1-1-2009

A model-based approach to measuring denitrification and greenhouse gas production in lakes

Juliet Falco Ajambo-Doherty
Ryerson University

Follow this and additional works at: <http://digitalcommons.ryerson.ca/dissertations>

Part of the [Fresh Water Studies Commons](#)

Recommended Citation

Ajambo-Doherty, Juliet Falco, "A model-based approach to measuring denitrification and greenhouse gas production in lakes" (2009).
Theses and dissertations. Paper 970.

This Thesis is brought to you for free and open access by Digital Commons @ Ryerson. It has been accepted for inclusion in Theses and dissertations by an authorized administrator of Digital Commons @ Ryerson. For more information, please contact bcameron@ryerson.ca.

TD
427
N3
A33
2009

**A MODEL-BASED APPROACH TO
MEASURING DENITRIFICATION AND
GREENHOUSE GAS PRODUCTION IN LAKES**

by

Juliet Falco Ajambo-Doherty

Bachelor of Science, Ryerson University, 2007

is reproduce this thesis by photocopying or by di...
of other institutions or individuals for the purpose

A thesis
presented to
Ryerson University

in partial fulfillment of the
requirements for the degree of
Master of Science
in the program of Molecular Science

Toronto, Ontario, Canada, 2009
© Juliet Ajambo-Doherty 2009

Author's Declaration

I hereby declare that I am the sole author of this thesis.

I authorize Ryerson University to lend this thesis to other institutions or individuals for the purpose of scholarly research.

I further authorize Ryerson University to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

A MODEL-BASED APPROACH TO MEASURING DENITRIFICATION AND GREENHOUSE GAS PRODUCTION IN LAKES

Juliet Falco Ajambo-Doherty

Master of Science, Molecular Science, Ryerson University, 2009

Abstract

An existing whole-system model based on changes in dissolved N_2 concentration was modified for lentic systems. Field validation was carried out at Christie Lake in Dundas, ON and Turtle Pond in Stoney Creek, ON (Canada). New model inputs included air temperature, atmospheric pressure, relative humidity, wind velocity, and Schmidt number. Monte Carlo analysis was integrated into the model to better constrain error in model estimates of denitrification, whole-system metabolism, and greenhouse gas production. Denitrification rates ranged from -419 - $4415 \mu\text{mol N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ in Christie Lake and from 10 - $74 \mu\text{mol N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ in Turtle Pond. N_2O production ranged from 915 - $10,635 \text{ nmol N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ in Christie Lake and from -344 - $131 \text{ nmol N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ in Turtle Pond. The whole-system model allows for the examination of biogeochemical processes at ecologically significant temporal and spatial scales.

Acknowledgements

I first wish to express my sincere gratitude to my thesis supervisor, Dr. Andrew Laursen, for his guidance and constant encouragement throughout this study. The completion of this thesis would not have been possible were it not for his support and enthusiasm. I would also like to thank my graduate committee members, Dr. Vaduz Bostan and Dr. Mehrab Mehrvar for their valuable suggestions and constructive advice. To Ryerson University and the Molecular Science program, thank you for the opportunity to complete this course of study. Sincere thanks are extended to the following people: Joseph Bautista, Scott Robertson, and Monisa Nandi for their aid with laboratory and field work, Aryo Maradona for programming and facilitation to a modeling environment change to MatLab, Shawn McFadden and Sylvia O'Sullivan for technical support in the Ryerson University Analytical Centre, Dr. Roxane Maranger and her team at the Université de Montréal for providing access and training to the their mass inlet membrane spectrometer, and to Conservation Hamilton for access to the field sites used. For funding for this project, I would like to thank Ryerson University and NSERC.

I would especially like to thank my mother, Oliver Mukasa, and my brother, Edward Ojambo, with whose guidance and unwavering faith in me I am continually able to succeed. You have imparted upon me a love for learning for which I am forever grateful. Words cannot express how much I love you. I am also thankful for the love and support of my husband Alan Doherty and his incredible patience during the final stretch of this journey. Last but not least, to all of my friends who have supported me through all the trials and tribulations of life, you know who you are.

TABLE OF CONTENTS

	Page
AUTHOUR'S DECLARATION	iii
ABSTRACT	iv
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vi
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS	xv
CHAPTER	
1 INTRODUCTION	1
1.1 Why look at nitrogen?	3
1.1.1 Nutrient Management	4
1.2 Denitrification	5
1.2.1 Measuring denitrification	9
1.2.1.1 Enclosure Experiments	10
1.2.1.2. Open system approach	10
1.2.1.2. Technology	11
1.3 Global Warming	16
1.3.1 Nitrous Oxide (N ₂ O)	17
1.4 Modeling	19
1.4.1 Open Channel N ₂ Method	20
1.4.2 Model Development – Lentic Systems	22
1.4.2.1 Model Inputs	23

1.4.2.2. Sampling	24
1.4.3 Model Modification	25
1.4.3.1 Stratification	29
1.4.3.2 Monte Carlo Analysis	30
1.5 Rationale	30
1.5.1 Objectives	31
1.5.2 Contributions to Science	32
2 METHODOLOGY	34
2.1 Site Description	34
2.1.1 Christie Lake	34
2.1.2 Turtle Pond	35
2.2 Sample Collection	35
2.2.1 Christie Lake	35
2.2.1.1 Water Column Sampling	35
2.2.1.2. Benthic Sampling	37
2.2.2 Turtle Pond	38
2.3 Sample Analyses	39
2.3.1 Nitrogen and Oxygen gas – MIMS	39
2.3.2 Nitrous Oxide – GC-ECD	40
2.3.3 Nitrate - Cadmium Reduction Method	40
2.3.4 Ammonia - Phenate Method	41
2.3.5 Phosphate - Ascorbic Acid Method	42
2.4 Determining Denitrification	42
2.5 Modeling	43
2.5.1 Modeling Environment	44

2.5.1.1 Christie Lake	45
2.5.1.2 Turtle Pond	46
2.5.2 Model Inputs	46
2.5.3 Modeling Equations	47
2.5.3.1 Pressure and Relative Humidity Correction	48
2.5.3.2 First-Order Gas Exchange Rates (K)	50
2.5.4 Sources of Error and Analysis	51
3 RESULTS AND DISCUSSION	53
3.1 Christie Lake	53
3.1.1 Climate Data	53
3.1.2 Water Column Gas Concentrations	58
3.1.3 Model Output for Gas Rates – Multiple Strata Approach, 2 time Points (June – July)	61
3.1.4 Model Output for Gas Rates – Single Parcel Approach, 2 time Points	62
3.1.5 Model Output for Gas Rates – Steady State Approach, Multiple Strata	63
3.1.6 Model Output for Gas Rates – Steady State Approach, Single Parcel	64
3.1.7 Benthic Chamber Gas Rates for N ₂ , O ₂ , and N ₂ O	67
3.1.8 Nutrient Data	68
3.2 Turtle Pond	77
3.2.1 Climate Data	77
3.2.2 Assumptions of Relative Humidity	90
3.2.3 Model Output for Gas Rates (N ₂ , O ₂ , and N ₂ O)	92
3.2.4 Nutrient Data	94
4 CONCLUSIONS AND RECOMMENDATIONS	97

4.1	Summary and Conclusions	97
4.2	Strengths and Limitations	100
4.3	Relevance and Recommendations	101
4.4	Directions for Future Research	102
APPENDICES		103
APPENDIX A:	Protocol for the UV-VIS Spectrophotometer	103
APPENDIX B:	Protocol for the GC-ECD	106
APPENDIX C:	Equilibrium concentration calculation for each gas (prior to correction)	109
APPENDIX D:	Schmidt numbers calculations	110
APPENDIX E:	Sample nitrous oxide chromatograph	111
APPENDIX F:	Christie Lake raw data	118
APPENDIX G:	Turtle Pond raw data	120
APPENDIX H:	MatLab program explanation	122
REFERENCES		123

LIST OF TABLES

	Page
Table 1.1: Atmospheric lifetime, GWPs and RFs of various green house gases.	19
Table 3.1: Summary of three approaches used to estimate denitrification for Christie Lake (chambers, model, and nitrogen balance)	72
Table 3.2: Summary nutrient production rates for Turtle Pond	96
Table G.1: Christie Lake raw data	120
Table H.2: Turtle Pond raw data	122

LIST OF FIGURES

	Page
Figure 1.1: Relationship of production/fishery yield and increased nutrient loading	2
Figure 1.2: Nitrogen cascade	3
Figure 1.3: Nitrogen cycle	6
Figure 1.4: Decrease of nitrogen transfer due to denitrification	6
Figure 1.5: Global nitrous oxide emissions, 1980s (15 Tg N/y)	17
Figure 1.6: Original model inputs	22
Figure 1.7: Solving for denitrification based on the original model	23
Figure 1.8: Lagrangian sampling for the original model	24
Figure 1.9: Modified model inputs	25
Figure 1.10: Nitrous oxide inventory determination	27
Figure 2.1: Christie Lake field site	34
Figure 2.2: Turtle Pond field site	35
Figure 2.3: Grab sampling method	36
Figure 2.4: Benthic chambers used for enclosure sampling	37
Figure 2.5: Relationship between wind velocity and piston velocity	50
Figure 3.1: Christie Lake climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for June 21-22, 2008	54
Figure 3.2: Christie Lake climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for June 21-July 25, 2008	55
Figure 3.3: Christie Lake climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for July 24-25, 2008	56
Figure 3.4: Christie Lake climate histograms of air temperature, relative	

humidity, atmospheric pressure, water temperature, and wind speed for October 3-4, 2008	57
Figure 3.5: Christie Lake dissolved N ₂ concentrations for June, July and October, 2008	58
Figure 3.6: Christie Lake dissolved O ₂ concentrations for June, July and October, 2008	60
Figure 3.7: Christie Lake dissolved N ₂ O concentrations for June, July and October, 2008	61
Figure 3.8: Christie Lake Modeled Gas Production Rate for June 21-July 25, 2008 - multiple strata approach, 2 time points	62
Figure 3.9: Christie Lake modeled gas production rate for June 21-July 25, 2008 - single parcel approach, 2 time points	63
Figure 3.10: Christie Lake modeled gas production rates for June, July and October 2008 - steady state approach, multiple strata	64
Figure 3.11: Christie Lake modeled nitrogen production rates for June, July, and October, 2008 - steady state approach, single parcel	65
Figure 3.12: Christie Lake modeled oxygen production rates for June, July, and October, 2008 - steady state approach, single parcel	65
Figure 3.13: Christie Lake modeled nitrogen oxide production rates for June, July, and October, 2008 - steady state approach, single parcel	67
Figure 3.14: Christie Lake benthic chamber gas flux for June, July and October, 2008	67
Figure 3.15: Christie Lake depth profiles and benthic flux of nitrate for June, July and October, 2008	68
Figure 3.16: Christie Lake depth profiles and benthic flux of ammonia for June, July and October, 2008	69
Figure 3.17: Christie Lake depth profiles and benthic flux of phosphate for June, July and October, 2008	70
Figure 3.18: Christie Lake depth profile of DIN:DIP for June, July and October 2008	71
Figure 3.19: Summary of three approaches used to estimate denitrification in Christie Lake	76
Figure 3.20: Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for November 02-11, 2007	79
Figure 3.21: Turtle Pond climate histograms of air temperature, relative	

humidity, atmospheric pressure, water temperature, and wind speed for November 11-18, 2007	80
Figure 3.22: Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for November 18-28, 2007	81
Figure 3.23: Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for January 10-26, 2008	82
Figure 3.24: Turtle Pond water temperature histograms for ice-covered period	83
Figure 3.25: Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for August 11-18, 2008	84
Figure 3.26: Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for August 18-28, 2008	85
Figure 3.27: Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for August 28-September 04, 2008	86
Figure 3.28: Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for September 04-12, 2008	87
Figure 3.29: Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for September 12-20, 2008	88
Figure 3.30: Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for September 20-28, 2008	89
Figure 3.31: Expected distribution of relative humidity	91
Figure 3.32: Actual distribution of relative humidity relative to wind speed for Turtle Pond	92
Figure 3.33: Modeled gas production for Turtle Pond, winter 2007- fall 2008	93
Figure 3.34: Summary nutrient production rates for Turtle Pond	95

Figure F.1: Sample nitrous oxide chromatograph, part 1

118

Figure F.2: Sample nitrous oxide chromatograph, part 2

119

LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviations	Term
Ar	argon
CH ₄	methane
CO ₂	carbon dioxide
DIN	dissolved inorganic N
DNRA	dissimilatory nitrate reduction to ammonium
GC	gas chromatography
GC-ECD	gas chromatography coupled with an electron capture detector
GC-TCD	gas chromatography coupled with a thermal conductivity detector
GHG	greenhouse gas
GWP	Global Warming Potential
IPCC	Intergovernmental Panel on Climate Change
MIMS	membrane inlet mass spectrometry
MS	mass spectrometry
N	nitrogen
N ₂	nitrogen gas
N ₂ O	nitrous oxide
NO ₂ ⁻	nitrite
NO ₃ ⁻	nitrate
NRC	National Research Council
O ₂	oxygen gas
P	phosphorus
PO ₄ ⁻	phosphate
RF	radiative forcing

Sc _X	Schmidt number coefficient of X
Δ time	time between measurements
UV-VIS	ultra violet/visible

CHAPTER 1

INTRODUCTION

A US National Research Council Report in 2000 asked the question “What links such seemingly diverse problems as red tides, fish kills, outbreaks of shellfish poisonings, degradation of water quality, coral reef destruction, and the Gulf of Mexico’s ‘dead zone’?” (National Research Council, 2000), concluding that the answer was nitrogen. Transforming elemental nitrogen to an available form of reactive nitrogen (Nr) requires a significant amount of energy in order to break the triple bond that holds the two N atoms together.

Biological Nitrogen Fixation

Only a small number of specialized nitrogen fixing microbes are able to perform biological nitrogen fixation and until recently, this greatly limited the amount of Nr in the aquatic environment (Galloway et al., 2003). However, the introduction of Nr at local, regional, and global scales by anthropogenic sources now exceeds the rate of biological nitrogen fixation in natural ecosystems (Galloway et al., 2003; Davidson and Seitzinger, 2006; Paerl, 2006). The environmental increase of Nr is due primarily to N fertilizer production and fossil fuel combustion used to support the food and energy demands of human population growth (Rabalais, 2002; Galloway et al., 2003; Davidson and Seitzinger, 2006; Groffman et al., 2009b).

The amount of anthropogenic Nr has increased up to 50 times from historical levels, from 15 to 165 Tg of N per year, for the period between 1860 and 2000. This has resulted in negative effects on both human and environmental health (Bernot et al., 2003; Galloway et al., 2003).

Figure 1.1 Relationship of production/fishery yield and increased nutrient loading (Rabalais, 2002).

Increases in N_r have been shown to cause: respiratory illness, cancer, and cardiac disease in humans due to the aerosols formed; acidification and loss of biodiversity in lakes and streams; and eutrophication, hypoxia, loss of biodiversity, and habitat degradation in coastal ecosystems (Galloway et al., 2003). Nutrient loading, in general, has contributed to trophic enrichment of many aquatic ecosystems, including many with globally important fisheries (Figure 1.1). Of the ten ecosystems listed in Figure 1.1, N loading is believed most directly responsible for trophic status change in eight (phosphorus loading has been the primary cause of eutrophication in the Laurentian Great Lakes). N_r is now regarded as the principal anthropogenic threat to coastal ecosystems (Howarth et al., 2000; National Research Council, 2000; Rabalais, 2002; Galloway et al., 2003).

1.1 Why look at nitrogen?

Figure 1.2. Nitrogen cascade (modified from Rabalais, 2002).

Nitrogen and phosphorus are most commonly the limiting nutrients in coastal ecosystems and freshwater ecosystems, respectively, but it is the ratio of the two that determines the relative importance of each in limiting primary production in a variety of ecosystems such as lakes, reservoirs, and streams (Hecky et al., 1993; Cornwell et al., 1999; National Research Council, 2000; Rabalais, 2002; Hessen et al., 2004; Davidson and Seitzinger, 2006). The Redfield ratio is the molecular ratio of carbon, nitrogen and phosphorus in phytoplankton biomass (106 C:15 N:1 P) when nutrients are not limiting (Redfield, 1934). Marine and estuarine ecosystems are often N-limited because the N:P ratio is typically below the Redfield ratio of 15:1, thus nitrogen is relatively more limiting for growth (production of new biomass). Freshwater ecosystems are commonly P-limited because the N:P ratio is greater than the Redfield ratio of 15:1, thus P is relatively more limiting for growth. Surface freshwater ecosystems play a role in the movement

of reactive forms of nitrogen and phosphorus from land and waterscapes towards estuaries and the coastal ocean (Figure 1.2) (Rabalais, 2002; Galloway et al., 2003). Since estuarine primary productivity is characteristically limited by N_r , nitrate (NO_3^-) overloading results in major perturbations in coastal ecosystems (Drescher and Brown, 2006). Thus, nitrogen represents the largest pollution problem in coastal waters and a great threat to coastal ecosystems (Howarth et al., 2006).

1.1.1 Nutrient Management

The desire to bolster and maintain healthy interactions between ecosystems and social systems has resulted in calls for further research and the advocacy of management practices that focus on this goal (Carpenter et al., 1999). One possible solution to nitrogen loading is to decrease anthropogenic sources. Programs such as the Helsinki Commission for the Baltic Sea, the Oslo-Paris Commission for the North Sea, and the Chesapeake Bay Program have been established in an effort to decrease nutrient over-enrichment. However, efforts such as these are often based on poorly informed goals of specific percentage reduction, without knowing what amount of reduction would really be effective and the time that such action would take (Boesch, 2002). Without understanding how nitrogen is processed during transport within a drainage network, it is difficult to develop an effective plan to achieve a reduction target. In order for legislation and reduction programs to be effective, an understanding of the processes involved, as well as the biogeochemistry of the system in question is required. To produce legislation that is meaningful, we need to be able to predict how biogeochemical processes will occur. Only then can we develop unique nutrient management plans applicable to differing and individual systems.

Nutrient management practices require a sound knowledge of the nutrient cycles that take place in the system being protected (Cornwell et al., 1999). In the case of nitrogen over-enrichment, it is practical to know that not all of the excess Nr released into the environment makes its way to coastal waters; some portion is removed by denitrification (Boesch, 2002). Nutrient management practices can be geared towards enhancing the conversion of Nr to nitrogen gas in both aquatic and terrestrial systems (Boesch, 2002; Davidson and Seitzinger, 2006; Seitzinger et al., 2006). Enumerating rates of denitrification is critical in order to predict the effects of agricultural and industrial land-use changes on downstream ecosystems. Yet many methods for measuring denitrification have been found to have large uncertainties, therefore the amount of Nr that is converted back to N₂ during denitrification and anammox is one of the largest uncertainties of the N cycle (Galloway et al., 2004; Davidson and Seitzinger, 2006; Böhlke et al., 2009).

1.2 Denitrification

Classical Denitrification

Multiple processes are involved in nitrogen cycling in aquatic systems and these include nitrogen fixation, nitrification, dissimilatory nitrate reduction to ammonium (DNRA), anammox and denitrification (Figure 1.3) (Saunders and Kalff, 2001; Francis et al., 2007). The removal of nitrogen and its products from the water column occurs by such processes as denitrification, sediment sorption, and plant/microbial uptake. However, of these processes, denitrification is the only permanent mechanism of nitrogen removal converting it to the form of nitrogen gas and nitrous oxide. Nitrogen gas and nitrous oxide are lost from the water column through re-equilibration with the atmosphere.

Figure 1.3. Nitrogen cycle (Francis et al., 2007).

Denitrification, the anaerobic reduction of nitrogen oxides (NO_3 and NO_2) to nitrogenous gases (NO , N_2O and N_2), is an important Nr sink in the global nitrogen budget, especially in waters with high concentrations of anthropogenic nutrients and ultimately determines N availability (Seitzinger, 1988; Cornwell et al., 1999; Bernot et al., 2003; Drescher and Brown, 2006; Piña-Ochoa and Álvarez-Cobelas, 2006; Gruber and Galloway et al., 2008; Böhlke et al., 2009). As water travels through a drainage network from wetlands and headwater streams to larger streams and rivers, passing through network lakes, and ultimately estuaries and coastal ocean, nitrogen is cycled among its various forms and there is great potential for loss of Nr from the biosphere due to

Figure 1.4. Decrease of nitrogen transfer due to denitrification (modified from Galloway et al., 2003; Seitzinger et al., 2006).

denitrification (Galloway et al., 2003; Böhlke et al., 2009) (Figure 1.4). Characterizing denitrification through precise and accurate methods of measurement is essential to understanding how ecosystems handle increased N loads (Bernot et al., 2003; Drescher and Brown, 2006).

What is known is that denitrification requires carbon and nitrate as substrates, the absence or scarcity of oxygen, is influenced by phosphorus content, temperature, and light, and that it occurs in almost all ecosystems including terrestrial, freshwater, coastal, and oceanic (Seitzinger, 1988; Galloway, 2003; Davidson and Seitzinger, 2006; Groffman et al., 2009b). In aquatic ecosystems, denitrification predominantly occurs in the top 2–5 cm of the sediment layer which is often anoxic below the first few millimeters, even if the overlying water is well oxygenated (Galloway, 2003). Empirical models also suggest that hydrologic characteristics, such as depth (Smith et al., 1997; Alexander et al., 2000), discharge (Alexander et al., 2002a), and water residence time (Alexander et al., 2000; Alexander et al., 2002b; Howarth et al., 2006; Seitzinger et al., 2006) affect nitrogen loss, presumably through denitrification. Laursen and Seitzinger (2004) found site-specific relationships between discharge and denitrification, measured explicitly as N_2 production. However, while advances in whole-system measurements of denitrification have given us the ability to study river reaches (Laursen and Seitzinger, 2002a; McCutchan et al., 2003) and salt wedges moving inland in estuaries (Kana et al., 2006), studies still lack reliable quantitative estimates of denitrification rates at ecologically relevant temporal and spatial scales across landscapes and waterscapes (Davidson and Seitzinger, 2006; Seitzinger, 2006).

Why study lakes?

The residence time of water (Howarth et al., 2006), and more specifically, residence time / depth (Seitzinger et al., 2006) is an important factor controlling the amount of Nr removal achieved in aquatic systems. The effect of water residence time is directly related to the reaction time that bacteria in sediments have to convert Nr to various nitrogenous forms before it is transported to an adjacent downstream system (Galloway et al., 2003). Not surprisingly then, a smaller proportion of the Nr inputs are retained in a river reach (5% to 20%) compared with many lakes and reservoirs (10% to ~90%) resulting in greater amounts of Nr conversion to N gas in lakes and reservoirs (Galloway et al., 2003). Due to their relatively long water residence time, and the resulting opportunity for enhanced particle settling and nutrient processing, lakes are recognized as systems where extensive denitrification and N burial can occur (Harrison, 2009). Model runs for the mid-1990s indicate that lentic systems are indeed important sinks for N, with small lakes (~50 km²) representing half the global total for conversion of Nr to N₂ via denitrification (Groffman et al., 2009b).

These models of Nr removal are based on total N removal, with most estimates based on N mass balance, rather than explicit measurements of denitrification, as the tools do not yet exist to measure denitrification in lakes at a whole system scale. Given this, we cannot yet say whether lakes are acting as long-term temporary sinks of N, reservoirs that can return N to the drainage network, or as permanent sinks within networks, removing N by denitrification. Further, without the tools to assess denitrification at the whole-lake scale, we cannot determine what other aspects of lakes (i.e. physical, chemical, and biological factors) are important determinants of N-loss via

denitrification in these potentially important sinks. Knowing the importance of these other determinants will be crucial in developing effective nitrogen export reduction plans.

