Denitrification and Greenhouse Gas Dynamics in

Lakes Receiving Atmospheric Nitrogen Deposition

by

Michelle Lynn McCrackin

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

ARIZONA STATE UNIVERSITY

December 2010

Denitrification and Greenhouse Gas Dynamics in

Lakes Receiving Atmospheric Nitrogen Deposition

by

Michelle Lynn McCrackin

has been approved

October 2010

Graduate Supervisory Committee:

James J. Elser, Chair
Nancy B. Grimm
Sharon J. Hall
Hilairy E. Hartnett
Valeria Souza

ACCEPTED BY THE GRADUATE COLLEGE

ABSTRACT

The global transport and deposition of anthropogenic nitrogen (N) to downwind ecosystems are significant and continue to increase. Indeed, atmospheric deposition can be a significant source of N to many watersheds, including those in remote, unpopulated areas. Bacterial denitrification in lake sediments may ameliorate the effects of N loading by converting nitrate (NO_3^-) to N_2 gas. Denitrification also produces nitrous oxide (N_2O), a potent greenhouse gas. The ecological effects of atmospheric N inputs in terrestrial ecosystems and the pelagic zone of lakes have been well documented; however, similar research in lake sediments is lacking. This project investigates the effects N of deposition on denitrification and N_2O production in lakes. Atmospheric N inputs might alter the availability of NO_3^- and other key resources to denitrifiers. Such altered resources could influence denitrification, N_2O production, and the abundance of denitrifying bacteria in sediments. The research contrasts these responses in lakes at the ends of gradients of N deposition in Colorado and Norway. Rates of denitrification and N_2O production were elevated in the sediments of lakes subject to anthropogenic N inputs. There was no evidence, however, that N deposition has altered sediment resources or the abundance of denitrifiers. Further investigation into the dynamics of nitric oxide, N_2O, and N_2 during denitrification found no difference between deposition regions. Regardless of atmospheric N inputs, sediments from lakes in both Norway and Colorado possess

considerable capacity to remove NO_3^- by denitrification. Catchment-specific properties may influence the denitrifying community more strongly than the rate of atmospheric N loading. In this regard, sediments appear to be insulated from the effects of N deposition compared to the water column. Lastly, surface water N_2O concentrations were greater in high-deposition lakes compared to low-deposition lakes. To understand the potential magnitude of deposition-induced N_2O production, the greenhouse gas inventory methodology of Intergovernmental Panel on Climate Change was applied to available datasets. Estimated emissions from lakes are 7–371 Gg N y^{-1}, suggesting that lakes could be an important source of N_2O.

ACKNOWLEDGMENTS

When I decided to leave my career in corporate finance, I knew that I wanted to do something different. I planned to study conservation biology until I took an introductory ecology class and learned about the nitrogen cycle. Since then my trajectory changed and I haven't looked back. I am grateful to a number of people and organizations that have supported me over the last few years and made my PhD possible.

I thank my advisor, Dr. Jim Elser, for giving me the opportunity to conduct fieldwork in Norway and the Colorado Rocky Mountains. Without his support and direction, the work presented here would not have been possible. I especially appreciate his energy and enthusiasm for science. The rest of my committee, Drs. Nancy Grimm, Sharon Hall, Hiliary Hartnett, and Valeria Souza, provided helpful advice and feedback. I also want to recognize and thank Nancy for taking me into her laboratory and introducing me to scientific research and collaboration, and for encouraging me to pursue graduate work in ecology.

Field and laboratory work could not have been completed without the efforts of Marcia Kyle, Cathy Kochert, David Huber, Erin Seybold, Melanie Engstrom, and Angela Peace. I am grateful for cultural advice and assistance with field and laboratory logistics provided by Trine Holm, Dag Hessen, Tom Andersen, and Peter Dörsch during my Fulbright exchange at Universitetet i Oslo in Norway. I also thank the staff of the Mountain Research Station, Niwot Ridge Long Term Ecological Research Program,

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction

Human activities, such as fossil fuel combustion and agriculture,

have increased concentrations of nitrogen (N) in the atmosphere and

resulted in the long-distance transportation of N pollutants (Galloway

1998). Indeed, atmospheric N deposition can be the dominant source of

new N to watersheds, even in remote and unpopulated regions. Such

elevated N inputs to lakes can have significant ecological effects such as

reduced water quality, changes in the composition of fish communities,

and shifts in nutrient limitation of algae and zooplankton (MEA 2005, Elser

et al. 2009). Denitrification is a microbial process that can ameliorate

these effects by converting NO_3^- into inert di-nitrogen (N_2) gas.

Denitrification also produces nitrous oxide (N_2O), a greenhouse gas that is

296 times more potent than carbon dioxide according to the International

Panel on Climate Change (IPCC 2006). The US Environmental Protection

Agency has also declared that greenhouse gases such as N_2O are

harmful to human health (EPA 2009). Thus, while the environmental

benefit of denitrification is the removal of excess N inputs, this process

might also increase atmospheric concentrations of N_2O.

Why study denitrification in lakes?

Freshwater quality

Americans place substantial value on the ecosystem services

provided by lakes, including clean drinking water, recreation, and sense of

place (Carson and Mitchell 1993, Wilson and Carpenter 1999). Economic losses from nutrient loading to US freshwaters exceed $2 billion annually (Dodds et al. 2009). Consequently, natural resource managers are concerned with water quality. Denitrification may mitigate the eutrophying effects of anthropogenic N inputs by permanently removing N that may otherwise be retained by biotic uptake and assimilation (Seitzinger et al. 2006). Microorganisms capable of denitrification are widely found in terrestrial and aquatic environments where concentrations of oxygen are low and where organic carbon and NO_3^- are available as substrates. The global atmospheric N deposition rate is expected to increase substantially in the next few decades (Galloway et al. 2004). Thus, investigating the effects of N deposition on lakes is critical as such elevated inputs can have significant negative ecological effects. Lakes have only been recently recognized as biogeochemical sinks for N and it is essential to understand the factors that influence denitrification in lake sediments (Harrison et al. 2009).

<center>Nitrous oxide production</center>

Interest in denitrification not only arises from its potential to mitigate the effects of N loading, but also due to its production of N_2O. The global warming potential of N_2O is nearly 300 times greater than that of CO_2 (Forster et al. 2007). In addition, N_2O is considered the most significant ozone-depleting substance currently being emitted (Ravishankara et al. 2009). The current atmospheric concentration of N_2O is 319 ppb and it is

<center>2</center>

increasing 0.3% per year due primarily to human activities (Galloway 1998, Nevison et al. 2007). The proportion of N lost as N_2O during denitrification is small, generally less than 1% of total gaseous production (Knowles 1982). However, elevated N loading increases both total and relative emissions of N_2O compared to N_2 via enhanced denitrification (Seitzinger and Nixon 1985). Indeed, 90% of N_2O production by rivers and estuaries (1.2 Tg N y^{-1}) is estimated to result from anthropogenic N loading (Seitzinger et al. 2000, Seitzinger et al. 2006). Nitrous oxide emissions from lakes have not been quantified even though lakes cover the same global surface area as rivers and have the potential for greater N_2O production due to their longer water residence times.

Research approach and considerations

The effects of anthropogenic N inputs on denitrification and related N_2O emissions have been well studied in terrestrial and marine ecosystems, however similar research in lakes is lacking. The overall objectives of this dissertation are to understand how atmospheric N pollution has altered biogeochemical processes in lake sediments. This research contrasts responses between lakes at the ends of two gradients of N deposition, in Norway and Colorado (USA), to address the questions: How has atmospheric N deposition altered background rates of denitrification and related greenhouse gas production in lake sediments? How has N deposition altered resource limitation of sediment

denitrification? What is the effect of atmospheric N deposition on the abundance of denitrifying bacteria and denitrification kinetics?

Following this introduction there are five chapters that are presented as separate manuscripts and a summary chapter. The second chapter investigates denitrification and N_2O production in sediments and the responses of denitrification to short-term resource enrichment. The third chapter examines denitrification kinetics and the abundance of NO_3^-- and nitrite-reducing bacteria. The fourth chapter describes the temporal dynamics of nitric oxide, N_2O, and N_2 production during denitrification in sediments. The fifth chapter investigates responses of pelagic and benthic bacteria to experimental resource enrichment. The sixth chapter describes greenhouse gas dynamics in lakes. The final chapter summarizes the findings of the dissertation research, compares responses between lakes in Norway and Colorado, and compares sediments responses to those of soils that receive atmospheric N deposition.

LITERATURE CITED

Carson, R. T. and R. C. Mitchell. 1993. The value of clean water: the public's willingness-to-pay for boatable, fishable, and swimmable quality water. Water Resources Research **29**:2445-2454.

Dodds, W. K., W. W. Bouska, J. L. Eitzmann, T. J. Pilger, K. L. Pitts, A. J. Riley, J. T. Schloesser, and D. J. Thornbrugh. 2009. Eutrophication of U.S. freshwaters: analysis of potential economic damages. Environmental Science & Technology **43**:12-19.

Elser, J. J., T. Andersen, J. S. Baron, A. K. Bergstrom, M. Jansson, M. Kyle, K. R. Nydick, L. Steger, and D. O. Hessen. 2009. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science **326**:835-837.

EPA. 2009. Endangerment and Cause or Contribute Findings for Greenhouse Gases under Section 202(a) of the Clean Air Act http://www.epa.gov/climatechange/endangerment.html.

Forster, P., V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D. W. Fahey, J. Haywood, J. Lean, D. C. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, M. Schulz, and R. Van Dorland. 2007. Changes in atmospheric constituents and in radiative forcing.*in* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Climate Change 2007: the Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK; New York, NY, USA.

Galloway, J. N. 1998. The global nitrogen cycle: changes and consequences. Environmental Pollution **201**:15-24.

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

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

IPCC. 2006. Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme.*in* H. S. Eggleston, L. Buendia, K. Miwa, T. Ngara, and K. Tanabe, editors. IGES, Hayama, Japan.

Knowles, R. 1982. Denitrification. Microbiological Reviews **46**:43-70.

MEA. 2005. Millenium Ecosystem Assessment: Ecosystems and human well-being: wetlands and water synthesis. WRI, Washington, DC.

Nevison, C. D., N. M. Mahowald, R. F. Weiss, and R. G. Prinn. 2007. Interannual and seasonal variability in atmospheric N_2O. Global Biogeochemical Cycles **21**:GB3017, doi:3010.1029/2006GB002755.

Ravishankara, A. R., J. S. Daniel, and R. W. Portmann. 2009. Nitrous oxide: the dominant ozone-depleting substance emitted in the 21st century. Science **326**:123-125.

Seitzinger, S., C. Kroeze, and R. Styles. 2000. Global distribution of N_2O emissions from aquatic systems: Natural emissions and anthropogenic effects. Chemosphere **2**:267-279.

Seitzinger, S. P., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, and C. Tobias. 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecological Applications **16**:2064-2090.

Seitzinger, S. P. and S. W. Nixon. 1985. Eutrophication and the rate of denitrification and N_2O production in coastal marine sediments. Limnology and Oceanography **30**:1332-1339.

Wilson, M. A. and S. R. Carpenter. 1999. Economic valuation of freshwater ecosystem services in the United States: 1971-1997. Ecological Applications **9**:772-783.

CHAPTER 2

Atmospheric Nitrogen Deposition Influences Denitrification and Nitrous

Oxide Production in Lakes (Norway)

ABSTRACT

Microbially mediated denitrification is an important process that

may ameliorate the effects of nitrogen (N) loading by permanently

removing excess N inputs. In this study, we measured the rate of

denitrification and nitrous oxide (N_2O) production during denitrification in

sediments from thirty-two Norwegian lakes at the high and low ends of a

gradient of atmospheric N deposition. Denitrification and N_2O production

rates averaged 41.7 and 1.1 N μmol m^{-2} h^{-1}, respectively, for high-

deposition lakes. There was no detectable denitrification or N_2O

production in low-deposition lakes. Epilimnetic nitrate concentration was

strongly correlated with denitrification rate (R^2 = 0.67). We also measured

the denitrification rate in response to experimental additions of organic

carbon, nitrate, and phosphorus. Experimental nitrate additions stimulated

denitrification in sediments of all lakes, regardless of N deposition level. In

fact, the rate of denitrification in nitrate-amended treatments was the same

magnitude for lakes in both deposition areas. These findings suggest that

lake sediments possess considerable capacity to remove nitrate and that

this capacity has not been saturated under conditions of chronic N

loading. Further, nitrous oxide was nearly 3% of the total gaseous product

8

during denitrification in high-deposition lakes, a fraction that is comparable to polluted marine sediments. Our findings suggest that while lakes play an important role in N removal in the landscape, they may be a source of N_2O emissions, especially in areas subject to elevated N inputs.

INTRODUCTION

As humans continue to dominate the global nitrogen cycle, denitrification plays an important role in the landscape by removing excess N inputs that may otherwise be available for uptake, transformation, and transport and, thus, impacts on receiving ecosystems (Galloway et al. 2004, Schlesinger 2009). Denitrification (microbial conversion of nitrate to inert atmospheric nitrogen gas) also potentially influences global climate through the ancillary production of nitrous oxide, a potent greenhouse gas (Knowles 1982). Heterotrophic microorganisms capable of denitrification are widely distributed in terrestrial and aquatic environments. As land-based N inputs cascade from soils to rivers, lakes, groundwater, and ultimately, estuaries and coastal areas, denitrification reduces downstream N loading (Galloway et al. 2003, Seitzinger et al. 2006). Aquatic ecosystems are particularly vulnerable to N-induced eutrophication, as primary production is frequently limited by N in both marine and fresh waters (Elser et al. 2007). Through removal and storage of nutrients, lakes reduce the transport of N from land to the ocean, (Seitzinger et al. 2006). In lakes, denitrification rates are greatest in the

9

sediments because of favorable conditions for facultatively anaerobic denitrifying bacteria. Yet lakes are often overlooked in efforts to quantify both global denitrification rates and N_2O emissions (Seitzinger et al. 2000, Seitzinger et al. 2006). Consequently, we have an incomplete understanding of the contributions of lake sediments to denitrification and have little information about the resources potentially controlling denitrification in lakes. We also do not have a good idea about the magnitude of N_2O production in lakes compared to other ecosystems. Here, we measured rates of denitrification and N_2O production in lakes across a gradient of atmospheric N inputs and tested for resource limitation of denitrification.

Even in unpopulated areas, lakes are subject to anthropogenic influences via atmospheric N deposition. Human activities have increased atmospheric concentrations of oxidized nitrogen (Galloway 1998), resulting in the long-distance transport and deposition of biologically available N species. The magnitude of these inputs is substantial and increasing. Globally, rates of atmospheric N deposition relative to 1990 are expected to double by 2050, to 125 Tg N/y (Galloway et al. 2004). Most investigations into the effects of N deposition focus on terrestrial ecosystems and there are limited data on the responses of lakes and reservoirs (hereafter as "lakes"). A recently published model by Harrison et al. (2009) estimated that, globally, lakes remove 19.7 Tg N/y from the

landscape and that small lakes (<50 km^2) are responsible for nearly half of this removal. This is significant given that lakes cover less than 3% of global surface area (Seitzinger et al. 2006). Further, as the rate of anthropogenic N loading increases, the rate of denitrification in soils and marine sediments generally increases (Firestone et al. 1980, Seitzinger and Nixon 1985). We similarly hypothesized that atmospheric N deposition influences denitrification in lake sediments.

The effectiveness of denitrification in removing nitrate may eventually become limited by the availability of other key resources, such as phosphorus (P). Indeed, such limitations might help explain why anthropogenic N inputs can accumulate to high levels in receiving waters. While aerobic bacteria in aquatic ecosystems are often limited by P (Cotner et al. 1997), little is known about the effects of P supply on denitrification. The strongest evidence for P limitation of heterotrophic microbial respiration comes from experimental additions of P to relatively N-rich soils (Cleveland and Townsend 2006). Fertilization of soils with P resulted in substantial carbon dioxide losses via soil respiration. In forest and riparian soils, researchers have observed no effect of P enrichment on denitrification rates (Ullah and Zinati 2006) while P enrichment reduced denitrification rates in salt marsh sediments (Sundareshwar et al. 2003). Kaste and Lyche-Solheim (2005) estimated by mass balance that denitrification was a significant sink for elevated atmospheric N inputs in a

11

P-fertilized lake, relative to an unenriched lake. While this investigation suggests that P plays a role in regulating N retention, P limitation of denitrification in lake sediments was not directly tested. We measured denitrification rates and tested for resource limitation of denitrification in sediments, which includes, to our knowledge, the first direct test of P limitation of short-term denitrification rates. We hypothesized that denitrification would be limited by phosphorus in sediments of lakes subject to atmospheric N deposition because phosphorus is the next likely resource to limit heterotrophic microbes after organic carbon and nitrate.

Interest in denitrification arises not only from its potential role in ameliorating excess N inputs to ecosystems, but also due to its production of N_2O, a greenhouse gas that has increased 18% since pre-industrial times (Galloway 1998, IPCC 2007). Nitrous oxide emissions from rivers are estimated to account for ~20% of emissions from all aquatic ecosystems, excluding lakes (Seitzinger et al. 2000, Seitzinger et al. 2006). Surprisingly, N_2O emissions from lakes have not been quantified even though lakes cover the same global surface area as rivers. Production of N_2O by soils and marine sediments increases as the rate of anthropogenic N loading increases because of increased denitrification (Firestone et al. 1980, Seitzinger and Nixon 1985). The proportion of N lost as N_2O during denitrification (hereafter, "relative N_2O production") is small, generally less than 1% of total gaseous production. In terrestrial

soils and aquatic sediments ecosystems, relative N_2O production is known to be influenced by the rate of N loading, availability of organic carbon, pH, and the concentration of dissolved oxygen (Firestone et al. 1980, Seitzinger 1988). We hypothesized that atmospheric N deposition influences both N_2O fluxes and the relative production of N_2O because of increased denitrification and elevated concentrations of nitrate, respectively.

Here we report responses of denitrification in lake sediments across a gradient of N deposition. Given the six-fold difference in N loading rates at the extreme ends of the gradient, we expected the denitrification rate to be greater in high-deposition areas compared to low-deposition areas. We also expected limitation of denitrification by P to be more frequent in high-deposition lakes than in low-deposition lakes. Lastly, we predicted the rate of N_2O production and proportion of gaseous flux as N_2O in high-deposition lakes to exceed that of lakes in the low-deposition region.

