Nitrogen retention in freshwater ecosystems

by

Darla L. Saunders

Department of Biology
McGill University
Montreal, Quebec
Canada

March, 2000

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the Master of Science

0-612-64444-8

Thesis Abstract

Given the prominent role of nitrogen in the eutrophication of aquatic systems, recent increases in nitrogen loading to freshwaters are of concern. A comparison of nitrogen retention in freshwaters in North America and Europe shows that wetlands retain the greatest proportion of their nitrogen load, followed by lakes and then rivers. A comparison of the relative importance of nitrogen retention mechanisms found denitrification to be responsible for the greatest proportion followed by nitrogen sedimentation and then uptake by aquatic plants. A more in-depth examination of denitrification in the littoral sediments of Lake Memphremagog in Quebec, using the N_2 flux technique, found an average denitrification rate of 111 μmol N m^{-2} h^{-1}. Denitrification rates were positively related to the % organic matter of the sediment, temperature and macrophyte biomass density and negatively related to depth. These results, in combination with a review of the literature indicate that denitrification rates are higher in littoral than profundal sediments.

Résumé de Thèse

Étant donné le rôle proéminent de l'azote dans l'eutrophisation des systèms aquatiques, l'augmentation récente de la charge d'azote qui entre dans l'eau douce est inquiétante. Une comparaison de la retention annuelle d'azote dans les eaux douces d'Amérique du Nord et d'Europe démontre que les milieux humides retiennent la proportion la importante de leur charge d'azote, étant suivie par les lacs et les rivères, respectivement. Une comparaison de la contribution relative des méchanismes de retention d'azote a démontré que la dénitrification était la plus importante, étant suivie par la sédimentation et l'entreposage par les plantes aquatiques, respectivement. Une analyse plus intensive de la dénitrification des sédiments littoraux du Lac Memphrémagog au Québec, en utilisant la technique de flux du N_2, a permis de trouver un taux moyen de dénitrification de 111 µmol N m^{-2} h^{-1}. Le taux de dénitrification était en relation positive avec le pourcentage de matière organique dans les sédiments, la temperature et la densité de la biomasse des macrophytes, et en relation négative avec la profondeur. Ces résultats, en combinaison avec une revue de la littérature, indiquent que les taux de dénitrification sont élevés dans les sédiments littoraux comparativement aux sédments profonds.

Table of Contents

List of Tables

Chapter 1. Nitrogen retention in wetlands, lakes and rivers.

Chapter 2. Sediment denitrification rates in Lake Memphremagog, Canada-USA.

List of Figures

Chapter 1. Nitrogen retention in wetlands, lakes and rivers.

Chapter 2. Sediment denitrification rates in Lake Memphremagog, Canada-USA

Preface

The Faculty of Graduate Studies and Research of McGill University requires that the following text be reproduced in full in the preface.

> Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly-duplicated text of one or more published papers. These texts must be bound as an integral part of the thesis.
>
> If this option is chosen, connecting texts that provide logical bridges between the different papers are mandatory. The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, results of a series of papers must be integrated.
>
> The thesis must still conform to all other requirements of the "Guideline for Thesis Preparation". The thesis must include: A Table of Contents, an abstract in English and French, an introduction which clearly states the rational and objectives of the study, a comprehensive review of the literature, a final conclusion and summary, and a thorough bibliography or reference list.
>
> Additional material must be provided where appropriate (e.g. in appendices) and in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.
>
> In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. Supervisors must attest to the accuracy of such statements at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of all the authors of the co-authored papers.

This thesis is written in manuscript style and is currently in review for publication. Chapters 1 and 2 have been submitted to the journals *Hydrobiologia* and *Water Research*, respectively. I was responsible for the field sampling and the laboratory and data analyses. Both the thesis and the manuscript have been written entirely by me. Dr. Jacob Kalff, my supervisor and co-author on the manuscript, provided advice and assistance throughout the development of the project and also critiqued and edited the manuscript.

Acknowledgements

I would like to thank Dr. Jacob Kalff, my supervisor, for giving me the opportunity to become a graduate student in his lab. I have benefited enormously from his patience, kindness and support. His enthusiasm for limnology is contagious and I have learned a great deal from him.

I would also like to thank my other committee members, Dr. Joe Rasmussen and Dr. Neil Price, for their support and guidance. Their comments and criticisms of my thesis at its various stages of development were extremely helpful and I am grateful to have had their input.

Numerous technicians, field assistants and work-study students have assisted me in the field and the laboratory. I would like to thank Christy Deslauriers, Ian Hatton, Audrey Roburn, Alya'a Sammak, Anton Pitts and Myriam Fillion for their assistance. I would also like to thank Dr. Roger Knowles for kindly allowing me to use his laboratory and its equipment for my introduction to gas chromatography. Mike Daoust, of the Department of Chemistry, generously loaned me a gas chromatograph for my first summer of sampling.

While at McGill, I have worked with wonderful a group of graduate students. I would particularly like to thank Adrian deBruyn for his help with statistical analyses, his amazing proof-reading abilities and his advice in times of need. Neil Rooney also deserves special recognition for his support and for his skills as the official and unofficial field station manager.

I would like to thank my parents, Darrell and Rubena, and my sister, Nancy, for their unwavering support and belief in my abilities. It was my father who first taught me about the mystery and magic of the natural world, and thereby inspired in me a lifelong interest in ecology.

Finally I would like to thank Kirk Roach, my partner, for his encouragement and his boundless enthusiasm for science. Not only did he assist me in the lab, several times late into the night, when all other assistants had fled, he did it smiling.

General Introduction

Nitrogen is one of the world's most abundant elements and as a main constituent of protein and genetic material, it is essential to all life. Its abundance is in some ways misleading, however, in that the vast majority of nitrogen is biologically unavailable. Atmospheric N_2 is by far the largest pool of global nitrogen yet it remains unavailable to most organisms except for those prokaryotes capable of nitrogen fixation (Howarth et al., 1988). This paradox of scarcity despite abundance has led many scientists to study nitrogen dynamics in terrestrial, marine and freshwater ecosystems. Limnologists, in particular, have examined the role of nitrogen and phosphorus as limiting nutrients to primary production in freshwater systems (Elser et al., 1990; Downing and McCauley, 1992).

In the last century, humans have approximately doubled the transfer of nitrogen from atmospheric to biologically available pools (Vitousek et al., 1997). This dramatic increase is the direct result of fossil fuel combustion, cultivation of nitrogen-fixing crops and agricultural fertilization. Because many ecosystems have evolved under nitrogen limited conditions, a sudden increase in the amount of biologically available nitrogen has the potential to bring about profound changes. For example, increased nitrogen inputs have been observed to cause shifts in plant and algal species composition, increases in productivity, changes in foodweb structure, and anoxic conditions in aquatic systems (as reviewed by Seitzinger and Kroeze, 1998).

Freshwater systems are subject to increased supplies of nitrogen not only from direct inputs but also from terrestrial runoff. It is currently estimated that 20% of the nitrogen applied to watersheds by humans enters adjacent freshwater systems (Moffat, 1998). Total nitrogen fluxes in riverine systems are thought to have increased from pre-industrial times by 2- to 20-fold (Howarth et al., 1996). Increased loading of nitrogen to freshwaters is of particular concern for several reasons. Ecosystem alterations such as changes in community productivity or composition may occur as a result of elevated nitrogen concentrations. Increased nitrogen loads may also contribute to the acidification of freshwater, directly through nitric acid deposition and also indirectly where biological uptake of ammonium produces hydrogen ions

(Vitousek et al., 1997). Finally, the prominent role of nitrogen in the eutrophication of freshwater systems is cause for concern (Elser et al., 1990; Downing and McCauley, 1992; Jansson et al., 1994; Moffat,1998).

Despite increasing nitrogen loads, local and downstream concentrations of nitrogen are somewhat reduced in freshwaters by natural retention mechanisms. Nitrogen retention is most simply defined as the difference between the amount of nitrogen carried in the inflow and the outflow of a water body. Nitrogen retention, therefore, refers to the nitrogen removed from the water column as it passes through a wetland, lake or river. Three processes contribute to nitrogen retention in freshwaters: denitrification, nitrogen sedimentation and uptake by aquatic plants. Denitrification occurs when facultative anaerobic bacteria use nitrate (NO_3^-) or nitrite (NO_2^-) as terminal electron acceptors in the oxidation of organic matter, thereby producing N_2 or N_2O gas (Knowles, 1982). In this process, nitrogen is removed from the water body through its release into the atmosphere. Nitrogen sedimentation occurs when particulate matter containing nitrogen becomes incorporated into the sediment. Nitrogen can also be removed from the water column through uptake by aquatic plants. This form of nitrogen retention is seasonal however, as plants re-release nitrogen back into the water column at the end of the growing season (Mitsch and Gosselink, 1986).

Nitrogen retention has been studied in numerous freshwater systems (e.g. Andersen, 1974; Knight et al., 1993; Jansson et al., 1994; Mengis et al., 1997). The capacity of freshwaters to retain nitrogen is important for two reasons. First, patterns of nitrogen retention provide some indication of how freshwaters are responding to increased nitrogen inputs. Secondly, freshwater systems often act as buffers between terrestrial and marine systems. This means that they have the potential to reduce nitrogen fluxes to coastal environments which are particularly sensitive to nitrogen eutrophication (Jansson et al., 1994).

In general, researchers have found that nitrogen retention increases with nitrogen loading (Jensen et al., 1990; Gale et al., 1993; Windolf et al., 1996). There are indications, however, that retention efficiency (% of input retained) decreases with very large increases in nitrogen loading (e.g. Nichols, 1983). Reduced retention efficiency may due to the higher hydraulic loads typically associated with large increases in nitrogen loading (Nichols, 1983). Increased hydraulic loads cause shorter water residence times and therefore allow less time for nitrogen removal mechanisms to occur.

The observed relationship between hydraulic loading and nitrogen retention efficiency suggests that freshwater systems with different water flow characteristics have different nitrogen retention capacities. River systems typically flush much more rapidly than do either wetlands or lakes (Ryder and Pesenddorfer, 1989; Mitsch and Gosselink, 1986). Wetlands are characteristically shallow ecosystems, with higher sediment to water ratios than in lakes or rivers (Mitsch and Gosselink, 1986). Despite these recognized differences, there have been no comparisons of the magnitude of nitrogen retention and its component mechanisms among freshwater ecosystems.

Denitrification is typically considered to be the most important mechanism of nitrogen retention in freshwaters (Seitzinger, 1988; Jansson et al., 1994). It is the most complete form of removal in that nitrogen is actually eliminated from the water body and released into the atmosphere. In addition to their role in the nitrogen cycle of aquatic systems, denitrifying bacteria play an important role in the carbon cycle by oxidizing organic matter (Andersen, 1977; Christensen et al., 1990). By reducing atmospheric deposits of nitric acid, denitrifiers can also buffer against lake acidification (Rudd et al., 1990).

Denitrification can be measured in a variety of ways including mass balance modeling, acetylene inhibition, ^{15}N technique and N_2 flux (Christensen and Sørensen, 1986; Seitzinger et al., 1993; van Luijn et al., 1996). In the mass balance method, denitrification is determined by difference. External inputs of nitrogen from streams, watershed runoff, precipitation and, occasionally, nitrogen fixation and groundwater, are quantified, as is the removal of nitrogen through outflow and burial in the sediment (Messer and Brezonik, 1983; Seitzinger, 1988). These two terms, input and output, are then subtracted to calculate an average denitrification rate for the whole system. The other techniques used to measure sediment denitrification do so directly by analyzing sediment cores. Both the acetylene inhibition method and the ^{15}N technique use acetylene or ^{15}N, to label the products of denitrification (Christensen and

Sørensen, 1986; Seitzinger, 1988). This labeling process permits N_2 produced by denitrifiers to be differentiated from atmospheric N_2 gas. Both techniques, however, routinely underestimate denitrification rates (Seitzinger et al., 1993). The N_2 flux technique is the most accurate technique available for measuring denitrification because it requires the least deviation from *in situ* conditions (Nielsen, 1992; Seitzinger et al., 1993; van Luijn et al., 1996). In this method sediment cores are incubated in an environment free of atmospheric N_2 gas thereby permitting N_2 produced by denitrification to be identified.

