NITROUS OXIDE PRODUCTION IN THE GRAND RIVER, ONTARIO, CANADA: NEW INSIGHTS FROM STABLE ISOTOPE ANALYSIS OF DISSOLVED NITROUS OXIDE

by

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AUTHOR’S DECLARATION

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

I understand that my thesis may be made electronically available to the public.
Nitrous oxide ($N_2O$) is a powerful greenhouse gas, and its atmospheric concentration is increasing dramatically. $N_2O$ is produced through the microbially-mediated processes of nitrification and denitrification. Since these processes have difference substrates and isotopic enrichment factors, stable isotope analysis ($\delta^{15}N$ and $\delta^{18}O$) of $N_2O$ can be used to study the production of this important greenhouse gas.

Although production in rivers accounts for a significant portion of the global $N_2O$ budget, the isotopic composition of $N_2O$ from this source is poorly characterized. Most of the previous work using stable isotopes of $N_2O$ has been conducted in terrestrial or oceanic environments, and only one published study has measured $\delta^{15}N$ and $\delta^{18}O$ of $N_2O$ produced in a riverine environment. The purpose of this research project was to use stable isotope analysis to characterize the processes responsible for $N_2O$ production in the Grand River, Ontario, Canada, and to determine the spatial and temporal variability of the isotopic composition of the $N_2O$ flux.

To meet the study objectives, an offline “purge and trap” method was developed to collect and purify dissolved $N_2O$ for stable isotope analysis. Using this method, $\delta^{15}N$ and $\delta^{18}O$ analysis of dissolved $N_2O$ is possible for samples with concentrations as low as 6 nmol $N_2O/L$.

Due to the isotopic effects of gas exchange and the back flux of tropospheric $N_2O$, there is a complex relationship between the $\delta^{15}N$ and the $\delta^{18}O$ of source, dissolved, and emitted $N_2O$ in aquatic environments. A simple box model (SIDNO – Stable Isotopes of Dissolved Nitrous Oxide) was developed to properly interpret isotopic data for dissolved $N_2O$. Using this model, it was determined that the isotopic composition of emitted $N_2O$ is much more representative of $N_2O$ production in aquatic environments than the isotopic composition of dissolved $N_2O$. If the concentration, $\delta^{15}N$ and $\delta^{18}O$ of dissolved $N_2O$ are measured, the magnitude and isotopic composition of the $N_2O$ flux can be calculated.

Sampling downstream of the major wastewater treatment plants (WWTPs) on the Grand River indicates that nitrification and denitrification in the river are strongly tied to diel changes in dissolved oxygen (DO) concentration. During the day, when DO concentrations are high, nitrification or nitrifier-denitrification is the dominant $N_2O$ production pathway, with sediment denitrification also contributing to $N_2O$ production. At night, when DO concentrations are low, denitrification in the sediments and at the sediment / water interface is the dominant production pathway. Using the SIDNO model, $N_2O$ produced during the day was found to have a $\delta^{15}N$ of -22‰ and a $\delta^{18}O$ of 43‰. $N_2O$ produced at night had a $\delta^{15}N$ of -30‰ and a $\delta^{18}O$ of 30‰. The isotopic composition of $N_2O$ emitted from the Grand River is dominated by night-time production downstream of the Waterloo and Kitchener WWTPs during the summer. The flux and time weighted annual average isotopic composition of $N_2O$ emitted from the Grand River is -18.5‰ and 32.7‰ for $\delta^{15}N$ and $\delta^{18}O$ respectively. These values are significantly more depleted than the only other published data for riverine $N_2O$ production. If the Grand River is representative of global riverine $N_2O$ production, these results will have significant implications for the global isotopic budget for atmospheric $N_2O$. 


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Chapter 1: Introduction

1.1 Introduction

Global climate change is becoming a great concern. Anthropogenic emissions of greenhouse gases absorb long wave radiation reflected by the surface of the earth, trapping heat in the atmosphere (Harrington 1987). Nitrous oxide (N\textsubscript{2}O) is a very potent greenhouse gas, with a global warming potential 310 times that of CO\textsubscript{2} over a 100 year timescale (Denman et al. 2007). N\textsubscript{2}O is also a concern because it is destructive to stratospheric ozone and it has a 114 year lifetime in the atmosphere (Montzka et al. 1999).

The concentration of N\textsubscript{2}O in the atmosphere has been steadily increasing over the last 250 years, from a pre-industrial concentration of 270 to 320 ppbv today, an increase of approximately 50 ppbv (Denman et al. 2007). Recently, the increase in N\textsubscript{2}O concentration has been directly observed by GAGE/AGAGE atmospheric monitoring stations around the globe (Figure 1.1 – Prinn et al. 1990, Prinn et al. 2000). Tropospheric N\textsubscript{2}O concentrations have been increasing linearly over the last three decades at a rate of approximately 0.26%/year (Denman et al. 2007).
Figure 1.1: Average monthly tropospheric N$_2$O concentrations measured at GAGE/AGAGE stations, indicating an average annual increase of 0.26% yr$^{-1}$ (Prinn et al 1990, Prinn et al 2000).

Globally, anthropogenic sources account for 6.7 Tg N/ year of the total 17.7 Tg N/year of N$_2$O emissions to the atmosphere. Currently, best estimates indicate that emissions from rivers, estuaries, and near shore marine environments account for 25% of the total anthropogenic source to the atmosphere (Denman et al. 2007). However, the uncertainty of this estimate is high, and the true value may range between 7% to 61% (Denman et al. 2007). Therefore, aquatic systems are quite important for the production of N$_2$O on a global scale. However, very little research has been done to study the processes responsible for N$_2$O production in these systems.
1.2 N₂O Production in the Nitrogen Cycle

Globally, approximately 65% of N₂O emissions are produced through microbial processes in soils (Bouwman 1990). Generally, these processes fall into two main categories, nitrification and denitrification. N₂O production during nitrification and denitrification was first described by a “leaky pipe” model (Firestone & Davidson 1989, Zafiriou 1990), where N₂O is a by-product (in the case of nitrification) or an intermediate (in the case of denitrification) of the major processes (Figure 1.2). This leaky pipe model may be an oversimplification of N₂O production in natural systems but it is a useful conceptual model.

Figure 1.2: N₂O production in the nitrogen cycle. N₂O can be produced through both nitrification and denitrification. Oxygen isotopic exchange with water can occur at multiple locations in the cycle. The oxidation state of nitrogen throughout the cycle is shown on the bottom scale.

1.2.1 Nitrification

Nitrification is the aerobic oxidation of ammonium (NH₄⁺) to produce nitrate (NO₃⁻). The first part of nitrification is termed nitrosification, where NH₄⁺ is oxidized to
form nitrite (NO$_2^-$) through a hydroxylamine (NH$_2$OH) intermediate. The NO$_2^-$ is then excreted by the nitrosifying microorganisms and further oxidized by other microorganisms to produce NO$_3^-$ in a process termed nitrite oxidation.

In most environments, nitrification is conducted by chemolithotrophic ammonium oxidizing bacteria and nitrite oxidizing bacteria (Hayatsu et al 2008) in order to provide energy for cell growth and metabolism. The ammonium oxidizing bacteria consist of three main genera (*Nitrosomonas, Nitrosopira, and Nitrosococcus*), while the major nitrite oxidizing bacteria belong to four genera (*Nitrobacter, Nitrospina, Nitrococcus, Nitrospira*).

Recently, research has shown that nitrification is carried out by a much more diverse set of microorganisms than previously thought. The enzyme necessary for nitrification (ammonia monooxygenase) has recently been discovered in archaean microorganisms (Hayatsu et al 2008). For example Crenarchaeota have been shown to play a major role in oceanic nitrification (Venter et al. 2004). *Nitrosopumilus maritimus*, a chemolithoautotrophic species of Crenarchaeota, has been shown to use NH$_4^+$ as its sole energy source while maintaining a similar growth rate to nitrifying bacteria (Hayatsu et al. 2008). Additionally, nitrification may be carried out by heterotrophic bacteria and fungi, though in heterotrophic organisms nitrification does not yield energy and does not contribute to cell growth.

Although nitrifying microorganisms are generally slow growing, they have the ability to process large quantities of nitrogen in the environment, due to the high energy requirements for CO$_2$ fixation. For example, *Nitrosomonas* species process approximately 35 moles of NH$_4^+$ for every mole of CO$_2$ fixed (Sprent 1987).
$N_2O$ is produced as a by-product of nitrification through two possible pathways. The first is through the oxidation of the $NH_2OH$ intermediate. $NH_2OH$ is the first reaction product during the oxidation of $NH_4^+$. During nitrification, most of the $NH_2OH$ is further oxidized to produce $NO_2^-$. However, some of the $NH_2OH$ is oxidized through a side reaction to produce $N_2O$. It is likely that $N_2O$ is formed through the spontaneous decomposition of an unstable intermediate (HNO) during the oxidation of $NH_2OH$ to form $NO_2^-$ (Hayatsu et al. 2008).

A second possible nitrification $N_2O$ production pathway during nitrification has been termed “nitrifier – denitrification” in the literature. In this pathway, $NH_4^+$ is oxidized to form $NO_2^-$, but before it is excreted from the microbial cell, the $NO_2^-$ is reduced to form $N_2O$. There is evidence that this reaction becomes favourable when the oxygen supply for nitrification becomes limited. Nitrifier –denitrification may also be used by microorganisms as a method to remove toxic $NO_2^-$ from the cell (Cho et al. 2006).

Nitrification can take place in a variety of environments, provided there is an adequate supply of $NH_4^+$ substrate and oxygen. Nitrification has been shown to increase in agricultural soils in response to fertilization events (Perez et al. 2001). Nitrification also occurs in aquatic systems such as rivers, especially when stimulated by inputs of $NH_4^+$ from sources such as agriculture or municipal wastewater effluent (Garnier et al. 2006, Garnier et al. 2007). In most cases, nitrification can only take place when pH is greater than 6, however, some species of *Nitrosospira* may have adapted to grow in acidic soils, down to pH 3 (Hayatsu et al. 2008).
1.2.2 Denitrification

Denitrification is the step-wise reduction of NO$_3^-$ through NO$_2^-$, NO, N$_2$O and finally to N$_2$. N$_2$O is an obligate intermediate during this process, and therefore, denitrification has the potential to produce large quantities of N$_2$O (Bremner 1997).

As with nitrification, a diverse group of organisms have the ability to perform denitrification. Heterotrophic bacteria, including *Pseudomonas*, *Bacillus* and *Paracoccous* species, are responsible for much of the denitrification activity in soils (Bouman 1990). Many of these bacterial species are facultative aerobes, and only denitrify when oxygen becomes depleted (Bremner 1997). Often, the lack of oxygen triggers the production of denitrification enzymes in these microbes (Sprent 1987).

Many fungi species also contribute significantly to denitrification in nature, both aerobically and anaerobically (Hayatsu et al. 2008). These fungal species contain many of the same denitrifying enzymes that have been studied extensively in the heterotrophic denitrifying bacteria. For example, the fungal species *Fusarium oxysporum* and *Fusarium solani* have been shown to reduce nitrate and produce N$_2$O in cultures with low O$_2$ concentration (Hayatsu et al 2008).

Recently, it has been discovered that several archaea species, such as *Pyrobaculum aerophilum* and *Haloferax denitrificans* have the ability to denitrify (Cabello et al. 2004). However, there are differences between the archaea and bacteria species in terms of the structure and regulation of the denitrifying enzymes (Hayatsu et al 2008). The importance of archaeal microorganisms for denitrification in most natural systems is currently unknown (Hayatsu et al 2008).
Denitrifying bacteria and fungi are widely distributed in nature, and present in large numbers in most soils (Bremner 1997). As a result, denitrification nearly always takes place in NO$_3^-$ containing soils when O$_2$ becomes limited (Bremner 1997).

During denitrification, the ratio of N$_2$:N$_2$O produced can vary widely depending on several factors, including soil pH, soil moisture content, redox potential, temperature, and NO$_3^-$ and organic carbon concentration (Bouman 1990). Additionally, higher N$_2$:N$_2$O ratios can result from denitrification in soils if the N$_2$O is unable to escape from the system before it is reduced by other microbes (Bremner 1997). Some microbial organisms lack the ability to reduce N$_2$O to N$_2$; as a result more N$_2$O can be produced by such microbial communities (Bouman 1990). High levels of nitrate in the soil have been shown to inhibit the reduction of N$_2$O to N$_2$ (Bremner 1997). Therefore, the N$_2$:N$_2$O ratio is related to the overall level of anoxia in the system, with highly anoxic, low nitrate systems producing less N$_2$O than environments containing more oxygen and nitrate.

1.2.3 Abiotic Sources of N$_2$O in Soils.

Biological processes are by far the largest source of N$_2$O in nature; however, it is possible that some N$_2$O is produced through abiotic chemical reactions (Bouman 1990). One such reaction is the chemical decomposition of NH$_2$OH. As mentioned previously, NH$_2$OH is an intermediate species in the process of nitrification. Laboratory experiments have indicated that NH$_2$OH undergoes rapid decomposition in sterile soil to form N$_2$O and N$_2$ (Bremner 1997). However, there is no evidence that NH$_2$OH is released from microbial cells during nitrification, and it has not been measured in significant concentrations in natural soils. Therefore it is unlikely that chemical decomposition of NH$_2$OH is a significant source of N$_2$O in the environment (Bremner 1997).
A second abiotic process that produces N$_2$O is termed chemo-denitrification. This process occurs when NO$_3^-$ in the soil reacts with organic compounds, in particular lignin and its breakdown products (Sprent 1987). This reaction forms unstable compounds that can abiotically decompose to form N$_2$O and other nitrogen compounds (Bouman 1990). The amount of N$_2$O that is produced through chemo-denitrification is small compared to the other products of this reaction, such as N$_2$ and NO (Bremner 1997). Also, it is unlikely that NO$_3^-$ is present in sufficient concentrations for this reaction to be significant (Sprent 1987). If the rates of chemo-denitrification are high, it would be expected that the N$_2$:N$_2$O ratio would be much greater than in systems where the chemo-denitrification rate is insignificant. Since chemo-denitrification is a relatively slow process compared to biological denitrification, and the major products of this reaction are N$_2$ and NO, it is unlikely that chemo-denitrification contributes significantly to N$_2$O production in the environment.

1.3 Stable Isotope Fractionation Associated with N$_2$O Production

Stable isotope ratio analysis of N$_2$O provides insights into the processes responsible for its production. Stable isotope ratios can be determined for both nitrogen and oxygen atoms in the N$_2$O molecule. Nitrogen has two stable isotopes, $^{15}$N and $^{14}$N. $^{14}$N is much more abundant and accounts for 99.632% of the nitrogen atoms in atmospheric N$_2$ (Kaiser 2002). Oxygen has three stable isotopes ($^{18}$O, $^{17}$O, and $^{16}$O). The relative abundances of these stable isotopes in ocean water are 0.201%, 0.038% and 99.761% respectively (Kaiser 2002).
Nitrogen and oxygen form N$_2$O molecules with different combinations of stable isotopes. The most common isotopologues of N$_2$O are: $^{14}$N$^{14}$N$^{16}$O, $^{15}$N$^{14}$N$^{16}$O, $^{14}$N$^{15}$N$^{16}$O, and $^{14}$N$^{14}$N$^{18}$O. The other isotopic combinations are statistically rare (Kaiser 2002).

Stable isotope data is usually expressed in delta ($\delta$) notation in units of per mil ($‰$) according to Equation 1.1, where:

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000$$

(1.1)

$R_{\text{sample}}$ and $R_{\text{standard}}$ are the stable isotope ratios of the sample and standard, respectively. N$_2$O is introduced into an isotope ratio mass spectrometer (IRMS) under a vacuum and given a positive charge through ionization. The N$_2$O$^+$ is then focused into a beam and accelerated by an electric field. The beam is redirected by a magnetic field, causing it to split into several beams based on the mass/charge (m/z) ratio of the ions. The relative intensity of these beams is measured by Faraday cup detectors, with the intensity being a function of the abundance of the various isotopologues in the sample.

Both nitrogen and oxygen isotope ratios are changed during many important biological processes. Isotopic fractionation occurs when a particular elemental isotope is favoured in the products of a reaction (Kendall and Aravena 2000). Isotopic fractionation occurs in both reversible equilibrium and irreversible kinetic reactions. The fractionation associated with irreversible kinetic reactions is generally more important during low temperature biological reactions, and therefore dominates during the production of N$_2$O (Kendall and Aravena 2000). Kinetic fractionation factors are much more variable than equilibrium fractionation factors and depend heavily on the environmental conditions under which the reaction takes place (Kendall and Aravena 2000).
Generally speaking, for biologically facilitated kinetic reactions, compounds containing the lighter isotope react faster, and therefore the products of the reaction tend to have less of the heavy isotopes compared to the reactants (Kendall and Aravena 2000). For example, during the denitrification of nitrate to form N$_2$O, the $\delta^{15}$N value of the N$_2$O that is formed is more negative than the original nitrate. For kinetic isotopic fractionation, an isotopic fractionation factor between the reaction product and substrate ($\alpha_{p,s}$) can be defined (Equation 1.2) (Kendall & Aravena 2000), where

$$\alpha_{p,s} = \frac{R_p}{R_s}$$

(1.2)

$R_p$ and $R_s$ are the stable isotope ratios of the reaction product and substrate, respectively.

Isotopic fractionation can also be expressed as a kinetic isotope enrichment factor ($\varepsilon_{p,s}$), (Equation 1.3) (Kendall & Aravena 2000).

$$\varepsilon_{p,s} = 1000 \times (\alpha_{p,s} - 1)$$

(1.3)

While the fractionation factor ($\alpha_{p,s}$) and the enrichment factor ($\varepsilon_{p,s}$) are the most accurate ways to express isotopic fractionation, occasionally in the published literature, these terms are approximated using Equation 1.4 (Kendall & Aravena 2000), where

$$\varepsilon_{p,s} \sim \Delta = \delta_p - \delta_s$$

(1.4)
\( \delta_p \) and \( \delta_s \) are the isotopic delta values in the product and substrate, respectively. When isotopic fractionation is small, \( \Delta \) is a good approximation for \( \epsilon \), but the error associated with \( \Delta \) increases when fractionation is large.

Many studies have reported isotopic fractionation factors associated with \( \text{N}_2\text{O} \) production through nitrification and denitrification (Table 1.1). In many cases, it is not clear whether the isotopic fractionation is reported as \( \Delta \) or \( \epsilon \), therefore, a distinction is not made between the two in Table 1.1.
### Table 1.1: Isotopic fractionation factors for $^{15}$N and $^{18}$O available in the published literature.

<table>
<thead>
<tr>
<th>Organism or Community</th>
<th>Substrate</th>
<th>Product</th>
<th>$\varepsilon$ or $\Delta$ (product - substrate) ($%$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mineralization</strong></td>
<td>-</td>
<td>Organic N</td>
<td>NH$_4^+$</td>
<td>+/-1</td>
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<tr>
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<td>NH$_4^+$</td>
<td>NO$_3^-$</td>
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<tr>
<td>(observed range in field studies)</td>
<td>Nitrosomonas europaea</td>
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<td>NO$_2^-$</td>
<td>-37</td>
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<td>soil microbial community</td>
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<td>NH$_4^+$</td>
<td>N$_2$O</td>
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</tr>
<tr>
<td>Nitrosomonas europaea</td>
<td>NO$_2^-$</td>
<td>N$_2$O</td>
<td>-38</td>
<td>-</td>
</tr>
<tr>
<td>Nitrosomonas multiformis</td>
<td>NO$_2^-$</td>
<td>N$_2$O</td>
<td>-24</td>
<td>-</td>
</tr>
<tr>
<td>Nitrosomonas multiformis</td>
<td>NH$_2$OH</td>
<td>N$_2$O</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Nitrosomonas europaea</td>
<td>NO$_2^-$</td>
<td>N$_2$O</td>
<td>-20</td>
<td>-</td>
</tr>
<tr>
<td>Nitrosomonas multiformis</td>
<td>NO$_2^-$</td>
<td>N$_2$O</td>
<td>-3</td>
<td>-</td>
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<tr>
<td><strong>Denitrification</strong></td>
<td>soil microbial community</td>
<td>NO$_3^-$</td>
<td>N$_2$</td>
<td>-38</td>
</tr>
<tr>
<td>soil microbial community</td>
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<td>NO$_3^-$</td>
<td>N$_2$O</td>
<td>-13</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 1.1:** Isotopic fractionation factors for $^{15}$N and $^{18}$O available in the published literature.
1.3.1 Nitrification

All of the published data on isotopic fractionation associated with N$_2$O production through nitrification was obtained from laboratory incubation experiments using pure culture organisms (e.g. Sutka et al 2003, 2004, 2006), or soil microbial communities (Perez et al 2006). Therefore, since these are the only data available, researchers have attempted to apply these fractionation factors to observations made in field environments.

N$_2$O produced through nitrification tends to be very depleted in $^{15}$N. Nitrogen enrichment factors for nitrification typically range from -47‰ to -20‰ (Table 1.1). However, Perez et al (2006) observed a very large fractionation (-112‰ to -102‰) in lab incubations of soils collected from the Brazilian Amazon. These very large nitrogen enrichment factors were obtained by mass balance calculations from various experimental treatments (where nitrification was inhibited or not inhibited). These extreme nitrogen enrichment factors for nitrification have not yet been confirmed by other lab or field studies.

Observed nitrogen enrichment factors for nitrifier-denitrification are generally greater than those observed for the hydroxylamine oxidation pathway (Table 1.1). In fact, several studies by Sutka et al (2003, 2004, 2006) have observed near zero or positive fractionation associated with the hydroxylamine oxidation pathway. The nitrogen fractionation factors associated with nitrification can be highly variable, depending largely on the metabolic pathway and the particular microorganisms involved.

There are very few data available on the oxygen isotopic composition of N$_2$O produced through nitrification. Historically, laboratory incubation studies have not reported the $\delta^{18}$O of N$_2$O produced through nitrification. However, several field based
studies have measured and reported the $\delta^{18}O$-N$_2$O values. Wahlen & Yoshinari (1985a) observed a $\delta^{18}O$ of 24‰ for N$_2$O produced through nitrification in a manure-fertilized field. These same authors measured $\delta^{18}O$ of 23, 22, and 36‰ in N$_2$O produced through nitrification at a sewage treatment plant (Wahlen & Yoshinari 1985b). Perez et al (2001) measured $\delta^{18}O$ of N$_2$O ranging from 20.5 to 28.5‰ produced during nitrification in a Mexican agricultural field fertilized with 150 kg N/ha as urea. These published results indicate that the $\delta^{18}O$ of N$_2$O produced through nitrification is typically close to that of atmospheric oxygen ($\delta^{18}O -$ O$_2$ = 23.5‰).

During nitrification of NH$_4^+$, the first oxygen atom added to form NH$_2$OH is obtained from atmospheric O$_2$ (Hollocher et al. 1981, Andersson & Hooper 1983). The two additional oxygen atoms needed to form NO$_3^-$ are obtained from ambient water (Aleem et al. 1965, Andersson & Hooper 1983, Kumar et al. 1983, Hollocher 1984). Therefore, it is expected that NO$_3^-$ produced through nitrification should have a $\delta^{18}O$ value that reflects a 1/3 contribution from atmospheric O$_2$ and a 2/3 contribution from ambient water. Given this relationship, the $\delta^{18}O$ of N$_2$O produced through nitrification should be different depending on whether the N$_2$O was produced through the hydroxylamine oxidation or nitrifier-denitrification pathway. The fact that the observed $\delta^{18}O$ – N$_2$O is typically similar to atmospheric O$_2$ during nitrification might suggest that hydroxylamine oxidation is the dominant pathway. However, there are several complicating factors. First, fractionation could occur during the cellular uptake of O$_2$ from the environment, and the $\delta^{18}O$ of NH$_2$OH should actually be greater than that of atmospheric O$_2$. Fractionation of O$_2$ during cellular uptake has been used as an indicator of respiration in aquatic environments (Venkiteswaran et al. 2007). Secondly, although
\( \text{N}_2\text{O} \) produced through the nitrifier-denitrification pathway incorporates some oxygen from ambient water (which would have a \( \delta^{18}\text{O} \) much less than atmospheric \( \text{O}_2 \)), a significant positive enrichment would be expected for \( ^{18}\text{O} \) as oxygen atoms are removed from \( \text{NO}_2^- \) to produce \( \text{N}_2\text{O} \). This could theoretically produce \( \text{N}_2\text{O} \) that has a \( \delta^{18}\text{O} \) value that is similar to atmospheric \( \text{O}_2 \) merely through coincidence. Lastly, recent research has shown that significant isotopic exchange of oxygen occurs between \( \text{N}_2\text{O} \) precursors and ambient water during nitrification and denitrification (Kool et al. 2007). This exchange of oxygen atoms would alter the \( ^{18}\text{O} \) signature of the resulting \( \text{N}_2\text{O} \), and make it very difficult to accurately determine the origin of the oxygen atoms.

### 1.3.2 Denitrification

As is the case with nitrification, all of the studies used to determine the isotopic fractionation effects associated with \( \text{N}_2\text{O} \) production through denitrification have been conducted using laboratory incubation experiments. Most of these studies have used pure cultures; however, several studies have used incubated soils containing natural microbial communities (Table 1.1), and thus may be more representative of the isotopic fractionation expected in the natural environment.

Generally, the nitrogen fractionation factors for \( \text{N}_2\text{O} \) produced through denitrification are less than those for nitrification. Typical values range from -30 to -9‰, though recently a few studies have observed values that fall outside this range (-45‰, Perez et al. 2006; -39‰, Toyoda et al. 2005; -37‰, Sutka et al. 2006). There is a great amount of variability in the nitrogen fractionation factors observed between different species in the pure culture incubation experiments. Therefore, the isotopic fractionation
associated with denitrification is highly dependent on the microbial species involved.

Several recent studies (Hayatsu et al. 2008, Irabar et al. 2008) have shown that the microbial communities responsible for denitrification in natural undisturbed environments are much more complex than previously thought, and that only a small fraction of the microorganisms present are able to be grown in laboratory cultures. This has implications for applying fractionation factors obtained through laboratory incubation studies to the field environment.

An additional complicating factor associated with laboratory incubation experiments is the fact that most of these studies used acetylene (C$_2$H$_2$) to inhibit nitrification and N$_2$O reduction to N$_2$. Although, in theory, the use of C$_2$H$_2$ should allow researchers to isolate N$_2$O production through denitrification, this situation is not representative of the natural environment and the effect on the observed isotopic fractionation is unknown. Also, the presence of C$_2$H$_2$ has been shown to enhance the chemical oxidation of NO (2NO + O$_2$ → NO$_2$(g)) by at least three orders of magnitude when O$_2$ is present in trace concentrations (Bollmann & Conrad 1997a, Bollmann & Conrad 1997b, McKenney et al. 1997). If this chemical oxidation artificially removes a significant amount of NO from the denitrification sequence, it could have a significant effect on the $\delta^{15}$N and $\delta^{18}$O of the N$_2$O produced.

In comparison to nitrogen fractionation, very little is known about oxygen fractionation effects during denitrification. Denitrification should have a very strong $^{18}$O enrichment effect, because five oxygen atoms are removed for every molecule of N$_2$O produced. This should leave the remaining oxygen atoms very enriched in $^{18}$O; however, for the few studies that have reported oxygen fractionation factors for denitrification, the
range in values is tremendous (-54 to +40‰ Table 1.1). The disparity between what is expected and the observed values can be explained by isotopic exchange of oxygen atoms between ambient water and N₂O precursors during denitrification (Kool et al 2007). Isotopic exchange of oxygen can readily occur during the NO₂⁻ to NO and NO to N₂O reduction steps, with exchange rates ranging from <10 to 100% (Kool et al. 2007). The isotopic exchange of oxygen alters the ¹⁸O/¹⁶O ratio in the N₂O that is produced, making it very difficult to use the δ¹⁸O values to determine the source and processes involved in N₂O production. The degree of oxygen isotopic exchange has been shown to vary greatly depending on the microorganism and the type of denitrifying enzymes used (Kool et al 2007). Different microbial communities would likely facilitate different oxygen isotopic exchange rates, and produce N₂O with differing δ¹⁸O values, even given identical environmental conditions and initial substrate compositions.

**1.3.3 N₂O Consumption**

Extracellular N₂O consumption (reduction to N₂), causes the δ¹⁵N and δ¹⁸O of N₂O produced by denitrification to become more enriched than that observed during laboratory incubation studies. Several published studies have attempted to quantify the isotopic fractionation associated with N₂O consumption using laboratory incubations (Table 1.1). Typically these experiments involve providing the microbial organisms with a N₂O substrate in the absence of oxygen, while monitoring the isotopic composition of the residual N₂O as it is reduced to N₂. The nitrogen and oxygen fractionation factors for N₂O consumption range from -27‰ to -1‰ and -5‰ to -42‰ respectively (Table 1.1). Although the range for these fractionation factors is quite large, several studies have
observed a relatively constant ratio of 1:2.5 for $\delta^{15}$N:$\delta^{18}$O evolution during N$_2$O consumption (Vieten et al. 2007, Menyailo & Hungate 2006, Mandernack et al. 2000). This effect is analogous to the characteristic 2:1 enrichment observed for $\delta^{15}$N:$\delta^{18}$O of residual NO$_3^-$ during reduction by denitrification (Mariotti et al 1988, Böttcher et al 1990, Smith et al. 1991, Aravena & Robertson 1998, Cey et al. 1999, Mengis et al. 1999).

Therefore, this relationship could potentially be used as a characteristic indicator of N$_2$O consumption in a natural system. Furthermore, since the characteristic ratios are different between N$_2$O consumption and NO$_3^-$ substrate consumption, $\delta^{15}$N and $\delta^{18}$O values can be used to distinguish between these two processes, both of which can affect measured N$_2$O isotope ratios.

1.4 Field Studies using $\delta^{15}$N and $\delta^{18}$O of N$_2$O

There is a tremendous amount of scatter in the $\delta^{15}$N and $\delta^{18}$O values that have been measured for N$_2$O from various field environments (Figure 1.3). However, some generalizations can be made about the data. Most of the N$_2$O collected from terrestrial environments is more depleted with respect to $^{15}$N and $^{18}$O compared to the isotopic signature of tropospheric N$_2$O. Generally, the terrestrial N$_2$O isotopic data falls between -40‰ and +5‰ for $\delta^{15}$N and +20‰ and +45‰ for $\delta^{18}$O, compared to the tropospheric N$_2$O composition of 6.72 (+/- 0.12)‰ and 44.62 (+/- 0.21)‰ for $\delta^{15}$N and $\delta^{18}$O, respectively (Kaiser et al 2003). Most of the studies conducted in terrestrial environments have been in fertilized agricultural systems (Kim & Craig 1993, Well et al 2005, Yamulki et al 2001, Perez et al. 2001, Bol et al 2003, Tilsner 2003, Van Groenigen et al. 2005, Rock et al 2007), though some data has also been collected from natural forest soils (Kim
& Craig 1993, Perez et al 2000). There is not a clear distinction in the \(\delta^{15}N\) and \(\delta^{18}O\) values between samples collected from agricultural and natural soils.

Figure 1.3: Summary of \(N_2O\) stable isotope data collected by various studies in terrestrial and aquatic environments. One point is an outlier, and did not fit on the scales of this plot (Shallow Groundwater, Germany\(^9\) \(\delta^{15}N = 86.1\,‰\), \(\delta^{18}O = 89.8\,‰\)). References: 1 – Kim & Craig (1993), 2 – Mandernack et al. (2000), 3 – Well et al. (2005), 4 – Yamulki et al. (2001), 5 – Perez et al. (2000), 6 – Perez et al. (2001), 7 – Bol et al. (2003), 8 – Tilsner et al. (2003), 9 – Van Groenigen et al. (2005), 10 – Rock et al. (2007), 11 – Kim & Craig (1990), 12 – Naqvi et al. (1998), 13 – Yoshanari et al. (1997), 14 – Westley et al (2006), 15 – Dore et al. (1998), 16 – Boontanon et al. (2000), 17 – Kaiser et al. (2003)
In contrast, N₂O stable isotope data collected from aquatic environments tends to be enriched with respect to $^{15}\text{N}$ and $^{18}\text{O}$ compared to the values for tropospheric N₂O (Kaiser et al 2003). Again, there is a great amount of scatter in the aquatic N₂O data, with $\delta^{15}\text{N}$ generally ranging between -5‰ and +20‰, and $\delta^{18}\text{O}$ generally ranging between +40‰ and +80‰. Most of the aquatic N₂O studies have been conducted in marine environments (Kim & Craig 1990, Naqvi et al 1998, Yoganani et al 1997, Westley et al 2006, Dore et al 1998). Most of the data from marine samples have $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values that are very similar to the values for tropospheric N₂O (Kaiser et al 2003). This is likely due to the isotopic effects of gas exchange with the atmosphere and low ambient N₂O concentrations in near surface ocean samples (e.g. 118% saturation in the Black Sea, Westley et al. 2006).

The one study that measured $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of N₂O dissolved in groundwater reported very wide ranging values. Well et al (2005) measured the stable isotope composition of N₂O dissolved in shallow groundwater beneath an experimental agricultural plot in Germany. The researchers observed $\delta^{15}\text{N}$ values ranging between -41.6‰ and +86.1‰, and $\delta^{18}\text{O}$ values ranging between 23.4‰ and 89.8‰. This extremely wide data range has not been observed in any other field sites.