METHODS

Study site

We sampled 32 lakes located in southern Norway between June and August 2007. Norway receives atmospheric pollution from industrial regions of Europe and total (wet and dry) N deposition rates are 10-20 kg N ha^{-1} y^{-1} in southwest regions of the country, decreasing to less than 4 kg

13

N ha^{-1} y^{-1} in east-central areas (Tørseth and Semb 1998, Klein and Benedictow 2006) (Fig. 1). We sampled 16 lakes each at the high and low ends of the N deposition gradient that spans over 400 km. The gradient encompasses a large number of unpopulated and sparsely vegetated watersheds, making it well suited to evaluate the influence of atmospheric deposition without confounding influences of variable land use history and vegetation type. In each region, we chose lakes with similar surface area, depth, and landscape position. Exposed rock, moss, and scattered trees, including Norway spruce (*Picea abies*) and aspen (*Populus tremula*), characterized the watersheds, which were largely undeveloped with the exception of sheep grazing and occasional seasonal cottages. Mean annual precipitation is approximately 1,700 mm in high-deposition areas and 700 mm in low-deposition areas (http://biogeo.berkeley.edu/ worldclim1_4/grid/ curprec_30s_esri). Mean elevation of the sampled lakes was 230 and 993 m above sea level in regions receiving high and low rates of atmospheric N deposition, respectively. Watershed percent tree cover data was generated from MODerate-resolution Imaging Spectroradiometer (MODIS) datasets (Hansen et al. 2003). See Table 1 for a summary of individual lake characteristics.

Field sampling and laboratory procedures

Lakes were visited during summer 2007; dates of sampling for each lake are given in Table 1. Fieldwork was staggered so that sampling of

lakes in high- and low-deposition regions was not strongly skewed by date. High-deposition lakes were sampled in late June and late July, while low-deposition lakes were sampled in early July and early August. Lakes in high-deposition regions were thermally stratified while most lakes in low-deposition regions were not, due largely to differences in mean lake depth between regions.

Lakes were sampled from an inflatable boat. The deepest part of the lake was found using a portable echo sounder after which a vertical profile of temperature and dissolved oxygen was made at ~1-m intervals using a YSI model 85 temperature-oxygen probe (YSI, Yellow Springs, Ohio, USA). Four independent water samples were taken using a battery-powered submersible pump fitted with tubing to take in water at 1 - 1.5 m depth. Water samples were frozen until analysis for dissolved organic C (DOC) on a Shimadzu TOC 5000 (Shimadzu, Kyoto, Japan). Water pH was determined using an Accumet AR10 pH meter and nitrate + nitrite (hereafter: NO_3-N) concentration was measured on a Metrohm 761 Compact Ion Chromatograph (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at the Kiowa Environmental Chemistry Laboratory (University of Colorado-Boulder, USA). Concentrations of total P in the study lakes were generally very low. To reduce the detection limit, oxidized TP samples were concentrated 5-fold using the method of

Solorzano and Sharp (1980) after which P concentrations were analyzed

using the ammonium molybdate method (APHA 2005).

Surface sediments were collected using a LaMotte dredge from a

water depth of approximately 10 m or at the maximum lake depth if the

lake was less than 10 m deep. Sediments were returned to the laboratory

and processed within four hours of collection. Denitrification rates were

measured using the acetylene inhibition method (Yoshinari and Knowles

1976). For each lake, six analytical replicate 100-g subsamples of

homogenized sediments were slurried with 80 ml of lake water collected

from above the sediments using a Van Dorn sampler. Bottles were purged

of oxygen with nitrogen gas (N_2). Acetylene was added to half of the

bottles (three per lake) to block the reduction of N_2O to N_2. After vigorous

shaking, we collected 10-mL samples from the headspace volume (about

550 mL) at the onset of anoxic conditions (0 h) and at 4 and 12 h.

Incubations were conducted at 15°C in dark conditions. Gas samples were

analyzed for N_2O on a Varian CP-3800 gas chromatograph (Varian, Palo

Alto, California, USA) with an electron-capture detector. The production of

N_2 and N_2O by denitrification was determined as the accumulation of N_2O

during incubations amended with acetylene. Nitrous oxide produced by

denitrification was determined as the accumulation of N_2O for incubations

that were not amended with acetylene (Garcia-Ruiz et al. 1998, Rudaz et

al. 1999). Relative N_2O production was determined as the N_2O flux for

16

incubations without acetylene divided by the N_2O flux for incubations with acetylene for the 0 – 4 h and 4 – 12 h periods. Denitrification and N_2O production were converted to an areal basis and reported as $\mu mol\ m^{-2}\ h^{-1}$: rates measured on the basis of mass of dry sediment (N $\mu mol\ g^{-1}\ h^{-1}$) were multiplied by the bulk density of the top 7 cm of sediment (g/m^2) determined for each lake (Richardson et al. 2004). The dredge collected sediments from an area of 221 cm^2 to a depth of 7 cm.

We also conducted a resource enrichment experiment to determine if the instantaneous sediment denitrification rate was limited by organic carbon, nitrate, or phosphate. For each lake, three analytical replicate 50-g subsamples of homogenized sediments were slurried with 40 mL of one of five different incubation media. The incubation media were: distilled water as a control, 100 mg organic carbon (oC, as dextrose and acetate)/L, 100 mg nitrate (NO_3^-)-N/L, 13.84 mg phosphate (PO_4^3)-P/L, or a combined medium that included oC, NO_3^- and PO_4^{3-} (CNP) at the concentrations just described. To prevent the *de novo* synthesis of nitrate reductase enzymes, chloramphenicol was added to a concentration of 10 mg/L to all incubation media. We induced anoxia by purging oxygen from the slurries with ultra high purity N_2 gas and blocked the reduction of N_2O to N_2 using acetylene. We collected 3.5-mL initial samples and final samples at the end of 4 h incubation at 15°C in dark conditions, both after vigorous shaking. Gas samples were analyzed for N_2O on a Shimadzu

17

14A gas chromatograph with an electron-capture detector. Denitrification rates for resource enrichment assays are reported on the basis of dry sediment mass.

Sediment water content was determined as mass loss after drying subsamples at 105°C for 48 h and organic matter (OM) content was determined as mass loss on ignition at 550°C for 4 h. Total C and N contents of dried sediments were measured with a Perkin-Elmer CHN elemental analyzer (PerkinElmer, Waltham, Massachusetts, USA). Phosphorus content of combusted sediment was measured colorimetrically following extraction with 0.5 M hydrochloric acid using the acid molybdate technique (APHA 2005, Lukkari et al. 2007).

Statistical analysis

We performed *t* tests to compare organic matter, water content, and sediment nutrient pools between lakes in high- and low-deposition regions. We used Mann-Whitney U tests to compare relative N_2O fluxes between lakes in high- and low-deposition regions, as the assumption of normality was not met. Multiple linear regression was used to identify relationships among sediment response variables and predictor variables. For rates of denitrification, N_2O production, and relative N_2O production, predictor variables included water nitrate concentrations, water pH, sediment organic matter, sediment C:N ratio, and watershed percent tree cover. Models were selected by considering all subsets on the basis of

adjusted R^2 and Mallows' Cp (Cetin and Erar 2006). We evaluated multi-

colinearity using tolerance values and selected final models for which

tolerance values were >0.5 for all predictor variables. When necessary,

response and predictor variables were transformed to improve normality.

One-way analysis of variance (ANOVA) was used to test the significance

of responses of the denitrification rate to resource enrichment treatments.

Student's t and Mann-Whitney U tests were calculated in Excel 2008

Version 12.1.7. Multiple linear regression analysis and ANOVA were

performed using JMP (SAS Institute, Inc., Cary, NC, USA) Version 5.0.1.2,

with α = 0.05.

RESULTS

Bulk sediment and water characteristics

There were no significant differences in sediment water content

(data not shown), OM, total C, N, and P content, or ratios of C:N, C:P, and

N:P between lakes in high- and low-deposition regions (P > 0.05). Bulk

sediment characteristics for all lakes are reported in Table 2. Water

column NO_3^- concentrations in lakes receiving elevated N inputs were

greater than that for lakes in low-deposition regions, which were generally

undetectable (Table 2). There was no correlation between water NO_3^-

concentrations and sampling dates (R^2=0.06, P > 0.30). The concentration

of DOC was greater in surface waters of lakes in high-deposition regions

compared to those in low-deposition regions (P < 0.0001). There was no

19

difference in the concentration of total dissolved P across the N deposition gradient. Characteristics of surface lake waters for all lakes are reported in Table 2.

Denitrification rates and resource limitation of denitrification

The accumulation of gaseous products of denitrification (N_2-N and N_2O-N) was greater for high-deposition lakes compared to low-deposition lakes at both the 4 h and 12 h time periods (Fig. 2A). For high-deposition lakes, the mean denitrification rate during the 0 – 4 h period was greater than that during the 4 – 12 h period (Table 3). There were no detectable differences in concentrations of N_2O between the three sampling time points for incubations of lakes in low-deposition areas; thus, we were unable to determine a denitrification rate for these lakes. In high-deposition lakes, the denitrification rate during the 0 – 4 h period was strongly correlated with the concentration of nitrate in surface waters (y = -0.2 + 1.3 * NO_3^-, R^2 = 0.67, P < 0.001, Fig. 3). Multiple regression analysis for high-deposition lakes revealed that denitrification was best predicted by water NO_3^- concentrations, pH, and sediment C:N for the 0 – 4 h period (y =1.07 * NO_3^- + 0.18 * pH - 1.81 * C:N + 1.17, R^2 = 0.70, P < 0.001) and by water nitrate concentrations, sediment C:N, and percent tree cover for the 4 – 12 h period (y = 1.55 * NO_3^- - 6.81 * C:N + 1.38 * tree cover + 6.33, R^2 = 0.68, P < 0.001).

In the resource enrichment experiments, denitrification rate increased significantly over the control in the nitrate and the combined CNP treatments for all lakes and these treatments did not differ from one another (P < 0.001, Fig 4). Mean denitrification rates in the oC and PO_4^{3-} treatments did not differ from the control. Further, while the denitrification rate for lakes in low-deposition areas was undetectable in ambient samples and in resource enrichment treatments lacking NO_3^-, the denitrification rate after experimental NO_3^- additions was the same for lakes in high- and low-deposition areas.

Nitrous oxide fluxes and relative production of nitrous oxide

Mean concentrations of N_2O were greater in incubations of sediments from high-deposition lakes compared to low-deposition lakes for all time periods (Fig. 2B). There was net N_2O production in the 0 – 4 h period and net N_2O consumption during the 4 – 12 h period for high-deposition lakes (Table 3). For low-deposition lakes, there was no difference in concentrations of N_2O for assays incubated with and without acetylene at the 0, 4, and 12 h sampling points and, thus, we were unable to determine a flux rate for these lakes (Fig. 2C). The N_2O flux from sediments in high-deposition lakes was predicted by water column concentrations of NO_3^- and percent tree cover for the 0 – 4 h period (y = 0.95 * NO_3^- + 0.76 * tree cover – 1.56, R^2 = 0.51, P = 0.004, Fig. 2D). For the 4 – 12 h period, N_2O flux was predicted by sediment C:N, OM, and

water pH (y = -4.78 * C:N + 5.06 * OM - 0.25 * pH + 5.80, R^2 = 0.61, P <

0.01). For lakes in high-deposition regions, relative production of N_2O

(fraction of overall flux attributable to N_2O) decreased from 0.025 to -0.020

between the 0 – 4 h and 4 – 12 h period, indicating a shift from N_2O

production to consumption (Table 3, Fig. 2B). Nitrogen gas was the

dominant product of denitrification for the duration of the incubation. The

relative production of N_2O during the 0 – 4 h period was strongly predicted

by water column concentrations of NO_3- and the watershed percent of tree

cover of (y = 0.33 * NO_3^- + 0.51 * tree cover – 0.68, R^2 = 0.43, P < 0.01).

For the 8 – 12 h period, relative N_2O production was not correlated with

any of the predictor variables.

DISCUSSION

Factors limiting denitrification in lake sediments

It has been suggested that lakes are sentinels of nitrogen

saturation in terrestrial ecosystems subject to chronic atmospheric N

deposition (Williamson et al. 2008). As land-based anthropogenic N inputs

are transported across the landscape, lakes perform an important

ecosystem function by reducing the export of nutrients to downstream

ecosystems. Consequently, it is crucial to understand the role of

denitrification in lake sediments in mitigating N loading and in producing a

potent greenhouse gas. Our hypothesis that denitrification in lake

sediments is influenced by available NO_3^- and thus by the rate of

atmospheric N loading was strongly supported both in correlative analysis

(Fig. 3) and in the short-term enrichment experiments (Fig. 4). The

denitrification rate for lakes in high-deposition areas was greatest during

the first 4 h of the incubation and decreased during the 4-12 h period,

possibly resulting from the depletion of NO_3^-. Interestingly, when NO_3^- was

made available in non-limiting concentrations, the denitrification rate in

sediments from lakes in low-deposition areas was the same as that of

lakes in high-deposition regions. These findings indicate that lake

sediments possess considerable capacity for removing NO_3^- associated

with atmospheric N deposition and that this capacity has not been

saturated even at the relatively high levels of N deposition present in

southwestern Norway. Our work also adds to the large dataset of N

cycling in streams (Mulholland et al. 2008) in supporting the conclusion

that NO_3^- concentration is the single most important predictor of

denitrification.

Our hypothesis that P limitation influences rate of denitrification

under high N deposition was not supported (Fig. 4). While experimental P

additions substantially increase soil respiration of carbon dioxide via

aerobic heterotrophic microbial respiration (Cleveland and Townsend

2006, Gnankambary et al. 2008), instantaneous rates of anaerobic

denitrification in lakes do not appear to be similarly limited. Longer-term

enrichment bioassays that would permit population-level responses by

denitrifiers might yield different insights, but our experiments suggest that the ability of lake sediments to remove N via denitrification does not diminish due to the onset of secondary limitations on denitrifying bacteria.

Nitrous oxide production during denitrification

Our measured N_2O flux from denitrification was within the ranges reported in the literature for marine sediments, agricultural streams, and a eutrophic lake (Seitzinger and Nixon 1985, Wang et al. 2006, Beaulieu et al. 2008). The hypothesis that N_2O production increases with N inputs was supported. These findings indicate that denitrification in lake sediments respond to N loading in a similar manner as do marine sediments and soils subject to elevated N inputs (Seitzinger and Nixon 1985, Ullah and Zinati 2006). Compared to terrestrial ecosystems, however, we have a more limited understanding of the roles played by nitrification and denitrification in N_2O production in aquatic environments. In coastal areas, estuaries, and streams subject to anthropogenic N loading, researchers have found that denitrification produces more N_2O than nitrification (Jensen et al. 1984, Seitzinger 1988, Beaulieu et al. 2008). Similarly, nitrate fertilization of littoral sediments from a boreal eutrophic lake increased N_2O production by as much as 100 times, while ammonium additions to had no effect on N_2O flux, suggesting a low nitrification capacity (Liikanen et al. 2003).

Although the sediments of lakes we sampled were not likely anoxic at the sediment-water interface, our observations may be typical of deeper sediments. Studies of agricultural soils have identified two phases of denitrification in soils following the onset of anoxic conditions (Firestone and Tiedje 1979, Smith and Tiedje 1979). Responses during the first phase, lasting 1 – 3 h after the start of anoxic incubation, reflect preexisting denitrifying enzymes and thus provide more information about the activity of the microbial community. Responses during the second phase, starting at 4 – 8 h of incubation, reflect enzyme synthesis by the denitrifiers but no significant population growth due to limitation by organic carbon. Consequently, the dominant product of denitrification shifts from N_2 during the first phase to N_2O during the transition between phases because of a lag in the synthesis of enzymes that reduce N_2O. Firestone and Tiedje (1979) noted that N_2 again becomes the dominant product during the second phase. Interestingly, the temporal pattern we observed for lakes in high-deposition areas did not follow that observed in agricultural soils. Rather, N_2 remained the dominant product for the duration of the incubation. Sediment denitrifiers in lakes may not experience as significant a time lag in synthesizing nitrous oxide after the onset of anoxic conditions, as observed in soils. Such a difference may result from differences in redox environment between sediments, which

are fairly stable, compared to soils, which experience distinct cycles of wetting and drying.

For marine sediments, relative N_2O production during denitrification is thought to between 0.001 and 0.01 (Seitzinger et al. 2000). We observed relative N_2O production of 0.025 for high-deposition lakes, just below values between of 0.03 – 0.06 that have been reported for heavily polluted marine areas (Nishio et al. 1983, Seitzinger and Nixon 1985). Our findings suggest that sediments of lakes and coastal areas respond similarly to chronic N loading in terms of the gaseous products of denitrification. Understanding the relative production of N_2O during denitrification is important not only for understanding underlying biogeochemical processes, but also for predicting N_2O emissions at broader spatial scales. To date, efforts to quantify global fluxes of N_2O from aquatic ecosystems have not included lakes.

Whole-lake estimates of denitrification and nitrous oxide emissions

Lakes play an important role by removing reactive N from the landscape through storage in sediments or biomass, and through permanent removal via denitrification. Models of N removal in aquatic ecosystems, however, do not distinguish between these potential fates of N (Kelly et al. 1987, Wollheim et al. 2008, Harrison et al. 2009). For the lakes we sampled, there was no evidence that N is accumulating in sediments of high-deposition areas (Table 2), nor did we observe

differences in seston N content between lakes in high- and low-deposition

areas (data not shown). Rather, our results suggest that denitrification is

the most important pathway for N removal in lakes.

To evaluate the portion of N inputs that is removed from

ecosystems, and hence, is unavailable for uptake, transformation, or

transport, denitrification rates must be translated from the measurement

scale to broader scales. In the first effort to quantify global lake

denitrification rates, Seitzinger et al. (2006) estimated that lakes contribute

to removal of 7-16% of terrestrial N loading. Water residence time explains

much of the variability in the fraction of N removed from aquatic

ecosystems by denitrification (Weyhenmeyer et al. 2007, Harrison et al.