Direct measurement techniques, such as N_2 flux, have several advantages compared to the mass balance approach. Mass balance modeling is best suited to whole systems comparisons and considerable error may be associated with each estimation of the input and output terms (Seitzinger, 1988). Direct measurement techniques, on the other hand, permit site specific analysis. This means that site specific environmental variables may be measured and tested for their relationship to denitrification rates. In addition, denitrification rates in different areas of a water body, such as the productive littoral zone, can be determined.

The littoral zone is of particular interest to limnologists for a number of reasons. It is in the shallow, shoreline region of lakes that macrophyte beds are found. Macrophytes communities affect a wide range of abiotic factors, including temperature, water flow, nutrient fluxes and sedimentation rates, and also affect biotic interactions (Carpenter and Lodge, 1986; Benoy and Kalff, 1999). The disproportionately high metabolic rates of littoral sediments (den Heyer and Kalff, 1998) point to higher denitrification rates in this zone. This hypothesis is supported by data showing that denitrification rates are higher in vegetated than in unvegetated sediments (e.g. Christensen and Sørensen, 1986; Olsen and Andersen, 1994). However, a study of the nature of the relationship between macrophytes and denitrification is lacking. Further study of denitrification in the littoral zone of freshwater lakes would help to clarify the role of littoral sediments in whole-lake nitrogen fluxes.

The aim of this thesis is to answer several questions related to nitrogen dynamics in freshwater systems. In Chapter 1, I hypothesize that nitrogen retention in freshwater systems is a predictable fraction of the total nitrogen load. To test this notion, I compile a data set of nitrogen retention in freshwater systems in North America and Europe from the literature. I then compare differences in nitrogen retention among three types of freshwater ecosystem: wetlands, lakes and rivers. I also assess the relative importance of different nitrogen retention mechanisms (denitrification, nitrogen sedimentation and uptake by aquatic plants) in these systems. In Chapter 2, I hypothesize that sediment denitrification rates can be predicted using macrophyte and sediment characteristics. I also hypothesize that littoral sediments make a disproportionate contribution to whole lake denitrification. To test these predictions, I measure sediment denitrification rates in the littoral zone of Lake Memphremagog in Quebec, Canada. I examine the relationship of aquatic plant communities and sediment characteristics to denitrification. I also use these findings in combination with data collected from the literature to make predications about the relative contribution of denitrification in littoral and profundal sediments.

References

Andersen, J. M., 1974. Nitrogen and phosphorous budgets and the role of sediment in six shallow Danish lakes. Arch. Hydrobiol. 74: 528-550.

Andersen, J. M., 1977. Importance of the denitrification process for the rate of degradation of organic matter in the sediments. In H. L. Golterman (ed.) Interactions between sediments and fresh water: proceedings of an international symposium held at Amsterdam, the Netherlands, September 6-10, 1976. Dr. W. Junk B. V. Publishers, the Hague. pp. 357-362.

Benoy, G. A. and Kalff, J., 1999. Sediment accumulation and Pb burdens in submerged macrophyte beds. Limnol. Oceangr. 44: 1081-1090.

Carpenter, S. R. and D. M. Lodge., 1986. Effects of submerged macrophytes on ecosystem processes. Aquat. Bot. 26: 341-370.

Christensen, P. B. and J. Sørensen, 1986. Temporal variation of denitrification activity in plant-covered littoral sediment from Lake Hampen, Denmark. Appl. Environ. Microbiol. 51: 1174-1179.

Christensen, P. B., L. P. Nielsen, J. Sorensen and N. P. Revsbech, 1990. Denitrification in nitrate-rich streams: diurnal and seasonal variation related to benthic oxygen metabolism. Limnol. Oceanogr. 35: 640-651.

den Heyer, C. and J. Kalff, 1998. Organic matter mineralization rates in sediments: A within- and among-lake study. Limnol. Oceanogr. 43: 695-705.

Downing, J. A. and E. McCauley, 1992. The nitrogen: phosphorous relationship in lakes. Limnol. Oceangr. 37: 936-945.

Elser, J. J., E. R. Marzolf and C. R. Goldman, 1990. Phosphorous and nitrogen limitation of phytoplankton growth in the freshwaters of North America: A review and critique of experimental enrichments. Can. J. Fish Aquat. Sci. 47: 1468-1477.

Gale, P. M., K. R. Reddy and D. A. Graetz, 1993. Nitrogen removal from reclaimed water applied to constructed and natural wetland microcosms. Wat. Environ. Res. 65: 162-168.

Howarth, R. W., R. Marino, J. Lane and J. J. Cole, 1988. Nitrogen fixation, estuarine, and marine ecosystems. 1. Rates and importance. Limnol. Oceangr. 33: 669-687.

Howarth, R. W., G. Billen, D. Swaney, A. Townsend, N. Jaworski, K. Lajtha, J. A. Downing, R. Elmgren, N. Caraco, T. Jordan, F. Berendse, J. Freney, V. Kudeyarov, P. Murdoch and Z. Zhao-Liang, 1996. Regional nitrogen budgets and riverine N and P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. Biogeochem. 35: 75-139.

Jansson, M., R. Andersson, H. Berggren and L. Leonardson, 1994. Wetlands and lakes as nitrogen traps. Ambio 23: 320-319.

Jensen, J.P., P. Kristensen and E. Jeppesen, 1990. Relationships between nitrogen loading and in-lake nitrogen concentrations in shallow Danish lakes. Verh. int. Ver. Limnol. 24: 201-204.

Knight, R. L., R. W. Ruble, R. H. Kadlec and S. Reed, 1993. Wetlands for wastewater treatment: Performance database. In G. A. Moshiri (ed.) Constructed wetlands for water quality improvement. Lewis Publishers, Ann Arbour, USA. pp. 35-58.

Knowles, R., 1982. Denitrification. Microbiol. Rev. 46: 43-70.

Mengis, M., R. Gächter and B. Wehrli, 1997. Nitrogen elimination in two deep eutrophic lakes. Limnol. Oceanogr. 42: 1530-1543.

Messer, J. and P. L. Brezonik, 1983. Comparison of denitrification rate estimation techniques in a large, shallow lake. Water Res. 17: 631-640.

Mitsch, W. J. and J. G. Gosselink, 1986. Wetlands. Van Nostrand Reinhold Company Inc., New York, USA.

Moffat, A. S., 1998. Global nitrogen overload problem grows critical. Science 279: 988-989.

Nichols, D. S., 1983. Capacity of natural wetlands to remove nutrients from wastewater. J. Wat. Poll. Contr. Fed. 55: 495-505.

Nielsen, L. P., 1992. Denitrification in sediment determined from nitrogen isotope pairing. FEMS Mircobiol. Ecol. 86: 357-362.

Olsen, K. R. and F. Anderson, 1994. Nutrient cycling in shallow, oligotrophic Lake Kvie, Denmark. Hydrobiologia 275/276: 255-265.

Ryder, R. A. and J. Pesendorfer, 1989. Large rivers are more than flowing lakes: a comparative review. In D. P. Dodge (ed.) Proceedings of the International Large River Symposium. Can. Spec. Publ. Fish Aquat. Sci. 106: 65-85.

Rudd, J. W., C. A. Kelly, D. W. Schindler and M. A. Turner, 1990. A comparison of the acidification efficiencies of nitric and sulfuric acids by tow whole-lake addition experiments. Limnol. Oceangr. 35: 663-679.

Seitzinger, S. P., 1988. Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. Limnol. Oceangr. 33: 702-724.

Seitzinger, S. P., L. P. Neilsen, J. Caffrey and P. B. Christensen, 1993. Denitrification measurements in aquatic sediments: A comparison of three methods. Biogeochemistry 23: 147-167.

Seitzinger, S. P. and C. Kroeze, 1998. Global alteration of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems. Global Biogeochem. Cycles. 12: 93-113.

van Luijn, F., P. C. M. Boers and L. Lijklema, 1996. Comparison of denitrification rates in lake sediments obtained by the N_2 flux method, the ^{15}N isotope pairing technique and the mass balance approach. Water Res. 30: 893-900.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger and D. W. Tilman, 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecol. Appl. 7: 737-750.

Windolf, J., E. Jeppesen, J. P. Jensen and P. Kristensen, 1996. Modelling of seasonal variation in nitrogen retention and in-lake concentration: a four-year mass balance study in 16 shallow Danish lakes. Biogeochemistry 33: 25-44.

Chapter 1.

Nitrogen retention in wetlands, lakes and rivers

Abstract

As human activities continue to alter the global nitrogen cycle, the ability to predict the impact of increased nitrogen loading to freshwater systems is becoming more and more important. Nitrogen retention is of particular interest because it is through its combined processes (denitrification, nitrogen sedimentation and uptake by aquatic plants) that local and downstream nitrogen concentrations are reduced. Here we compare the magnitude of nitrogen retention and its components in wetlands, lakes and rivers. We show that wetlands retain the highest proportion of total nitrogen loading, followed by lakes and then rivers. The differences in the proportion of N retained among systems is explained almost entirely by differences in water discharge. Denitrification is the primary mechanism of nitrogen retention, followed by nitrogen sedimentation and uptake by aquatic plants.

Introduction

During the last century human activities have dramatically changed the global nitrogen (N) cycle. Practices such as agricultural fertilization, fossil fuel combustion and the clearing and conversion of land have dramatically increased the supply of nitrogen to freshwaters (Jansson et al., 1994a; Vitousek et al., 1997; Moffat, 1998). Elevated concentrations of nitrogen in freshwater are of concern for several reasons. Nitrogen plays a prominent role in the eutrophication of aquatic systems (Moffat, 1998). Increasing nitrate (NO_3^-) concentrations are of particular concern because of associated human health risks (Focht and Verstraete, 1977). Finally, nitrate is known to contribute to lake acidification (Kelly et al., 1990). Given the negative impact of increasing nitrogen loads, the mechanisms by which freshwater systems can reduce local and downstream nitrogen concentrations are becoming increasingly important.

Nitrogen retention is the difference between N inputs and N outputs to a given freshwater system. Three processes contribute to nitrogen retention: denitrification, sedimentation and uptake by aquatic plants. Denitrification is the process whereby facultative anaerobic bacteria produce N_2 or N_2O gas by using nitrate (NO_3^-) or nitrite (NO_2^-) as terminal electron acceptors (Knowles, 1982). Denitrifying bacteria release N_2 into the atmosphere thereby permanently removing it from aquatic systems. Nitrogen is also retained when particulate matter becomes incorporated into the sediment. Lastly, macrophytes influence nitrogen cycling by taking up and storing nitrogen in their shoots and roots during the growing season (Hill, 1986).

It has been well established that nitrogen retention increases with nitrogen loading in aquatic systems (Jensen et al., 1990; Gale et al., 1993; Jansson et al., 1994a; Windolf et al., 1996). A study by Fleischer and Stibe (1991) found that nitrogen loading was an excellent predictor ($r^2 = 0.94$, $p<0.05$, $n=50$) of nitrogen retention in lakes, rivers and wetlands in Europe. Differences in this relationship among these three types of waterbodies have, however, been largely unexplored. By identifying which systems retain nitrogen most efficiently, management strategies can utilize natural retention capacities more effectively (Jansson et al., 1994a). For example, wetlands are increasingly being used to protect aquatic systems against N-rich wastewaters (Mitsch and Gosselink, 1986).

A comparison of the components of nitrogen retention also has the potential to yield predictions useful for lake management. Denitrification is particularly important as it results in a permanent removal of nitrogen from freshwater. The relative importance of this mechanism, therefore, will determine whether observed nitrogen retention is a long term or seasonal sink.

To address these issues, we examine differences in nitrogen retention among wetlands, lakes and rivers. We further assess the mechanisms of nitrogen retention and their relative importance. Finally, we relate these findings to their impact on ecosystem processes.

Methods

Nitrogen retention

Total nitrogen (TN) retention and loading data were compiled from the literature for 23 wetlands, 23 lakes and 5 rivers in North America and Europe (Table 1-1). All data were taken from mass balance studies in which nitrogen retention was calculated by subtracting total nitrogen (TN) outputs (g m^{-2} y^{-1}) from TN inputs (g m^{-2} y^{-1}). Due to the characteristically high water discharge rates (m^3 s^{-1}) of rivers, the nitrogen loads of these systems were dramatically higher than those of wetlands and lakes. To facilitate comparison among systems, nitrogen load and retention were standardized by dividing by the water discharge. Discharge data were not available for seven sites (Table 1-1).