1.2.1 Measuring denitrification

A number of approaches have been applied in aquatic and marine systems to detect the production of N_2 due to denitrification. These approaches fall into two general categories: enclosure experiments designed to measure N_2 fluxes and open system measurements of ambient N_2 (Groffman et al., 2006). Both approaches measure net denitrification which is the difference between gross denitrification and gross N fixation (Groffman et al., 2006).

Denitrification is a difficult process to measure accurately due to: methodological limitations, its high spatial and temporal variability, the high background concentration in the aquatic environment of its dominant end product, N_2 , and the involvement of several forms of nitrogen as reactants and products in denitrification and other nitrogen cycle processes which complicates accounting of fluxes (Cornwell et al., 1999; Groffman et al., 2006; Tonitto et al., 2009). Heterogeneity in the variables that control for denitrification in both space and time results in the great heterogeneity observed in denitrification rates (Kulkarni et al., 2008; Böhlke et al., 2009). Current methods used to measure denitrification are problematic for a variety of reasons: they change the substrate concentrations of reactants (e.g. nitrate or carbon), they disturb the physical environment that the process occurs in (e.g. flow of water across surface, and therefore diffusive delivery of oxygen and nitrate), they lack sensitivity, or they are prohibitively costly in time (e.g. N mass balance) and expense (e.g. large-scale ^{15}N tracer experiments)

(Seitzinger et al., 1993; Cornwell et al., 1999; Laursen and Seitzinger, 2002a; Groffman et al., 2006).

1.2.1.1 Enclosure Experiments

Enclosure experiments are typically used for studies involving the sediment layer, the primary site of denitrification activity (Groffman et al., 2006). In aquatic systems, the method usually involves either an *in situ* approach with benthic chambers (e.g. Ryden et al., 1979; Devol, 1991; Laursen and Seitzinger, 2002b) or an *ex situ* approach with sediment cores (e.g. Seitzinger et al., 1980; Seitzinger et al., 1993; Joye and Pearl, 1994; Seitzinger and Giblin, 1996; Kana et al., 1998; Cornwell et al., 1999; An et al., 2001) with dissolved N_2 concentrations in the overlying water column measured repeatedly through time. The N_2 flux is determined from the rate of change in N_2 concentration (ΔN_2). If nitrogen to argon ratio ($N_2:Ar$) data are used, ΔN_2 is determined from $\Delta N_2:Ar$ (Kana et al., 1994; Groffman et al., 2006). Rates that are determined in centimeter and hour scale experiments must be extrapolated to kilometer and month scales for ecosystem studies of denitrification resulting in built-in uncertainty at assessing the environmental heterogeneity of the system (Groffman et al., 2006; McCutchan et al., 2003). An alternative approach that integrates large scale systems is the *in situ* measurement of N_2 in open systems where water samples are taken from the environment in question (Groffman et al., 2006).

1.2.1.2 Open system approach

In situ measurement studies involve the measurement of N_2 in ambient water produced by denitrification. The difficulty lies in the high background aqueous concentration of N_2

(equilibrium concentrations in freshwater are 449 and 822 $\mu\text{mol/L}$ for 30°C and 0°C, respectively) and consideration of physical processes related to mixing, air exchange, and water volume in contact with the area of denitrification (Groffman et al., 2006). Several different systems have been studied using *in situ* $\text{N}_2:\text{Ar}$ measurements beginning with Richards and Benson (1961) in the marine environment followed by Codispoti et al. (2001). While these studies noted $\text{N}_2:\text{Ar}$ anomalies and attributed these anomalies to denitrification, no attempts were made to model denitrification from these anomalies. Anomalies in $\text{N}_2:\text{Ar}$, specifically temporal changes in these anomalies in a parcel of water, were first used for denitrification estimates in open-systems by Laursen and Seitzinger (2002a) in a model-based approach that accounted for re-equilibration of dissolved gases. A similar study used time-series measurements of diel $\text{N}_2:\text{Ar}$ concentrations measured at a single location, correcting for air exchange and groundwater discharge (McCutchan et al., 2003). Denitrification based on this basic modeling approach has since been measured in a variety of lotic systems (e.g. Laursen and Seitzinger, 2004; Yan et al., 2004; Kana et al., 2006; Smith et al., 2008), however until this study no model-based approach has been adapted to lentic systems.

1.2.1.3 Technology

Measurement technologies play an important role in these studies and dissolved N_2 has been measured using gas chromatography coupled with a thermal conductivity detector (GC-TCD) and mass spectrometry (MS) (Groffman et al., 2006). The availability of inexpensively-priced mass spectrometers that offer much higher precision and the development of membrane inlet interfaces to allow direct extraction of gases from water samples has resulted in MS being used more frequently than GC-TCD (Groffman et al., 2006). However, advances in the direct

quantification of denitrification are often slow coming due to a lack of knowledge transfer between scientists of different disciplines or the inhibitory effect of cost (Davidson and Seitzinger, 2006). Although measurements of $N_2:Ar$ in open systems go back to the 1950s (Richards and Benson, 1961), the mass spectrometry technique was not available to many laboratories until very recently (Groffman et al., 2006). GC measurements of dissolved N_2 were applied in the 1960s (Reeburgh, 1969) and 1970s (Barnes et al., 1975) on sediment pore water samples, which exhibited large gradients with depth. The first direct flux measurements on sediment cores were done by Seitzinger et al. (1980) using a technique involving the purging of N_2 with helium, followed by measurements of new N_2 evolved. The introduction of the relatively simple membrane inlet mass spectrometry (MIMS) technique (Kana et al., 1994) coupled to low-cost MS detectors led to growing interest in the development of both core incubation techniques (Kana et al., 1998; Cornwell et al., 1999) and open system measurements (Laursen and Seitzinger, 2002a; McCutchan et al., 2003) for quantifying N_2 production based on changes in $N_2:Ar$ ratios.

Acetylene (C_2H_2) Inhibition

The C_2H_2 inhibition method is a simple and widely used technique for measuring denitrification rates in aquatic and terrestrial sediments based on the inhibition of the transformation of N_2O to N_2 (Sørensen, 1978; Bernot et al., 2003; Groffman et al., 2006). N_2O , which becomes the terminal product of denitrification, is easily measured due to low atmospheric concentrations and the ease with which it can be measured using gas chromatography coupled with an electron capture detector (GC-ECD) (Cornwell et al., 1999; Groffman et al., 2006). The total N_2O flux is equivalent to the denitrification rate. However, major limitations of this

technique include the incomplete blockage of the N_2O transformation to N_2 , the concurrent inhibition of nitrification which may result in underestimation of coupled nitrification-denitrification rates (Seitzinger et al., 1993; Cornwell et al., 1999; Bernot et al., 2003; Groffman et al., 2006), and penetration of acetylene into undisturbed sediments, usually alleviated by performing slurry incubations. Modifications of the technique, using parallel incubations in which nitrification is specifically inhibited have improved the technique (Bernot et al., 2003), however acetylene block is still best used as a measure of denitrification potential rather than actual rates.

^{15}N Stable Isotope Tracer Experiments

The nitrogen isotope pairing technique was developed by Nielsen (1992) to measure denitrification in sediments. Nitrogen has two stable isotopic forms, ^{14}N and ^{15}N , that denitrifying bacteria can use indiscriminately. In the method developed by Nielsen, water overlying sediments in the experimental system is enriched with $^{15}\text{NO}_3^-$ (Nielsen, 1992). If denitrification takes place and is solely supported by the $^{15}\text{NO}_3^-$ introduced to the overlying water, then the N_2 that evolves will be of the form $^{15}\text{N}^{15}\text{N}$ (Nielsen, 1992; Rysgaard et al., 1993). However, if the denitrification that takes place is also supported by internal nitrification processes, then the N_2 that evolves will be of the fractionated form $^{14}\text{N}^{15}\text{N}$ ($^{14}\text{NO}_3^-$ from the naturally occurring NO_3^-) and $^{14}\text{N}^{14}\text{N}$ (Nielsen, 1992; Rysgaard et al., 1993). The formation of single-labeled ($^{14}\text{N}^{14}\text{N}$ and $^{15}\text{N}^{15}\text{N}$) and double-labeled ($^{14}\text{N}^{15}\text{N}$) N_2 by denitrification is measured by mass spectrometry and the $^{14}\text{N}:^{15}\text{N}$ is indicative of the NO_3^- source(s) supporting denitrification (Nielsen, 1992; Rysgaard et al., 1993). Although this method has the highest precision it can be costly and

difficult to interpret due to isotopic fractionation by co-occurring nitrogen cycling processes (Groffman et al., 2006).

Nitrogen Mass Balance

The mass balance approach is a comprehensive method based on the conservation of mass principle (National Research Council, 1978). When creating a mass balance budget, the identity and magnitude of all the inputs, outputs, and reservoirs of an element or compound in a defined, bounded area are accounted for in order to study the behaviour of the substance in the system (Boyer et al., 2006). Mass balance models provide insight into interactions between components of the study system (Boyer et al., 2006), the fate of nutrients as they are transported (Boynton et al., 1985), and data needs or gaps (National Research Council, 1978). Major limitations of this method are that a relatively constant stoichiometry of organic matter is assumed based on the Redfield ratio (Groffman et al., 2006) and a complete nitrogen budget of the system in question is required (e.g. Smith et al., 2008). The assumption of constant stoichiometry is not as robust in freshwater systems where phytoplankton have been shown to be less constrained in their elemental ratios than marine phytoplankton (e.g. Hecky et al., 1993; Elser and Hamilton, 2007). The need for a complete nitrogen budget is a minor issue when dealing with core samples, however, when scaling up to the whole system, the issue becomes time, labour and cost intensive as knowledge of various factors are required (e.g. nutrient pool size for each species of nitrogen, hydrology, tributaries, etc.). In addition, uncertainties associated with a large number of variables (e.g. pool sizes of all N-forms and flows of material) are additive (Smith et al., 2008), leading to large uncertainties in the estimates of N-loss.

Membrane inlet mass spectrometry (MIMS)

Kana et al. (1994) described a method for measuring denitrification rates that involves measuring N_2 concentrations in water samples by membrane inlet mass spectrometry (MIMS). This technique is suitable for estimates of ambient denitrification rates, given its relatively rapid set-up and incubation time, and speed of measurement (Cornwell et al., 1999). This method has several advantages over other methods, including rapid throughput (~20 to 30 samples per hour), lack of sample water preparation (i.e. no degassing step), small sample size (≤ 15 ml), and precise measurement of concentration and gas isotope ratio data (coefficient of variation $< 0.03\%$ for $N_2:Ar$ ratios) (Kana et al., 1994; Kana et al., 1998). However, the method only provides the net change of N_2 not information pertaining to the responsible processes; the method does not discriminate between denitrification and anammox, and nitrogen fixation occurring simultaneously could lead to an underestimate of denitrification. The method also relies on the assumption that Ar is at equilibrium and measurements are dependent on knowledge of gas exchange within the system (Laursen and Seitzinger, 2002a).

Summary of Techniques

There is no single perfect method for measuring denitrification that is best applied to all scenarios (Seitzinger et al., 1993; Piña-Ochoa and Álvarez-Cobelas, 2006). Stable isotope pairing is best suited for core incubations or very small open systems due to the costs associated with this method (e.g. Böhlke et al., 2004). Acetylene inhibition may be a reasonable approach in highly reducing environments where potential for nitrification is negligible (e.g. Sørensen, 1978). Nitrogen mass balance provides a more complete picture than other methods but can often be time, labour and cost intensive due to the complexity of accounting for all the necessary

variables (e.g. Nixon et al., 1995) and propagation of error leads to very imprecise estimates. Membrane inlet mass spectrometry (or isotope ratio mass spectrometry) probably supplants GC-based approaches as they alleviate the need to first strip N_2 (and the many problems associated with this), and offers much greater precision. MIMS based approaches are most applicable to large systems (as opposed to isotope pairing) and offer advantages over whole-system mass balance approaches for the reasons mentioned in section 1.2.1.3. For this reason, this study aims to develop a lentic whole-system modeling approach based on high precision measurements of $N_2:Ar$, so that estimates of denitrification based on changes in $N_2:Ar$ anomalies through time can be made.

1.3 Global Warming

Reactive N in the form N_2O contributes to global climate change (Wang et al., 1976; Huttunen et al., 2003a). Nitrogen oxides can also have impacts on human and ecosystem health (Cowling et al., 1998; Wolfe and Patz, 2002; Galloway et al., 2003). A great deal of attention has been directed to the intermediate gaseous products of denitrification, NO and N_2O , because of their importance in processes of ozone production and consumption, and radiative forcing (Davidson and Seitzinger, 2006). The Intergovernmental Panel on Climate Change (IPCC) employs two metrics for quantitative comparison of the potential impact of different climate change agents (IPCC, 2001). The first is *radiative forcing* (RF), which gives the change in radiative budget (in Wm^{-2}) of the surface-troposphere system following a perturbation by a climate change mechanism (Fuglestad et al., 2003). The other is the *Global Warming Potential* (GWP), which is defined as the warming influence of a gas over a set time period relative to that of carbon dioxide related to infrared absorption and atmospheric lifetime (IPCC, 2001). Increases

in the concentration of greenhouse gases (GHGs) released into the atmosphere have resulted in the increase in the average temperature of the Earth's near-surface air and oceans since the mid-twentieth century (IPCC, 2001). This is known as global warming.

Freshwaters are important sources of the GHGs nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2). Nitrous oxide is mainly produced during denitrification as an intermediate of the reduction of nitrate (NO_3^-) to molecular nitrogen (N_2). Some N_2O is also formed though nitrification by the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) (Bremner and Blackmer, 1978).

1.3.1 Nitrous Oxide

Increased nitrogen loading leads to an increase in denitrification and subsequently, an increase in nitrous oxide at a rate of 0.2–0.3% per year (IPCC, 2001; Beaulieu et al., 2008). Globally, denitrification is estimated to produce 6.3 Tg of N_2O in soils and 4.7 Tg N_2O in oceans each year, causing the release

of this gas into the atmosphere (Figure 1.5) (IPCC, 2001). Agricultural soil management is estimated to produce the same amount of nitrous oxide. Direct emissions of N_2O from agricultural soils and animal feedlots are fairly well studied but less is known about the indirect emissions of N_2O from groundwater, streams, lakes and rivers receiving N-rich drainage water from agricultural soils (Beaulieu, 2008). Seitzinger & Kroeze (1998) modeled N_2O emissions

Figure 1.5. Global nitrous oxide emissions, 1980s (15 Tg N/y) (modified from IPCC, 2001).

from rivers and concluded that flowing water in lotic systems are responsible for 20% of global anthropogenic N₂O emissions.

Rivers have been included in recent global estimates of the aquatic N₂O emissions (e.g. Seitzinger and Kroeze, 1998; Seitzinger et al., 2000), but lakes are not considered significant sources of N₂O emissions and are often excluded (Mengis et al., 1997; Seitzinger and Kroeze, 1998; Huttunen et al., 2003a; Wang et al., 2009). The dismissal of lakes in N₂O emission studies creates uncertainty in regional and global estimates as well as modeling results (Seitzinger and Kroeze 1998; Seitzinger et al., 2000; Wang et al., 2009). It is also incongruous with the understanding that the geomorphic properties of lakes make them efficient systems for N removal by denitrification, the primary source of N₂O (Harrison et al., 2009). Justification for observing lakes for N₂O production is also evident when looking at Arctic lakes with permanent ice cover which have been shown to contain extremely high concentrations of dissolved N₂O (Priscu et al., 1996; Priscu et al., 2008). Priscu et al. (2008) reported a N₂O concentration of 43.3 $\mu\text{mol N.L}^{-1}$ (>700,000% saturation with respect to air) for Lake Bonney (McMurdo Dry Valleys, Antarctica) which represents one of the highest concentrations for a natural aquatic system.

N₂O is a relatively stable GHG that may play a significant role in atmospheric photochemical reactions that contribute to stratospheric ozone destruction (Crutzen, 1970; Hahn and Crutzen, 1982), although the evidence for this remains unclear. Net N₂O emissions are variable in space and time, and uncertainty in field-scale budgets is large, especially when fluxes are measured using chambers (Chapuis-Lardy et al., 2007). Nitrous oxide, although not released in nearly the same volume and with one tenth the radiative forcing as the more infamous carbon

dioxide (IPCC, 2007), has a far greater global warming potential so it takes far less nitrous oxide to produce the same warming effects as carbon dioxide (Table 1.1) (IPCC, 2001; Fuglestedt et al., 2003; Galloway et al., 2008).

Table 1.1. Atmospheric lifetime, GWPs and RFs of various greenhouse gases (modified from IPCC, 2001).

Gas	Atmospheric Lifetime (yrs)	100-year GWP	20-year GWP	500-year GWP	Radiative Forcing (W.m ⁻²)
Carbon dioxide (CO ₂)	50-200	1	1	1	1.66
Methane (CH ₄)	12+-3	21	56	7	0.48
Nitrous oxide (N ₂ O)	120	310	280	170	0.16
HFC-23	264	11,700	9,100	9,800	0.0033
HFC-125	33	2,800	4,600	920	0.0009
HFC-134a	15	1,300	3,400	420	0.0055
HFC-143a	48	3,800	5,000	1,400	
HFC-152a	2	140	460	42	0.0004
HFC-227ea	37	2,900	4,300	950	
HFC-236fa	209	6,300	5,100	4,700	
HFC-4310mee	17	1,300	3,000	400	
CF ₄	50,000	6,500	4,400	10,000	
C ₂ F ₆	10,000	9,200	6,200	14,000	0.0008
C ₄ F ₁₀	2,600	7,000	4,800	10,100	
C ₆ F ₁₄	3,200	7,400	5,000	10,700	
SF ₆	3,200	23,900	16,300	34,900	0.0029

1.4 Modeling

Approaches to modeling denitrification vary widely from mass balance, to stoichiometry and regression analysis but uncertainty remains in all estimations of denitrification rates (Davidson and Seitzinger, 2006). Uncertainty in denitrification models stems from the fact that small areas (“hotspots”) and brief periods (“hot times”) frequently account for a high percentage of the denitrification activity that occurs in both terrestrial and aquatic ecosystems (Davidson and Seitzinger, 2006; Groffman et al., 2009b). Denitrification measurements made at small spatial and temporal scales, and in laboratory conditions are not suitable for scale up to ecosystem levels

and provide poor quantitative understanding of *in situ* rates (Davidson and Seitzinger, 2006). A whole system approach integrates over large temporal and spatial scales to minimize the uncertainty brought about by the phenomena of “hotspots” and “hot times” (Cornwell et al., 1999). To preserve ecological relevance, denitrification measurements should be made within the context of other biological, chemical, and physical processes within the ecosystem being studied (Davidson and Seitzinger, 2006). An approach based on understanding the response of denitrification to changing temperature, salinity, overlying water NO_3^- , organic loading, and other limiting factors would have more predictive power than a denitrification measurement program alone (Cornwell et al., 1999).

1.4.1 Open Channel N_2 Method

Previous approaches to measuring denitrification in aquatic systems (i.e. N_2 production and gas-tight chambers) were adapted for *in situ* denitrification measurements using benthic chambers (e.g., Devol, 1991; Laursen and Seitzinger, 2002b). However, the use of benthic chambers has several limitations. The first is that physical and chemical gradients may be altered which in turn can influence rates of benthic processes such as denitrification (Jahnke et al., 2000). The second limitation of benthic chambers is the uncertainty associated with scaling up from a small surface area (Laursen and Seitzinger, 2002a). Stable N isotope tracers and mass balance approaches also have their limitations thus a new method was developed to allow for a more direct measurement of *in situ* denitrification in rivers (Laursen and Seitzinger, 2002a; McCutchan et al., 2003) through the use of MIMS (Kana et al., 1994).

The open-channel N_2 method provides integrated, whole-system estimates of denitrification in flowing waters based on the net production of N_2 , and corrects for atmospheric exchange (Laursen and Seitzinger, 2002a). Laursen and Seitzinger (2002a and 2004) used a two-station approach wherein a single parcel of water was followed and sampled through time. This provides spatial representation of denitrification rates (Baulch et al., In Press). McCutchan et al. (2003) used a one station, or diel approach employing a time for space substitution. In that model, temporal patterns of concentration and solubility at an unsampled upstream location are assumed to be the same as those measured in the same parcel of water as it passes through the downstream sampling station (McCutchan et al., 2003).

The open-channel N_2 method also provides a direct measure of denitrification via its endproduct N_2 . The advantages of this method are that it measures denitrification at ecological scales that are spatially (Laursen and Seitzinger, 2002a; McCutchan et al., 2003) and temporally (Laursen and Seitzinger, 2002a) relevant, it can be adapted to estimate the *in situ* production of other biogenic gases simultaneously (e.g. N_2O and CH_4) (Laursen and Seitzinger, 2004), it is a relatively simple and inexpensive method, and it is applicable to rivers of various sizes. However, open channel methods for measuring N_2 production in rivers (Laursen & Seitzinger, 2002a; McCutchan et al., 2003) do not discriminate between denitrification and anammox. The assumption is that the total N_2 production in rivers is due primarily to denitrification (Laursen and Seitzinger, 2004) since anammox is considered of limited importance in estuarine and freshwater systems (Schubert et al., 2006). Open channel methods also do not discriminate between denitrification and nitrogen fixation thus denitrification may be underestimated in systems where rates of N-fixation are high, and are not amendable for use in cascading mountain

streams (McCutchan et al., 2003) where gas re-equilibration rates are high and entrainment of N_2 is high (gas bubbles become injected and collapse resulting in abnormally high N_2 concentrations). The greatest limitation of the open-channel method deals with method uncertainty as discussed by Baulch et al. (In Press).

1.4.2 Model Development – Lentic Systems

The original riverine model (Laursen and Seitzinger, 2002a) was developed to address the shortfalls of traditional methods used to study biogeochemical processes in rivers. The advantages a model-based whole-system approach has over traditional methods include temporal relevance, spatial relevance (the whole system is analyzed versus the very limited surface area of the chambers or the cores), a reduction in field sampling time (i.e. no need for installation of instrumentation, minimal preparation time), a reduction in the cost associated and the supplies needed, no sampling artefacts introduced by installing chambers (e.g. changes in hydrology), and minimal training required to use of low-tech equipment.

However, modifying the original model to lentic systems presented challenges of its own as new considerations had to be factored in. The reactants and products of many biogeochemical processes (such as denitrification) are gases and in open systems, these gases re-equilibrate with the atmosphere. The original model estimated re-equilibration on the basis of a piston velocity and the disequilibrium of dissolved gases in water, with equilibrium dissolved gas concentrations being estimated from water temperature and salinity (Weiss et al., 1970). However, the equilibrium also depends on the partial pressure of the gas in the atmosphere, and therefore on

air temperature, atmospheric pressure, and relative humidity, all of which change rapidly. In the work described in this thesis, the model is modified to include these important parameters.

1.4.2.1 Model Inputs

The original model had the following measured inputs: channel depth, channel width, channel velocity, Δ time (time between measurements), initial N_2 concentration, initial equilibrium N_2 , water temperature, the first order exchange rate (K_{N_2}), and denitrification rate (Figure 1.6). The derived inputs included: channel volume, sediment surface area, water surface area, and $[d(\text{equilibrium } N_2)]/dt$ (Figure 1.6) (Laursen and Seitzinger, 2002a).

- Model Inputs

<u>Measured</u>	<u>Derived</u>
• Channel depth	• Channel volume
• Channel width	• Sediment surface area
• Δ time	• Water surface area
• Initial N_2 concentration	• $\frac{d(\text{equilibrium } N_2)}{dt}$
• Initial equilibrium N_2	
• Average temperature	
• K_{N_2}	
• Denitrification rate	

Figure 1.6. Original model inputs.