2009). Seitzinger et. al (2006) summarized this relationship as:

(1)   % N removed by denitrification = 23.4 *

water residence time (months) $^{0.204}$

While water residence time for the lakes we sampled is not known,

comparable lakes in Norway have residence times between 0.1 – 1 year

(Berge et al. 1997, Kaste et al. 2003). Applying these residence times to

formula (1), we estimate that 24 – 39% of N inputs are removed by

denitrification. Given the 10 – 20 kg N ha^{-1} y^{-1} deposited atmospherically

to lake surfaces, we further estimate that denitrification removes 2.4 – 7.8

kg N ha^{-1} y^{-1} (or 1.8 – 5.8 μmol m^{-2} h^{-1}). For high-deposition lakes, we

observed a mean denitrification rate of 41.7 μmol m^{-2} h^{-1} (Table 3), which

is an order magnitude greater than the "top down" estimate of denitrification based on water residence time. While the denitrification rates we measured for high-deposition lakes were within published range for lakes (Pina-Ochoa and Alvarez-Cobelas 2006), the difference between our rates and those based on residence time underscores the challenge of extrapolating and modeling measurements made at the scale of hours to the whole lake (Groffman et al. 2009). We may have overestimated denitrification by performing assays in the laboratory where conditions were more favorable for denitrifiers compared to *in situ* lake conditions. For example, our slurries disturbed the sediment profile for the top 7 cm that we sampled and allowed for greater diffusion of NO_3^- in lake water to active sites of denitrification within the sediments. Further, incubation temperatures were about 5°C warmer than lake temperatures at the sampling depth. Thus, assuming a Q_{10} value of 2, we may have overestimated denitrification rates by ~40%. We also induced anoxia in the sediment slurries, whereas sediment conditions were likely oxic at the sediment-water interface and suboxic or anoxic only for deeper sediments. On the other hand, the anoxic conditions we induced and our use of acetylene may have underestimated denitrification rates. In many sediments, nitrification is an important source of NO_3^- to denitrification. However, nitrification requires oxygen and is inhibited by acetylene (Groffman et al. 2006). The lack of measurable denitrification in low-

deposition lakes may result from tight coupling of nitrification and denitrification compared to lakes subject to anthropogenic N loading. Acetylene may also incompletely block the reduction of N_2O to N_2 and lead to an underestimation of denitrification. Because of these factors we will use estimated denitrification rates of $2.4 - 7.8$ kg N ha^{-1} y^{-1}, based on water residence time and N deposition rates as discussed above, to estimate lake N_2O emissions.

Understanding relative N_2O flux during denitrification is important not only for understanding underlying biogeochemical processes, but also for predicting N_2O emissions at broad spatial scales. Based on Seitzinger and Kroeze (1998), global fluxes of N_2O produced by denitrification in aquatic ecosystems can be estimated as:

(2) N_2O flux (kg N ha^{-1} y^{-1}) = emission factor * denitrification rate

        (kg N ha^{-1} y^{-1})

The emission factor is based on ratios of nitrous oxide and nitrogen gas fluxes observed in studies of estuarine and coastal marine sediments. Seitzinger and Kroeze (1998) used a factor of 0.03 for aquatic areas (excluding lakes) with external N loading rates >10 kg N ha^{-1} y^{-1}, compared to the mean relative N_2O production of 0.025 that we measured in high-deposition lakes (Table 3). Applying an emission factor of 0.025 to the top down denitrification estimate of $2.4 - 7.8$ kg N ha^{-1} y^{-1} in formula (2), we predict nitrous oxide production is $0.06 - 0.19$ kg N ha^{-1} y^{-1} (or 0.05

29

– 0.15 μmol m^{-2} h^{-1}) for lakes in high-deposition areas. This is an order of magnitude greater than the rate of N$_2$O production we measured (Table 3), likely for the same reasons that our measured rate of denitrification may be overestimated, as previously discussed. While there limited data on N$_2$O emissions for lakes, fluxes between 0.06 – 0.19 kg N ha^{-1} y^{-1} are in the range of N$_2$O emissions reported for eutrophied European lakes (Mengis et al. 1997, Huttunen et al. 2003). Further direct measurements of N$_2$O fluxes are needed to better understand how anthropogenic N loading is altering gas exchange between lakes and the atmosphere.

Conclusion

While there are uncertainties in scaling plot-level flux rates to whole-lake, or larger, scales, our findings are consistent with a growing body of literature suggesting that small lakes play an important role in N removal in the landscape. The magnitude of denitrification emissions from lakes may be substantial and should be considered in global estimates. Global N$_2$O emissions from rivers are estimated at 1.1 Tg N y^{-1}, greater than combined emissions from estuaries and coastal areas (Seitzinger et al. 2000). Given that denitrification rates and the surface area of lakes and rivers are comparable (Seitzinger et al. 2006), current global N$_2$O emissions from aquatic ecosystems may be significantly underestimated.

Atmospheric N inputs to Norway are expected to increase >30% by 2100 (Hole and Engardt 2008) and similar trends are likely in other areas

depending on the alteration of precipitation patterns by climate change. Our findings suggest that lake sediments have the capacity to respond to current and future N loading rates to remove much of this anthropogenic N. However, in doing so the lakes appear to become a potentially important source of a potent greenhouse gas, N_2O. Consequently, we need a better understanding of how elevated N loading affects denitrifying communities, denitrification rates, and N_2O emissions in lakes. Such understanding may allow us to predict lake responses to N loading at broader spatial scales, to estimate the role of lakes in processing of N as it transits from the atmosphere to the ocean, and to take lake biogeochemical processing into account with respect to greenhouse gas dynamics.

ACKNOWLEDGEMENTS

LITERATURE CITED

APHA. 2005. Standard methods for the examination of water and wastewater, 21st edition. American Public Health Association, Washington, D.C.

Beaulieu, J. J., C. P. Arango, S. K. Hamilton, and J. L. Tank. 2008. The production and emission of nitrous oxide from headwater streams in the Midwestern United States. Global Change Biology **14**:878-894.

Berge, D., E. Fjeld, A. Hindar, and O. Kaste. 1997. Nitrogen retention in two Norwegian watercourses of different trophic status. Ambio **26**:282-288.

Cetin, M. and A. Erar. 2006. A simulation study on classic and robust variable selection in linear regression. Applied Mathematics and Computation **175**:1629-1643.

Cleveland, C. C. and A. R. Townsend. 2006. Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. Proceedings of the National Academy of Sciences of the United States of America **103**:10316-10321.

Cotner, J. B., J. W. Ammerman, E. R. Peele, and E. Bentzen. 1997. Phosphorus-limited bacterioplankton growth in the Sargasso Sea. Aquatic Microbial Ecology **13**:141-149.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters **10**:1-8.

Firestone, M. K., R. B. Firestone, and J. M. Tiedje. 1980. Nitrous oxide from soil denitrification: factors controlling its biological production. Science **208**:749-751.

Firestone, M. K. and J. M. Tiedje. 1979. Temporal change in nitrous-oxide and dinitrogen from denitrification following onset of anaerobiosis. Applied and Environmental Microbiology **38**:673-679.

Galloway, J. N. 1998. The global nitrogen cycle: changes and consequences. Environmental Pollution **201**:15-24.

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

Garcia-Ruiz, R., S. N. Pattinson, and B. A. Whitton. 1998. Denitrification in river sediments: relationship between process rate and properties of water and sediment. Freshwater Biology **39**:467-476.

Gnankambary, Z., U. Stedt, G. Nyberg, V. Hien, and A. Malmer. 2008. Nitrogen and phosphorus limitation of soil microbial respiration in two tropical agroforestry parklands in the south-Sudanese zone of Burkina Faso: The effects of tree canopy and fertilization. Soil Biology & Biochemistry **40**:350-359.

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

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

Hansen, M. R., J. R. DeFries, M. Townshend, M. Carroll, C. Dimiceli, and R. Sohlberg. 2003. Vegetation Continuous Fields MOD44B. 2001 Percent Tree Cover, Collection 3. University of Maryland, College Park, Maryland.

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

Hole, L. and M. Engardt. 2008. Climate change impact on atmospheric nitrogen deposition in northwestern Europe: A model study. Ambio **37**:9-17.

Hole, L. L. and K. Tørseth. 2002. Deposition of major inorganic compounds in Norway 1978-1982 and 1997-2001: status and trends. Norwegian Ministry of the Environment.

Huttunen, J. T., J. Alm, A. Liikanen, S. Juutinen, T. Larmola, T. Hammar, J. Silvola, and P. J. Martikainen. 2003. Fluxes of methane, carbon dioxide and nitrous oxide in boreal lakes and potential anthropogenic effects on the aquatic greenhouse gas emissions. Chemosphere **52**:609-621.

IPCC. 2007. Climate Change 2007: The physical basis - summary for policy makers. World Meteorological Organization/United Nations Environmental Programme, Paris.

Jensen, H. B., K. S. Jorgensen, and J. Sorensen. 1984. Diurnal-variation of nitrogen cycling in coastal, marine-sediments .2. Nitrous-oxide emission. Marine Biology **83**:177-183.

Kaste, Ø. and A. Lyche-Solheim. 2005. Influence of moderate phosphate addition on nitrogen retention in an acidic boreal lake. Canadian Journal of Fisheries and Aquatic Sciences **62**:312-321.

Kaste, Ø., J. L. Stoddard, and A. Henriksen. 2003. Implication of lake water residence time on the classification of Norwegian surface water sites into progressive stages of nitrogen saturation. Water Air and Soil Pollution **142**:409-424.

Kelly, C. A., J. W. M. Rudd, R. H. Hesslein, D. W. Schindler, C. T. Dillon, C. T. Driscoll, S. A. Gherini, and R. E. Hecky. 1987. Prediction of biological acid neutralization in acid-sensitive lakes. Biogeochemistry **3**:129-140.

Klein, H. and A. Benedictow. 2006. Transboundary air pollution by main pollutants (S, N, O_3) and PM: Norway.*in* Norwegian Meteorological Institute, editor. Programme for Monitoring and Evaluation of the Long-range Transmission of Air Pollutants in Europe.

Knowles, R. 1982. Denitrification. Microbiological Reviews **46**:43-70.

Liikanen, A., E. Ratilainen, S. Saarnio, J. Alm, P. J. Martikainen, and J. Silvola. 2003. Greenhouse gas dynamics in boreal, littoral sediments under raised CO_2 and nitrogen supply. Freshwater Biology **48**:500-511.

Lukkari, K., H. Hartikainen, and M. Leivuori. 2007. Fractionation of sediment phosphorus revisited. I: Fractionation steps and their biogeochemical basis. Limnology and Oceanography: Methods **5**:433-444.

Mengis, M., R. Gachter, and B. Wehrli. 1997. Sources and sinks of nitrous oxide (N_2O) in deep lakes. Biogeochemistry **38**:281-301.

Mulholland, P. J., A. M. Helton, G. C. Poole, R. O. Hall, S. K. Hamilton, B. J. Peterson, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, W. K. Dodds, S. E. G. Findlay, S. V. Gregory, N. B. Grimm, S. L. Johnson, W. H. McDowell, J. L. Meyer, H. M. Valett, J. R. Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, L. T. Johnson, B. R. Niederlehner, J. M. O'Brien, J. D. Potter, R. W. Sheibley, D. J. Sobota, and S. M. Thomas. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature **452**:202-206.

Nishio, T., I. Koike, and A. Hattori. 1983. Estimates of denitrification and nitrification in coastal and estuarine sediments. Applied and Environmental Microbiology **45**:444-450.

Pina-Ochoa, E. and M. Alvarez-Cobelas. 2006. Denitrification in aquatic environments: a cross-system analysis. Biogeochemistry **81**:111-130.

Richardson, W. B., E. A. Strauss, L. A. Bartsch, E. M. Monroe, J. C. Cavanaugh, L. Vingum, and D. M. Soballe. 2004. Denitrification in the Upper Mississippi River: rates, controls, and contribution to nitrate flux. Canadian Journal of Fisheries and Aquatic Sciences **61**:1102-1112.

Rudaz, A. O., E. Walti, G. Kyburz, P. Lehmann, and J. Fuhrer. 1999. Temporal variation in N_2O and N_2 fluxes from a permanent pasture in Switzerland in relation to management, soil water content and soil temperature. Agriculture Ecosystems & Environment **73**:83-91.

Schlesinger, W. H. 2009. On the fate of anthropogenic nitrogen. PNAS **106**:203-208.

Seitzinger, S., C. Kroeze, and R. Styles. 2000. Global distribution of N_2O emissions from aquatic systems: Natural emissions and anthropogenic effects. Chemosphere **2**:267-279.

Seitzinger, S. P., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, and C. Tobias. 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecological Applications **16**:2064-2090.

Seitzinger, S. P. and S. W. Nixon. 1985. Eutrophication and the rate of denitrification and N_2O production in coastal marine sediments. Limnology and Oceanography **30**:1332-1339.

Smith, M. S. and J. M. Tiedje. 1979. Phases of denitrification following oxygen depletion in soil. Soil Biology & Biochemistry **11**:261-267.

Solorzano, L. and J. H. Sharp. 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnol. Oceanogr. **25**:754-758.

Sundareshwar, P. V., J. T. Morris, E. K. Koepfler, and B. Fornwalt. 2003. Phosphorus limitation of coastal ecosystem processes. Science **299**.

Tørseth, K. and A. Semb. 1998. Deposition of nitrogen and other major inorganic compounds in Norway, 1992-1996. Environmental Pollution **102**:299-304.

Ullah, S. and G. M. Zinati. 2006. Denitrification and nitrous oxide emissions from riparian forest soils exposed to prolonged nitrogen runoff. Biogeochemistry **81**:253-267.

Wang, H. J., W. D. Wang, C. Q. Yin, Y. C. Wang, and J. W. Lu. 2006. Littoral zones as the "hotspots" of nitrous oxide (N_2O) emission in a hyper-eutrophic lake in China. Atmospheric Environment **40**:5522-5527.

Weyhenmeyer, G. A., E. Jeppesen, R. Adrian, L. Arvola, T. Blenckner, T. Jankowski, E. Jennings, P. Noges, T. Noges, and D. Straile. 2007. Nitrate-depleted conditions on the increase in shallow northern European lakes. Limnology and Oceanography **52**:1346-1353.

Williamson, C. E., W. K. Dodds, T. K. Kratz, and M. A. Palmer. 2008. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Frontiers in Ecology and the Environment **6**:247-253.

Wollheim, W. M., C. J. Vorosmarty, A. F. Bouwman, P. Green, J. Harrison, E. Linder, B. J. Peterson, S. P. Seitzinger, and J. P. M. Syvitski. 2008. Global N removal by freshwater aquatic systems using a spatially distributed, within-basin approach. Global Biogeochemical Cycles **22**:Gb2026, doi:2010.1029/2007GB002963.

Yoshinari, T. and R. Knowles. 1976. Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochemical and Biophysical Research Communications **69**:705-710.

Table 1. Study lakes according to N deposition level.

Lake	Sample date	Elevation (masl)	Lake surface area (km^2)[1]	Lake depth (m)	Tree cover %
High-deposition Lakes					
Berse	7/28/07	216	0.37	21.6	40
Brynesland	7/29/07	228	0.54	46.0	29
Dypingstjørni	7/31/07	177	0.08	13.4	32
Eigelivatnet	8/1/07	69	0.29	18.3	52
Eptelandvatnet	6/22/07	305	0.61	22.6	49
Furevatnet	7/21/07	146	1.16	14.1	33
Glypstadvatnet	8/1/07	281	0.36	20.7	43
Helleren	6/17/07	168	0.18	26.5	28
Krokavatnet	6/22/07	310	0.11	14.1	66
Nordravatnet	6/26/07	334	0.21	20.4	56
Revsvatnet	6/20/07	178	1.41	10.1	43
Saglandsvatnet	7/25/07	128	0.38	22.9	36
Skjævelandvnt	6/25/07	294	0.48	18.9	44
Stølsjørni	7/25/07	189	0.06	13.7	26
Svartavatnet	7/22/07	450	0.26	18.6	44
Ulsvatnet	6/16/07	174	0.09	17.7	22
Low-deposition lakes					
Bolvatnet	8/13/07	1,039	0.38	6.7	3
Djupen	7/6/07	951	0.74	7.6	12
Flaksjøen	8/14/07	941	1.43	7.9	3
Goppollvatnet	7/11/07	994	1.47	18.3	7
Hamntjønna	8/8/07	996	0.07	13.7	4
Hornsjøen	7/10/07	878	0.83	9.1	21
Langjtønna	8/17/07	1,020	0.09	15.8	3
Langrumpa	8/8/07	939	0.14	1.7	3
Ljosvatnet	7/15/07	827	0.39	5.8	35
Musvoltjønna	8/9/07	1,280	0.04	9.7	35
Muvatnet	8/18/07	1,141	0.45	7.6	8
Ner-Åst	7/8/07	978	0.54	9.1	7
Nevelvatnet	7/10/07	913	0.59	10.7	18
Reinsvatnet	7/6/07	961	3.88	6.4	18
Settningen	8/10/07	1,095	0.08	11.0	12
Vasjøen	7/8/07	935	1.01	5.5	17

[1] http://arcus.nve.no/website/nve/viewer.htm

Table 2. Average values (and standard error, s.e.) for various water and sediment parameters for the study lakes.