Components of N retention

To determine the importance of denitrification relative to nitrogen sedimentation and uptake by aquatic plants, we analyze data from TN mass balance studies of lakes in Europe and North America (Table 1-2). Lakes were the only water bodies for which both sedimentation and denitrification rates were readily available.

Results

Nitrogen retention

Total nitrogen loading is an excellent predictor of TN retention for wetlands and lakes (Table 1-3, Figure 1-1). An ANCOVA showed that, on average, wetlands retain approximately twice as much TN as lakes for a given N load ($p<0.001$). The relationship between TN loading and retention was not significant for rivers (Table 3), presumably because the large differences among their discharge results in a highly variable TN loading and water residence time. Regression analysis indicates that, on average, wetlands retain 64% of the TN loading, lakes 34% and rivers 2%. Average water discharge was 0.1 $m^3 s^{-1}$, 0.7 $m^3 s^{-1}$ and 18.6 $m^3 s^{-1}$ in wetlands, lakes and rivers respectively. ANCOVA shows that after standardization to water discharge, the relationship between TN loading and retention is extremely strong (Table 1-3, Figure 1-2). Furthermore, there is no longer a significant difference among wetlands, lakes and rivers in the proportion of N retained (Figure 1-2).

Components of N retention

Denitrification was twice as effective as nitrogen sedimentation in preventing nitrogen from being exported downstream (paired ANOVA, $p<0.005$) (Table 1-3, Figure 1-3). Denitrification accounted for an average 63% of the TN retention, while sedimentation was responsible for 37%.

Discussion

N retention

For a given TN load, wetlands retain almost twice the amount of nitrogen as lakes (Table 1-3, Figure 1-1). In general, the proportion of N retained by rivers is minimal. Once differences in water discharge have been taken into consideration, however, there are no longer significant differences in the nitrogen retention capacities of wetlands, lakes and rivers (Figure 1-2).

The principle reason why water discharge affects the percentage of nitrogen loading retained is that discharge serves as surrogate measure for water residence time.

Water residence time (or renewal rate) is defined here as the ratio of discharge to volume of the system (Mitsch and Gosselink, 1986). The greater the discharge, the shorter the time it will take for a water body to completely flush (Abrahamsson and Håkanson, 1998). Lower discharge rates and longer water residence times provide greater opportunities for sediment-water contact, thereby promoting retention processes such denitrification and sedimentation (Nichols, 1983; Svendsen and Kronvang, 1993; Hammer and Knight, 1994; Windolf et al., 1996; Sand-Jensen, 1998). Increased water residence times in wetlands are due, in part, to the dense stands of aquatic plants that characterize these ecosystems (Brix, 1997; Eriksson and Weisner, 1997; Benoy and Kalff, 1999). Aquatic plants increase nitrogen retention through vegetative uptake and provide favorable conditions for sedimentation and denitrification (Reddy et al., 1989; Brix, 1997; Benoy and Kalff, 1999). The importance of water residence time to nitrogen retention is supported by the strong, positive relationship observed between the two variables in Danish lakes ($r^2=0.79$, $p<0.05$, $n=16$) (Windolf et al, 1996).

Nitrogen retention in freshwater has a significant impact on ecosystem processes and the importance of nitrogen as a limiting nutrient in aquatic systems is increasingly recognized (Elser et al., 1990; Downing and McCauley, 1992). Low N:P loading ratios are characteristic of waste-water and runoff from disturbed catchments (Nichols, 1983; Downing and McCauley, 1992). The receiving waters, therefore, are more likely to be nitrogen limited. Nutrient rich systems characterized by nitrogen limitation commonly have noxious blooms of blue-green algae, resulting in fish kills, beach closures, and increased water treatment costs (Downing and McCauley, 1992; Findlay et al., 1994). While the amount of N relative to P (N:P ratio) is particularly low in wastewater and in runoff from disturbed catchments, absolute loads ($g\ N\ m^{-2}\ y^{-1}$) are higher. Given that N retention increases with N loading, N:P ratios will decrease, further increasing the possibility of nitrogen-fixing cyanobacterial blooms in eutrophic freshwaters (Downing and McCauley, 1992).

Components of N retention

In the lakes examined, denitrification was the primary mechanism (63%) of nitrogen retention (Figure 1-3). A review of 69 shallow, Danish lakes similarly found denitrification to account for the majority (77%) of TN removal (Jensen et al, 1990). Available evidence from other freshwaters supports the conclusion that denitrification is the primary mechanism by which N is removed. Denitrification has been observed to be an order of magnitude larger than sedimentation in both experimental and natural wetlands (Brinson et al., 1984; Van Oostrom, 1995). In the Danish River, Gjern Å, denitrification was calculated to exceed sedimentation on an annual basis by a factor of 2 to 3 (Svendsen and Kronvang, 1993). In general, the proportion of TN retention accounted for by denitrification in rivers must be higher than in lakes. River turbulence is sufficiently high that typically little or no sediment accumulates relative to wetlands and lakes (Ryder and Pesendorfer, 1989). Furthermore, in agricultural areas rivers tend to receive higher proportions of their TN loading as nitrate, which is not subject to significant sedimentation (Jansson et al., 1994a).

The importance of denitrification in freshwaters has implications beyond the process of nitrogen retention. Denitrifying bacteria play an important role in the carbon cycle of aquatic systems by oxidizing organic matter. Wherever NO_3^- is present in concentrations similar to those of dissolved oxygen, denitrification will contribute significantly to the carbon mineralization budget (Christensen et al., 1990; Andersen, 1977). Up to 50% of the carbon mineralized in eutrophic freshwaters has been attributed to denitrifier activity (Andersen et al., 1977). Denitrification can also buffer against lake acidification by reducing nitric acid concentrations (Rudd et al., 1990). With increasing nitric acid additions, denitrification rates have been observed to increase dramatically, whereas other nitrogen retention processes remained the same (Rudd et al., 1990).

Studies of TN retention in freshwaters usually overlook uptake and retention by aquatic plants. This has been justified by the assumption that macrophytes represent a small and temporary nitrogen sink (Reddy and D'Angelo, 1994; Nichols, 1983). While studies calculating nitrogen retention in lakes do not normally take macrophytes into account, TN budgets of wetlands and rivers occasionally do. Such studies provide important information as to the relative importance of vegetative uptake as a retention mechanism. Removal by denitrification (3.0-3.3 g N m^{-2} d^{-1}) was far greater than either sedimentation (0.16-0.27 g N m^{-2} d^{-1}) or plant uptake (0.19-0.33 g N m^{-2} d^{-1}) in three experimental New Zealand wetlands (Van Oostrom, 1995). In a natural floodplain swamp, uptake of nitrogen by vegetation (.3 g m^{-2} d^{-1}) was small in comparison with retention by denitrification (1.3 g m^{-2} d^{-1}) and sedimentation (.6 g m^{-2} d^{-1}) (Brinson et al., 1984). In rivers, the relative importance of vegetative uptake also appears to be small. Macrophyte uptake was calculated to be an order of magnitude lower than other nitrogen retention processes in a lowland Danish river (Svendsen and Kronvang, 1993). Uptake of nitrogen by benthic algae and macrophytes accounted for only 15% of nitrate removal from Duffin Creek, Ontario (Hill, 1979). Researchers studying nitrogen dynamics in a New Zealand stream in concluded that, in the long-term, stream channel vegetation acted primarily to modify nitrogen export rather than retain it (Cooper and Cooke, 1984). It is evident that while the importance of vegetative assimilation varies, it tends to be minor compared to other nitrogen retention processes.

Despite the relatively small contribution of macrophyte uptake as a nitrogen retention mechanism, aquatic plants also affect nitrogen cycling indirectly. By retaining N during the growing season, aquatic plants can influence the growth of phytoplankton by sequestering nitrogen during the period when it is in highest demand. Nutrient assimilation by macrophytes also affects sedimentation rates by contributing particulate matter to sediments during their senescence (Hill, 1986). Aquatic plants increase sedimentation rates by decreasing water velocity and increasing water retention time (Brix, 1997; Eriksson and Weisner, 1997; Sand-Jensen, 1998; Benoy and Kalff, 1999). Finally, macrophytes create an ideal environment for denitrification by increasing the supply of potentially rate-limiting organic carbon and nitrate to denitrifying bacteria (Reddy et al., 1989; Weisner et al., 1994; Brix, 1997). The presence of aquatic plants, therefore, has a significant indirect impact on nitrogen retention in rivers, lakes and wetlands.

Conclusion

As nitrogen loading to freshwater systems increases as a result of human activities, the ability to predict the resulting impact is becoming more and more important. Wetlands retain the highest proportion of total nitrogen loading, followed by lakes and then distantly, by rivers. The observed differences in retention capacity are explained almost entirely by differences in water discharge. The low retention capacities of rivers are of particular concern, because these systems are often subject to high nitrogen loading from agricultural drainage basins and point source loading from urban areas. This also allows them to serve as major sources of nitrogen to downstream lakes and wetlands. The problem has surely been exacerbated by the canalization of rivers and the draining of wetlands for agricultural and other purposes. These activities prevent wetlands from serving as major sites of denitrification.

Our findings show that denitrification is the principal mechanism of nitrogen retention. The majority of nitrogen retained in freshwaters, therefore, will be permanently removed through release of N_2 into the atmosphere. Although nitrogen sedimentation and uptake by aquatic plants are responsible for a smaller proportion of N retention, these processes significantly contribute, both directly and indirectly, to nitrogen cycling in freshwaters.

Acknowledgements

The authors would like to thank Adrian deBruyn, Guillaume Changnon and Neil Rooney for helpful suggestions and critical review of an earlier draft of the manuscript. Christy Deslauriers and Audrey Roburn provided technical assistance. This research was supported by a David Stewart McGill University Fellowship to D.S and grants from NSERC and FCAR to J.K. and J. Rasmussen.

References

Abrahamsson, O. and L. Håkanson, 1998. Modelling seasonal flow variability of European rivers. Ecol. Model. 114: 49-58.

Ahlgren, I., F. Sorensson, T. Waara and K. Vrede, 1994. Nitrogen budgets in relation to microbial transformation in lakes. Ambio 23: 367-377.

Andersen, J. M., 1974. Nitrogen and phosphorous budgets and the role of sediment in six shallow Danish lakes. Arch. Hydrobiol. 74:528-550.

Andersen, J. M., 1977. Importance of the denitrification process for the rate of degradation of organic matter in lake sediments. In H. L. Golterman, (ed.), Interactions between sediments and fresh water: proceedings of an international symposium held at Amsterdam, the Netherlands, September 6-10, 1976. Dr. W. Junk B. V. Publishers, the Hague: 357-362.

Benoy, G. and J. Kalff, 1999. Sediment accumulation and Pb burdens in submerged macrophyte beds. Limnol. Oceanogr. 44: 1081-1090.

Brinson, M. M., H. D. Bradshaw and E. S. Kane, 1984. Nutrient assimilative capacity of an alluvial floodplain swamp. J. Appl. Ecol. 21: 1041-57.

Brix, H., 1997. Do macrophytes play a role in constructed treatment wetlands? Wat. Sci. Tech. 35: 11-17.

Christensen, P. B., L. P. Nielsen, J. Sorensen and N. P. Revsbech, 1990. Denitrification in nitrate-rich streams: diurnal and seasonal variation related to benthic oxygen metabolism. Limnol. Oceanogr. 35: 640-651.

Cooper, A. B. and J. G. Cooke, 1984. Nitrate loss and transformation in 2 vegetated headwater streams. N. Z. J. Mar. Freshwater Res. 18: 441-450.

Devito, K. J., P. J. Dillon and B. D. Lazerte, 1989. Phosphorus and nitrogen retention in five Precambrian shield wetlands. Biogeochemistry 8: 185-204.

Dørge, J., 1994. Modelling nitrogen transformations in freshwater wetlands. Estimating nitrogen retention and removal in natural wetlands in relation to their hydrology and nutrient loadings. Ecol. Model. 75/76: 409-420.

Downing, J. A. and E. McCauley, 1992. The nitrogen: phosphorus relationship in lakes. Limnol. Oceanogr. 37: 936-945.

Elser, J. J., E. R. Marzolf and C. R. Goldman, 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. Can. J. Fish. aquat. Sci. 47: 1468-1477.

Eriksson, P. G. and S. E. B. Weisner, 1997. Wetlands and aquatic processes. J. Environ. Qual. 26: 905-910.