There is currently only one published study that measured the isotopic ratios of dissolved N₂O produced in a river (Boontanon et al 2000). These researchers measured the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of N₂O in the Bang Nara River in Thailand on several occasions between November 1997 and January 1998. They observed $\delta^{15}\text{N}$ values ranging between -3.8‰ and 15.6‰ and $\delta^{18}\text{O}$ values ranging between 36.6‰ and 63.8‰. Boontanon et al (2000) observed a change in both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values with time in their study. Initially,
the values were enriched in to \(^{15}\text{N}\) and \(^{18}\text{O}\) compared to tropospheric \(\text{N}_2\text{O}\). However, partway through the study the values became more depleted, before returning to the enriched values. The authors attributed this change in \(\delta^{15}\text{N}\) and \(\delta^{18}\text{O}\) to a change from \(\text{N}_2\text{O}\) production through denitrification to production through nitrification. However, this conclusion is not consistent with other studies (Perez et al. 2001, Bol et al. 2003), which have shown that the \(\delta^{15}\text{N}\) and \(\delta^{18}\text{O}\) values of \(\text{N}_2\text{O}\) produced through nitrification are usually much lower than the values observed by Boontanon et al. (2000).

Several studies have also used \(\delta^{15}\text{N}\) and \(\delta^{18}\text{O}\) of \(\text{N}_2\text{O}\) to distinguish between nitrification and denitrification in field environments. For example, Perez et al. (2001) measured the \(\delta^{15}\text{N}\) and \(\delta^{18}\text{O}\) of \(\text{N}_2\text{O}\), \(\text{NH}_4^+\), and \(\text{NO}_3^-\) in agricultural soils following fertilization and irrigation. These researchers found that the observed instantaneous \(^{15}\text{N}\) enrichment factor between \(\text{N}_2\text{O}\) and its precursors (\(\text{NH}_4^+\) and \(\text{NO}_3^-\)) was a good indicator of nitrification and denitrification, and closely matched values obtained from laboratory studies (Table 1.1). Bol et al. (2003) measured the \(\delta^{15}\text{N}\) and \(\delta^{18}\text{O}\) of \(\text{N}_2\text{O}\) produced from a grassland soil in the UK after an application of fertilizer. The authors of this study also measured the \(\text{N}_2\text{O}/\text{N}_2\) production ratio, and used this data to identify three phases of \(\text{N}_2\text{O}\) production (phase 1, nitrification > denitrification; phase 2 denitrification > nitrification; phase 3, denitrification >> nitrification). Bol et al. (2003) found that the \(\delta^{15}\text{N}\) and \(\delta^{18}\text{O}\) data independently confirmed this conclusion, as the \(\delta^{15}\text{N}\) and \(\delta^{18}\text{O}\) values increased as \(\text{N}_2\text{O}\) production became dominated by denitrification rather than nitrification.

Although some studies have had success in distinguishing nitrification from denitrification using \(\delta^{15}\text{N}\) and \(\delta^{18}\text{O}\) of \(\text{N}_2\text{O}\), the large range in \(^{15}\text{N}\) and \(^{18}\text{O}\) enrichment factors for nitrification and denitrification (Table 1.1) can lead to ambiguous results. For
example Tilsner et al. (2003) measured N₂O produced by grassland soils in Bavaria, Germany, both before and after application of organic and mineral fertilizers. These authors observed a large amount of scatter in the δ¹⁵N and δ¹⁸O values, and were not able to use the isotopic data to determine the dominant N₂O production process. The authors attributed scatter in the data to a high spatial heterogeneity in N₂O production processes in the soil. However, using laboratory incubations of the same soil, Tilsner et al. (2003) were able to determine that denitrification was likely the dominant N₂O production process at the site.

1.5 Research Objectives

The primary goal of this thesis project was to characterize N₂O production in the Grand River, Ontario, Canada, in terms of dominant production pathways and isotopic composition of the N₂O produced. This was achieved through the stable isotope analysis of dissolved N₂O, NO₃⁻ and NH₄⁺. To achieve this goal, four main objectives were addressed.

The first objective was to develop a method to measure the stable isotope ratio of dissolved N₂O. Although there are several online methods available in the literature (e.g. Ostrom et al 2000, Westley et al 2006), these methods were not practical for this study due to the large sample processing time and the requirement for a dedicated mass spectrometer. Therefore, a new offline method was developed and tested to ensure accuracy for isotopic analysis of dissolved N₂O.

The second objective was to develop a computer model to simulate the stable isotope dynamics of dissolved N₂O in systems that are open to gas exchange with the
atmosphere. Since N₂O is highly soluble, the invasion of N₂O from the atmosphere has a significant effect on the δ¹⁵N and δ¹⁸O of dissolved N₂O, especially when the dissolved concentrations approach equilibrium saturated conditions. Additionally, there are kinetic and equilibrium fractionation factors for nitrogen and oxygen during N₂O gas exchange with the atmosphere. As a result, the relationship between the δ¹⁵N and δ¹⁸O values of dissolved N₂O, produced N₂O, and emitted N₂O is not simple. A box model was created using Stella modeling software to elucidate this relationship.

The third objective was to determine the dominant N₂O production processes in the Grand River, making use of stable isotope analysis of N₂O, NO₃⁻ and NH₄⁺. From previous work and continuous monitoring by the Grand River Conservation Authority (GRCA), it was known that the concentration of dissolved oxygen (DO) in the river follows strong diel cycles during the summer months. Other studies (Clough et al. 2007, Harrison et al. 2005) have shown that nitrogen cycling processes and N₂O production can be influenced by the diel oxygen cycle. It was therefore expected that N₂O production in the Grand River would also be affected by the diel DO cycle. By monitoring the concentrations of DO, N₂O, NO₃⁻, NH₄⁺ and N₂, it was possible to examine how nitrification and denitrification respond to the DO concentration in the river. The N₂O stable isotope model developed earlier was then used to determine in-situ enrichment factors for N₂O production through nitrification and denitrification.

The fourth objective was to fully characterize the isotopic composition of N₂O emitted from the Grand River to the atmosphere. Since many other studies have observed a wide range in the δ¹⁵N and δ¹⁸O values of N₂O produced in field environments (Figure 1.3), it was expected that the isotopic composition of the N₂O flux would be variable,
both spatially and temporally. Currently there is very little known about the isotopic composition of N$_2$O produced in riverine systems. Current estimates indicate that the N$_2$O emissions from rivers, estuaries and coastal zones total 1.7 Tg N/year globally, and may be as high as 2.9 Tg N/year (Denman et al. 2007). This compares to the global anthropogenic N$_2$O source of 6.7 Tg N/year, and the total global N$_2$O emission rate of 17.7 Tg N/year (Denman et al. 2007). In spite of the relative importance of this N$_2$O source, only one published study has measured the isotopic composition of dissolved N$_2$O in a river (Boontanon et al 2000). Isotopic models can be used to determine the relative importance of various sources of N$_2$O to the atmosphere (Rahn & Wahlen 2000). Since rivers are an important source of N$_2$O to the atmosphere, it is necessary to characterize the isotopic composition of this source in order to refine the global N$_2$O budget, and determine the true contribution of riverine N$_2$O to the total global emissions.
Chapter 2:

Site Description and Methodology

2.1 Grand River Watershed

The Grand River is the largest river in southern Ontario. It is approximately 300 km long, and drains an area of 7000 km$^2$ into Lake Erie (Figure 2.1). As of 2001, approximately 720 000 people were living in the basin; this figure is projected to increase to 1 220 000 by 2031 (Table 2.1). The cities of Waterloo, Kitchener, Cambridge and Guelph form the urban centre of the watershed, accounting for approximately 72% of the total basin population. Projected growth in the Region of Waterloo is expected to be particularly extensive, with the population expanding by approximately 60% by 2031 (Table 2.1). The Grand River is an important source of drinking water, supplying approximately 500 000 people in the watershed.
Figure 2.1: Map of the Grand River Watershed.

<table>
<thead>
<tr>
<th>Region</th>
<th>Projected Population (000's)</th>
<th>2001</th>
<th>2011</th>
<th>2021</th>
<th>2031</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wellington County</td>
<td></td>
<td>85</td>
<td>91</td>
<td>269</td>
<td>321</td>
</tr>
<tr>
<td>City of Guelph</td>
<td></td>
<td>110</td>
<td>132</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Region of Waterloo</td>
<td></td>
<td>456</td>
<td>526</td>
<td>623</td>
<td>729</td>
</tr>
<tr>
<td>Brant County</td>
<td></td>
<td>35</td>
<td>39</td>
<td>157</td>
<td>173</td>
</tr>
<tr>
<td>City of Brantford</td>
<td></td>
<td>94</td>
<td>102</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>780</td>
<td>890</td>
<td>1,049</td>
<td>1,223</td>
</tr>
</tbody>
</table>

Table 2.1: Projected population growth in the Grand River Watershed (Ministry of Public Infrastructure Renewal, 2006)
The Grand River is heavily impacted by both diffuse and point-source inputs of nitrogen. Agriculture is the primary land use in the watershed, and 26 wastewater treatment plants (WWTPs) currently discharge effluent to the Grand River or its tributaries. Six of these WWTPs are required to report discharge data to the Federal National Pollutant Release Inventory (Table 2.2, NPRI 2007). Of these six, the Kitchener and Waterloo WWTPs contribute approximately 30% of the total WWTP nitrogen load to the river. Of all the WWTPs, the Kitchener plant releases the most $\text{NH}_4^+$ to the river, 74% of the total.

![Table 2.2: Mass of nitrogen and phosphorous pollutants released by WWTP effluent in the Grand River Watershed. Data provided by NPRI (2007).](image)

Downstream of the urban centre, the river is overwhelmed by the input of nutrients, and there is heavy macrophyte growth in the channel. The heavy macrophyte growth contributes to very large diel swings in dissolved oxygen (DO) concentration, especially during the hot summer months. During the summer months, night-time DO concentrations at this location often fall below the water quality target of 4 mg/L set by the Grand River Conservation Authority. This DO problem is further compounded by the fact that the effluent from the Kitchener and Waterloo WWTPs contain high
concentrations of ammonium (NH$_4^+$). These two major WWTPs are not designed to nitrify the effluent within the plant, and instead rely on the river to oxidize the NH$_4^+$ to nitrate (NO$_3^-$). Nitrification of WWTP derived NH$_4^+$ further consumes the limited DO in the river and contributes to poor river health.

2.1.1 Climate

The Grand River Watershed is located within the Warm Summer Continental (Dfb) Köppen climatic region (Ackerman 1941). Typical monthly mean temperatures and precipitation amounts for the Grand River Basin are summarized in Figure 2.2. These values are based on 30 year historical weather data collected at the Waterloo Wellington Airport (43°27’20.09”N, 80°23’08.29”W). The data was supplied by Environment Canada (2004).

![Figure 2.2: 30 Year Climate history as recorded at Waterloo – Wellington Airport](image)
During the study period (May 2006 to May 2008), there were several periods where the weather in the basin deviated significantly from the 30 year climate average. Figure 2.3 illustrates the recorded monthly mean daily temperature as compared to the 30 year average over the course of this study. The temperature data was recorded at the University of Waterloo weather station (43°28’24.58”N, 80°33’25.95”W). Data from the University of Waterloo weather station was used because recent data is easily accessible, though the station lacks a long-term historical record. There were several periods where the mean monthly temperature was significantly higher than normal, especially the summer and early winter in 2006 (Figure 2.3). February 2007 was significantly colder than normal. Temperatures otherwise closely followed the 30-year monthly mean.

Figure 2.3: Monthly mean daily temperature data, as recorded at the University of Waterloo weather station. The 30 – year monthly mean data was collected at the Waterloo-Wellington airport.
Figure 2.4 is a plot of the total monthly precipitation measured at the University of Waterloo weather station over the course of this study (May 2006 to May 2008). As indicated, precipitation patterns were quite variable during this time period. Several months during 2006 recorded significantly higher amounts of precipitation compared to the 30 year monthly mean (Figure 2.4). Also, the summer and fall of 2007 were significantly drier than normal. The below normal precipitation led to very low flow conditions during this time period. Above normal amounts of precipitation fell during the winter of 2007-2008. The bulk of this precipitation fell as snow, and led to large snowmelt events during two major thaw periods in January and April 2008.

Figure 2.4: Monthly total precipitation data, as recorded at the University of Waterloo weather station. The 30 – year monthly mean data was collected at the Waterloo-Wellington airport.
2.1.2 Hydrology

Flow conditions in the Grand River are carefully controlled throughout the year by a series of 8 major dams and reservoirs. The Shand dam is the largest control structure on the Grand River. The Shand Dam was constructed in 1942, and was one of the first hydrologic control structures built on the Grand River. Generally, the various dams and reservoirs are operated to buffer the high river flows during snowmelt and heavy precipitation events, and to also supplement the low river flows during the summer months. Therefore, the reservoirs typically follow an annual operation cycle of filling during the early spring snowmelt, and slowly draining throughout the summer.

Since the flow in the river is highly regulated, it follows a predictable pattern throughout the year (Figure 2.5). River flows are lowest during the summer months, typically ranging between 10 – 20 m$^3$/sec at Galt, Ontario (HYDAT 2005). River flow is usually highest during the annual spring snowmelt event, which typically occurs during the months of March or April (Figure 2.5). Since the spring snowmelt event typically occurs in a sharp peak, it is not well represented in Figure 2.5. The typically peak snowmelt flow ranges between 170 to 680 m$^3$/sec (taken as the range between the 10$^{th}$ and 90$^{th}$ percentiles of the peak flow value during the period from March 1 to April 30, flow data collected from 1943 to 2004 inclusive - HYDAT 2005).
2.2 Methodology

2.2.1 Water Chemistry

Samples for general water chemistry analyses were collected in 120 mL plastic bottles. Samples were kept cold until brought back to the lab, usually less than 6 hours after they were collected. Water samples were then filtered with 0.45 μm membrane syringe filters and separated into two 40 mL amber vials. One of the vials was acidified to approximately pH 5 with 10% H₂SO₄. Both vials were then placed into cold storage (4°C) until analysis (usually within 1-2 weeks).
2.2.2 Nitrate Concentration

Sample water was taken from the non-acidified amber vials for analysis of anion concentrations. These samples were analyzed using a Dionex ICS-90 ion chromatography machine, equipped with an IonPac AS14A column and AS40 automated sampler. Samples were corrected to a calibration curve created from standards run at the same time as the samples. Precision for this analysis was +/- 0.05 mg N/L.

2.2.3 DOC Concentration

Samples were analyzed for dissolved organic carbon (DOC) using a Rosemount-Dohrmann high temperature total carbon analyzer equipped with an autosampler system. DOC is determined by a measurement of total carbon, after inorganic carbon is removed from the sample by sparging with phosphoric acid. Samples were corrected to a calibration curve created from standards analyzed during the same run as the samples. The precision associated with this analysis is typically +/- 0.2 mg C/L.

2.2.4 Ammonium Concentration

Ammonium concentration was analyzed using an automated colourometric method. Briefly, reagent solutions (containing sodium salicylate, sodium nitro-prusside, sodium hydroxide, potassium sodium tartrate tetrahydrate, di-sodium hydrogen phosphate, and sodium salt) are added to the samples. These reagents react with ammonium present in the sample to form a colour, the intensity of which depends on the concentration of ammonium. The concentration of NH$_4^+$ in the original sample is then
determined by measuring the absorption of 660 nm light through the samples. The amount of absorption at this wavelength is proportional to the amount of ammonium present in the sample. The data was then corrected against a calibration curve created from standards analyzed at the same time as the samples. The detection limit for this method is 0.01 mg N-NH$_4^+$/L and the precision associated with this analysis is +/- 0.005 mg N-NH$_4^+$/L.

2.2.5 Dissolved Nitrous Oxide Concentration

Samples for determination of dissolved N$_2$O concentration were collected in 60 mL glass serum bottles with red Vacutainer™ (Benton-Dickson) stoppers. The bottles were filled underwater, with as little disturbance as possible, in order to prevent degassing of the sample. The bottles were then capped with no headspace, using a hypodermic needle to pierce the stopper and release excess pressure and air bubbles during capping. The samples were injected with 0.2 mL of saturated HgCl$_2$ solution to inhibit biological activity in the samples during transport and storage. The samples were kept cold until they were brought back to the lab, usually in less than 6 hours. The samples were then put into cold storage (4°C) until they were analyzed (usually within 2-3 weeks).

N$_2$O concentrations were determined using a headspace equilibrium technique and a Varian CP-3800 gas chromatograph. The gas chromatograph was equipped with a Combi-Pal autosampler, 2m x 1/8" SS column packed with Hayesep D 80/100 mesh, and an ECD. P-5 mix (95% Ar, 5% CH$_4$) was used as the carrier gas.

Samples were first prepared by injecting 10mL of He into the bottle while removing 5mL of sample water, creating a headspace. The positive pressure thus created
inside the bottle, allowed headspace gas to be removed without creating a vacuum.
Bottles were then gently agitated on an orbital shaker for 90 minutes to allow the
dissolved gasses to come into equilibrium with the headspace. Finally, N₂O concentration
analyzed by injection of 2.5 mL headspace samples into the Varian CP-3800. Headspace
centration were calculated using a calibration curve created by analyzing certified
gas standards run in conjunction with the samples. The concentration of dissolved N₂O in
the original sample is then calculated using Henry’s Law, taking into account changes in
temperature and pressure between sample collection and analysis (Sander 1999; Lide &
Fredrikse 1995). The detection limit for this method is approximately 6.5 nmol – N₂O / L
and the error associated with this analysis is approximately +/- 5% at 8.5 nmol – N₂O / L.

2.2.6 Isotopic Analysis of Nitrate

Samples for isotopic analysis of nitrate were collected in 1L plastic bottles. These
samples were kept cold until they were brought back to the lab, usually less than 6 hours
after they had been collected. Once the samples had been returned to the lab, they were
promptly frozen until analysis.

These samples were analyzed for the isotopic signature of nitrate using the
method described in Environmental Geochemistry Lab Technical Procedure 30.1. In
brief, anion exchange columns are used to strip out the nitrate from a large volume of
water. The nitrate is then removed from the columns by eluting them with 10% HCl. The
solution is neutralized by the addition of silver oxide (AgO). This forms soluble silver
nitrate (AgNO₃) and a solid precipitate of silver chloride (AgCl₂). The solution is
decanted and filtered to remove any fine particles of AgCl₂ and subsequently frozen.
Following freeze-drying, the solid AgNO$_3$ is stored in amber vials until analysis by EA-IRMS to determine the $\delta^{15}$N and $\delta^{18}$O values of the nitrate in the original sample.

**2.2.7 Isotopic Analysis of Ammonium**

Samples for the isotopic analysis of NH$_4^+$ were collected in 250mL plastic bottles. The pH of these samples was adjusted to 5 – 6 in the field using a 10% sulphuric acid solution. The samples were kept on ice until they were returned to the laboratory (typically within 6 hours). Once returned to the laboratory, samples were promptly frozen until analysis.

$\delta^{15}$N – NH$_4^+$ analysis was conducted using a diffusion technique (Murray 2008, modified from Spoelstra et al. 2006). Briefly, an acidified quartz filter disk contained in a polytetrafluoroethylene (PTFE) packet was placed in a 60 mL serum bottle containing approximately 20 mL of sample water. The pH of the sample was adjusted with a buffer solution to convert the NH$_4^+$ in the sample to NH$_3$ gas. The serum bottle was capped and placed on a stir plate for approximately 10 days, after which the quartz filter disks were removed, freeze dried, and analyzed for $\delta^{15}$N – NH$_4^+$ at the University of Waterloo Environmental Isotope Laboratory (UW-EIL).

**2.2.8 Dissolved Oxygen Concentration**

Dissolved oxygen concentration was measured using the Winkler Titration technique (Azide modification, APHA 1995). Samples were collected in duplicate using glass BOD bottles with ground glass stoppers. The samples were fixed in the field using solutions containing manganese chloride, sodium hydroxide, sodium iodide and sodium azide. The bottles were kept cold and sealed with Parafilm to ensure they would not be
disturbed until they were returned to the lab, usually in less than 6 hours after sampling. 
At the lab, the samples were titrated following the standard Winkler titration technique, 
using sodium thiosulphate. The sodium thiosulphate solution was calibrated by titrating 
against a standard solution containing potassium iodide, potassium bi-iodate and 
sulphuric acid. The detection limit for this method is 0.2 mg – O₂ /L and the precision 
associated with this analysis is +/- 0.2 mg – O₂ /L.
Chapter 3:

A “Purge & Trap” Method to Extract Dissolved Nitrous Oxide for Stable Isotope Analysis

3.1 Introduction

Nitrous oxide (N$_2$O) is a powerful greenhouse gas with a global warming potential 310 times that of CO$_2$. Atmospheric concentrations have been increasing steadily during the last ~150 years by approximately 0.25%/year (Denman et al. 2007). Therefore understanding N$_2$O production in the environment is the focus of widespread research effort. N$_2$O is largely produced through microbially-mediated processes of nitrification and denitrification (Zafiriou 1990). Since isotopic fractionation factors are different for these two processes, isotopic analysis of N$_2$O can be used to determine the dominant N$_2$O production pathways (Wada & Ueda 1996, Perez et al. 2001). Furthermore, stable isotope ratios of different N$_2$O sources may be useful when quantifying the relative importance of various atmospheric sources (Stein & Yung 2003).

Many researchers have measured the isotopic composition of gaseous N$_2$O produced by cultured microorganisms (e.g. Sutka et al. 2006). Also, several studies have measured gaseous N$_2$O emitted from natural soils in field environments (e.g. Perez et al. 2001, Kim & Craig 1993, Mandernack et al. 2000). N$_2$O is highly soluble ($K_H = 0.025$ M·atm$^{-1}$ at 20°C) and is also produced and transported in aquatic environments. Current estimates indicate approximately 25% of the total anthropogenic N$_2$O flux is produced in aquatic environments such as rivers, estuaries, and coastal zones (Denman et al. 2007). Other researchers have measured stable isotope ratios of dissolved N$_2$O in aquatic systems, mostly in marine environments (e.g. Kim & Craig 1990, Yoshinari et al. 1997, ...
Popp et al. 2002). Only a very small number of studies have measured isotopic ratios of dissolved N\textsubscript{2}O in freshwater environments (Wahlen & Yoshinari 1985, Boontanon et al. 2000).

Several techniques for dissolved N\textsubscript{2}O analysis are described in the literature. Ostrom et al. (2000) and Westley et al. (2006) used a modified apparatus (Dore et al. 1998) designed for the online analysis of dissolved methane (Sansone et al 1997). Tsunogai et al (2008) also used a modified apparatus originally designed for the analysis of dissolved methane (Tsunogai et al 2000). In brief, this technique involves transferring a water sample to an extraction chamber where it is sparged with helium to remove the dissolved N\textsubscript{2}O, which is then cryogenically trapped by liquid nitrogen. The sample is then transferred through a complex series of valves and traps to a GC-IRMS. Other online methods (Casciotti et al 2002, Coplen et al 2004) have been developed for the automated isotopic analysis of nitrate samples. In these methods, nitrate is converted to N\textsubscript{2}O through bacterial denitrification and is sparged from small (~20mL) samples and transferred to a mass spectrometer for analysis.

Although the above mentioned online methods can be advantageous in certain situations, there are several disadvantages for using these methods for the routine isotopic analysis of dissolved N\textsubscript{2}O. The methods developed for the isotopic analysis of NO\textsubscript{3} by bacterial denitrification to N\textsubscript{2}O (Casciotti et al 2002, Coplen et al 2004) are not applicable to the isotopic analysis of dissolved N\textsubscript{2}O because they operate at N\textsubscript{2}O concentrations much higher than found in the natural environment. The minimum sample size for the method developed by Dore et al (1998) is 1 nmol – N\textsubscript{2}O. However, using a similar method, Tsunogai et al (2008) specifies a minimum sample size of 20 nmol - N.
This is equivalent to 1-2 L of water for dissolved N\textsubscript{2}O samples near atmospheric equilibrium.

The analysis time required to process each sample using these online methods makes them impractical for most researchers. For example, the online extraction apparatus developed by Dore et al (1998) and used by several others requires 45 minutes per sample. Likewise, the latest method of Tsunogai et al. (2008) requires 30 minutes to process each sample.

Lastly, the current online methods are only useful to researchers that have access to a dedicated mass spectrometer for dissolved N\textsubscript{2}O analysis. Many laboratories would benefit from a simple offline method that would allow them to process a large number of dissolved samples quickly. The extracted N\textsubscript{2}O could then be stored for later isotopic analysis, or if the laboratory does not have access to a mass spectrometer, shipped elsewhere for analysis.

Presented here is a simple offline method for extracting dissolved N\textsubscript{2}O from freshwater samples for stable isotope analysis ($^{15}$N/$^{14}$N and $^{18}$O/$^{16}$O) from samples containing as little as 3 nmol N\textsubscript{2}O. This limit is much lower than that obtained by Tsunogai et al (2008), Casciotti et al (2002) and Coplen et al (2004). The sensitivity of this method is almost equal to that of Dore et al (1998), but the sample processing time is much faster with our method. Samples can be processed in as little as 10 minutes, and the simplicity of our method makes it feasible to construct multiple purge and trap stations, allowing several samples to be processed simultaneously. Additionally, processing samples offline allows for the stable storage of extracted N\textsubscript{2}O, which can then be shipped to external laboratories for isotopic analysis.
3.2 Sampling Method

Water samples for dissolved N$_2$O isotopic analysis are collected in either 160mL serum (Wheaton # 223748) or 500mL media (Corning # 1395-500) bottles, depending on the expected concentration. Sample bottles are filled and capped underwater to prevent the entrapment of air. Groundwater samples may be collected by pumping and filling the bottles to overflowing; however, care must be taken to prevent degassing or introduction of air into the sample. A red rubber stopper (Vacutainer™) is used for serum bottles and a black rubber lyophilization stopper (Wheaton # 224100-503) is used for media bottles. Once capped, samples are preserved by injection of a saturated HgCl$_2$ solution (2 mL HgCl$_2$ solution per litre of sample). Samples are stored at 4°C until they are extracted (typically within 72 hours).

3.3 Extraction Apparatus

A “purge and trap” system (Figure 3.1) is used for isolating dissolved N$_2$O. Dissolved N$_2$O gas is extracted directly from the original sample bottle by bubbling with ultra-pure helium (He), at a flow rate of 300mL/min for 10 – 20 minutes (depending on sample size). A stainless steel frit (3 micron pore size – Aimark Travers Ltd # 459500) is used to increase the extraction efficiency of the helium stream. The frit is attached to 1/16” ID stainless steel tubing threaded through a butyl-blue rubber stopper (Belco Glass Inc # 2048-11800). The sample bottle is attached to the system by uncapping the sample and inserting the stopper with the frit. Due to the high solubility of N$_2$O and the low equilibrium fractionation factors for $^{15}$N and $^{18}$O (Inoue & Mook 1994), there is
insignificant and inconsequential loss of N₂O during attachment of the sample bottle to the purge and trap system.

Figure 3.1: Schematic flow diagram of purge and trap system.
Valve A: Needle Valve to control He flow rate (Swagelok #SS-SS1)
Valve B: 3-way plastic stopcock with luer fittings (Kontes Part # KT420163-4503)
Valve C: 2-way valve (Swagelok #SS-41GS1)
Valve D: 3-way valve (Swagelok #SS-41XS1)
Valve E: Mininert valve with luer fittings (Vici #654051)
Valve F: Mininert valve with luer fittings (Vici #654051)
Nafion Dryer (Permapure #MD-110-24P-4)

The extracted gas passes through several stages of purification before N₂O is trapped cryogenically. A water trap is used to prevent liquid water from reaching the downstream nafion dryer and chemical traps. The water trap consists of a small glass vial fitted with a rubber stopper. The nafion membrane is permeable with respect to water, but impermeable to other gases. The gas stream is “dried” by a counter-current flow of dry air across the nafion membrane. The chemical trap contains Carbosorb to remove CO₂ and magnesium perchlorate to remove any residual water vapour. N₂O is trapped cryogenically in a storage vessel cooled with liquid nitrogen. The storage vessel consists
of a 20mL round bottom vial (Restek #21162), containing a 3 cm layer of pyrex wool in between two layers of glass beads, and capped with a butyl-blue rubber stopper and crimp seal. Preliminary method development indicated that, under high rates of He flow (>100mL/min), cryogenic trapping of N₂O is generally inefficient using a simple coiled loop of tubing. For other cryogenic trapping methods, glass fibre thimbles have been used to improve trapping efficiency (Brenninkmeijer 1991, Brenninkmeijer & Röckmann 1996). With our method, the pyrex wool and glass beads greatly increase the surface area, and allow for extremely efficient cryogenic trapping of N₂O, even under high rates of flow (> 300 mL/min). Before use, storage vessels are evacuated to ~0.1 mBar to remove any N₂O. A vessel can be re-used several times provided it is re-evacuated between uses. N₂O samples are stored in these vessels in darkness at room temperature. Repeat analysis of extracted samples yields the same isotope values after 5 months of storage indicating no sample degradation occurs under these conditions.

### 3.4 Extraction Method

Valves B, C and D are first positioned to allow He to flow through all lines and purge any air from the system (Figure 3.2a). After 30 seconds, valve C is closed, with valves B and D remaining in position to allow the He flow to bypass the metal frit and flow directly through the traps (Figure 3.2b). A storage vessel is attached to the system using a 1 inch, 23 gauge needle attached to valve E. The needle is positioned so its tip is protruding about 3mm through the stopper, close to the vial wall; this positioning helps prevent the needle from freezing up and becoming plugged. A 4 inch, 22 gauge needle (Air-Tite Products Co. #N224) attached to valve F is inserted through the storage vessel
stopper, and pushed to the bottom of the vial. First valve E, then valve F is opened, and the storage vessel is immersed in liquid nitrogen to the top of the pyrex wool layer. The stopper is removed from the sample bottle, and the stopper with the attached metal frit is inserted into the bottle. The line connecting valve B to the metal frit is disconnected at valve B, and a 20mL syringe is attached to the line using a luer lock fitting. A 15mL He headspace is created in the sample bottle by momentarily opening valve C, and removing the excess water through the syringe. The syringe is removed, and the line reattached. Valves B and D are positioned to allow He to flow through the metal frit in the sample bottle and through the various traps (Figure 3.2c). The sample is purged at a flow rate of 300mL/min for 10 minutes for 160mL bottles or 20 minutes for 500mL bottles. He flow rate is monitored at the outlet and is adjusted using the needle valve (valve A). Occasionally, heat may need to be applied to the top of the storage vessel to prevent freezing and plugging of the inlet needle. After the sample has been purged, valves B and D are positioned to allow the He flow to bypass the sample bottle, and to flush any remaining N\textsubscript{2}O from the lines and traps (Figure 3.2b). After 30 seconds, valve E is closed, and any non-condensable gases are removed using a hand vacuum pump attached at valve F. Once a vacuum of approximately 0.3 Bar is achieved, valve F is closed and the storage vessel is removed from the liquid nitrogen bath. After the storage vessel has reached room temperature, the needles are removed, and 20mL of He is injected into the vial to create a positive pressure.
3.5 Concentration and Isotopic Analysis of N\textsubscript{2}O

Water samples were analyzed for dissolved N\textsubscript{2}O concentration with a headspace equilibrium technique using a Varian CP-3800 gas chromatograph. The gas chromatograph is equipped with an ECD and P-5 mix (95% Ar, 5% CH\textsubscript{4}) is used as the carrier gas. Samples are bracketed by N\textsubscript{2}O standards with known concentration (0.1, 0.5, 1.0 and 10 ppm N\textsubscript{2}O).

Extracted N\textsubscript{2}O samples in storage vessels are analyzed for $\delta^{15}$N and $\delta^{18}$O by injection of 3 – 15 nmol of N\textsubscript{2}O into a GV Trace Gas preconcentrator system, attached to a GV Isoprime mass spectrometer at the EIL-UW. Injection volumes are dependant on initial dissolved N\textsubscript{2}O concentration. The Trace Gas system further purifies the sample and chromatographically separates N\textsubscript{2}O from any remaining trace CO\textsubscript{2}. Raw molecular ratios (mass 44, 45 and 46) from the mass spectrometer are converted to isotopic ratios ($^{15}$N/$^{14}$N and $^{18}$O/$^{16}$O) using the data correction method described by Kaiser et al. (2003). Due to the lack of available internationally recognized N\textsubscript{2}O isotope standards, N\textsubscript{2}O
isotopic data is corrected using internal standards bracketing the isotopic ratios of nearly all natural samples (EGL-5: 10ppm N₂O, δ¹⁵N = 2.76‰, δ¹⁸O = 40.38; HAN2O-2: ~1700 ppm N₂O, δ¹⁵N = -92.64‰, δ¹⁸O = 20.52‰). δ¹⁵N is reported relative to atmospheric N₂, and δ¹⁸O is reported relative to VSMOW.