Lake	Surface Water				Sediment						
	pH	NO$_3^-$-N µmol/L	DOC µmol/L	TDP µmol/L	Total C mmol/g	Total N mmol/g	Total P mmol/g	C:N	C:P	N:P	Organic matter
Berse	6.30	14.4	163.5	0.09	8.7	0.60	0.10	14.5	88	6.0	0.24
Brynesland	6.65	45.3	165.0	0.13	10.9	0.92	0.27	11.8	40	3.4	0.27
Dypingstjørni	6.59	20.3	221.3	0.14	20.4	1.12	0.45	18.2	45	2.5	0.40
Eigelivatnet	6.43	35.1	112.7	0.08	14.5	0.95	0.37	15.3	40	2.6	0.32
Eptelandvatnet	5.50	13.6	105.2	0.08	9.4	0.62	0.07	15.3	134	8.8	0.25
Furevatnet	6.71	29.4	236.6	0.13	11.5	0.78	0.13	14.8	89	6.0	0.23
Glypstadvatnet	6.04	33.6	89.7	2.17	7.8	0.54	0.15	14.4	53	3.7	0.21
Helleren	5.55	26.8	91.1	0.08	14.1	0.99	0.24	14.2	59	4.1	0.28
Krokavatnet	6.22	10.6	140.5	0.07	13.7	0.81	0.11	17.0	129	7.6	0.33
Nordravatnet	6.51	16.8	84.8	0.09	5.9	0.31	0.08	18.9	71	3.7	0.18
Revsvatnet	6.62	11.2	158.8	0.15	18.2	0.97	0.19	18.7	96	5.1	0.37
Saglands	6.67	38.9	72.5	0.16	8.2	0.59	0.10	14.0	83	5.9	0.22
Skjæveland	6.73	10.4	151.6	0.08	9.3	0.69	0.20	13.5	46	3.4	0.27
Stølsjørni	5.76	28.3	76.8	0.08	13.9	0.87	0.15	15.9	92	5.8	0.30
Svartava	5.93	7.8	109.3	0.15	12.2	0.73	0.14	16.7	90	5.4	0.30
Ulsvatnet	5.48	22.6	83.3	0.13	12.4	0.83	0.14	14.9	87	5.8	0.26
Mean	6.23	22.8	128.9	0.24	11.95	0.77	0.18	15.5	78	5.0	0.28
s.e.	0.12	3.0	13.0	0.13	0.99	0.05	0.03	0.5	7.6	0.5	0.02

Table 2, continued. Results of statistical test indicate if there is a significant difference between deposition regions.

Nonsignificant results indicated by n.s.

| | Surface Water | | | | Sediment | | | | | | |
Lake	pH	NO₃⁻-N µmol/L	DOC µmol/L	TDP µmol/L	Total C mmol/g	Total N mmol/g	Total P mmol/g	C:N	C:P	N:P	Organic Matter
Bolvatnet	6.28	<0.04	421.4	0.19	12.5	0.81	0.13	15	97	6.3	0.28
Djupen	6.74	<0.04	233.1	0.14	14.4	0.90	0.15	16	97	6.0	0.32
Flaksjøen	6.81	<0.04	289.2	0.13	9.5	0.68	0.12	14	78	5.6	0.26
Goppollvnet	6.80	<0.04	147.2	0.13	9.3	0.67	0.12	14	78	5.6	0.21
Hamntjønna	6.19	<0.04	210.5	0.28	6.9	0.62	0.15	11	45	4.0	0.19
Hornsjøen	6.55	0.13	498.9	0.30	12.5	0.81	0.22	15	61	4.0	0.28
Langtjønna	6.96	<0.04	447.7	0.20	9.9	0.76	0.07	13	144	11.0	0.22
Langrumpa	6.94	<0.04	151.5	0.16	6.3	0.52	0.10	12	61	5.0	0.17
Ljosvatnet	6.78	<0.04	395.9	0.56	25.5	1.06	0.22	24	115	4.8	0.46
Musvoltjønna	6.54	<0.04	387.7	0.16	13.8	0.71	0.06	20	218	11.2	0.30
Muvatnet	7.50	<0.04	207.9	0.13	7.4	0.52	0.07	14	114	8.1	0.18
Ner-Åst	6.72	<0.04	332.5	0.18	14.6	1.07	0.30	14	48	3.5	0.30
Nevelvatnet	6.53	<0.04	218.6	0.16	7.8	0.65	0.18	12	44	3.6	0.18
Reinsvatnet	6.77	0.13	198.4	0.16	7.7	0.55	0.20	14	39	2.7	0.20
Settningen	7.01	<0.04	83.3	0.19	3.8	0.31	0.07	13	54	4.3	0.11
Vasjøen	6.73	<0.04	373.3	0.20	20.6	1.23	0.24	17	85	5.1	0.39
Mean	6.7	0.1	287.3	0.20	11.41	0.74	0.15	15	86	5.7	0.25
s.e.	0.3	0.0	123.1	0.11	1.44	0.06	0.02	0.8	13	0.6	0.02
high vs. low deposition	high < low	high > low	high < low	high = low	high = low	high = low	high = low	high = low	high = low	high = low	high = low
P	<0.001	<0.001	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

41

Table 3. Average rates (and standard error, s.e.) of N_2O production and denitrification for the study lakes.

High-deposition Region

Time period (h) Lake	N_2O-N μmol m⁻² h⁻¹ 0 - 4	4 - 12	Denitrification N μmol m⁻² h⁻¹ 0 - 4	4 - 12
Berse	0.6	-1.1	59.2	24.8
Brynesland	2.9	-1.1	114.1	102.0
Dypingstjørni	0.0	0.0	31.6	0.8
Eigelivatnet	1.2	-0.2	41.5	17.3
Eptelandvatnet	0.0	-0.7	23.4	2.4
Furevatnet	0.7	-2.1	61.4	29.6
Glypstadvatnet	1.3	0.3	52.8	54.0
Helleren	0.3	-1.1	34.6	32.8
Krokavatnet	1.0	0.0	10.5	4.5
Nordravatnet	0.8	-0.7	21.2	6.5
Revsvatnet	0.0	-0.3	21.6	1.7
Saglandsvatnet	6.8	-0.5	87.0	44.4
Skjævelandvatnet	0.0	-0.5	29.1	24.8
Stølsjørni	0.6	0.0	47.6	22.8
Svartavatnet	0.0	0.0	2.4	-
Ulsvatnet	0.4	0.0	29.0	2.0
Mean	1.1	-0.5	41.7	24.7
s.e.	0.4	0.2	7.4	7.0

Low-deposition Region

Time period (h) Lake	N_2O-N μmol m⁻² h⁻¹ 0 - 4	4 - 12	Denitrification N μmol m⁻² h⁻¹ 0 - 4	4 - 12
Bolvatnet	-	-	-	-
Djupen	-	-	-	0.2
Flaksjøen	-	-	0.3	-
Goppollvatnet	-	-	-	-
Hamntjønna	-	-	-	-
Hornsjøen	0.3	-	-	-
Langjtønna	-	-	2.8	0.6
Langrumpa	-	-	-	-
Ljosvatnet	-	-	-	-
Musvoltjønna	-	-	-	-
Muvatnet	-	-	-	-
Ner-Åst	-	-	-	-
Nevelvatnet	-	0.6	-	-
Reinsvatnet	-	-	-	-
Setningen	-	-	0.3	-
Vasjøen	-	-	-	-
Mean	0.0	0.1	0.2	0.0
s.e.	0.0	0.1	0.4	0.1

Figure 1. Location of study sites in Norway. Fieldwork was conducted in two regions that encompass a gradient of atmospheric nitrogen deposition. Nitrogen deposition is greatest in extreme southwestern Norway, with rates of 10-20 kg N ha^{-1} y^{-1}, and decreases to less than 4 kg N ha^{-1} y^{-1} in northeastern areas (Hole and Tørseth 2002). Circles denote lakes sampled in high deposition areas and lakes sampled in low deposition areas are denoted by triangles.

43
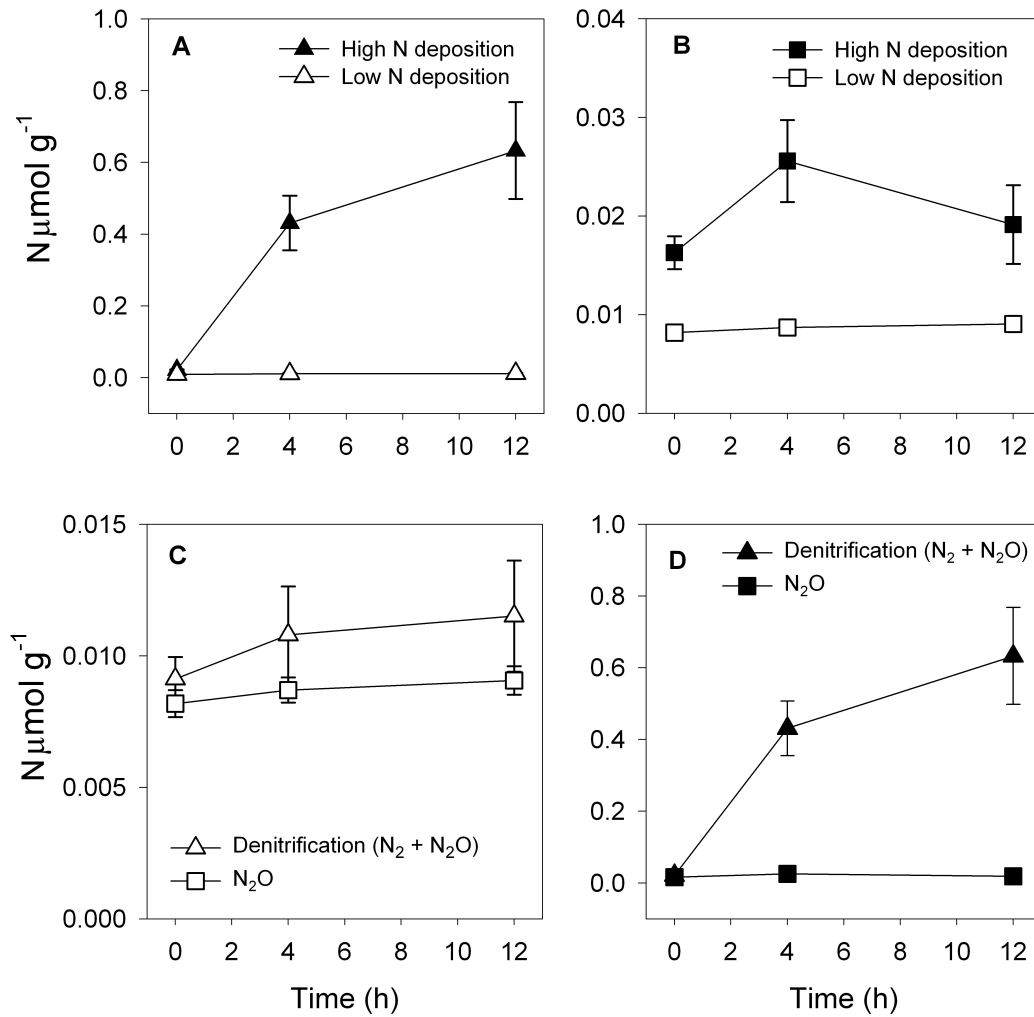
Figure 2. Comparisons of N_2O production following the onset of anoxic conditions between N deposition regions in incubations (A) amended with acetylene and (B) without acetylene and N_2O production in incubations with and without acetylene for (C) low-deposition lakes and (D) high-deposition lakes. Solid symbols indicate lakes in high-deposition areas and open symbols indicate lakes in low-deposition areas. Triangles indicate incubations amended with acetylene and squares indicate incubations without acetylene.
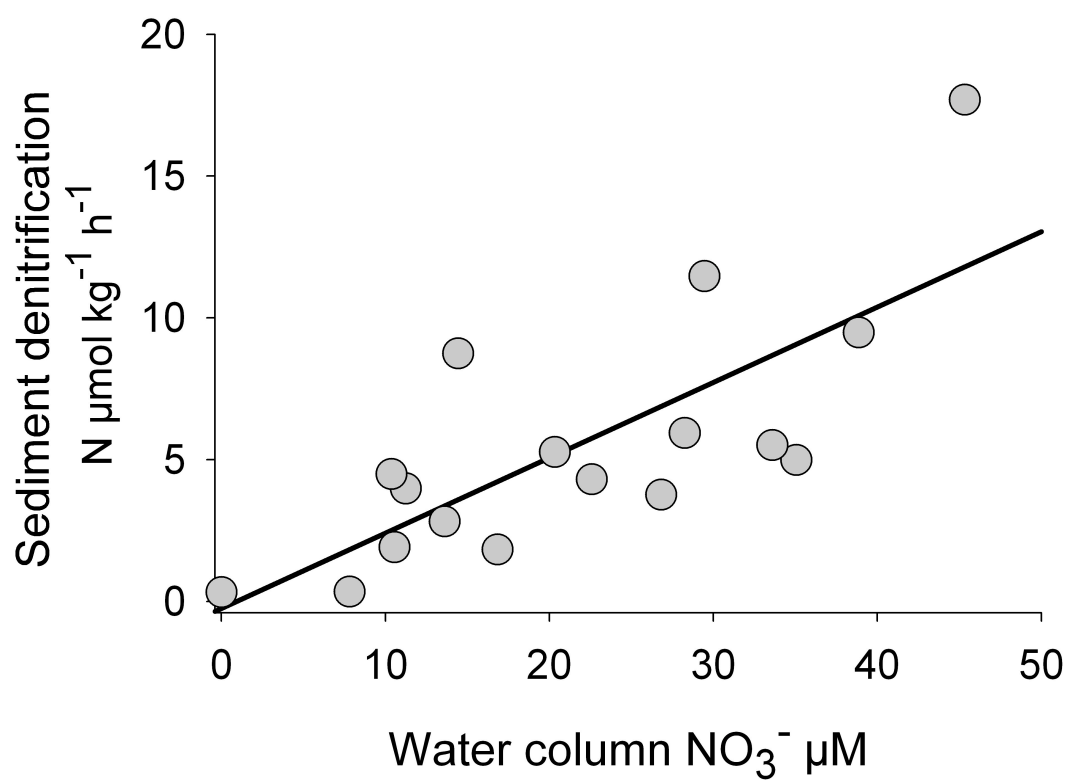
Figure 3. Relationship between sediment denitrification rate and water column concentrations of nitrate for lakes in the high nitrogen deposition region (n = 16 lakes).
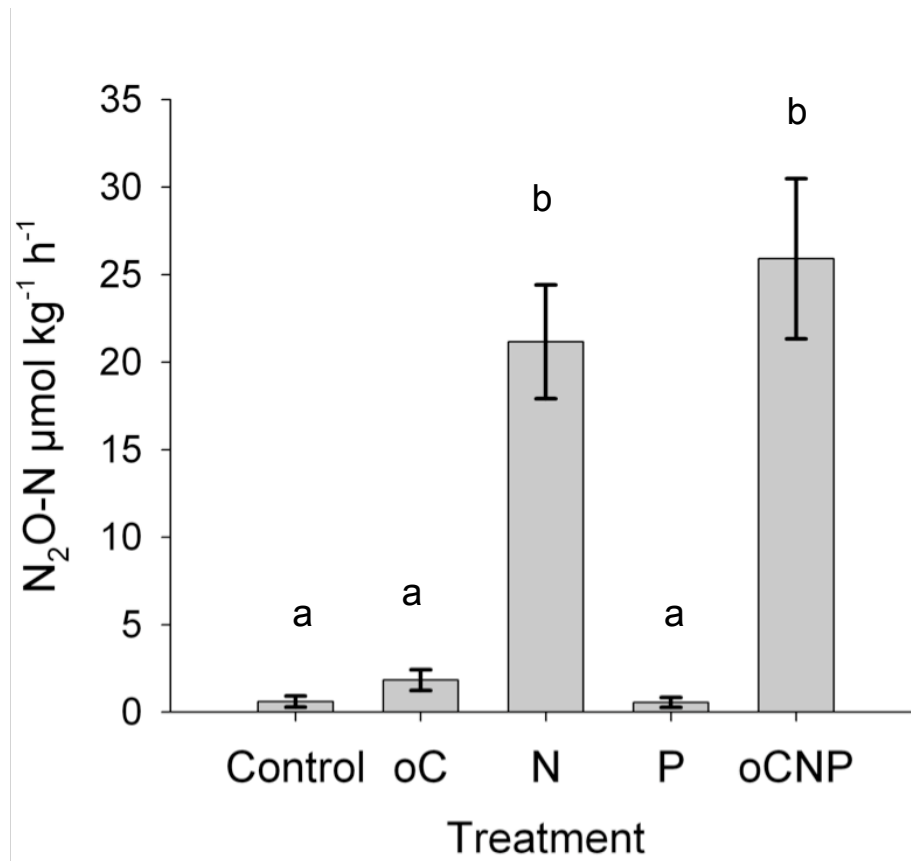
Figure 4. Denitrification rate in response to experimental resource enrichments. Bars are means +/- 1 SE (n = 32 lakes). Letters above bars indicate significant differences between means (P < 0.001)

CHAPTER 3

Kinetics Of Denitrification and the Abundance Of Denitrifying Bacteria in

High-Elevation Lakes Receiving Atmospheric Nitrogen Deposition

(Colorado, USA)

ABSTRACT

The transport and deposition of anthropogenic nitrogen (N) to downwind ecosystems is significant and continues to increase. Indeed, atmospheric deposition can be the dominant source of new N to watersheds. Microbially mediated denitrification in lake sediments may ameliorate the effects of N loading by permanently removing such inputs. We measured denitrification in sediments collected from alpine and subalpine lakes in the Rocky Mountains of Colorado (USA) receiving elevated (5-8 kg N ha^{-1} yr^{-1}) or low (< 2 kg N ha^{-1} yr^{-1}) levels of atmospheric N deposition. The nitrate (NO_3^-) concentration was significantly greater in high-deposition lakes (11.3 µmol L^{-1}) compared to low-deposition lakes (3.3 µmol L^{-1}), but denitrification did not differ between high- and low-deposition regions. We estimate that the sampled lakes are capable of removing a significant portion of N inputs via denitrification in sediments. We also conducted a dose-response experiment to determine whether chronic N loading has altered sediment denitrification capacity. Under the Michaelis-Menten model, the maximum denitrification rate and half saturation constant were 765 µmol N m^{-2} h^{-1}

47

and 293 µmol L^{-1} NO$_3^-$, respectively, for all lakes. We estimated the abundances of nitrate- and nitrite-reducing bacteria and found no difference between high- and low-deposition lakes. The abundances of these bacteria were most strongly related to available light and bulk sediment resources. Our findings support a growing body of evidence that lakes play an important role in N removal and, furthermore, suggest that current levels of N deposition have not saturated the capacity for sediment denitrification.