Findlay, D.L., R. E. Heckey, L. L. Hendzel, M. P. Stainton and G. W. Regehr, 1994. Relationship between N_2-fixation and heterocyst abundance and its relevance to the nitrogen budget of Lake 227. Can. J. Fish. aquat. Sci. 51: 2254-2266.

Fleischer, S. and L. Stibe, 1991. Drainage basin management - reducing river transported nitrogen. Verh. int. Ver. Limnol. 24: 1753-1755.

Focht, D. D. and W. Verstraete, 1977. Biochemical ecology of nitrification and denitrification. Advan. Microb. Ecol. 1: 135-214.

Gale, P. M., K. R. Reddy and D. A. Graetz, 1993. Nitrogen removal from reclaimed water applied to constructed and natural wetland microcosms. Wat. Environ. Res. 65: 162-168.

Hammer, D. A. and R. L. Knight, 1994. Designing constructed wetlands for nitrogen removal. Wat. Sci. Tech.29: 15-27.

Hill, A. R., 1979. Denitrification in the nitrogen budget of a river ecosystem. Nature 281: 291-292.

Hill, B. H., 1986. The role of aquatic macrophytes in nutrient flow regulation in lotic ecosystems. In B. G. Isom (ed), Rationale for sampling and interpretation of ecological data in the assessment of freshwater ecosystems, ASTM STP 894. American Society for Testing and Materials, Philadelphia: 157-167.

Jansson, M., R. Andersson, H. Berggren and L. Leonardson, 1994a. Wetlands and lakes as nitrogen traps. Ambio 23: 320-325.

Jansson, M., L. Leonardson and J. Fejes, 1994b. Denitrification and nitrogen retention in a farmland stream in southern Sweden. Ambio 23: 326-331.

Jensen, J.P., P. Kristensen and E. Jeppesen, 1990. Relationships between nitrogen loading and in-lake nitrogen concentrations in shallow Danish lakes. Verh. int. Ver. Limnol. 24: 201-204.

Jensen, J. P., E. Jeppesen, P. Kristensen, P. B. Christensen and M. Søndergaard, 1992. Nitrogen loss and denitrification as studied in relation to reductions in nitrogen loading in a shallow hypertrophic lake (Lake Søbygård, Denmark). Int. Rev. ges. Hydrobiol. 77: 29-42.

Kaushik, N. K., J. B. Robinson, P. Sain, H. R. Whiteley and W. Stammers, 1975. A quantitative study of nitrogen loss from water of a small, spring-fed stream.

Proceeding of the 10th Canadian Symposium 1975 Water Pollution Research Canada: 110-117.

Kelly, C. A., J. W. M. Rudd and D. W. Schindler, 1990. Acidification by nitric acid-future considerations. Wat. Air Soil Pollut. 50: 49-61.

Knight, R. L., R. W. Ruble, R. H. Kadlec and S. Reed, 1993. Wetlands for wastewater treatment: performance database. In Moshiri, G. A. (ed.), Constructed wetlands for water quality improvement. Lewis Publishers, Michigan: 35-58.

Knowles, R, 1982. Denitrification. Microbiol. Rev. 46: 43-70.

Mengis, M., R. Gachter and B. Wehrli, 1997. Nitrogen elimination in two deep eutrophic lakes. Limnol. Oceanogr. 42: 1530-1543.

Messer, J. and P. L. Brezonik, 1983. Comparison of denitrification rate estimation techniques in a large, shallow lake. Wat. Res. 17: 631-640.

Mitsch, W. J. and J. G. Gosselink, 1986. Wetlands. Van Nostrand Reinhold, New York, 722 pp.

Moffat, A. S., 1998. Global nitrogen overload problem grows critical. Science 279: 988-989.

Molot, L. A. and P. J. Dillon, 1993. Nitrogen mass balances and denitrification rates in central Ontario lakes. Biogeochemistry 20: 195-212.

Nichols, D. S., 1983. Capacity of natural wetlands to remove nutrients from wastewater. J. Wat. Poll. Contr. Fed. 55: 495-505.

Olsen, K. R. and F. Anderson, 1994. Nutrient cycling in shallow, oligotrophic Lake

Kvie, Denmark. Hydrobiologia 275/276: 255-265.

Owens, M., J. Garland, I. Hart and G. Wood, 1972. Nutrient budgets in rivers. Symp. zool. Soc. Lond. 29: 21-40.

Reddy, K. R., W. H. Patrick, Jr. and C. W. Lindau, 1989. Nitrification-denitrification at the plant root-sediment interface in wetlands. Limnol. Oceanogr. 34: 1004-1013.

Reddy, K. R. and E. M. D'Angelo, 1994. Soil processes regulating water quality in wetlands. In Mitsch, W. J. (ed.), Global wetlands: old world and new. Elsevier Science, B.V., New York: 309-324.

Rudd, J. W. M., C. A. Kelly, D. W. Schindler and M. A. Turner, 1990. A comparison of the acidification efficiencies of nitric and sulfuric acids by two whole-lake addition experiments. Limnol. Oceanogr. 35: 663-679.

Ryder, R. A. and J. Pesendorfer, 1989. Large rivers are more than flowing lakes: a comparative review. In Dodge, D. P. (ed.) Proceedings of the International Large River Symposium. Can. Spec. Publ. Fish Aquat. Sci. 106: 65-85

Sand-Jensen, K., 1998. Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams. Freshwat. Biol. 39: 663-679.

Seitzinger, S. P., 1986. The effect of pH on the release of phophorous from the Potomac River sediments. Academy of Natural Sciences of Philadelphia, Division of Environmental Research, Report No. 86-8F, Philadephia, 50 pp.

Smith, S. V., S. Serruya, Y. Geifman and T. Berman, 1989. Internal sources and sinks of water, P, N, Ca, and Cl in Lake Kinneret, Israel. Limnol. Oceanogr. 34: 1202-1213.

Svendsen, L. M. and B. Kronvang, 1993. Retention of nitrogen and phosphorus in a

Danish lowland river system: implications for the export from the watershed. Hydrobiologia 251: 123-135.

Van Oostrom, A.J., 1995. Nitrogen removal in constructed wetlands treating nitrified meat processing effluent. Wat. Sci. Tech. 32: 137-147.

Verry, E. S. and D. R. Timmons, 1982. Waterbourne nutrient flow through an upland-peatland watershed in Minnesota. Ecology 63: 1456-1467.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger and D. G. Tilman, 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7: 737-750.

Vollenweider, R. A., 1971. Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. Organisation for economic co-operation and development, Paris, France. 61 pp.

Weisner, S. E. B., P G. Eriksson, W. Granell and L. Leonardson, 1994. Influence of macrophytes on nitrate removal in wetlands. Ambio 23: 363-366.

Windolf, J., E. Jeppesen, J. P. Jensen and P. Kristensen, 1996. Modelling of seasonal variation in nitrogen retention and in-lake concentration: a four-year mass balance study in 16 shallow Danish lakes. Biogeochemistry 33: 25-44.

Table 1-1: Sources of total nitrogen loading and retention data (Figures 1 and 2).
* indicates that water discharge data was not available.

	Site	Location	Source
Lakes	Blue Chalk	Canada	Molot and Dillon, 1993
	Chub	Canada	"
	Crosson	Canada	"
	Dickie	Canada	"
	Harp	Canada	"
	Plastic	Canada	"
	Red Chalk	Canada	"
	* Okeechobee	USA	Messer and Brezonik, 1983
	Bryup Langsø	Denmark	Andersen, 1974
	Kvind	Denmark	"
	Kul	Denmark	"
	Salten Lang	Denmark	"
	Halle	Denmark	"
	Stigsholm	Denmark	"
	Kvie	Denmark	Olsen and Andersen, 1994
	Søbygård	Denmark	Jensen et al., 1992
	Vallentuna	Sweden	Ahlgren et al., 1994
	Norrviken	Sweden	"
	* Hallwilersee	Switzerland	Vollenweider, 1971
	* Pfäffikersee	Switzerland	"
	* Kinneret	Israel	Smith et al., 1989
	Baldegg	Switzerland	Mengis et al., 1997
	Zugg	Switzerland	"

Table 1-1: Continued. Sources of total nitrogen loading and retention data (Figures 1 and 2). * indicates that water discharge data was not available.

	Site	Location	Source
Wetlands	Harp 4-Beaver pond	Canada	Devito et al., 1989
	Plastic-Conifer swamp	Canada	"
	Paint -Sedge fen	Canada	"
	Clermont Plot L	USA	Knight et al., 1993
	Clermont Plot M	USA	"
	Clermont Plot H	USA	"
	Pottsburg Creek	USA	"
	Eastern Service Area1	USA	"
	Cypress Domes	USA	"
	Reedy Creek WTS1	USA	"
	Reedy Creek OFWTS	USA	"
	Ironbridge	USA	"
	Boot	USA	"
	Apalachicola	USA	"
	Boggy Gut	USA	"
	Central Slough	USA	"
	Bear Bay	USA	"
	Hurtsboro	USA	"
	Hamilton	USA	"
	* Marcell Forest Bog	USA	Verry and Timmons, 1982
	* Tarr River Floodplain	USA	Brinson et al., 1984
	* Rabis Baek Riparian Zone	Denmark	Dørge, 1994
	* Syvbaek	Denmark	"
Rivers	Gjern River	Denmark	Svendsen and Kronvang, 1993
	Swift's Brook	Canada	Kaushik et al., 1975
	River Raan	Sweden	Jansson, 1994b
	Potomac River	USA	Seitzinger, 1986
	Great Ouse	England	Owens et al., 1972
	River Trent	England	"

Table 1-2: Sources of denitrification and nitrogen sedimentation data (Figure 3).

Lake	Location	Source
Blue Chalk	Canada	Molot and Dillon, 1993
Chub	Canada	"
Crosson	Canada	"
Dickie	Canada	"
Harp	Canada	"
Plastic	Canada	"
Red Chalk	Canada	"
Okeechobee	Florida	Messer and Brezonik, 1983
Bryup Lang	Denmark	Andersen, 1974
Kvind	Denmark	"
Kul	Denmark	"
Salten Lang	Denmark	"
Halle	Denmark	"
Stigsholm	Denmark	"
Kvie	Denmark	Olsen and Andersen, 1994
Søbygård	Denmark	Jensen et al., 1992
Vallentuna	Sweden	Ahlgren et al., 1994
Norrviken	Sweden	"
Aegerisee	Switzerland	Vollenweider, 1971
Hallwilersee	Switzerland	"
Kinneret	Israel	Smith et al., 1989
Baldegg	Switzerland	Mengis et al., 1997
Zugg	Switzerland	"

Table 1-3: Regression equations describing the relationship between nitrogen retention (x) and load (y) in wetlands, lakes and rivers.

	N	Regression equation	r^2	SEE	p
Wetlands	23	$y = 0.42 + 0.64x$	0.82	13.8	<0.001
Lakes	23	$y = 2.53 + 0.34x$	0.80	8.3	<0.001
Rivers	5	$y = 145.6 + 0.02x$	0.10	206.0	>0.050
Wetlands, lakes and rivers	43	$y = (10^{(1.00\,(\log(x\,/\,\text{water discharge}))-0.39)})/(\text{water discharge})$	0.92	0.4	<0.001

Figure 1-1: Nitrogen retention as a function of nitrogen loading in wetlands and lakes.

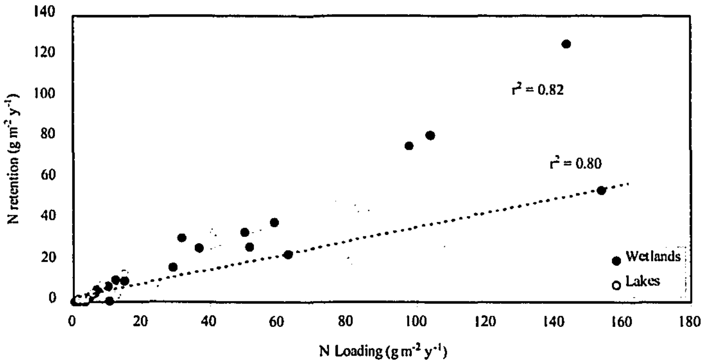

140
120
100
80
60
40
20
0
N retention (g m⁻² y⁻¹)
0
20
40
60
80
100
120
140
160
180
N Loading (g m⁻² y⁻¹)
r² = 0.82
r² = 0.80
● Wetlands
○ Lakes

Figure 1-2: Log nitrogen retention standardized for water discharge as a function of log nitrogen loading standardized for water discharge in wetlands, lakes and rivers. Dotted lines indicate the 95% confidence interval about the mean.