3.5.1 Calibration of Internal Isotope Standards

Internal N₂O standards are calibrated against tropospheric N₂O, which has been thoroughly characterized as having an isotopic composition of δ¹⁵N = (6.72 ± 0.12) ‰ and δ¹⁸O = (44.62 ± 0.21) ‰ (Kaiser et al. 2003). Tropospheric N₂O samples were collected in 500mL sample bottles by filling with air from the CEIT building rooftop at the University of Waterloo. Kaiser et al. (2003) did not observe local source contamination of tropospheric N₂O in samples taken from the rooftop of the Max Planck Institute of Chemistry in Mainz, Germany. Since there are no known point sources of N₂O nearby, air samples obtained at the University of Waterloo are assumed to be representative of tropospheric N₂O. N₂O was extracted from sample bottles using the same method for dissolved N₂O samples. The only difference in procedure was the attachment of sample bottles to the system using a pair of needles in place of the metal frit assembly, thereby allowing sample bottles to remain sealed during N₂O extraction. Once the tropospheric N₂O was trapped in storage vessels, care was taken to ensure that any non-condensable gases were purged from the vessels while they were still submerged in liquid nitrogen. Due to the low vapour pressure of oxygen (O₂) at the temperature of liquid nitrogen (-196 °C), a vacuum of less than 0.2 Bar is needed to ensure complete removal of any liquid O₂ formed (Lide 2008). A mechanical vacuum pump was used to
achieve a vacuum greater than 0.001 Bar. Internal N\textsubscript{2}O standards were analyzed on the Trace Gas system during the same run as the tropospheric N\textsubscript{2}O samples. Calibration used a simple calculation adapted from Werner and Brand (2001), and the $\delta^{15}$N and $\delta^{18}$O values of tropospheric N\textsubscript{2}O determined by Kaiser et al. (2003).

Tropospheric N\textsubscript{2}O collected and analyzed on different dates (over a period of 5 months with varying wind directions) produced the same results (n=15, standard deviation: $\delta^{15}$N=0.2‰, $\delta^{18}$O=0.5‰). This reproducibility further supports the assumption that air samples collected at the University of Waterloo are not affected by local sources of N\textsubscript{2}O, and are thus representative of tropospheric N\textsubscript{2}O composition.

### 3.6 Method Testing

Method accuracy was tested by extracting N\textsubscript{2}O from degassed ultra-pure de-ionized water (Nanopure\textsuperscript{TM}) spiked with internal N\textsubscript{2}O gas standards of known isotopic compositions (EGL-6: 1000 ppm N\textsubscript{2}O, $\delta^{15}$N = 1.04‰, $\delta^{18}$O=40.30‰; EGL-5: 10 ppm N\textsubscript{2}O, $\delta^{15}$N = 2.76‰, $\delta^{18}$O=40.38‰). Prior to spiking with N\textsubscript{2}O isotope standards, Nanopure\textsuperscript{TM} water was degassed by sparging with He for 20min, followed by application of a vacuum (0.2 Bar) while being stirred for 2 hours. A 60mL sample of degassed water was subsequently analyzed for N\textsubscript{2}O concentration and was found to be less than the detection limit (<1.5 nmol/L). A 160mL serum bottle was filled with degassed water, and a 10mL helium headspace was created. EGL-6 standard gas (5.5mL) was injected into the headspace and the bottle placed on an orbital shaker for 2 hours to dissolve the N\textsubscript{2}O. The resulting dissolved N\textsubscript{2}O solution was diluted with degassed Nanopure\textsuperscript{TM} water to produce a solution with a N\textsubscript{2}O concentration representative of natural samples (approximately 30 -
40 nmol/L). Five 500mL sample bottles were filled with the mixture, and the N\textsubscript{2}O subsequently extracted using the described method. To determine extraction efficiency, samples were extracted using increasing purging times. The concentration of dissolved N\textsubscript{2}O remaining in the bottle, and the isotopic composition of the extracted N\textsubscript{2}O was measured for each purging duration.

The isotopic composition of the extracted N\textsubscript{2}O was within error of that of the standard gas (Figure 3.3a). Because of the slight equilibrium enrichment factors and the high solubility of N\textsubscript{2}O, the dissolved N\textsubscript{2}O was expected to be enriched by 0.07‰ and 0.11‰ for δ\textsuperscript{15}N and δ\textsuperscript{18}O with respect to the standard gas (Inoue & Mook 1994). The δ\textsuperscript{15}N of the extracted N\textsubscript{2}O was consistently slightly higher than expected, however the maximum observed difference between the extracted N\textsubscript{2}O and the standard gas (0.6‰) was similar to the precision for δ\textsuperscript{15}N of this analysis. The isotopic composition of extracted N\textsubscript{2}O was not affected by incomplete sample recovery. For example, even with a sample extraction efficiency of only 70%, the isotopic composition of the extracted N\textsubscript{2}O was not different from the N\textsubscript{2}O standard.

**Figure 3.3:** N\textsubscript{2}O extractions of degassed water spiked with EGL-6 N\textsubscript{2}O isotope standard
To determine the optimal purging time for 160mL bottles a similar procedure was followed and only N₂O concentrations were measured (Figure 3.3b). Isotopic composition of N₂O extracted from 160mL serum bottles for the optimal time of 10 minutes was again the same as the source gas (EGL-5 standard gas - Table 3.1). Based on these results, purge times of 10 and 20 minutes are used for extracting dissolved N₂O from 160mL and 500mL samples, respectively.

A second experiment was conducted to test the stability of dissolved N₂O samples in long-term storage. Bottles were filled with samples of Nanopure™ or water from the Grand River downstream of Kitchener, Ontario. Nanopure™ water samples contained naturally high levels of N₂O, due to high dissolved N₂O concentrations in municipal tap water in Waterloo, Ontario. Samples without a headspace were stored in 160mL serum bottles capped with red rubber (Vacutainer™) stoppers. Samples were preserved with varying amounts of HgCl₂ saturated solution, at concentrations greater than and less than the concentration typically used for sample preservation (0 to 3.125 mL solution / L sample). Duplicate and triplicate samples were extracted and analyzed at intervals ranging from 2 weeks to 2 months (Table 3.2). There was no observable change in δ¹⁵N and δ¹⁸O of N₂O over the storage period for any treatments.

The results of the method testing also provide a measure of the precision of this method, not only within a single run, but also between runs. The average standard deviation for both sample sets indicates the average precision for the isotopic analysis of N₂O is 0.4‰ and 0.8‰ for δ¹⁵N and δ¹⁸O respectively.
Table 3.1: Results of the test extractions using degassed Nanopure™ water spiked with EGL-5 in 160mL serum bottles. Recovery is calculated by measuring the sample peak height on the mass spectrometer, calibrated against reference standards of known concentration.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Purging Time (minutes)</th>
<th>N₂O Concentration (nmol/L)</th>
<th>δ¹⁵N-N₂O (‰)</th>
<th>δ¹⁸O-N₂O (‰)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGL-5 Spiked Water</td>
<td>10</td>
<td>38.6</td>
<td>2.8</td>
<td>40.4</td>
<td>103.6</td>
</tr>
<tr>
<td>EGL-5 Spiked Water</td>
<td>10</td>
<td>38.6</td>
<td>2.4</td>
<td>40.2</td>
<td>71.9</td>
</tr>
<tr>
<td>EGL-5 Spiked Water</td>
<td>10</td>
<td>42</td>
<td>2.7</td>
<td>40.7</td>
<td>101.8</td>
</tr>
<tr>
<td>EGL-5 Spiked Water</td>
<td>10</td>
<td>42</td>
<td>3.0</td>
<td>40.5</td>
<td>81.7</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td>40.30</td>
<td>2.7</td>
<td>40.5</td>
<td>89.7</td>
</tr>
</tbody>
</table>

Table 3.2: Results of the sample storage experiment. Recovery is calculated by measuring the sample peak height on the mass spectrometer, calibrated against reference standards of known concentration. Recovery could not be accurately calculated for the Nanopure samples because only a partial sample was injected into the mass spectrometer.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Storage Time (Days)</th>
<th>Preservative (mL of HgCl₂ soln/L)</th>
<th>N₂O Concentration (nmol/L)</th>
<th>δ¹⁵N-N₂O (‰)</th>
<th>δ¹⁸O-N₂O (‰)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanopure</td>
<td>0</td>
<td>0</td>
<td>220</td>
<td>-12.9</td>
<td>-13.9</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>0</td>
<td>0</td>
<td>220</td>
<td>-14.0</td>
<td>-12.2</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>0</td>
<td>0</td>
<td>220</td>
<td>-13.4</td>
<td>-12.5</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>14</td>
<td>0</td>
<td>218</td>
<td>-13.6</td>
<td>-13.0</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>14</td>
<td>0</td>
<td>218</td>
<td>-13.7</td>
<td>-12.5</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>14</td>
<td>0</td>
<td>218</td>
<td>-12.8</td>
<td>-13.3</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>40</td>
<td>0</td>
<td>210</td>
<td>-12.9</td>
<td>-13.0</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>40</td>
<td>0</td>
<td>210</td>
<td>-13.1</td>
<td>-13.2</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>40</td>
<td>0</td>
<td>210</td>
<td>-12.5</td>
<td>-12.8</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>14</td>
<td>1.25</td>
<td>205</td>
<td>-12.4</td>
<td>-12.0</td>
<td>N/A</td>
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<tr>
<td>Nanopure</td>
<td>14</td>
<td>1.25</td>
<td>205</td>
<td>-12.7</td>
<td>-16.1</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>14</td>
<td>1.25</td>
<td>205</td>
<td>-13.5</td>
<td>-12.8</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>40</td>
<td>1.25</td>
<td>213</td>
<td>-12.7</td>
<td>-13.6</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>40</td>
<td>1.25</td>
<td>213</td>
<td>-12.9</td>
<td>-13.6</td>
<td>N/A</td>
</tr>
<tr>
<td>Nanopure</td>
<td>40</td>
<td>1.25</td>
<td>213</td>
<td>-13.0</td>
<td>-13.3</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td>213</td>
<td>-13.1</td>
<td>-13.2</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

|                  | Standard Deviation   | 5.6                               | 0.47                     | 0.97         |
| Grand River       | 0                   | 1.25                              | 24.2                     | -0.5         | 40.6         | 103.3        |
| Grand River       | 0                   | 1.25                              | 24.2                     | -0.4         | 40.7         | 96.1         |
| Grand River       | 14                  | 1.25                              | 25                       | -0.6         | 40.4         | 96.8         |
| Grand River       | 14                  | 1.25                              | 25                       | -1.1         | 40.4         | 90.9         |
| Grand River       | 40                  | 1.25                              | 26.2                     | -0.6         | 40.5         | 93.7         |
| Grand River       | 40                  | 1.25                              | 26.2                     | -1.0         | 40.5         | 88.8         |
| Grand River       | 68                  | 1.25                              | 22                       | -1.0         | 39.5         | 104.9        |
| Grand River       | 68                  | 1.25                              | 22                       | -1.8         | 38.2         | 97.0         |
| Grand River       | 14                  | 3.125                             | 24.9                     | -0.9         | 40.0         | 99.8         |
| Grand River       | 14                  | 3.125                             | 24.9                     | -1.1         | 40.3         | 93.2         |
| Grand River       | 40                  | 3.125                             | 25.7                     | -1.0         | 39.5         | 92.5         |
| Grand River       | 40                  | 3.125                             | 25.7                     | -1.0         | 40.3         | 93.2         |
| Grand River       | 68                  | 3.125                             | 22.6                     | -1.0         | 40.2         | 97.1         |
| Grand River       | 68                  | 3.125                             | 22.6                     | -1.0         | 39.9         | 104.8        |
| **Average**       |                     | 24.4                               | -0.9                     | 40.1         | 96.6         |

|                  | Standard Deviation   | 1.50                              | 0.34                     | 0.65         |
|                  | Precision (average standard deviation) | 0.4                          | 0.8                      |              |

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3.7 Field Data

Samples of dissolved N\textsubscript{2}O were collected at two field sites in Southern Ontario, Canada and analyzed using the method described above (Table 3.3).

The Grand River stretches over 300 km and drains a watershed of 6800 km\textsuperscript{2}. The watershed is predominantly agricultural and 26 municipal wastewater treatment plants (WWTPs) discharge effluent into the river and its tributaries before the river drains into Lake Erie. Nitrogen inputs from both agriculture and the WWTPs contribute to elevated NO\textsubscript{3}\textsuperscript{-} concentrations and the production of riverine N\textsubscript{2}O. Water samples were collected from one site upstream of a major urban centre on 53 different occasions between May 2006 and June 2007. NO\textsubscript{3}\textsuperscript{-} concentrations ranged from 0.8 to 6.5 mg-N/L and dissolved N\textsubscript{2}O concentrations from 8.6 to 44 nmol/L. CH\textsubscript{4} concentration was elevated in these samples (57 to 740 nmol/L), reflecting production in anoxic river sediments. $\delta^{15}$N – N\textsubscript{2}O of the river samples ranged from -5.7 to 6.4‰, and $\delta^{18}$O – N\textsubscript{2}O ranged from 38.2 to 48.8‰. Analyses of duplicate samples indicate an average precision of 0.2 and 0.3‰ for $\delta^{15}$N and $\delta^{18}$O, respectively. These results indicate that our extraction method is reliable for the isotopic analysis of samples with low concentrations of dissolved N\textsubscript{2}O.

**Table 3.3:** Stable isotope analysis of dissolved N\textsubscript{2}O from two field sites in Southern Ontario, Canada.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Nitrate concentration (mg N/L)</th>
<th>N\textsubscript{2}O Concentration (nmol/L)</th>
<th>$\delta^{15}$N – N\textsubscript{2}O (range)</th>
<th>$\delta^{18}$O – N\textsubscript{2}O (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putnam Groundwater</td>
<td>0.5 to 61</td>
<td>48 to 28,000</td>
<td>-31.2‰ to 23.4‰</td>
<td>35.7‰ to 74.3‰</td>
</tr>
<tr>
<td>Grand River</td>
<td>0.8 to 6.5</td>
<td>8.6 to 44</td>
<td>-5.7‰ to 6.4‰</td>
<td>38.2‰ to 48.8‰</td>
</tr>
</tbody>
</table>
Groundwater samples were obtained from piezometers (1.5 to 4m below ground surface) in a groundwater plume highly contaminated with NO$_3^-$ that originated from a large manure composting facility near Putnam, Ontario (Robertson & Schiff 2008). Dissolved N$_2$O samples were collected along a transect of multilevel piezometers (n=36) within the groundwater plume in September 2006. In addition to high nitrate concentrations (ranging from 0.5 to 61 mg-N/L), concentrations of dissolved CH$_4$ and CO$_2$ were also high, ranging from 20 to 15,500 nmol/L, and 0.2 to 4.2 mmol respectively.

Dissolved N$_2$O concentration was very high in these samples, ranging from 48 to 27,600 nmol/L. The $\delta^{15}$N – N$_2$O values of these samples ranged from -31.2 to 23.4‰, and the $\delta^{18}$O – N$_2$O values ranged from 33.7 to 74.3‰. Analyses of N$_2$O in duplicate groundwater samples yielded an average precision of 0.6 and 0.9‰ for $\delta^{15}$N and $\delta^{18}$O, respectively. Although the overall precision for the groundwater samples was slightly lower than for the river samples, groundwater samples are more susceptible to degassing or introduction of air. These results indicate that our extraction method is suitable for the extraction of N$_2$O from samples containing elevated levels of nitrate, CH$_4$ and CO$_2$.

The isotope data on N$_2$O from the Grand River covers a narrow range of $\delta^{15}$N and $\delta^{18}$O values, while the data collected at the groundwater site exhibits a wide range in both isotope ratios (Table 3.3). $\delta^{18}$O - N$_2$O values from the groundwater site tends to be more enriched in $^{18}$O than the Grand River $\delta^{18}$O – N$_2$O values. Also, the very large range in $\delta^{15}$N values at the Putnam site indicates that the $\delta^{15}$N of the nitrogen source and N$_2$O production processes are variable within the groundwater plume.

The very narrow range in $\delta^{15}$N and $\delta^{18}$O values from the Grand River N$_2$O samples indicates the nitrogen source and processes of N$_2$O production at this site are
relatively constant with time. Although supersaturated with respect to atmospheric equilibrium, N₂O in the Grand River has a similar isotopic composition to tropospheric N₂O ($\delta^{15}N = 6.72, \delta^{18}O = 44.62$) (Kaiser et al. 2003) likely reflecting the importance of gas exchange with the atmosphere. River samples with the highest concentration of N₂O were the most distinct from the isotopic composition of tropospheric N₂O, indicating that, at higher dissolved N₂O concentrations, the isotopic exchange of tropospheric N₂O and river water becomes less noticeable.

3.8 Conclusions

There is limited data published on the isotopic composition of N₂O in aqueous systems, partially due to the difficulty in preserving and preparing samples for analysis. The purge and trap method described here allows for the simple and precise determination of the stable isotope composition of dissolved N₂O. The technique provides an inexpensive and effective method for researchers to expand their capacity for analysis of dissolved N₂O. This method is especially useful for researchers who do not have direct access to a mass spectrometer, allowing them to ship the extracted N₂O samples to an external isotopic laboratory for analysis. Furthermore, our method allows for a substantial increase to sample throughput as compared to current published methods. Although this method was tested for freshwater, there are no known reasons to prevent the method from being applied to marine samples.

While it is possible to calibrate internal standards against tropospheric N₂O, an internationally recognized N₂O isotopic standard needs to be developed. Such a standard
would ensure the compatibility and consistency of N₂O isotope analysis between laboratories, allowing for the direct comparison of field data generated worldwide.

The method was shown to be reliable for the extraction and analysis of dissolved N₂O from field samples at relatively low concentrations (as low as 8.6 nmol – N₂O/L). Elevated concentrations of dissolved NO₃⁻, CH₄ and CO₂ do not interfere with the isotopic analysis of dissolved N₂O using this method.

Field data indicates the isotopic composition of dissolved N₂O varies widely within and between field sites. Systems open to the atmosphere are strongly influenced by gas exchange, indicating the isotopic exchange of tropospheric N₂O may be an important control on the isotopic composition of dissolved N₂O at low concentrations (< 15 to 30 nmol/L). Stable isotope analysis of dissolved N₂O has strong potential for identifying N₂O sources and production processes in aquatic systems.
Chapter 4:
A Dynamic Model to Determine the δ\textsubscript{15}N and δ\textsubscript{18}O of Dissolved Nitrous Oxide in Response to Tropospheric Gas Exchange

4.1 Introduction

Nitrous oxide (N\textsubscript{2}O) is a powerful greenhouse gas (310 times more potent than CO\textsubscript{2} over a 100-year timeline) and its concentration has been increasing in the atmosphere at a rate of 0.25% year\textsuperscript{-1} over the last 150 years (Denman et al. 2007). N\textsubscript{2}O is produced through both nitrification and denitrification, therefore, it is a useful indicator of these processes in the environment (Stein & Yung 2003). Since the various microbial pathways of N\textsubscript{2}O production have different isotopic enrichment factors, the isotopic analysis of N\textsubscript{2}O can potentially distinguish N\textsubscript{2}O produced through these various pathways (Wada & Ueda 1996). Recent studies have measured the isotopic ratios of N\textsubscript{2}O produced in soil environments (e.g. Mandernack et al. 2000, Perez et al. 2001 and Bol et al. 2003). N\textsubscript{2}O is also produced in aquatic environments, though most studies of the isotopic composition of N\textsubscript{2}O in aquatic systems have been limited to the oceans (Kim & Craig 1990, Naqvi et al. 1998, Yohanari et al. 1997, Westley et al. 2006, Dore et al. 1998). Although N\textsubscript{2}O production in rivers and estuaries is a significant portion of the global N\textsubscript{2}O budget (approximately 1.5 Tg – N/year, Kroeze et al. 2005), only one study has reported δ\textsubscript{15}N and δ\textsubscript{18}O of dissolved N\textsubscript{2}O in a river (Boontanon et al. 2000).

The isotopic composition (\textsuperscript{15}N/\textsuperscript{14}N and \textsuperscript{18}O/\textsuperscript{16}O) of dissolved N\textsubscript{2}O is affected by gas exchange, and therefore is not equal to the isotopic composition of N\textsubscript{2}O produced in that environment. Also, the isotopic composition of dissolved N\textsubscript{2}O is not indicative of the
N₂O flux emitted to the atmosphere, due to the kinetic fractionation factors associated with N₂O evasion (Inoue and Mook 1994). Previous work to study the isotopic composition of N₂O produced in aqueous systems has not considered the isotopic effects of gas exchange (e.g. Boontanon et al. 2000).

The isotopic ratios of N₂O emitted through diffusive gas exchange with the atmosphere are controlled by the isotopic ratios of dissolved N₂O and kinetic gas exchange fractionation factors, while the dissolved N₂O isotopic composition is controlled by the composition of the source N₂O, the equilibration with N₂O from the atmosphere, and the evasion of dissolved N₂O to the atmosphere. This paper will elucidate the relationship between the isotopic ratios of source, dissolved, and emitted N₂O, to allow for proper interpretation of dissolved N₂O isotope data.

4.4.1 Stable Isotopes of N₂O

The most abundant isotopologues of N₂O are \(^{14}\text{N}^{14}\text{N}^{16}\text{O}, ^{15}\text{N}^{14}\text{N}^{16}\text{O}, ^{14}\text{N}^{15}\text{N}^{16}\text{O}\) and \(^{14}\text{N}^{14}\text{N}^{18}\text{O}\). The isotopic ratios, \(^{15}\text{N}/^{14}\text{N}\) and \(^{18}\text{O}/^{16}\text{O}\), are as follows:

\[
15R = \frac{[^{15}\text{N}^{14}\text{N}^{16}\text{O}] + [^{14}\text{N}^{15}\text{N}^{16}\text{O}]}{2[^{14}\text{N}^{14}\text{N}^{16}\text{O}]} = \frac{[^{15}\text{N}_2\text{O}]}{[^{14}\text{N}_2\text{O}]} \tag{4.1}
\]

\[
18R = \frac{[^{14}\text{N}^{14}\text{N}^{18}\text{O}]}{[^{14}\text{N}^{14}\text{N}^{16}\text{O}]} = \frac{[\text{N}_2^{18}\text{O}]}{[\text{N}_2^{16}\text{O}]} \tag{4.2}
\]

Where \([^{14}\text{N}^{14}\text{N}^{16}\text{O}],[^{15}\text{N}^{14}\text{N}^{16}\text{O}],[^{14}\text{N}^{15}\text{N}^{16}\text{O}]\) and \([^{14}\text{N}^{14}\text{N}^{18}\text{O}]\) represent the concentrations of the various N₂O isotopologues. Note that \(^{15}R\) is the bulk \(^{15}\text{N}/^{14}\text{N}\) ratio and represents an average ratio for the two \(^{15}\text{N}\) isotopomers. Although it is possible to measure the isotopic ratio of the two \(^{15}\text{N}\) isotopomers independently (Toyoda & Yoshida 1999, Brenninkmeijer & Rockmann 1999), the gas exchange fractionation factors are not affected by the intramolecular distribution of \(^{15}\text{N}\) (Inoue and Mook 1994). Using the bulk
$^{15}$R greatly simplifies the gas exchange fractionation calculations. Also, many laboratories do not have the capability to measure the intramolecular distribution of $^{15}$N, while analysis of the bulk $^{15}$N/$^{14}$N of N$_2$O is much more accessible.

Isotopic ratios are reported as $\delta$ values in permil ($\permil$) units, relative to air – N$_2$ and Vienna Standard Mean Ocean Water (VSMOW).

$$\delta^{15}N = \left( \frac{R_{\text{sample}}^{15}}{R_{\text{AIR-N}_2}^{15}} - 1 \right)$$  \hspace{1cm} (4.3)

$$\delta^{18}O = \left( \frac{R_{\text{sample}}^{18}}{R_{\text{VSMOW}}^{18}} - 1 \right)$$  \hspace{1cm} (4.4)

### 4.2 Dynamic Isotope Model for Dissolved N$_2$O

A simple three box model (SIDNO, Stable Isotopes of Dissolved Nitrous Oxide) was created using Stella modeling software (version 9.0.1, http://www.iseesystems.com) in order to study the relationships between the isotopic ratios of source, dissolved and emitted N$_2$O. This model is an adaptation of the isotopic gas exchange portion of the PoRGy model (Venkiteswaran et al. 2007), which successfully simulated the stable isotope dynamics of dissolved oxygen in response to photosynthesis, respiration and gas exchange in aquatic ecosystems. One box in SIDNO is used for the total mass of dissolved N$_2$O and two additional boxes in the model for the dissolved masses of the heavy isotopologues ($^{15}$N$_2$O, and N$_2^{18}$O – Figure 4.1). The depth and surface area of the boxes in the model are set by the user. This allows a dissolved concentration to be calculated from the mass contained in each box and its volume.
Figure 4.1: A simplified representation of the SIDNO model. The three boxes represent dissolved masses of bulk N₂O and heavy isotopologues (¹⁵N₂O, N₂¹⁸O). The flows from the left represent N₂O production from the aquatic source, and the two-way flows on the right represent gas exchange. The ratios between the masses in the boxes are used to calculate the isotopic ratios of dissolved N₂O. The flow rates for bulk N₂O, ¹⁵N₂O, and N₂¹⁸O in and out of the respective boxes are not equal, and the ratios between the flows are the various isotopic ratios of source and emitted N₂O.

4.2.1 Stable Isotope Dynamics of N₂O Production

In SIDNO, the masses and magnitude of the flows of ¹⁵N₂O and N₂¹⁸O relative to bulk N₂O are used to calculate the isotopic composition of source, dissolved and emitted N₂O. For example, the isotopic ratios of dissolved N₂O in the model are as

\[ ^{15}R_{dissolved} = \frac{[^{15}N_2O]_{dissolved}}{[N_2O]_{dissolved}} = \frac{[^{15}N_2O]_{dissolved}}{[N_2O]_{dissolved} - [^{15}N_2O]_{dissolved}} \]  \hspace{1cm} (4.5)

\[ ^{18}R_{dissolved} = \frac{[N_2^{18}O]_{dissolved}}{[N_2^{16}O]_{dissolved}} = \frac{[N_2^{18}O]_{dissolved}}{[N_2^{16}O]_{dissolved} - [N_2^{18}O]_{dissolved}} \]  \hspace{1cm} (4.6)

Where [N₂O_dissolved], [¹⁵N₂O_dissolved], and [N₂¹⁸O_dissolved] are the concentrations of these species in their respective boxes in the model (Figure 4.1). Since the model is mass
based, the concentration is calculated by dividing the mass in each box by a user defined volume.

In SIDNO, \( \text{N}_2\text{O} \) production is represented by three separate inflows, one for bulk \( \text{N}_2\text{O} \) production, and two for the less abundant isotopologues (Figure 4.1). Bulk \( \text{N}_2\text{O} \) inflow rate and its isotopic composition is set by the user, and the inflows to the dissolved \( ^{15}\text{N}_2\text{O} \) and \( \text{N}_2^{18}\text{O} \) boxes are

\[
\text{Production Rate } ^{15}\text{N}_2\text{O} = \text{Production Rate } \text{N}_2\text{O} \times \left( \begin{array}{c}
^{15}\text{R}_\text{source} \\
1 + ^{15}\text{R}_\text{source}
\end{array} \right) \tag{4.7}
\]

\[
\text{Production Rate } \text{N}_2^{18}\text{O} = \text{Production Rate } \text{N}_2\text{O} \times \left( \begin{array}{c}
^{18}\text{R}_\text{source} \\
1 + ^{18}\text{R}_\text{source}
\end{array} \right) \tag{4.8}
\]

Where Production Rate \( \text{N}_2\text{O} \), Production Rate \( ^{15}\text{N}_2\text{O} \), and Production Rate \( \text{N}_2^{18}\text{O} \) are the inflow rates to the bulk \( \text{N}_2\text{O} \), \( ^{15}\text{N}_2\text{O} \) and \( \text{N}_2^{18}\text{O} \) boxes, respectively.

### 4.2.2 Stable Isotope Dynamics of Gas Exchange

The isotopic composition of the gas exchange flux is controlled by the kinetic fractionation factors for evasion (\( \alpha_{ev} = R_{\text{evaded}}/R_{\text{dissolved}} \)) and invasion (\( \alpha_{in} = R_{\text{invaded}}/R_{\text{gas}} \)) of \( \text{N}_2\text{O} \) (Inoue & Mook 1994, Table 4.1). These two factors are related to the equilibrium fractionation factor by the following relationship \( \alpha_{eq} = (R_{\text{gas}}/R_{\text{aq\_solution}}) = (\alpha_{ev}/\alpha_{in}) \).

**Table 4.1:** Kinetic and equilibrium fractionation factors for gas exchange of \( \text{N}_2\text{O} \) (Inoue & Mook 1994).

<table>
<thead>
<tr>
<th></th>
<th>( \alpha_{eq} )</th>
<th>( \alpha_{in} )</th>
<th>( \alpha_{ev} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Invasion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ^{15}\alpha_{in} )</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ^{18}\alpha_{in} )</td>
<td>0.9992</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Evasion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ^{15}\alpha_{ev} )</td>
<td>0.9993</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ^{18}\alpha_{ev} )</td>
<td>0.9981</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Equilibrium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ^{15}\alpha_{eq} )</td>
<td>0.99925</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ^{18}\alpha_{eq} )</td>
<td>0.99894</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
These fractionation factors are not temperature dependent over the range of 0 to 44.5°C (Inoue and Mook 1994). The equilibrium fractionation factors ($\alpha_{eq}^{15}$ and $\alpha_{eq}^{18}$) can be used to predict the isotopic ratios of dissolved N$_2$O for a solution in equilibrium with the atmosphere. The $\delta^{15}$N and $\delta^{18}$O of tropospheric N$_2$O is 6.72‰ +/- 0.12 and 44.62‰ +/- 0.21, respectively (Kaiser et al. 2003). An equilibrium saturated solution will be slightly enriched in $^{15}$N and $^{18}$O relative to tropospheric N$_2$O, with a $\delta^{15}$N and $\delta^{18}$O of 7.48‰ and 45.73‰, respectively.

The net N$_2$O flux from the atmosphere into the dissolved phase can be calculated (equation 4.9).

$$\text{N}_2\text{O Flux} = K[P_{N_2O}K_h - [N_2O]_{dissolved}]$$ (4.9)

Where the N$_2$O flux is calculated in mol·m$^{-2}$·h$^{-1}$, K is the gas exchange coefficient (m·h$^{-1}$), $P_{N_2O}$ is the partial pressure of tropospheric N$_2$O (atm), $K_h$ is the Henry's constant for N$_2$O (mol·atm$^{-1}$·m$^{-3}$), and $[N_2O]_{dissolved}$ is the dissolved concentration of N$_2$O (mol·m$^{-3}$). In SIDNO, $P_{N_2O}$ is constant, while the value of K is set by the user, and the value for $K_h$ is calculated as a function of water temperature, which is also set by the user (adapted from Gevantman 2008):

$$K_h = 55394 \times e^{\left(-60.7467 + \frac{8882.8}{T} + 21.2531 \ln \frac{T}{100}\right)}$$ (4.10)

where T is water temperature (K) and $K_h$ is expressed in mol·atm$^{-1}$·m$^{-3}$.

Gas exchange is a two-way process, and the net N$_2$O flux is the difference between the invasion rate of gaseous N$_2$O into solution, and the evasion rate of dissolved N$_2$O into the gas phase. Since the concentration of tropospheric N$_2$O ($P_{N_2O}$) is relatively
constant with time, the absolute invasion rate of gaseous N$_2$O into solution is also constant with time (assuming K and K$_h$ are constant) and is not influenced by the concentration of dissolved N$_2$O. However, the net flux rate (the difference between the invasion and evasion rates) does depend on the concentration of dissolved N$_2$O. The evasion rate of N$_2$O out of solution is a first order function of dissolved N$_2$O concentration. When a solution is at equilibrium with the atmosphere, the invasion and evasion rates will be equal, and the net flux will be zero.

As with the bulk N$_2$O flux, the flux of the heavy isotopologues ($^{15}$N$_2$O and N$_2^{18}$O) can be calculated by including the kinetic fractionation factors for N$_2$O (adapted from Venkiteswaran et al. 2007)

\[
\text{Flux }^{15}\text{N}_2\text{O} = K\left[\alpha_{in}^{15}\text{P}_{15\text{N}_2\text{O}}K_h - \alpha_{ev}^{15}\text{N}_2\text{O}_{\text{dissolved}}\right]
\]

(4.12)

\[
\text{Flux }^{18}\text{N}_2\text{O} = K\left[\alpha_{in}^{18}\text{P}_{18\text{N}_2\text{O}}K_h - \alpha_{ev}^{18}\text{N}_2\text{O}_{\text{dissolved}}\right]
\]

(4.13)

Where, P$_{15\text{N}_2\text{O}}$ and P$_{18\text{N}_2\text{O}}$ are calculated by multiplying the partial pressure of tropospheric N$_2$O (atm) by $^{15}$N/$^{14}$N and $^{18}$O/$^{16}$O ratios of tropospheric N$_2$O. The $^{15}$N and $^{18}$O isotopic ratios of the N$_2$O flux can then be calculated:

\[
R_{\text{flux}}^{15} = \frac{\text{Flux }^{15}\text{N}_2\text{O}}{\text{Flux }^{14}\text{N}_2\text{O}} = \frac{\text{Flux }^{15}\text{N}_2\text{O}}{[\text{Flux }N_2\text{O} - \text{Flux }^{15}\text{N}_2\text{O}]}
\]

(4.14)

\[
R_{\text{flux}}^{18} = \frac{\text{Flux }^{18}\text{N}_2\text{O}}{\text{Flux }^{16}\text{N}_2\text{O}} = \frac{\text{Flux }^{18}\text{N}_2\text{O}}{[\text{Flux }N_2\text{O} - \text{Flux }^{18}\text{N}_2\text{O}]}
\]

(4.15)

4.2.3 Model Testing

To test the ability of SIDNO to reproduce observed data, input parameters were set to replicate a series of experiments that were conducted by Inoue & Mook (1994) to
derive empirical kinetic enrichment factors for N\textsubscript{2}O gas exchange. In these experiments, degassed water was exposed to N\textsubscript{2}O gas in a sealed container for varying lengths of time. At the end of each experiment, the N\textsubscript{2}O concentration and isotopic composition in the gaseous and dissolved phases were measured.