INTRODUCTION

The global rate of atmospheric nitrogen deposition is expected to reach 125 Tg N y^{-1} by 2050, a seven-fold increase since preindustrial times (Galloway et al. 2004). Ecosystems even in unpopulated areas are subject to anthropogenic influences via the long distance transport of these pollutants (Wolfe et al. 2006). Saturation occurs when N is delivered to ecosystems in excess of biological demand and NO$_3^-$ leaches from the ecosystem (Aber et al. 1998, Tietema 1998). Thus, increases in N deposition will perpetuate saturating conditions for systems already subject to chronic N loading and may push other ecosystems to saturation. As N leaches from soils to the oceans, denitrification (the microbial conversion of NO$_3^-$ to inert di-nitrogen gas) plays an important role in permanently removing N and mitigates N loading to coastal ecosystems (Galloway et al. 2003, Seitzinger et al. 2006).

Lentic ecosystems, such as lakes and reservoirs, have recently been recognized as important biogeochemical sinks for N. Indeed, lakes may remove nearly 20 Tg N y^{-1} from watersheds globally, which represents ~30% of estimated inputs to surface waters (65 Tg N y^{-1}, Wollheim et al. 2008, Harrison et al. 2009). Such N removal capacity is significant considering that lakes occupy 3% of the land surface. Potential fates for N in lakes include permanent removal by denitrification, long-term storage in sediments, or temporary storage in biomass. Of these mechanisms, denitrification may often account for the majority of N removed globally from these ecosystems (Saunders and Kalff 2001b). Here we report on denitrification in sediments of high-elevation Colorado lakes receiving low (< 2 kg ha^{-1} yr^{-1}) or elevated (> 6 kg ha^{-1} yr^{-1}) inputs of atmospheric N deposition and estimates of the role of these lakes in reducing downstream N loading.

Given the expected increases in anthropogenic N inputs and the susceptibility of remote ecosystems to atmospherically deposited N, it is important to understand the limits to denitrification. Just as terrestrial ecosystems may experience N saturation (Aber et al. 1998), aquatic ecosystems may similarly become prone to increased N exports. Models of N saturation were originally developed for terrestrial ecosystems and more recently have been applied to streams (Bernot and Dodds 2005, Earl et al. 2006). Indeed, there is strong evidence that the efficiency of removal

of NO_3^- by denitrification in streams decreases with increasing N loading rate (Mulholland et al. 2008). We directly tested the denitrification capacity of lake sediments by performing NO_3 dose-response experiments. We expected a negative relationship between denitrification capacity and N deposition rate if chronic atmospheric N deposition had produced a saturating effect as suggested for temperate forests.

Microbial communities influence biogeochemical processes that are critical to N cycling in soils and lake sediments. Chronic N loading has altered the composition and function of microbial communities in soils, reducing respiration rates and increasing N mineralization rates (Lovett and Rueth 1999, Bowden et al. 2004, Wallenstein et al. 2006a). McCrackin and Elser (2010) found sediment denitrification was greater in lakes receiving elevated N deposition, however, there was no difference in potential denitrification (assays amended with non-limiting concentrations of NO_3^- and organic carbon, oC) between lakes at the extreme ends of a gradient of N deposition, despite a five-fold difference in atmospheric N loading rates (< 4 to 20 kg N ha^{-1} y^{-1}). These data suggest that sediment function has not been affected; however, the composition of denitrifying communities per se has not been directly analyzed in lakes subject to long-term N deposition. Here we contrast the abundance of denitrifying bacteria in sediments between lakes in two regions with differing levels of atmospheric N inputs.

In addition to understanding how denitrification varies between lakes, there is interest in understanding how denitrification varies *within* a given lake. In terrestrial ecosystems, denitrification is known to vary at small scales, both temporally and spatially, making it a particularly difficult process to measure (Groffman et al. 2006). Such variability likely exists in lake sediments as well, because factors that influence denitrification – concentrations of oxygen, oC, NO_3^- – will fluctuate as the balance between production and respiration shifts with the availability of light. In littoral sediments where denitrification is tightly linked with nitrifying bacteria as a source of NO_3^-, benthic periphyton may suppress nitrification—and hence denitrification—by out-competing nitrifying bacteria for ammonium (NH_4^+, Risgaard-Petersen 2003). As light declines in deeper water layers, corresponding decreases in the abundance of periphyton may allow nitrifiers and denitrifiers to more successfully compete for N. Indeed, a study of marine sediments found that denitrification increased with water depth and that rates of denitrification and gross primary production were strongly negatively related (Sundbäck et al. 2004). Accordingly, we hypothesized that microbially mediated N cycling in lakes is influenced by water depth because of competition from microalgae. We tested this hypothesis by quantifying the abundance of bacterial capable of NO_3^- and nitrite (NO_2^-) reduction in 16 lakes and in one lake along a water depth gradient.

METHODS

Study site

We sampled 20 lakes located in the Rocky Mountains of Colorado (USA) between June and August 2008. The eastern slopes of the Rocky Mountains near Niwot Ridge Long Term Ecological Research site (NWT) and the Loch Vale Watershed Research site (LVW) in Rocky Mountain National Park receive atmospheric N deposition from fossil fuel combustion and agricultural sources in Denver and eastern Colorado (Nanus et al. 2003, Burns 2004). The rate of inorganic N (NO_3^- + NH_4^+) deposition has increased over the past 20 years to 6-8 kg ha^{-1} y^{-1} from background levels of < 2 kg ha^{-1} y^{-1} (Fig. 5; data from the National Atmospheric Deposition Program, nadp.sws.uiuc.edu). Lakes in central and western Colorado near the Rocky Mountain Biological Laboratory (RMBL, Gothic, CO) and the Mountain Studies Institute (MSI, Silverton, CO) receive < 2 kg ha^{-1} y^{-1} atmospheric inputs of N. Lakes near NWT and LVW are considered to be in the high-deposition region and lakes near RMBL and MSI are considered to be in the low-deposition region. Selected lakes were within 6 km of a trailhead. Mean annual temperatures at high elevations are < 2°C and the lakes are generally covered with ice between November and June (Baron et al. 2000). Catchment vegetation below the tree line (~3,300 m) is characterized by Englemann spruce and subalpine fir forests. Alpine tundra is found above the tree line. With the

exception of Estes Lake, the sampled lakes were small, generally ~ 0.1 km^2, and the watersheds were unpopulated.

The sampled lakes occupy geologically diverse bedrock (Kent and Porter 1980). In the vicinity of Rocky Mountain National Park, Precambrian-age granite, gneiss, and schist dominate the underlying geology. Near RMBL the geologic parent materials include Mesozoic sedimentary rocks and Paleozoic metamorphics and intrusives. The San Juan Mountains surrounding MSI are characterized by extensive volcanic deposits and felsic gneisses and granites of the Uncompahgre formation.

Field sampling and laboratory procedures

We sampled 10 lakes in both the high- and low-deposition regions (Table 4). Each lake was visited once during summer 2008. Fieldwork was staggered so that sampling of lakes in high- and low-deposition regions was not strongly skewed by date. High-deposition lakes were sampled in late June and late July 2008 and low-deposition lakes were sampled in early July and early August 2008. An inflatable boat was used for sampling each lake. The depth of the lake was measured with a hand-held echo sounder. Water temperature and dissolved oxygen at the sampling depth were measured with a YSI model 85 temperature-oxygen probe (YSI, Yellow Springs, Ohio, USA). Water was collected just above the sediments using a battery-powered submersible pump and then filtered with Pall A/E glass fiber filters. Water samples were frozen until analysis

53

for dissolved organic carbon (DOC) on a Shimadzu TOC 5000 (Shimadzu Corporation, Kyoto, Japan) and for nitrate plus nitrite (hereafter: NO_3^-) concentration on a Lachat Quick Chem 8000 autoanalyzer (Hach Corporation, Loveland, Colorado, USA).

Photosynthetically active radiation (PAR) was measured at the surface and at 10 m or at the maximum lake depth if <10 m using a LI-COR data-logger and model LI-192 Underwater Quantum Sensor (LI-COR Corporation, Lincoln, NE, USA). The light extinction coefficient (k) was calculated as $k = -z * L_N(I_Z/I_0)$, where I_Z is the irradiance at 10 m or the maximum lake depth, I_0 is the irradiance at the lake surface, and z was the depth at which irradiance was measured.

Surface sediments were collected using a LaMotte dredge from a water depth of approximately 10 m or at the maximum lake depth if the lake was < 10 m. The dredge collected sediments from an area of 221 cm^2 to a depth of ~7 cm. Sediments were returned to the laboratory and processed within 24 hours of collection. Denitrification was measured using the acetylene inhibition method (Yoshinari and Knowles 1976). To estimate the background rate of denitrification, three replicate 100 g subsamples of homogenized sediments from each lake were slurried with 80 mL of water collected from above the sediments. Bottles were purged of oxygen with nitrogen gas (N_2) and acetylene was added to block the reduction of N_2O to N_2. After vigorous shaking, we collected 10-mL

samples from the headspace volume (about 550 mL) at the onset of anoxic conditions (0 h) and at 4 h. Incubations were conducted at 4°C (estimated annual temperature at sediment depth) in dark conditions. Gas samples were analyzed for N_2O on a Varian CP-3800 gas chromatograph (Agilent Technologies, Santa Clara, CA, US) with an electron-capture detector. The denitrification rate was determined as the production of N_2O during the incubations on the basis of dry sediment mass and was converted to an areal basis using the sediment bulk density for each lake (Richardson et al. 2004).

We conducted a dose-response experiment to evaluate the capacity of sediments to denitrify additional NO_3^- inputs. For each lake, three replicate 50-g subsamples of homogenized sediments were slurried with 40 mL of one of six different incubation media. The incubation media were: distilled water as a control, 0.07 mg nitrate-N L^{-1} (NO_3^--N, 5 µmol L^{-1}), 0.7 mg NO_3^--N L^{-1} (50 µmol L^{-1}), 7.0 mg NO_3^--N L^{-1} (500 µmol L^{-1}), 70 mg NO_3^--N L^{-1} (5,000 µmol L^{-1}), and a potential denitrification medium (oCNP) consisting of 100-mg oC (dextrose and acetate) L^{-1}, 100 mg NO_3^--N L^{-1} (7,143 µmol L^{-1}), 13.84 mg phosphate (PO_4^{3-})-P L^{-1} (450 µmol L^{-1}, for N:P ratio of 16). We induced anoxia and amended the slurries with acetylene as described above. We collected 3.5-mL initial samples and final samples at the end of the 4-h incubation at 17°- 20°C in dark conditions, both after vigorous shaking. These slurries were incubated at

room temperature because we interested in maximal denitrification rates, not rates that are representative of *in situ* conditions, and for comparison with previous studies (McCrackin and Elser 2010). Gas samples were analyzed for N_2O on a Shimadzu 14A gas chromatograph (Shimadzu, Kyoto, Japan) with an electron-capture detector. Denitrification in response to experimental resource enrichments is reported on the basis of dry sediment mass and was converted to an areal basis using the sediment bulk density for each lake.

We investigated the influence of atmospheric N deposition and individual lake characteristics on NO_3^-- and NO_2^--reducers (together as denitrifiers) in a subset of 16 lakes by estimating the culturable populations of these organisms using a most probable number (MPN) technique (Staley and Griffin 1981). We further investigated the spatial variability of these organisms for Little Molas Lake along a water depth gradient. To determine the MPN of denitrifiers, we suspended 4-g subsamples of homogenized sediment in 25 mL of 25% Ringer's solution, forming a slurry from which 100 µL was inoculated into each well of a 96-well micro-titer plate by 10-fold serial dilutions (Johnson et al. 2007). Prior to inoculation with sediment slurry, 100 µL of NO_3^- broth (9.9 mmol NO_3^-/L, Difco Laboratories, Detroit, MI, USA) was added to each well. Plates were incubated in a dark, anoxic environment in a vacuum desiccator for one week. Positive growth was determined by the addition of diphenylamine to

test for the presence of NO_3^- and N(1-napthyl)-ethylene-diamine-dihydrochloride to test for the presence of NO_2^-. Wells that indicated NO_2^- were scored positive for NO_3^--reducing bacteria and wells that indicated the absence of NO_3^- and NO_2^- were scored positive for NO_2^--reducing bacteria. Scores were translated to the MPN of each microorganism using a probability table developed by Rowe et al. (1977).

Sediment water content was determined as mass loss after drying subsamples at 105°C for 48 h and organic matter (OM) content was determined as mass loss on ignition at 550°C for 4 h. Total C and N content of dried sediments were measured with a PerkinElmer CHN elemental analyzer (Perkin-Elmer, Waltham, MA). Total phosphorus content of combusted sediment was measured colorimetrically following extraction with 0.5 M hydrochloric acid using the acid molybdate technique (Lukkari et al. 2007).

Statistical analysis

We performed t tests or, where the assumption of normality was not met, Mann-Whitney *U* tests to compare water concentrations of NO_3^-, DOC, sediment organic matter, sediment nutrient pools, MPN of denitrifiers, and gas fluxes from slurry incubations between lakes in high- and low-deposition regions. Under the assumption that the denitrification process is a single step that converted NO_3^- to $N_2O + N_2$, the Michaelis-Menten model was used to determine dose-response relationships of

57

denitrification to experimental additions of NO_3^- (Betlach and Tiedje 1981, Silvennoinen et al. 2008):

(1)   Denitrification rate = $(V_{max} * [NO_3^-]) / (K_m + [NO_3^-])$

Here the denitrification rate is the production of N_2O plus N_2, V_{max} is the maximum denitrification rate, and K_m is the NO_3^- concentration that results in 50% of the denitrification rate, also referred to as the affinity constant (Laverman et al. 2006). The parameters V_{max} and K_m were estimated by fitting the "ligand-binding, one-site saturation model" in SigmaPlot Version 10 (SSI, San Jose, CA, USA) to measured denitrification rates. Parameters were estimated separately for each lake, separately for lakes in the high- and low-deposition regions, and together for all sampled lakes.

Multiple-linear regression was used to identify relationships among predictor variables and background denitrification, potential denitrification, and the MPN of NO_3^-- and NO_2^--reducers. Predictor variables included water concentrations of NO_3^- and DOC, sediment organic matter, sediment C, N, and P content, ratios of sediment C:N and N:P, the PAR light extinction coefficient, and bacterial abundances. Models were selected by considering all subsets on the basis of adjusted R^2 and AIC. We evaluated multi-colinearity and selected final models for which tolerance values were > 0.5 for all predictor variables. When necessary, response and predictor variables were transformed to improve normality.

Statistical tests were performed using JMP (SAS Institute, Inc.) Version 8.0.1, with α = 0.05.

RESULTS

Water and bulk sediment characteristics

The mean concentration of NO_3^- was greater in high-deposition lakes compared to low-N deposition lakes at 11.3 µmol L^{-1} and 3.3 µmol L^{-1}, respectively (Table 4). High- and low-deposition lakes did not differ in DOC concentrations, which averaged 0.64 mmol L^{-1} across all lakes. There were no significant differences in sediment organic matter, total C, N, and P content, or ratios of C:N, C:P, and N:P between lakes in high- and low-deposition regions (P > 0.05). Lakes in the low-deposition region were generally thermally stratified whereas lakes in the high-deposition region were not. All lakes were oxic at the depth where sediments were collected.

Dose-response experiment

Among the study lakes, the denitrification rate in response to the maximum dose of NO_3^- was between 9 and 1,600 µmol N m^{-2} h^{-1} with a mean rate of 700 µmol N m^{-2} h^{-1} ($\pm$ 117 SE, Table 5). Assuming Michaelis-Menten kinetics, V_{max} was 765 µmol N m^{-2} h^{-1} and K_m was 293 µmol L^{-1} NO_3^- across all lakes (R^2 = 0.98, P = 0.001; Fig. 6). The half-saturation constants, K_m, for the study lakes were negatively related to sediment OM content (Table 6). The fitted V_{max} for high-deposition lakes was

59

comparable to that for low-deposition lakes, at 797 and 700 μmol N m^{-2} h^{-1}, respectively. When the Michaelis-Menten model was fit to the data for each deposition region separately, the half-saturation (or NO_3^--affinity) constant for high-deposition lakes was nearly half that of low-deposition lakes (448 and 207 μmol L^{-1} NO_3^-, respectively); however, these values were not statistically different (P > 0.05).

<center>Denitrification in sediments</center>

In slurries amended with lake water, denitrification ($N_2 + N_2O$) was undetectable in nine of the sampled lakes (Table 5). For all lakes, the mean denitrification rate was 4.4 μmol N m^{-2} h^{-1} ($\pm$ 3.3 SE). Where denitrification was observed, the mean rate was 8.4 μmol N m^{-2} h^{-1} ($\pm$ 6.3 SE) with a range of 0.7 to 61.9 μmol N m^{-2} h^{-1}. There was no difference in mean background rates of denitrification in lakes between high- and low-deposition regions (P > 0.05). The concentration of dissolved NO_3^- was the strongest predictor of denitrification (Table 6, Fig. 7).

The mean rate of potential denitrification (in response to non-limiting concentrations of oC, NO_3^-, and P) was nearly 200 times greater than the mean rate of denitrification in assays amended with lake water (Table 5). There was no difference in potential denitrification potential between high- and low-deposition lakes, which averaged 924 μmol N m^{-2} h^{-1} ($\pm$ 159 SE). The rate of potential denitrification across all lakes was negatively related to the sediment C:P ratio (Table 6).

Most probable number of denitrifying bacteria

The abundances of sediment denitrifiers varied between lakes, but did not differ between deposition regions ($P > 0.05$, Table 7). The mean abundances of NO_3^-- and NO_2^--reducing bacteria at the maximum measured depths were 38 ($\pm$ 7 SE) x 10^4 and 15 ($\pm$ 5 SE) x 10^2 bacteria g^{-1} dry sediment, respectively, across all lakes (Table 4). Regression analysis revealed that the MPN of NO_3^--reducing microbes was negatively related to available light and positively related to sediment OM. The abundance of NO_2^--reducing bacteria was negatively related with the sediment C:N ratio (Table 6). Intensive sampling in Little Molas Lake at 0.5, 1, 2, and 6 m, yielded no statistically significant depth variation in terms of abundances of bacteria capable of NO_3^-- and NO_2^--reduction; however, the MPN generally increased from 0.5 to 6 m water depth (Table 7).