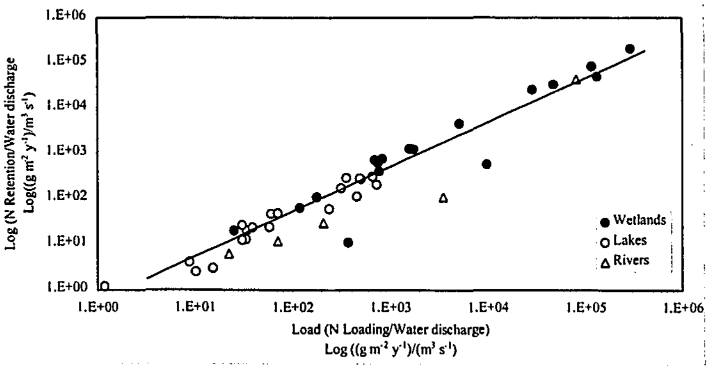
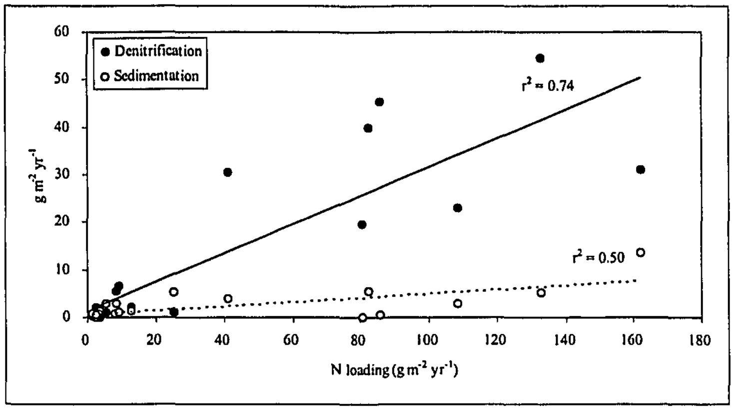

1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
Log (N Retention/Water discharge
Log((g m^{2} y^{-1})/m^{3} s^{-1})
1.E+00
1.E+01
1.E+02
1.E+03
1.E+04
1.E+05
1.E+06
Load (N Loading/Water discharge)
Log ((g m^{-2} y^{-1})/(m^{3} s^{-1})
● Wetlands
○ Lakes
△ Rivers

Figure 1-3: Lake denitrification and nitrogen sedimentation as a function of nitrogen loading.



60
50
40
30
20
10
0
g m⁻² yr⁻¹
● Denitrification
○ Sedimentation
r² = 0.74
r² = 0.50
0
20
40
60
80
100
120
140
160
180
N loading (g m⁻² yr⁻¹)

Chapter 2.

Sediment denitrification rates in Lake Memphremagog, Canada-USA.

Abstract

Denitrification is the most important mechanism of nitrogen retention in aquatic systems. Research into the spatial variability of sediment denitrification has been relatively rare. Here, we use the N_2 flux technique to measure sediment denitrification rates at 19 littoral and 1 profundal site in Lake Memphremagog. Littoral denitrification rates were highly variable with an average rate of 111 μmol N m^{-2} h^{-1}. Littoral denitrification rates were positively related to temperature ($r^2=0.66$, $p<0.01$), % organic matter ($r^2=0.31$, $p<0.05$) and macrophyte biomass density and negatively related to depth. These results in combination with an analysis of the literature and a predictive model created from literature data relating denitrification rates to site depth show that the littoral zone dominates whole lake denitrification.

Introduction

Denitrification plays a major role in the nitrogen (N) cycle of aquatic systems. In this process, facultative anaerobic bacteria transform nitrate or nitrite into nitrogen gas which escapes into the atmosphere (Knowles, 1982). Because nitrogen and phosphorus have the lowest supply: demand ratio of the nutrients found in freshwaters, the removal of nitrogen by denitrifiers can limit primary production (Seitzinger, 1988). Furthermore, the ability of denitrifying bacteria to reduce high levels of ambient N is becoming increasingly important as N loads to freshwater increase due to human activities.

Denitrification primarily occurs in the sediment (Seitzinger, 1988). Sediment denitrification rates are highly variable both among systems (Seitzinger, 1988) and over time within systems (e.g. Christensen and Sørensen, 1986; Olsen and Andersen, 1994; Ahlgren et al., 1994). Research into spatial variation within lakes is rare (Messer and Brezonik, 1983; Ahlgren et al., 1994), with denitrification and other sediment measurements largely restricted to the structurally less complex pelagic zone rather than the littoral zone. Littoral sediments are particularly heterogeneous with respect to numerous abiotic and biotic conditions (Lodge et al., 1988; den Heyer and Kalff, 1998). While this patchiness in sediment structure and metabolism has discouraged littoral zone research, it is likely to influence sediment denitrification rates.

A number of environmental factors have been shown to influence denitrification and may therefore contribute to its spatial variation in freshwater sediments. Bacterial activity is stimulated by an increase in water temperature (e.g. Messer and Brezonik, 1983; van Luijn et al., 1999) and is disproportionately high in warm littoral zones (den Heyer and Kalff, 1998). Both nitrate and organic matter supply can be rate limiting for denitrifiers (Seitzinger, 1988). Sediment redox potential has also been shown to affect denitrification rates, with higher rates in more reduced sediments (van Kessel, 1977). In addition, denitrification rates are typically higher in vegetated than in unvegetated sediments (Christensen and Sørensen, 1986;

Olsen and Andersen, 1994). Aquatic plants can serve as a direct source of organic carbon and as traps for particulate matter from the open water. Furthermore, plant roots release oxygen into the sediment, thereby increasing the sediment redox potential and creating more favorable conditions for nitrate production through nitrification (Christensen and Sørensen, 1986; Ottosen et al., 1999).

The goal of the present study was to measure denitrification rates in the littoral and profundal zone of a single lake and to test the hypothesis that denitrification rates are a function of plant and sediment conditions and decline with depth. To this end, we measured sediment denitrification rates at 19 littoral and 1 profundal site in the Quebec portion of Lake Memphremagog. Cores collected from an additional 6 profundal sites to compare with the littoral samples were lost due to a malfunction of the refrigeration equipment. We chose to use the N_2 flux not only because of its superior accuracy relative to other direct measurement techniques (Christensen and Sørensen, 1986; Seitzinger et al., 1993; van Luijn et al., 1996) but also because it requires the least deviation from in situ conditions. Examining numerous sites in the littoral zone allowed us to determine the spatial variability of sediment denitrification. We focused on littoral sediments because their disproportionately high metabolic rates relative to the profundal zone (den Heyer and Kalff, 1998) suggest that a large proportion of whole lake denitrification occurs in shallow waters.

Methods

Sediment samples were collected from Green Bay and Sargent Bay in the oligotrophic portion of Lake Memphremagog (45°00'N, 72°10'W) located in the Eastern Townships of southern Quebec (Figure 2-1, Table 2-1). The lake is long (45 km) and relatively narrow (1-4 km) and extends from Quebec across the Canada-USA border into Vermont. Lake Memphremagog has been the subject of extensive limnological studies, many of which have focused specifically on the littoral zone (e.g. Duarte and Kalff, 1986; Benoy and Kalff, 1999).

Sampling was conducted from June to August, 1999 by SCUBA divers. Littoral sites (1-3 m) were selected to provide a range of plant densities and sediment characteristics. Prior to sample collection, water depth and temperature were measured. At each site a single 30x30 cm quadrat was placed on the sediment and any macrophytes growing within that area were collected. Two sediment cores were taken from within each quadrat using acrylic core tubes (diameter=6 cm, length=70 cm). The single useable profundal sample was obtained using a gravity corer. Immediately after collection, all samples were brought back to the laboratory for analysis.

Sediment denitrification rates were measured using the N_2 flux technique on intact sediment cores (Seitzinger et al., 1993; Nowicki, 1994). One sediment core from each site was designated as the anoxic 'control' and the second was used as the oxic standard. Because denitrification does not occur under completely anoxic conditions, the anoxic control cores served as a measure of the magnitude of sediment degassing rates, allowing rates of total N_2 flux in the oxic chambers to be corrected for the background flux (Nowicki, 1994). The use of anoxic control cores allowed measurements to begin soon after core collection, thereby eliminating the need for a long pre-incubation degassing period and reducing the deviation from in situ conditions. Denitrification rates were calculated as the N_2 flux from the oxic core (denitrification and sediment degassing) minus the N_2 flux from its anoxic counterpart (sediment degassing).

In the laboratory, the top 5 cm of each sediment core (surface area 25.5 cm^2) was extruded and placed into a gas-tight glass incubation chamber. The chambers were similar to those described by Seitzinger et al. (1980) but had only two stopcocks, a single sampling port and slightly different dimensions (surface area 25.5 cm^2, height 25.5 cm). To ensure that both anoxic and oxic cores had equal N_2 de-gassing rates, all sediments were placed under deep vacuum (approximately 30 Torr) for two minutes prior to incubation. Pressure in the chambers was equalized using a He-filled, gas-tight bag. To ensure that denitrification did not occur in anoxic cores (making sediment degassing the only source of N_2 flux) 4 ml of formalin was injected into each anoxic sediment core (Kaplan et al., 1979). Subsequently, each core was covered with 460 ml of ambient water that had been sparged with either a mixture of 80% He and

20%O_2 (oxic cores) or pure He (anoxic cores) to remove any dissolved N_2. These same gas mixtures were used to flush the 70 ml gas headspace at the top of each incubation chamber.

Measurements of N_2 and O_2 concentrations in the gas phase of each chamber were made by withdrawing gas samples through the sampling ports. Replicate 2 ml samples were withdrawn using a gas-tight syringe which was pre-flushed with He. All gas samples were analyzed using a Varian 4500 gas chromatograph with a thermal conductivity detector and a 3.2 mm stainless steel column (1.8 m) packed with a Å molecular sieve (80-100 mesh size). Denitrification rates are based on headspace samples collected on the fifth day of incubation (48 hours after the last water change and headspace flush).

Sediment cores were incubated at near in situ temperatures (maximum difference of ±3°C; +7°C for the single profundal core). Where incubation temperatures differed from in situ temperatures by more than 1°C, a Q_{10} correction for temperature was applied to measured denitrification rates. We used a Q_{10} value of 2.16, based on the average of reported temperature-based variation (Q_{10} range: 1.0 to 3.4) in freshwater sediment denitrification rates (as reviewed in Seitzinger, 1988; Pfenning and McMahon, 1996; Holmes et al., 1996).

In addition to sediment denitrification rates, several other variables were measured at each site. Macrophytes were rinsed to remove loose sediment and invertebrates. At this time, any attached roots were pinched off. Samples were then spun dry in a lettuce spinner to remove excess water and weighed to determine wet plant biomass. Plant height was calculated as the average height of the dominant macrophyte species and used to calculate plant biomass density (plant biomass/plant height), a measure of the distribution of plant biomass in the water column. After final gas samples were taken from the glass incubation chambers, sediment redox potential (E_h) was measured in the sediments of oxic chambers using an Oakton redox probe. The probe was inserted 2.5 cm into each sediment core and read when the E_h reading stabilized (approx. 10 min). To determine the sediment water content, sediments from the oxic chambers were dried at 75°C until a constant weight was achieved. Triplicate subsamples (2-7 g) of the dried sediment were then burned at 550°C overnight to determine the loss on ignition as an estimate of the organic content of the sediment.

To determine the relationship between sediment denitrification rates and sediment characteristics, regression and principle components analysis were performed using SYSTAT software (Version 8.0, 1998). Where appropriate, variables were log transformed to improve homoscedasticity of the variance. The single profundal core was excluded from these analyses.

Results

Denitrification rates in the littoral sediments of Lake Memphremagog ranged between 8 to 340 μmol N m^{-2} h^{-1}, averaging 111 μmol N m^{-2} h^{-1} (Table 2-2). Summary statistics of site characteristics show that a wide variety of plant biomass densities and sediment types were sampled (Table 2-3).