The initial dissolved N\textsubscript{2}O concentration and production rate were both set to zero. The model was then run and the model output was allowed to come into steady state. The results were then superimposed on the Inoue & Mook experimental results (Figure 4.2).

As expected, the modeled dissolved N\textsubscript{2}O concentration and isotopic ratios increased in response to gas exchange. The model output closely matched the experimental results. The model fit to the experimental data is not accurately reflected by the r\textsuperscript{2} values (r\textsuperscript{2}= 0.76 for \(\delta\textsuperscript{15}N\) and 0.78 for \(\delta\textsuperscript{18}O\)), since there is a large amount of scatter in the experimental data. When the dissolved N\textsubscript{2}O concentration was close to zero, the isotopic composition of the invading N\textsubscript{2}O was a function solely of the kinetic fractionation factors for invasion of N\textsubscript{2}O \((\alpha_{in}^{15}=1.000 \text{ and } \alpha_{in}^{18}=0.9992)\) As a result, the initial isotopic composition of dissolved N\textsubscript{2}O was identical to the \(\delta^{15}N\) of tropospheric N\textsubscript{2}O, but was slightly depleted with respect to \(\delta^{18}O\). As the dissolved N\textsubscript{2}O approached the equilibrium saturation, the isotopic composition became slightly enriched in both \(\delta^{15}N\) and \(\delta^{18}O\) with respect to tropospheric N\textsubscript{2}O. At chemical and isotopic equilibrium, the rates of N\textsubscript{2}O invasion and evasion are equal for each isotopologue. Therefore, the isotopic composition of dissolved N\textsubscript{2}O at equilibrium is determined by the equilibrium fractionation factors, defined by the ratios of the evasion and invasion fractionation factors. \((\alpha_{eq}^{15} = \alpha_{ev}^{15}/\alpha_{in}^{15} = 0.99925 \text{ and } \alpha_{eq}^{18} = \alpha_{ev}^{18}/\alpha_{in}^{18} = 0.99894)\). The model successfully simulates the kinetic and equilibrium stable isotope fractionation
during both equilibrium and non equilibrium gas exchange under the experimental conditions of Inoue & Mook (1994).

Figure 4.2: Comparing the model output to the experimental data of Inoue and Mook (1994). $R^2 = 0.76$ for $\delta^{15}N$ and $0.78$ for $\delta^{18}O$. Precision of measurements for the experimental data is ± 0.05‰ for $\delta^{15}N$ and ± 0.1‰ for $\delta^{18}O$.

4.3 Model Predictions

The SIDNO model was used to make predictions about the stable isotope dynamics of $N_2O$ in a variety of situations that may be encountered in aquatic environments. These predictions will help to elucidate the relationship between source, dissolved, and emitted $N_2O$ in these environments.

4.3.1 Gas Exchange Trajectories

SIDNO was used to investigate the relationship between the stable isotope composition of dissolved and emitted $N_2O$ in the absence of $N_2O$ production. This was
done to provide insight into how the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of dissolved N$_2$O and the emitted N$_2$O flux change with time as a supersaturated N$_2$O solution is allowed to come to equilibrium with the atmosphere. The output from two model runs with the same initial N$_2$O concentration but different initial isotopic compositions of dissolved N$_2$O is shown in Figure 4.3.

As the initially supersaturated solution comes to equilibrium with the atmosphere, the dissolved N$_2$O concentration decreases, and the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ approach the equilibrium saturated values. The instantaneous values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of the emitted N$_2$O remains relatively constant with time when the solution remains very supersaturated (>300% saturation), but increases rapidly as the concentration approaches 100% saturation. Because the light isotopologue diffuses out of solution faster than the heavy isotopologue, the instantaneous values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of the emitted N$_2$O are more negative than the instantaneous dissolved composition. The cumulative composition of the emitted N$_2$O does not necessarily have the same $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of the initial dissolved N$_2$O. The total mass of N$_2$O emitted is not equal to the mass originally in solution, because the concentration is not zero at equilibrium. The various isotopologues of N$_2$O will reach equilibrium independently of each other, and therefore the total mass emitted for each isotopologue will depend on the initial concentration and isotopic ratios relative to the equilibrium values. However, if the initial concentration of dissolved N$_2$O is high (>1000% saturation), these effects will be minor, and the cumulative isotopic composition of emitted N$_2$O will be similar to the initial composition of dissolved N$_2$O.

The gas exchange coefficient $K$ does not affect the isotopic gas exchange trajectories, only the speed at which the solution reaches equilibrium.
The curvature of the trajectories on the $\delta^{15}N$ vs $\delta^{18}O$ plot (Figure 4.3C) is influenced by the initial $N_2O$ concentration. Qualitatively, a higher initial concentration will result in more curvature. This curvature is related to the difference in slope of the dissolved $\delta^{15}N$-$N_2O$ vs concentration curve (Figure 4.3A) relative to the $\delta^{18}O$-$N_2O$ vs concentration curve (Figure 4.3B).

**Figure 4.3**: $\delta^{15}N$ and $\delta^{18}O$ trajectories for dissolved and emitted $N_2O$ in two supersaturated solutions with zero $N_2O$ production. Initial dissolved isotopic values for the two dissolved $N_2O$ solutions were $\delta^{15}N = -50‰$, $\delta^{18}O = 10‰$, and $\delta^{15}N = -10‰$, $\delta^{18}O = 30‰$. Both runs used an initial dissolved $N_2O$ concentration of 1500‰ saturation. Note that in the $\delta^{15}N$ vs. $\delta^{18}O$ plot, the dissolved $N_2O$ curves do not pass through the tropospheric $N_2O$ value due to the small equilibrium isotope effect.

### 4.3.2 Steady State Production

During the steady state production of $N_2O$ (i.e. at a constant rate and with a constant isotopic composition), the isotopic composition of the emitted $N_2O$ is equal to the isotopic composition of the $N_2O$ source once steady state is achieved. However, the
isotopic composition of dissolved N₂O is not equal to that of the source (or emitted) N₂O, because it is altered by equilibration with tropospheric N₂O. The isotopic contribution of tropospheric N₂O becomes more important as the dissolved concentration approaches the equilibrium saturated value.

SIDNO was used to simulate the δ¹⁵N and δ¹⁸O of dissolved N₂O in a system open to gas exchange with the atmosphere with production of N₂O at a constant rate and isotopic composition. The model output for concentration, δ¹⁵N and δ¹⁸O was allowed to come to steady state and this procedure was repeated several times, altering the production rate but keeping the isotopic composition of the source constant.

The equilibration with tropospheric N₂O had a dramatic effect on the δ¹⁵N and δ¹⁸O of dissolved N₂O, especially at low concentrations (Figure 4.4). As production rate and concentration increases, the dissolved δ¹⁵N and δ¹⁸O in the model approach a value (represented by a “*”) that is not equal to that of the source. At very high production rates, the contribution of tropospheric N₂O is insignificant relative to the production rate of N₂O, however the δ¹⁵N and δ¹⁸O of dissolved N₂O are still slightly enriched relative to the source because the heavy isotopologues are preferentially retained in solution. This effect is controlled by α¹⁵_{ev} and α¹⁸_{ev}, as a result, the δ¹⁵N and δ¹⁸O values for dissolved N₂O will always be offset by at least 0.7‰ and 1.9‰ respectively from that of the source.
Figure 4.4: The relationship between $\delta^{15}N$, $\delta^{18}O$ and N$_2$O concentration in a system at steady state with constant N$_2$O production and open to gas exchange with the atmosphere. The point marked with a (*) represents the minimum difference between the isotopic composition of dissolved and source N$_2$O. The point at 100% saturation is the equilibrium value, the $\delta^{15}N$ and $\delta^{18}O$ of this point is controlled by the isotopic composition of tropospheric N$_2$O and the equilibrium enrichment factors.

4.3.3 Variable Production of N$_2$O

Compared to steady state production, the relationship between $\delta^{15}N$ and $\delta^{18}O$ of source, dissolved and emitted N$_2$O are much more complicated when N$_2$O production is variable. N$_2$O production can be variable with respect to either production rate or isotopic composition, or both. In many environments, N$_2$O production is not constant. The N$_2$O production processes nitrification and denitrification are sensitive to redox conditions. In many aquatic systems, the redox conditions are highly variable, due to diel changes in dissolved oxygen concentration driven by photosynthesis during the day and respiration at night. For example, Laursen & Seitzinger (2004) observed diel changes in the
denitrification rate in the Iroquois River and Sugar Creek (Midwestern USA), and found that the denitrification rates were consistently higher during the day compared to the night. Also, since the processes of nitrification and denitrification are sensitive to redox conditions, the dominant N$_2$O production process can change in response to the diel oxygen cycle. For example, Harrison et al. (2005) observed a diel change from nitrification during the day to denitrification during the night in a subtropical eutrophic stream. Since the fractionation factors and substrates are different for these two processes, the isotopic composition of N$_2$O produced in such systems is not constant. In many situations, it is likely that both the N$_2$O production rate and the isotopic composition of the source would vary with time in response to the diel oxygen cycle.

To simulate the variable production of N$_2$O driven by diel changes in dissolved oxygen concentration, various scenarios were run while changing either production rate or isotopic composition or both over diel time periods. These input parameters with diel variation were driven by a sine function with a 24 hour period, so as to mimic the diel DO cycle. The range used for production rate in the model was based on a review of relevant literature data (Table 4.2). The published data on N$_2$O flux rates was used as a proxy indicator of N$_2$O production rates in theses environments. In the following scenarios, the N$_2$O production rates ranged from 1 to 5 µmol/m$^2$/hour (Table 4.3), which is intermediate in range between the diel variation in N$_2$O flux observed by Clough et al. (2007) and Harrison et al (2005).

The value of K was varied between 0.1 and 0.3 m/day (Table 4.3). These values were within the range that have been observed in other studies (Table 4.2). The combination of production rates and K values were chosen to produce N$_2$O
concentrations ranging between 150 to 500 % saturation (Table 4.3). This concentration range was selected because it falls within the range of published data (Table 4.2) and the isotope effects of gas exchange on dissolved N₂O are the greatest in this range (Figure 4.4).

The range in δ¹⁵N and δ¹⁸O used as the input values for the N₂O source (Table 4.3) falls well within the range of published values for N₂O collected in various field studies (Rock et al 2007). For scenarios where the isotopic composition of the source N₂O was variable, the sine function for δ¹⁵N and δ¹⁸O was synchronized, with maximum and minimum values coinciding. This was done for simplicity, and since δ¹⁵N and δ¹⁸O are independent values, any combination of source values would give the same result.

The following parameters were held constant across comparisons, unless otherwise stated: temperature of 20°C, depth of 1m, and surface area subject to gas exchange of 1m². Model scenarios were run until the output parameters reached dynamic steady state (i.e., model parameters were not constant over the 24 hour period, but repeated in successive diel cycles). Model results are summarized in Table 4.4.
Table 4.2: Summary of relevant published data on N₂O production in aquatic environments.

<table>
<thead>
<tr>
<th>Location</th>
<th>N₂O Concentration (range)</th>
<th>N₂O Flux (range)</th>
<th>K (range)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LII River, New Zealand</td>
<td>201 to 404</td>
<td>1.35 to 17.9</td>
<td>0.13 to 0.82</td>
<td>Clough et al. 2006</td>
</tr>
<tr>
<td>LII River, New Zealand</td>
<td>402 to 644</td>
<td>0.46 to 0.89</td>
<td>14.76</td>
<td>Clough et al. 2007</td>
</tr>
<tr>
<td>Agricultrual Stream, UK</td>
<td>100 to 630</td>
<td>0 to 37.5</td>
<td>-</td>
<td>Reay et al. 2003</td>
</tr>
<tr>
<td>Bang Nara River, Thailand</td>
<td>170 to 2000</td>
<td>-</td>
<td></td>
<td>Boontanon et al. 2000</td>
</tr>
<tr>
<td>Seine River, France</td>
<td>-</td>
<td>2.2 to 5.2</td>
<td>0.04 to 0.06</td>
<td>Garnier et al. 2006</td>
</tr>
<tr>
<td>Canal Two, Yaqui Valley, Mexico</td>
<td>100 to 6000</td>
<td>0 to 34.9</td>
<td>0.3 to 0.6</td>
<td>Harrison et al. 2005</td>
</tr>
<tr>
<td>Grand River, Ontario, Canada</td>
<td>100 to 8540</td>
<td>0 to 69.8</td>
<td>0.06 to 0.35</td>
<td>Rosamond et al (Unpublished Data)</td>
</tr>
</tbody>
</table>

Table 4.3: Summary of input parameters SIDNO model scenarios for non-steady state production of N₂O. If an input parameter was variable with time, the maximum and minimum values are given. *Maximum production rate coincides with the most depleted δ¹⁵N and δ¹⁸O values of the source. **Maximum production rate coincides with the most enriched δ¹⁵N and δ¹⁸O values of the source.

<table>
<thead>
<tr>
<th>N₂O Source</th>
<th>Scenario #</th>
<th>K (m/h)</th>
<th>Production Rate (µmol/m²/h)</th>
<th>(δ¹⁵N, δ¹⁸O)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variable Production Rate, Constant Isotopic Composition of Source</strong></td>
<td>1</td>
<td>0.3</td>
<td>1 to 5</td>
<td>(-50, 10)</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>1 to 5</td>
<td>(-50, 10)</td>
<td></td>
</tr>
<tr>
<td><strong>Constant Production Rate, Variable Isotopic Composition of Source</strong></td>
<td>3</td>
<td>0.3</td>
<td>3</td>
<td>(-50, 10) to (-10, 30)</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>3</td>
<td>(-50, 10) to (-10, 30)</td>
<td></td>
</tr>
<tr>
<td><strong>Variable Production Rate, Variable Isotopic Composition of Source</strong></td>
<td>5*</td>
<td>0.3</td>
<td>1 to 5</td>
<td>(-50, 10) to (-10, 30)</td>
</tr>
<tr>
<td>6**</td>
<td>0.3</td>
<td>1 to 5</td>
<td>(-50, 10) to (-10, 30)</td>
<td></td>
</tr>
<tr>
<td>7*</td>
<td>0.1</td>
<td>1 to 5</td>
<td>(-50, 10) to (-10, 30)</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.4: Summary of SIDNO output for model scenarios simulating non-steady state production of N₂O. If an output parameter was variable with time, the maximum and minimum values are given. *Maximum production rate coincides with the most depleted δ¹⁵N and δ¹⁸O values of the source. **Maximum production rate coincides with the most enriched δ¹⁵N and δ¹⁸O values of the source. Δδ¹⁵N and Δδ¹⁸O are the maximum difference between the range of source N₂O and the range for the model output parameter.

<table>
<thead>
<tr>
<th>Scenario #</th>
<th>Concentration</th>
<th>Dissolved N₂O</th>
<th>Emitted N₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% SAT</td>
<td>(δ¹⁵N, δ¹⁸O)</td>
<td>Δδ¹⁵N</td>
</tr>
<tr>
<td>Variable Production Rate, Constant Isotopic Composition of Source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>153 to 263</td>
<td>(-27.8, 24.6) to (-12.1, 34.4)</td>
<td>37.9</td>
</tr>
<tr>
<td>2</td>
<td>345 to 501</td>
<td>(-38.1, 18.4) to (-32.8, 22.1)</td>
<td>17.2</td>
</tr>
<tr>
<td>Constant Production Rate, Variable Isotopic Composition of Source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>208</td>
<td>(-19.6, 29.4) to (-3.7 to 37.4)</td>
<td>30.4</td>
</tr>
<tr>
<td>4</td>
<td>423</td>
<td>(-26.1, 24.8) to (-15.1, 30.3)</td>
<td>23.9</td>
</tr>
<tr>
<td>Variable Production Rate, Variable Isotopic Composition of Source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5*</td>
<td>153 to 263</td>
<td>(-25.8, 25.6) to (-1.2, 39.8)</td>
<td>24.2</td>
</tr>
<tr>
<td>6**</td>
<td>153 to 263</td>
<td>(-11.2, 33.8) to (-5.0, 36.1)</td>
<td>38.8</td>
</tr>
<tr>
<td>7*</td>
<td>345 to 501</td>
<td>(-31.6, 21.6) to (-18.4, 29.3)</td>
<td>18.4</td>
</tr>
</tbody>
</table>

In scenario #1 (Table 4.3), the isotopic composition of source N₂O was held constant and production rate was variable. An example of such a system may be N₂O production through denitrification in sediments in a river with abundant NO₃⁻.

Denitrification rates in rivers have been observed to fluctuate in response to the diel dissolved oxygen cycle (Laursen & Seitzinger 2004). If the fractionation factors for denitrification are constant and not dependent on rate, the resulting N₂O production rate would be variable, but the isotopic composition of the produced N₂O would be constant.
In model scenario #1, the maximum concentration lagged approximately 2.75 hours behind the maximum N\textsubscript{2}O production rate. The values for the emitted N\textsubscript{2}O were relatively constant and always nearly equal to that of the source within (within 0.4‰ for $\delta^{15}$N and 1.1‰ for $\delta^{18}$O, Figure 4.5, Table 4.4). However, the $\delta^{15}$N and $\delta^{18}$O values for dissolved N\textsubscript{2}O were more variable, varying by approximately 16‰ for $\delta^{15}$N and 10‰ for $\delta^{18}$O respectively. These results indicate that a change in the isotopic composition of dissolved N\textsubscript{2}O can be driven simply by a change in production rate (and therefore concentration) rather than by a change in the isotopic signature of the source.

Figure 4.5: Model scenario #1, the isotopic composition of dissolved and emitted N\textsubscript{2}O with a variable production rate and constant isotopic composition of the source. Note, figure 4.5 D, the data points for emitted N\textsubscript{2}O are masked by the data point for source N\textsubscript{2}O.

In scenario #2, the effect of altering K was investigated. When K was altered in this scenario, the isotopic composition of emitted N\textsubscript{2}O remained relatively constant and
nearly equal to that of the source (Table 4.4). However, as K was decreased, the lag time between the maximum N₂O production rate and the maximum N₂O concentration increased. Also, since the dissolved N₂O concentration is a function of the ratio of production rate to K, the system was able to sustain a higher N₂O concentration for the same production rate. Therefore, the isotopic composition of dissolved N₂O was closer to that of the source, since the contribution of tropospheric N₂O was not as significant relative to the total mass of dissolved N₂O present. Also, the total range for δ¹⁵N and δ¹⁸O in dissolved N₂O was reduced when compared to scenario #1.

SIDNO was used to investigate the stable isotope dynamics of dissolved N₂O in the case where the production rate is constant, but the isotopic composition of the source is changing with time. In rivers or lakes that do not have a strong diel DO cycle, denitrification in the sediments would be expected to produce N₂O at a generally constant rate. However, the isotopic composition of the source N₂O may change if that of the nitrate substrate is not constant with time. For example, many studies have shown that the isotopic composition of residual nitrate becomes more enriched in both δ¹⁵N and δ¹⁸O as it is consumed during denitrification (e.g. Mengis et al. 1999). The following model runs were used to simulate this situation.

In model scenario #3, when the N₂O production rate was held constant, and the isotopic signature of the source was variable with time, the isotopic composition of the emitted N₂O was again much closer to the composition of the source compared that of the dissolved N₂O (Figure 4.6, Table 4.4). The difference between the maximum and minimum δ¹⁵N and δ¹⁸O values for the emitted and source N₂O was 4.7‰ and 2.3 ‰.
respectively. However, since the N₂O flux is driven by the dissolved composition, the emitted δ¹⁵N and δ¹⁸O also lagged 2.75 hours behind that of the source. Since the system is at dynamic steady state, the average δ¹⁵N and δ¹⁸O of the emitted N₂O is identical to the average isotopic composition of the source. This must be true in all cases to conserve mass of N₂O produced.

To examine the effects of varying K it was reduced from 0.3 to 0.1 m/hour in model scenario #4 (Figure 4.7, Table 4.4). All other parameters were the same as model scenario #3.

The isotopic values for the emitted N₂O are centred between those of the source N₂O, but the values for the dissolved N₂O are offset by some amount towards the δ¹⁵N and δ¹⁸O values for tropospheric N₂O (Figure 4.7 D). This offset is the same as seen in steady state N₂O production (Figure 4.4). In scenario #4, the average δ¹⁵N and δ¹⁸O values for the source and the dissolved N₂O, follow a similar pattern as seen in Figure 4.4. The relationship between these average values for source and dissolved N₂O depend on the concentration of dissolved N₂O, and therefore the relative importance of equilibration with tropospheric N₂O compared to the production of N₂O. The slope of the line connecting the dissolved N₂O isotope data points is always the same as the slope of the line connecting the source data points. Therefore, if the slope of the line connecting the dissolved N₂O isotope data points does not trend through the value for tropospheric N₂O, there must be a variation in the isotopic composition of the source N₂O.

Again, in scenario #4, since concentration is a function of the ratio of production rate to K, the effect of reducing K was to support a higher concentration with the same production rate, and to cause the δ¹⁵N and δ¹⁸O of the dissolved N₂O to be closer to that
of the source as compared to the previous example. Increasing the production rate while keeping K fixed would have produced the same effect. Reducing K also tends to dampen the response of the instantaneous $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of the emitted N$_2$O to changes in the isotopic composition of the source. The lag time between the isotopic composition of the source and emitted N$_2$O increased as K decreased. Also the difference between the maxima and minima of the source and emitted $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ increased. In scenario #4, lag time increased to 4.75 hours, and the maximum difference between the source and emitted $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ maxima and minima increased to 12.8 and 6.4 respectively.

Figure 4.6: Model scenario #3 - Isotopic composition of dissolved and emitted with a constant production rate and variable isotopic composition of the source.
Figure 4.7: Model scenario #4 - Isotopic composition of dissolved and emitted N\textsubscript{2}O with a constant production rate and variable isotopic composition of the source. K is reduced from 0.3 m/h to 0.1 m/h.

To simulate a system alternating between two N\textsubscript{2}O production processes (such as nitrification and denitrification) that have different rates of N\textsubscript{2}O production and different isotopic compositions, the model was run with both the production rate and the isotopic composition variable with time (model scenario #5). The production rate was timed so that the maximum production rate coincided with the most depleted $\delta^{15}$N and $\delta^{18}$O values for the source N\textsubscript{2}O.

The resulting N\textsubscript{2}O concentrations were identical to those in model scenario #1, with the maximum concentration lagging approximately 2.75 hours behind the maximum production rate (Figure 4.8, Table 4.4). The relationship between the $\delta^{15}$N and $\delta^{18}$O of the
dissolved and emitted N\textsubscript{2}O were more complex than in the other model scenarios. The shape of the dissolved and emitted N\textsubscript{2}O $\delta^{15}$N and $\delta^{18}$O curves were altered from a true sine curve. The lag time between the maximum $\delta^{15}$N and $\delta^{18}$O of the source and that of the dissolved and emitted N\textsubscript{2}O (when the production rate was at a minimum) was 3.75 hours. The lag time between the minimum $\delta^{15}$N and $\delta^{18}$O of the source and that of the dissolved and emitted N\textsubscript{2}O (when the production rate was at a maximum) was only 2.25 hours. The difference between the source and most positive emitted $\delta^{15}$N was 8.0‰, while the difference between the minimum values was only 3.1‰. Similarly, the difference between the source and the maximum $\delta^{18}$O for the emitted was 3.4‰, and for the minimums it was 1.3‰. In short, the isotopic composition of the emitted N\textsubscript{2}O was closer to that of the source during periods coinciding with high production rates and thus higher concentrations, compared to periods of low production rates. However, the flux-weighted average $\delta^{15}$N and $\delta^{18}$O of emitted N\textsubscript{2}O is equal to the average source composition weighted by production rate, because the system was at dynamic steady state.

To determine the effects of a high production rate with an isotopically enriched N\textsubscript{2}O source, the timing of the maximum production rate was altered to coincide with the most enriched $\delta^{15}$N and $\delta^{18}$O values of the source in model scenario #6. All other parameters remained the same as in model scenario #5 (Table 4.4). The resulting pattern for the $\delta^{15}$N and $\delta^{18}$O of dissolved was very different than in model scenario #5 (Figure 4.9, Table 4.4). The dissolved N\textsubscript{2}O concentrations were identical to the model scenario #5, but the $\delta^{15}$N and $\delta^{18}$O of dissolved N\textsubscript{2}O was nearly constant. The relationship between the isotopic composition of emitted and source N\textsubscript{2}O was the same as in the
previous example (scenario #5). The isotopic composition of the emitted N\(_2\)O was close to the source values, though it lagged behind by several hours.

In scenario #6, the \(\delta^{15}\)N and \(\delta^{18}\)O curves of the dissolved N\(_2\)O are greatly dampened by the equilibration with tropospheric N\(_2\)O. In the previous example (Figure 4.8), the equilibration with tropospheric N\(_2\)O and the isotopic composition of the source had an additive effect, and increased the amplitude of the oscillations of \(\delta^{15}\)N and \(\delta^{18}\)O in the dissolved N\(_2\)O. However, in scenario #6 (Figure 4.9), the two effects move the \(\delta^{15}\)N and \(\delta^{18}\)O of dissolved N\(_2\)O in opposite directions, and the result is a nearly constant \(\delta^{15}\)N and \(\delta^{18}\)O of dissolved N\(_2\)O.

To determine the effects of a lower K on model scenario #5, K was reduced from 0.3 to 0.1 m/hour in scenario #7. This had the effect of increasing the dissolved N\(_2\)O concentration compared to model scenario #5, and damping the oscillations in the \(\delta^{15}\)N and \(\delta^{18}\)O of the dissolved and emitted N\(_2\)O (Table 4.4, Figure 4.10).

In scenario #7, the lower K also dampened the oscillation in the \(\delta^{15}\)N and \(\delta^{18}\)O of the N\(_2\)O, and the lag time between the isotopic composition of emitted and source N\(_2\)O increased. As a result, there is a greater difference between the isotopic composition of the emitted and source N\(_2\)O than in model scenario #5 (Figure 4.10). As in scenario #5, the flux-weighted average \(\delta^{15}\)N and \(\delta^{18}\)O of emitted N\(_2\)O is equal to the average source composition weighted by production rate.
Figure 4.8: Model scenario #5 - Isotopic composition of dissolved and emitted N₂O with a variable production rate and variable isotopic composition of the source. Maximum production rate is in sync with the most depleted δ¹⁵N and δ¹⁸O of the source.
Figure 4.9: Model scenario #6 - Isotopic composition of dissolved and emitted N\textsubscript{2}O with a variable production rate and variable isotopic composition of the source. Maximum production rate is in sync with the least depleted $\delta^{15}$N and $\delta^{18}$O of the source.
Figure 4.10: Model scenario #7 - Isotopic composition of dissolved N\textsubscript{2}O and emitted N\textsubscript{2}O with a variable production rate and variable isotopic composition of the source. Maximum production rate is in sync with the most depleted $\delta^{15}$N and $\delta^{18}$O of the source. K is reduced from 0.3 m/h to 0.1 m/h.

4.4 Discussion & Conclusions

The relationship between the isotopic composition of dissolved, source and emitted N\textsubscript{2}O is complex for aquatic systems that are open to gas exchange with the atmosphere. The SIDNO model was able simulate this relationship, and provided additional insight into the stable isotope dynamics of dissolved N\textsubscript{2}O.

For a solution with no N\textsubscript{2}O production at equilibrium with tropospheric N\textsubscript{2}O, the isotopic composition of dissolved N\textsubscript{2}O will be slightly enriched with respect to tropospheric N\textsubscript{2}O. At equilibrium, the rates of N\textsubscript{2}O invasion into solution and evasion out of solution are equal. Therefore, the offset between the dissolved N\textsubscript{2}O isotopic
composition and that of tropospheric N$_2$O is dictated by the ratios between the kinetic enrichment factors for evasion and invasion.

In a system at steady state with respect to rate of N$_2$O production and its isotopic composition, the $\delta^{15}$N and $\delta^{18}$O of dissolved N$_2$O are not directly indicative of the isotopic composition of the source N$_2$O. The difference between the isotopic composition of dissolved and source N$_2$O increases as the N$_2$O concentration decreases. This is because the equilibration with tropospheric N$_2$O becomes more important relative to the input of source N$_2$O from N$_2$O production. However, even at concentrations higher than 1000% saturation (resulting from either high production rates, low K, or both), the $\delta^{15}$N and $\delta^{18}$O of the dissolved N$_2$O will not be equal to that of the source, and will approach a value that is offset from the source composition by 0.7‰ and 1.9‰ for $\delta^{15}$N and $\delta^{18}$O respectively. This offset is a function of the $\alpha_{ev}^{15}$ and $\alpha_{ev}^{18}$ values, as determined by Inoue & Mook (1994). However, for a system at steady state with respect to N$_2$O production rate and the isotopic composition of the source, isotopic composition of the emitted N$_2$O is identical to that of the source, and is easily calculated from the concentration, $\delta^{15}$N and $\delta^{18}$O of dissolved N$_2$O (equations 4.11 to 4.14).

Calculating the isotopic composition of emitted N$_2$O also provides information about the composition of the source N$_2$O in systems that are not at steady state. If the concentration changes with time, the N$_2$O production rate must also change with time (provided K and temperature are constant). However, a change in $\delta^{15}$N or $\delta^{18}$O of dissolved N$_2$O did not necessarily indicate a change in the isotopic composition of the source (Figure 4.5). If the change in $\delta^{15}$N and $\delta^{18}$O of dissolved N$_2$O is only a result of a change in N$_2$O concentration, the data points for dissolved N$_2$O on a $\delta^{15}$N vs. $\delta^{18}$O plot
will trend towards the equilibrium saturated value as the concentration falls. Additionally, a constant $\delta^{15}N$ and $\delta^{18}O$ of dissolved N$_2$O does not indicate a constant isotopic composition of the source (Figure 4.9).