The calculated N_2 concentrations are a function of the previous N_2 concentration plus the amount of new N_2 produced (equal to the denitrification rate times the sediment surface area), minus the N_2 lost due to re-equilibration. Denitrification rates are estimated by modifying the denitrification rate (as a model input) such that the model predicted final *in situ* N_2 concentration matches the measured final *in situ* N_2 concentration (Figure 1.7). N_2 flux is area-specific rate of

N_2 lost to the atmosphere and is a function of the depth of the system (h) and the first order exchange rate (k_{N_2}) multiplied by the previous N_2 concentration, minus the N_2 equilibrium concentration for the time step in question.

- **$N_2 \text{ conc} = (N_2 \text{ conc}' + N_2 \text{ prod}) - N_2 \text{ lost}$ Model Output**
- where:
 - $N_2 \text{ conc}'$ = concentration from previous time step
 - $N_2 \text{ prod}$ = denitrification rate * sediment surface area
 - $N_2 \text{ lost}$ = $hk_{N_2}[(N_2 \text{ conc}' + N_2 \text{ prod}) - N_2 \text{ equil}]$
 - $N_2 \text{ lost}$ due to re-equilibration
 - $N_2 \text{ equil}'$ = equilibrium N_2 concentration at current time step given current temperature

Figure 1.7. Solving for denitrification based on the original model.

1.4.2.2 Sampling

Lagrangian sampling, wherein the properties of a single marked particle are sampled over a period Λ , was used for the measurement of N_2 flux rates in the original whole-system approach developed by Laursen and Seitzinger (2002a). Figure 1.8 shows how a parcel of water was measured. The first step is to repeatedly measure the same parcel of water as it moves downstream over time. This is done in order to measure the change in the concentration of nitrogen as a N_2 :Ar ratio within that parcel of water. The second step is to determine how quickly N_2 goes back into the atmosphere by using volatile tracers to determine first order gas exchange rates. The third step is to use the model-based approach (figures 1.6 and 1.7) to solve for denitrification rate based on gas exchange rate, changes in N_2 concentration during riverine transport, and physical characteristics of the system (such as width and depth).

Figure 1.8. Lagrangian sampling for the original model.

1.4.3 Model Modification

The modified model used for this research project included the following measured parameters which affect the partial pressure and gas exchange rate of a gas [see explanation below]: air temperature, atmospheric pressure, relative humidity, wind velocity, and Schmidt number coefficient (Figure 1.9). Channel depth, width and velocity will be replaced with lake depth, while denitrification becomes a model output. Several inputs to the model are held constant including: initial N_2 concentration, final N_2 concentration, time interval, and water column depth. Other inputs to the model were variable over the measurement interval and these included: water temperature, air temperature, atmospheric pressure, relative humidity, wind velocity, and the Schmidt number. The model is then run repeatedly, with a new value of each variable input selected with each iteration. The selection of values for a variable model input was based on the distribution of measured values for that variable over the sampling interval.

- Model Inputs

Measured

- Channel depth
- Channel width
- Δ time
- Initial N_2 concentration
- Initial equilibrium N_2
- Average temperature
- K_{N_2}

Derived

- Channel volume
- Sediment surface area
- Water surface area
- $\frac{d(\text{equilibrium } N_2)}{dt}$

- Denitrification rate

- Air temperature
- Atmospheric pressure
- Relative humidity
- Wind speed
- Schmidt number coefficient

= Modifications
All affect partial pressure

Figure 1.9. Modified model inputs.

The concentration of a dissolved gas is directly proportional to the partial pressure of the gas (Henry's Law) (Mackay and Shiu, 1981). Partial pressure is reported with respect to dry air; however pressure is dependant on moisture content which is a function of temperature and relative humidity (Equation 1.1).

$$p_{\text{moist}}^X = p_{\text{dry}}^X \cdot \left[1 - \frac{p^{\text{H}_2\text{O}}}{P} \right]$$

Equation 1.1

Where P is equal to the total atmospheric pressure, $p^{\text{H}_2\text{O}}$ is equal to the partial pressure of water vapour ($= e^{(77.3450 + 0.0057 T - 7,235 / T) / T^{8.2}}$), e is the constant Euler's number, and T is the temperature of the moist air in degrees K. Relative humidity is inversely proportional to pressure and temperature (which is directly proportional to the saturation vapour pressure). Water temperature also affects gas saturation levels and gas exchange rates (Weiss, 1970). The solubility of a gas is inversely proportional to temperature because increasing temperature results

in increased kinetic energy of the gas molecules causing bonds to break and gas molecules to escape.

Gas flux across the air-water interface is determined by molecular transfer across a diffusive layer of thickness Δz . Turbulence in the form of wind reduces Δz which in turn increases the transfer velocity, k (Liss and Slater, 1974; Wanninkhof, 1992). Gas flux can be estimated indirectly with equation 1.2:

$$F = k(C_w - \alpha C_a)$$

Equation 1.2

Where k is the gas transfer velocity, C_w and C_a are the gas concentrations in water and air, respectively, near the interface, and α is the Ostwald solubility coefficient. The piston velocity (k) is a function of turbulence at the interface, the momentum diffusivity of the water (ν), and the diffusion coefficient or mass diffusivity of the gas (D). The ratio of ν and D is referred to as the Schmidt number ($Sc = \nu/D$) which varies for a gas as a function of temperature. For a smooth liquid interface, k is proportional to $Sc^{-2/3}$ (Deacon, 1977) and for an interface with waves, k is proportional to $Sc^{-1/2}$ (Ledwell, 1984). Thus wind speed and Schmidt number are important variable additions as they directly affect gas exchange rates (Wanninkhof, 1992; Laursen and Seitzinger, 2005).

Lentic systems present different sampling considerations than rivers in that one is now dealing with a more static body of water that is not typically well mixed, and which is often thermally stratified. Therefore, assumptions made in the riverine model of a well-mixed parcel of water moving downstream a predictable distance over a given time do not apply to lakes. When

applying the whole-system approach to lakes, instead of sampling one parcel of water repeatedly over a period of time, many parcels of water are sampled (depth defined strata) throughout the water column.

Figure 1.10. Nitrous oxide inventory determination.

The data can then be plotted as shown in Figure 1.10 (theoretical data used) to determine the excess of the measured gas that is not accounted for solely by equilibration with the atmosphere. Plotted on the x-axis are the recorded temperature, measured N₂O concentration and the calculated equilibrium concentration. On the y-axis is the depth creating a depth profile. When the area between the measured and equilibrium concentrations is integrated, it represents an inventory of surplus gas (disequilibrium) at a single point in time. The change in this inventory over time can be used to determine a rate of change in the inventory. By simultaneously estimating flux to the atmosphere, a production rate of the gas can be estimated. In this example, the inventory of excess N₂O is shown by the shaded area. The model is then used to determine the rate of production necessary to explain the surplus of N₂O given the rate of atmospheric

exchange from the top stratum. Alternatively, the model can determine the rate of gas production that would be needed to generate the observed surplus if the surplus were to represent a steady-state.

1.4.3.1 Stratification

Special considerations had to be made when modifying the original model from a lotic system to a lentic system and this included accounting for stratification. Stratification is generally produced by gradients in temperature and/or salinity. Stratification caused by temperature gradation is due to solar heating of surface waters which causes the warmer, less dense surface water to sit on top of colder water, with a thermocline in between (Hutchinson, 1938; Fee et al., 1996; Wetzel, 2001; Kim, 2006). Stratification is greatest in the summer when the surface waters are exposed to the greatest amount of warming. Wind is the primary means of mixing the different layers but as the thermocline increases greater energy is required to cause mixing. As a consequence, very little mixing of warm water and cold water occurs. This results in the epilimnion (the surface layer) and hypolimnion (the bottom, cooler layer) exhibiting different physiochemical characteristics (Hutchinson, 1938; Kim, 2006). During fall and as winter approaches, the surface waters will cool and eventually become denser than the deeper waters which causes the two layers to overturn and mixing to occur. Stratification is minimal during the winter as there are minute temperature gradients (Wetzel, 2001; Kim, 2006). If air temperature continues to drop, ice formation will occur. The process is repeated in the springtime with the warming of the ice-cover which produces dense water that then sinks below causing mixing to occur. This seasonal stratification can cause “hotspots” and “hot times” resulting in a greater need for a more robust model to handle the heterogeneity of the system examined.

1.4.3.2 Monte Carlo Analysis

A statistical model was developed for the estimation of denitrification rates, metabolic rates and N₂O flux rates by applying a Monte Carlo sampling algorithm to field observations to create probability distributions given different climate variables. With deterministic modeling, which was applied in the original model, single-point estimates are chosen from field observations to produce a few, discrete estimations. Monte Carlo simulation uses random sampling of probability distribution functions as model inputs (here water temperature, air temperature, atmospheric pressure, relative humidity, wind velocity, and the Schmidt number) to produce hundreds or thousands of possible outcomes based on probability that account for the full range of field observations (Tonitto et al., 2009). Since estimations of denitrification rates, metabolic rates and N₂O production rates are based on model inputs that vary over a broad range across spatial and temporal scales, this modeling approach is an empirical tool for approximating the response to diverse environmental conditions (Tonitto et al., 2009). A recent review of whole-system measurements of denitrification in rivers has pointed out the need for Monte Carlo analysis in these models to better constrain error estimates associated with model output (Baulch et al, In Press). Monte Carlo analysis has been added as a component of the model developed here when modifying riverine models for lentic application.

1.5 Rationale

The rationale behind this research project is the development of a new approach for the measurement of denitrification at the whole-lake scale that is ecologically relevant at spatial and temporal scales with better estimation and constraint of error. The proposed method would also be advantageous in that prohibitive factors such as cost, level of expertise, time consumption,

and propagation of error due to scaling up found in other methods would be reduced. This would allow a research program to determine denitrification (and, simultaneously other biogeochemical processes such as greenhouse gas production) in a larger number of lakes than would be possible using more traditional approaches. As a result, a research team can begin addressing more significant ecological questions regarding physical, chemical, and biological drivers of denitrification at the whole-system to watershed scale

1.5.1 Objectives

The present study had four objectives involved with this research project. The first was the modification of an existing whole-system model that was developed by Laursen and Seitzinger (2002a) for application in lentic systems. Climate parameters such as wind velocity, water depth, water and air temperature, relative humidity, and atmospheric pressure, were included in the model calculations to improve estimates of gas re-equilibration over those made in the 2002 model. The flux rates and/or concentrations of dissolved N_2 , O_2 , and N_2O were determined.

The second objective was field validation of the modified whole-system model by comparing results obtained with the enclosure method of chamber use to the *in situ* approach. It was hypothesized that the new method would provide a faster, simpler and more robust method for measuring denitrification and other biogeochemical processes at ecologically relevant temporal and spatial scales, but if there was not correspondence between the rates measured in whole-system versus benthic chamber approaches, the open system approach may need further modification before it can be adopted.

The third objective was the development of an annual budget of N_2O production within a single aquatic system. This was achieved by performing a high frequency of measurements for a period of one year. It was hypothesized that frequent and continuous sampling would provide a more complete and accurate budget because both variability and trends due to seasonal, climate and ice-cover changes would be recorded and accounted for, providing a greater understanding of the components that affect biogeochemical cycling on temporally and spatially relevant scales.

The fourth objective was the development and use of a Monte Carlo sampling algorithm within the model to better constrain error in model estimates of denitrification, whole-system metabolism, and greenhouse gas production. It was hypothesized that the use of probability distributions to generate rate loss or gain estimates would result in the decrease of uncertainty of model predictions because the full range of field observations and the distributions of these observations, instead of discrete observations used in deterministic models, would be used to determine a range of estimates that reflect changing environmental conditions.

1.5.2 Contributions to Science

A new and robust tool will be developed that allows for the examination of biogeochemical processes at ecologically significant temporal and spatial scales. It will emphasize the need to better constrain error and nutrient budget estimations through the use of Monte Carlo analysis, and will improve on previous models by better modeling gas re-equilibration by inclusion of additional atmospheric data.

The major implications of this research project will be the application of this tool to allow for cross-system comparison and the application of this for the measurement of other biogeochemical processes.

CHAPTER 2

MATERIALS AND METHODS

Sample collection was performed at Christie Lake (Conservation Hamilton, Dundas, ON, Canada) and Turtle Pond (Stoney Creek, ON, Canada). Experiments were conducted in the environmental laboratories of Drs. Andrew Laursen, Lynda McCarthy, and Vadim Bostan (Ryerson University, Toronto, ON, Canada), the Ryerson University Analytical Centre (Ryerson University, Toronto, ON, Canada), and the laboratory belonging to Dr. Roxane Maranger (Université de Montréal, Montreal, PQ, Canada).

2.1 Site Description

2.1.1 Christie Lake

Christie Lake (Figure 2.1) is a reservoir that is part of Conservation Hamilton property located in Dundas, ON, Canada (latitude: 43.2878 N and longitude: 80.0278 W) with an average depth of ~3.5m and a surface area of ~10 ha. The site was chosen in consultation with Conservation

Figure 2.1. Christie Lake field site.

Hamilton and Conservation Halton. The ideal study site was a lake that had substantial depth and would reliably stratify and after consultation, this was believed to be the deepest and most relatively stable body of water under Conservation Authority jurisdiction. However, the study site was much shallower than estimated, and with a wet year and high flow through, it did not stratify well. Sampling occurred in June, July and October, 2008. The sampling dates were June 21-22, July 24-25, and October 3-4, of 2008. Reservoirs are artificial lakes created to hold water for various uses ranging from recreational to flood control. These 'lakes' undergo a good deal of

mixing and cycling between oxic and anoxic conditions due to the removal and re-introduction of the water from these systems. This property of the system made it a good choice for method validation as “hotspots” and “hot times” are endemic to this system.

2.1.2 Turtle Pond

Turtle Pond (Figure 2.2) is an inland wetland constructed in the 1920s and located in Stoney Creek, ON, Canada (latitude: 43.2431 and longitude: 79.7310) with an average depth of ~1.3m and a surface area of ~1 ha.

Figure 2.2. Turtle Pond field site.

Wetlands are lands where water saturation is the dominant factor that determines the nature of the soil (hydric soil) and the types of plant and animal communities living in or on the surface of the soil (EPA, 1995). They cover a large range of differing climatic and geographic regions across the biosphere and are regions of great biodiversity (EPA, 1995). Sampling in Turtle Pond occurred on a weekly or bi-weekly interval during a period from November, 2007 to September, 2008 with an interruption from March to August of 2008. The sampling dates were as follows: November 2, 11, 18 and 28, 2007; January 10, 26, February 3, 16, 23, March 2, August 11, 18, 28, September 4, 12, 20 and 28, 2008.

2.2 Sample Collection

2.2.1 Christie Lake

2.2.1.1 Water Column Sampling

A Niskin-type sampler was used to collect discrete water samples at 0.5m intervals ranging from 0.5m below the water surface to 3.5m below the water surface (Figure 2.3). The Niskin-

type sampler was made of a Plexiglas cylinder that was closed at each end by a rubber force cups connected by rubber tubing. The two cups were placed in the armed position by short loops of wire and released underwater by a messenger sent down a length of rope that had depth measurements on it. The sampler was then pulled up and water samples retrieved immediately to minimize exposure to the atmosphere. Sample containers were gently over-

Figure 2.3. Grab sampling method.

filled by gravity with a tube inserted into the bottom of the vessel delivering a minimum of 3x the vessel volume. Water samples for N_2 and O_2 analysis were placed in gas-tight 5mL Exetainers (Labco International Inc., Houston, TX, U.S.A.) and preserved with a small volume of $ZnCl_2$ at a final concentration of 0.1% vol/vol. The samples were sealed immediately and preserved in a way so as not to change pH which can change the solubility of CO_2 in turn changing the partial pressure of the gases.

Nutrient samples were filtered using a Whatman GF/F filter (nominal pore size = $0.7\mu m$), placed in 50mL polypropylene centrifuge tubes, and stored in a cooler filled with ice while in the field. Samples were then transferred to a freezer within 8 hours of sampling and stored at $-20^\circ C$ until analysis. Samples for N_2O analysis were prepared by injecting 10mL of water collected into 30mL serum vials that had been previously flushed with nitrogen gas (purging with N_2 for 10 volumes of the vessel). The samples were allowed to re-equilibrate and 5mL of the headspace was withdrawn and injected into gas-tight 5mL Exetainers that had previously been flushed with N_2 as above. Sample vessels for all gases were stored immersed in water at a temperature $\sim 1^\circ C$

below *in situ* temperature. If the samples were stored at a temperature higher than the *in situ* temperature, degassing and formation of a gas bubble in the sample could have resulted. The samples were also stored under water so that if the volume of the sample changed and a vacuum was created, water would have been sucked in versus air. Sample collection is the most important step because it is where there is the most potential for error to occur. The most important thing to consider is the entrainment of air bubbles which is not desired. The presence of an air headspace or formation of a pocket due to degassing can result in the stripping of nitrogen preferentially over Ar, resulting in a decrease in N₂:Ar in water during storage.

2.2.1.2 Benthic Sampling

Figure 2.4. Benthic chambers used for enclosure sampling.

Samples were collected using benthic chambers at the sediment-water interface (Fig. 2.4). The chambers were manufactured from acrylic, were round in shape with a flat top and an open bottom, and had a volume of 39L, with a surface area of 0.196m². The chambers contained a propeller for mixing, and a sampling port connected to tubing to facilitate sample withdrawal. The chambers were deployed using SCUBA and sediment resuspended during installation was allowed to settle for a minimum of two hours before sample collection commenced. A transect line was placed with the chambers to aid in placement and locating the chambers in times of poor

water clarity. For sample withdrawal, 25mL gas-tight glass syringes were attached to the chambers via tubing. Mixing of water inside the chamber with the water entering the chamber inlet was prevented through the use of a nitrile bag that was fastened to the inlet port within the chamber. The nitrile bag is used to prevent pressure changes and formation of headspace in the chamber (Laursen and Seitzinger, 2002b). As water was removed from the sampling port, the corresponding volume was replaced by water flowing into the inlet and filling the nitrile bag.

Immediately after surfacing, the water samples were transferred to the appropriate vessels for storage and analysis at a later date. Samples for N₂:Ar were transferred to Exetainers using a short piece of Tygon tubing (Saint-Gobain Performance Plastics, Akron, OH, U.S.A.) inserted to the bottom of the vessel. The plunger was depressed very slowly to prevent an increase in pressure inside the tube and the consequent increase in solubility of N₂. Sample storage was the same as that for water column sampling: nutrient samples were filtered then frozen; water samples for N₂ and O₂ analysis were preserved then stored in water ~ 1°C below the *in situ* temperature; and samples for N₂O analysis were prepared by injection of water into N₂ flushed serum vials and collection of the headspace (please refer to section 2.2.1.1 for greater detail). Climate data was obtained from Environment Canada's historical weather data for the weather station located at Hamilton International Airport (Climate Data Online http://www.climate.weatheroffice.ec.gc.ca/climateData/canada_e.html).

2.2.2 Turtle Pond

A Niskin-type sampler was used to collect discrete water samples near the surface (~0.2m depth) and a meter below the surface of the water. Sample collection and storage were as

previously described in sections 2.2.1.1 and 2.2.1.2. Climate data was obtained from a weather station (WS-161OU-IT-11, La Crosse Technology Ltd., La Crosse, WI, U.S.A.) that remained on-site through out the sampling periods. During periods of ice cover an auger was used to bore a hole through the ice and samples were collected by deploying the sampler through the hole. Disruptions in sampling (from late November to early January) were due to unsafe ice conditions.

2.3 Samples Analyses

2.3.1 Nitrogen and Oxygen gas – MIMS

Dissolved N_2 and O_2 concentrations were determined using a modified version of the MIMS method developed by Kana et al. (1994). A stainless steel capillary draws up the water sample into a semi-permeable membrane that is placed in glass tubing under high vacuum. Gaseous molecules are stripped from the water and swept towards the mass spectrometer for ionization and detection at m/z values of 28, 32, and 40 for N_2 , O_2 and Ar respectively. N_2 and O_2 concentrations were not directly measured but were calculated from $N_2:Ar$ and $O_2:Ar$ concentrations since it has been found that $N_2:Ar$ measurements have smaller errors associated with them ($<0.03\%$ coefficient of variation) than N_2 measurements alone ($<0.10\%$ coefficient of variation) (Kana et al., 1994). Instrument calibration was performed with deionized water standards that were atmospherically equilibrated, with known salinity and temperature (Kana et al., 1994). The standards were maintained at a constant temperature of $18.0^\circ C$ in the same thermostated bath that contained the membrane apparatus and stirred slowly to prevent entrainment of bubbles (Kana et al., 2006). Dissolved gas concentrations were determined from

equations derived by Weiss (1970) and the instrument signal was adjusted to match the calculated values (Kana et al., 1994; Kana et al., 2006).

2.3.2 Nitrous Oxide – GC-ECD

N₂O concentrations were measured by gas chromatography coupled with an electron capture detector (GC-ECD) (AutoSystem XL, PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA, U.S.A.) with a Plot 1010 column (Supelco Inc., Bellefonte, PA, U.S.A.) after dissolved gases were allowed to equilibrate with the N₂ headspace. The ECD uses a radioactive Beta emitter (electrons) to ionize some of the carrier gas and produce a current between a biased pair of electrodes. When organic molecules with electronegative functional groups (i.e. nitro groups) pass by the detector, they capture some of the electrons and reduce the current measured between the electrodes. The temperature settings were 375°C for the ECD, 35°C isothermal for the oven, and 185°C for the injector. The injection volume was 1.00mL, two injections were done for each sample with an average of the results taken, and a standard gas was analyzed after every 10 samples. Injections were performed with a split ratio of 0 and the carrier gas was helium at a flow rate of 30 mL/min. N₂O flux was calculated from the linear change of its concentration in the chamber volume as a function of time and was expressed in units of nmol N.m⁻².h⁻¹.

2.3.3 Nitrate - Cadmium Reduction Method

Nitrate (plus nitrite) concentrations were determined using a modified version of the cadmium reduction method developed by Wood et al. (1967). In the cadmium reduction method, nitrate is reduced to nitrite in the presence of cadmium granules treated with copper sulfate. A

cadmium column was prepared by packing commercially available Cd granules that were made to be copper-coated by treating them with a dilute solution of HCl followed by swirling with CuSO_4 (APHA, 1995). Tubing was attached to both ends of the column, with one end connected to a peristaltic pump and the other end for the collection of the eluted, reduced sample. The peristaltic pump was used for sample withdrawal from the sample vessel into the column. The total nitrite eluted was then reacted with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride under acidic conditions to form a highly coloured azo dye that has an absorption peak at 543 nm. Absorbance was then determined within a 2 hour period using UV-VIS (ultra violet/visible) spectrophotometry (Lambda 20, PerkinElmer Life And Analytical Sciences, Inc., Waltham, MA, U.S.A.). The original nitrate concentration was determined with the aid of a standard curve.

All glassware used for nitrate analysis (and the analysis of ammonia and phosphate) was pre-combusted in a muffle furnace at 500°C for 2 hours, washed with 5% HCl prior to use, and then rinsed three times with Millipore-Q water.

2.3.4 Ammonia - Phenate Method

Ammonia concentrations were determined using a modified version of the phenate method developed by Solorzano (1969). In the phenate method, hypochlorite and phenol react with ammonia in the presence of a sodium nitroprusside catalyst to form indophenol. Indophenol is an intensely blue compound that has a strong absorption peak at 640 nm. Absorbance of the resulting indophenol was determined within a 24 hour period using UV-VIS spectrophotometry

(Lambda 20, PerkinElmer Life And Analytical Sciences, Inc., Waltham, MA, U.S.A.). The original ammonia concentration was then determined with the aid of a standard curve.