Individually, only % organic matter ($r^2=0.31$, $p<0.05$) and temperature ($r^2=0.66$, $p<0.01$) were significantly related to denitrification in the littoral zone (Table 2-4, Figures 2-2 and 2-3). The relationship to temperature was sufficiently strong that even rates uncorrected for incubation temperature (Q_{10}) were significantly related ($r^2=0.51$, $p<0.01$). Site variables were then used in combination in an attempt to create stronger predictive relationship. The combination of % organic matter and redox potential ($r^2=0.64$, $p<0.01$) was the only set of variables which produced a significant relationship (Table 2-4), but it was no better than that of temperature alone.

Principle components analysis was used to further explore the relationship between denitrification and environmental factors (Figure 2-4). Factors 1 and 2 explained 30% and 23% of the total variance, respectively. Factor 1 alone captured 82% of the total variance of sediment denitrification while factor 2 contributed a negligible 0.3%. Organic matter content of the sediment, temperature and macrophyte biomass density were positively related to sediment denitrification and water depth was negatively related. Redox and macrophyte biomass were not linked to denitrification rates.

Discussion

The average rate of denitrification measured in the littoral sediments of Lake Memphremagog was surprisingly high. Our rates are comparable to those typically found in shallower and more eutrophic systems (Table 5). In addition, our measurements, taken from 19 littoral sites, show denitrification rates to be highly variable. Only three other studies have expressly measured the spatial variability of sediment denitrification in lakes (Messer and Brezonik, 1983, Ahlgren et al., 1994, Roy et al., 1994). These studies similarly showed denitrification rates to exhibit considerable variation within systems. The variability observed within the sediments of Lake Memphremagog provides an ideal opportunity to explore which environmental factors influence denitrification rates.

Denitrification rates are positively related to temperature in the littoral sediments, with temperature alone explaining 66% of the variation in denitrification (Table 2-4, Figures 2-3 and 2-4). Temperature is long recognized as an important factor in regulating microbial activity in both water and sediment. Denitrification rates, measured in other locations, have also exhibited higher rates at warmer temperatures (Seitzinger, 1988). The strong effect noted here over a very small range (18-24°C) is a particularly convincing testimony to the importance of temperature.

The increase in denitrification with increasing sediment organic matter content (Figures 2-2 and 2-4) has been previously reported for denitrifying bacteria (Seitzinger, 1988; van Luijn et al, 1999). In general, heterotrophic bacterial production has a strong positive relationship with sediment organic matter (Sander and Kalff, 1993). It is argued that organic matter influences denitrification rates because its mineralization supplies the ammonia for nitrification which in turn increases nitrate supply (Seitzinger, 1988). But, as denitrifying bacteria also mineralize organic matter as part of their metabolic processes (Knowles, 1982), organic matter may also influence denitrification rates more directly. Sediments that are rich in organic matter typically have lower redox potentials and indeed, these variables are significantly related in the littoral sediments of Lake Memphremagog ($r^2=0.53$, $p<0.01$). While the addition of redox potential improved the regression of organic matter and denitrification (Table 2-4), redox alone was not related to denitrification rates (Figure 2-4). This suggests that redox contributed to the predictive model of denitrification by explaining some of the variation associated with sediment organic matter.

In addition to the relationships revealed by regression analysis, PCA showed that macrophyte biomass density is related to sediment denitrification (Figure 2-4). Denitrification rates are typically higher in sediments with plants than those without (Christensen and Sørensen, 1986; Olsen and Andersen, 1994). Not only do aquatic plants supply denitrifiers with organic carbon, a potentially rate limiting energy source, but their biomass density is a excellent indicator of the rate of sedimentation (Benoy and Kalff, 1999). Moreover, macrophytes create more favorable conditions for nitrification, and subsequently denitrification, by releasing oxygen into the sediment through their roots (Ottosen et al., 1999). Macrophyte density, in particular, may be important because the compact plant forms characterized by high biomass densities have been observed to stimulate rhizosphere coupled nitrification-denitrification (Ottosen et al., 1999).

Despite the various ways in which macrophytes are known to affect sediment denitrification, neither macrophyte biomass nor biomass density were significant related to denitrification in our regression analysis. The importance of these two environmental variables appears to be masked by the strong influence of temperature. It should also be noted that the above-sediment portion of the plants was removed prior to incubation, which might have reduced rhizosphere coupled nitrification-denitrification. Finally, macrophyte biomass density was calculated only for the small area from which sediment cores were collected. The best predictor of sedimentation rates, however, is the average biomass density of the entire macrophyte bed (Benoy and Kalff, 1999) which integrates small scale differences among cored sites. The significant regression between organic matter and denitrification appear to reflect the importance of plants to sediment denitrification.

It is remarkable that even over a 2 m depth range, site depth and sediment denitrification were negatively related (Figure 2-4). Site depth is strongly and negatively correlated with organic mineralization rates in a number of lakes near Lake Memphremagog (den Heyer and Kalff, 1998). That denitrification in the present study is less strongly linked with depth is surely due to the vastly smaller depth range (1-3 m) than those examined for mineralization rates (1-35 m).

Site depth can be expected to be negatively linked to denitrification for several reasons. Profundal lake sediments are typically colder than their shallow counterparts, particularly in stratified temperate lakes. Furthermore, profundal sediments are composed of particles that experience a much longer transit time in the water column and are therefore subject to greater organic matter decomposition and nutrient recycling than their littoral counterparts (den Heyer and Kalff, 1998). This yields a lower quality and quantity of nitrogen and organic matter in sediment particles in the profundal zone.

Assuming the demonstrated link between depth and sediment metabolism to be at least roughly indicative of the pattern in denitrification, the littoral denitrification rates in Lake Memphremagog and elsewhere can be expected to be higher in the warm littoral than those in the cold profundal zone. Supporting this view is the observation that our littoral rates are among the highest in the literature, which is largely based on whole lake or profundal measurements (Table 2-5). Our single profundal sample (20 m) is, furthermore, among our lowest measured (Table 2-2).

The notion that denitrification rates are typically much higher in littoral than profundal sediments is supported by the literature. Sediment cores collected from the deepest part of Lake Baldegg, Switzerland (66 m) yielded denitrification rates ranging from 38 to 50 μmol N m^{-2} h^{-1} (Mengis et al., 1997). Yet, a mass balance of the lake as a whole reported an average denitrification rate of 254 μmol N m^{-2} h^{-1} (Mengis et al., 1997). The authors proposed the discrepancy to be due to much higher rates of sediment denitrification in the littoral zone. Secondly, a direct comparison of denitrification rates in the profundal and littoral sediments of Lake Norrviken, Sweden (z_{max}=11 m) reported littoral denitrification to be significantly higher (Ahlgren et al., 1994). Less direct evidence for higher littoral denitrification comes from a literature analysis of mass balance data showing that shallow freshwater systems retain a higher proportion of their total nitrogen load and experience higher rates of denitrification than deeper systems (Saunders and Kalff, in review).

A predictive model developed from the combined findings of two separate studies provides yet further evidence for the disproportionate importance of littoral denitrification. Seitzinger (1994) found denitrification rates in freshwater wetlands to be strongly correlated with sediment oxygen consumption (SOC) rates (r^2=0.91, n=15) while Campbell (1984) noted SOC rates to be significantly related to site depth (r^2=0.42, n=59). If the intercept of the relationship between SOC and denitrification in wetlands is set to zero, the two predictive relationships can be combined and the rate of sediment denitrification at varying lake depths estimated (Figure 2-5). While the combination of the two models can only be exploratory, the resulting predictive model provides an indication of how denitrification rates can be expected to change with depth. As denitrifying bacteria are affected by many of the same constraints (e.g. temperature, organic matter, nutrients) that influence bacterial metabolism in general, it is likely that a pattern of decreasing SOC with site depth also applies to sediment denitrification. Indeed, the relationship observed by den Heyer and Kalff (1998) between depth and organic carbon mineralization rates in a series of lakes near Memphremagog is almost identical to the denitrification model (Figure 2-5). Lastly, the average littoral and single profundal denitrification rates measured in Lake Memphremagog are well predicted by the two models (Figure 2-5). Our data combined with those from the literature support the conclusion drawn for sediment metabolism (den Heyer and Kalff, 1998) that littoral rates are higher than their profundal counterparts and that most whole lake denitrification occurs in the littoral zone.

Conclusion

In summary, littoral zone denitrification rates (1-3 m) in oligotrophic Lake Memphremagog, averaging 111 μmol N m^{-2} h^{-1}, are approximately 10-fold greater than the average rate calculated for oligotrophic lakes in the literature (Table 2-5). The measured littoral and single profundal rates are of the same magnitude as the rates predicted using a denitrification model created from literature data (Figure 2-5). The modeled decline with depth is supported by the only two other studies that measured or calculated both a littoral and profundal denitrification rate. Even within the littoral there are indications of decline in denitrification with depth (Figure 2-4). However, over the 2 m littoral zone depth range, water temperature was the single best indicator of denitrification (r^2=0.66, Figure 2-2) followed by organic matter (r^2=0.31, Figure 2-3). These results, combined with an analysis of the literature, allow the conclusion that littoral zones make a disproportionately high contribution to whole lake denitrification.

Acknowledgements

The authors would like to thank Adrian deBruyn and Neil Rooney for helpful suggestions and critical review of an earlier draft of the manuscript. Christy Deslauriers, Ian Hatton, Audrey Roburn, Alya'a Sammak, Anton Pitts, Myriam Fillion and Kirk Roach provided technical assistance. Roger Knowles and Mike Daoust provided gas chromatography equipment. This research was supported by a David Stewart McGill University Fellowship to D.S. and grants from NSERC and FCAR to J.K. and J. Rasmussen.

References

Ahlgren I., F. Sorensson, T. Waara and K. Vrede, 1994. Nitrogen budgets in relation to microbial transformation in lakes. Ambio 23: 367-377.

Andersen, J. M., 1971. Nitrogen and phosphorus budgets and the role of the sediments in six shallow Danish lakes. Arch. Hydrobiol. 74: 528-550.

Benoy, G. and J. Kalff, 1999. Sediment accumulation and Pb burdens in submerged macrophyte beds. Limnol. Oceanogr. 44: 1081-1090.

Campbell, P. J., 1984. Laboratory measurements and predictions of sediment oxygen consumption. M. Sc. Thesis. Biology Dept., McGill University, Montreal, Canada.

Chan, Y. K. and N. E. R. Campbell, 1980. Denitrification in Lake 227 during summer stratification. Can. J. Fish. Aquat. Sci. 37: 506-512.

Christensen, P. B. and J. Sørensen, 1986. Temporal variation of denitrification activity in plant-covered littoral sediment from Lake Hampen, Denmark. Appl. Environ. Microbiol. 51: 1174-1179.

den Heyer, C. and J. Kalff, 1998. Organic matter mineralization rates in sediments: A within- and among-lake study. Limnol. Oceanogr. 43: 695-705

Duarte, C. M. and J. Kalff, 1986. Littoral slope as a predictor of the maximum biomass of submerged macrophyte communities. Limnol. Oceangr. 31: 1072-1080.

Gardner, W. S., T. F. Nalepa and J. M. Malczyk, 1987. Nitrogen mineralization and denitrification in Lake Michigan sediments. Limnol. Oceangr. 32: 1226-1238.

Höhener, P. and R. Gächter, 1993. Prediction of dissolved inorganic nitrogen (DIN) concentrations in deep, seasonally stratified lakes based on rates of DIN input and N removal processes. Aquat. Sci. 55: 112-131.

Holmes, R. M., J. B. Jones, Jr., S. G. Fisher and N. B. Grimm, 1996. Denitrification in a nitrogen-limited stream ecosystem. Biogeochemistry 33: 125-146.

Jensen J. P., E. Jeppesen, P. Kristensen, P. B. Christensen and M. Søndergaard, 1992. Nitrogen loss and denitrification as studied in relation to reductions in nitrogen loading in a shallow hypertrophic lake (Lake Søbygård, Denmark). Int. Rev. ges. Hydrobiol. 77: 29-42.

Kaplan, W., I. Valiela and J. M. Teal, 1979. Denitrification in a salt marsh ecosystem. Limnol. Oceangr. 24: 726-734.

Knowles, R., 1982. Denitrification. Microbiol. Rev. 46: 43-70.

Lodge, D. M., J. W. Barko, D. Strayer, J. M. Melack, G. G. Mittlebach, R. W. Howarth, B. Menge and J. E. Titus, 1988. Spatial heterogeneity and habitat interactions in lake communities. In S. R. Carpenter (ed.) Complex interactions in lake communities. Springer, New York. pp.181-208.