When the $\delta^{15}N$ and $\delta^{18}O$ of the source N$_2$O is variable, the relationship of the emitted N$_2$O to the composition of the source becomes complicated. The composition of the emitted N$_2$O will lag behind that of the source, and the amplitude of the $\delta^{15}N$ and $\delta^{18}O$ fluctuations will be dampened relative to the source. The amount of lag and dampening is a function of concentration and flux. Qualitatively; the isotopic composition of emitted N$_2$O will be nearly equal to that of the source if the residence time of dissolved N$_2$O is small relative to the period of source variability. The residence time is the average length of time that a dissolved N$_2$O molecule will remain in solution from the time it is produced to the time it is emitted to the atmosphere, and is defined as the areal N$_2$O concentration / N$_2$O flux. The residence time can be approximated by the ratio between mean depth and K if the N$_2$O concentration is substantially greater than atmospheric saturation. If the mean depth is low and the K is high, the residence time will be short, and the isotopic composition of the emitted N$_2$O will be close to that of the source. As the residence time increases, the isotopic composition of the emitted N$_2$O will increasingly lag behind and be dampened relative to the isotopic composition of the variable source. In the most extreme case, the variability in the $\delta^{15}N$ and $\delta^{18}O$ of the emitted N$_2$O will be dampened to the point that they are nearly constant, and the system will appear to be at steady state. At this point, the isotopic composition of the emitted N$_2$O is equal to the average isotopic composition of the source.
In aquatic environments, the instantaneous isotopic composition of $N_2O$ emitted to the atmosphere is easily calculated if the water temperature, concentration, $\delta^{15}N$ and $\delta^{18}O$ of dissolved $N_2O$ are measured. The $\delta^{15}N$ and $\delta^{18}O$ of dissolved $N_2O$ is not directly representative of either the isotopic composition of $N_2O$ produced or emitted to the atmosphere. Thus, the calculated $\delta^{15}N$ and $\delta^{18}O$ of the emitted $N_2O$ should be used to draw important conclusions regarding $N_2O$ production in aquatic systems (Table 4.5). The flux weighted $\delta^{15}N$ and $\delta^{18}O$ of emitted $N_2O$ provides the average production rate weighted isotopic composition of the $N_2O$ source in aquatic environments. If the $\delta^{15}N$ and $\delta^{18}O$ of emitted $N_2O$ are constant with time, either the isotopic composition of the source must also be constant, or the residence time of $N_2O$ in the aquatic system is very long. However, if the calculated $\delta^{15}N$ and $\delta^{18}O$ of emitted $N_2O$ is variable with time, the isotopic composition of the source must also be variable with time. Depending on the $N_2O$ production rate and the value of $K$, range of values for $\delta^{15}N$ and $\delta^{18}O$ of the emitted $N_2O$ will be smaller than that of the source. Also, $N_2O$ residence time (again dependant on production rate and $K$) will determine the lag time between the isotopic composition of the emitted $N_2O$ and the source. However, the timing of the maximum and minimum values for $\delta^{15}N$ and $\delta^{18}O$ of emitted $N_2O$ relative to maximum and minimum dissolved $N_2O$ concentrations indicates how the isotopic composition of the source changes with production rate. For example, if the emitted $N_2O$ is most depleted when the concentration is the highest, this indicates that the source is most depleted when the production rate is relatively high.
### Table 4.5: Implications of the SIDNO results.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observation</th>
<th>Implications</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of Dissolved N\textsubscript{2}O</td>
<td>Constant with time</td>
<td>The N\textsubscript{2}O production rate is constant with time (if K and temperature are also constant). The N\textsubscript{2}O flux to the atmosphere is equal to the production rate. This may not be true if the concentration is close to atmospheric equilibrium.</td>
<td>Scenarios 3 and 4</td>
</tr>
<tr>
<td></td>
<td>Variable with time</td>
<td>The N\textsubscript{2}O production rate is variable with time (if concentration change cannot be explained by change in K or temperature). The average N\textsubscript{2}O flux to the atmosphere is equal to the average production rate.</td>
<td>Scenarios 1 and 2</td>
</tr>
<tr>
<td>δ\textsuperscript{15}N and δ\textsuperscript{18}O of Dissolved N\textsubscript{2}O</td>
<td>Constant with time</td>
<td>The observation is inconclusive. At concentrations near atmospheric equilibrium, isotopic composition of dissolved N\textsubscript{2}O will approximate tropospheric N\textsubscript{2}O, regardless of source values. A constant isotopic signature of dissolved N\textsubscript{2}O that is different from tropospheric N\textsubscript{2}O can indicate either a constant source (if production rate is constant), or a variable source.</td>
<td>Scenario 6</td>
</tr>
<tr>
<td></td>
<td>Variable with time, slope of data points trends through value for tropospheric N\textsubscript{2}O</td>
<td>The change in δ\textsuperscript{15}N and δ\textsuperscript{18}O of dissolved N\textsubscript{2}O is likely a result of a change in concentration, but it is possible that the source is variable with time, if the δ\textsuperscript{15}N and δ\textsuperscript{18}O values of the source also trend through the value for tropospheric N\textsubscript{2}O.</td>
<td>Scenario 1</td>
</tr>
<tr>
<td></td>
<td>Variable with time, slope of data points does not trend through value for tropospheric N\textsubscript{2}O</td>
<td>The change in δ\textsuperscript{15}N and δ\textsuperscript{18}O of dissolved N\textsubscript{2}O is likely the result of a change in the isotopic composition of the source.</td>
<td>Not Shown</td>
</tr>
<tr>
<td>Calculated δ\textsuperscript{15}N and δ\textsuperscript{18}O of Emitted N\textsubscript{2}O</td>
<td>Constant with Time</td>
<td>The isotopic composition of the source is constant with time, and equal to the calculated value for emitted N\textsubscript{2}O</td>
<td>Scenarios 1 and 2</td>
</tr>
<tr>
<td></td>
<td>Variable with Time</td>
<td>The isotopic composition of the source is variable with time. The range in δ\textsuperscript{15}N and δ\textsuperscript{18}O of emitted N\textsubscript{2}O is the minimum for the range in that of the source. The flux weighted average δ\textsuperscript{15}N and δ\textsuperscript{18}O of emitted N\textsubscript{2}O is equal to the production weighted average source values.</td>
<td>Scenarios 3 to 7</td>
</tr>
<tr>
<td>Residence Time Relative to Variability of Source</td>
<td>Long</td>
<td>The changes in N\textsubscript{2}O concentration and δ\textsuperscript{15}N and δ\textsuperscript{18}O of emitted N\textsubscript{2}O will be dampened relative to, and lag behind, that of the source</td>
<td>Scenarios 2, 4, and 7</td>
</tr>
<tr>
<td>(Need to independently determine K)</td>
<td>Short</td>
<td>The changes in N\textsubscript{2}O concentration and δ\textsuperscript{15}N and δ\textsuperscript{18}O of emitted N\textsubscript{2}O will be indicative of changes in the source</td>
<td>Scenarios 1, 3, 4 and 5</td>
</tr>
</tbody>
</table>
SIDNO can potentially be used to back calculate the K value, N\textsubscript{2}O production rate and isotopic composition of the N\textsubscript{2}O source in aquatic environments. If physical properties, such as depth and temperature, are entered into the model, SIDNO can be used to fit the measured field data (concentration, $\delta^{15}$N and $\delta^{18}$O of dissolved N\textsubscript{2}O) by adjusting the N\textsubscript{2}O source parameters. In this way, SIDNO could be used to estimate K, the N\textsubscript{2}O production rate, and the source $\delta^{15}$N and $\delta^{18}$O. SIDNO could easily be adapted to simulate the stable isotope dynamics of other dissolved gases, such as CH\textsubscript{4}.
Chapter 5:

Diel Changes in Nitrous Oxide Production in the Grand River, Ontario, Canada

5.1 Introduction

The amount of biologically available nitrogen in the environment is increasing; anthropogenic sources release more than 160 Tg of reactive nitrogen each year, compared to 250 Tg/year of natural nitrogen fixation (Gruber & Galloway 2008). Much of this nitrogen is released to rivers through diffusive sources (e.g. agriculture) or point sources (e.g. municipal wastewater treatment). This excess nitrogen leads to eutrophication in freshwater and coastal zones (Dumont et al 2005, Thornber et al 2008).

To understand the fate of natural and anthropogenic nitrogen in river systems, it is necessary to study the biological nitrogen processing occurring in these environments. Methods have been developed to measure the in-situ rates of riverine denitrification (Laursen & Seitzinger 2002). Nitrification also takes place in oxic river waters and sediments, and the production of nitrate (NO$_3^-$) can further stimulate denitrification in the anoxic river sediments (Laursen & Seitzinger 2004). Since nitrous oxide (N$_2$O) is produced through both of these processes, rivers are an important source of this powerful greenhouse gas (Clough et al 2006, Cole & Caraco 2001, McMahon & Dennehy 1999).

The relative importance of nitrification and denitrification in a riverine system has been shown to vary spatially in an agriculturally influenced stream (Kemp & Dodds 2002). Recent studies on the Seine River in France have shown that nitrification and
denitrification rates can vary spatially as well as seasonally (Garnier et al 2006, Garnier et al 2007).

The concentration of dissolved oxygen (DO) in rivers can vary widely over diel cycles, driven by changing rates of photosynthesis and respiration (Venkiteswaran et al. 2007 and references within). Since nitrogen cycling processes depend on redox conditions in the system, it would be expected that nitrogen cycling in many rivers is linked to the diel oxygen cycle. Laursen & Seitzinger (2004) measured rates of denitrification in the Iroquois River and Sugar Creek (Midwestern USA) and found that these rates were consistently higher during the day than at night. The authors attributed this finding to increased nitrification during the day in response to elevated DO and temperature, providing NO$_3^-$ for denitrification in the river sediments. Harrison et al (2005) also studied diel changes in nitrogen cycling in a eutrophic subtropical stream. The authors observed a complete change in redox conditions in the stream from day to night, with high DO concentrations in the day due to photosynthesis, and hypoxic conditions at night due to excessive respiration. The large diel changes in redox conditions resulted in a reduction in N$_2$O emitted during the night-time period and a change in the form of nitrogen transported downstream. Most recently, Clough et al (2007) studied the diel changes in nitrogen cycling in the LII river in New Zealand. These authors did not observe the dramatic changes in nitrogen cycling observed by Harrison et al (2005). This is likely due to the fact that the DO cycle was not as extreme at this location, and thus the redox conditions in the river did not vary enough to produce a large change in the nitrogen speciation observed. However, these authors did observe a peak in N$_2$O saturation (though absolute concentration remained relatively constant) during the
late afternoon, which subsequently declined at night. The small diel changes in N₂O saturation did not translate into a significant diel change in N₂O flux to the atmosphere.

Stable isotope analysis is a useful tool to study nitrogen cycling, and numerous studies have used δ¹⁵N and δ¹⁸O analysis of NO₃⁻ and δ¹⁵N analysis of ammonium (NH₄⁺) to study nitrogen cycling in the environment, including river systems (e.g. Sebilo et al 2006). Recently, more studies have employed δ¹⁵N and δ¹⁸O analysis of N₂O as an indicator of nitrogen cycling in soils, (e.g. Perez et al 2001; Bol et al 2003); however, to our knowledge, only one study (Boontanon et al 2005) has measured the stable isotope ratios of N₂O in a river. If N₂O produced through nitrification and denitrification has a different isotopic composition, the isotopic analysis of dissolved N₂O is useful to study diel changes in the riverine nitrogen cycle; however, currently no published studies have done so.

Researchers often make use of isotopic enrichment factors for N₂O produced through denitrification and nitrification that have been obtained in laboratory culture studies (e.g. Sutka et al. 2006, Toyoda et al. 2005, Casciotti et al. 2002). However, the in-situ microbial community in a natural environment may be very different from the microbial species used in these laboratory culture studies (Iribar et al 2008, Amann et al 1995). As a result, the enrichment factors for nitrification and denitrification in the natural environment may be significantly different than those obtained during laboratory studies.

The purpose of this chapter is to investigate the nitrogen cycling processes and N₂O production in the Grand River. The first objective was to determine the dominant processes responsible for riverine N₂O production, and how these processes are
influenced by diel changes in dissolved oxygen concentration. Previous work conducted at the University of Waterloo (Terra Jamesion, Gao Chen, and Tim Kuntz – unpublished data) and continuous DO monitoring by the Grand River Conservation Authority has shown that the magnitude of DO fluctuations at certain locations in the river is much greater than those measured by Clough et al (2007) in the LII River, and thus it was expected that a greater change in nitrogen cycling processes and N\textsubscript{2}O production would be observed in the Grand River.

The second objective of this study was to determine if stable isotope analysis of NO\textsubscript{3}\textsuperscript{-}, NH\textsubscript{4}\textsuperscript{+} and N\textsubscript{2}O can provide greater insight into N\textsubscript{2}O production in the Grand River. Very little data is available in the published literature regarding the isotopic composition of N\textsubscript{2}O produced in riverine environments, and this study will help to characterize this important source of N\textsubscript{2}O to the atmosphere.

The final objective was to use the SIDNO model to simulate N\textsubscript{2}O production in the Grand River. The SIDNO model will determine the apparent δ\textsubscript{15}N and δ\textsubscript{18}O of N\textsubscript{2}O produced through separate processes in the river. In this study, the SIDNO model output was used to provide a measure of the apparent enrichment factors for N\textsubscript{2}O produced through riverine denitrification that have not previously been measured in-situ.

5.2 Study Site

The Grand River is the largest river in southern Ontario. It is approximately 300 km long, and drains an area of approximately 7000 km\textsuperscript{2} into Lake Erie (Figure 5.1). As of 2001, approximately 720 000 people were living in the watershed, this figure is projected to increase to 1 220 000 by 2031 (Ministry of Public Infrastructure Renewal 2006). The
Grand River is a source of drinking water for approximately 500,000 people in the watershed.

The Grand River is heavily impacted by both diffuse and point-source inputs of nitrogen. Agriculture is the primary land use in the watershed, and 26 wastewater treatment plants (WWTPs) currently discharge effluent to the Grand or its tributaries. The cities of Kitchener and Waterloo form the urban centre of the watershed, and the Kitchener and Waterloo WWTPs contribute 10% of the total NO$_3^-$ load and 83% of the total NH$_4^+$ load of the six major WWTPs in the watershed (NPRI 2007). Sampling for this study was conducted approximately 6 km downstream of the Kitchener WWTP (Figure 5.1). To provide additional background data, samples were collected concurrently at a site approximately 0.5 km upstream of the Waterloo WWTP (Figure 5.1).

Downstream of the urban centre, the river receives heavy nutrient input, resulting in
dense macrophyte growth in the channel. In the summer, macrophyte photosynthesis results in elevated DO concentrations during the day, and community respiration results in decreased DO concentrations at night (Terra Jamesion and Gao Chen– unpublished data). During the summer months, night-time DO concentrations at this location often fall below the water quality target set by the Grand River Conservation Authority. This DO problem is further compounded by the fact that the effluent from the Kitchener and Waterloo WWTPs contain high concentrations of ammonium (NH$_4^+$). These two major WWTPs are not operated to nitrify the effluent within the plant, and instead rely on the river to oxidize the NH$_4^+$ to nitrate (NO$_3^-$). Nitrification of NH$_4^+$ further consumes the limited DO in the river and contributes to poor river health.

5.3 Methods

Sampling for this study was conducted over a period of 28 hours on June 26 to June 27, 2008. Samples were collected for NH$_4^+$, NO$_3^-$, N$_2$, N$_2$O, CH$_4$, DO and DOC concentration approximately every 1.5 hours. At the same time these concentration samples were collected, pH measurements were taken using a YSI meter (650 MDS), fitted with a 600 QS Multi-Parameter Water Quality Monitor probe. Samples for dissolved gas concentration were collected in 60mL serum bottles (except for dissolved N$_2$ samples, which were collected in 160mL bottles), capped with no headspace with a baked red Vacutainer stopper and preserved with 0.2mL of saturated HgCl$_2$ solution. All samples were kept on ice until they were transported back to the University of Waterloo. Chemistry samples were filtered to 0.45µm and the NH$_4^+$ samples were acidified to pH 5 using H$_2$SO$_4$. All samples were stored at 4°C until they were analyzed.
Photosynthetically active radiation (PAR) and barometric pressure were measured continuously at the downstream sampling location using a HOBO Micro Station datalogger (Onset Computer Corporation). DO, conductivity, and water temperature were measured continuously at the downstream location using a Hydrolab MiniSonde 4a.

Samples for $\delta^{15}$N and $\delta^{18}$O analysis of and NO$_3^-$, and $\delta^{15}$N analysis of NH$_4^+$ were collected every 3-5 hours. NH$_4^+$ isotope samples were acidified on site to pH 5 using H$_2$SO$_4$. Samples for $\delta^{15}$N and $\delta^{18}$O of N$_2$O were collected every 1.5 hours along with the concentration samples. N$_2$O isotope samples were collected in 160mL serum bottles, capped with no headspace with red Vaccutainer stoppers and preserved with 0.4 mL of saturated HgCl$_2$ solution. All isotope samples were kept on ice until they were transported back to the University of Waterloo. N$_2$O samples were stored at 4°C and NH$_4^+$ and NO$_3^-$ samples were frozen until further processing.

NH$_4^+$ concentrations were measured using a Technicon Auto Analyzer and an automated salicylate procedure, with a method precision of +/- 0.005 mg N/L (Technicon Industrial Method No. 329-74 W/B). NO$_3^-$ concentrations were measured using a Dionex ICS 90 ion chromatograph, with a precision of +/- 0.05 mg N/L.

Dissolved gasses (with the exception of DO) were measured using a headspace equilibration technique. A 5 mL headspace was created in sample bottles by injection of ultra high purity helium. Sample bottles were then placed on an orbital shaker for 2 hours to equilibrate. Analysis was conducted with a Varian 3800 GC equipped with TCD, FID and ECD. Method precision for N$_2$O and CH$_4$ was +/- 5%. Method precision for N$_2$ analysis was +/- 7%.
DO concentration in discreet samples was measured by Winkler titration with sodium azide modification (APHA 1995). Method precision was +/- 0.2 mg O$_2$/L. The results of the Winkler titrations were used to calibrate the continuous DO measurements obtained by the Hydrolab datalogger.

$\delta^{15}$N – NH$_4^+$ analysis was conducted using a diffusion technique (Murray 2008, modified from Spoelstra et al. 2006). Briefly, an acidified quartz filter disk contained in a polytetrafluoroethylene (PTFE) packet was placed in a 60 mL serum bottle containing approximately 20 mL of sample water. The pH of the sample was adjusted with a buffer solution to convert the NH$_4^+$ in the sample to NH$_3$ gas. The serum bottle was capped and placed on a stir plate for approximately 10 days, after which the quartz filter disk was removed, dried, and analyzed for $\delta^{15}$N – NH$_4^+$ at the University of Waterloo Environmental Isotope Laboratory (uwEILAB). Precision for this technique was +/- 0.3‰.

$\delta^{15}$N and $\delta^{18}$O analysis of NO$_3^-$ was conducted using the silver nitrate method described by Spoelstra et al. (2004). Samples were pre-concentrated by evaporation, and nitrate was stripped from the samples using an anion exchange resin. Nitrate was eluted from the resin using an HCl solution, and converted to AgNO$_3$ by addition of silver oxide. The concentrated samples were freeze dried, and the resulting AgNO$_3$ was stored in amber vials until isotopic analysis at the uwEILAB. Precision for this technique was +/- 0.5‰ for $\delta^{15}$N and +/- 1‰ for $\delta^{18}$O.

$\delta^{15}$N and $\delta^{18}$O of dissolved N$_2$O was measured by first extracting the N$_2$O with helium and cryogenic trapping and subsequent analysis by IRMS as described in Chapter 3. Precision for this method was +/- 0.4‰ for $\delta^{15}$N and +/- 0.8‰ for $\delta^{18}$O.
5.3.1 SIDNO Model

The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of $\text{N}_2\text{O}$ produced in the river are not necessarily the same as the measured values for dissolved $\text{N}_2\text{O}$. Specifically, gas exchange with tropospheric $\text{N}_2\text{O}$ and the mixing of sources makes it particularly difficult to interpret dissolved $\text{N}_2\text{O}$ isotopic data. For this reason, a simple box model was developed using Stella Modeling software to determine the relationship between the stable isotope composition of source, dissolved and emitted $\text{N}_2\text{O}$. This model is described in detail in Chapter 4, however a few small modifications were made for this study to better represent the theoretical framework for nitrogen cycling in the Grand River.

In brief, there are three major reservoirs in the model, representing the dissolved isotopologues of $\text{N}_2\text{O}$ ($\text{N}_2\text{O}$, $^{15}\text{N}_2\text{O}$, and $^{18}\text{N}_2\text{O}$). The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of dissolved $\text{N}_2\text{O}$ are calculated from the ratios of masses in each of these boxes. The production of $\text{N}_2\text{O}$ is modeled by adding mass to each box at a rate dictated by the $\text{N}_2\text{O}$ production rate and isotopic ratios of the $\text{N}_2\text{O}$ source. These parameters are set by the user. Gas exchange is modeled using the kinetic and equilibrium gas exchange fractionation factors determined by Inoue and Mook (1994). $\text{N}_2\text{O}$ in each reservoir is allowed to exchange with a tropospheric $\text{N}_2\text{O}$ pool, with a fixed concentration and isotopic ratios as determined by Kaiser et al (2003), Prinn et al (1990), Prinn et al (2000).

The model described in Chapter 4 was modified to better simulate conditions in the Grand River at the study site. First of all, a new function was added to the model to simulate the flow of the river. In this study, it is assumed that most of the $\text{N}_2\text{O}$ production in the river occurs downstream of the Kitchener WWTP effluent outfall. This assumption is justified by samples collected upstream of the Waterloo and Kitchener WWTPs; these
samples had N$_2$O concentrations much lower than the downstream samples, with a nearly constant concentration, $\delta^{15}$N and $\delta^{18}$O values (average values: concentration = 220 % SAT, $\delta^{15}$N = 5.3 ‰, $\delta^{18}$O = 44.6 ‰). From continuous streamflow and conductivity measurements provided by the Grand River Conservation Authority, it is known that the travel time during summer baseflow from the Kitchener WWTP outfall to the sampling site is approximately 2 hours. Calculations indicate that the average residence time of a dissolved N$_2$O molecule in the river (areal N$_2$O concentration / flux rate) is also approximately 2 hours. The short residence time coupled with the low upstream concentration means that the N$_2$O measured at the sampling location is most representative of production downstream of the Kitchener WWTP. Therefore, for the purpose of this study, the river was modeled as a well mixed reservoir with a turnover time equal to the hydraulic travel time between the Kitchener WWTP and the sampling site. An extra inflow and outflow was added to each box in the model, such that the turnover time of each reservoir was 2 hours. The N$_2$O concentration and isotopic composition of the inflow was dictated by the average values observed Kitchener WWTP effluent outfall during this sampling period, assuming complete mixing of the effluent in the river.

The second modification made to the model was to allow for two separate source N$_2$O processes. This allows a separate characterization of the dominant N$_2$O source for the oxic period during the day and dominant source during the hypoxic period at night. The model was designed to allow the N$_2$O production rate from these two processes to vary, but the $\delta^{15}$N and $\delta^{18}$O of each process was fixed.
The temperature parameter in the model was set to correspond with the field measurements. The gas exchange constant (K) was determined with the PoRGy model (Venkiteswaran 2007) using DO concentration and $\delta^{18}$O – DO measurements collected on four separate occasions at the sampling location during May to October 2006. The average K value calculated using this method was 0.2 m/hour. Determining K using the PoRGy model is advantageous compared to other methods because it integrates at the whole-reach scale, and is not influenced by small scale features in the river. The average depth of the river was known to be approximately 0.4 m at the site.

5.3.2 Calculations of $N_2O$ Flux and Isotopic Composition of Emitted $N_2O$

The net $N_2O$ flux from the dissolved phase to the atmosphere is calculated as follows,

$$N_2O\text{ Flux} = K \left( [N_2O]_{dissolved} - P_{N_2O} K_H \right)$$

(5.1)

Where the $N_2O$ flux is calculated in mol·m$^{-2}$·h$^{-1}$, K is the gas exchange coefficient (m·h$^{-1}$), $P_{N_2O}$ is the partial pressure of tropospheric $N_2O$ (atm), $K_H$ is the Henry’s constant for $N_2O$ (mol·atm$^{-1}$·m$^{-3}$), and $[N_2O]_{dissolved}$ is the dissolved concentration of $N_2O$ (mol·m$^{-3}$). The value for $K_H$ is calculated as a function of water temperature, (adapted from Gevantman 2008):

$$K_H = 55394 \times e^{\left(-60.7467 + \frac{8882.8}{T} + 21.2531 \ln \frac{T}{100}\right)}$$

(5.2)
Where \( T \) is water temperature (K) and \( K_h \) is expressed in mol·atm\(^{-1}\)·m\(^{-3}\).

As with the bulk N\(_2\)O flux, the flux of the heavy isotopologues (\( ^{15}\)N\(_2\)O and N\(_2^{18}\)O) can be calculated by including the kinetic fractionation factors for N\(_2\)O (adapted from Venkiteswaran et al. 2007) 

\[
\text{Flux} \ ^{15}\text{N}_2\text{O} = K \left( \alpha_{\text{in}}^{15} P_{^{15}\text{N}_2\text{O}} K_h \ - \alpha_{\text{ev}}^{15} [^{15}\text{N}_2\text{O}]_{\text{dissolved}} \right) \\
\text{Flux} \ N_2^{18}\text{O} = K \left( \alpha_{\text{in}}^{18} P_{N_2^{18}\text{O}} K_h \ - \alpha_{\text{ev}}^{18} [N_2^{18}\text{O}]_{\text{dissolved}} \right) 
\]

(5.3)

(5.4)

Where, \( P_{^{15}\text{N}_2\text{O}} \) and \( P_{N_2^{18}\text{O}} \) are calculated by multiplying the partial pressure of tropospheric N\(_2\)O (atm) by \( ^{15}\text{N}/^{14}\text{N} \) and \( ^{18}\text{O}/^{16}\text{O} \) ratios of tropospheric N\(_2\)O. \( \alpha_{\text{in}}^{15} \), \( \alpha_{\text{ev}}^{15} \), \( \alpha_{\text{in}}^{18} \), and \( \alpha_{\text{ev}}^{18} \) are the kinetic fractionation factors for N\(_2\)O invasion and evasion, as determined experimentally by Inoue and Mook (1994).

The \(^{15}\text{N}\) and \(^{18}\text{O}\) isotopic ratios of the N\(_2\)O flux can then be calculated as the ratio between the flux rates of the various isotopologues:

\[
R_{\text{flux}}^{15} = \frac{\text{Flux} \ ^{15}\text{N}_2\text{O}}{\text{Flux} \ ^{14}\text{N}_2\text{O}} = \frac{\text{Flux} \ ^{15}\text{N}_2\text{O}}{[\text{Flux} \ N_2\text{O} - \text{Flux} \ ^{15}\text{N}_2\text{O}]} \\
R_{\text{flux}}^{18} = \frac{\text{Flux} \ N_2^{18}\text{O}}{\text{Flux} \ N_2^{16}\text{O}} = \frac{\text{Flux} \ N_2^{18}\text{O}}{[\text{Flux} \ N_2\text{O} - \text{Flux} \ N_2^{18}\text{O}]} 
\]

(5.5)

(5.6)

**5.4 Results**

During the sampling period and the proceeding week, the river flow was generally constant at the study location at approximately 10 m\(^3\)/sec. The air temperature during the
sampling period ranged from 16.7°C to 32.0°C, measured at the University of Waterloo weather station (17.3 km from the sampling location). The University of Waterloo weather station did not record any precipitation during the sampling period, and the most recent rainfall was 6 days prior to sampling when 7 mm of rain was recorded.

The observed diel variation in DO was large, ranging from approximately 140% saturation during the late afternoon to 10% saturation just before sunrise (Figure 5.2). The DO concentration dropped off slightly in the late afternoon, likely due to a reduction in photosynthesis in response to intermittent cloud cover, which can be seen in the PAR data. The water temperature ranged from approximately 24°C to 29°C, the maximum temperature occurring in the early evening. The pH and conductivity of the river also followed a diel cycle, ranging from 7.7 to 8.7 (highest during the day) and 670 to 850 µS/cm (highest during the night) respectively. The first few pH measurements are missing due to a malfunction of the YSI probe. The diel cycles of DO, pH, temperature and conductivity measured here are typical of the diel variability observed by continuous water quality monitoring by the Grand River Conservation Authority at this location during the summer months.

Large diel changes in concentration were also observed for NO$_3^-$, NH$_4^+$, N$_2$, N$_2$O and CH$_4$ (Figure 5.2). NO$_3^-$ ranged from 1.4 to 2.7 mg N/L, with the peak concentration occurring just before sunset, and the minimum occurring just after sunrise. Similarly, NH$_4^+$ concentrations ranged from 0.07 to 1.1 mg N/L, the peak concentration occurring just after sunrise and the minimum concentration occurring during midday. The dissolved gasses N$_2$, N$_2$O and CH$_4$ were always above saturation during the sampling period, indicating the river is a source of these gases to the atmosphere. The concentration of
N$_2$O and CH$_4$ peaked during the night, at 8,500 % saturation and 30,700 % saturation respectively. Day time concentrations of both N$_2$O and CH$_4$ were significantly lower, at approximately 990 % saturation and 21,200 % saturation respectively. N$_2$ concentration also peaked during the night at approximately 160 % saturation, though there is much more scatter in the N$_2$ data compared to the other gases. This is because there is more error associated with the analysis of dissolved N$_2$ by the headspace equilibration technique. DOC concentration was relatively constant, ranging from 6.6 to 7.6 mg C/L, with a slight increasing trend over the sampling period.

The concentrations of NO$_3^-$, NH$_4^+$ and N$_2$O in effluent from the two WWTPs upstream of the site were generally constant during the sampling period, however a few samples had abnormally low concentrations of NH$_4^+$ and N$_2$O (Table 5.1). This data has been reported in more detail elsewhere (Rosamond 2008). The contribution of wastewater effluent to river flow was calculated using chloride concentrations as a conservative tracer. The average chloride concentration upstream and downstream of the WWTPs was 23 and 93 mg/L, respectively. Chloride concentrations in the WWTP effluent averaged 255 and 442 mg/L for the Waterloo and Kitchener plants, respectively. Assuming that the volume of effluent for each plant is proportional to the populations of Waterloo and Kitchener, the effluent outflow contributed 6.2% and 13.1% of the river flow at the downstream location, respectively during the sampling period. However it is possible that this calculation overestimates the WWTP contribution, due to the input of chloride to the Grand River from urban use of road salts.
Table 5.1: NO$_3^-$, NH$_4^+$, and N$_2$O concentrations measured in the WWTP effluent during the sampling period.

<table>
<thead>
<tr>
<th>Location</th>
<th>Date/Time</th>
<th>NO$_3^-$ (\text{mg N/L})</th>
<th>NH$_4^+$ (\text{mg N/L})</th>
<th>N$_2$O (%\text{SAT})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterloo WWTP</td>
<td>26/06/2007 13:55</td>
<td>2.2</td>
<td>9.6</td>
<td>5,120</td>
</tr>
<tr>
<td>Waterloo WWTP</td>
<td>26/06/2007 16:06</td>
<td>1.7</td>
<td>5.2</td>
<td>5,370</td>
</tr>
<tr>
<td>Waterloo WWTP</td>
<td>26/06/2007 17:37</td>
<td>1.7</td>
<td>5.2</td>
<td>4,801</td>
</tr>
<tr>
<td>Waterloo WWTP</td>
<td>27/06/2007 7:06</td>
<td>0.8</td>
<td>8.8</td>
<td>3,425</td>
</tr>
<tr>
<td>Waterloo WWTP</td>
<td>27/06/2007 8:49</td>
<td>1.1</td>
<td>0.5</td>
<td>213</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td>1.5</td>
<td>5.9</td>
<td>3,786</td>
</tr>
<tr>
<td>Kitchener WWTP</td>
<td>26/06/2007 12:44</td>
<td>0.0</td>
<td>24.2</td>
<td>781</td>
</tr>
<tr>
<td>Kitchener WWTP</td>
<td>26/06/2007 15:08</td>
<td>0.2</td>
<td>23.3</td>
<td>9,486</td>
</tr>
<tr>
<td>Kitchener WWTP</td>
<td>26/06/2007 16:49</td>
<td>0.2</td>
<td>21.9</td>
<td>10,257</td>
</tr>
<tr>
<td>Kitchener WWTP</td>
<td>27/06/2007 6:19</td>
<td>-</td>
<td>27.9</td>
<td>12,989</td>
</tr>
<tr>
<td>Kitchener WWTP</td>
<td>27/06/2007 8:03</td>
<td>0.2</td>
<td>26.7</td>
<td>14,103</td>
</tr>
<tr>
<td>Kitchener WWTP</td>
<td>27/06/2007 10:34</td>
<td>-</td>
<td>27.4</td>
<td>537</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td>0.1</td>
<td>25.2</td>
<td>8,025</td>
</tr>
</tbody>
</table>

The concentration of DO, NO$_3^-$, NH$_4^+$ and N$_2$O measured at the upstream site did not have the strong diel variation seen in the downstream site (Table 5.2). The concentration of NH$_4^+$ measured in the river upstream of the WWTPs was insignificant compared to that of NO$_3^-$. N$_2$O concentrations measured upstream of the WWTPs were always much less than the concentrations measured at the downstream location. CH$_4$ concentrations were similar at the two locations, with night time concentrations slightly higher than during the day.
Table 5.2: The diel variation of measured parameters upstream of the WWTPs as compared to those measured at the downstream location. Day values are an average of the data collected from 12:00 to 15:00, and night values are an average of the data collected from 2:00 to 5:00.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Upstream</th>
<th>Downstream</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td>Night</td>
</tr>
<tr>
<td>DO (% saturation)</td>
<td>140</td>
<td>64</td>
</tr>
<tr>
<td>NO\textsubscript{3}\textsuperscript{-} (mg N/L)</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>NH\textsubscript{4}\textsuperscript{+} (mg N/L)</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>N\textsubscript{2}O (% saturation)</td>
<td>190</td>
<td>270</td>
</tr>
<tr>
<td>N\textsubscript{2} (% saturation)</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>CH\textsubscript{4} (% saturation)</td>
<td>22,300</td>
<td>26,500</td>
</tr>
</tbody>
</table>

The stable isotope composition of NO\textsubscript{3}, NH\textsubscript{4}\textsuperscript{+} and N\textsubscript{2}O also followed a diel cycle during the sampling period (Figure 5.3). The $\delta^{15}$N – N\textsubscript{2}O reached a peak of -6‰ during the day, and a minimum of -27‰ at night. The $\delta^{18}$O – N\textsubscript{2}O was also variable, ranging from 28 to 35 ‰. $\delta^{18}$O – N\textsubscript{2}O was generally higher during the day compared to the nighttime values, however, there was a sudden decrease in $\delta^{18}$O values at midday. This decrease also corresponded to a smaller decrease in $\delta^{15}$N – N\textsubscript{2}O. The $\delta^{15}$N and $\delta^{18}$O values of dissolved N\textsubscript{2}O were closest to the isotopic composition of tropospheric N\textsubscript{2}O ($\delta^{15}$N = 6.72, $\delta^{18}$O = 44.62, Kaiser et al. 2003) during the day when the dissolved N\textsubscript{2}O concentration was at a minimum. The $\delta^{15}$N and $\delta^{18}$O values for N\textsubscript{2}O measured in the Grand River were more negative than the only other published values for riverine N\textsubscript{2}O ($\delta^{15}$N ranging from -3.8‰ to 15.6‰, $\delta^{18}$O ranging from 36.6‰ to 63.8‰, Boontanon et al. 2000).
The $\delta^{15}$N of NH$_4^+$ ranged between 22.9‰ and 35‰ during the sampling period. The $\delta^{15}$N – NH$_4^+$ exhibited an increasing trend during the day, while NH$_4^+$ concentrations were low. The $\delta^{15}$N-NH$_4^+$ peaked early in the night, just as the NH$_4^+$ concentrations began to rise.

