, B., Calvert, S.E. (1998). Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by a marine diatom. *Limnol. Oceanogr.*, 43(2), 215-224

Watmough, S.A., Aherne, J., Alewell, C., Arp, P., Bailey, S., Clair, T., Dillon, P., Duchesne, L., Eimers, C., Fernandez, I., Forester, N., Larssen, T., Miller, E., Mitchell, M., Page, S. (2005). Sulphate, nitrogen and base cation budgets at 21 forested catchments in Canada, The United States, and Europe. *Environmental Monitoring and Assessment*, 109, 1-36

Williard, K.W.J., DeWalle, D.R., Edwards, P.J., Sharpe, W.E. (2001).  $^{18}\text{O}$  isotopic separation of stream nitrate sources in mid-Appalachian forested watersheds. *Journal of Hydrology*, 252, 174-188

Wolfe, B., Kling, H.J., Brunskill, G.J., Wilkinson, P. (1994). Multiple dating of a freeze core from Lake 227, an experimentally fertilized lake with varved sediments. *Can. J. Fish. Aquat. Sci.*, 51, 2274-2285

Xiao H.Y., Liu, C.Q. (2002). Sources of nitrogen and sulphur in wet deposition at Guiyang, Southwest China. *Atmospheric Environment*, 36, 5121-5139

Zhang, L., Altabet, M.A., Wu, T., Hadas, O. (2007). Sensitive measurement of  $\text{NH}_4^+ ^{15}\text{N}/^{14}\text{N}$  ( $^{15}\text{NH}_4^+$ ) at natural abundance levels in fresh and saltwaters. *Anal. Chem.*, 79, 5297-5303