2.3.5 Phosphate - Ascorbic Acid Method

Phosphate concentrations were determined using a modified version of the ascorbic acid method developed by Murphy and Riley (1962). In the ascorbic acid method, ammonium molybdate and potassium antimonyl tartrate react in an acid medium with orthophosphate-phosphorus to form an antimonyl-phospho-molybdate complex that is then reduced to an intensely blue-coloured complex by ascorbic acid. Absorbance of the molybdenum blue complex was determined within a half hour period using UV-VIS spectrophotometry (Lambda 20, PerkinElmer Life And Analytical Sciences, Inc., Waltham, MA, U.S.A.). The original phosphate concentration was then determined with the aid of a standard curve.

2.4 Determining Denitrification

For the purposes of this study three approaches were taken to measure denitrification. The first approach employed the use of benthic chambers which has inherent uncertainty due to extrapolation of rates that were determined in meter and hour scales to kilometer and month scales for ecosystem studies of denitrification (McCutchan et al., 2003; Groffman et al., 2006). The second approach was the use of a whole-system approach that used *in situ* measurements from the water column in combination with MatLab modeling (see section 2.5 for further details). This approach relies heavily on the ability to precisely measure dissolved N_2 against a high background concentration while contending with the physical processes of mixing and air exchange (Groffman et al., 2006). Both approaches determined denitrification by measuring N_2

production through the use of MIMS. The third approach employed stoichiometry based on the Redfield ratio (106 C:15 N:1 P) (Redfield, 1934). The assumption was made that when organic matter with a known elemental ratio (Redfield) is decomposed using O_2 , there will be a set of products with a defined elemental composition. The consumption of O_2 was expected to be related to two processes, respiration (and mineralization of organic N) and nitrification. An assumption was made that all ammonium was oxidized to nitrate. Oxygen consumption was then apportioned between these two processes. The portion of O_2 consumption ascribed to respiration was estimated to produce 15mol inorganic N for every 100 mol O_2 consumed, assuming 1mol O_2 consumed per 1mol CO_2 produced. The difference between the expected change in dissolved inorganic N (DIN) concentration change due to remineralization and the observed change in DIN concentration was then used to calculate denitrification as N loss.

2.5 Modeling

The general modeling structure was adapted from Laursen and Seitzinger (2002a, 2005). Denitrification was determined to be the change in the dissolved N_2 concentration (DN_2) required to achieve the observed change in N_2 , from time = n to time = $n + 1$ (t = time), given K_{N_2} (K = first-order gas transfer rate). In other words, denitrification was determined by the excess in N_2 not accounted for by atmospheric exchange alone. Excess N_2 was determined by first modeling the expected change in N_2 concentration if no denitrification took place (i.e. due only to re-equilibration with the atmosphere). Denitrification was then calculated based on the N_2 production necessary to explain the excess N_2 observed in the measured concentration in comparison to the equilibrium concentration. Changes in dissolved N_2 were modeled using 1-hour time steps, allowing for changes at each time step in K_{N_2} due to variations in water

temperature by recalculating the Schmidt number coefficient of N_2 (Sc_{N_2}) and equilibrium N_2 concentration. The determination of the net consumption of O_2 as well as the net production of N_2O followed the same modeling structure.

2.5.1 Modeling Environment

Another modification to the original model is the environment used to generate estimations. A switch was made from the software program Excel (Microsoft Corp., Redmond, WA, U.S.A.) to MatLab (The MathWorks, Inc., Natick, MA, U.S.A.). The software program MatLab was used to randomly and iteratively sample through Monte Carlo analysis initial N_2 concentration, final N_2 concentration, time interval, and water column depth against the distributions that were calculated for each of the following inputs: water temperature, air temperature, atmospheric pressure, relative humidity, wind velocity, and the Schmidt number coefficient ranging between $-\frac{1}{2}$ and $-\frac{2}{3}$. For each of the above input variables, distributions of the same sample size were generated by creating an alternate matrix of random values with the same distribution frequency as the original input variable. MatLab then read in the values from each variable matrix line by line with each iteration. Normal distribution was confirmed with a K-S Lilliefors test for normality using Systat (Systat Software Inc., Chicago IL, U.S.A.). The model was run with one hour time steps and 1000 iterations. MatLab generated 1000 unique solutions for denitrification rate based on the values of input variables. Reported denitrification rates are based on the mean $\pm$ 95% confidence intervals from this population of 1000 unique solutions. Oxygen consumption and N_2O production were similarly modeled. Different approaches were used to the run the model (see sections 2.5.1.1 and 2.5.1.2).

2.5.1.1 Christie Lake

For Christie Lake, the first method used to run the model was the determination of DN_2 for each stratum of water followed by the summation of the results. This was achieved by entering the measured N_2 concentration for the top layer of water (0.5m) at the beginning and end of the specified time period as model inputs for initial and final values of N_2 concentration into MatLab. The remaining strata (represented at 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5m below the surface of the water) were treated as though no diffusive loss to the atmosphere occurred. The model generated values for DN_2 were then averaged and the mean value summed with the calculated values for DN_2 for the remaining strata.

The second method used to run the model for Christie Lake was the determination of DN_2 when treating the lake as one parcel of water. This was achieved by averaging the N_2 concentrations over the depth profile (e.g. measured values for N_2 obtained at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5m below the surface of the water) for the specified time period. The average values (for water column N_2 on June 21-22 and July 24-25) were then entered into MatLab as model inputs for initial and final values of N_2 , respectively, to produce DN_2 .

The third method used for Christie Lake was based on the assumption of a pseudo steady state. In this approach, values of dissolved N_2 were assumed to have reached a steady state between production and loss to the atmosphere. Therefore, the values measured for N_2 were entered as both the initial and final concentrations in a model with a one week run. This form of the model was run both as discrete parcels (approach 1) and with the column treated as a single parcel (approach 2).

2.5.1.2 Turtle Pond

The method used to run the model for Turtle Pond was the determination of DN_2 by entering the measured N_2 concentration at the beginning and end of a specified time period as model inputs for initial and final values of N_2 concentration into MatLab. Turtle Pond was not modeled by stratum as it was well mixed (based on measured chemical parameters near the surface and the bottom).

2.5.2 Model Inputs

The original model had the following measured inputs: channel depth, channel width, channel velocity, Δ time, initial N_2 concentration, initial equilibrium N_2 , water temperature and K_{N_2} , and denitrification rate. Derived inputs for the model included: channel volume, sediment surface area, water surface area, and $[d(\text{equilibrium } N_2)]/dt$ (Laursen and Seitzinger, 2002a). The modified model used for this research project added the following measured parameters which affect the partial pressure and gas exchange rate of a gas: air temperature, atmospheric pressure, relative humidity, and wind velocity. Channel depth, width and velocity were replaced with lake depth, while denitrification became a model output. The inputs that remained constant during the model simulation were initial N_2 concentration, final N_2 concentration, time interval, and water column depth. The inputs that were entered as distributions were: water temperature, air temperature, atmospheric pressure, relative humidity, wind velocity, and the Schmidt number coefficient.

2.5.3 Modeling Equations

General Equation:

$$N_2(t_{n+1}) = [N_2(t_n) + \text{new } N_2(\text{interval } t_n \text{ to } t_{n+1})] - N_2 \text{ lost}(\text{interval } t_n \text{ to } t_{n+1})$$

[units in mmol N.m⁻³]

Equation 2.1

Nitrogen concentration will be calculated using one hour time steps. For each time step, the N₂ concentration was determined as a function of the previous N₂ concentration (at time n) added to amount of N₂ that was produced (at time interval n to n+1) and subtracting the N₂ that was lost from the system, the N₂ flux (at time interval n to n+1). The initial N₂ concentration was measured using MIMS.

where...

- new N₂ (interval t_n to t_{n+1}) = (denitrification/depth)/2

Equation 2.2

[units in mmol N.m⁻³]

The new N₂ produced is assumed to be solely from net denitrification. Nitrogen production is based on denitrification rate (units of mmol N.m⁻².h⁻¹ over 1h) divided by depth (units of m) and further divided by 2. The division by 2 accounts for 2 mmol N denitrified for every 1 mmol of new N₂ created.

- N₂ lost (interval t_n to t_{n+1}) = [N₂ flux (interval t_n to t_{n+1})] /depth

Equation 2.3

[units in mmol N.m⁻³]

The N₂ lost is assumed to be due to atmospheric exchange and is calculated as the N₂ flux (at time interval n to n+1) (units of mmol N.m⁻².h⁻¹ over 1h) divided by depth (units of m).

- $N_2 \text{ flux} = h \times K_{N_2} (\text{in situ } N_2 - \text{equilibrium } N_2)$

Equation 2.4

[h has units of m, K has units of h^{-1} , concentrations have units of $mmol\ N.m^{-3}$]

The N_2 flux (at time interval n to n+1) is the first order exchange rate times depth times the in situ N_2 concentration minus the equilibrium N_2 concentration (see section 2.4.1.2).

and...

- $\text{Equilibrium } N_2 = f(\text{water temperature, partial pressure } N_2 \text{ in atm})$

Gas specific equilibrium concentrations (units of μM or $mmol.m^{-3}$) were calculated according to the Weiss model (Weiss, 1970) and the equations can be found in Appendix C. These equilibrium gas concentrations were corrected for partial pressures of atmospheric gasses according to equation 2.5 (see section 2.5.3.1).

- $\text{Partial pressure } N_2 = f(rH, \text{atmospheric pressure, air temperature})$

so...

- Model Output = denitrification

- Solve by modifying the denitrification rate such that model predicted *in situ* N_2 matches the measured *in situ* N_2

2.5.3.1 Pressure and Relative Humidity Correction

The corrected equilibrium gas concentrations were calculated by multiplying the equilibrium gas concentrations (based on water temperature) by equation 2.5.

$$= (A/101.3) \times \underbrace{[0.7584882 \times (B/100) + 0.7584882]}_{\text{partial pressure term}} / 0.7584882$$

Equation 2.5

A = measured atmospheric pressure (units in kPa)

Atmospheric pressure was recorded at discrete time steps with the use of a climate data logger or climate data acquired from Environment Canada. The value 0.7584882 is the nitrogen specific partial pressure (units in atm) given 1 atm, at a temperature of 0°C, and assuming 100% relative humidity.

$$B = (0.6292 \times 2.718282^{0.0636 AT}) - (RH/100 \times 0.6292 \times 2.718282^{0.0636 AT})$$

The B term is incorporated into the partial pressure term to correct for the assumption of 100% relative humidity. The first half of equation B ($0.6292 \times 2.718282^{0.0636 AT}$) calculates the partial pressure of water vapour as a function of air temperature (AT, in °C) at 100% relative humidity. The second half of equation B ($RH/100 \times 0.6292 \times 2.718282^{0.0636 AT}$) calculates partial pressure as a function of air temperature and relative humidity. At 0°C, relative humidity is 0%, $B/100 = 0$ and the partial pressure term is equal to 1. At temperatures > 0°C, relative humidity is > 0%, $B/100 \neq 0$ and the partial pressure term is < 1. Air temperature was recorded at discrete time steps with the use of a climate data logger or climate data acquired from Environment Canada.

RH = measured relative humidity (units of %)

Relative humidity was recorded at discrete time steps with the use of a climate data logger or climate data acquired from Environment Canada. Relative humidity was measured as a function of height (units in cm) above the water surface. The relative humidity data logger was

placed at 0, 10, 20, 50, and 80 cm above the surface of the water. The purpose was to determine if there was a relationship between relative humidity (units in %) and distance from the surface of the water. This was intended to test the assumption that relative humidity approached 100% near the water surface. This is important as many models of gas exchange assume 100% humidity at the air-water interface. As relative humidity affects partial pressure, this assumption affects modeled equilibrium gas concentrations in water. Further, wind velocity was measured to determine if it influenced the relationship between relative humidity and height above the water column. The data generated could be used ultimately to correct for relative humidity at the water surface as a function of relative humidity measured at a given height and wind velocity.

2.5.3.2 First-Order Gas Exchange Rates (K)

Figure 2.5. Relationship between wind velocity and piston velocity. (Laursen and Seitzinger, 2005).

First order exchange rates were calculated from piston velocities. Piston velocities (k_{600} , Schmidt number of 600) were calculated from an empirical relationship described by Laursen and Seitzinger (2005) (Figure 2.5). Gas specific first order exchange rates were calculated according to equation 2.6.

$$K_{N2} = \frac{(Sc_{N2}/600)^n}{K_{600}} / \text{depth} \quad \text{Equation 2.6}$$

[units of h^{-1}]

Where ...

$K_{600} = k_{600}/\text{depth (cm)}$

n = Schmidt number coefficient (allowed to vary from $-1/2$ to $-2/3$)

The Schmidt number is the ratio of momentum diffusivity to mass diffusivity and is a function of water temperature. Schmidt numbers for specific gases were calculated according to equations in Appendix D.

2.5.4 Sources of Error and Analysis

Monte Carlo Analysis (MCA) is a method based on computational algorithms that estimates a range of outcomes, given a set of variable conditions, to capture parameter variability (Miller, 2006). MCA is frequently used to quantify variability and uncertainty by using probability distributions of defined variables to generate a range of possible output distributions (Miller, 2006). The number of iterations and possible output estimations is defined by the user. The switch to MatLab allowed for a change from a deterministic model to a stochastic model with greater complexity in the computational algorithms required to run the MCA simulations.

The necessity for the use of MCA is its function as a tool to better define uncertainty and therefore aid in constraining error (Miller, 2006; Baulch et al., In Press).

Although past research has attempted to constrain the error in reported nutrient cycling rates, the methods used have differed greatly ranging from the assumption that the error was additive to assuming the error was analytical in nature (Baulch et al., In Press). In an encouraging shift, MCA has been used to constrain error (e.g. McCutchan et al., 2003) but the method is only as good as the number of variables considered that are sources of error (Baulch et al., In Press). Method uncertainty results from errors in measured variables and derived variables that are not understood or accounted for (Baulch et al., In Press). Error associated with air temperature, water temperature, atmospheric pressure, and wind velocity due to instrument precision and frequency of measurements was accounted for in the model by entering these variables as distributions of the recorded field values. The variable distributions were then treated with MCA to produce a range of estimates based on the variable conditions. Error due to uncertainty in the Schmidt number coefficient was also accounted for in the model by varying the Schmidt number coefficient from $-\frac{1}{2}$ to $-\frac{2}{3}$ as a variable distribution. Possible sources of error for this study that were not accounted for include: (1) Measurement error of dissolved gas concentrations due to limits of instrument precision; (2) Measurement error of depth; and (3) Uncertainty in K, based on piston velocity (Figure 2.5).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Christie Lake

3.1.1 Climate Data

For June 21-22, 2008, the mean air temperature was 18.3°C, the mean relative humidity was 77.6%, the mean atmospheric pressure was 98.5kPa, the mean water temperature was 18.9°C, and the mean wind speed was 3.0m/s (figure 3.1). For June 21- July 25, 2008, the mean air temperature was 19.7°C, the mean relative humidity was 74.6%, the mean atmospheric pressure was 98.5kPa, the mean water temperature was 19.9°C, and the mean wind speed was 3.1m/s (figure 3.2). For July 24-25, 2008, the mean air temperature was 20.1°C, the mean relative humidity was 70.0%, the mean atmospheric pressure was 98.8kPa, the mean water temperature was 21.0°C, and the mean wind speed was 3.3m/s (figure 3.3). For October 3-4, 2008, the mean air temperature was 7.4°C, the mean relative humidity was 78.2%, the mean atmospheric pressure was 99.0kPa, the mean water temperature was 20.9°C, and the mean wind speed was 2.2m/s (figure 3.4). Although the period from June 21-July 25, 2008 was not continuously sampled, it was included for the two time point modeling approaches, while the short-term climate data sets were (figures 3.1 and 3.3) for steady-state modeling approaches.

Figure 3.1 Christie Lake climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for June 21-22, 2008

Figure 3.2 Christie Lake climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for June 21-July 25, 2008

Figure 3.3 Christie Lake climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for July 24-25, 2008

Figure 3.4 Christie Lake climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for October 3-4, 2008

3.1.2 Water Column Gas Concentrations

For all sampling periods during the months of June, July and October, there appears to be a body of warmer water at a depth of ~1.5m to 2.0m below the surface of the water (Figures 3.5). This lens of warmer water was most likely due to incoming water flowing from the stream as there was no thermocline and little evidence of stratification. This was an unexpected result as the study site was chosen based on consultation to mimic lake conditions. Instead, since it was a wet year, the study site mimicked a poorly mixed river. This increased the difficulties encountered in trying to model this system.

Figure 3.5 Christie Lake dissolved N_2 concentrations for June, July and October, 2008

Dissolved N_2 concentrations were greatest during all months at the bottom of the depth profile, decreasing in the lens of warmer water, then increasing nearer the surface of the water, as anticipated based on water temperature (Figure 3.5). N_2 concentrations were also greater during June and October than for the month of July (Figure 3.5). The data supports the conclusion that dissolved N_2 concentrations were related to temperature and closely tracked equilibrium concentrations. In all months, there was a net excess of N_2 (greater total dissolved N_2 than anticipated based on temperature) which can be anticipated in a system that is denitrifying.

O_2 concentrations were greatest during October, followed by June then July (Figure 3.6). For all three months, O_2 concentration was below saturation, indicative of net respiration in the system. Sources of dissolved oxygen in the aquatic environment include the atmosphere and photosynthesis, which is influenced by plant and algal biomass, light intensity and water temperature. Loss of dissolved oxygen is due to respiration, decay by aerobic bacteria and decomposition of dead and decaying matter in sediments (Atobatele and Ugwumba, 2008). Nutrient enrichment stimulates plant and algal growth and often results in a mass influx of particulate organic matter to the sediments. The decomposition of this labile organic matter by aerobic microorganisms leads to a rapid acceleration of oxygen consumption and increased rates of N remineralization (Cornwell et al., 1999; Atobatele and Ugwumba, 2008).

Figure 3.6 Christie Lake dissolved O_2 concentrations for June, July and October, 2008

N_2O concentrations were recorded for the months of June and July with higher concentrations in July (Figure 3.7). For both months, the net measured concentrations were well above calculated equilibrium concentrations. In June the dissolved N_2O concentration was greatest near the sediment and decreased toward the surface. This is suggestive of N_2O being derived primarily from denitrification in sediments. In July concentrations reached maxima near

the surface and at a depth of 2.5m. This suggests a substantial portion of N₂O in July may have been derived from nitrification in the water column.

Figure 3.7 Christie Lake dissolved N₂O concentrations for June, July and October, 2008

3.1.3 Model Output for Gas Rates – Multiple Strata Approach, 2 time

Points (June – July)

The following values were obtained with the model approach that employed multiple strata at two time points for the time period from June 21, 2008 to July 25, 2008: $4,415 \pm 996 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$ for N₂ gas rate production, $-10,890 \pm 4805 \mu\text{mol O}_2.\text{m}^{-2}.\text{h}^{-1}$ for O₂ gas rate production, representing the consumption of O₂, and $10,635 \pm 1969 \text{ nmol N.m}^{-2}.\text{h}^{-1}$ for N₂O gas rate production (Figure 3.8).

Figure 3.8. Christie Lake modeled gas production rate for June 21-July 25, 2008
- multiple strata approach, 2 time points

3.1.4 Model Output for Gas Rates – Single Parcel Approach, 2 time points

The following values were obtained with the model approach that employed the single parcel approach at two time points for the time period from June 21, 2008 to July 25, 2008: - $419.4 \pm 11.2 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$ for N₂ gas rate production, $-5,347.6 \pm 46.8 \mu\text{mol O}_2.\text{m}^{-2}.\text{h}^{-1}$ for O₂ gas rate production, and $957.2 \pm 10.4 \text{ nmol N.m}^{-2}.\text{h}^{-1}$ for N₂O gas rate production (Figure 3.9).

**Figure 3.9. Christie Lake modeled gas production rate for June 21-July 25, 2008
- single parcel approach, 2 time points**

3.1.5 Model Output for Gas Rates – Steady State Approach, Multiple Strata

The values obtained with the steady state model approach that employed multiple strata were for the time period from June 21-22, 2008, July 24-25, 2008, and October 3-4, 2008 (Figure 3.10). For nitrogen flux the values were $3,017 \pm 139 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$ in June, and $3,589 \pm 146 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$ for July, and $4,298 \pm 175 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$ for October. For oxygen flux the values were $-2,993 \pm 95 \mu\text{mol O}_2.\text{m}^{-2}.\text{h}^{-1}$ in June, $-15,114 \pm 440 \mu\text{mol O}_2.\text{m}^{-2}.\text{h}^{-1}$ in July, and -

$12,964 \pm 723 \mu\text{mol O}_2\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ for October. For nitrous oxide flux the values were $2,567 \pm 54 \text{ nmol N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ in June, and $1,194 \pm 31 \text{ nmol N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ in July.

Figure 3.10. Christie Lake modeled gas production rate for June, July and October 2008 - steady state approach, multiple strata

3.1.6 Model Output for Gas Rates – Steady State Approach, Single Parcel

The values obtained with the steady state model approach that employed the single parcel were for the time period from June 21-22, 2008, July 24-25, 2008, and October 3-4, 2008. For nitrogen flux the values were $649.4 \pm 11.5 \mu\text{mol N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ in June, and $896.7 \pm 10.0 \mu\text{mol N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ in July (Figure 3.11). For oxygen flux the values were $-3,442.9 \pm 27.0 \mu\text{mol O}_2\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ in

June, $1,979.5 \pm 16.3 \mu\text{mol O}_2.\text{m}^{-2}.\text{h}^{-1}$ in July, and $-2,198.1 \pm 20.0 \mu\text{mol O}_2.\text{m}^{-2}.\text{h}^{-1}$ for October (Figure 3.12). For nitrous oxide flux the values were $915.4 \pm 6.5 \text{ nmol N}.\text{m}^{-2}.\text{h}^{-1}$ in June, and $957.2 \pm 10.4 \text{ nmol N}.\text{m}^{-2}.\text{h}^{-1}$ in July (Figure 3.13).

Figure 3.11. Christie Lake modeled nitrogen production rates for June, July, and October, 2008
- steady state approach, single parcel

**Figure 3.12. Christie Lake modeled oxygen production rates for June, July, and October, 2008
- steady state approach, single parcel**

3.1.7 Benthic Chamber Gas Rates for N₂, O₂, and N₂O

Figure 3.14. Christie Lake benthic chamber gas flux for June, July and October, 2008

Benthic chamber gas flux rates for Christie Lake were obtained for June, July and October of 2008 (Figure 3.14). Nitrogen flux values were $57 \pm 32 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$ in June, $58 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$ in July (based on one chamber), and $199 \pm 98 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$ for October. For oxygen flux, the values were $-745 \pm 146 \mu\text{mol O}_2.\text{m}^{-2}.\text{h}^{-1}$ in June, $-1,005 \pm 94 \mu\text{mol O}_2.\text{m}^{-2}.\text{h}^{-1}$ in July, and $-1,746 \pm 824 \mu\text{mol O}_2.\text{m}^{-2}.\text{h}^{-1}$ for October. For nitrous oxide flux the values were $11 \pm 3 \text{ nmol N.m}^{-2}.\text{h}^{-1}$ June, and $57 \pm 5 \text{ nmol N.m}^{-2}.\text{h}^{-1}$ in July (based on one chamber).

3.1.8 Nutrient Data

Figure 3.15. Christie Lake depth profiles and benthic flux of nitrate for June, July and October, 2008

Nitrate concentrations decreased from the months of June to July to October (Figure 3.15) which is consistent with nitrogen loss, either by incorporation, or as suggested by the measured and modeled N₂ production rates, with high rates of denitrification. On net, sediments were a source of nitrate in June. Net consumption of nitrate by sediments was seen in July and October. It is worth noting that nitrate flux was 2-3 orders of magnitude lower than N₂ flux, indicating that

denitrification and nitrification were in near balance in Christie Lake sediments throughout this study.