The $\delta^{15}$N – NO$_3^-$ also displayed a diel variation. The $\delta^{15}$N – NO$_3^-$ ranged between 0.9‰ to 11.7‰. The $\delta^{15}$N – NO$_3^-$ followed the same trend as the NO$_3^-$ concentration, with the highest value for $\delta^{15}$N corresponded to the highest concentration, and lowest value for $\delta^{15}$N corresponded to the lowest concentration. The $\delta^{18}$O – NO$_3^-$ was more consistent than the $\delta^{15}$N, and ranged between -5.0‰ to -1.5‰, with the most negative value occurring during the day.
Figure 5.2: Physical and chemical parameters monitored during the sampling period. Time scale runs from noon June 25, 2007 to noon June 27, 2007. A – DO (% SAT), B – NH$_4^+$ (mg N/L), C – NO$_3^-$ (mg N/L), D – N$_2$O (%SAT), E – N$_2$ (%SAT), F – CH$_4$ (% SAT), G – Photosynthetically active radiation (PAR - µE), H – water temperature (°C), I – pH, J – electrical conductivity (µS/cm), K – dissolved organic carbon (DOC – mg C/L).
Figure 5.3: Results of isotopic analysis of water samples collected from the sampling site. Time scale runs from noon June 25, 2007 to noon June 27, 2007. A – \( \delta^{15}N \) of NH\(_4^+\), B – \( \delta^{15}N \) of NO\(_3^-\), C – \( \delta^{18}O \) of NO\(_3^-\), D – \( \delta^{15}N \) of N\(_2\)O, E – \( \delta^{18}O \) of N\(_2\)O.

5.5 Discussion

5.5.1 Sources of Nitrogen

The major inputs of nitrogen to the study reach of the Grand River are NH\(_4^+\) from WWTP effluent (Table 5.1), and NO\(_3^-\) loading from upstream sources (mainly agricultural runoff, Table 5.2). As observed during concurrent sampling at the WWTPs, NO\(_3^-\) concentrations in the effluent from both plants were low, while NH\(_4^+\) concentrations were elevated and generally constant (Table 5.1). Concurrent sampling in the Grand
River upstream of Waterloo WWTP effluent outfall indicated that the contribution of 
$NH_4^+$ from upstream sources was negligible (Table 5.2). However, upstream sources of 
$NO_3^-$ were significant. The concentration of $NO_3^-$ measured upstream of the Waterloo 
WWTP was nearly constant, ranging from 1.2 mg N/L to 1.4 mg N/L (Table 5.2).

Nitrification of $NH_4^+$ released from the WWTPs was an additional source of $NO_3^-$ 
measured at the downstream location. Evidence for nitrification in the river was given by 
the increase in $NO_3^-$ concentration from upstream of the WWTPs (approximately 1.3 mg 
N/L) to downstream of the WWTPs (ranging from 1.4 to 2.7 mg N/L), since the only 
significant source of nitrogen between these points was $NH_4^+$ in WWTP effluent (Figure 
5.4). The general rise in $\delta^{15}N$ - $NH_4^+$ during the day is consistent with $NH_4^+$ consumption 
through nitrification. The $\delta^{15}N$ –$NH_4^+$ reached a maximum value just before the 
concentration began to rise, and quickly dropped again as the concentration recovered to 
previous levels.

The results of $\delta^{15}N$ and $\delta^{18}O$ analysis of $NO_3^-$ also suggests nitrification is a 
significant source of $NO_3^-$ downstream of the WWTPs. Using the expected nitrogen 
fractionation factors for $NO_3^-$ production through nitrification (-29‰ to -12‰, Shearer & 
Kohl 1986, Kendall 1998), and the observed $\delta^{15}N$ values for $NH_4^+$ measured in the 
WWTP effluent, the $\delta^{15}N$ value of $NO_3^-$ produced through nitrification in the Grand 
River should range between -24.9‰ and 4‰. Furthermore, using the 1/3 $O_2$, 2/3 $H_2O$ 
rule for the oxygen isotopic composition of $NO_3^-$ produced through nitrification, the 
expected range in $\delta^{18}O$ of $NO_3^-$ can also be calculated (Aleem et al. 1965, Andersson & 
Hooper 1983, Kumar et al. 1983, Hollocher 1984). The $\delta^{18}O$ of DO was measured to 
range between 8.3‰ to 25.2‰ during the day, with the minimum value corresponding to
the maximum DO concentration (data not shown). Furthermore, it is known that the $\delta^{18}O$ of H$_2$O in the river is typically around -10‰. Therefore the expected range of $\delta^{18}O$ in NO$_3^-$ produced through nitrification is -3.9‰ to 1.7‰, with the more negative values corresponding to NO$_3^-$ production when the DO concentration and expected nitrification rate are at a maximum. Therefore, the $\delta^{15}N$ – NO$_3^-$ values measured at the downstream location are greater than would be expected from nitrification of WWTP derived NH$_4^+$ alone. The $\delta^{18}O$ – NO$_3^-$ values are slightly lower than expected, but are mostly consistent with NO$_3^-$ production through nitrification.

The concentration of dissolved inorganic nitrogen (DIN, calculated as [NO$_3^-$] + [NH$_4^+$]) measured downstream of the WWTPs is less than would be expected considering only the upstream input of NO$_3^-$ and the NH$_4^+$ from the WWTP effluent (Figure 5.4). Accounting for dilution, the DIN concentration downstream of the WWTPs would be expected to range from 4.6 to 5.6 mg N/L. However, the measured concentration ranged from 2.0 to 2.8 mg N/L, indicating that a significant amount of nitrogen is lost in the study reach of the river. It is possible that the difference between the expected and actual DIN concentration is due to an overestimation of the contribution of WWTP effluent to river flow.

DIN concentrations were generally lower during the day than at night. Although the magnitude of the variations in DIN concentration measured in the river are similar to those expected from the variation in WWTP effluent composition, the timing is incompatible and does not explain the observations (Figure 5.4). Therefore, other processes must be responsible for the change in DIN concentration.
Figure 5.4: Dissolved inorganic nitrogen (DIN) measured downstream of the WWTPs compared to upstream DIN inputs. Time scale runs from noon June 25, 2007 to noon June 27, 2007. WWTP DIN is calculated from concentrations of NO$_3^-$ and NH$_4^+$ measured in WWTP effluent and corrected for dilution. The dotted lines represent the range of DIN concentration expected at the downstream location considering only the upstream and WWTP DIN inputs and ignoring any losses.

During the day, the loss of DIN through the volatilization of NH$_3$ would have been particularly important. Near midday on July 26, 2007, the pH of the river water was at a maximum of 8.7, and the water temperature was 27.6°C. Under these conditions 25% of the total ammonia would be in the form of NH$_3$. The NH$_3$ would then be available for loss through gas exchange with the atmosphere. At night, when the pH and water temperature were lower (pH = 7.7, temperature = 25.3°C, Figure 5.2 H, I), only 3% of the total ammonia would be in the form of NH$_3$, reducing the rate of NH$_3$ loss through volatilization.
Other processes may also be important for the loss of DIN in the study reach. NH$_4^+$ can be lost through plant uptake or adsorption to suspended sediment. However, these processes have no effect on the $\delta^{15}$N of NH$_4^+$, and the high values measured in the river at the downstream location (Figure 5.3) indicate that these processes are not the major pathway for DIN loss in the study reach. Although nitrification also consumes NH$_4^+$ and causes elevated $\delta^{15}$N values, these values remain high even during the night when nitrification rates would be expected to be minimal. DIN may also be lost to the atmosphere as N$_2$O or N$_2$ during denitrification. The elevated concentrations of these two gasses (Figure 5.2) above the excess expected from diel changes in temperature at the upstream and downstream sites indicate that denitrification is a significant process for the removal of DIN from the Grand River.

Dissolved N$_2$O from WWTP effluent would have contributed significantly to the dissolved N$_2$O measured at the downstream location, especially during the day when river N$_2$O concentrations were low. The hydraulic transport time in the Grand River from the Waterloo and Kitchener WWTPs to the sampling location was approximately 8 and 2 hours, respectively. Using the observed range of dissolved N$_2$O measured in the WWTP effluent (Table 5.1), the range in expected concentrations of WWTP derived N$_2$O can be calculated for the sampling location by considering both dilution and gas exchange. Assuming that the effluent was uniformly mixed with the river, the average river depth was 0.4 m, the gas exchange coefficient (K) was 0.2 m/hour (calculated previously for the reach using the PoRGY model, Venkiteswaran et al. 2007), and no riverine N$_2$O production, the expected concentration of WWTP derived N$_2$O at the sampling location would range between 9.3 and 66.2 nmol N$_2$O/L. This corresponds to a range in the excess
N₂O concentration ([N₂O] – [N₂O]_{saturated}) of 1.8 to 58.7 nmol/L. This excess N₂O was almost entirely derived from the Kitchener WWTP. The contribution of N₂O from the Waterloo WWTP was insignificant, since all almost the N₂O from this source was lost to gas exchange with the atmosphere during the 8 hour travel time from the effluent outfall to the sampling location.

It is possible to calculate the expected contribution of WWTP derived N₂O to the total dissolved N₂O measured in the river, using the 2 hour hydraulic travel time from the Kitchener WWTP to the sampling site. During the day when the measured N₂O concentration in the river was low, WWTP derived N₂O accounted for approximately 4% to 28% of the excess N₂O at the downstream sampling location. Using the maximum N₂O concentration measured in the WWTP effluent, WWTP derived N₂O accounted for approximately 9% of dissolved N₂O in the river at its peak night time concentration.

Therefore, the contribution of WWTP effluent derived N₂O to that measured in the river was variable, ranging from <5% to approximately 30% of the excess dissolved N₂O. These estimates represent a maximum contribution of WWTP derived N₂O, since the estimates of effluent discharge rates may have been influenced by the contribution of chloride from urban runoff.

Although the concentration of dissolved N₂O in the WWTP effluent was not measured during the night period, it is extremely unlikely that the large increase in dissolved N₂O at the downstream location during the night could be explained by an increase in WWTP N₂O. To reach the maximum dissolved N₂O concentration of 8,540% saturation (640 nmol/L) measured in the river, the concentration of N₂O in the effluent
would have to exceed 180,000% saturation (13,500 nmol N$_2$O/L), which is almost 14 times higher than the maximum observed effluent N$_2$O concentration.

5.5.2 Diel Variability in Nitrogen Cycle Processes

The observed variation in concentrations of DO, NO$_3^-$, NH$_4^+$, N$_2$, N$_2$O and CH$_4$ cannot be explained by changes in upstream or WWTP input. The diel variation in the DO concentration was caused by photosynthesis during the day, and respiration at night. Many redox sensitive measured parameters varied concurrently with changes in the DO concentration at the downstream sampling site (Figure 5.5). Thus redox conditions in the river were likely affected by the diel variation in DO concentration.

**Figure 5.5**: The concentration of NO$_3^-$, NH$_4^+$ and CH$_4$ downstream of the WWTPs plotted against the concentration of DO. This indicates that changes in redox conditions are driven by changes in DO concentration.
The dominant N\textsubscript{2}O production pathways were likely different during the day when DO concentrations were high, than at night when DO concentrations were low. The diel change in N\textsubscript{2}O concentration and isotopic composition (Figures 5.2 and 5.3) likely resulted from a change in the N\textsubscript{2}O production pathway. The separate characterization of N\textsubscript{2}O produced during the day and night can provide further insight into the diel variation of nitrogen cycling in the Grand River.

5.5.3 Stable Isotope Composition of Riverine N\textsubscript{2}O Production

The isotopic composition of emitted N\textsubscript{2}O is more representative of the N\textsubscript{2}O source than the $\delta^{15}$N and $\delta^{18}$O measured for dissolved N\textsubscript{2}O in aquatic environments open to gas exchange with the atmosphere. The average residence time of a N\textsubscript{2}O molecule in solution (defined as the areal concentration / flux rate) was approximately 2 hours at the sampling location during the study period. Therefore, the residence time is short relative to the period of variability (diel, approximately 24 hours), and the isotopic composition of the N\textsubscript{2}O flux will closely match that of the N\textsubscript{2}O source (See Chapter 4). The isotopic composition of the N\textsubscript{2}O flux can be calculated from the dissolved concentration, $\delta^{15}$N and $\delta^{18}$O of N\textsubscript{2}O (Figure 5.6, equations 5.1 – 5.6). Downstream of the WWTPs, the isotopic composition of the emitted N\textsubscript{2}O was very similar to that of the dissolved N\textsubscript{2}O (Figure 5.3 D, E) because the concentration of dissolved N\textsubscript{2}O was high. N\textsubscript{2}O emitted at night had a $\delta^{15}$N ranging from -28‰ to -22‰, and a $\delta^{18}$O ranging from 26‰ to 29‰. N\textsubscript{2}O emitted during the day had a $\delta^{15}$N ranging from -15‰ to -9‰, and a $\delta^{18}$O ranging from 25‰ to 32‰.
Figure 5.6: The isotopic composition of the N₂O emitted to the atmosphere from the Grand River during the sampling period. Due to the high dissolved N₂O concentration, the isotopic composition of emitted N₂O was very similar to the dissolved. Time scale runs from noon June 25, 2007 to noon June 27, 2007. A – δ¹⁵N of emitted N₂O. B – δ¹⁸O of emitted N₂O. C – δ¹⁵N vs. δ¹⁸O of emitted N₂O indicating a difference in the isotopic composition of N₂O emitted during the day compared to the night. Isotopic composition of tropospheric N₂O was determined by Kaiser et al. (2003).

The diel change in δ¹⁵N and δ¹⁸O of emitted N₂O indicates that the isotopic composition of the N₂O source must have varied on a diel basis. This is consistent with the diel change in redox conditions and suggests the dominant N₂O production pathways are different during the day compared to the night.

Using the modified SIDNO model (Chapter 4), it was possible to further characterize the isotopic composition of N₂O produced in the Grand River. The modified SIDNO model allowed for the isolation of N₂O production in the river, by assuming a constant WWTP effluent N₂O source. Additionally, the modified SIDNO model allowed for the separate characterization of day time and night time N₂O production. This provided additional insight into the diel variation in riverine nitrogen cycling.
Since the diel change in $\text{N}_2\text{O}$ production appeared to be driven by the diel change in DO concentration, the timing of peak day and night $\text{N}_2\text{O}$ production was set to match the timing of maximum and minimum DO concentration. The duration of the day time $\text{N}_2\text{O}$ production was set to correspond to the time period that the DO concentration was greater than 20% saturation. The peak of the nighttime $\text{N}_2\text{O}$ production was set to occur when the DO concentration was low, and the timing was adjusted to match the nighttime peak in $\text{N}_2\text{O}$ concentration.

SIDNO produced a best fit for the field data by setting the peak daytime $\text{N}_2\text{O}$ production rate 50 $\mu\text{mol} \text{N}_2\text{O}/\text{m}^2/\text{hour}$, and the peak night time $\text{N}_2\text{O}$ production rate to 250 $\mu\text{mol} \text{N}_2\text{O}/\text{m}^2/\text{hour}$ (Figure 5.7). $\text{N}_2\text{O}$ produced during the day was found to have a $\delta^{15}\text{N}$ of -22‰ and a $\delta^{18}\text{O}$ of 43‰. $\text{N}_2\text{O}$ produced at night was found to have a $\delta^{15}\text{N}$ of -30‰ and a $\delta^{18}\text{O}$ of 30‰.
**Figure 5.7:** Model output compared to measured field data. Day time N₂O production parameters: Peak N₂O production rate = 50 µmol N₂O/m²/hour, δ¹⁵N = -22‰, δ¹⁸O = 43‰. Night time N₂O production parameters: Peak N₂O production rate = 250 µmol N₂O/m²/hour, δ¹⁵N = -30‰, δ¹⁸O = 30‰.
In general, the modeled data matches the field data quite well for dissolved N\textsubscript{2}O concentration and $\delta^{15}$N. However, the model does not fit well to the $\delta^{18}$O values of dissolved N\textsubscript{2}O. The model predicted a drop in $\delta^{18}$O of dissolved N\textsubscript{2}O shortly after the DO concentration began to rise. However, the field data indicates that $\delta^{18}$O actually increased at that time. This is likely explained by a change in the amount of N\textsubscript{2}O released through the WWTP effluent. The WWTP N\textsubscript{2}O input is assumed to be constant in the model, but it is known that the N\textsubscript{2}O output from the Kitchener WWTP was low in the morning of June 26, 2007 (Table 5.1). Therefore, the model overestimated the WWTP contribution at that time, and since the average $\delta^{18}$O of the WWTP derived N\textsubscript{2}O is much less than that produced in the river (average WWTP N\textsubscript{2}O $\delta^{18}$O = 15.2‰), the model underestimated the value for $\delta^{18}$O at that time. For the same reason, the model also slightly overestimated the $\delta^{15}$N value for dissolved N\textsubscript{2}O at the same time (average WWTP N\textsubscript{2}O $\delta^{15}$O = 4.4‰). The model also does not recreate the day time drop in dissolved N\textsubscript{2}O $\delta^{18}$O values.

Although the model was not able to recreate the $\delta^{18}$O of N\textsubscript{2}O produced during the day, the fit to the night time field data was much better. This indicates that the $\delta^{18}$O of N\textsubscript{2}O produced at night was constant, and variations in WWTP output were not significant. Therefore, it can be assumed that the night time $\delta^{15}$N and $\delta^{18}$O values for produced N\textsubscript{2}O determined using SIDNO are representative of the natural system. However, for day time N\textsubscript{2}O production, only the $\delta^{15}$N value should be considered, since the model was unable to recreate the day time $\delta^{18}$O field data.
5.5.4 Daytime $\text{N}_2\text{O}$ Production Pathways

Since the dissolved $\text{N}_2\text{O}$ concentrations measured at the downstream location are significantly greater than upstream of the WWTPs, and cannot be explained by the input from the WWTP effluent alone, significant daytime $\text{N}_2\text{O}$ production must have occurred in the study reach of the Grand River. There is evidence that denitrification occurred continuously in the river sediments. Denitrification can only occur in anoxic zones or microsites. The elevated $\text{CH}_4$ concentration during the day (minimum concentration of 20,000% saturation) indicated that there were anoxic zones within the river sediments, even when DO concentrations were high. Also, the $\text{N}_2$ concentration remained supersaturated above the concentration expected from the diel temperature variation at the sampling location. Since $\text{N}_2$ is the final product of denitrification, these observations indicated that anoxic conditions existed in the river sediments and denitrification was actively occurring under these conditions.

The amount of $\text{N}_2\text{O}$ produced by denitrification in the river sediments likely varied in response to the DO concentration. When the DO concentration in the river was high, the anoxic zone would have retreated deeper within the sediments, slowing the rate of $\text{NO}_3^-$ diffusion into the active denitrification zone, and reducing the amount of $\text{N}_2\text{O}$ produced. Also, a deep active denitrification zone would slow the diffusion of $\text{N}_2\text{O}$ out of the sediments, providing more time for $\text{N}_2\text{O}$ to be further reduced to $\text{N}_2$ and reducing the total amount of $\text{N}_2\text{O}$ released. When the DO concentration began to fall in the late afternoon and evening, the anoxic zone would move closer to the sediment surface, increasing the amount of $\text{N}_2\text{O}$ produced and released.
The daytime concentrations of DO, NO$_3^-$, N$_2$ and CH$_4$ were similar upstream and downstream of the WWTPs (Table 5.2). Therefore, it is likely that the N$_2$O production rates through sediment denitrification were similar at these two locations; however the daytime N$_2$O concentration was much higher at the downstream site (Table 5.2). N$_2$O derived from WWTP effluent likely accounts for a maximum of 30% of the daytime excess dissolved N$_2$O observed at the downstream site. N$_2$O derived from sediment denitrification would only account for an additional 7% of the daytime excess N$_2$O if the day time denitrification rates are similar upstream and downstream of the WWTPs.

Although additional input of organic matter from the WWTP and decaying plant matter may enhance anoxic conditions and denitrification in the sediments at the downstream site, the similar daytime CH$_4$ and N$_2$ concentrations at the upstream and downstream locations indicate that this was not an important factor. Therefore, an additional process is responsible for the elevated daytime N$_2$O concentrations at the downstream site.

Since nitrification was actively occurring during the day, it represents an additional pathway for N$_2$O production. Models developed to simulate N$_2$O production in the natural environment have indicated that generally the amount of N$_2$O produced through nitrification is low compared to the total amount of nitrogen processed. For example, the PnET-N-DNDC model was developed to simulate N$_2$O production in forest soils (Li et al. 2000). In this model, the N$_2$O production rate in nitrification is only 0.06% of the gross nitrification rate. Other models, such as RIVERSTRAHLER, used in the Lower Seine River and estuary have indicated that the nitrification N$_2$O production rate may be as high as 1-2% of the gross nitrification rate (Garnier et al. 2007). However, the RIVERSTRAHLER model suggests that N$_2$O production through nitrification only
reaches the maximum rate through the nitrifier-denitrification pathway, when DO concentrations are below 1.5 mg/L, and nitrite (NO₂⁻) concentrations are greater than 1-4 mg N/L. Although daytime DO concentrations were much greater than 1.5 mg/L at the downstream location, it is possible that low DO concentrations were present at the sediment/water interface. NO₂⁻ concentrations were not measured in this study, but since it is an intermediate in both nitrification and denitrification, it may have been present in significant concentrations at the sediment/water interface. The excess daytime dissolved N₂O that cannot be attributed to WWTP effluent or sediment denitrification sources is approximately 55 to 80 nmol/L. Assuming that most of the nitrification and N₂O production occurs immediately downstream of the WWTP outfall, this corresponds to approximately 0.28% to 0.42% of the total NH₄⁺ nitrogen converted to NO₃⁻ at the peak nitrification rate. These rates are less than the maximum nitrification N₂O production rates described by the RIVERSTRAHLER model (Garnier et al. 2007), and may be consistent with production through nitrification or the nitrifier-denitrification pathway.

The δ¹⁵N of N₂O produced during the day (determined with the SIDNO model δ¹⁵N = -22‰) is consistent with N₂O production through nitrification or nitrifier-denitrification. Using published enrichment factors and the observed day time range for δ¹⁵N of NH₄⁺, the δ¹⁵N of N₂O produced through nitrification or nitrifier-denitrification would be expected to range from -23‰ to -5‰ (Sutka et al. 2006, Yoshida 1988), but may be as low as -87‰ (Perez et al. 2006). However, this result cannot be used to rule out N₂O production through sediment denitrification. Using published enrichment factors, the δ¹⁵N of N₂O produced through denitrification would be expected to range

The day time drop in δ¹⁸O values is likely an indicator of N₂O production through nitrification or nitrifier-denitrification. Other studies have shown that the δ¹⁸O of N₂O produced through nitrification has a similar value to ambient molecular oxygen (Perez et al. 2006, Yoshinari & Wahlen 1985). The δ¹⁸O values for DO were observed to vary widely during the day, ranging from 8.3‰ at the maximum DO concentration, to 25.2‰ as the DO concentration decreased in the late afternoon and evening. This would account for the day time variation in δ¹⁸O of N₂O and suggest that nitrification or nitrifier-denitrification is the dominant N₂O production process during the day.

### 5.5.5 Night-time N₂O Production

During the night period, when the DO concentration was very low, the N₂O production rate and concentration increased dramatically at the downstream location. The isotopic composition of N₂O produced at night was different than during the day (Figures 5.5, 5.6) indicating that the dominant production pathway was also different than during the day.

When the DO concentration dropped below 1.5 mg/L, the denitrification rate increased dramatically, as indicated by the rapid increase in N₂O and N₂ concentrations. As the DO concentration fell, the river sediments became more anoxic, and it is likely that the entire sediment layer, including the sediment / water interface was completely anoxic. This would have allowed N₂O production from denitrification to proceed at a high rate.
It is possible that denitrification occurred not only within the river sediments, but within the water column when the DO concentration was very low at night. Since $\text{N}_2\text{O}$ production through denitrification in the water column is not limited by the diffusion of $\text{NO}_3^-$ into the river sediments, its onset has the potential to produce a rapid change in the dissolved $\text{N}_2\text{O}$ concentration. It is difficult to distinguish between denitrification in the water column and in the sediments on the basis of the measured $\text{N}_2\text{O}$, $\text{NO}_3^-$ and $\text{N}_2$ concentrations alone. However, denitrification activity was likely higher in the sediments than in the water column, due to the greater number of denitrifying organisms that would have been present in the sediments and sediment surface.

The $\text{N}_2\text{O}$ concentration began to fall shortly before sunrise while the DO concentration was low and the denitrification rate was expected to remain high. This is potentially due to the consumption of extracellular $\text{N}_2\text{O}$ through reduction to $\text{N}_2$. However, the results of the stable isotope analysis of dissolved $\text{N}_2\text{O}$ rules out this possibility. Other studies have shown that $\text{N}_2\text{O}$ reduction causes a relative enrichment of $\delta^{18}\text{O}$ compared to $\delta^{15}\text{N}$ in the residual $\text{N}_2\text{O}$ (Vieten et al. 2007, Menyailo & Hungate 2006, Mandernack et al. 2000), however, this enrichment was not seen in the dissolved $\text{N}_2\text{O}$ as the concentration fell. Therefore, the drop in $\text{N}_2\text{O}$ production when the DO concentration was at its lowest was likely a reduction in the $\text{N}_2\text{O}:\text{N}_2$ ratio of the denitrification products. More anoxic redox conditions induce a drop in the $\text{N}_2\text{O}:\text{N}_2$ ratio. If more $\text{N}_2\text{O}$ is reduced to $\text{N}_2$ without first being released from the microbial cells during denitrification, it is likely that there is no relative enrichment related to this process. Therefore, the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ – $\text{N}_2\text{O}$ data supports the hypothesis that the drop in $\text{N}_2\text{O}$
concentration was due to a reduction in the ratio of N\textsubscript{2}O:N\textsubscript{2} released during denitrification, rather than a reduction in extracellular N\textsubscript{2}O to N\textsubscript{2}.

The very large increase in the N\textsubscript{2} concentration measured at night is consistent with an increase in the denitrification rate when the DO concentration was low. However, the amount of NO\textsubscript{3}\textsuperscript{-} that would be consumed to produce the observed N\textsubscript{2} increase from the daytime minimum to the night time maximum is approximately 3.5 mg N/L, which is more than the maximum NO\textsubscript{3}\textsuperscript{-} concentration measured in the river. Therefore, the measured night time N\textsubscript{2} concentrations may have been elevated by entrapped air bubbles during sampling, and may not be representative of the actual N\textsubscript{2} concentrations in the river. A better estimate of the night time denitrification rate can be calculated using the N\textsubscript{2}O concentrations, since entrapped air in the sample bottles would not cause a significant change in the measured concentration. The maximum N\textsubscript{2}O concentration at night was 640 nmol N\textsubscript{2}O/L, which is equivalent to approximately 0.02 mg N/L. Using the RIVERSTRAHLER model, Garnier et al. (2007) determined that the N\textsubscript{2}O:N\textsubscript{2} production ratio for denitrification in the Seine River ranged between 1:10 and 1:5. If it can be assumed that the N\textsubscript{2}O:N\textsubscript{2} ratios are similar in the Grand River, this relates to a maximum 0.2 mg N/L of NO\textsubscript{3}\textsuperscript{-} consumed during maximum denitrification. Therefore, the drop in NO\textsubscript{3}\textsuperscript{-} concentration observed downstream of the WWTPs at night resulted from the lack of NO\textsubscript{3}\textsuperscript{-} production through nitrification, rather than from increased consumption through denitrification.

The high denitrification rates at night do not appear to cause an increase in the $\delta^{15}$N and $\delta^{18}$O values of the NO\textsubscript{3}\textsuperscript{-} measured in the river. Using enrichment factors determined by Mengis et al. (1999), the consumption of 0.2 mg N/L NO\textsubscript{3}\textsuperscript{-} should have
increased the NO$_3^-$ $\delta^{15}$N by approximately 4.3‰, and the $\delta^{18}$O by approximately 2.8‰. However, if most of the denitrification occurred in the sediments, it would be limited by the diffusion of NO$_3^-$ into the active zone. Any NO$_3^-$ in the active zone of the sediments would be completely consumed, and since there is no residual, sediment denitrification would likely not cause an increase in the $\delta^{15}$N and $\delta^{18}$O values of NO$_3^-$ in the water column. Therefore, since $\delta^{15}$N and $\delta^{18}$O of NO$_3^-$ did not increase at night, this likely indicates most of the denitrification likely occurred in the sediments or at the sediment / water interface, rather than in the water column.

Nitrification activity during the night was minimal, and was likely not a significant source of N$_2$O production when DO concentrations were low. At night, when DO concentrations were less than 15% saturation, it was likely that the entire sediment layer, including the sediment / water interface, was completely anoxic. Most nitrifying organisms downstream of the WWTPs are likely attached to the sediment surface. Since water column NH$_4^+$ concentrations are significant only in the short reach downstream (within approximately 10 km) of the WWTPs, nitrifying organisms would likely not survive in the water column, as they would be quickly carried away from the NH$_4^+$ substrate source. Therefore, the nitrifying organisms were likely under completely anoxic conditions during the night period, and nitrification activity would cease until DO concentrations increased again shortly after sunrise.

Other observations are also consistent with a cessation of nitrification activity during the night period. As the DO concentration fell, the NO$_3^-$ concentration measured at the downstream location also fell to the background concentration measured at the upstream site. Also, during the night, $\delta^{15}$N values for both NO$_3^-$ and NH$_4^+$ became less
positive. If NO$_3^-$ was no longer being produced from nitrification of a high $\delta^{15}$N NH$_4^+$ substrate, both the concentration and $\delta^{15}$N of NO$_3^-$ would have decreased. The decrease in $\delta^{15}$N NH$_4^+$ is also consistent with a lack of nitrification at night.

The diel variation in nitrogen cycling processes can be summarized by a conceptual model (Figure 5.8). The dominant processes responsible for N$_2$O production in the Grand River downstream from the WWTPs varied in response to the diel DO concentration change. When DO concentrations were high, N$_2$O was produced mainly through nitrification or nitrifier-denitrification, and to a lesser extent by denitrification in the river sediment. During the night, when DO concentrations were low, denitrification was the dominant N$_2$O production pathway, occurring in the sediments and at the sediment / water interface.
Figure 5.8: Conceptual model of the diel variability of nitrogen cycling in the Grand River. The major nitrogen transformations are thought to occur either in the sediments, in the water column, or at the sediment water interface (SWI). A – Nitrogen cycling during periods of high dissolved oxygen concentrations, typically during the day due to photosynthesis. B – Nitrogen cycling during periods of low dissolved oxygen, typically at night due to high respiration and a lack of photosynthesis. The thickness of the arrows represents the relative important of each pathway or source.
5.5.6 Implications

This study is one of the first to measure $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of dissolved N$_2$O produced in a river. Boontanon et al (2000) measured $\delta^{15}\text{N}$ –N$_2$O ranging from -3.8‰ to 15.6‰ and $\delta^{18}\text{O}$ ranging from 36.6‰ to 63.8‰ in the Bang Nara River in Thailand, while the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of N$_2$O produced in the Grand River was generally more negative. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values for emitted N$_2$O can be calculated for both rivers (equations 5.1 – 5.6, Figure 5.9). The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values for emitted N$_2$O from the Bang Nara River were calculated using N$_2$O % saturation values inferred from the data published by Boontanon et al. (2000). The flux weighted average for N$_2$O produced in the Grand River was -21.6‰ and 24.8‰ for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, respectively. The flux weighted average for N$_2$O produced in the Bang Nara River (Boontanon et al. 2000) is more positive for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ (-0.1‰ and 41.1‰, respectively). It is not clear if this difference is a reflection of a difference in the N$_2$O production pathways between these two rivers (i.e. the dominance of nitrification, denitrification or other processes such as anamox), or if there are other factors involved. For example, the isotopic composition of the substrates (NO$_3^-$ and NH$_4^+$) were not measured by Boontanon et al. (2000), so it is not possible to directly compare enrichment factors for N$_2$O production. The values for the Grand River are influenced by the contribution of WWTP derived N$_2$O, which had a isotopic composition that was much different than the values for in-river N$_2$O production determined using the SIDNO model (average $\delta^{15}\text{N} =4.4‰$, average $\delta^{18}\text{O} =15.2‰$, n = 4 , measured at the Kitchener WWTP). However, the large difference in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of N$_2$O between these two studies suggests that there is likely a large range of the isotopic composition of
N$_2$O produced in rivers worldwide, and much more work is necessary to fully characterize the composition of N$_2$O produced in these environments.