DISCUSSION

Nitrogen removal in lakes

In catchments subject to N loading, seasonal patterns of NO_3^- export are an indicator of N saturation (Aber et al. 1998, Stoddard et al. 1999). Nitrogen saturation begins with increased seasonal NO_3^- concentrations in stream water exiting the watershed. Later stages occur when NO_3^- concentrations are elevated in all seasons, with no decline during the growing season, and where watershed NO_3^- exports exceed

inputs. Symptoms of advanced stages of N saturation have been observed in catchments of the Colorado Rocky Mountains and atmospheric N deposition have been linked with elevated NO_3^- concentrations in high-elevation lakes and streams (Williams et al. 1996, Burns 2004). Recent models have found that lakes are significant sinks for N, removing an average of 0.3 mol N m^{-2} y^{-1} through denitrification and burial in the sediment (Harrison et al. 2009). Here, we investigated the role that lakes play in removing N from high-elevation ecosystems. We measured potential denitrification and conducted dose-response experiments to investigate the upper capacity for N removal by denitrification.

We found that sediments possess considerable capacity for denitrification regardless of the atmospheric N loading rate (Table 5). Potential denitrification did not differ between regions, consistent with experiments conducted with sediments of Norwegian lakes across a gradient of deposition (McCrackin and Elser 2010). The mean potential denitrification rate for Colorado lakes was three times that of Norwegian lakes. The difference between studies could be due to the underlying denitrifying communities because in Norway the N deposition rate is two to three times greater than that in Colorado (Tørseth and Semb 1998). Further, the potential rate of denitrification was comparable to the maximum denitrification rate (in response to additions of 5000 μmol

L^{-1} NO_3^-), and both were up to three orders of magnitude greater than

denitrification measured in response to additions of lake water. This

observation and the large NO_3^- half-saturation concentration (K_m,), which

was ~35 times greater than current mean lake water NO_3^- concentrations,

suggest that denitrifying communities are functioning well below their

maximum potential rates. These results are not strictly comparable

because of temperature differences between the incubations but are

consistent with evidence that NO_3^- often limits denitrification in sediments

(Seitzinger et al. 2006, Mulholland et al. 2008). Denitrification in sediments

of highly N-loaded lakes may be more likely to saturate than the

unproductive lakes that we sampled. Thus, it would be interesting to

compare our findings to denitrification kinetics in sediments of lakes in

agricultural landscapes.

The NO_3^--affinity constant for all lakes in the dose-response

experiment, 298 µmol L^{-1} NO_3^-, is comparable to values reported in the

literature for soils and sediments but greater than that measured in pure

cultures (Table 8, Oren and Blackburn 1979, Betlach and Tiedje 1981, Yu

et al. 2006). In pure cultures, generally low values for K_m reflect the

absence of diffusion barriers between NO_3^- and active sites of enzymes.

Results from pure cultures might also reflect a bias of isolated taxa that

are selected for fast growth and high affinity for NO_3^- (Laverman et al.

2006). Half-saturation constants < 50 µmol L^{-1} have also been reported for

lake and coastal marine sediments, although these low values might reflect enhanced microbial access to NO_3^- and oC resulting from stirring or shaking sediment slurries during incubation (Koike et al. 1978, Oremland et al. 1984). Silvennoinen et al. (2008) reported K_m of 20 µmol L^{-1} using sediment cores from a eutrophied river. In this case, the low half-saturation concentration was attributed to a bacterial community with a high affinity for NO_3^-. Thus, differences in K_m values between pure cultures and environmental samples likely reflect both the effects of the physical structure of sediment or soil as a diffusional barrier and the innate affinity of microorganisms for NO_3^-. The magnitude of the K_m that we measured suggests that, while our slurry method disturbed the sediment structure at the onset of the experiment, the lack of shaking during the course of the incubation may result in conditions similar to that of intact sediment. Alternatively, the denitrifiers in sediments of lakes we sampled may have lower affinity for NO_3^- compared to those in other ecosystems.

In Colorado lakes and elsewhere, N deposition has reduced pH, changed the composition and biomass of the diatom community, altered the stoichiometric ratios of N to P in the water column, and shifted phytoplankton nutrient limitation (Baron et al. 2000, Elser et al. 2009). Future population growth in metropolitan Denver or agricultural intensification in eastern Colorado will result in increased N inputs to the Front Range of the Colorado Rocky Mountains and will further stress

ecosystems that currently show signs of N saturation (Burns 2004).

Catchment storage and removal of inorganic N is between 21% and 97%,

varying largely due to differences in forest and soil cover (Sickman et al.

2002), although lakes weren't specifically considered. Musselman and

Slauson (2004) surveyed high-elevation lakes in Colorado and found that

NO_3^- concentrations decreased between lake inlets and outlets. While the

sediments of the lakes we sampled show considerable denitrification

capacity, the actual role that sediments play in N removal depends on lake

depth and water residence time in addition to the actual denitrification rate

(Kelly et al. 1987). Lake N removal has been observed to correlate

negatively with lake depth and positively with water residence time (Dillon

and Molot 1990, Molot and Dillon 1993). The denitrification rate we

measured in high-deposition lakes was at the low end of that reported for

other lakes (Pina-Ochoa and Alvarez-Cobelas 2006), although such rates

may not be comparable because of differences in N inputs or methods.

Still, these data permit us to make a preliminary estimate of the magnitude

of N removal via denitrification.

We estimated the fraction of N that may be removed in the sampled

lakes based on the background denitrification rate as in Harrison et al.

(2009) as $R_N = 1 - \exp(-V_f / H)$, where V_f is the piston velocity for N (m y^{-1})

in lake sediments and H is the areal water discharge from the lake,

hydraulic load (m y^{-1}). Using the method of Kelly et al. (1987), the piston

velocity was estimated as V_f = denitrification rate/$[NO_3^-]$, with the measured denitrification rate and NO_3^- concentration for each lake. The mean piston velocity was 2.1 m y^{-1}, with a range of 0 – 23.8 m y^{-1}, comparable to values reported by Harrison and colleagues (2009). The hydraulic load was estimated as in Wollheim and Vorosmarty (2006) as H = $\bar{z}/\tau$, where the mean lake depth, $\bar{z}$ (m), was estimated as one-third of the maximum depth of each lake and the water residence time, τ (y), was assumed to be 0.083 (1 month) or 1 y, which is in the range of residence times reported for lakes in areas where we sampled.

Nitrate concentrations in lakes in the Colorado Rocky Mountains vary seasonally due to the timing of snowmelt and biological assimilation (Campbell et al. 2002). In the Loch Vale watershed, stream water NO_3^- concentrations have been found to vary between 10 and 35 μmol L^{-1} seasonally (Baron and Campbell 1997). Concentrations tend to peak in lake May, decrease during June and July, and increase in the fall. Assuming lakes follow a similar pattern, the timing of our sampling likely missed peak NO_3^- concentrations. We expect the denitrification rate would increase with additional NO_3^-; thus, variability in concentrations contributes to uncertainty in our estimates. Indeed, temporal variation in sediment denitrification has been attributed to seasonal changes in dissolved NO_3^- concentrations (Hasegawa and Okino 2004). Consequently, we calculated

a 95% confidence interval (CI) of N removal based on the standard error of mean R_N to reflect uncertainty and variability in these parameters.

In the case of extremely short (1 month) residence times, we estimate that the sampled lakes could remove an average 11% (4 – 25% CI) of N inputs across all lakes, 18% (10 – 38% CI) for high-deposition lakes and < 1% (0 – 2% CI) for low-deposition lakes. For a 1 y residence time, denitrification could remove an average 26% (9 – 43% CI) for all lakes, 41% (10 – 72% CI) for high- deposition lakes, and 9% (0 – 18% CI) for low-deposition lakes. There are many uncertainties in scaling from laboratory measurements of denitrification to whole-lake estimates of N removal. To refine these estimates, existing models of N removal (e.g. Harrison et al. 2009) could be modified and downscaled. Further work is needed to understand the *in situ* denitrification rate and how it varies within and between lakes, and the seasonality of NO_3^- concentrations. Obtaining such data from remote, high-elevation lakes is challenging. While our estimates are approximate, they suggest that denitrification in lake sediments can be an important biogeochemical sink for N in lakes receiving atmospheric deposition.

Atmospheric N deposition is the dominant source of new N to watersheds in the Front Range of the Colorado Rocky Mountains (Sickman et al. 2002). The source of NO_3^- to sediment denitrifiers, however, is not clear. In Colorado, NO_3^- and NH_4^+ are 61% and 39%,

respectively, of N deposition (Baron et al. 2000). Thus, deposition can deliver NO_3^- to the lake directly or through watershed runoff, and indirectly through nitrification resulting from elevated NH_4^+ inputs. Indeed, isotopic studies have found that nitrification in talus slopes is a significant source of NO_3^- to streams in the Colorado Front Range (Campbell et al. 2002, Nanus et al. 2008). In lake sediments, the extent to which nitrification and denitrification are coupled depends on concentrations of NO_3^- in bottom waters (Dong et al. 2000, Seitzinger et al. 2006). Where NO_3^- concentrations are < 10 μM, over 90% of denitrification may tightly coupled with nitrification. Where NO_3^- concentrations are > 10 μM, such as in high-deposition lakes, sedimentary denitrification may predominantly consume NO_3^- that diffuses from the water column.

Due to their underlying geology, lakes in the high-deposition region are sensitive to atmospheric deposition (Clow et al. 2003, Nanus et al. 2009). Increased pH and decreased acid neutralizing capacity have been observed in surface water of lakes in the Colorado Front Range. Indeed, Elser and colleagues (2009) sampled lakes in the same regions and reported that pH averaged 6.5 in high-deposition lakes and 7.1 in low-deposition lakes. The optimum pH range for denitrification is between 7 and 8 and there is generally a positive relationship between pH and denitrification rates (Knowles 1982). Interestingly, whole-lake studies found that experimental acidification enhanced, not repressed,

denitrification rates (Rudd et al. 1988). Accordingly, it is not clear whether reduced pH in high-deposition lakes had a positive or negative effect on our denitrification measurements.

Another factor that could have influenced our results is thermal stratification. Lakes in the low deposition region tended to be thermally stratified, while lakes in the high-deposition region were not, likely because lakes were deeper in the former region. Stratification may have reduced sedimentation rates by trapping particles above the thermocline (Håkanson and Jansson 2002). Previous work found that sediment denitrification was not limited by oC so it is unlikely that stratification influenced the MPN results or denitrification measured in response to the addition of lake water (McCrackin and Elser 2010). Additionally, all of the sampled lakes are unproductive and we found no evidence of anoxia in the hypolimnion. Lastly, we only collected water from above the sediments, so we do not know how concentrations of NO_3^- and DOC differ between the surface and at depth.

Abundance and distribution of denitrifiers

Denitrification is a difficult process to measure because of limitations in current methods and because of inherent temporal and spatial variability in the process itself (Groffman et al. 2006). Aquatic ecosystems, such as lakes, are considered "hot spots" for denitrification compared to terrestrial ecosystems because of the role of water in

stimulating favorable conditions for denitrification. Difficulties in measuring denitrification are compounded in lakes because of the challenge of collecting intact sediments for laboratory study or of making *in situ* measurements of sediment processes in deep water. Consequently, very little is known about how microorganisms and biogeochemical processes are distributed between lakes or across sediments within a lake.

  Despite chronic differences in N inputs, we found no difference in the abundance of denitrifying bacteria between high- and low-deposition lakes. The Front Range of the Colorado Rocky Mountains receives < 8 kg N ha^{-1} y^{-1} atmospherically. This rate is less than that the eastern US or Europe, which are as high as 11- 20 kg N ha^{-1} y^{-1} (Tørseth and Semb 1998, Bergstrom and Jansson 2006), but this level of N loading has produced measurable changes in high-elevation ecosystems in Colorado. The ratio of C:N in foliage of old-growth forests has decreased and soil N content and N mineralization rates have increased (Baron et al. 2000). Perhaps the current N deposition rate in the Front Range is insufficient to alter the sediment microbial abundance and function as has been observed in soils. For example, both fungal and bacterial biomass decreased 20% in hardwood forest soils receiving more than double (20 kg N ha^{-1} y^{-1}) the N inputs of the Front Range (DeForest et al. 2004). Interestingly, a recent meta-analysis of N fertilization studies found that bacterial biomass did not change significantly in response to N additions,

although N-cycling bacteria were not specifically considered (Treseder 2008). Alternatively, sediments may insulate bacterial communities from adverse effects of N deposition that have been observed in the water column.

In terms of within-lake spatial variability, we expected the MPN of denitrifers to be greater in profundal compared to shallow sediments. Indeed, we found that the light extinction coefficient was a co-predictor of the MPN of NO_3^--reducers, suggesting available light can influence bacterial abundance. Results of our more intensive sampling in Little Molas Lake are consistent with this relationship, in that the MPN of NO_3^--reducing bacteria generally increased moving from 0.5 to 6 m. In addition to less competition for resources by periphyton, bacteria in deep sediments also experience a more stable thermal regime and are not subject to seasonal changes, such as freeze-thaw cycles, as are microbes in shallow sediments (Neilson et al. 2001, Sharma et al. 2006). Furthermore, abundances of NO_3^-- and NO_2^--reducing bacteria were related to sediment resources. This result, taken together with the relationship to available light, suggests that these bacteria are more strongly influenced by environmental conditions than by anthropogenic N inputs.

We found no relationship between the abundance of denitrifying bacteria and denitrification process rates across the lakes we sampled.

Rather, the denitrification rate was best predicted by water NO_3^- concentrations (and not the MPN of NO_3^-- or NO_2^--reducers) and MPN's were related to lake and sediment characteristics. Wallenstein and colleagues (2006b) proposed that ephemeral conditions such as temperature and concentrations of oxygen and NO_3^- influence the instantaneous denitrification rate, while long-term environmental conditions influence the composition of denitrifying community. Indeed, this proposal is consistent with our observations. While we expected the abundances of denitrifiers to increase with depth, the littoral areas of lakes have been found to contribute disproportionately to denitrification and organic matter mineralization compared to profundal areas (Ahlgren et al. 1994, den Heyer and Kalff 1998, Saunders and Kalff 2001a). In addition, a study of Lake Constance (bordered by Austria, Switzerland, and Germany) found that bacterial biomass was 2 to 5 times greater in profundal sediments but bacterial metabolic activities were an order of magnitude greater in littoral sediments (Sala and Gude 2006). In these studies, differences in responses rates were attributed warmer temperatures higher quality substrates in shallow areas. Thus, perhaps in lake sediments, connecting bacterial biomass and community structure to denitrification process rates will not lead to improved mechanistic models of denitrification. Rather, identification of the environmental factors that

most influence sedimentary denitrification (e.g. temperature, oxygen, NO_3^-) could be a focus of future research efforts.

Most probable number techniques measure the growth of culturable bacteria and, thus, underestimate abundances of *in situ* populations. Also, certain members of the bacterial community may respond disproportionately to the incubation conditions. Thus, the application of molecular techniques would be useful to further characterize the abundance and composition of denitrifiers and investigate possible deposition-related effects. We compared our results to those determined with similar methods and found that the abundances of lake sediment denitrifiers were within the ranges reported for desert, riparian, and prairie ecosystems, but less than that for grazed and agricultural influenced soils (Table 9). The MPN's may not be directly comparable because of differences in incubation conditions. It is notable, however, that despite significant differences in environmental conditions across ecosystems (such as the quality of oC and the temperature and moisture regimes) the abundances of denitrifiers are fairly comparable. Unlike nitrification, which is limited to few taxa, denitrification can be performed by biochemically diverse microorganisms (Knowles 1982). Indeed, denitrifiers have flexible metabolic pathways and use electron acceptors other than N oxides, perhaps allowing them to develop similarly sized communities in a wide range of ecosystems.

In summary, our data suggest that atmospheric N deposition has not altered the function of denitrifying microbial communities or the abundance of NO_3^-- and NO_2^--reducing bacteria. While lakes in the Colorado Front Range receive less atmospheric N deposition than those in other areas, they may be more sensitive to the effects of deposition because of exposed bedrock and the lack of vegetation (Clow et al. 2003). The effects of N deposition appear more pronounced in the water column of lakes compared to sediments, when one considers such phenomena as shifts in the community structure, abundance, and nutrient limitation of phytoplankton, as well as changes in lake water chemistry (Wolfe et al. 2001, Elser et al. 2009, Hessen et al. 2009). Further, lakes might play an important role in removing N from ecosystems via denitrification in sediments. Our findings suggest that even if atmospheric N deposition rates increase substantially, lake sediments possess significant capacity to remove much of this anthropogenic N.

ACKNOWLEDGMENTS

74

LITERATURE CITED

Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forests; hypotheses revised. Bioscience **48**:921-934.

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

Baron, J. S. and D. H. Campbell. 1997. Nitrogen fluxes in a high elevation Colorado Rocky Mountain basin. Hydrological Processes **11**:783-799.

Baron, J. S., H. M. Rueth, A. M. Wolfe, K. R. Nydick, E. J. Allstott, J. T. Minear, and B. Moraska. 2000. Ecosystem responses to nitrogen deposition in the Colorado Front Range. Ecosystems:352-368.

Bergstrom, A. K. and M. Jansson. 2006. Atmospheric nitrogen deposition has caused nitrogen enrichment and eutrophication of lakes in the northern hemisphere. Global Change Biology **12**:635-643.

Bernot, M. J. and W. K. Dodds. 2005. Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems **8**:442-453.

Betlach, M. R. and J. M. Tiedje. 1981. Kinetic explanation for accumulation of nitrite, nitric-oxide, and nitrous-oxide during bacterial denitrification. Applied and Environmental Microbiology **42**:1074-1084.

Bowden, R. D., E. Davidson, K. Savage, C. Arabia, and P. Steudler. 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. Forest Ecology and Management **196**:43-56.

Burns, D. A. 2004. The effects of atmospheric nitrogen deposition in the Rocky Mountains of Colorado and southern Wyoming, USA - a critical review. Environmental Pollution **127**:257-269.

Campbell, D. H., C. Kendall, C. C. Y. Chang, S. R. Silva, and K. A. Tonnessen. 2002. Pathways for nitrate release from an alpine watershed: Determination using delta N-15 and delta O-18. Water Resources Research **38**:9.