Figure 3.16. Christie Lake depth profiles and benthic flux of ammonia for June, July and October, 2008

Ammonia water column concentrations also decreased from the months of June to July to October (Figure 3.16). Sediments were generally a source of ammonium, likely due to remineralization of deposited organic matter, and helps explain the generally higher concentrations of ammonium near the sediment. However, as indicated by both nitrate and ammonium loss over time, and high measured and modeled rates of denitrification, the sediments were overall a sink for N in this system.

Phosphate water column concentrations followed the same trend, decreasing from June to July to October (Figure 3.17). Benthic chamber measurements showed that sediments were a sink for phosphate throughout the study. The month of July saw the greatest variation within water column measurements, with highest concentrations associated with the warm water lens.

This would be consistent with a riverine source of phosphate enriched, warm water flowing through the system at mid-depth.

Figure 3.17 Christie Lake depth profiles and benthic flux of phosphate for June, July and October, 2008

The ratio of dissolved inorganic nitrogen to phosphorus increased from June to October (Figure 3.18). In June and July, the ratio was below the Redfield ratio of N:P = 15:1 (Figure 3.18). Under these conditions nitrogen is more likely to limit primary production than phosphorus. By October, however, N:P is much greater than 15:1, suggesting a switch to phosphorus limitation. Although the study site was found to have elevated rates of denitrification, ultimately it is most likely the effect of sediments on sequestering P that limits denitrification. The sediments act as a sink for P, thus limiting primary production. As P becomes increasingly limiting with the progression of the season, less organic material from algal biomass is produced resulting in less organic nitrogen deposition to sediments, less remineralization, nitrification, and finally a decrease in denitrification of this N. N:P has been found to be high in oligotrophic lakes and very low in eutrophic lakes, declining with increasing phosphorus concentrations (Downing and McCauley, 1992). The observed N:P also reflects the source of nutrients in that high ratios (oligotrophic lakes) are due to natural sources of N and P,

while low ratios (mesotrophic and eutrophic lakes) are due to anthropogenic inputs of N and P (Downing and McCauley, 1992).

Figure 3.18. Christie Lake depth profile of DIN:DIP for June, July and October 2008

Table 3.1 is a summary of the three approaches used to estimate denitrification rates in Christie Lake. The nitrogen balance approach (based on expected N remineralized given the modeled oxygen consumption rate) (represented in yellow) was closer in value to the benthic chamber (represented in blue) approach than were estimates from the N_2 modeling approach (represented in green). All of the N_2 model-based approaches were substantially higher than the chamber-based approach, with the exception of the single-parcel approach integrated over the June-July interval.

Table 3.1 Summary of three approaches used to estimate denitrification for Christie Lake (chambers, model, and nitrogen balance)

	June-July ($\mu\text{mol N or P/m}^2/\text{h}$)		June ($\mu\text{mol N or P/m}^2/\text{h}$)		July ($\mu\text{mol N or P/m}^2/\text{h}$)		October ($\mu\text{mol N or P/m}^2/\text{h}$)	
Δ Nitrate	-1.16							
Δ Ammonia	-16.25							
Δ Total DIN	-17.41							
Δ Phosphate	0.73							
Δ DIN:DIP	-23.65							
	June-July ($\mu\text{mol N or O}_2/\text{m}^2/\text{h}^{-1}$)		June ($\mu\text{mol N or O}_2/\text{m}^2/\text{h}^{-1}$)		July ($\mu\text{mol N or O}_2/\text{m}^2/\text{h}^{-1}$)		October ($\mu\text{mol N or O}_2/\text{m}^2/\text{h}^{-1}$)	
	multiple strata	single parcel	Steady state, multiple strata (average)	Steady state, single parcel (average)	Steady state, multiple strata (average)	Steady state, single parcel (average)	Steady state, multiple strata (average)	Steady state, single parcel (average)
Oxygen consumption	10890.84	5347.64	2993.00	3442.90	15114.00	-1979.50	12964.00	2198.10
O ₂ loss due to respiration	9540.74	4684.71	2621.97	3016.09	13240.36	-1734.11	11356.89	1925.61
Predicted N remineralization	1350.10	662.93	371.03	426.81	1873.64	-245.39	1607.11	272.49
Δ Total DIN	17.41	17.41						
Estimated nitrogen loss	1367.51	680.34	371.03	426.81	1873.64	-245.39	1607.11	272.49
Modeled denitrification	4415.00	-419.40	3016.83	649.40	3589.36	896.70	4298.92	685.20
Chamber denitrification	57.5 (average)		57		58		199	
Chamber oxygen	875 (average)		745		1005		1746	

A possible reason the N₂ based approaches yielded estimates much higher than the chambers could be due to there not being enough spatial resolution in sampling close to the water surface. Sampling was performed at every 0.5m, but if the top parcel was not well-mixed, then the dissolved N₂ concentration at the surface, where gases are exchanged with the atmosphere, may have been much closer to equilibrium concentrations. As a result, estimates of N₂ flux, based on $K(\text{N}_2 \text{ measured} - \text{N}_2 \text{ equilibrium})$, and consequently denitrification, would have been overestimated. If, with greater spatial resolution in the top parcel, measured gas concentrations near the surface were closer to the equilibrium concentrations, the modeled value for N₂ flux would decrease. As an example, on June 21-22, the equilibrium N₂ concentration for the top parcel (based on temperature) was 535.95 μM and the measured N₂ concentration was 556.43 μM . In a simple sensitivity analysis, the measured N₂ concentration was changed to approach the

equilibrium concentration with the following initial N_2 concentrations used for the top parcel in model runs: 550, 545, 540, and 535 μM . These new initial inputs to the model resulted in denitrification rates of 2839.5, 2288.6, 1775.4, and 1687.8 $\mu mol\ N.m^{-2}.h^{-1}$. If final measured N_2 concentrations for July 24-25 were similarly allowed to approach equilibrium, model estimates of denitrification would further decrease.

Another possible reason N_2 -based approaches yielded higher estimates is that benthic chambers could severely underestimate denitrification rates. This could result from several causes. First, there is the inherent uncertainty in extrapolation from a small area (the surface area of the chamber) to the ecosystem scale. The occurrence of hot spots and hot times has been introduced as a limitation in extrapolating chamber measurements to whole ecosystems. Similarly, chambers could be deployed in "cold spots" that would underestimate denitrification when rates are extrapolated to whole systems. Second, disturbance of the sediment layer and disruption of redox boundaries upon deployment of the chamber are givens. The installation of a chamber, however delicately performed, will resuspend sediment. This is particularly true with fine grained sediments. While particle size was not measured, Christie Lake has very fine, silty sediments. The disruption of redox gradients can reduce processes such as denitrification that require anoxic conditions. The third, and perhaps most likely reason chamber approaches might underestimate denitrification comes from the entrainment of air bubbles. N_2 partitions more rapidly into these bubbles than Ar. This would reduce the rate at which $N_2:Ar$ increases within the water, leading to underestimation of denitrification. The problem with chambers is that it is very hard to get rid of bubbles and even the smallest of bubbles which may be difficult to see can influence $N_2:Ar$ -based measurements.

As for the nitrogen balance approach, there are two possible explanations for its proximity to the denitrification rate estimates produced with the benthic chamber approach. The first is that O_2 is a much easier gas to accurately measure changes over time than N_2 , due to the stronger disequilibrium of O_2 than N_2 (Figure 3.5 versus Figure 3.6). We are typically measuring larger changes in O_2 compared with N_2 , so the level of precision needed is much lower. Measurements that require lower precision, or where concentration changes through time are large relative to background concentrations (such as for N_2O and CH_4) are less sensitive to gas partitioning into air bubbles. Therefore, a more robust estimation of O_2 consumption can be made compared to denitrification. However, more accurate estimations of denitrification rates based on the stoichiometric approach could be made if the elemental ratio of phytoplankton was measured versus assumed. Freshwater phytoplankton have been shown to be less constrained in their elemental ratios than marine phytoplankton, which maintain the Redfield ratio more consistently (e.g. Hecky et al., 1993), hence there is more potential for error when applying stoichiometric techniques in freshwaters (Groffman et al., 2006).

Another observation based on comparison of approaches is that modeling denitrification by treating the water column as a single parcel, rather than modeling each depth stratum, resulted in closer agreement to benthic chamber measurements (Figure 3.19). The reason for this is that most of the water column is relatively close to equilibrium. The depth stratum representing the greatest disequilibrium is the surface stratum where the atmospheric exchange occurs. Treating the entire water column as a single parcel uses mean measured N_2 and mean equilibrium N_2 integrated over the water column. As most depth strata are near equilibrium, this approach reduces the driving influence of the surface layer in stratum by stratum modeling.

Seasonal trends

The physiochemical properties of aquatic ecosystems change in composition and concentration on a seasonal, diurnal or even hourly basis (Atobatele and Ugwumba, 2008). The spring thaw signals a transitional period for ice-covered water bodies. Physical forcings change dramatically between ice cover and open water, creating large and rapid changes in lake biology and geochemistry (Baehr and DeGrandpre, 2004). As the ice and snow cover diminishes, light penetration stimulates both phytoplankton growth and convective mixing (Baehr and DeGrandpre, 2004). Surface water temperatures continue to warm and causing the surface water to sink, due to a change in water density, or to be mixed by winds to greater depths, which results in an isothermal water column (Baehr and DeGrandpre, 2004). With further solar heating, the water column becomes thermally stratified, isolating the bottom water from the atmosphere until turnover occurs again, usually in the fall (Baehr and DeGrandpre, 2004).

Lakes have demonstrated large seasonal variability in denitrification rates with the highest levels occurring during late spring to early summer (Pinã-Ochoa and Álvarez-Cobelas, 2006). This seasonal summer maximum may be a result of increased nitrate availability after a springtime peak (Kana et al. 1998; David et al., 2006), increased temperatures due to summer climate (David et al., 2006; Atobatele and Ugwumba, 2008; Groffman, 2009a; Sobota et al., 2009), and reduced oxygen concentrations (Pinã-Ochoa and Álvarez-Cobelas, 2006). For many systems, the dissolved O_2 concentration decreases with warming water temperatures (Groffman, 2009a; Wang et al., 2009). With a shift in redox potential in the sediments due to decreasing oxygen concentrations, there is an increase in the flux of NH_4^+ and PO_4^{3-} into the overlying water (Rabalais, 2002). Nitrification rates have been shown to increase with increasing temperatures

which results in a summer maxima for NH_4 concentrations in the water column (Kemp et al., 1990).

General seasonal trends were observed for denitrification estimates obtained with the benthic chamber approach, the steady state – multiple strata model approach, and the steady state – single parcel model approach (Figure 3.19). Although the general trend in most temperate aquatic systems is for denitrification rates to be greatest during late spring to early summer (e.g. David et al., 2006) the greatest denitrification rates for Christie Lake were observed in mid-summer to fall. The reason for this could, in part, be due to the unseasonably cool spring and early summer as well as relatively high early fall water temperatures. It is also worth noting that water column nitrate concentrations were low throughout the study period (Figure 3.15). Denitrification rates might have been higher earlier in the spring, before measurements were performed, if the spring flush resulted in elevated nitrate concentrations.

Figure 3.19 Summary of three approaches used to estimate denitrification in Christie Lake (2008)

3.2 Turtle Pond

3.2.1 Climate Data

For November 2-11, 2007, the mean air temperature was 5.4°C, the mean relative humidity was 76.7%, the mean atmospheric pressure was 101.8kPa, the mean water temperature was 8.9°C, and the mean wind speed was 0m/s (figure 3.20). For November 11-18, 2007, the mean air temperature was 6.1°C, the mean relative humidity was 74.6%, the mean atmospheric pressure was 101.6kPa, the mean water temperature was 6.9°C, and the mean wind speed was 0m/s (figure 3.21). For November 18-28, 2007, the mean air temperature was 2.0°C, the mean relative humidity was 78.5%, the mean atmospheric pressure was 102.3kPa, the mean water temperature was 3.6°C, and the mean wind speed was 0.2m/s (figure 3.22). For January 10-26, 2008, the mean air temperature was 2.4°C, the mean relative humidity was 73.2%, the mean atmospheric pressure was 101.4kPa, the mean water temperature was 3.9°C, and the mean wind speed was 0.3m/s (figure 3.23). During periods of ice cover, only the water temperature was recorded. The mean water temperature was 3.5°C for November 28-January 10, 2008, 1.2°C for January 26-February 3, 2008, 1.5°C for February 3-16, 2008, 1.3°C for February 16-23, 2008, and 1.3°C for February 23-March 2, 2008 (figure 3.24). For August 11-18, 2008, the mean air temperature was 23.4°C, the mean relative humidity was 70.5%, the mean atmospheric pressure was 101.4kPa, the mean water temperature was 22.0°C, and the mean wind speed was 0.0m/s (figure 3.25). For August 18-28, 2008, the mean air temperature was 20.6°C, the mean relative humidity was 75.5%, the mean atmospheric pressure was 101.9kPa, the mean water temperature was 23.3°C, and the mean wind speed was 0.0m/s (figure 3.26). For August 28-September 04, 2008, the mean air temperature was 22.3°C, the mean relative humidity was 73.9%, the mean atmospheric pressure was 101.7kPa, the mean water temperature was 22.3°C, and the mean wind

speed was 0.0m/s (figure 3.27). For September 04-12, 2008, the mean air temperature was 16.5°C, the mean relative humidity was 80.7%, the mean atmospheric pressure was 98.9kPa, the mean water temperature was 21.4°C, and the mean wind speed was 3.1m/s (figure 3.28). For September 12-20, 2008, the mean air temperature was 16.6°C, the mean relative humidity was 82.4%, the mean atmospheric pressure was 98.9kPa, the mean water temperature was 22.7°C, and the mean wind speed was 3.1m/s (figure 3.29). For September 20-28, 2008, the mean air temperature was 15.9°C, the mean relative humidity was 79.9%, the mean atmospheric pressure was 99.7kPa, the mean water temperature was 21.8°C, and the mean wind speed was 3.1m/s (figure 3.30).

Figure 3.20. Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for November 02-11, 2007.

Figure 3.21. Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for November 11-18, 2007.

Figure 3.22. Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for November 18-28, 2007.

Figure 3.23. Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for January 10-26, 2008.

Figure 3.24. Turtle Pond water temperature histograms for ice-covered period.

Figure 3.25. Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for August 11-18, 2008.

Figure 3.26. Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for August 18-28, 2008.

Figure 3.27. Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for August 28-September 04, 2008.

Figure 3.28. Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for September 04-12, 2008.

Figure 3.29. Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for September 12-20, 2008.

Figure 3.30. Turtle Pond climate histograms of air temperature, relative humidity, atmospheric pressure, water temperature, and wind speed for September 20-28, 2008.

3.2.2 Assumptions of Relative Humidity

Previous open system models (Cole et al., 1994; Cole and Caraco, 2001; Laursen and Seitzinger, 2002a, McCutchan et al., 2003) have made assumptions about the water saturation level of the air above the surface of the water. The assumption is that relative humidity increases to 100% saturation the closer one gets to surface of the water (or the air-water interface). Therefore the exchange of gasses across the air-water interface is assumed to be from water to air at 100% humidity. This is an important assumption as relative humidity affects partial pressures of gases in air, and therefore, equilibrium concentrations in water when gasses re-equilibrate between water and this moist air mass. The expected distribution of relative humidity, if the 100% saturation assumption is correct, is illustrated in Figure 3.31a. Relative humidity data generating patterns such as shown in Figure 3.31a can be log transformed (natural log) and fit to a regression, the slope of which is the first-order decay coefficient (K) describing the shape of the curve. The assumption that the air mass is at 100% relative humidity would be supported by an observation that this slope (or K) is negative and non-zero wherever the relative humidity of the air mass measured well above the water column is less than 100%. Further, if shape of this curve (and, therefore, K) could be expected to relate to wind velocity. A high wind velocity would tend to favour a well-mixed air mass above the water with a sharp boundary where relative humidity increases sharply to 100% saturation. In contrast, a low wind velocity would be expected to favour a poorly mixed air mass above the water column and a more gradual transition to 100% relative humidity at the air-water interface (Figure 3.31b).

Relative humidity measurements were conducted at fixed points above the water surface for two purposes, 1) to determine if the assumption that $rH\%$ is 100% at the air-water interface is

valid. If it is, water would always equilibrate with an air mass at 100% saturation, and there would be no need to include rH% as a model input. 2) To determine if rH% can be estimated at the air water interface as a function of wind velocity if the assumption of rH% = 100 at the air-water interface is not supported. Results of rH% measurements taken under a wide range of bulk air rH% and wind velocity conditions revealed two important patterns. The first is that K was not related to wind velocity (Figure 3.32). Therefore, rH% at the air-water interface cannot be estimated at the air-water interface based on a bulk air rH% and wind velocity. Second and more importantly, there is little support for K values being negative and significantly different than zero. This suggests that rH% measured in bulk air, such as by placing an rH% probe 1 meter above the water surface, provides a reasonable approximation of rH% at the air-water interface. This rH% measurement is an appropriate model input as the assumption that rH% is 100% at the air-water interface was not supported.

Figure 3.31. Expected distribution of relative humidity.

Figure 3.32. Actual distribution of relative humidity relative to wind speed for Turtle Pond.

3.2.3 Model Output for Gas Rates (N₂, O₂, and N₂O)

In the late autumn, 2007, Turtle Pond alternated between net N₂O production and N₂O consumption, with rates ranging between -344 nmol N.m⁻².h⁻¹ during Nov.02-11 and 131 nmol.N m⁻².h⁻¹ during Nov. 11-18 (Appendix H). During most measurement periods, Turtle Pond was a net N₂O producer, with production spiking during the late summer – early autumn, 2008 (Figure 3.33a).

Nitrogen and oxygen gas production were only measured during the late summer – Early autumn, 2008 (Appendix H). N₂ production during this period was between 10 and 74 μmol N.m⁻².h⁻¹ (Figure 3.33b). Oxygen production was variable with net consumption during the period August 18 – 28 (-3021 μmol O₂.m⁻².h⁻¹) and net production during the other sampling intervals (2045 to 3076 μmol O₂.m⁻².h⁻¹) (Figure 3.33c).

Figure 3.33 Modeled gas production for Turtle Pond, winter 2007- fall 2008.

3.2.4 Nutrient Data

Nitrate production was greatest during the summer period from August 11-September 28, 2008 and the lowest during the early winter period of November, 2007 (Figure 3.34a). Ammonium production was greatest in early winter of 2007 and late summer of 2008 with the greatest decline during winter of 2007 (Figure 3.34b). Phosphate production was greatest during the winter of 2007/2008, especially after the period of the first ice-thaw, and the greatest decline was seen at the start of winter in November, 2007 (Figure 3.34c). Generally, sediments were a strong sink for nutrients in late autumn, while tending to be a source (at least for nitrate) in summer and early autumn (Table 3.2).

N:P was greatest September 04-12, 2008 at 164.8 and the least November 28-January 10, 2008 at 20.62 which represents a significant amount of total nitrogen (NO_3 and NH_4) in relation to phosphorus (PO_4). Winter of 2007/8 saw the smallest values which represents an increase in the amount of phosphorus relative to total nitrogen. N:P was above the Redfield ration of 15:1 on all dates (Table 3.2). Generally, nutrient ratios suggest that this system is phosphorus limited during much of the year. Based on this, phosphorus availability is likely to limit the production of organic matter, deposition of this organic matter to sediments, and processing of organic N (including denitrification) in sediments.

Figure 3.34. Summary nutrient production rates for Turtle Pond, 2007-2008.

Table 3.2. Summary nutrient production rates for Turtle Pond

Date	NO ₃ (μmol.m ⁻² .d ⁻¹)	NH ₄ (μmol.m ⁻² .d ⁻¹)	PO ₄ (μmol.m ⁻² .d ⁻¹)	DIN:DIP
Nov 02-11, 2007	51.70	68.93	40.64	29.08
Nov 11-8	-39.03	-24.07	0.41	42.38
Nov 18-28	-2.99	43.27	5.02	22.15
Nov 28-Jan 10	1.28	-1.63	-0.47	20.62
Jan10 - 26, 2008	9.26	6.45	-0.13	30.22
Jan 26-Feb 03	-15.88	-19.21	-4.25	66.54
Feb 03- 16	-3.09	18.60	0.94	74.81
Feb 16-23	18.45	16.99	-0.43	58.96
Feb 23-Mar 02	-24.35	-66.54	-0.15	51.77
Aug 11-18	10.42	-7.63	0.65	
Aug 18-28		8.01	-0.83	
Aug 28-Sep 04		33.19	1.21	
Sep 04-12	98.75	-21.34	0.27	164.80
Sep 12-20		20.11	1.30	
Sep 20-28	178.75	-33.07	-2.70	74.18

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

4.1 Summary and Conclusions

This study had four objectives the first of which was the modification of an existing whole-system model (Laursen and Seitzinger, 2002a) for application in lentic systems. This was achieved by utilizing a MatLab environment to integrate new model inputs (air temperature, atmospheric pressure, relative humidity, wind velocity). The second objective was field validation of the modified whole-system model by comparing results obtained with denitrification measured in benthic chambers. Field validation was carried out at Christie Lake in Dundas, ON and Turtle Pond in Stoney Creek, ON (Canada). The third objective was the development of an annual budget of N_2O production within a single aquatic system, Turtle Pond. The fourth objective was the development and use of a Monte Carlo sampling algorithm within the model to better constrain error in model estimates of denitrification, whole-system metabolism, and greenhouse gas production.

Three approaches were used to estimate denitrification rates in Christie Lake; the nitrogen balance approach, the benthic chamber approach and the N_2 model-based approach. The nitrogen balance approach ($20\text{--}1,747 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$) was closest in value to the chamber approach ($57\text{--}199 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$) (Table 3.1). All of the N_2 model-based approaches ($-419\text{--}4,415 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$) were substantially higher than the chamber-based approach, with the exception of the single-parcel approach integrated over the June-July interval ($-419 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$) (Table 3.1).

General seasonal trends were observed for denitrification estimates; highest rates for Christie Lake were observed in mid-summer to fall (Figure 3.19). N_2O flux rates were calculated to be $11\text{-}57\text{ nmol N.m}^{-2}.\text{h}^{-1}$ for benthic chambers and $915\text{-}10,635\text{ nmol N.m}^{-2}.\text{h}^{-1}$ for the model-based approaches in Christie Lake. Nutrient ratios suggested that Turtle Pond is phosphorus limited during much of the year as is expected for freshwater systems. Denitrification rates determined by the N_2 model-based approach ranged between $10\text{-}74\text{ }\mu\text{mol N.m}^{-2}.\text{h}^{-1}$ in Turtle Pond. N_2O flux rates were calculated to be $-344\text{-}131\text{ nmol N.m}^{-2}.\text{h}^{-1}$ in Turtle Pond.