Figure 5.9: Calculated values for $\delta^{15}$N and $\delta^{18}$O of emitted N$_2$O from the Grand River compared to the data of Boontanon et al (2000). Values for day time and night time N$_2$O production determined using the SIDNO model are also shown, along with the average value for WWTP derived N$_2$O. The value for tropospheric N$_2$O was determined by Kaiser et al. (2003).

Since denitrification was the dominant N$_2$O production process during the night, the SIDNO model output can be used to determine apparent *in-situ* enrichment factors for N$_2$O production through denitrification in a river environment. Most of the published enrichment factors for these processes are based on laboratory culture experiments (e.g. Sutka et al. 2006, Toyoda et al. 2005, Casciotti et al. 2002), and may not be representative of N$_2$O production in the natural environment. Other studies have shown that only approximately 0.25% of the total microbial population in freshwater environments can be grown in laboratory cultures (Amann et al 1995). But by comparing
the model output for $\delta^{15}$N and $\delta^{18}$O of $N_2O$ produced in the river to the measured $\delta^{15}$N and $\delta^{18}$O of the NO$_3^-$ precursor, it is possible to infer the apparent in-situ enrichment factors for denitrification in the Grand River. During the night, the measured $\delta^{15}$N for NO$_3^-$ ranged between 0.8‰ and 4.4‰. The measured $\delta^{18}$O for NO$_3^-$ ranged between -3.2‰ and -1.4‰. Using the best fit values for $\delta^{15}$N and $\delta^{18}$O of $N_2O$ produced through denitrification, a range of $\varepsilon$ values can be calculated for $^{15}$N and $^{18}$O. For $N_2O$ production through denitrification, $\varepsilon^{15}$N ranged between -34.4‰ to -30.8‰, and $\varepsilon^{18}$O ranged between 31.4‰ to 33.2‰.

The apparent nitrogen enrichment factors are more negative than those typically seen for denitrification in published literature. Although several laboratory incubation studies have measured $\varepsilon^{15}$N values for denitrification greater than -30‰ (Tilsner et al. 2003, Perez et al. 2006, Sutka et al. 2006, Toyoda et al. 2005, and Yoshida et al. 1988), $\varepsilon^{15}$N values for denitrification in many other studies have generally been smaller (typically ranging from -30‰ to -10‰, Menyailo & Hungate 2006, Wada et al. 1991, Barford et al. 1999, Schumidt and Voekelius 1989). The apparent $\varepsilon^{18}$O values for denitrification are more positive than most published values, though Casciotti et al. (2002) measured a $\varepsilon^{18}$O value of 40‰ for denitrification in a laboratory culture. It should be noted that the apparent enrichment factors measured in the Grand River are could be affected by $N_2O$ consumption by extracellular reduction to $N_2$, this would cause the apparent enrichment factors to be more positive than the true values. In addition, most laboratory culture experiments inhibit the reduction of $N_2O$ to $N_2$, which would change the observed enrichment factor.
The SIDNO results can also be used to calculate enrichment factors for N$_2$O produced through nitrification during the day, however, this calculated value is likely influenced by the contribution of N$_2$O from denitrification in the river sediments. The δ$^{15}$N of N$_2$O produced during the day was approximately -22‰, and the δ$^{15}$N of NH$_4^+$ in the WWTP effluent ranged from 4.1‰ to 16.0‰. Therefore, the apparent in-situ enrichment factor for nitrification (ε$^{15}$N) ranged between -26‰ to -38‰. This value is less negative than most published values available in the literature (-112‰ to -35‰, Perez et al. 2006, Sutka et al. 2006, Yoshida 1988).

The results of this study also have implications for other studies that are aimed at the assessment of water quality. Most often, routine sampling of rivers for water quality monitoring is conducted during the day. However, the results presented here clearly indicate that this may often be inadequate to accurately characterize the concentrations of several important compounds. For example, the Canadian Water Quality Guideline for un-ionized ammonia (NH$_3$) is 19 µg/L (CCME 2007). Under the conditions observed during the sampling period (pH range 7.7 to 8.7, temperature range 25.3°C to 27.6°C), this limit corresponds to NH$_4^+$ concentrations ranging from 0.01 to 0.6 mg N/L. Our results indicate that these limits are consistently exceeded in the Grand River at the sampling location. However, since nitrification rates are typically greatest during the day when DO concentrations are highest, the concentration of NH$_4^+$ could fall below the threshold value during the day when NH$_4^+$ concentrations are low, and exceed the limit at night when the NH$_4^+$ concentration rises. Routine daytime water quality sampling would not capture this variability.
This study also has implications for studies of greenhouse gas emissions. Concentrations of N₂O and CH₄ in the Grand River are much higher at night. Greenhouse gas emissions from the Grand River would be significantly underestimated if only daytime concentrations were used. The opposite trend was seen by Harrison et al (2005) who found that the emission of N₂O from a subtropical eutrophic stream was much less at night than during the day. Our results also differ from that of Clough et al (2007) who did not observe a significant diel change in N₂O emission. In rivers with a large diel variation in DO concentrations, diel sampling is necessary to properly characterize nitrogen cycling and greenhouse gas production.

5.6 Summary & Conclusions

This study is one of the first to characterize the isotopic composition of N₂O produced in a river, and the only study to measure the diel variability in δ¹⁵N and δ¹⁸O of riverine N₂O. N₂O produced during the day was isotopically distinct from N₂O produced at night, indicating a diel shift in the dominant N₂O production pathways. Using the SIDNO model, it was possible to determine the isotopic composition of N₂O produced in the river, as the measured dissolved values were influenced by gas exchange with tropospheric N₂O and the contribution of N₂O derived from WWTP effluent. Using the model, it was determined that the N₂O produced in the river during the day (dominated by nitrification or nitrifier-denitrification) had a δ¹⁵N of approximately -22‰, and a δ¹⁸O of approximately 43‰. At night, the N₂O production rate was much greater than during the day, and was likely dominated by denitrification. The δ¹⁵N of N₂O produced at night was approximately -30‰, while the δ¹⁸O was approximately 30‰.
The results of this study indicate that the nitrogen cycling processes in the Grand River are linked to diel variations in DO concentration. Other studies have also observed a link between riverine nitrogen cycling and diel variations in DO concentration. Harrison et al. (2005) observed a diel shift in nitrogen cycling processes in a canal in the Yaqui Valley, Mexico. However, in contrast to the Grand River, Harrison et al. (2005) observed that denitrification peaked early at night but then ceased, due to the total consumption of NO$_3^-$.

In the Grand River, denitrification and N$_2$O production occurred at a high rate throughout the night, indicating that the DO concentration did not drop sufficiently to cause the total denitrification of all the NO$_3^-$ in the water column and sediments.

Another study on the LII River in New Zealand (Clough et al. 2007) also observed diel changes in N$_2$O production in response to changes in DO concentration. However, the diel range of DO concentration was much greater in the Grand River than was observed in the LII River (Clough et al. 2007). This related to a greater diel response in nitrogen cycling in the Grand River as compared to the LII River. The results of these studies clearly indicate that riverine nitrogen cycling responds to diel variation in DO concentration. Diel shifts in riverine nitrogen cycling are highly sensitive to the magnitude of the diel DO concentration range.

The results of this study indicate that the isotopic analysis of N$_2$O can provide additional information not available from concentration data alone. Although the isotopic enrichment factors for nitrification and denitrification often overlap, making a clear interpretation difficult, a change in the isotopic composition of produced N$_2$O is a conclusive indicator that the dominant production pathways have also changed. Also, the
$\delta^{18}O$ of N$_2$O may be a useful indicator for nitrification in environments with a strong diel variation in $\delta^{18}O$ of DO, since the $\delta^{18}O$ of N$_2$O produced through nitrification will respond to this variation. Additionally, the isotopic analysis of N$_2$O provides information regarding the importance of extracellular consumption of N$_2$O, allowing the relative importance of this pathway to be determined. The isotopic analysis of N$_2$O will prove valuable in future studies of nitrogen cycling and N$_2$O production in aquatic environments.
Chapter 6:

Stable Isotope Ratios of Nitrous Oxide Emissions from the Grand River, Ontario, Canada

6.1 Introduction

Nitrous oxide (N\textsubscript{2}O) is a powerful greenhouse gas, approximately 310 times more potent than CO\textsubscript{2} on a 100-year timescale (Denman et al 2007). The concentration of N\textsubscript{2}O in the atmosphere has steadily risen over the last 250 years (Denman et al 2007), and currently the atmospheric concentration is 320 ppbv, increasing at a rate of 0.26%/year (Prinn et al 1990, Prinn et al 2000). N\textsubscript{2}O is produced by microbial transformations of reactive nitrogen (nitrification & denitrification) in the environment. Due to artificial nitrogen fixation, the amount of reactive nitrogen in the environment has increased dramatically over the last 250 years, coinciding with an increase in N\textsubscript{2}O production in the environment (Denman et al 2007).

Stable isotope analysis is a powerful tool that can provide a deeper understanding of sources and processes responsible for the production of N\textsubscript{2}O. For example, several studies have used $\delta^{15}$N and $\delta^{18}$O analysis of N\textsubscript{2}O to distinguish between nitrification and denitrification processes in the field (e.g. Bol et al. 2003, Perez et al 2001). Another use of stable isotope analysis of N\textsubscript{2}O is the isotopic characterization of global N\textsubscript{2}O sources and sinks, for the calculation of atmospheric N\textsubscript{2}O budgets.

Rahn & Wahlen (2000) developed a simple model of the isotopic composition of atmospheric N\textsubscript{2}O. If the stable isotope ratios of the major N\textsubscript{2}O sources are known, it is possible to determine the relative contribution of these sources using their model. Rahn &
Wahlen determined that the difference between the preindustrial and modern values for tropospheric N$_2$O is approximately 1.9‰ and 2.4 ‰ for $\delta^{15}$N and $\delta^{18}$O respectively, with $\delta^{15}$N decreasing by approximately 0.03‰/year. The most recent and thorough isotopic characterization of tropospheric N$_2$O places the current values at 6.72 (+/- 0.12) ‰ and 44.62 (+/- 0.21) ‰ for $\delta^{15}$N and $\delta^{18}$O, respectively (Kaiser et al 2003). At this level of precision, it will be several years before a change in $\delta^{15}$N and $\delta^{18}$O of atmospheric N$_2$O will be detected. Since the work of Rahn & Wahlen (2000), many more studies have been conducted to characterize the isotopic ratios of N$_2$O emitted from both terrestrial and oceanic environments. The results of these studies are summarized in Figure 6.1.

The global isotopic model of atmospheric N$_2$O developed by Rahn & Wahlen (2000) only considered terrestrial and oceanic sources of N$_2$O. More recent work has suggested that rivers and estuaries are a major source of N$_2$O to the atmosphere. Kroeze et al (2005) and Nevison et al. (2004) estimate that rivers and estuaries contribute between 0.5 to 2.9 Tg N/year of N$_2$O production. This compares to an estimated total of 9.4 Tg N/year and 3.8 Tg N/year from soil and oceanic sources (Denman et al 2007).

Although this evidence suggests that rivers and estuaries are a significant global source of N$_2$O to the atmosphere, the isotopic composition of this N$_2$O is poorly constrained. To date, only one other published study has reported the $\delta^{15}$N and $\delta^{18}$O values for N$_2$O produced in a river (Boontanon et al 2000).

To further complicate matters, the isotopic composition of N$_2$O emitted to the atmosphere from rivers is not equal to the isotopic composition of dissolved N$_2$O, due to the equilibration with tropospheric N$_2$O in such systems (Chapter 4). Additionally, several studies have shown that the N$_2$O flux and N$_2$O production processes in rivers can
change dramatically over diel time periods, in response to changes in redox conditions brought on by diel variations in dissolved oxygen (DO) concentrations (Harrison et al 2005, Clough et al 2007, Chapter 5). For these reasons, the $\delta^{15}$N and $\delta^{18}$O values of N$_2$O emitted from rivers is particularly difficult to characterize.

The purpose of this study is to characterize the isotopic composition of N$_2$O emitted to the atmosphere from the Grand River, Ontario, Canada, and to provide insights into the processes responsible for the variability observed. It is expected that the isotopic signature of N$_2$O in river systems will vary spatially and temporally, on seasonal and diel timescales. This information will be invaluable for the refinement of the global N$_2$O isotopic budget (Rahn & Wahlen 2000), and will greatly improve the understanding of N$_2$O production in rivers.
6.2 Study Site

The Grand River is the largest river in southern Ontario, Canada. It is approximately 300 km long, and drains an area of 7000 km² into Lake Erie (Figure 6.2). In 2001, the population in the watershed was approximately 720 000, and is expected to increase by about 60% by 2031 (Ministry of Public Infrastructure Renewal 2006).

Figure 6.2: Map of the Grand River watershed and study area. River flow direction is generally north to south. Additional information about sampling points and wastewater treatment plants is given in Tables 6.1 & 6.2.

The Grand River is heavily impacted by both diffuse and point-source inputs of nitrogen. Agriculture is the primary land use in the watershed, and 26 wastewater treatment plants (WWTPs) currently discharge effluent to the Grand or its tributaries. The cities of Waterloo, Kitchener, Guelph and Cambridge form the urban centre of the watershed, and the WWTPs for these cities are the major source of municipal effluent to the river. Most of the sampling for this study was conducted at six major sampling points...
in the central watershed, located upstream and downstream of these major WWTPs (Figure 6.2). Some additional data was collected from the lower reaches of the Grand River (LG - Figure 6.2), between the communities of York and Dunville. Information about the sampling locations and major WWTPs are summarized in Tables 6.1 & 6.2.

**Table 6.1:** Summary of sampling sites. Location names in the first column refer to labels in Figure 6.2.

<table>
<thead>
<tr>
<th>Sampling Location</th>
<th>Watercourse</th>
<th>Geographical Coordinates</th>
<th>Description</th>
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<tbody>
<tr>
<td>WM</td>
<td>Grand River</td>
<td>43°35'07.77&quot;N, 80°28'53.51&quot;W</td>
<td>Upstream of urban centre</td>
</tr>
<tr>
<td>BP</td>
<td>Grand River</td>
<td>43°28'54.54&quot;N, 80°28'53.32&quot;W</td>
<td>Upstream of Waterloo and Kitchener WWTPs</td>
</tr>
<tr>
<td>BL</td>
<td>Grand River</td>
<td>43°23'07.80&quot;N, 80°23'09.51&quot;W</td>
<td>Downstream of Waterloo and Kitchener WWTPs</td>
</tr>
<tr>
<td>GM</td>
<td>Grand River</td>
<td>43°16'36.73&quot;N, 80°20'47.15&quot;W</td>
<td>Downstream of urban centre</td>
</tr>
<tr>
<td>SP-A</td>
<td>Speed River</td>
<td>43°32'04.47&quot;N, 80°15'03.81&quot;W</td>
<td>Upstream of Guelph WWTP</td>
</tr>
<tr>
<td>SP-B</td>
<td>Speed River</td>
<td>43°29'03.06&quot;N, 80°16'54.16&quot;W</td>
<td>Downstream of Guelph WWTP</td>
</tr>
<tr>
<td>LG</td>
<td>Grand River</td>
<td>43°01'18&quot;N, 79°53'30&quot;W to 42°54'7&quot;N, 79°37'52&quot;W</td>
<td>Low energy reaches of the Grand River, located between the communities of York and Dunville</td>
</tr>
</tbody>
</table>

**Table 6.2:** Nutrient loading from the major WWTPs in the Grand River watershed. Letters in the first column refer to labels in Figure 6.2. Data provided by NPRI (2007).

<table>
<thead>
<tr>
<th>WWTP</th>
<th>Mass Discharged through Effluent</th>
<th>Total N tonnes/yr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH$_4^+$</td>
<td>NO$_3^-$</td>
</tr>
<tr>
<td>A Waterloo</td>
<td>77.8</td>
<td>196.4</td>
</tr>
<tr>
<td>B Kitchener</td>
<td>633.8</td>
<td>35.3</td>
</tr>
<tr>
<td>C Preston</td>
<td>3.5</td>
<td>42.3</td>
</tr>
<tr>
<td>D Galt</td>
<td>2.2</td>
<td>242</td>
</tr>
<tr>
<td>E Guelph</td>
<td>6.3</td>
<td>1831</td>
</tr>
<tr>
<td>(not shown)</td>
<td>Brantford</td>
<td>134.9</td>
</tr>
<tr>
<td>Total</td>
<td><strong>858.5</strong></td>
<td><strong>2402.4</strong></td>
</tr>
</tbody>
</table>

**6.3 Methods**

River water samples were collected periodically at the various sampling locations during the period of May 2006 to January 2008. Sampling at all locations (with the
exception of LG) was usually conducted every 2-3 weeks; however sampling was
typically more intensive during the summer months. Sampling was also more intensive
during and following high river flow periods (daily sampling during snowmelt and storm
events), in order to capture the variability in N₂O fluxes associated with these hydrologic
events.

Intensive diel sampling was conducted in June 2007 at four sites (BP, BL, SP-A,
SP-B) over two separate 28 hour periods, with samples collected every 1.5 hours. Diel
sampling was conducted at the BP and BL sites on June 26 – 27, 2007, and at the SP-A
and SP-B sites on June 23 – 24, 2007. This diel sampling was conducted to characterize
the changes in δ¹⁵N and δ¹⁸O of N₂O produced in the river in response to the diel
dissolved oxygen (DO) cycles in the river at these locations. These four sites were chosen
to contrast N₂O production upstream and downstream of the major WWTPs in the
watershed (Figure 6.2, Table 6.2).

Samples for the measurement of dissolved N₂O concentration were collected in
50mL glass serum bottles (Wheaton # 223745), and capped with no headspace
underwater with a red rubber stopper (Vaccutainer™). Stoppers are baked at 60°C
overnight before use to limit out gassing into the samples. Samples for the isotopic
analysis of N₂O were collected in 500mL glass media bottles (Corning # 1395-500), and
similarly capped underwater with no headspace using a black rubber lyophilization
stopper (Wheaton # 224100-503). All samples were both preserved with saturated HgCl₂
solution (2mL solution / L sample) and kept on ice until they were transported back to the
lab, typically within 4 hours of collection. Samples were kept in cold storage (4°C) until
they were analyzed (typically within 2 days of collection).
Dissolved N\textsubscript{2}O concentrations were determined using a headspace equilibration technique. A headspace was created in the sample bottles by injection of ultra-high purity helium using a syringe and hypodermic needle, while extracting sample water with a second syringe and needle. The samples were then placed on an orbital shaker for 1.5 hours to bring the dissolved phase into equilibrium with the headspace. N\textsubscript{2}O concentration in the headspace was then determined using a Varian CP-3800, equipped with an ECD detector. The analytical error for dissolved N\textsubscript{2}O concentration using this method is approximately ±5%.

Stable isotope ratios of dissolved N\textsubscript{2}O were obtained using the purge and trap method described by Thuss et al (2008a). Briefly, dissolved N\textsubscript{2}O is sparged from the water samples using a fast flowing stream of ultra-pure helium. Water vapour and CO\textsubscript{2} is stripped from the resulting gas mixture using a nafion membrane and chemical traps. N\textsubscript{2}O is trapped cryogenically in a vial containing pyrex wool and glass beads in a liquid nitrogen bath. The extracted N\textsubscript{2}O is then analyzed by CF-IRMS using a GV Trace-Gas preconcentrator system coupled with a GV Isoprime mass spectrometer. The analytical precision for both δ\textsubscript{15}N and δ\textsubscript{18}O is approximately ±0.5‰.

N\textsubscript{2}O fluxes from the river to the atmosphere were calculated from the dissolved concentration using gas exchange coefficients determined with the PoRGy model (Venkiteswaran et al 2007). N\textsubscript{2}O concentrations and flux rates for the Grand River during the study period are reported in more detail elsewhere (Rosamond 2008).

The isotopic ratios of the N\textsubscript{2}O flux to the atmosphere were calculated using the isotopic and concentration data for dissolved N\textsubscript{2}O, as described in Chapter 4. Since gas exchange is a two-way equilibrium process, there is a continuous invasion of
tropospheric N\textsubscript{2}O into solution, even when the net N\textsubscript{2}O flux is in the opposite direction.

This calculation accounts for the isotopic effects of the isotopic equilibration with tropospheric N\textsubscript{2}O, as well as the equilibrium and kinetic fractionation factors for N\textsubscript{2}O gas exchange determined by Inoue & Mook (1994). The stable isotope ratios of the N\textsubscript{2}O flux (R\textsubscript{15}\text{flux} and R\textsubscript{18}\text{flux}) are simply the ratios between the flux rates of the heavy and light isotopologues of N\textsubscript{2}O:

$$ R_{\text{flux}}^{15} = \frac{\text{Flux}^{15}\text{N}_2\text{O}}{\text{Flux}^{14}\text{N}_2\text{O}} = \frac{\text{Flux}^{15}\text{N}_2\text{O}}{[\text{Flux} \text{N}_2\text{O} - \text{Flux}^{15}\text{N}_2\text{O}]} $$

$$ R_{\text{flux}}^{18} = \frac{\text{Flux} \text{N}_2^{18}\text{O}}{\text{Flux} \text{N}_2^{16}\text{O}} = \frac{\text{Flux} \text{N}_2^{18}\text{O}}{[\text{Flux} \text{N}_2\text{O} - \text{Flux} \text{N}_2^{18}\text{O}]} $$

The fluxes (mol·m\textsuperscript{-2}·h\textsuperscript{-1}) for bulk N\textsubscript{2}O and the heavy isotopologues can be calculated as follows:

$$ \text{Flux N}_2\text{O} = K(P(\text{N}_2\text{O})K_h - [\text{N}_2\text{O}]_{\text{dissolved}}) $$

$$ \text{Flux}^{15}\text{N}_2\text{O} = K \left[ \alpha^{15}_{\text{in}} P(\text{N}_2\text{O})K_h \left( \frac{\text{R}_{\text{gas}}^{15}}{1 + \text{R}_{\text{gas}}^{15}} \right) - \alpha^{15}_{\text{ev}} [\text{N}_2\text{O}]_{\text{dissolved}} \left( \frac{\text{R}_{\text{dissolved}}^{15}}{1 + \text{R}_{\text{dissolved}}^{15}} \right) \right] $$

$$ \text{Flux} \text{N}_2^{18}\text{O} = K \left[ \alpha^{18}_{\text{in}} P(\text{N}_2\text{O})K_h \left( \frac{\text{R}_{\text{gas}}^{18}}{1 + \text{R}_{\text{gas}}^{18}} \right) - \alpha^{18}_{\text{ev}} [\text{N}_2\text{O}]_{\text{dissolved}} \left( \frac{\text{R}_{\text{dissolved}}^{18}}{1 + \text{R}_{\text{dissolved}}^{18}} \right) \right] $$

Where \(P(\text{N}_2\text{O})\) is the partial pressure of tropospheric N\textsubscript{2}O (atm - as determined by Prinn et al. 1990, Prinn et al. 2000), \(\text{R}_{\text{gas}}^{15}\) and \(\text{R}_{\text{gas}}^{18}\) are the isotopic ratios of tropospheric N\textsubscript{2}O (as determined by Kaiser et al 2003), \(\alpha^{15}_{\text{in}}, \alpha^{18}_{\text{in}}, \alpha^{15}_{\text{ev}}, \) and \(\alpha^{18}_{\text{ev}}\) are the kinetic fractionation factors for N\textsubscript{2}O gas exchange (as determined by Inoue & Mook 1994), and
\([N_2O]_{\text{dissolved}}\) is the dissolved concentration of \(N_2O\) (mol·m\(^{-3}\)). \(K_h\) (mol·atm\(^{-1}\)·m\(^{-3}\)) is Henry’s constant for \(N_2O\), and can be calculated empirically as follows (adapted from Gevantman 2008):

\[
K_h = 55394 \times e^{-60.7467 + \frac{2882.8}{T} + 21.2531 \ln \frac{T}{100}}
\]  

(6.6)

Where \(T\) is water temperature in K. Although the gas exchange coefficient (K - m·h\(^{-1}\)) is unknown for equations 6.3 - 6.5, the isotopic ratios of the \(N_2O\) flux depend only on the relative flux rates between the major isotopologues, and the term is eliminated by substitution into equations 6.1 & 6.2.

The error associated with the calculated \(\delta^{15}N\) and \(\delta^{18}O\) values of the \(N_2O\) flux is greater than the error for \(\delta^{15}N\) and \(\delta^{18}O\) of dissolved \(N_2O\), since the calculation also depends on the dissolved concentration which has an associated error value.

The error for the calculated isotopic ratios of the \(N_2O\) flux was calculated by varying the \(\delta^{15}N\) and \(\delta^{18}O\) values for dissolved \(N_2O\) by \(\pm 0.5\%e\), and the dissolved concentration values by \(\pm 5\%\). The maximum variation in the calculated values for \(\delta^{15}N\) and \(\delta^{18}O\) were taken as the error. For most data points, the error associated with this calculation was \(< 2\%e\). Data points with an error greater than \(5\%e\) were rejected.

To calculate the annual average isotopic composition of the bulk \(N_2O\) flux from the central Grand River, only data from 2007 were included in the analysis. The \(\delta^{15}N\) and \(\delta^{18}O\) data were weighted by flux rate and time. Flux rates for each data point were calculated using the dissolved \(N_2O\) concentration and representative gas exchange rates.
calculated using the PoRGy gas exchange model (Rosamond et al 2008, Venkiteswaran et al 2007). The annual average isotopic composition of N₂O flux from the LG sites was not calculated due to insufficient data for this analysis.

6.4 Results

During the period from May 2006 to January 2008, 616 river samples were collected for dissolved N₂O concentration analysis (Figure 6.3). Of these samples, 268 were analyzed to determine the δ¹⁵N and δ¹⁸O of dissolved N₂O (Figure 6.4). In 40 of these samples, the dissolved N₂O concentration was too low to accurately calculate the isotopic composition of the N₂O flux, so it was possible to determine the δ¹⁵N and δ¹⁸O of the N₂O flux on only 228 samples (Figure 6.5). Generally, the calculated δ¹⁵N and δ¹⁸O values for the N₂O flux become less accurate as the concentration approaches 100% saturation. Typically, the rejected data points had concentrations that were less than 130% saturation. Therefore the rejected data points represented the lowest 15% of the measured N₂O concentrations, and the magnitude of the N₂O flux for these data points was insignificant.

There is a significant difference in δ¹⁵N and δ¹⁸O values for the dissolved N₂O and the N₂O flux to the atmosphere (Figure 6.6). The difference was most pronounced in samples with N₂O concentration less than 1000% saturation. The δ¹⁵N and δ¹⁸O values for dissolved N₂O range from -35.3 to 8.2‰ and 22.7 to 59.1‰, respectively. In contrast, the δ¹⁵N and δ¹⁸O values for the N₂O flux are more spread out, ranging from -57.4 to 8.7‰ and 11.3 to 77.1‰, respectively. This indicates that the isotopic composition of dissolved N₂O in the Grand River is largely controlled by the equilibration with
tropospheric N\textsubscript{2}O, resulting in $\delta^{15}$N and $\delta^{18}$O values that approach the composition of tropospheric N\textsubscript{2}O ($\delta^{15}$N = 6.72 +/- 0.12‰ and $\delta^{18}$O = 44.62 +/- 0.21‰; Kaiser et al 2003) as the concentration decreases. Since the isotopic composition of the N\textsubscript{2}O emitted to the atmosphere (Figure 6.5) is most representative of the isotopic composition of N\textsubscript{2}O produced in the river at steady state, (Chapter 4) the discussion in this chapter will focus on the calculated $\delta^{15}$N and $\delta^{18}$O values for the N\textsubscript{2}O flux.

There is a large amount of spatial variation in the $\delta^{15}$N and $\delta^{18}$O – N\textsubscript{2}O data collected from the Grand River. There is a clear difference in N\textsubscript{2}O concentration and isotopic composition from upstream of the Waterloo and Kitchener WWTPs (WM & BP sites) compared to data collected immediately downstream (BL site). N\textsubscript{2}O from the BL site is the most depleted in both $^{15}$N and $^{18}$O, with most of the flux values ranging from -30 to -10‰, and 20 to 30‰ for $\delta^{15}$N and $\delta^{18}$O (Table 6.3). The N\textsubscript{2}O concentration is also the greatest at the BL site, with dissolved concentrations as high as 8,500% saturation (Figure 6.3, Table 6.3). These differences in N\textsubscript{2}O concentration and isotopic composition are a result of nutrient input to the Grand River from the upstream WWTPs.

High nutrient concentrations downstream of the Waterloo and Kitchener WWTPs, cause heavy macrophyte growth in the river channel. Also, these treatment plants (especially the Kitchener WWTP, Table 6.2) release large amounts of NH\textsubscript{4}\textsuperscript{+} (Table 6.2), and rely on nitrification in the river oxidize the NH\textsubscript{4}\textsuperscript{+} to NO\textsubscript{3}\textsuperscript{-}. Respiration and nitrification result in very low night-time concentrations of dissolved oxygen (DO) downstream of the Waterloo and Kitchener WWTPs, which stimulate high rates of N\textsubscript{2}O production through denitrification (Chapter 5). The discharge of municipal WWTP
effluent into the Grand River therefore has a clear effect on the downstream nitrogen cycling and N₂O production.

The type of wastewater treatment and the quality of the effluent released to the river also has an effect on the isotopic composition and concentration of N₂O in the river. Although SP-B is also directly downstream of a major WWTP (Guelph), the N₂O isotopic composition and production rate at this location is very different than at BL (downstream of Waterloo and Kitchener WWTPs). While dissolved N₂O concentrations at BL reached a maximum of 8,500% saturation, the maximum concentration observed at SP-B was only 515% saturation (Table 6.3). The isotopic composition of the N₂O flux at SP-B is also more enriched in ¹⁵N and ¹⁸O compared to BL (Table 6.3). While the nitrogen released by the Waterloo and Kitchener WWTPs is predominantly in the form of NH₄⁺, the Guelph plant fully nitrifies the wastewater before it is released to the river, and NO₃⁻ is the predominant nitrogen species released. Therefore, the discharge of effluent from the Guelph WWTP likely does not stimulate nitrification in the river, though the release of NO₃⁻ has the potential to stimulate denitrification. However, the concentration of DO at SP-B does not fall below 4 mg/L, making very large increases in denitrification rate unlikely. Compared to the large difference in N₂O concentration and isotopic composition upstream and downstream of the Waterloo and Kitchener WWTPs (BP and BL), the concentration and isotopic composition of N₂O in the Speed River upstream and downstream of the Guelph WWTP (SP-A and SP-B) are quite similar (Table 6.3). Although the addition of NO₃⁻ and organic nitrogen from the Guelph WWTP has the potential to affect the downstream nitrogen cycling, there is no significant change in N₂O production.
Although GM is also located downstream of several major WWTPs (Figure 6.2), the N\textsubscript{2}O concentrations (and thus N\textsubscript{2}O production rates) are similar to the upstream sites (Figure 6.3). However, the isotopic composition of the N\textsubscript{2}O flux is intermediate between the BL site and the rest of the upstream sites (Figure 6.5). Although the N\textsubscript{2}O is similar to the upstream sites, the nitrogen source and N\textsubscript{2}O production is influenced by the WWTPs upstream of this location. Previous work by the Grand River Conservation Authority has determined that there is significant groundwater discharge to the Grand River upstream of the GM site. The additional groundwater dilutes the nitrogen nutrients in the river, leading to an improvement in water quality. Macrophyte growth in the river channel at GM is much less than at BL, and thus the diel change in DO concentration is also much less at this location. Although the NO\textsubscript{3}^- concentration at GM remain elevated with respect to the sites upstream of the WWTPs (WM and BP), the heavy NH\textsubscript{4}^+ load from the Waterloo and Kitchener WWTPs is fully nitrified far upstream of the GM site. Therefore, effluent from these treatment plants does not affect N\textsubscript{2}O production at this location to the same extent as the BL location.

The δ\textsuperscript{15}N and δ\textsuperscript{18}O values from the samples collected at the LG sites are the most positive compared to data collected at all other sites (Table 6.3). The isotopic composition of the N\textsubscript{2}O flux from the LG sites is very similar to that of N\textsubscript{2}O collected from oceanic waters (Figure 6.1). The gradient of the river is very low at the LG sites, and a small dam in Dunville, Ontario greatly increases river depth and hydraulic residence time throughout this reach. Since the river water is much deeper and more stagnant at these sites, dissolved N\textsubscript{2}O remains in the water column relatively longer before it is released to the atmosphere through gas exchange. Therefore, there is a greater
potential for dissolved N\textsubscript{2}O to be consumed through reduction to N\textsubscript{2} gas. Several studies have shown that $\delta^{18}$O tends increase faster than $\delta^{15}$N in residual N\textsubscript{2}O during reduction to N\textsubscript{2} gas, and there is typically a 1:2 ratio for $\delta^{15}$N:$\delta^{18}$O during this process (Vieten et al. 2007, Menyailo & Hungate 2006, Mandernack et al. 2000). The $\delta^{15}$N and $\delta^{18}$O values for the N\textsubscript{2}O flux from the LG sites trends along this 1:2 line. Other studies that have measured the isotopic composition of N\textsubscript{2}O from deep or stagnant water (oceanic studies, Figure 6.1, Green Lake – Wahlen & Yoshinari 1985, Bang Nara River – Boontanon et al 2000), have observed $\delta^{15}$N and $\delta^{18}$O values which are generally more positive compared to N\textsubscript{2}O from terrestrial sources. It is likely that high $\delta^{15}$N and $\delta^{18}$O values are characteristic of dissolved N\textsubscript{2}O in deep or stagnant water sites due to N\textsubscript{2}O reduction to N\textsubscript{2}.