Mengis M., R. Gachter and B. Wehrli, 1997. Nitrogen elimination in two deep eutrophic lakes. Limnol. Oceanogr. 42: 1530-1543.

Messer, J. and P. L. Brezonik, 1983. Comparison of denitrification rate estimation techniques in a large, shallow lake. Wat. Res. 17: 631-640.

Molot, L. A. and P. J. Dillon, 1993. Nitrogen mass balances and denitrification rates in central Ontario lakes. Biogeochemistry 20: 195-212.

Nowicki, B. L., 1994. The effect of temperature, oxygen, salinity, and nutrient enrichment on estuarine denitrification rates measured with a modified nitrogen gas flux technique. Estuar. Coast. Shelf S. 38: 137-156.

Olsen, K. R. and F. Anderson, 1994. Nutrient cycling in shallow, oligotrophic Lake Kvie, Denmark. Hydrobiologia 275/276: 255-265.

Ottosen, L. D. M., N. Risgaard-Petersen and L. P. Neilsen, 1999. Direct and indirect measurements of nitrification and denitrification in the rhizosphere of aquatic macrophytes. Aquat. Microb. Ecol. 19: 81-91.

Pfenning, K. S. and P. B. McMahon, 1996. Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments. Jour. Hydrol. 187: 283-295.

Roy, R., P. Legende, R. Knowles and M. N. Charlton, 1994. Microbial. Ecology. 27: 123-141.

Sander, B. C. and J. Kalff, 1993. Factors controlling bacterial production in marine and freshwater sediments. Microb. Ecol. 26: 79-99.

Saunders D. L. and J. Kalff, in review. Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia.

Seitzinger, S. P., S. Nixon, M. E. Q. Pilson and S. Burke, 1980. Denitrification and N_2O production in near-shore marine sediments. Geochimic et Cosmochimica Acta. 44: 1853-1860.

Seitzinger S. P., 1988. Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. Limnol. Oceanogr. 33: 702-724.

Seitzinger, S. P., L. P. Neilsen, J. Caffrey and P. B. Christensen, 1993. Denitrification measurements in aquatic sediments: A comparison of three methods. Biogeochemistry 23: 147-167.

Seitzinger S. P., 1994. Linkages between organic matter mineralization and denitrification in eight riparian wetlands. Biogeochemistry 25: 19-39.

van Kessel, J. F., 1977. Factors affecting the denitrification rate in two water sediment systems. Wat. Res. 11: 259-267.

van Luijn, F., P. C. M. Boers and L. Lijklema, 1996. Comparison of denitrification rates in lake sediments obtained by the N_2 flux method, the ^{15}N isotope pairing technique and the mass balance approach. Wat. Res. 30: 893-900.

van Luijn, F., P. C. M. Boers, L. Lijklema and J. P. R. A. Sweerts, 1999. Nitrogen fluxes and processes in sandy and muddy sediments from a shallow eutrophic lake. Wat. Res. 33: 33-42.

Table 2-1: Characteristics of Lake Memphremagog, Sargent Bay, Quebec.

Characteristic	
Mean Depth (m)	20
Maximum Depth (m)	107
Total Phosphorous (μg/L)	15
Total Nitrogen (μg/L)	280

Table 2-2: Denitrification rates in sediments of Lake Memphremagog.

Site	Depth (m)	Denitrification μmol N m^{-2} h^{-1}
1	1.0	8
21	1.0	312
2	1.2	16
5	2.1	88
6	2.1	59
3	2.5	39
16	2.7	122
7	2.5	15
8	2.5	52
9	2.5	116
10	2.5	73
11	2.5	128
12	2.5	142
18	2.5	340
19	2.5	182
22	2.5	52
23	2.5	124
20	3.0	178
13	3.4	69
4	20.0	15

Table 2-3: Summary statistics for the littoral sites in Lake Memphremagog.

Variable	Mean	Minimum	Maximum	SD
Plant Biomass (g wet weight m^{-2})	328	0	1292	376
Plant Biomass Density (g m^{-3})	1532	0	8615	1913
E_h	-307	-462	-54	118
% water content	46	20	71	21
% organic matter	8	1	25	7
In situ temperature (°C)	22	18	24	0.1
Depth	3	1	2	1

Table 2-4: Predictive relationship of sediment denitrification rates (y) to % organic matter (OM), temperature (T) and redox potential (Eh).

Variable	Relationship	r^2	p
% Organic matter	$\ln(y) = 4.07 + 0.06 \cdot OM$	0.31	<0.05
Temperature	$\ln(y) = -5.12 + 0.43 \cdot T$	0.66	<0.01
% Organic matter and Redox	$\ln(y) = 5.00 + 0.13 \cdot OM + 0.005 \cdot Eh$	0.64	<0.01

Table 2-5: Denitrification rates (μmol N m^{-2} h^{-1}) in lake sediments. Trophic status of the lake - o: oligotrophic, m: mesotrophic, e: eutrophic. Method – NF: (N-mineralization – (N-assimilation + N-sediment-water exchange)), N2: N_2 flux method, AI: acetylene inhibition, 15N: ^{15}N technique, MB: mass balance. References - [1]reviewed in Seitzinger, 1988; [2]reviewed by van Luijn, 1997

Lake	Location	z_{mean} (m)	Trophic Status	Method	Denitrification (μmol N m^{-2} h^{-1})	Reference
Kvie	Denmark	1	o	NF	17-58	Olsen and Andersen, 1994
Ägerisse	Switzerland	49	o	MB	7	as reviewed in Höhener and Gächter, 1993
Türlersee	Switzerland	14	o	MB	23	“
Blue Chalk	Canada	8	o	MB	7	Molot and Dillon, 1993
Chub	Canada	9	o	MB	1	“
Crosson	Canada	9	o	MB	1	“
Dickie	Canada	5	o	MB	9	“
Harp	Canada	13	o	MB	13	“
Plastic	Canada	8	o	MB	2	“
Red Chalk	Canada	14	o	MB	5	“
Memphremagog	Canada	20	o	N2	8-340	This study
Michigan	US	84	o	N2	12-51	Gardner *et al.*, 1987[1]
Lacawac	US	-	o-m	N2	50	Seitzinger, 1988
Ernest	US	-	o-m	N2	56	"
Hampen	Denmark	-	o-m	AI	5-50	Christensen and Sørensen, 1986[1]
Hampen	Denmark	-	o-m	^{15}N	24-30	Ottosen *et al.*, 1999
Boden-Obersee (Constance)	Austria, Germany, Switzerland	99	m	MB	125	as reviewed in Höhener and Gächter, 1993
Okeechobee	US	3	e	AI	2-25	Messer and Brezonik, 1983[1]
ELA 227	Canada	4	e	^{15}N	42-58	Chan and Campbell, 1980[1]
Nuldernauw	Netherlands	2	e	N2	42-214	van Luijn, 1996

Table 2-5: Continued. Denitrification rates (μmol N m^{-2} h^{-1}) in lake sediments. Trophic status of the lake - o: oligotrophic, m: mesotrophic, e: eutrophic. Method – NF: (N-mineralization – (N-assimilation + N-sediment-water exchange)), N2: N_2 flux method, AI: acetylene inhibition, 15N: ^{15}N technique, ND: NO_3^- decrease, MB: mass balance. References - [1]reviewed in Seitzinger, 1988; [2]reviewed by van Luijn, 1997

Lake	Location	z_{mean}	Trophic Status	Method	Denitrification (μmol N m^{-2} h^{-1})	Reference
Pfäffiker	Switzerland	17	e	MB	68	as reviewed in Höhener and Gächter, 1993
Greifen	Switzerland	19	e	MB	168-306	"
Baldegger	Switzerland	33	e	MB	74-200	"
Sempacher	Switzerland	44	e	MB	82	"
Alpnacher	Switzerland	21	e	MB	37	"
Baldegg	Switzerland	33	e	MB	254	Mengis et al., 1997
Zugg	Switzerland	84	e	MB	46	"
Vilhelmsborg	Denmark	-	-	N2	191-343	Seitzinger et al., 1993
Søbygärd	Denmark	1	e	MB	324-637	Jensen et al., 1992
Bryup Langsø	Denmark	2	e	MB	326	Andersen, 1971[1]
Kvind	Denmark	2	e	MB	244-260	"
Kul	Denmark	2	e	MB	163-195	"
Salten Langsø	Denmark	4	e	MB	0-18	"
Halle	Denmark	3	e	MB	359-383	"
Stigsholm	Denmark	1	e	MB	148-171	"
Vallentuna	Sweden	3	e	MB	5	Ahlgren et al., 1994
Norrviken	Sweden	5	e	MB	8	"

Figure 2-1: Map of Lake Memphremagog, Canada-USA (45°00'N, 72°10'W). The smaller box encloses the study sites: Green Bay and Sargent Bay.

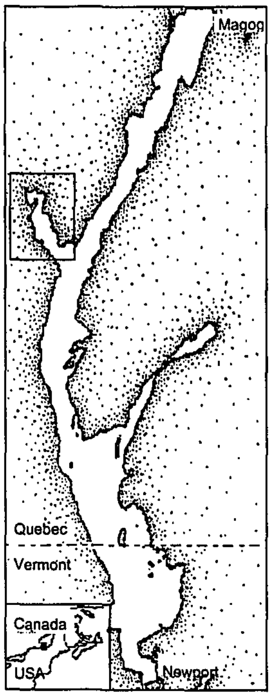

Magog
Quebec
Vermont
Canada
USA
Newport

Figure 2-2: Sediment denitrification (μmol N m^{-2} h^{-1}) as a function of % organic matter of the sediment in the littoral zone of Lake Memphremagog.

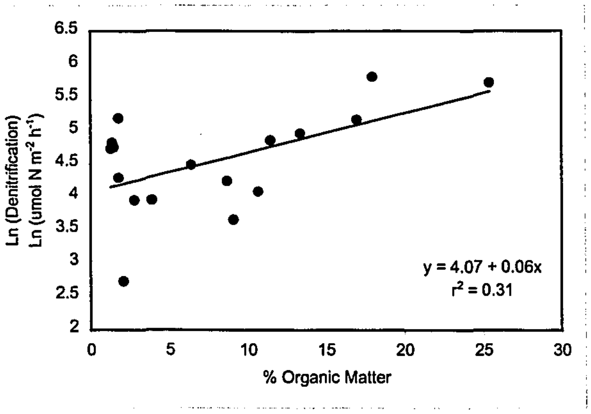

6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
Ln (Denitrification)
Ln (umol N m-2 h-1)
0
5
10
15
20
25
30
% Organic Matter
y = 4.07 + 0.06x
r2 = 0.31

Figure 2-3: Sediment denitrification (μmol N m^{-2} h^{-1}) as a function of *in situ* temperature (°C) in the littoral zone of Lake Memphremagog.

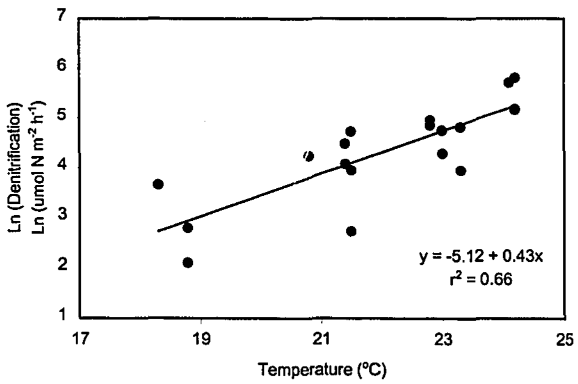

7
6
5
4
3
2
1
Ln (Denitrification)
Ln (umol N m-2 h-1)
17
19
21
23
25
Temperature (°C)
y = -5.12 + 0.43x
r2 = 0.66

Figure 2-4: PCA of site characteristics of littoral sediment in Lake Memphremagog.