Numerous methods have been used to measure denitrification rates which have made comparison of the many measurements difficult. In addition, the physical and chemical characteristics that might affect nitrogen removal are unique to each site (Seitzinger, 2008). However, a general comparison will be made for to determine if the results obtained with this study are comparable to other sites. Seitzinger reported denitrification rates in lake sediments of $2\text{-}171\text{ }\mu\text{mol N.m}^{-2}.\text{h}^{-1}$ with rates obtained mainly by acetylene block and nitrate loss (Seitzinger, 1988). Molot and Dillon (1993) used a N:P mass balance approach to estimate denitrification rates of $777\text{ mg N.m}^{-2}.\text{y}^{-1}$ (or $6.3\text{ }\mu\text{mol N.m}^{-2}.\text{h}^{-1}$) in Dickie lake to $1210\text{ mg N.m}^{-2}.\text{y}^{-1}$ (or $9.9\text{ }\mu\text{mol N.m}^{-2}.\text{h}^{-1}$) in Harp lake (Molot and Dillon, 1993). Tomaszek et al., (1997) reported *in vitro* denitrification rates of $40\text{-}135\text{ }\mu\text{mol N.m}^{-2}.\text{h}^{-1}$ in October 1993 and from $66\text{-}428\text{ }\mu\text{mol N.m}^{-2}.\text{h}^{-1}$ in May and July 1994 for sediments of Old Woman Creek, Huron, Ohio (a Lake Erie coastal wetland) using *in vitro* N_2 -flux method with intact cores. Saunders and Kalff (2001b) reported

extremely high denitrification rates using N_2 flux technique on intact sediment cores in the littoral zone (1-3m) of Lake Memphremagog, Quebec to be on average $111,000 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$, approximately 10x more than the average literature rates calculated for oligotrophic lakes (Saunders and Kalff, 2001b). Hasegawa and Okino (2004) reported denitrification rates for Lake Suwa, Japan ranging from $0-1800 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$ using the acetylene block method. They also reported a distinct seasonal trend of relatively high rates in spring and winter, and low rates in summer (Hasegawa and Okino, 2004). The denitrification rate estimates obtained in this study fall within range of literature values and suggest that small reservoirs and ponds can be important N sinks.

Mengis et al. (1997) sampled 15 lakes, reporting average N_2O fluxes, found that they did not contribute significantly to the atmospheric N_2O budget (Mengis et al., 1997). Liikanen et al. (2002) studied Lake Kevätön, Finland and also found N_2O fluxes to be negligible in their study as well as studies focusing on other Finnish lakes (Liikanen, 2002). In contrast, Huttunen et al. (2001) reported mean N_2O flux rates of $0.35 \text{ mg N.m}^{-2}.\text{h}^{-1}$ (or $25 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$) for Lake Ernest, Finland and $0.04 \text{ mg N.m}^{-2}.\text{h}^{-1}$ (or $2.8 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$) for Lake Lacawac, Finland (Huttunen et al., 2001). Huttunen et al. (2003a) reported mean N_2O flux rates of -2.1 to $8.2 \text{ mg N.m}^{-2}.\text{h}^{-1}$ (or -150 to $585 \mu\text{mol N.m}^{-2}.\text{h}^{-1}$) for freshwater lakes and reservoirs in Finland (Huttunen et al., 2003a). The N_2O flux rate estimates obtained with this study are within the range of literature values supporting previous research that states that freshwater lakes are potentially significant sources of N_2O (Huttunen et al., 2001; Huttunen et al., 2003a). However,

very few studies have been undertaken that recorded an annual N_2O budget which is of special interest because of the potential for ice-covered lakes to release a large amount of N_2O to the atmosphere after a spring thaw. Also, given that $\sim 0.3\%$ (4.6 million km^2) of the earth's continental surface is covered by water, with the majority being aquatic systems smaller than 1 km^2 , the total contribution by freshwater systems has been far underestimated.

4.2 Strengths and Limitations

The strengths of this study were the use of Monte Carlo analysis to better estimate rates and constrain error and therefore uncertainty in the rates determined and the use of multiple approaches to modeling to again better estimate rates. The use of MIMS which can detect minute changes in N_2 was a valuable analytical tool without which denitrification rates would be extremely hard to determine due to the high background concentration of N_2 in the aquatic environment. The proposed method is advantageous in that prohibitive factors such as cost, level of expertise, time consumption, and propagation of error due to scaling up found in other methods have been greatly reduced. The proposed method is also amendable to the simultaneous determination of other biogeochemical process (e.g. CH_4 and CO_2 production).

Limitations of this study included the dependency on benthic chambers for method validation which have inherent uncertainty in extrapolation from a small area to the ecosystem scale as well as the possible entrainment of air bubbles which can cause severe underestimation of denitrification. The ideal study site was a lake that had substantial depth and would reliably stratify. Christie Lake was suggested as an ideal study site and was thought to be $\sim 10\text{m}$ in depth. However, the study site was much shallower than estimated ($\sim 3.5\text{m}$ in depth). As a result, there

was little evidence of stratification and due to a year of high precipitation; the study site mimicked a poorly mixed river which increased the difficulties encountered in trying to model this system. Another limitation is the assumed elemental ratio of phytoplankton based on the Redfield ratio made with the stoichiometric approach based on oxygen consumption. Freshwater phytoplankton have been shown to be less constrained in their elemental ratios hence the potential for error (Hecky et al., 1993).

4.3 Relevance and Recommendations

A new tool was developed that allows for the examination of biogeochemical processes at ecologically relevant temporal and spatial scales and for cross-system comparison. This new method is better able to constrain error and nutrient budget estimations through the use of Monte Carlo analysis. It has also provided a means to improve on previous estimates by better modeling gas re-equilibration through inclusion of additional atmospheric data.

To further enhance this new method, sampling should occur in smaller increments near the surface of the water where the greatest disequilibrium occurs due to atmospheric exchange. This would provide better spatial resolution on the depth profiles. To reduce possible error in the stoichiometric approach, the elemental ratio of phytoplankton should be measured instead of assumed. Once again, great care must be taken when using benthic chambers to minimize the chance of trapping bubbles which would result in underestimation of denitrification rates. To further reduce the sources of error within the model and uncertainty in model estimates, the model input variables of dissolved gas concentrations, depth, and piston velocity should be entered as distributions within the model. With regards to study sites, although Christie Lake did

not provide the desired depth profile it was protected and subject to minimal disturbance (e.g. recreational and commercial boats) and there was a fair bit of knowledge of the study site (i.e. tributaries, flooding and draining times). For future studies, a similar system should be used with the requisite depth requirement.

4.4 Directions for Future Research

Future research should include greater frequency of sampling at both spatial and temporal scales within a single system for field validation. This should then be followed with further field validation in a variety of lakes using both the N_2 model-based approaches as well as the stoichiometric approach applied in this study. Additional greenhouse gases should be studied (i.e. CO_2 and CH_4) to increase data used to determine the importance of lakes as sources of GHGs. This new method could then be used in combination with other methods (e.g. mass balance) to determine physical, chemical, and biological drivers of biogeochemical processes at the whole-system scale.

APPENDIX A

INSTRUCTIONS FOR THE UV-VIS SPECTROPHOTOMETER

A. Creating a method:

1. Turn on the computer and log-in.
2. Double-click UV WinLab 2.85.04 software icon on the desktop OR select the software from the programs listed once you chose the **START** button.
3. In the menu bar, click on **Application** and select **Wave Prog...** to open the Wave program editor. Alternatively, select the **Wave Prog** icon in the toolbar. Enter the instrument parameters for each of the three pages within the Wave program editor as desired.

a) Wave Prog Page (Wavep):

In the **Wave Prog** method editor, click on the **Wavep.** tab to set the parameters that are relevant to the instrument and the method ie. Cycle time, number of cycles, number of wavelengths, autoprnt, autosave and method info.

Wavep. tab

- Set the **number of wavelengths** to 1
- Set the **wavelength** to desired wavelength in nm
- Set the **number of cycles** to 1
- Set the **cycle time** to 10s
- Set the **Autosave** to On
- Set the **Autoprnt** to On
- Type in reagent name analysis under **Method info**:

b) Instrument Page (Inst):

In the **Wave Prog** method editor, click on the **Inst.** tab to enter sample information and to enter or calculate a concentration factor i.e. dilution factor, number of samples, sample identities, etc.

Inst. tab

- Set the **ordinate mode** to A
- Set the **response** to 2s
- Set the **Lamp UV** to Off
- Set the **Lamp Vis** to On
- Set the **Slit** to 2.00nm

c) Sample Page (Sample):

In the **Wave Prog** method editor, click on the **Sample** tab to set the parameters that control measurements at the desired wavelength(s) and data output i.e. ordinate mode, lamp UV (on or off), Lamp Vis(on or off), Slit width, and Scan speed.

Sample tab

- Set the **Result Filename** to the first sample number or identity
- Set the **calculation factor** to Factor
- Set the **number of samples** to the desired number
- Provide sample information

4. Click once on the **X** at the top right-hand corner of the **Wave Prog** window to open a **Method SaveAs** window. Enter the desired file name in box labeled **Filename:** and click once on the **OK** icon.

B. Running a Sample:

1. Turn on spectrophotometer (Perkin Elmer Lambda 20) for a minimum of 15 minutes to warm-up. The switch is located on the top, right-hand corner of the instrument and will light-up a green colour when turned on.
2. Flip-up top left-hand panel to view instrumentation screen.
3. Turn on computer and log-in.
4. Double-click UV WinLab 2.85.04 software icon on the desktop OR select the software from the programs listed once you chose the **START** button.
5. Select the **Wp** tab from the **Methods** window. Double click on the desired method name.
6. Fill two matched cuvettes with the reagent blank (RB). Wipe the cuvettes clean with tissue wipes (ie. Kimwipes) and ensure that there are no air bubbles to scatter light. If using frosted cuvettes, ensure that the frosted sides of the cuvette are not in the beam path.
7. Lift the panel on the bottom right-hand side of the instrument. Place one cuvette into the reference compartment (located in the rear) and the other into the sample compartment (located in the front). Replace the panel cover.
8. Click once on the **Autozero** tab in the toolbar. A **Sample Change** window will appear and the **Autozero** tab will turn red in colour. Click once on the **OK** tab.
9. Once the auto zeroing is complete, the **Start** tab will turn green in colour and measurements can begin.
10. Lift the panel on the bottom right-hand side of the instrument. Remove the cuvette in the sample compartment (located in the front), empty the contents, rinse with Millipore water and refill with sample. If creating a standard curve, begin with lowest concentration. Replace the panel cover.
11. Click once on the **Start** tab. A **Sample Change** window will appear. Click once on the **OK** tab. The results will appear in a **Result Window**.
12. When you are ready to analyze the next sample repeat steps #10 and #11.
13. When sampling is complete, close the **Result Window** by clicking on the **X** in the top right-hand corner, close the **Method Window** by clicking on the **X** in the top right-hand corner, and then close the **UV WinLab Window** by clicking on the **X** in the top right-hand corner.

APPENDIX B

INSTRUCTIONS FOR THE GC-ECD

A. Creating a method:

1. Turn on the computer and log-in.
2. Double-click Turbochrom Navigator 6.1.2 (tcnav) software icon on the desktop OR select the software from the folder labeled Turbochrom Client-Server Ver6.1.2 under the programs tab listed once you chose the **START** button.
3. Under the **Instruments** panel, ensure that GC (not LC) is selected under the **All** tab.
4. Under the **Build** panel, click once on the **Method** icon. A **Startup** window will appear. To create a new method, select **Create a new method** and click once on **OK**. (Alternatively, you can choose to select **Load method stored on disk** to modify an already existing method in which case you would change the desired parameters and save the method under a new name.)
5. Click on **Instrument** under the toolbar select **Data Channels** and a **Data Acquisition** window will appear.

Under the **Data Channels** tab select the following:

- Set **Data Channel** to B
- Set **Source** to DetB
- Set **Set Data Rate** to By sampling rate (pts/s) 12.5
- Set **Data Storage** to Store all data from run

Under the **Real-time Plot** tab select the following:

- Set **Offset (mV)** to 0
- Set **Full scale (mV)** to 1000.000
- Set **Number of pages** to 1
- Click once on the **OK** tab to close the **Data Acquisition** window

6. Click on **Instrument** under the toolbar select **Control Options** and a **Instrument Control** window will appear.

Under the **Autosampler** tab select the following:

- Set **Injection source** to Manual

Under the **Oven/Inlets** tab select the following:

- Click once on the **Oven ramp** tab and set the **Oven Ramp rate, temperature and hold** to desired parameters
- Click once on the **Inj B ramp** tab and set the **Inj B Ramp rate, temperature and hold** to desired parameters
- Under Heated zone setpoints (°C), set **Injector A** to OFF and **Injector B** to On
- Set **Coolant** to OFF

- Set **Oven Max Temp (°C)** to 25°C below the manufacturers' recommended max temp for the column to be used
- Set **Oven Equil time (min)** to 1.0

Under the **Carrier** tab select the following:

- Click once on the **Carrier A** tab and set the **Oven Ramp rate** to 0, **temperature** to 0 and **hold** to 999
- Click once on the **Carrier A** tab and set the **Oven Ramp rate, temperature** and **hold** to desired parameters
- Set **Column Length (m)** to manufacturers' provided information
- Set **Column Diameter (µm)** to manufacturers' provided information
- Set **Column Vacuum comp** to OFF
- Set **Split control Mode** to either Ratio or Flow
- Set **Split control Ratio (n:1)** to desired parameters
- Set **Split control Flow (mL/min)** to desired parameters

Under the **Detectors** tab select the following:

Detector A – FID (although not using, must be set or instrument will not work)

- Set **Temp** to 200 and **Range** to 20
- Set **Time constant** to 200
- Set **H2** to 45.0 mL/min and **Air** to 450.0 mL/min
- Set **Attenuation** to 0 and **Offset** to 5.0mV

Detector B – ECD (although not using, must be set or instrument will not work)

- Set **Temp** to desired temperature and **Range** to 1
- Set **Time constant** to 200
- Set **Autozero** to On
- Set **Make-up Gases** 30mL/min
- Set **Attenuation** and **Offset** to desired parameters

Under the **Instrument Timed Events** tab select the following:

- Set **Time** to 0
- Set **Event** to ATT1
- Set **Value** to 0
- Click once on the **OK** tab to close the **Instrument Control** window

7. Click once on the **File** tab on the toolbar and select **Save As...** and a **Documentation** window will appear. Type in a description of your method and click once on the **OK** tab.

8. A **Turbochrom File-Save-As** window will appear. Type in a file name for your method and click once on the **OK** tab.

B. Running a Sample:

1. Turn on the hydrogen, air, helium, and nitrogen gas cylinders located behind the instrument.
2. Check the status of the septum and change if necessary (every 40 injections).
3. Turn on the Gas Chromatographer (Perkin Elmer AutoSystem XL) and ensure you hear the flame light.
4. Turn on the computer and log-in.
5. Double-click Turbochrom Navigator 6.1.2 (tcnv) software icon on the desktop OR select the software from the folder labeled Turbochrom Client-Server Ver6.1.2 under the programs tab listed once you chose the **START** button.
6. Under the **Instruments** panel, ensure that GC (not LC) is selected under the **All** tab.
7. Under the **Instrument** panel, click once on the **Set-up** icon. A **Setup Instrument** window will appear. Under **setup type** select Method. Under **Method**, click once on the file folder icon and a **Turbochrom File-Select** window will appear. Find method and double click on it to select it. Note: If you are unable to locate your method, ensure that the Data Path is correct.
8. Type in a descriptive title under the **Base file name** and click once on the **OK** icon.
9. The font in the **Status** panel will appear red and then green. To view the current status of the instrument click once on the **Details** icon. You are ready to inject a sample when all parameters read as Ready versus NRDY. Ensure that the instrument panel also reads as Ready.
10. Inject a desired volume of sample using a gas tight syringe into the rear injector port and IMMEDIATELY upon withdrawal of the syringe from the instrument press the **RUN** tab on the instrument panel. The font in the **Status** panel will appear blue and Sampling will appear under GC where it previously read as Ready.
11. Click once on the **Real-Time Plot** icon to open the **Real-Time Plot** window and view the chromatogram as it appears. Select the **Rescale Plot** icon in the toolbar to change the chromatogram view (i.e. the time and scale options).
12. Continue injections as desired as long as the sample run time permits. Ensure that ample time is permitted for the sample to elute prior to the next sample injection.
13. When sampling is completed, click once on the **Run** icon and select **Stop Run...** or select **Cancel Run...** if you do not wish to keep the results and print a chromatogram. The run may be stopped once the expected peaks have eluted, or once the total run time has elapsed. The font in the **Status** panel will appear red and it will read as No Method under GC.
14. Close the software window by clicking once on the **X** on the upper right-hand side of the window and then log-off the computer.

APPENDIX C

EQUILIBRIUM CONCENTRATION CALCULATION FOR EACH GAS (PRIOR TO CORRECTION)

- $[N_2] \text{ equilibrium} = 0.2103528 T_w^2 - 18.54774237 T_w$

[units of μmol]

Equation C.1

- $[O_2] \text{ equilibrium} = 0.1295 T_w^2 - 10.8739 T_w + 455.1543$

[units of μmol]

Equation C.2

- $[N_2O] \text{ equilibrium} = 0.000007713 T_w^2 - 0.000615967 T_w + 0.018249814$

[units of μmol]

Equation C.3

- $[CH_4] \text{ equilibrium} = 0.000001504 T_w^2 - 0.000123881 T_w + 0.004706$

[units of μmol]

Equation C.4

Where T_w = the measured water temperature in $^{\circ}\text{C}$

APPENDIX D

SCHMIDT NUMBERS CALCULATIONS

Schmidt number = the ratio of momentum diffusivity to mass diffusivity.

- for $N_2 = 1970.7 - (131.45 \times T_w) + (4.139 \times T_w^2) - (0.052106 \times T_w^3)$

[dimensionless]

Equation D.1

- for $O_2 = 1800.6 - (120.1 \times T_w) + (3.7818 \times T_w^2) - (0.047608 \times T_w^3)$

[dimensionless]

Equation D.2

- for $N_2O = 2055.6 - (137.11 \times T_w) + (4.3173 \times T_w^2) - (0.05435 \times T_w^3)$

[dimensionless]

Equation D.3

Where T_w = the measured water temperature in °C

APPENDIX E

SAMPLE NITROUS OXIDE CHROMATOGRAPH

Page 1 of 2

Software Version	: 6.1.2.0.1:D19	Date	: 1/8/09 10:40:40 AM
Operator	: manager	Sample Name	:
Sample Number	:	Study	:
AutoSampler	: BUILT-IN	Rack/Vial	: 0/0
Instrument Name	: GC	Channel	: B
Instrument Serial #	: 610N9072715	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 27.76 min
Sampling Rate	: 12.5000 pts/s		
Volume Injected	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 1/8/09 11:50:49 AM	Cycle	: 1

Raw Data File : \\Poweredge\E drive\TC\dennis\n2o standard run001-20090108-115057.raw
 Result File : \\Poweredge\E drive\TC\dennis\n2o standard run001-20090108-104040.rst
 Inst Method : \\poweredge\E drive\TC\dennis\juliet_method_split from \\Poweredge\E drive\TC\dennis\n2o standard run001-20090108-104040.rst
 Proc Method : \\Poweredge\E drive\TC\dennis\juliet_method_split
 Calib Method : \\Poweredge\E drive\TC\dennis\juliet_method_split
 Sequence File : \\poweredge\E drive\TC\dennis\n2o standard run.seq

DEFAULT REPORT

Peak #	Time [min]	Area [μV·s]	Height [μV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	0.113	114793.23	10697.50	0.15	0.15	BB	10.7309
2	0.749	111073.86	2975.45	0.15	0.15	BB	37.3300
3	1.615	16377433.26	989132.17	21.58	21.58	BE	16.5574
4	2.181	524455.38	13830.08	0.69	0.69	EB	37.9214
5	3.624	126390.53	11677.03	0.17	0.17	BB	10.8239
6	5.771	103690.57	9699.23	0.14	0.14	BB	10.6906
7	6.286	82509.41	3308.01	0.11	0.11	BB	24.9423
8	7.281	15960673.53	987823.83	21.04	21.04	BE	16.1574
9	7.828	923185.17	18220.33	1.22	1.22	EV	50.6679
10	9.280	170319.82	13737.85	0.22	0.22	VB	12.3979
11	11.815	109757.23	10267.65	0.14	0.14	BB	10.6896
12	12.353	100991.21	3580.01	0.13	0.13	BB	28.2098
13	13.321	15161638.41	987623.92	19.98	19.98	BE	15.3516
14	13.860	701270.71	14157.77	0.92	0.92	EV	49.5326
15	15.320	373396.80	31619.19	0.49	0.49	VB	11.8092
16	15.889	113199.87	10585.97	0.15	0.15	BB	10.6934
17	16.376	78086.57	3724.80	0.10	0.10	BB	20.9640
18	17.395	15354215.89	987039.87	20.24	20.24	BE	15.5558
19	17.939	957106.91	14722.31	1.26	1.26	EV	65.0107
20	19.389	500673.33	36421.89	0.66	0.66	VV	13.7465
21	19.772	206092.23	16523.86	0.27	0.27	VB	12.4724
22	20.286	89034.87	3886.31	0.12	0.12	BB	22.9099
23	21.305	3689039.14	304516.95	4.86	4.86	BB	12.1144
24	23.268	1175256.22	86026.16	1.55	1.55	BB	13.6616
25	24.081	106535.19	3889.78	0.14	0.14	BB	27.3885
26	25.067	1622538.36	134749.58	2.14	2.14	BB	12.0411
27	27.021	1041356.30	87702.49	1.37	1.37	BB	11.8737
		75874713.98	4.80e+06	100.00	100.00		

N₂O
Standard

1 ppm = 0.35.7
 5 ppm = 11.7, 15.8
 10 ppm = 19.6, 23.4

$$y = 102736x + 6160.6$$

$$R = 0.9988$$

Figure F.1. Sample nitrous oxide chromatograph, part 1.

Chromatogram

Sample Name : \Poweredge\E drive\TC\dennis\120 standard run001-20090108-115057.raw
 Sample #:
 Page 1 of 1
 Date : 1/8/09 10:40:41 AM
 Method : juliet_method_split.mth
 Time of Injection: 1/8/09 11:50:49 AM
 Start Time : 0.00 min End Time : 27.76 min Low Point : -49.69 mV High Point : 993.88 mV
 Scale Factor: 1.0 Plot Offset: -49.69 mV Plot Scale: 1043.6 mV

Figure F.2. Sample nitrous oxide chromatograph, part 2.

APPENDIX F

CHRISTIE LAKE RAW DATA

Table G.1 Christie Lake raw data.