**Figure 6.3:** Dissolved N\textsubscript{2}O concentrations measured at the seven sampling locations on the Grand and Speed Rivers during the study period. Bars represent median values, 10\textsuperscript{th}, 25\textsuperscript{th}, 75\textsuperscript{th}, and 90\textsuperscript{th} percentiles. Individual points represent outliers which fall outside the 10\textsuperscript{th} and 90\textsuperscript{th} percentiles. Note the log scale.
Figure 6.4: $\delta^{15}$N and $\delta^{18}$O values of dissolved N$_2$O measured at the seven sampling locations on the Grand and Speed Rivers during the study period. Analytical precision for $\delta^{15}$N and $\delta^{18}$O is approximately 0.5‰. $(Kaiser$ et al. 2003).}

Figure 6.5: Calculated $\delta^{15}$N and $\delta^{18}$O values of the N$_2$O flux to the atmosphere from the seven sampling locations on the Grand and Speed Rivers during the study period. Values are calculated using equations 6.1-6.6, and the $\delta^{15}$N, $\delta^{18}$O and concentration values of dissolved N$_2$O. Data points were rejected where the calculation error exceeded 5‰ for $\delta^{15}$N or $\delta^{18}$O. The large cluster of BP points to the left of the tropospheric N$_2$O point were all collected during a 28 hour period $(Kaiser$ et al. 2003).
Table 6.3: Summary of dissolved N\(_2\)O concentration, and \(\delta^{15}\)N and \(\delta^{18}\)O values for dissolved and flux N\(_2\)O from the seven sampling locations on the Grand and Speed Rivers during the study period. Concentration data only includes samples for which \(\delta^{15}\)N and \(\delta^{18}\)O analysis was also conducted.

<table>
<thead>
<tr>
<th>Sampling Location</th>
<th>Concentration (% saturation)</th>
<th>Concentration (‰)</th>
<th>Concentration (‰)</th>
<th>n</th>
<th>Concentration (‰)</th>
<th>Concentration (‰)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM</td>
<td>105 to 115</td>
<td>-2 to 6</td>
<td>36 to 48</td>
<td>39</td>
<td>-18 to 8</td>
<td>11 to 53</td>
<td>29</td>
</tr>
<tr>
<td>BP</td>
<td>100 to 300</td>
<td>-35 to 2</td>
<td>24 to 46</td>
<td>68</td>
<td>-23 to 10</td>
<td>29 to 54</td>
<td>58</td>
</tr>
<tr>
<td>BL</td>
<td>120 to 8540</td>
<td>-6 to 9</td>
<td>23 to 49</td>
<td>68</td>
<td>-50 to -9</td>
<td>11 to 43</td>
<td>64</td>
</tr>
<tr>
<td>GM</td>
<td>105 to 330</td>
<td>-29 to 4</td>
<td>34 to 50</td>
<td>36</td>
<td>-24 to 1</td>
<td>17 to 52</td>
<td>29</td>
</tr>
<tr>
<td>SP-A</td>
<td>190 to 390</td>
<td>-4 to 6</td>
<td>39 to 54</td>
<td>26</td>
<td>-9 to 4</td>
<td>33 to 59</td>
<td>25</td>
</tr>
<tr>
<td>SP-B</td>
<td>105 to 515</td>
<td>-19 to 5</td>
<td>23 to 49</td>
<td>24</td>
<td>-25 to 3</td>
<td>16 to 9</td>
<td>23</td>
</tr>
<tr>
<td>LG</td>
<td>115 to 460</td>
<td>0 to 8</td>
<td>42 to 59</td>
<td>17</td>
<td>-10 to 9</td>
<td>33 to 82</td>
<td>13</td>
</tr>
</tbody>
</table>
**Figure 6.6:** The difference in the isotopic composition of dissolved N\textsubscript{2}O and calculated flux N\textsubscript{2}O as a function of concentration. The difference is most pronounced when the concentration is less than 1000 % saturation. A – $\delta^{15}$N\textsubscript{dissolved} – $\delta^{15}$N\textsubscript{flux} vs. concentration. B – $\delta^{18}$O\textsubscript{dissolved} – $\delta^{18}$O\textsubscript{flux} vs. concentration.

### 6.4.1 Seasonal Variability

N\textsubscript{2}O concentration and fluxes were generally much higher in the warmer months (May to October) compared to the colder months of the year (November to April) (Figure 6.7, Rosamond 2008). Since N\textsubscript{2}O is produced as a result of biological processes, it is not
surprising that $N_2O$ production rates are greater with increased water temperatures. Similarly, the $\delta^{15}N$ and $\delta^{18}O$ values of the $N_2O$ flux to the atmosphere also vary seasonally.

Typically, there is a greater range of $\delta^{15}N$ and $\delta^{18}O$ values for $N_2O$ produced during the warm months as compared to the cold months (Figures 6.8 & 6.9, Table 6.4). The difference in the $\delta^{15}N$ and $\delta^{18}O$ values of the $N_2O$ flux between the various sampling locations is also most pronounced during the warm months (Figure 6.8, Table 6.4).

Generally, the $N_2O$ flux is more depleted in $^{15}N$ and $^{18}O$ during the cold months of the year (November to April), and values are generally more consistent between the various sampling locations. The $\delta^{15}N$ of NO$_3^-$ collected upstream and downstream of the major WWTPs at various times throughout the year was quite consistent, ranging between 7.4 and 11.2‰ (Table 6.5). Given that the isotopic composition of the nitrogen source is relatively constant throughout the year, the differences are a result of changes in isotopic enrichment factors for $N_2O$ production only.

The NO$_3^-$ concentration in the river is typically greater in the winter months as compared to the summer months. Since fractionation is expected to be greatest when the substrate is non-limiting, the change in NO$_3^-$ concentration does not explain the change in fractionation factors for $N_2O$ production. Based on laboratory incubation experiments, fractionation factors for $N_2O$ production are inversely proportional to the production rate when the substrate concentration is held constant (Mariotti et al. 1981, 1982). Since the $N_2O$ production rate is lower during the cold months, this effect partially explains the lower $\delta^{15}N$ and $\delta^{18}O$ values.
Figure 6.7: Average monthly N$_2$O flux rates from the four sampling locations in the central Grand River (Rosamond 2008). Fluxes are calculated using the concentration data and gas exchange rates obtained using the PoRGy model (Venkiteswaran et al 2007). Average monthly flux rates were not calculated for the SP-A, SP-B and LG sites due to insufficient data.

Figure 6.8: Calculated $\delta^{15}$N and $\delta^{18}$O values of the N$_2$O flux to the atmosphere from the seven sampling locations on the Grand and Speed Rivers for the period from May to October. These values are representative of N$_2$O produced during the warm months of the year. $^1$(Kaiser et al 2003).
Figure 6.9: Calculated δ¹⁵N and δ¹⁸O values of the N₂O flux to the atmosphere from the seven sampling locations on the Grand and Speed Rivers for the period from November to April. These values are representative of N₂O produced during the cold months of the year. *(Kaiser et al 2003).*

Table 6.4: Summary of the calculated δ¹⁵N and δ¹⁸O values of the N₂O flux to the atmosphere from the seven sampling locations on the Grand and Speed Rivers for the summer and winter periods. Summer is defined as the period from May to October, and winter is defined as the period from November to April.

<table>
<thead>
<tr>
<th>Sampling Location</th>
<th>Concentration (‰)</th>
<th>δ¹⁵N (‰)</th>
<th>δ¹⁸O (‰)</th>
<th>n</th>
<th>Concentration (‰)</th>
<th>δ¹⁵N (‰)</th>
<th>δ¹⁸O (‰)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM</td>
<td>105 to 315</td>
<td>-9 to 4</td>
<td>19 to 40</td>
<td>13</td>
<td>105 to 165</td>
<td>-16 to -2</td>
<td>30 to 36</td>
<td>5</td>
</tr>
<tr>
<td>BP</td>
<td>100 to 300</td>
<td>-23 to 8</td>
<td>28 to 54</td>
<td>41</td>
<td>105 to 250</td>
<td>-17 to -6</td>
<td>28 to 40</td>
<td>14</td>
</tr>
<tr>
<td>BL</td>
<td>170 to 8540</td>
<td>-37 to -9</td>
<td>18 to 37</td>
<td>43</td>
<td>120 to 540</td>
<td>-49 to -9</td>
<td>11 to 43</td>
<td>21</td>
</tr>
<tr>
<td>GM</td>
<td>140 to 305</td>
<td>-24 to 1</td>
<td>17 to 52</td>
<td>15</td>
<td>105 to 270</td>
<td>-22 to -7</td>
<td>24 to 40</td>
<td>12</td>
</tr>
<tr>
<td>SP-A</td>
<td>185 to 255</td>
<td>-9 to 4</td>
<td>42 to 59</td>
<td>20</td>
<td>230 to 390</td>
<td>-9 to -2</td>
<td>33 to 49</td>
<td>5</td>
</tr>
<tr>
<td>SP-B</td>
<td>280 to 515</td>
<td>-14 to 3</td>
<td>29 to 48</td>
<td>19</td>
<td>105 to 610</td>
<td>-25 to -8</td>
<td>16 to 36</td>
<td>5</td>
</tr>
<tr>
<td>LG</td>
<td>110 to 460</td>
<td>1 to 9</td>
<td>49 to 77</td>
<td>8</td>
<td>160 to 190</td>
<td>-10 to -6</td>
<td>33 to 44</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 6.5: A summary of the measured $\delta^{15}$N values for NO$_3^-$ in the Grand River, collected at various times throughout the year during the study period.

<table>
<thead>
<tr>
<th>Sampling Location</th>
<th>$\delta^{15}$N – NO$_3^-$ (%o)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
</tr>
<tr>
<td>BP</td>
<td>9.8</td>
</tr>
<tr>
<td>BL</td>
<td>9.3</td>
</tr>
<tr>
<td>GM</td>
<td>9.1</td>
</tr>
<tr>
<td>SP-B</td>
<td>8.7</td>
</tr>
</tbody>
</table>

6.4.2 Diel Variability

Previous work has shown that the production rate and isotopic composition of N$_2$O in the Grand River downstream of the Waterloo and Kitchener WWTPs (BL site) can vary greatly in response to diel changes in dissolved oxygen concentrations (Chapter 5). The results of diel sampling at three other sites (BP, SP-A, SP-B) in June 2007, indicate that these diel variations are present at other locations on the Grand River, but not to the same extent as at the BL site (Figure 6.10, 6.11).

As explained in Chapter 5, this diel variability is driven by changes in dissolved oxygen (DO) concentrations, which increase during the day due to photosynthesis, and decrease at night due to respiration (Figure 6.10). Since the N$_2$O production processes of nitrification and denitrification are highly sensitive to redox conditions (e.g. Harrison et al 2005), the relative importance of these processes for N$_2$O production is variable throughout the diel cycle. The diel DO concentration cycle is much more pronounced at BL than at the other three sites, leading to much greater variation in the rates of nitrification and denitrification at this location (Chapter 5). As a result, the diel variation
in NO$_3^-$ and NH$_4^+$ concentration is much more pronounced at BL than the other sites (Figure 6.10).

In general, N$_2$O production is greatest during the night when DO concentrations are lowest (Figure 6.11). Since NH$_4^+$ is only present in significant concentrations (greater than 0.1 mg N/L) at the BL site, denitrification in the river sediments is likely the dominant N$_2$O production process at BP, SP-A and SP-B. Denitrification activity in the river sediments will increase in response to lower DO concentrations, though it is possible that the N$_2$O:N$_2$ ratio could decrease. However, these results indicate that the net N$_2$O production rate increases as the DO concentration decreases. Although previous research has shown that fractionation factors for N$_2$O production are inversely proportional to production rate (Mariotti et al. 1981, 1982), this effect is not seen at BP and SP-B, assuming that the $\delta^{15}$N of NO$_3^-$ is relatively constant (Table 6.6).

The gas exchange rate at SP-A is very high, due to a series of small dams located immediately upstream of the sampling location. For this reason, the DO concentration did not vary greatly over the diel cycle. As a result, the N$_2$O production rate and isotopic composition at SP-A remained relatively constant over the diel time period.
Figure 6.10: Diel variations in NO$_3^-$, NH$_4^+$ and DO concentration at four sampling locations measured in June 2007. Sampling at BP and BL was conducted over 28 hours from June 26 – 27, and sampling at SP-A and SP-B was conducted over 28 hours from June 23 – 24.
Figure 6.11: Diel variations in N₂O concentration and δ¹⁵N and δ¹⁸O values of the N₂O flux to the atmosphere at four sampling locations measured in June 2007. Sampling at BP and BL was conducted over 28 hours from June 26 – 27, and sampling at SP-A and SP-B was conducted over 28 hours from June 23 – 24. All vertical scales are the identical, except for the N₂O concentration scale for BL.
6.5 Discussion

The annual average $\delta^{15}$N and $\delta^{18}$O values for WM, BP, GM, and SP-B are remarkably similar, ranging from -14‰ to -7‰ and 34.5‰ to 36.8‰, for $\delta^{15}$N and $\delta^{18}$O respectively (Table 6.6). These values are more negative with respect to $\delta^{15}$N and $\delta^{18}$O compared to published data from other aquatic environments (Figure 6.1). Although the average $\delta^{15}$N value for SP-A is similar to the others, it is not clear why the $\delta^{18}$O value is considerably higher (45.5‰). Since the $\delta^{18}$O of N$_2$O produced through denitrification is largely controlled by oxygen exchange with environmental H$_2$O (Kool et al. 2007), a small change in the extent of this exchange rate would have a great effect on the $\delta^{18}$O of N$_2$O produced. Since different microbial species have been shown to facilitate oxygen exchange at greatly different rates (Kool et al. 2007), a difference in the microbial communities between locations would lead to a change in the rate of oxygen exchange. Alternatively, the higher $\delta^{18}$O values could be a reflection of partial N$_2$O consumption in the river sediments (Vieten et al. 2007, Menyailo & Hungate 2006, Mandernack et al. 2000), however the $\delta^{15}$N value for SP-A is not significantly greater than at the other locations.

As expected, the average isotopic composition of the N$_2$O flux from the BL site is significantly different than that from all the other locations. The $\delta^{15}$N and $\delta^{18}$O values for this site are heavily influenced by the night-time N$_2$O production during the warm summer months. The most depleted N$_2$O (both in $^{15}$N and $^{18}$O) at the BL site is produced during the summer nights when DO concentrations are low and N$_2$O production rates are highest. As explained in Chapter 5, N$_2$O production at BL at night likely occurs through denitrification in the sediments or at the sediment water interface, while during the day
most of the N$_2$O at BL is produced through nitrification, with sediment denitrification also contributing to N$_2$O production. Since N$_2$O at BP is also likely produced by denitrification in the sediments, and the $\delta^{15}$N of NO$_3^-$ is similar upstream and downstream of the WWTPs (Table 6.5), these results indicate that the fractionation factors for N$_2$O production through denitrification upstream of the WWTPs is different than downstream of the WWTPs. This difference in enrichment factors is likely a result of a change in the microbial community responsible for denitrification upstream and downstream of the WWTPs.

The estimated total emission of N$_2$O to the atmosphere from the central Grand River was 15.5 tonnes in 2007. The annual average $\delta^{15}$N and $\delta^{18}$O values for N$_2$O emitted to the atmosphere from the central Grand River were -18.5‰ and 32.7‰ respectively. Since the total N$_2$O production in the Grand River is dominated by summertime production at the BL site (Figure 6.8), the total average isotopic composition of the N$_2$O flux from the Grand River most resembles that from the BL site. The flux and time weighted average values for $\delta^{15}$N and $\delta^{18}$O of N$_2$O produced in the Grand River are much more depleted than the values measured for N$_2$O in the Bang Nara River, Thailand (Boontanon et al 2000, Figure 6.12).
Table 6.6: Magnitude and isotopic composition of the $N_2O$ flux to the atmosphere from the six major sampling locations. Only data collected for 2007 was included in this analysis.

<table>
<thead>
<tr>
<th>Sampling Location</th>
<th>Surface area of Representative Reach (ha)</th>
<th>Total Annual $N_2O$ Emission (tonnes)</th>
<th>$\delta^{15}N$ (%)</th>
<th>$\delta^{18}O$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM</td>
<td>68</td>
<td>0.20</td>
<td>-11.3</td>
<td>36.8</td>
</tr>
<tr>
<td>BP</td>
<td>187.5</td>
<td>2.41</td>
<td>-7.0</td>
<td>36.6</td>
</tr>
<tr>
<td>BL</td>
<td>52.8</td>
<td>8.50</td>
<td>-26.3</td>
<td>29.3</td>
</tr>
<tr>
<td>GM</td>
<td>87.4</td>
<td>1.74</td>
<td>-14.0</td>
<td>34.5</td>
</tr>
<tr>
<td>SP-A</td>
<td>33.2</td>
<td>0.84</td>
<td>-6.7</td>
<td>45.5</td>
</tr>
<tr>
<td>SP-B</td>
<td>57.5</td>
<td>1.78</td>
<td>-7.5</td>
<td>35.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>486</strong></td>
<td><strong>15.5</strong></td>
<td><strong>-18.5</strong></td>
<td><strong>32.7</strong></td>
</tr>
</tbody>
</table>

Using only the available isotopic data for terrestrial and oceanic $N_2O$ production, Rahn & Wahlen (2000) estimated that the $\delta^{15}N$ of tropospheric $N_2O$ is decreasing by approximately 0.03‰/year. However, due to the current limitations in precision of $N_2O$ isotopic analysis, it will be several years before a clear trend can be directly observed (Kaiser et al. 2003). Since $N_2O$ production in rivers was not accounted for in this calculation, this estimate may be greatly altered by including $N_2O$ from this important source. However, using this very limited dataset, it is difficult to assess the impact of riverine $N_2O$ production on the global isotopic budget of atmospheric $N_2O$.

If the global riverine $N_2O$ production is similar in isotopic composition to the Bang Nara River, Thailand (Boontanon et al 2000), this source will have a very limited impact on the predicted negative trend for $\delta^{15}N$ of tropospheric $N_2O$ (~0.03‰/year Rahn & Wahlen 2000). However, if the average value obtained from the Grand River ($\delta^{15}N = -18.5$, $\delta^{18}O = 32.7$) is representative of global riverine $N_2O$ production, the $\delta^{15}N$ trend of tropospheric $N_2O$ would be greater than that predicted by Rahn & Wahlen (2000). Once the true trend in the $\delta^{15}N$ and $\delta^{18}O$ of tropospheric $N_2O$ is observed, the
isotopic composition of various global N$_2$O sources can be used to determine the relative significance of each source. Therefore, more work is needed to characterize the isotopic composition of riverine N$_2$O before the true isotopic contribution of this important source can be determined.

Figure 6.12: Flux and time weighted annual average $\delta^{15}$N and $\delta^{18}$O values of the N$_2$O flux for the Grand River compared to other published N$_2$O flux values. Only data collected for 2007 was included in this analysis. The average value for the Bang Nara River was calculated using the published $\delta^{15}$N, $\delta^{18}$O and concentration values published by Boontanon et al 2000. The heavy and light outlined ovals represent the range of published data for terrestrial and oceanic environments from Figure 6.1, respectively. (Boontanon et al 2000), (Rahn & Wahlen 2000) (Kaiser et al 2003).

6.6 Conclusions

The $\delta^{15}$N and $\delta^{18}$O of N$_2$O emitted to the atmosphere from the Grand River is highly variable, with variability dependent on location with respect to nutrient sources such as WWTPs. It is possible to determine the composition of the bulk annual N$_2$O flux, by calculating the $\delta^{15}$N and $\delta^{18}$O of the N$_2$O flux from measured values for dissolved
N$_2$O concentration, $\delta^{15}$N and $\delta^{18}$O. The isotopic composition of the bulk annual flux from the Grand River is significantly different than the only other reported value for riverine N$_2$O production (Boontanon et al 2000). If the isotopic composition of N$_2$O produced in the Grand River is representative of global riverine N$_2$O production, then Rahn & Wahlen (2000) likely have underestimated the trend for $\delta^{15}$N of tropospheric N$_2$O. Due to the current analytical precision for $\delta^{15}$N and $\delta^{18}$O analysis of tropospheric N$_2$O (+/- 0.12‰ for $\delta^{15}$N and +/- 0.21‰ for $\delta^{18}$O, Kaiser et al 2003), it will be several years before a clear trend can be detected. Additional research is needed to better characterize the isotopic composition of riverine N$_2$O production. This knowledge will enable researchers to better apportion global sources of N$_2$O and understand the production of this important greenhouse gas.
Chapter 7:

Summary of Conclusions

This project achieved several research objectives. The first was to develop an effective method to measure the stable isotope composition ($\delta^{15}$N and $\delta^{18}$O) of dissolved N$_2$O. A new simplified method was developed for the isolation of dissolved N$_2$O. This technique involved purging dissolved N$_2$O from samples using an ultra pure helium stream, and cryogenic trapping the resulting N$_2$O in sample vials using a liquid nitrogen bath. Using this method, it is possible to analyze samples at concentrations as low as 6 nmol N$_2$O/L with a 20 minute processing time. Although other published studies have used online methods to measure the $\delta^{15}$N and $\delta^{18}$O of dissolved N$_2$O, this offline method allows for a greater sample throughput, and will allow researchers who do not have access to a dedicated mass spectrometer to collect and store dissolved N$_2$O for offsite isotopic analysis.

The second objective was to characterize the complex relationship between the $\delta^{15}$N and $\delta^{18}$O values for produced, dissolved and emitted N$_2$O in aquatic systems. Due to the kinetic and equilibrium isotope effects associated with gas exchange, as well as the equilibration with tropospheric N$_2$O, the isotopic composition of dissolved N$_2$O is not equal to that of the source N$_2$O, or that of N$_2$O emitted to the atmosphere in aqueous systems open to gas exchange. To provide insight into the relationship between the stable isotope compositions of source, dissolved and emitted N$_2$O, a simple box model was
created using Stella modelling software (SIDNO – Stable Isotopes of Dissolved Nitrous Oxide).

Using this model, it was found that when the residence time of dissolved N\textsubscript{2}O is short compared to the period of variability, the isotopic composition of emitted N\textsubscript{2}O is much more representative (compared to dissolved N\textsubscript{2}O) of the isotopic composition of source N\textsubscript{2}O. For a N\textsubscript{2}O source with a constant $\delta^{15}$N and $\delta^{18}$O, a simple change in production rate (and therefore dissolved concentration) can produce a change in the $\delta^{15}$N and $\delta^{18}$O values of dissolved N\textsubscript{2}O, while the isotopic composition of the emitted N\textsubscript{2}O will remain nearly constant. Also, under certain conditions, the $\delta^{15}$N and $\delta^{18}$O values of dissolved N\textsubscript{2}O can remain nearly constant, even if the values for the source and emitted N\textsubscript{2}O are changing dramatically.

Using a simple set of equations, it is possible to calculate the isotopic composition of N\textsubscript{2}O emitted from an aquatic system if the concentration, $\delta^{15}$N and $\delta^{18}$O of dissolved N\textsubscript{2}O are measured. The isotopic composition of emitted N\textsubscript{2}O is a much more useful measurement than the isotopic composition of dissolved N\textsubscript{2}O, both for inferring the $\delta^{15}$N and $\delta^{18}$O of the N\textsubscript{2}O produced, as well as for global N\textsubscript{2}O isotopic budgets. Therefore, it is recommended that the $\delta^{15}$N and $\delta^{18}$O of the emitted N\textsubscript{2}O be calculated and reported for studies using stable isotope analysis of dissolved N\textsubscript{2}O.

In future research, the SIDNO model should be improved by the addition of a term to account for the consumption of N\textsubscript{2}O and reduction to N\textsubscript{2}. This would allow for a more accurate understanding of the stable isotope dynamics of dissolved N\textsubscript{2}O, especially in anoxic systems where N\textsubscript{2}O consumption is an important process.
The third objective of this research project was to characterize the processes responsible for N$_2$O production in the Grand River. Previously published studies have shown that nitrogen cycle processes and N$_2$O production in rivers is strongly tied to diel changes in dissolved oxygen concentration. This link was confirmed in the Grand River by continuous sampling over a 28 hour period downstream of the major wastewater treatment plants.

It was found that nitrification or nitrifier-denitrification dominated N$_2$O production during the day when dissolved oxygen concentrations were high. Sediment denitrification likely also contributed to N$_2$O production during the day. Denitrification occurred at night when dissolved oxygen concentrations were low, producing N$_2$O at a much higher rate than during the day. The SIDNO model was used to determine the diel variation in the isotopic composition of N$_2$O production. N$_2$O produced during the day had a $\delta^{15}$N value of -22‰ and a $\delta^{18}$O value of 43‰. N$_2$O produced during the night had a $\delta^{15}$N value of -30‰ and a $\delta^{18}$O value of 30‰.

Using the $\delta^{15}$N and $\delta^{18}$O values for N$_2$O, NO$_3^-$ and NH$_4^+$ in the river, apparent in-situ enrichment factors for N$_2$O production through nitrification and denitrification were calculated. For denitrification, the nitrogen enrichment factor ranged from -34‰ to -30.8‰, and the oxygen enrichment factor ranged from 31‰ to 33‰. The nitrogen enrichment factor for nitrification ranged between -50‰ to -46‰, though these values may be influenced by the contribution of N$_2$O from sediment denitrification. Since these enrichment factors are based on in-situ production, they are more representative of N$_2$O production than published enrichment factors obtained by laboratory cultures of single microbial species.
It is recommended that in future research, lab incubations of sediment and water from the Grand River be conducted to further investigate the relative importance of N$_2$O production in the sediments versus the water column. Isotopic data from these laboratory incubations could be used to further support the in-situ isotopic enrichment factors obtained using the SIDNO model.

This study fully characterized N$_2$O production in the Grand River downstream of the Waterloo and Kitchener WWTPs. Planned modifications to these treatment plants are expected to greatly improve the quality of effluent discharged to the Grand River within the next 10 years. It is recommended that further sampling be conducted after the improvements are completed in order to determine the importance of WWTP effluent quality on N$_2$O production and nitrogen cycling. The results of this research would have broad implications for river management and wastewater treatment in the future.

The final objective of this research project was to characterize the spatial and temporal variability of the isotopic composition of N$_2$O emitted from the Grand River. Although N$_2$O production in rivers accounts for a significant portion of the global N$_2$O budget, the isotopic composition of N$_2$O from this important source has not been well characterized. Only one other published study has measured $\delta^{15}$N and $\delta^{18}$O of N$_2$O produced in a river. The results of the present study indicate that the magnitude and isotopic composition of the N$_2$O flux to the atmosphere varies significantly both spatially and temporally in the Grand River.

Generally, N$_2$O fluxes are greatest immediately downstream of the major wastewater treatment plants. N$_2$O produced downstream of the Waterloo and Kitchener
WWTPs is more depleted in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ compared to upstream locations. The Guelph WWTP does not have the same effect on downstream $\text{N}_2\text{O}$ production. The major difference between the plants is that the Waterloo and Kitchener plants release large quantities of $\text{NH}_4^+$, while the Guelph plant only releases $\text{NO}_3^-$. These results indicate that the quality of wastewater effluent has a great effect on the downstream production of $\text{N}_2\text{O}$.

The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of $\text{N}_2\text{O}$ emitted from the sites on the lower Grand River were higher than any other location in the Grand River and were similar to the isotopic composition of $\text{N}_2\text{O}$ produced in oceanic environments. The lower Grand River is deeper and slower moving than the other locations studied, and $\text{N}_2\text{O}$ consumption is likely an important process in the lower reaches of the river. It is possible that high $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values are characteristic of $\text{N}_2\text{O}$ produced in deep water environments.

Overall, the magnitude and isotopic composition of the $\text{N}_2\text{O}$ flux from the Grand River is dominated by night-time production downstream of the Waterloo and Kitchener WWTPs during the summer months. The flux and time weighted annual average isotopic composition of $\text{N}_2\text{O}$ emitted from the Grand River was $-18.5‰$ and $32.7‰$ for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ respectively. This is significantly more depleted than $\text{N}_2\text{O}$ from the Bang Nara River, Thailand (the only other published data for riverine $\text{N}_2\text{O}$ production).

This study focused on $\text{N}_2\text{O}$ production in the middle and lower reaches of the Grand River. Future research should investigate $\text{N}_2\text{O}$ production and nitrogen cycling in the upper reaches and smaller tributaries in the Grand River watershed. Since the nitrogen sources and hydrologic conditions in these smaller watercourses are much different than that of the higher order reaches of the Grand River, it is expected that $\text{N}_2\text{O}$ production
and nitrogen cycling would differ in these areas as well. Also, this study did not investigate N₂O production in the major reservoirs located within the Grand River watershed. The deep water and long hydraulic retention times in these reservoirs likely allows for denitrification and N₂O consumption to remove nitrogen from the system. Future research should examine the role of these reservoirs in nitrogen cycling in the Grand River watershed.

Currently, this study is the most thorough characterization of the δ¹⁵N and δ¹⁸O of N₂O produced in a river environment. However, further research is needed to characterize the isotopic composition of global riverine N₂O production. Current research indicates that approximately 25% of global anthropogenic N₂O production occurs in rivers, estuaries and near shore coastal environments (Denman et al 2007); however the isotopic composition of this important source is poorly constrained. The data presented here indicates that the isotopic composition of N₂O produced in river environments is highly variable, both spatially and temporally. The isotopic composition of riverine N₂O production is likely also influenced by other environmental factors such as climate, water quality, and hydrology. Future research into riverine N₂O production should focus on characterizing the isotopic composition of N₂O in rivers other than the Grand River, and should focus on developing links between environmental conditions and δ¹⁵N and δ¹⁸O of N₂O. This research is needed to accurately determine the effect of riverine N₂O production on the global atmospheric N₂O isotopic budget. If global riverine N₂O production is similar in isotopic composition to that from the Grand River, the negative trend for δ¹⁵N and δ¹⁸O of tropospheric N₂O will be significantly greater in magnitude than previously predicted. The thorough characterization of global riverine N₂O
production will allow for a more accurate understanding of the relative importance of global N₂O sources and sinks.
References

Ackerman, EA. (1941) The Koppen Classification of Climates in North America. 

*Geographical Review.* 31(1): 105-111.


Andersson KK, Hooper AB (1983) O$_2$ and H$_2$O are each the source of one O in NO$_2$ produced from NH$_3$ by Nitrosomonas: $^{15}$N evidence. *Federation of European Biochemical Societies Letters.* 164, 236-240.


Bollmann A, Conrad R (1997a) Acetylene blockage technique leads to underestimation
of denitrification rates in oxic soils due to scavenging of intermediate nitric oxide.

*Soil Biology and Biochemistry* **29**(7):1067-1077


Coplen TB, Böhlke JK, Casciotti KL. (2004) Using dual-bacterial denitrification to improve $\delta^{15}$N determinations of nitrates containing mass-independent $^{17}$O. *Rapid Communications in Mass Spectrometry* 18:245-250


http://www.climate.weatheroffice.ec.gc.ca/climate_normals/results_e.html?Province=ONT%20&StationName=&SearchType=&LocateBy=Province&Proximity=25&ProximityFrom=City&StationNumber=&IDType=MSC&CityName=&ParkName=&LatitudeDegrees=&LatitudeMinutes=&LongitudeDegrees=&LongitudeMinutes=&NormalsClass=A&SelNormals=&StnId=4832&


Inoue HY, Mook WG, (1994) Equilibrium and kinetic nitrogen and oxygen isotope fractionations between dissolved and gaseous N$_2$O. *Chemical Geology (Isotope Geosciences Section)* **113**:135-148


NPRI (2007). National Pollutant Release Inventory, Environment Canada


Distinguishing nitrous oxide production from nitrification and denitrification on the
basis of isotopomer abundances. *Applied and Environmental Microbiology, 72*, 638-
644.


an extensively managed grassland in NE Bavaria, Germany. II. Stable isotope natural
abundance of N\textsubscript{2}O. *Biogeochemistry, 63*, 249-267.

Tsunogai U, Tadasuke K, Hirota A, Ohkubo SB, Komatsu DD, Nakagawa F (2008)

Sensitive determinations of stable nitrogen isotopic composition of organic nitrogen
though chemical conversion into N\textsubscript{2}O *Rapid Communications in Mass Spectrometry*
*22*:345-354

methane in deep-sea hydrothermal plume, Myojin Knoll Caldera, Izu-Bonin arc:
Implications for microbial methane oxidation in the oceans and applications to heat
flux estimation

Toyoda, S, Yoshida, N (1999) Determination of nitrogen isotopomers of nitrous oxide on
a modified isotope ratio mass spectrometer. *Analytical Chemistry 71*:4711-4718

Toyoda S, Mutobe H, Yamagishi H, Yoshida N, Tanji Y (2005) Fractionation of N\textsubscript{2}O
isotopomers during production by denitrifier. *Soil Biology & Biochemistry, 37*, 1535-
1545.