Clow, D. W., J. O. Sickman, R. G. Striegl, D. P. Krabbenhoft, J. G. Elliott, M. Dornblaser, D. A. Roth, and D. H. Campbell. 2003. Changes in the chemistry of lakes and precipitation in high-elevation national parks in the western United States, 1985-1999. Water Resources Research **39**.

DeForest, J. L., D. R. Zak, K. S. Pregitzer, and A. J. Burton. 2004. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Science Society of America Journal **68**:132-138.

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

Dillon, P. J. and L. A. Molot. 1990. The role of ammonium and nitrate retention in the acidification of lakes and forested catchments. Biogeochemistry **11**:23-43.

Dong, L. F., C. O. Thornton, D. B. Nedwell, and G. J. C. Underwood. 2000. Denitrification in sediments of the River Colne estuary, England. Marine Ecology-Progress Series **203**:109-122.

Earl, S. R., H. M. Valett, and J. R. Webster. 2006. Nitrogen saturation in stream ecosystems. Ecology **87**:3140-3151.

Elser, J. J., T. Andersen, J. S. Baron, A. K. Bergstrom, M. Jansson, M. Kyle, K. R. Nydick, L. Steger, and D. O. Hessen. 2009. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science **326**:835-837.

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

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

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

Håkanson, L. and M. Jansson. 2002. Principles of Lake Sedimentology. Blackburn Press, Caldwell, NJ, USA.

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

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

Hessen, D. O., T. Andersen, S. Larsen, B. L. Skjelkvale, and H. A. de Wit. 2009. Nitrogen deposition, catchment productivity, and climate as determinants of lake stoichiometry. Limnology and Oceanography **54**:2520-2528.

Johnson, S. L., S. Neuer, and F. Garcia-Pichel. 2007. Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands. Environmental Microbiology **9**:680-689.

Kelly, C. A., J. W. M. Rudd, R. H. Hesslein, D. W. Schindler, C. T. Dillon, C. T. Driscoll, S. A. Gherini, and R. E. Hecky. 1987. Prediction of biological acid neutralization in acid-sensitive lakes. Biogeochemistry **3**:129-140.

Kent, H. C. and K. W. Porter, editors. 1980. Colorado Geology. Rocky Mountain Association of Geologists, Denver.

Knowles, R. 1982. Denitrification. Microbiological Reviews **46**:43-70.

Koike, I., A. Hattori, and J. J. Goering. 1978. Controlled ecosystem pollution experiment - effect of mercury on enclosed water columns Marine Science Communications **4**:1-12.

Laverman, A. M., P. Van Cappellen, D. van Rotterdam-Los, C. Pallud, and J. Abell. 2006. Potential rates and pathways of microbial nitrate reduction in coastal sediments. Fems Microbiology Ecology **58**:179-192.

Lovett, G. M. and H. M. Rueth. 1999. Soil nitrogen transformations in beech and maple stands along a nitrogen deposition gradient Ecological Applications **9**:1330-1344.

Lukkari, K., H. Hartikainen, and M. Leivuori. 2007. Fractionation of sediment phosphorus revisited. I: Fractionation steps and their biogeochemical basis. Limnology and Oceanography: Methods **5**:433-444.

McCrackin, M. L. and J. J. Elser. 2010. Atmospheric nitrogen deposition alters denitrification and nitrous oxide production in lake sediments. Ecology **91**:528-539.

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

Mulholland, P. J., A. M. Helton, G. C. Poole, R. O. Hall, S. K. Hamilton, B. J. Peterson, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, W. K. Dodds, S. E. G. Findlay, S. V. Gregory, N. B. Grimm, S. L. Johnson, W. H. McDowell, J. L. Meyer, H. M. Valett, J. R. Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, L. T. Johnson, B. R. Niederlehner, J. M. O'Brien, J. D. Potter, R. W. Sheibley, D. J. Sobota, and S. M. Thomas. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature **452**:202-206.

Musselman, R. C. and W. L. Slauson. 2004. Water chemistry of high elevation Colorado wilderness lakes. Biogeochemistry **71**:387-414.

Nanus, L., D. H. Campbell, G. P. Ingersoll, D. W. Clow, and M. A. Mast. 2003. Atmospheric deposition maps for the Rocky Mountains. Atmospheric Environment **37**:4881-4892.

Nanus, L., M. W. Williams, D. H. Campbell, E. M. Elliott, and C. Kendall. 2008. Evaluating regional patterns in nitrate sources to watersheds in national parks of the Rocky Mountains using nitrate isotopes. Environmental Science & Technology **42**:6487-6493.

Nanus, L., M. W. Williams, D. H. Campbell, K. A. Tonnessen, T. Blett, and D. W. Clow. 2009. Assessment of lake sensitivity to acidic deposition in national parks of the Rocky Mountains. Ecological Applications **19**:961-973.

Neilson, C. B., P. M. Groffman, S. P. Hamburg, C. T. Driscoll, T. J. Fahey, and J. P. Hardy. 2001. Freezing effects on carbon and nitrogen cycling in northern hardwood forest soils. Soil Science Society of America Journal **65**:1723-1730.

Oremland, R. S., C. Umberfer, C. W. Culbertson, and R. L. Smith. 1984. Denitrification in San Francisco Bay intertidal sediments. Applied and Environmental Microbiology **47**:1106-1112.

Oren, A. and T. H. Blackburn. 1979. Estimation of sediment denitrification rates at insitu nitrate concentrations. Applied and Environmental Microbiology **37**:174-176.

Pina-Ochoa, E. and M. Alvarez-Cobelas. 2006. Denitrification in aquatic environments: a cross-system analysis. Biogeochemistry **81**:111-130.

Richardson, W. B., E. A. Strauss, L. A. Bartsch, E. M. Monroe, J. C. Cavanaugh, L. Vingum, and D. M. Soballe. 2004. Denitrification in the Upper Mississippi River: rates, controls, and contribution to nitrate flux. Canadian Journal of Fisheries and Aquatic Sciences **61**:1102-1112.

Risgaard-Petersen, N. 2003. Coupled nitrification-denitrification in autotrophic and heterotrophic estuarine sediments: on the influence of benthic microalgae. Limnology and Oceanography **48**:93-105.

Rowe, R., R. Todd, and J. Waide. 1977. Microtechnique for most-probable-number analysis. Applied and Environmental Microbiology **33**:675-680.

Rudd, J. W. M., C. A. Kelly, D. W. Schindler, and M. A. Turner. 1988. Disruption of the nitrogen-cycle in acidified lakes. Science **240**:1515-1517.

Sala, M. M. and H. Gude. 2006. Seasonal dynamics of pelagic and benthic (littoral and profundal) bacterial abundances and activities in a deep prealpine lake (L. Constance). Archiv Fur Hydrobiologie **167**:351-369.

Saunders, D. L. and J. Kalff. 2001a. Denitrification rates in the sediments of Lake Memphremagog, Canada-USA. Water Research **35**:1897-1904.

Saunders, D. L. and J. Kalff. 2001b. Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia **443**:205-212.

Seitzinger, S. P., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, and C. Tobias. 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecological Applications **16**:2064-2090.

Sharma, S., Z. Szele, R. Schilling, J. C. Munch, and M. Schloter. 2006. Influence of freeze-thaw stress on the structure and function of microbial communities and denitrifying populations in soil. Applied and Environmental Microbiology **72**:2148-2154.

Sickman, J. O., J. M. Melack, and J. L. Stoddard. 2002. Regional analysis of inorganic nitrogen yield and retention in high-elevation ecosystems of the Sierra Nevada and Rocky Mountains. Biogeochemistry **57**:341-374.

Silvennoinen, H., A. Liikanen, J. Torssonen, C. F. Stange, and P. J. Martikainen. 2008. Denitrification and nitrous oxide effluxes in boreal, eutrophic river sediments under increasing nitrate load: a laboratory microcosm study. Biogeochemistry **91**:105-116.

Staley, T. E. and J. B. Griffin. 1981. Simultaneous enumeration of denitrifying and nitrate reducing bacteria by a microtiter most-probable-number (MPN) procedure. Soil Biology & Biochemistry **13**:385-388.

Stoddard, J. L., D. S. Jeffries, A. Lukewille, T. A. Clair, P. J. Dillon, C. T. Driscoll, M. Forsius, M. Johannessen, J. S. Kahl, J. H. Kellogg, A. Kemp, J. Mannio, D. T. Monteith, P. S. Murdoch, S. Patrick, A. Rebsdorf, B. L. Skjelkvale, M. P. Stainton, T. Traaen, H. van Dam, K. E. Webster, J. Wieting, and A. Wilander. 1999. Regional trends in aquatic recovery from acidification in North America and Europe. Nature **401**:575-578.

Sundbäck, K., F. Linares, F. Larson, and A. Wulff. 2004. Benthic nitrogen fluxes along a depth gradient in a microtidal fjord: The role of denitrification and microphytobenthos. Limnology and Oceanography **49**:1095-1107.

Tietema, A. 1998. Microbial carbon and nitrogen dynamics in coniferous forest floor material collected along a European nitrogen deposition gradient. Forest Ecology and Management **101**:29-36.

Tørseth, K. and A. Semb. 1998. Deposition of nitrogen and other major inorganic compounds in Norway, 1992-1996. Environmental Pollution **102**:299-304.

Treseder, K. K. 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters **11**:1111-1120.

Wallenstein, M. D., S. McNulty, I. J. Fernandez, J. Boggs, and W. H. Schlesinger. 2006a. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. Forest Ecology and Management **222**:459-468.

Wallenstein, M. D., D. D. Myrold, M. K. Firestone, and M. Voytek. 2006b. Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. Ecological Applications **16**:2143-2152.

Williams, M. W., J. S. Baron, N. Caine, R. Sommerfeld, and R. Sanford. 1996. Nitrogen saturation in the Rocky Mountains. Environmental Science & Technology **30**:640-646.

Wolfe, A. P., J. S. Baron, and R. J. Cornett. 2001. Anthropogenic nitrogen deposition induces rapid ecological changes in alpine lakes of the Colorado Front Range (USA). Journal of Paleolimnology **25**:1-7.

Wolfe, A. P., C. A. Cooke, and W. O. Hobbs. 2006. Are current rates of atmospheric nitrogen deposition influencing lakes in the eastern Canadian arctic? Arctic, Antarctic, and Alpine Research **38**:465-476.

Wollheim, W. M. and C. J. Vorosmarty. 2006. Relationship between river size and nutrient removal. Geophysical Research Letters **33**:L06410, doi:06410.01029/02006GL025845.

Wollheim, W. M., C. J. Vorosmarty, A. F. Bouwman, P. Green, J. Harrison, E. Linder, B. J. Peterson, S. P. Seitzinger, and J. P. M. Syvitski. 2008. Global N removal by freshwater aquatic systems using a spatially distributed, within-basin approach. Global Biogeochemical Cycles **22**:Gb2026, doi:2010.1029/2007GB002963.

Yoshinari, T. and R. Knowles. 1976. Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochemical and Biophysical Research Communications **69**:705-710.

Yu, K. W., R. D. DeLaune, and P. Boeckx. 2006. Direct measurement of denitrification activity in a Gulf coast freshwater marsh receiving diverted Mississippi River water. Chemosphere **65**:2449-2455.

Table 4. Average (and standard error, s.e.) values for various water and sediment parameters for the study lakes.

	Sample date	Elevation (m)	Lake depth (m)	NO_3^- µmol L^{-1}	DOC mmol L^{-1}	Total C mmol g^{-1}	Total N mmol g^{-1}	Total P mmol g^{-1}	Organic Matter
High-deposition lakes									
Brainard	6/26/08	3,154	3	12.6	0.27	10.4	0.94	0.20	0.18
Dream	7/4/08	3,032	4	16.6	0.28	11.4	0.91	0.09	0.12
Estes	6/29/08	2,277	>20	6.4	0.45	4.7	0.33	0.09	0.28
Green Lake 1	7/25/08	3,421	9	9.5	0.33	10.1	0.84	0.10	0.13
Green Lake 2	7/25/08	3,416	5	15.6	0.43	5.5	0.40	0.13	0.09
Isabelle	6/30/08	3,314	40	19.3	0.39	3.5	0.27	0.08	0.07
Long	7/5/08	3,219	3	12.4	0.72	7.0	0.66	0.06	0.11
Mitchell	7/23/08	3,280	1.5	10.8	0.63	7.2	0.59	0.07	0.17
Nymph	7/4/08	2,965	1	5.2	0.55	19.3	1.15	0.06	0.23
Red Rock	6/26/08	3,112	1	4.3	0.93	18.8	1.45	0.10	0.16
Mean				11.3	0.50	9.8	0.75	0.10	0.15
s.e.				1.3	0.05	1.8	0.13	0.01	0.02

85

Table 4, continued. Results of statistical test comparing high and low deposition lakes are shown for each

parameter. Non-significant results are indicated by n.s.

	Sample date	Elevation (m)	Lake depth (m)	NO_3^- µmol L^{-1}	DOC mmol L^{-1}	Total C mmol g^{-1}	Total N mmol g^{-1}	Total P mmol g^{-1}	Organic Matter
Low-deposition lakes									
Andrews	7/11/08	3,284	6	3.4	1.44	2.4	0.19	0.13	0.19
Clear	7/10/08	3,633	>25	6.3	0.53	4.6	0.36	0.07	0.04
Dollar	8/6/08	3,059	5	0.4	0.60	15.1	1.04	0.08	0.18
Highland Mary	7/15/08	3,708	30	2.9	0.77	5.0	0.44	0.18	0.10
Irwin	8/7/08	3,148	5	0.5	0.35	5.3	0.49	0.07	0.11
Little Molas	7/8/08	3,329	6	4.1	0.84	4.6	0.36	0.07	0.18
Lost	8/6/08	3,010	10	3.3	0.18	6.9	0.61	0.08	0.09
Lost Slough	8/13/08	2,939	4	0.3	0.29	4.1	0.30	0.06	0.12
Potato	7/18/08	2,983	17	11.4	1.47	9.3	0.79	0.05	0.13
Spring Creek	8/12/08	3,040	8	0.3	1.45	7.7	0.62	0.11	0.12
Mean				3.3	0.79	6.5	0.52	0.09	0.13
s.e.				1.0	0.14	1.2	0.08	0.01	0.02
high vs. low deposition				high > low	high = low	high = low	high = low	high = low	high = low
P				0.001	n.s.	n.s.	n.s.	n.s.	n.s.

Table 5. Average denitrification rates (and standard error, s.e.) for the study lakes. Potential denitrification is in response to the addition of non-limiting concentrations of oC and NO_3^-. Maximum denitrification is in response to 5000 μM NO_3^- in the dose-response experiment. "Background" denitrification is in response to additions of lake water.

	Sample depth	Potential Denitrification (μmol N m^{-2} h^{-1})	Maximum Denitrification (μmol N m^{-2} h^{-1})	"Background" Denitrification (μmol N m^{-2} h^{-1})
High-deposition lakes				
Brainard	3	2,115	1,540	3.8
Dream	4	430	188	61.9
Estes	10	2,352	1,614	0.9
Green Lake 1	10	1,499	975	0.0
Green Lake 3	9	876	610	1.7
Isabelle	10	1,833	1,481	0.0
Long	3	733	514	7.0
Mitchell	1.5	505	461	0.0
Nymph	1	5	9	3.7
Red Rock	1	487	269	0.0
Mean		1,083	766	7.9
s.e.		269	199	6.4

87

Table 5, continued. Results of statistical test comparing high- and low-deposition lakes are shown

for each parameter; non-significant results are indicated by n.s.

	Sample depth	Denitrification Potential (μmol N m^{-2} h^{-1})	Denitrification Capacity (μmol N m^{-2} h^{-1})	"Background" Denitrification (μmol N m^{-2} h^{-1})
Low-deposition lakes				
Andrews	6	734	719	0.0
Clear	10	498	972	0.9
Dollar	5	1,005	559	0.0
Highland Mary	10	260	162	0.7
Irwin	5	1,826	1,071	--
Little Molas	6	460	402	0.7
Lost	10	1,439	1,357	0.0
Lost Slough	4	437	263	0.0
Potato	10	23	20	2.2
Spring Creek	8	961	854	0.0
Mean		764	638	0.5
s.e.		196	153	0.3
high vs. low deposition		high = low	high = low	high = low
P		n.s.	n.s.	n.s.

88

Table 6: Comparison of multiple-linear regression models for sediment fluxes and the abundances of denitrifiers. R^2 denotes goodness-of-fit values adjusted for the number of parameters in the model.

Response variable	Predictor variables	R^2	P	Equation
K_m	Sediment OM	0.26	0.02	Log K_m = -3.15 arcsine OM + 3.61
Denitrification rate μmol N m^{-2} h^{-1}	[NO$_3^-$]	0.22	0.04	Log denitrification rate = 0.42 * log [NO$_3^-$] + 0.07
[a]Denitrification rate μmol N m^{-2} h^{-1}	[NO$_3^-$]	0.48	0.03	Log denitrification rate = 1.67* log [NO$_3^-$] – 1.07
Potential denitrification rate μmol N kg h^{-1}	Sediment C:P	0.28	0.03	Log potential denitrification rate = -1.12 * log sediment C:P + 4.84
MPN NO$_2^-$ reducers	Sediment C:N	0.24	0.04	Log MPN denitrifiers = - 7.46 log sediment C:N + 10.87
MPN NO$_3^-$ reducers	[b]PAR, sediment OM	0.28	0.047	Log MPN NO$_3^-$ reducers = 3.80 * log PAR + 2.56 arcsine OM + 3.78

[a] Excludes 9 lakes with no measureable denitrification flux

[b]PAR is the light extinction coefficient, k (Eq. 1).

Table 7. Average (and standard error, s.e.) values of MPN of NO_3^-- and NO_2^--reducing bacteria for the study lakes.

High-deposition Lakes	Sample depth	PAR	Most Probable Number	
			Nitrate reducers g^{-1} (x10^4)	Nitrite reducers g^{-1} (x10^2)
Brainard	3	0.71	90.9	19.7
Dream	4	0.59	3.0	6.8
GL1	9	0.82	18.9	0.3
GL3	10	0.81	75.7	24.7
Isabelle	10	1.25	65.7	5.8
Long	3	0.89	38.1	30.4
Mitchell	1.5	0.22	30.4	71.1
Nymph	1	0.18	5.1	1.1
Red Rock	1	0.21	39.5	0.3
	Mean		40.8	17.8
	s.e.		10.9	8.1

Table 7, continued. Results of statistical test indicating whether there was a significant difference between deposition regions. Non-significant results indicated by n.s.