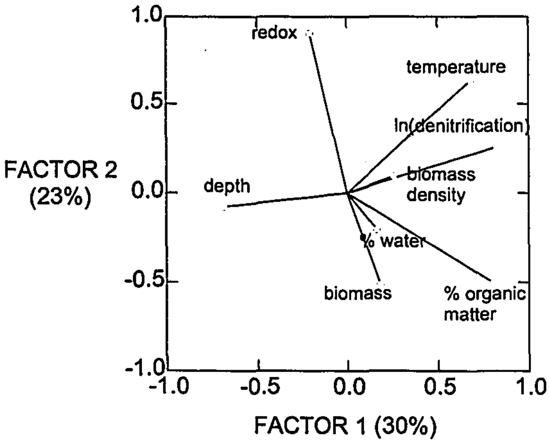
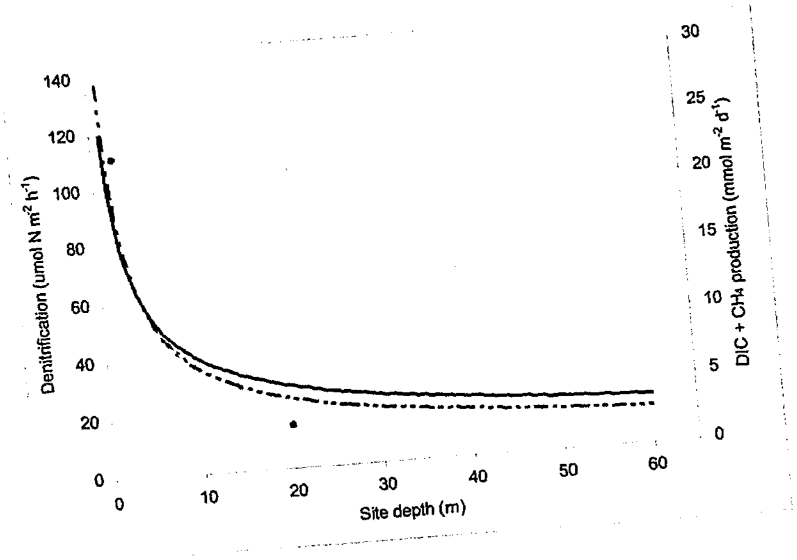

1.0
0.5
0.0
-0.5
-1.0
FACTOR 2 (23%)
-1.0
-0.5
0.0
0.5
1.0
FACTOR 1 (30%)
redox
temperature
ln(denitrification)
biomass density
depth
% water
biomass
% organic matter

Figure 2-5: Sediment denitrification and carbon release rates versus site depth. The solid line represents the estimated relationship between denitrification and site depth based on reported relationship between sediment denitrification and sediment oxygen consumption rate (SOC) (Seitzinger, 1994) (r^2=0.78, n=13, denitrification = -0.0658*(SOC); where SOC is measured in μmol m^{-2} h^{-1}) and the relationship between oxygen consumption rate and site depth (Campbell, 1984) (r^2=0.42, n=59, log(SOC/(1000*24))=1.64 - 0.48 * log(site depth); where SOC is measure in mmol m^{-2} d^{-1}). The dotted line represents the reported relationship between carbon release rates and site depth (den Heyer and Kalff, 1998) (r^2=0.62, n=25, $y=29.5*x^{-0.58}$). ● represents the average littoral and single profundal sediment denitrification rates measured in this study.


Denitrification (umol N m-2 h-1)
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
Site depth (m)
DIC + CH4 production (mmol m-2 d-1)
30
25
20
15
10
5
0

Appendices

Appendix A. Nitrogen loading and retention data

Appendix A consists of the nitrogen loading (g N m^{-2} y^{-1}), nitrogen retention (g N m^{-2} y^{-1}) and water discharge (m^3 s^{-1}) data for the wetlands, lakes and rivers in North America and Europe that were used in Chapter 1 (Figures 1-1 and 1-2). References for these sites are presented in Table 1-1 of Chapter 1.

System	Site	Nitrogen Load ($g\ N\ m^{-2}\ y^{-1}$)	Nitrogen Retention ($g\ N\ m^{-2}\ y^{-1}$)	Water Discharge ($m^3\ s^{-1}$)
Lakes	Blue Chalk	1.6	1.2	0.026
	Chub	2.8	1.1	0.048
	Crosson	3.5	1.3	0.103
	Dickie	2.8	1.6	0.083
	Harp	3.8	2.2	0.096
	Plastic	1.5	1.0	0.021
	Red Chalk	3.2	1.3	0.105
	Okeechobee	5.6	4.0	-
	Bryup Lang	82.6	45.4	0.169
	Kvind	162	44.5	0.223
	Kul	108.5	25.8	0.238
	Salten Lang	25.2	6.1	2.452
	Halle	85.8	45.9	0.274
	Stigsholm	80.7	19.5	0.337
	Kvie	2.7	2.1	0.008
	Søbygård	132.6	59.8	0.201
	Vallentuna	2.4	1.1	0.270
	Norrviken	8.2	1.6	0.536
	Hallwilersee	12.8	3.5	-
	Pfõffikersee	14.7	13.5	-
	Baldegg	40.9	34.4	1.338
	Zugg	8.5	8.5	7.197
Wetlands	Harp 4-Beaver pond	3.4	0.1	0.0092
	Plastic-Conifer swamp	1.5	0.0	-
	Paint -Sedge fen	10.5	0.6	0.0011
	Clermont Plot L	6.0	4.3	0.0001
	Clermont Plot M	14.8	10.3	0.0003
	Clermont Plot H	36.8	26.2	0.0001
	Pottsburg Creek	29	16.9	0.1625
	Eastern Service Area1	4.7	2.4	0.0394
	Cypress Domes	12.2	10.9	0.0004
	Reedy Creek WTS1	104.1	81.4	0.1400
	Reedy Creek OFWTS	143.7	126.5	0.0280
	Ironbridge	10.0	7.7	0.3960
	Boot	5.2	2.7	0.0103
	Apalachicola	31.7	31.3	0.0456
	Boggy Gut	50.0	34.2	0.0290
	Central Slough	98.0	75.9	0.0622
	Bear Bay	7.0	6.2	0.0085
	Hurtsboro	62.8	23.5	0.0005
	Hamilton	0.2	0.1	0.0003
	Marcell Forest Bog	1.3	0.6	-

System	Site	Nitrogen Load (g N m^{-2} y^{-1})	Nitrogen Retention (g N m^{-2} y^{-1})	Water Discharge (m^3 s^{-1})
Wetlands	Tarr River Floodplain	51.6	27.1	-
	Rabis Baek Riparian Zone	58.9	39.0	-
	Syvbaek	153.8	54.2	-
Rivers	Gjern River	75.6	12.1	1.06
	Swift's Brook	229.8	123.3	0.00
	River Raan	6333.3	190.0	1.80
	Potomac River	61.8	33.5	-
	Great Ouse	1936.0	267.0	9.26
	River Trent	1810.0	498.4	81.00

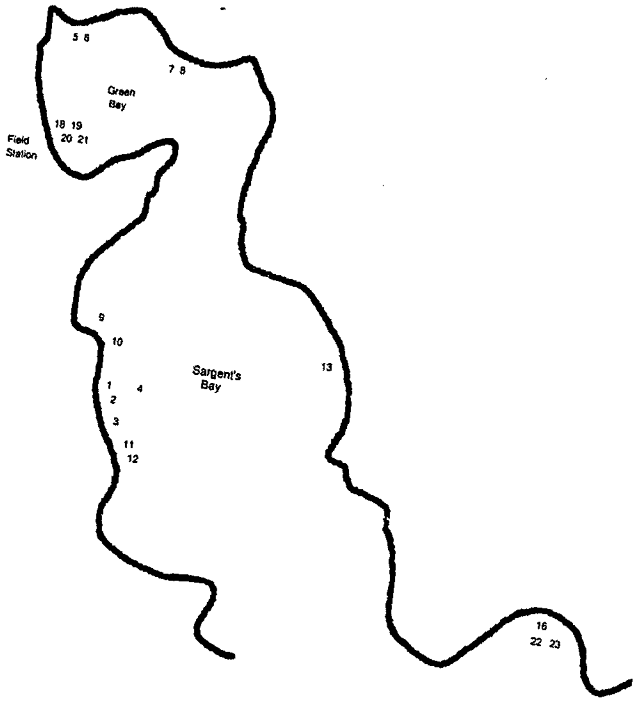
Appendix B. Denitrification and nitrogen sedimentation data

Appendix B consists of the nitrogen loading (g N m^{-2} y^{-1}), denitrification (g N m^{-2} y^{-1}) and nitrogen sedimentation (g N m^{-2} y^{-1}) data for the lakes in North America and Europe that were collected from the literature and used in Chapter 1 (Figure 1-3). References for these sites are presented in Table 1-1 of Chapter 1.

Site	Nitrogen Load (g N m^{-2} y^{-1})	Denitrification (g N m^{-2} y^{-1})	Nitrogen Sedimentation (g N m^{-2} y^{-1})
Blue Chalk	1.6	0.8	0.4
Chub	2.8	0.1	1.0
Crosson	3.5	0.1	1.2
Dickie	2.8	1.1	0.5
Harp	3.8	1.6	0.6
Plastic	1.5	0.3	0.7
Red Chalk	3.2	0.6	0.7
Okeechobee	5.6	1.0	3.0
Bryup Lang	82.6	40.0	5.4
Kvind	162	31.0	13.5
Kul	108.5	23.0	2.8
Salten Lang	25.2	1.0	5.1
Halle	85.8	45.5	0.4
Stigsholm	80.7	19.5	0
Kvie	2.7	2.0	0.1
Søbygård	132.6	54.5	5.3
Vallentuna	2.4	0.6	0.5
Norrviken	8.2	0.9	0.7
Aegerisee	0.9	0.5	0.5
Hallwilersee	12.8	2.2	1.2
Kinneret	9.1	6.4	1.1
Baldegg	40.9	30.4	4.0
Zugg	8.5	5.5	3.0

Appendix C. Site map

Appendix C consists of a figure detailing the locations where sediment samples were collected from Green Bay and Sargent Bay in Lake Memphremagog. Sample numbers correspond to those used in Chapter 2 and in Appendix D and E.


5 6
7 8
Green Bay
18 19
20 21
Field Station
9
10
Sargent's Bay
13
1
4
2
3
11
12
16
22 23

Appendix D. Site characteristics

Appendix D consists of the site characteristics of sediment samples collected from Lake Memphremagog for denitrification analysis. Macrophyte biomass (g m^{-2}), macrophyte biomass density (g m^{-3}), organic content of the sediment (% by weight), water content of the sediment (% by weight) and sediment redox potential are presented.

Site	Macrophyte Biomass (g m^{-2})	Macrophyte Biomass Density (g m^{-3})	Sediment Organic Matter (%)	Sediment Water (%)	Sediment Redox
1	23.8	476.2	-	-	-329
2	87.3	1091.3	-	-	-232
3	1292.2	8614.8	9.1	-	-302
4	0.0	0.0	15.6	-	-228
5	12.2	174.6	6.4	-	-278
6	21.8	181.7	10.7	48.7	-462
7	58.1	1162.9	2.1	61.5	-357
8	69.0	1380.0	3.9	28.7	-280
9	0.0	0.0	1.5	22.2	-118
10	202.8	3379.1	1.8	19.9	-260
11	862.1	1436.9	11.5	20.9	-413
12	502.1	1434.5	13.4	61.8	-364
13	858.0	7800.0	8.7	61.5	-436
16	89.1	523.9	1.3	-	-
18	564.6	1449.5	18.0	-	-447
19	72.9	800.7	1.8	70.9	-54
20	49.5	182.8	17.0	22.3	-404
21	410.8	2242.9	25.4	67.7	-416
22	710.8	1499.5	2.8	70.1	-186
23	355.1	2223.6	1.4	39.8	-187

Appendix E. Denitrification rates and temperatures

Appendix E consists of the *in situ* and incubation temperatures (°C) of the sediment cores used in Chapter 2. Measured and Q_{10} corrected denitrification rates (µmol N m^{-2} h^{-1}) are also presented.

Site	*In Situ* Temperature (°C)	Incubation Temperature (°C)	Measured Denitrification (μmol N m^{-2} h^{-1})	Q_{10} Corrected Denitrification (μmol N m^{-2} h^{-1})
1	18.8	20.0	9	8
2	18.8	20.0	18	16
3	18.3	20.0	44	39
4	13.0	20.0	26	15
5	21.4	23.6	105	88
6	21.4	23.6	70	59
7	24.5	23.6	18	15
8	21.5	23.6	61	52
9	23.0	25.5	140	116
10	23.0	25.5	88	73
11	22.8	25.5	158	128
12	22.8	25.5	175	142
13	20.8	22.5	79	69
16	21.5	22.5	122	113
18	24.2	24.4	340	340
19	24.2	24.4	182	182
20	24.2	24.4	178	178
21	24.1	24.4	312	312
22	23.3	23.4	52	52
23	23.3	23.4	124	124