Date		NH ₄ (μM)	NO ₃ (μM)	PO ₄ (μM)	N ₂ (μM)	O ₂ (μM)	N ₂ O (μM)
21-Jun-08	0.5	5.084	0.544	1.174	556.430	293.065	11.897
	1	5.429	0.664	1.379	545.795	270.022	11.087
	1.5	5.968	0.729	1.360	547.277	229.093	17.944
	2	6.584	0.738	1.553	517.609	217.232	11.036
	2.5	7.292	0.478	1.329	523.103	193.555	29.571
	3	8.799	0.820	1.733	548.965	159.262	26.938
	3.5	11.500	////	0.863	////	////	////
	blue	9.669	0.448	1.255	535.238	180.565	10.599
	green	15.396	0.533	1.199	535.195	170.200	35.186
	white	5.844	0.453	1.081	534.047	166.332	16.001
	red	7.240	0.512	1.099	533.578	171.951	21.224
22-Jun-08	0.5	5.552	0.700	1.118	523.239	262.315	9.951
	1	////	0.539	////	541.527	266.177	10.227
	1.5	6.149	0.639	1.273	536.220	257.247	14.772
	2	7.058	0.773	1.385	515.181	232.742	11.715
	2.5	8.721	0.593	1.807	515.363	171.683	13.425
	3	14.695	0.629	1.429	536.870	138.500	37.855
	3.5	10.844	0.475	1.565	558.278	113.815	40.011
	blue	12.305	0.466	1.329	534.468	121.227	17.790
	green	11.091	0.647	0.981	535.533	139.198	27.230
	white	10.234	0.580	1.280	540.778	110.809	22.996
	red	12.545	0.399	0.863	536.470	145.705	19.889
24-Jul-08	0.5	2.870	0.299	0.683	517.046	239.245	3.448
	1	2.812	0.177	////	520.681	241.319	20.015
	1.5	4.312	0.343	2.422	512.499	226.918	9.180
	2	2.558	0.315	5.714	520.292	230.998	38.896
	2.5	2.792	0.527	5.994	523.977	173.685	61.466
	3	3.617	0.328	0.429	529.732	162.431	7.976
	3.5	5.825	0.596	0.807	527.875	130.258	19.954
	am blue	7.065	0.453	0.497	525.474	166.276	24.476
	pm	5.234	0.454	0.609	521.928	152.543	83.898
	am red	4.318	0.484	0.683	525.764	169.147	42.430
	pm	11.383	0.357	0.497	526.509	102.122	26.655
	green	////	0.431	////	524.949	158.937	53.469
25-Jul-08	0.5	2.382	0.242	0.329	519.964	280.435	49.131
	1	2.760	0.327	0.832	521.044	281.688	22.440
	1.5	4.974	0.327	2.037	513.611	219.229	35.630
	2	11.519	0.294	0.412	516.713	160.408	36.219
	2.5	4.396	0.545	0.470	522.312	150.993	84.812
	3	4.123	0.448	0.621	530.438	148.014	37.206
	3.5	3.818	0.580	0.807	528.861	159.956	42.663
	blue	11.305	0.359	0.658	527.572	100.027	83.663
	red	10.318	0.303	0.224	554.970	96.109	37.786

Date		NH ₄ (μM)	NO ₃ (μM)	PO ₄ (μM)	N ₂ (μM)	O ₂ (μM)	N ₂ O (μM)
3-Oct-08	0.5	0.656	0.333	0.022	525.588	241.911	
	1	1.210	0.129	-0.002	518.163	238.694	
	1.5	0.088	0.146	0.006	514.642	233.201	
	2	1.197	0.141	0.031	514.159	234.635	
	2.5	1.036	0.215	0.078	519.787	238.004	
	3	1.742	0.288	0.108	534.217	239.202	
	am blue	1.173	0.139	0.574	515.213	224.712	
	pm	2.107	0.230	0.740	513.468	207.006	
	am green	2.140	0.287	0.712	520.270	274.163	
	pm	2.042	0.269	0.311	520.843	216.489	
	am white	3.224	0.215	1.598	512.962	208.358	
	pm	1.393	0.175	0.241	////	////	SAMPLES
	am red	4.927	0.298	4.810	526.124	279.625	LOST
	pm	1.567	0.151	0.732	524.266	246.699	
4-Oct-08	0.5	0.137	0.274	0.020	522.354	246.788	
	1	0.729	0.134	0.013	520.182	240.199	
	1.5	0.115	0.170	0.029	508.533	232.030	
	2	1.265	0.184	0.019	510.857	232.727	
	2.5	0.556	0.249	0.034	523.741	224.874	
	3	2.331	0.298	0.367	524.067	228.370	
	blue	2.702	0.110	0.350	521.352	164.280	
	green	1.940	0.227	0.417	////	////	
	white	0.669	0.197	0.287	536.123	276.171	
	red	2.855	0.251	0.197	533.831	277.736	

APPENDIX G

TURTLE POND RAW DATA

Table H.1 Turtle Pond raw data.

Date		NH ₄ (μM)	NO ₃ (μM)	PO ₄ (μM)	N ₂ (μM)	O ₂ (μM)	N ₂ O (μM)
2-Nov-07	Top	7.162	18.044	15.676			105.521
	Bottom	7.603	16.868	14.751			83.206
3-Nov-07	Top	8.485	19.000	16.428			0.218
	Bottom	21.059	17.897	15.561			23.465
11-Nov-07	Top 1	7.529	19.809	0.327			47.285
	Top 2	6.941	19.368	0.329			43.522
	Bottom 1	10.397	17.529	0.624			57.250
	Bottom 2	8.706	15.176	0.578			33.080
18-Nov-07	Top 1	4.662	15.912	0.682			50.853
	Top 2	7.309	9.147	0.607			31.904
	Bottom 1	-1.074	11.353	0.457			74.664
	Bottom 2	1.500	9.515	0.335			30.380
28-Nov-07	Top 1	10.737	17.789	1.624			23.377
	Top 2	12.382	16.813	1.757			53.476
	Bottom 1	15.079	5.024	1.318			16.541
	Bottom 2	15.276	6.488	1.422			15.916
10-Jan-08	Bottom 1	13.434	6.081	0.931			54.361
	Bottom 2	14.237	15.187	1.029			29.961
26-Jan-08	Bottom 1	17.322	16.976	1.081			33.780
	Bottom 2	14.309	18.033	0.798			53.935
3-Feb-08	Bottom 1	10.882	14.618	0.220			30.445
	Bottom 2	14.849	15.512	0.353			33.919
16-Feb-08	Top 1	8.928	22.341	0.723			48.796
	Top 2	9.730	20.146	0.636			34.489
	Bottom 1	11.612	13.154	0.555			24.490
	Bottom 2	23.401	15.431	0.486			18.849
23-Feb-08	Bottom 1	13.895	15.106	0.353			32.170
	Bottom 2	13.533	18.439	0.572			37.192
2-Mar-08	TP 1	3.526	9.902	0.497			16.169
	TP 2	3.461	16.163	0.382			24.198

April, May, June and July

01-Aug-08	TP 1	1.347					
	TP 2	2.421					
11-Aug-08		5.380	0.250	0.073	517.484	387.136	16.409
18-Aug-08		0.100		0.160	523.649	558.443	23.519
28-Aug-08		8.020		0.000	520.868	177.178	23.450
04-Sep-08		30.960		0.163	523.391	431.388	18.124
12-Sep-08		14.110	2.370	0.205	525.887	89.707	0.160
20-Sep-08		29.990		0.405			4.980
28-Sep-08		3.870	4.290	-0.010			18.124

APPENDIX H

MATLAB PROGRAM EXPLANATION

clc

In Matlab, the results are displayed in the Command window. Without 'clc', all previous results will be displayed. By having 'clc' (it stands for "clear screen"), only the result of the current run is displayed. This setting is done for clarity but can be disabled.

clear

By having 'clear', all previously stored data (if we previously assigned values to some variables) will be deleted. This is useful to prevent miscalculations.

% reading from Excel

```
DATA = xlsread('file name.xls');
```

Having a '%' sign prevents a line from being executed, therefore it is useful for creating headings or disabling a certain function for later use.

Having a semicolon (;) at the end of a code prevents the values of the variables from being displayed in the Command window. This is useful when assigning values to variables because it would be a hassle if they are all displayed. However, it might be useful to take the semicolon off during debugging to see if we have made a mistake somewhere.

'xlsread' lets us read an Excel file with a syntax similar to the line above.

'file name.xls' is the name of the Excel file being read, and it must be saved in the same folder as the Matlab file. The 'xlsread' function only reads numbers within the Excel file so if we have words (headings, titles, units) they will be read as 'NaN' (stands for 'Not a Number'). The function may also skip columns/rows completely if they are empty or do not contain a number. Therefore when initially performing xlsread, it is recommended to manually inspect the DATA matrix file.

Example

Original denitrification.xls in Excel:

	A	B	C	D	E	F	G	H
1	real time	site	time	measured N ₂	modeled N ₂		Schmidt coefficient	
2	6/25/99 21:29	IR03	0	475.23	475.23	490.56	-0.666666667	
3	6/26/99 18:02	IR07	1233	490.56	490.56			
4							462.9187608	
5	denitrification rate (mmol N ₂ /m ³ -h)	0.75088201				C _{N t=n-1}	C _{denitrif.}	
6	denitrification rate (mmol N ₂ /m ³ -h)	1.0812701	measured	measured		modeled	DeN2	Conc + DeN2
7			water			N ₂ conc	new N ₂	init total N ₂
8	Real time	time (hour)	temp (°C)	avg depth (m)	avg vel (m/min)	(mmol/m ³)	(mmol/m ³)	(mmol/m ³)
9	05/28/08 12:45:32.0	0	24.3	1.44	19.036	475.23	0.375441006	475.605441
10	05/28/08 12:46:32.0	1	24.2988372	1.44	19.036	475.951992	0.375441006	476.3274331
11	05/28/08 12:47:32.0	2	24.2976744	1.44	19.036	476.654872	0.375441006	477.0303133
12	05/28/08 12:48:32.0	3	24.2965116	1.44	19.036	477.339071	0.375441006	477.7145123
13	05/28/08 12:49:32.0	4	24.2953488	1.44	19.036	478.005012	0.375441006	478.3804528
14	05/28/08 12:50:32.0	5	24.294186	1.44	19.036	478.653109	0.375441006	479.0285498
15	05/28/08 12:51:32.0	6	24.2930233	1.44	19.036	479.283769	0.375441006	479.6592102
16	05/28/08 12:52:32.0	7	24.2918605	1.44	19.036	479.897392	0.375441006	480.2728335
17	05/28/08 12:53:32.0	8	24.2906977	1.44	19.036	480.494371	0.375441006	480.8698116
18	05/28/08 12:54:32.0	9	24.2895349	1.44	19.036	481.075088	0.375441006	481.4505288
19	05/28/08 12:55:32.0	10	24.2883721	1.44	19.036	481.639921	0.375441006	482.0153621
20	05/28/08 12:56:32.0	11	24.2872093	1.44	19.036	482.18924	0.375441006	482.5646815

How denitrification.xls looks after being read by Matlab:

Variable Editor - DATA

Stack: Base

DATA <1241x20 double>

	1	2	3	4	5	6	7	8
1	NaN	0	475.2300	475.2300	490.5600	-0.6667	NaN	101
2	NaN	1233	490.5600	490.5600	NaN	NaN	NaN	
3	NaN	NaN	NaN	NaN	NaN	462.9188	NaN	
4	0.7509	NaN	NaN	NaN	NaN	NaN	NaN	
5	1.0813	NaN	NaN	NaN	NaN	NaN	NaN	
6	NaN	NaN	NaN	NaN	NaN	NaN	NaN	
7	NaN	NaN	NaN	NaN	NaN	NaN	NaN	
8	0	24.3000	1.4400	19.0360	475.2300	0.3754	475.6054	
9	1	24.2988	1.4400	19.0360	475.9520	0.3754	476.3274	
10	2	24.2977	1.4400	19.0360	476.6549	0.3754	477.0303	
11	3	24.2965	1.4400	19.0360	477.3391	0.3754	477.7145	
12	4	24.2953	1.4400	19.0360	478.0050	0.3754	478.3805	
13	5	24.2942	1.4400	19.0360	478.6531	0.3754	479.0285	
14	6	24.2930	1.4400	19.0360	479.2838	0.3754	479.6592	
15	7	24.2919	1.4400	19.0360	479.8974	0.3754	480.2728	
16	8	24.2907	1.4400	19.0360	480.4944	0.3754	480.8698	
17	9	24.2895	1.4400	19.0360	481.0751	0.3754	481.4505	
18	10	24.2884	1.4400	19.0360	481.6399	0.3754	482.0154	
19	11	24.2872	1.4400	19.0360	482.1892	0.3754	482.5647	

% measured variables

```
C_INITIAL = DATA(1,3);
```

```
C_FINAL = DATA(2,3);
```

```
HOUR = DATA(size(DATA,1),1);
```

```
T_WATER1 = DATA(8:size(DATA,1),2);
```

```
DEPTH1 = DATA(8:size(DATA,1),3);
```

```
RH1 = DATA(8:size(DATA,1),13);
```

```
P1 = DATA(8:size(DATA,1),14);
```

```
T_AIR1 = DATA(8:size(DATA,1),15);
```

```
V_AIR1 = DATA(8:size(DATA,1),16);
```

```
n1 = DATA(1,6);
```

In the steps above, some variables are defined based on their values in the Excel file.

C_INITIAL is set to be the value in row 1, column 3 of the matrix DATA

C_FINAL is set to be the value in row 2, column 3 of the matrix DATA

size(DATA) will give the dimensions of the matrix DATA, the first entry being the row size, the second being the column size:

```
>> size(DATA)
```

```
ans =
```

```
1241
```

```
20
```

By specifying size(DATA,1), we only get the column size:

```
>> size(DATA,1)
```

```
ans =
```

```
1241
```

This technique is useful for selecting a large number of data points in the DATA matrix, so one doesn't have to keep changing the code if the amount of data points in the DATA matrix changes. In this example, T_WATER1 is set to be the values in rows 8 until the end (row 1241), column 2 of the matrix DATA

% defined variables

```
SIZE = size(T_WATER1,1);
```

```
ITER = 1000;
```

Here we define some more variables to help with the iterations

SIZE is set to equal the number of data points in the matrix T_WATER1 (typing 'size(T_WATER1,1)' in the Command window will give an answer of 1234)

ITER is set to the number of iterations we want

% randomize

% convert into new matrices of the same means and variances

% random(distribution type, mean, std dev, NUMBER OF DATA, column);

```

T_WATER2 = random('Normal', mean(T_WATER1), std(T_WATER1), size(T_WATER1,1),
1);
DEPTH2 = random('Normal', mean(DEPTH1), std(DEPTH1), size(DEPTH1,1), 1);
RH2 = random('Normal', mean(RH1), std(RH1), size(RH1,1), 1);
P2 = random('Normal', mean(P1), std(P1), size(P1,1), 1);
T_AIR2 = random('Normal', mean(T_AIR1), std(T_AIR1), size(T_AIR1,1), 1);
V_AIR2 = random('Normal', mean(V_AIR1), std(V_AIR1), size(V_AIR1,1), 1);
n2 = n1; %change later when n is not constant

```

In this step values are randomized by creating duplicates of the original matrices. The syntax used is *random('Type of Distribution', mean, standard deviation, number of rows, number of columns)*. For example, the matrix T_WATER2 is a duplicate of the matrix T_WATER1, both having the same distribution, mean, standard deviation, rows and columns (number of rows of T_WATER2 is equated to that of T_WATER1 using the SIZE syntax, and number of columns is set to 1).

```

% counter matrix
% randint( row(=number of runs) , column , [range]);
COUNT = randint(SIZE, ITER , [1,SIZE]);

```

For the purpose of the Monte Carlo simulation, a counter matrix is created which will become our instruction for data point picking. Using the syntax *randint(number of rows, number of column, [range])*, a matrix COUNT is created which has the same number of rows as our data points (number of rows = SIZE = 1234), columns equaling the desired number of iterations, and range of 1 to SIZE.

Here is an example of the COUNT matrix:

	1	2	3	4	5	6	7	8
1	1145	407	189	168	53	944	129	
2	321	332	1023	822	176	702	1097	
3	1028	267	913	1025	269	759	448	
4	217	947	123	832	960	727	560	
5	1086	451	1013	173	442	761	80	
6	346	191	281	468	32	296	1050	
7	1049	204	132	838	1029	977	345	
8	996	1103	818	637	449	871	1126	
9	234	1168	1178	1207	974	911	74	
10	1208	639	1006	331	1086	171	761	
11	314	23	770	1070	835	7	696	
12	961	518	406	462	673	912	825	
13	1001	151	343	169	1110	1001	414	
14	615	1175	537	103	123	697	47	
15	1131	334	433	912	113	69	353	
16	125	1103	1084	460	43	399	1150	
17	168	1105	8	470	401	1153	988	
18	1060	958	860	373	205	1053	409	

After that, the COUNT matrix is applied as a picking instruction within the duplicate matrices (not the original matrices but their copies which have the same distributions, means and standard deviations)

```
T_WATER = T_WATER2(COUNT);
DEPTH = DEPTH2(COUNT);
RH = RH2(COUNT);
P = P2(COUNT);
T_AIR = T_AIR2(COUNT);
V_AIR = V_AIR2(COUNT);
n = n2; %change later when n is not constant
```

In this example, the first column of T_WATER contains data points 1145, 321, 1028, ... of T_WATER2 and the second column of T_WATER contains data points 407, 332, 267, ... of T_WATER2. T_WATER has the same dimensions as COUNT.

% calculations

```
for N = 1:ITER
    C_DN2(N,1) = fzero(@objfun2, 1, [], C_INITIAL, C_FINAL, T_WATER(:,N),
        DEPTH(:,N), RH(:,N), P(:,N), T_AIR(:,N), V_AIR(:,N), n, HOUR);
end
```

Calculations are performed within a 'for' loop which has a counter N going from 1 up to the number of iterations (ITER, which in this example equals 1000). During the first run, N = 1 and hence the next line of coding becomes:

```
C_DN2(1,1) = fzero(@objfun2, 1, [], C_INITIAL, C_FINAL, T_WATER(:,1), DEPTH(:,1),
    RH(:,1), P(:,1), T_AIR(:,1), V_AIR(:,1), n, HOUR);
```

In the second run, N = 2 and the next line of coding becomes:

```
C_DN2(2,1) = fzero(@objfun2, 1, [], C_INITIAL, C_FINAL, T_WATER(:,2), DEPTH(:,2),
    RH(:,2), P(:,2), T_AIR(:,2), V_AIR(:,2), n, HOUR);
```

And so on until N = ITER

The function fzero finds the zero of a specific function, thus the rest of the calculations are done in a separate function m-file (refer to **objfun2.m** below). The syntax is *fzero(@name_of_file, initial guess, range, variables we want to input into @name_of_file)*. The file name is objfun2.m and in order to perform the calculations the initial concentration, final concentration, water temperature, depth, relative humidity, partial pressure, air temperature, wind speed, Schmidt number coefficient and duration of the experiment are needed. Therefore C_INITIAL, C_FINAL, T_WATER, DEPTH, RH, P, T_AIR, V_AIR, n, and HOUR are put in the matrices.

As explained above, some of the syntax changes as N increases from 1 to ITER. The colon in the parentheses means "all data" (very similar to typing size(matrix_name) like in previous examples, but this way time is saved, and it makes the code shorter and easier to read). So T_WATER(:,1) means all data in column 1 of matrix T_WATER, and T_WATER(:,2) means all data in column 2 of matrix T_WATER. Each column in T_WATER (and also DEPTH, RH, P,

T_AIR and V_AIR) are input into objfun2.m one at a time, and every iteration yields one denitrification rate. In the end, the total number of denitrification rates we have should equal to our iteration (= ITER = 1000).

% histogram

```
hist(C_DN2, mean(C_DN2)-3*std(C_DN2): std(C_DN2) : mean(C_DN2)+3*std(C_DN2))
```

As the final step, a histogram is created and the syntax is *hist(name_of_matrix_to_be_turned_into_a_histogram, lower bound : bin size : upper bound)*. In this example I set the upper and lower bound to be the mean plus and minus 3 standard deviations and bin size equalling the standard deviation.

```
MEAN_STDDEV = [mean(C_DN2) std(C_DN2)]
```

Finally a matrix is created containing the average and standard deviation of the denitrification rate. There is no semicolon at the end of this code, and thus the results are displayed in the Command window.

objfun2.m

```
function SOLVE = objfun2 (C_DN2, C_MODEL, C_FINAL, T_WATER, DEPTH, RH, P,  
T_AIR, V_AIR, n, HOUR)
```

```
for N = 1:HOUR
```

```
    CORR = (P(N,1) ./ 101.3) .* ((0.7584882 .* ((0.6292 .* exp(0.0636 .* T_AIR(N,1)))) -  
    ((RH(N,1) ./ 100) .* 0.6292 .* exp(0.0636 .* T_AIR(N,1))))/100) + 0.7584882) ./  
    0.7584882;
```

```
    C_EQUIL_STAR = 0.2103528 * (T_WATER(N,1) .* T_WATER(N,1)) - 18.54774237 *  
    T_WATER(N,1) + 815.40460148;
```

```
    C_EQUIL = C_EQUIL_STAR .* CORR;
```

```
    K600 = 0.526 * (V_AIR(N,1) .^ 2.191) .* 1 ./ (DEPTH(N,1) .* 100);
```

```
    SCN2 = 1970.7 - 131.45 .* T_WATER(N,1) + 4.139 .* T_WATER(N,1) .^ 2  
    - 0.052106 .* T_WATER(N,1) .^ 3;
```

```
KN2 = K600 .* (SCN2 ./ 600) .^ n;
%change n to n(N,1) if n is a matrix
```

```
C_FLUX = KN2 .* (C_MODEL + (C_DN2/2) - C_EQUIL);
```

```
C_MODEL = C_MODEL - C_FLUX + (C_DN2/2);
end
```

```
SOLVE = C_MODEL - C_FINAL;
end
```

The calculations in this function m-file are very similar to the calculations performed in Excel. There is another 'for' loop in this file and a counter N. Keep in mind that this counter N is different than the counter N from the denitrification_mc.m file as they are two different m-files. The N counter here is responsible for performing calculations row-by-row whereas the previous N counter is responsible for performing calculations column-by-column.

Some multiplications and divisions are written as '.*' and './' (having a period precede the sign) as opposed to just '*' and '/'. This is to prevent whole matrix multiplications/divisions to occur, and instead multiplications/divisions occur data point-by-data point:

$$\begin{array}{cc} \mathbf{A} & \mathbf{B} \\ \begin{bmatrix} 5 & 6 \\ 7 & 8 \end{bmatrix} & \begin{bmatrix} 1 & 2 \\ 3 & 4 \end{bmatrix} \end{array} \quad \mathbf{A} * \mathbf{B} = \begin{bmatrix} 5*1 + 6*3 & 5*2 + 6*4 \\ 7*1 + 8*3 & 7*2 + 8*4 \end{bmatrix} = \begin{bmatrix} 23 & 34 \\ 31 & 46 \end{bmatrix}$$

$$\mathbf{A} .* \mathbf{B} = \begin{bmatrix} 5*1 & 6*2 \\ 7*3 & 8*4 \end{bmatrix} = \begin{bmatrix} 5 & 12 \\ 21 & 32 \end{bmatrix}$$

C_MODEL is an intermediate concentration value as one goes through the calculations, and it is a function of C_DN2, C_MODEL, C_FINAL, T_WATER, DEPTH, RH, P, T_AIR, V_AIR, n and HOUR. Initially C_MODEL is set to equal to C_INITIAL. As we go from 1 to the total HOUR of our monitoring, C_MODEL changes and its final value should equal to C_FINAL. Therefore, C_MODEL is subtracted from C_FINAL the result should be zero. To obtain zero, one performs the fzero function with C_DN2 as our variable.

REFERENCES

- Alexander, R. B., Smith, R. A. and Schwarz, G. E. (2000). Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* **403**: 758–761.
- Alexander, R. B., Elliott, A. H., Shankar, U. and McBride, G. B. (2002a). Estimating the sources and transport of nutrients in the Waikato River basin, New Zealand. *Water Resources Research*, **38**: 1268–1290.
- Alexander, R. B., Johnes, P. J., Boyer, E. W. and Smith, R. A. (2002b). A comparison of methods for estimating the riverine export of nitrogen from large watersheds. *Biogeochemistry* **57–58**: 295–339.
- An, S. M., Gardner, W. S., and Kana, T.M. (2001) Simultaneous measurement of denitrification and nitrogen fixation using isotope pairing with membrane inlet mass spectrometry analysis. *Applied and environmental microbiology* **67**: 1171 -1178.
- APHA (1995) *Standard Methods for the Examination of Water and Wastewater*, 19th edn. American Public Health Association, Washington, DC.
- Atobatele, O. E. and Ugwumba, O. A. (2008) Seasonal variation in the physicochemistry of a small tropical reservoir (Aiba Reservoir, Iwo, Osun, Nigeria). *African Journal of Biotechnology* **7**: 1962-1971.
- Baehr, M. M. and DeGrandpre, M. D. (2004) In situ pCO₂ and O₂ measurements in a lake during turnover and stratification- Observations and modeling. *Limnology and Oceanography* **49**: 330-340.
- Barnes, R. O., Bertine, K. K., and Goldberg, E. D. (1975) N₂:Ar, nitrification and denitrification in southern California borderland basin sediments. *Limnology and Oceanography* **20**: 962–970.
- Bastviken, D., Ejlertsson, J., and Tranvik, L. (2002) Measurement of methane oxidation in lakes: A comparison of methods. *Environmental science & technology* **36**: 3354 -3361
- Baulch, H., Venkiteswaran, J., Dillon, P., and Maranger, R. (In press) Revisiting the application of open-channel estimates of denitrification. *Limnology and Oceanography: Methods*.
- Beaulieu, J. J., Arango, C. P., Hamilton, S. K., and Tank, J. L. (2008) The production and emission of nitrous oxide from headwater streams in the Midwestern United States. *Global Change Biology* **14**: 878–894.
- Bernot, M. J., Dodds, W. K., Gardner, W. S., McCarthy, M. J., Sobolev, D. and Tank, J. L. (2003) Comparing denitrification estimates for a Texas estuary by using acetylene inhibition and membrane inlet mass spectrometry. *Applied and Environmental Microbiology* **69**: 5950–5956.

- Böhlke J. K., Harvey, J.W., and Voytek, M. A. (2004) Reach-scale isotope tracer experiment to quantify denitrification and related processes in a nitrate-rich stream, mid-continent United States. *Limnology and Oceanography* **49**:821–838.
- Böhlke J. K., Antweiler, R. C., Harvey, J.W., Laursen, A. E., Smith, L. K., Smith, R. L., and Voytek, M. A. (2009) Multi-scale measurements and modeling of denitrification in streams. *Biogeochemistry* **93**:117–141.
- Boesch D. F. (2002) Challenges and opportunities for science in reducing nutrient over-enrichment of coastal ecosystems. *Estuaries* **25**: 744–758.
- Boyer, E. W., Alexander, R. B., Parton, W. J., Changsheng, L., Butterbach-Bahl, K., Donner, S. D., Skaggs, R. W., Del Grosso, S. J. (2006) Modeling denitrification in terrestrial and aquatic ecosystems at regional scales. *Ecological Applications* **16**: 2123–2142.
- Boynton, W. R., Garber, J. H., Summers, R., and Kemp, W. M. (1985) Inputs, transformations, and transport of nitrogen and phosphorus in Chesapeake Bay and selected tributaries. *Estuaries* **18**: 285-314.
- Bremner, J. M. and Blackmer A. M. (1978) Nitrous oxide: emission from soils during nitrification of fertilizer nitrogen. *Science* **199**: 295-296.
- Carpenter, S. R., Ludwig, D. and Brock, W. A. (1999) Management of eutrophication for lakes subject to potentially irreversible change. *Ecological Applications* **751**:
- Casamitjana, X., Serra, T., Colomer, J., Baserba, C. and Pérez-Losada, J. (2003) Effects of the water withdrawal in the stratification patterns of a reservoir. *Hydrobiologia* **504**: 21–28.
- Chapuis-Lardy, L., Wrage, N., Metay, A., Chottes, J.-L., and Bernoux, M. (2007) Soils, a sink for N₂O? A review. *Global Change Biology* **13**: 1–17.
- Codispoti, L. A., Brandes, J. A., Christensen, J. P., Devol, A. H., S. Naqvi, W. A., Pearl, H. W., and Yoshinari. T. (2001) The oceanic fixed nitrogen and nitrous oxide budgets: moving targets as we enter the anthropocene. *Scientia Marina* **65**:85–105.
- Cole, J. J., Caraco, N. F., Kling, G. W., and Kratz, T. K. (1994) Carbon dioxide supersaturation in the surface waters of lakes. *Science* **265**: 1568 – 1570.
- Cole, J. J. and Caraco, N. F. (2001) Emissions of nitrous oxide (N₂O) from a tidal, freshwater river, the Hudson River, New York. *Environmental science & technology* **35**: 991 -996.
- Cornwell, J. C., Kemp, W. M., Kana, T. M. (1999) Denitrification in coastal ecosystems: methods, environmental controls, and ecosystem level controls, a review. *Aquatic Ecology* **33**:41–54.

- Cowling, E. B., Erisman, J. W., Smeulders, S. M., Holman, S. C., Nicholson, B. M. (1998) Optimizing air quality management in Europe and North America: Justification for integrated management of both oxidized and reduced forms of nitrogen. *Environmental pollution* **102**: 599 -608.
- Crutzen, P. J. (1970) The influence of nitrogen oxides on the atmospheric ozone content. *Quarterly Journal of the Royal Meteorological Society* **96**: 320-325.
- David, M. B., Wall, L. G., Royer, T. V., and Tank, J. L. (2006) Denitrification and the nitrogen budget of a reservoir in an agricultural landscape. *Ecological Applications* **16**: 2177 -2190.
- Davidson, E. A. and Seitzinger, S. P. (2006) The enigma of progress in denitrification research. *Ecological Applications* **16**: 2057–2063.
- Deacon, E. L. (1977) Gas transfer to and across an air-water interface. *Tellus* **29**: 363-374.
- Devol, A. H. (1991) Direct measurements of nitrogen gas fluxes from continental shelf sediments. *Nature* **349**:319–321.
- Downing, J. A. and McCauley, E. (1992) The nitrogen- phosphorus relationship in lakes. *Limnology and Oceanography* **37**: 936 -945.
- Downing, J. A., Prairie, Y. T., Cole, J. J., Duarte, C. M., Tranvik, L. J., Striegl, R. G., McDowell, W. H., Kortelainen, P., Caraco, N. F., Melack, J. M., and Middelburg, J. J. (2006) The global abundance and size distribution of lakes, ponds, and impoundments. *Limnology and Oceanography*. **51**: 2388 -2397.
- Drescher, S. R. and Brown, S. D. (2006) Solid phase microextraction-gas chromatographic-mass spectrometric determination of nitrous oxide. *Journal of Chromatography A* **1133**: 300–304.
- U.S. Environmental Protection Agency (1995) America's wetlands: Our vital link between land and water. Office of Water, Office of Wetlands, Oceans and Watersheds. EPA843-K-95-001.
- Environment Canada, 2007. National Climate Data and Information Archive – Climate Data Online. [Online] (Modified on 09 Oct 2008) Available at: http://www.climate.weatheroffice.ec.gc.ca/climateData/canada_e.html [Accessed June and August 2009].
- Elser, J. J. and Hamilton, A. (2007) Stoichiometry and the New Biology: The Future Is Now. *PLoS Biololgy* **5**: 1403-1405.
- Fee, E. J., Hecky, R. E., Kasian, S. E. M., and Cruikshank, D. R. (1996) Effects of lake size, water clarity, and climatic variability on mixing depths in Canadian Shield lakes. *Limnology and oceanography* **41**: 912 -920.

Francis, C. A., Beman, J. M., and Kuypers, M. M. M. (2007) New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. *The ISME Journal* **1**: 19–27.

Fuglestad, J. S., Bernsten, T. K., Godal, O., Sausen, R., Shine, K. P., and Skodvin, T. (2003) Metrics of climate change- Assessing radiative forcing and emission indices. *Climatic Change* **58**: 267–331

Galloway, J. N., Aber, J. D., Erisman, J. W., Seitzinger, S. P., Howarth, R. W., Cowling, E. B., and Cosby, B. J. (2003). The nitrogen cascade. *BioScience* **53**:341–356.

Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., Karl, D. M., Michaels, A. F., Porter, J. H., Townsend, A. R., Vorosmarty, C. J. (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* **70**: 153–226.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z. C., Freney, J. R., Martinelli, L. A., Seitzinger, S. P., Sutton, M. A. (2008) Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **320**: 889–892.

Groffman, P. M., Altabet, M. A., Böhlke, J. K., Butterbach-Bahl, K., David, M. B., Firestone, M. K., Giblin, A. E., Kana, T. M., Nielsen, L. P., and Voytek, M. A. (2006). Methods for measuring denitrification: diverse approaches to a difficult problem. *Ecological Applications* **16**:2091–2122.

Groffman P. M., Butterbach-Bahl, K., Fulweiler, R. W., Gold, A. J., Morse, J. L., Stander, E. K., Tague, C., Tonitto, C., and Vidon, P. (2009a) Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. *Biogeochemistry* **93**:49–77.

Groffman, P. M., Davidson, E. A., and Seitzinger, S.P. (2009b) New approaches to modeling denitrification. *Biogeochemistry* **93**: 1 -5.

Gruber, N. and Galloway, J. N. (2008) An Earth-system perspective of the global nitrogen cycle. *Nature* **451**: 293–296.

Hahn J. and Crutzen P. J. (1982) The role of fixed nitrogen in atmosphere photochemistry. *Philosophical Transactions of the Royal Society of London, Series B* **296**: 521–541.

Harrison, J. A., Maranger, R. J., Alexander, R. B., Giblin, A. E., Jacinthe, P. A., Mayorga, E., Seitzinger, S. P., Sobota, D. J., Wollheim, W. M. (2009) The regional and global significance of nitrogen removal in lakes and reservoirs. *Biogeochemistry* **93**: 143 -157.

Hasegawa, T and Okino, T (2004) Seasonal variation of denitrification rate in Lake Suwa sediment. *Limnology* **5**: 33–39.

- Hecky, R. E., Campbell, P., and Hendzel L. L. (1993) The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. *Limnology and oceanography* **38**: 709-724.
- Hessen, D. O., Agren, G. I., Anderson, T. A., Elser, J. J., and de Ruiter, P. C. (2004) Carbon, sequestration in ecosystems: The role of stoichiometry. *Ecology* **85**: 1179-1192.
- Howarth, R. W., Swaney, D. P., Butler, T. J., and Marino, R. (2000) Climatic control on eutrophication of the Hudson River estuary. *Ecosystems* **3**: 210-215.
- Howarth, R. W., Swaney, D. P., Boyer, E. W., Marino, R., Jaworski, N., Goodale, C. (2006) The influence of climate on average nitrogen export from large watersheds in the Northeastern United States. *Biogeochemistry* **79**: 163-186.
- Hutchinson, G. E. (1938) Chemical Stratification and Lake Morphology. *Proceedings of the National Academy of Sciences of the United States of America* **24**: 63-69.
- Huttunen, J. T., Hammar, T., Alm, J., Silvola, J., and Martikainen, P. J. (2001) Greenhouse gases in non-oxygenated and artificially oxygenated eutrophied lakes during winter stratification. *Journal of Environmental Quality* **30**: 387- 394.
- Huttunen, J. T., Alm, J., Liikanen, A., Juutinen, S., Larmola, T., Hammar, T., Silvola, J., Martikainen, P. J. (2003a) Nitrous oxide flux to the atmosphere from the littoral zone of a boreal lake. *Journal of Geophysical Research – Atmospheres* **108**: ARTN 4421.
- Huttunen, J. T., Alm, J., Liikanen, A., Juutinen, S., Larmola, T., Hammar, T., Silvola, J., Martikainen, P. J. (2003b) Fluxes of methane, carbon dioxide and nitrous oxide in boreal lakes and potential anthropogenic effects on the aquatic greenhouse gas emissions. *Chemosphere* **52**: 609-621.
- IPCC: 2001: Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change [Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P. J., Dai, X., Maskell, K., and Johnson C. A. (eds.)] Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 881 pp.
- IPCC, 2007: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M. and Miller, H.L. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 996 pp.
- Jahnke, R. A., Nelson, J. R., Marinelli, R. L., and Eckman, J. E. (2000) Benthic flux of biogenic elements on the Southeastern US continental shelf: influence of pore water advective transport and benthic microalgae. *Continental shelf research* **20**: 109 -127.

- Joye, S. B. and Paerl, H. W. (1994) Nitrogen cycling in marine microbial mats: Rates and patterns of nitrogen fixation and denitrification. *Marine Biology* **119**: 285-295.
- Kana T.M., Darkangelo C., Hunt M.D., Oldham J.B., Bennett G.E. & Cornwell J.C. (1994) A membrane mass spectrometer for rapid high precision determination of N₂, O₂, and Ar in environmental water samples. *Analytical Chemistry* **66**: 4166–4170.
- Kana, T. M., Sullivan, M. B., Cornwell, J. C., and Groszkowski, K. M. (1998) Denitrification in estuarine sediments determined by membrane inlet mass spectrometry. *Limnology and Oceanography* **43**:334–339.
- Kana, T.M., Cornwell, J. C., Zhong, L. J. (2006) Determination of denitrification in the Chesapeake Bay from measurements of N₂ accumulation in bottom water. *Estuaries and coasts* **29**: 222 -231.
- Kemp W.M. , Sampou P., Caffrey J., Mayer M., Henriksen K., Boynton W. R. (1990) Ammonium recycling versus denitrification in Chesapeake Bay sediments. *Limnology and Oceanography* **35**:1545–1563
- Kim, C., Nishimura, Y., and Nagata, T. (2006) Role of dissolved organic matter in hypolimnetic mineralization of carbon and nitrogen in a large, monomictic lake. *Limnology and Oceanography* **51**: 70–78.
- Knowles, R. (1996) Denitrification: microbiology and ecology. *Life Support and Biosphere Science* **3**: 31–34.
- Kulkarni, M. V., Groffman, P. M., and Yavitt, J. B. (2008) Solving the global nitrogen problem: it's a gas! *Frontiers in Ecology and the Environment* **4**:199–206.
- Laursen, A. E. and Seitzinger, S. P. (2002a) Measurement of denitrification in rivers: an integrated, whole reach approach. *Hydrobiologia* **485**: 67-81.
- Laursen, A. E. and Seitzinger, S. P. (2002b) The role of denitrification in nitrogen removal and carbon mineralization in Mid-Atlantic Bight sediments. *Continental Shelf Research* **22**: 1397-1416.
- Laursen, A. E. and Seitzinger, S. P. (2004) Diurnal patterns of denitrification, oxygen consumption and nitrous oxide production in rivers measured at the whole-reach scale. *Freshwater Biology* **49**: 1448-1458.
- Laursen, A. E. and Seitzinger, S. P. Laursen (2005) Limitations to measuring riverine denitrification at the whole reach scale: effects of channel geometry, wind velocity, sampling interval, and temperature inputs of N₂-enriched groundwater. *Hydrobiologia* **545**:225–236

- Paerl, H. W. (2006) Assessing and managing nutrient-enhanced eutrophication in estuarine and coastal waters: Interactive effects of human and climatic perturbations. *Ecological Engineering* **26**: 40–54.
- Piña-Ochoa, E. and Álvarez-Cobelas, M. (2006) Denitrification in Aquatic Environments: A Cross-system Analysis. *Biogeochemistry* **81**: 111-130.
- Priscu, J. C., Downes, M. T., and McKay, C. P. (1996) Extreme supersaturation of nitrous oxide in a poorly ventilated Antarctic lake. *Limnology and oceanography* **41**: 1544 -1551.
- Priscu, J. C., Christner, B. C., Dore, J. E., Westley, M. B., Popp, B. N., Casciotti, K. L., and Lyons W. B. (2008) Supersaturated N₂O in a perennially ice-covered Antarctic lake: Molecular and stable isotopic evidence for a biogeochemical relict. *Limnology and oceanography* **53**: 2439 -2450.
- Rabalais N. (2002) Nitrogen in aquatic ecosystems. *Ambio* **31**:102–112.
- Redfield, A.C. (1934) On the proportions of organic derivatives in sea water and their relation to the composition of plankton. In R. J. Daniel, ed. *James Johnstone Memorial Volume*, University Press of Liverpool, pp. 176-192.
- Reeburgh, W. S. (1969) Observations of gases in Chesapeake Bay sediments. *Limnology and Oceanography* **14**:368–375.
- Richards, F. A. and Benson, B. B. (1961) Nitrogen/argon and nitrogen isotope ratios in two anaerobic environments, the Cariaco Trench in the Cari~ Sea and Dramsfjord, Norway. *Deep-Sea Research* **7**: 254-264.
- Ryden, J. C., Lund, L. J., Letey, J., and Focht, D. D. (1979) Direct measurement of denitrification loss from soils: II. Development and application of field methods. *Soil Science Society of America Journal* **43**:110–118.
- Rysgaard, S., Risgaard-Petersen, N., Nielsen, L. P., and Revsbech, N. P. (1993) Nitrification and denitrification in lake and estuarine sediments measured by the ¹⁵N dilution technique and isotope pairing. *Applied and environmental microbiology* **59**: 2093 -2098.
- Saunders, D. L., and Kalff, J. (2001a) Nitrogen retention in wetland, lakes and rivers. *Hydrobiologia* **443**:205–212.
- Saunders, D. L., and Kalff, J. (2001b) Denitrification rates in the sediments of Lake Memphremagog, Canada-USA. *Water research* **35**: 1897 -1904.
- Schubert, C. J., Durisch-Kaiser, E., Wehrli, B., Thamdrup, B., Lam, P., and Kuypers, M.M.M. (2006) Anaerobic ammonium oxidation in a tropical freshwater system (Lake Tanganyika). *Environmental Microbiology* **8**: 1857-1863.

- Ledwell, J. R. (1984) The variation of the gas transfer coefficient with molecular diffusivity, in *Gas Transfer at Water Surfaces*, edited by W. Brutsaert and G. H. Jirka, pp. 293-302, D. Reidel. Hingham, Mass.
- Liss, S. P. and Slater, P. G. (1974) Flux of gases across the air-sea interface. *Nature* **247**: 181-184.
- Mackay, D. and Shiu, W. Y. (1981) A Critical-Review of Henrys Law Constants for Chemicals of Environmental Interest. *Journal of physical and chemical reference data* **10**: 1175 -1199.
- Matisoff, G. and Neeson, T. M. (2005) Oxygen Concentration and Demand in Lake Erie Sediments. *Journal of Great Lakes research* **31**: 284 -295.
- McCutchan, J. H., Saunders, J. F., Pribyl, A. L., and Lewis, W. M. (2003) Open-channel estimation of denitrification. *Limnology and Oceanography: Methods* **1**: 74-81.
- Mengis M., Gächter R., Wehrli B., and Bernasconi S. (1997) Nitrogen elimination in two deep eutrophic lakes. *Limnology and Oceanography* **42**:1530-1543.
- Miller, S. A., Landis, A. E., and Theis, T. L. (2006) Use of Monte Carlo Analysis to Characterize Nitrogen Fluxes in Agroecosystems. *Environmental Science and Technology* **40**: 2324-2332.
- Molot, L. A. and Dillon, P. J. (1993) Nitrogen Mass Balances and Denitrification Rates in Central Ontario Lakes. *Biogeochemistry* **20**: 195 -212.
- Murphy, J. and Riley, J. (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27**: 31-.
- National Research Council (1978) Nitrates: an environmental assessment: a report. Scientific and technical assessments of environmental pollutants. Washington, D.C.: National Academy of Sciences.
- National Research Council (2000) Clean coastal waters: understanding and reducing the effects of nutrient pollution. Washington, DC: National Academy Press.
- Nielsen, L. P. (1992) Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microbiology Ecology* **86**: 357-362.
- Nixon, S. W., Granger, S. L., and Nowicki, B. L. (1995) An assessment of the annual mass balance of carbon, nitrogen, and phosphorus in Narragansett Bay. *Biogeochemistry* **31**: 15-61.
- Noges, P., Kangur, K., Noges, T., Reinart, A., Simola, H., and Viljanen, M. (2008) Highlights of large lake research and management in Europe. *Hydrobiologia* **599**: 259 -276.

- Seitzinger, S. P., Nixon, S., Pilson, M.E.Q., and Burke, S. (1980) Denitrification and N₂O production in near-shore marine sediments. *Geochimica et Cosmochimica Acta* **44**: 1853-1860.
- Seitzinger, S. P. (1988) Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. *Limnology and Oceanography* **33**:702-724.
- Seitzinger, S. P., Nielsen, L. P., Caffrey, J., Christensen, P.B. (1993) Denitrification Measurements in Aquatic Sediments: A Comparison of Three Methods. *Biogeochemistry* **23**: 147-167.
- Seitzinger, S. P. and Giblin, A. E. (1996) Estimating denitrification in North Atlantic continental shelf sediments. *Biogeochemistry* **35**: 235-260.
- Seitzinger, S. P., and Kroeze, C. (1998) Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems. *Global Biogeochemical Cycles* **12**: 93-113.
- Seitzinger, S. P., Kroeze, C., and Styles, R.V. (2000) Global distribution of N₂O emissions from aquatic systems: natural emissions and anthropogenic effects. *Chemosphere-Global Change Science* **2**:267-279.
- Seitzinger, S. P., Harrison, J. A., Böhlke, J. K., Bouwman, A. F., Lowrance, R. R., Peterson, B. J., Tobias, C. R., and van Drecht, G. (2006) Denitrification across landscapes and waterscapes: a synthesis. *Ecol Appl* **16**:2064-2090.
- Seitzinger, S. P. (2008) Nitrogen cycle - Out of reach. *Nature* **452**: 162-163.
- Smith, R. A., Schwarz, G. E., and Alexander, R. B. (1997) Regional interpretation of water-quality monitoring data. *Water Resources Research* **33**: 2781- 2798.
- Smith, T. E., Laursen, A. E., and Deacon, J. R. (2008) Nitrogen attenuation in the Connecticut River, northeastern USA; a comparison of mass balance and N-2 production modeling approaches. *Biogeochemistry* **87**: 311 -323.
- Sobota, D. J., Harrison, J. A., and Dahlgren, R. A. (2009) Influences of climate, hydrology, and land use on input and export of nitrogen in California watersheds. *Biogeochemistry* **94**: 43-62.
- Solorzano, L. (1969) Determination of ammonia in natural waters by the phenylhypochlorite method. *Limnology and Oceanography* **14**:799.
- Sørensen, J. (1978) Denitrification rates in a marine sediment as measured by the acetylene inhibition technique. *Applied and environmental microbiology* **36**:139 -143.

- Tomaszek, J. A., Gardner, W. S., and Johengen, T. H. (1997) Denitrification in sediments of a Lake Erie coastal wetland (Old Woman Creek, Huron, Ohio, USA). *Journal of Great Lakes research* 23: 403 -415.
- Tonitto, C., David, M. B. and Drinkwater, L. E. (2009) Modeling N₂O flux from an Illinois agroecosystem using Monte Carlo sampling of field observations. *Biogeochemistry* 93:31–48.
- Wang, W. C., Yung, Y. L., Lacis, A. A., Mo, T., and Hansen, J. E. (1976) Greenhouse effects due to man-made perturbations of trace gases. *Science* 194: 685–690.
- Wang, S. L., Liu C. Q., Yeager, K. M., Wan, G. J., Li, J., Tao, F. X., Lue, Y. C., Liu, F., Fan, C. X. (2009) The spatial distribution and emission of nitrous oxide (N₂O) in a large eutrophic lake in eastern China: Anthropogenic effects. *The Science of the total environment* 407: 3330 -3337.
- Wanninkhof, R. (1992) Relationship between wind speed and gas exchange over the ocean. *Journal of Geophysical Research* 97: 7373-7382.
- Weiss, R. F. (1970) The solubility of nitrogen, oxygen, and argon in water and seawater. *Deep Sea Research* 17: 721–735.
- Wetzel, R.G. 2001. *Limnology: Lake and River Ecosystems*. Third Edition. San Diego: Academic Press.
- Wolfe A. and Patz J.A. (2002) Nitrogen and human health: acute and long-term implications. *Ambio* 31:120–125.
- Wood, E.D., Armstrong, F.A.J. & Richards, F.A. (1967) Determinations of nitrate in sea water by cadmium-copper reduction to nitrite. *Journal of the Marine Biological Association of the United Kingdom* 47:23.
- Yan, W., Laursen, A. E., Wang, F., Sun, P., and Seitzinger, S. P. (2004) Measurement of Denitrification in the Changjiang River. *Environmental Chemistry* 1: 95-98.

④
Bk-61-260