Low-deposition Lakes	Sample depth	PAR	Most Probable Number	
			Nitrate reducers g^{-1} ($x10^4$)	Nitrite reducers g^{-1} ($x10^2$)
Andrews	0.5	0.11	2.2	0.8
Andrews	6	1.33	65.2	5.7
Clear	10	0.72	2.9	4.1
Dollar	5	1.17	40.4	0.5
Highland Mary	10	1.15	4.0	6.0
Irwin	5	0.65	15.2	5.5
Little Molas	0.5	0.12	5.5	5.2
Little Molas	1	0.16	5.4	5.4
Little Molas	2	0.26	7.5	9.8
Little Molas	6	0.81	65.9	3.1
Lost	10	0.84	57.0	24.2
Potato	10	0.90	32.0	46.2
	mean[1]		35.3	11.9
	s. e.[1]		9.9	5.9
high vs. low deposition			high = low	high = low
P			n.s.	n.s.

[1]Mean and standard error values and statistical analyses do not include samples from Andrews lake 0.5m depth and Little Molas lake depths 0.5, 1, and 2 m.

Table 8. Comparison of half-saturation constants for denitrification for

aquatic ecosystems.

Location	Incubation Temperature °C	Method	K_m (µmol N)	Reference
20 Colorado lakes, CO, USA	17-20	Sediment slurry, static	298	This study
Temmesjoki River, Finland	15	Intact sediment core	20	Silvennoinen et al. 2008
Barataria Basin Estuary, LA, USA	26-29	*in situ* chamber	433	Yu et al. 2006
Scheldt Estuary, Netherlands and Belgium	20	Sediment slurry, shaken	200-400	Laverman et al. 2006
San Francisco Bay, CA, USA	20	Sediment slurry, stirred	50	Oremland at al. 1984
N/A	30	Pure culture, stirred	<15	Betlach and Tiedje 1981
Kysing Fjord, Denmark	12	Sediment slurry	344	Oren and Blackburn 1979
Tokyo Bay, Japan	16	Sediment slurry, stirred	27	Koike et. al 1978

Table 9. Cross-ecosystem comparison of MPN of NO_3^-- and NO_2^--reducing bacteria. Instances where authors did not report separate abundances of NO_3^-- and NO_2^--reducing bacteria are shown as denitrifiers.

Reference	Location	Most probable number (cells g^{-1} dry sediment or soil)		
		Nitrate reducers	Nitrite reducers	Denitrifiers
This study	Lake sediment, USA	$0.2–9.1 \times 10^5$	$1–7.1 \times 10^3$	
McCrackin et al., 2008	Desert soil, USA	$0.3–1.5 \times 10^5$	$2–2.8 \times 10^2$	
Johnson et al., 2007	Desert crust, USA	$0.1–1.8 \times 10^4$	$1–7 \times 10^2$	
McCarty et al., 2007	Riparian soil, USA			$1–8 \times 10^5$
Laverman et al., 2006	Eutrophic lake sediment, Netherlands			[a]3.6×10^6 $– 3.6 \times 10^8$
Cannavo et al., 2002	Agricultural-influenced soils, France	$4–6.7 \times 10^6$	$2.4–4.2 \times 10^4$	
Cannavo et al., 2004	Agricultural-influenced soils, France		2.3×10^6	
Patra et al., 2005	Ungrazed grassland, France			$1.5–2.5 \times 10^6$
Florinsky et al., 2004	Prairie soil, Canada			$1.5–23.9 \times 10^4$
Sotomayor and Rice, 1996	Prairie soil, USA			$1 \times 10^2 – 1 \times 10^5$

[a] converted from areal to dry sediment mass basis assuming bulk density of 0.14 g dry sediment cm^{-3}.
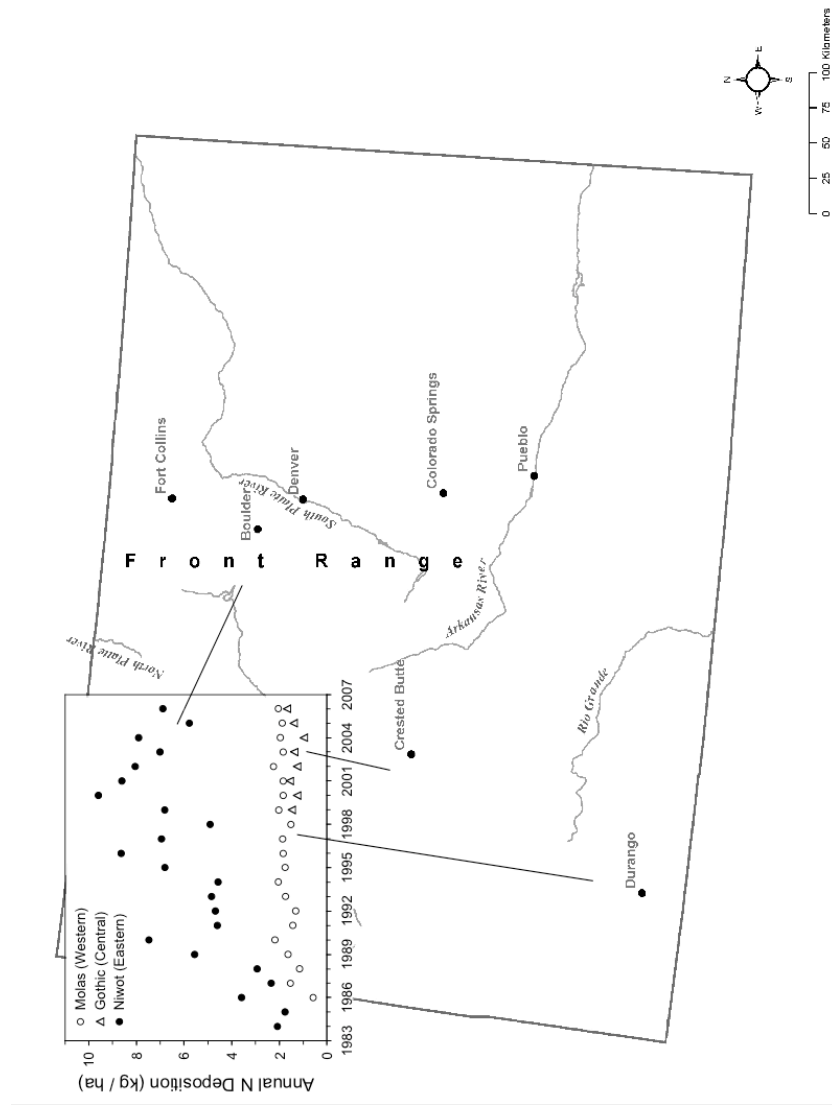
Figure 5. Locations of study sites in Colorado, USA. The inset figure shows the annual deposition (wet) of inorganic N at monitoring stations close to each of the three sampling regions.
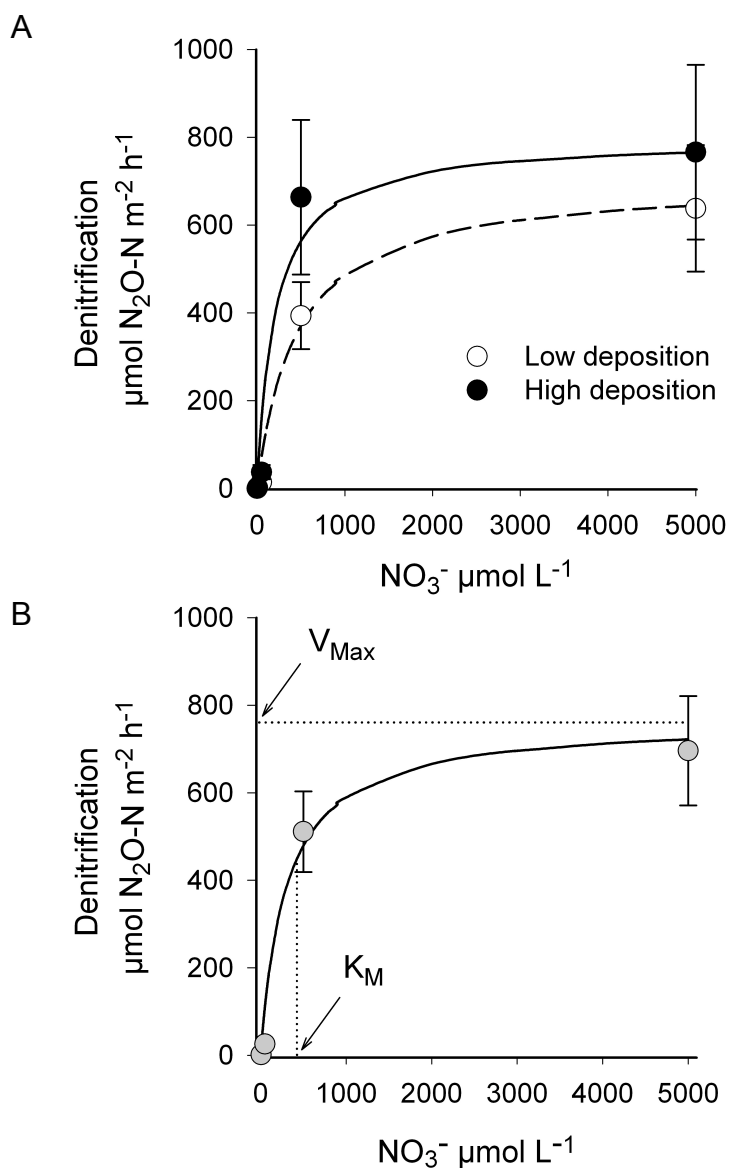
Figure 6. Results of dose-response experiments. (A) Points are the mean denitrification rates (± SE). Lines are the predicted denitrification rate using Eq. 1. (B) Points are the mean denitrification rate for all sampled lakes (± SE). The solid line shows the predicted denitrification rate using Eq. 1, where V_{max} is 765 μmol N m^{-2} h^{-1} and K_m is 293 μmol L^{-1} NO_3^-.
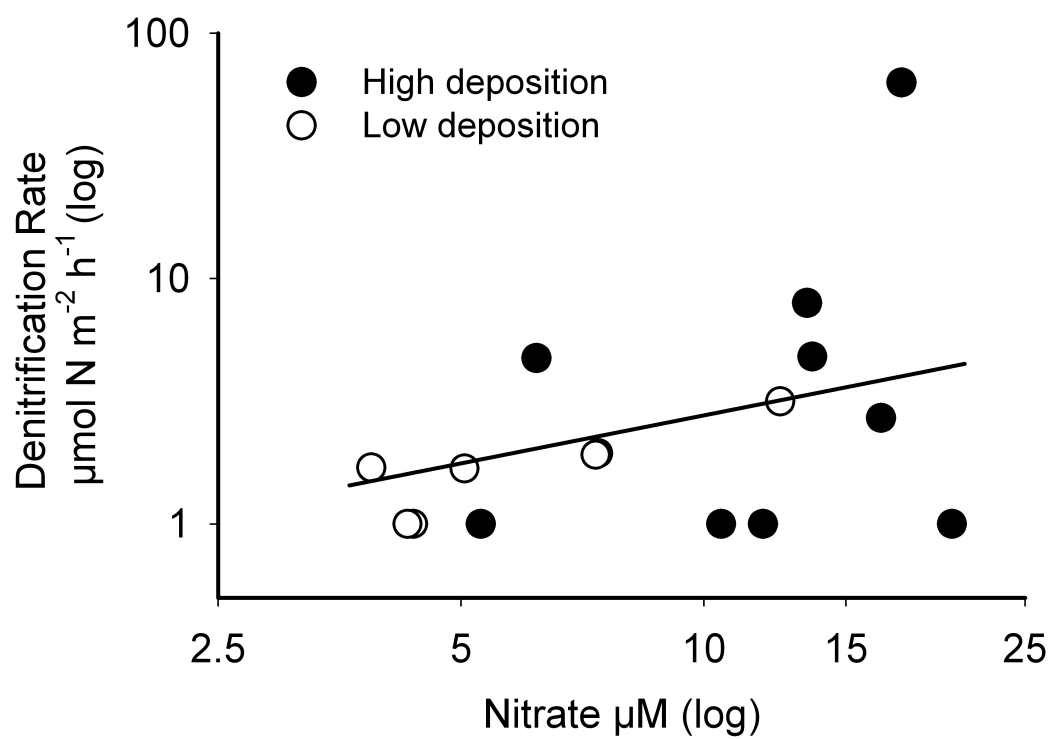
Figure 7. Relationship between denitrification and NO_3^- for lakes in the high- (solid circles, n = 10 lakes) and low-deposition (open circles, n = 10 lakes) regions (R^2 = 0.22, P = 0.04).

CHAPTER 4

Temporal Dynamics of Denitrification in Lake Sediments (Norway)

ABSTRACT

Lakes are important sinks for nitrogen (N) in the landscape via biological assimilation, sedimentation, and denitrification. Of these mechanisms, microbially mediated denitrification in sediments is considered the most important pathway by which N is removed from lakes. We investigated the dynamics of gases produced and consumed during denitrification to determine whether chronic N loading has affected the functional performance of denitrifying communities. Sediments were collected from 29 lakes that receive either high (10-20 kg N ha^{-1} y^{-1}) or low (<4 kg N ha^{-1} y^{-1}) levels of atmospheric N deposition in three regions of southern Norway and in the Arctic archipelago of Svalbard. Denitrification dynamics were investigated by incubation in an automated system that monitors nitric oxide (NO), nitrous oxide (N_2O), di-nitrogen (N_2), oxygen (O_2), and carbon dioxide (CO_2) production. There were no differences between deposition regions in terms of net cumulative production of NO, N_2O, and N_2 and total N denitrified after incubation for 64 h. There were differences in responses between lake sediments, suggesting that certain denitrifying communities are inherently more efficient than others in converting NO_3^- to N_2, perhaps due to community composition.

INTRODUCTION

Lakes are important sinks for N in the landscape. In areas that receive N from anthropogenic sources, lakes perform an important ecosystem service by reducing N loading to downstream ecosystems. A recently published model estimates that lakes and reservoirs remove nearly 20 Tg N y^{-1} from watersheds globally, which represents ~30% of estimated inputs to surface waters (Harrison et al. 2009). Potential fates of N are biological assimilation, burial in sediments, and denitrification (the microbial conversion of NO_3^- to N_2 gas). Of these mechanisms, denitrification is considered to be the most significant and it is the only permanent sink for NO_3^- (Saunders and Kalff 2001). Consequently, it is important to understand the factors that influence denitrification.

In lakes, denitrification occurs primarily in sediments where concentrations of oxygen are low and there is available organic carbon (oC) and NO_3^-. Obligate intermediate products of denitrification are nitrite (NO_2^-), NO, and N_2O. The kinetics of existing enzymes and the synthesis of new reductase enzymes influence the transient concentrations of NO and N_2O that accumulate during denitrification (Betlach and Tiedje 1981). Production of intermediates is also influenced by factors such as the composition of the denitrifying community, pH, or concentrations of oxygen, NO_3^-, NO_2^-, or NO (Firestone et al. 1979, Firestone et al. 1980, Cavigelli and Robertson 2000, Morley et al. 2008).

98

Biogeochemically and taxonomically diverse microorganisms are capable of using N oxides as electron acceptors (Knowles 1982). Differences in the composition of denitrifying communities have been observed along environmental gradients of salinity, NO_3^-, and pH (Santoro et al. 2006, Desnues et al. 2007). Thus, there is potential for sediment microbial communities to differ between lakes that receive either high or low levels of atmospheric nitrogen (N) deposition. Even in relatively remote areas, catchments are subject to anthropogenic influences from N compounds that are transported long distance in the atmosphere (Wolfe et al. 2006). Chronic N loading often results in acidification of surface water, shifts in dissolved nutrient concentrations and phytoplankton community composition, and altered food web dynamics (Bergstrom et al. 2005, Elser et al. 2009, Hessen et al. 2009). The ecological effects of N deposition on sediment processes are not well understood.

The objectives of this study were to contrast the functional performance of "intact" bacterial communities in lake sediments from different N loading regimes by comparing the dynamics of NO, N_2O, and N_2 under controlled laboratory conditions. A variety of microorganisms are capable of reducing NO_3^- to NO_2^- during respiration but fewer taxa have the ability to further reduce NO_2^- and other intermediates of denitrification (Knowles 1982). In addition, comparative genomics has revealed that there are often taxon-specific processes regulating each step of the

denitrification process (Rodionov et al. 2005). Hence, differences in community composition could result in different patterns of NO and N_2O production and reduction during denitrification. Sediments were collected from lakes across a gradient of atmospheric N deposition in Norway and Svalbard. Previous studies have found elevated denitrification in sediments of lakes that receive high levels of N deposition, but no differences in the potential denitrification rate (in response to non-limiting concentrations of organic carbon and NO_3^-) or in the abundance of denitrifying bacteria (McCrackin and Elser In revision). These studies involved bulk, end-point determinations of gas fluxes and thus may not detect differences between communities in terms of denitrification dynamics that could be revealed through the high-resolution monitoring of gases produced during denitrification that are presented here.

METHODS

Study Sites

During summer 2009, we sampled lakes located at the high and low ends of a gradient of N deposition that spans over 400 km across southern Norway. Norway receives atmospheric pollution from industrial regions of Europe and total (wet and dry) N deposition rates are 10-20 kg N ha^{-1} y^{-1} in southwest regions of the country, decreasing to less than 4 kg N ha^{-1} y^{-1} in east-central areas (Tørseth and Semb 1998, Klein and Benedictow 2006). Lakes near Egersund (58.35° latitude, 6.04° longitude),

in southwestern Norway, are considered to be in the high-deposition region, while lakes near Lillehammer (61.07° latitude, 10.27° longitude) and Atna (61.52° latitude, 10.27° longitude) are considered to be in the low-deposition region. In addition, we sampled two non-glacial lakes near Ny-Ålesund (78.93° latitude, 11.95 ° longitude) on the Svalbard archipelago. While there is evidence that some atmospheric N deposition reaches Arctic areas including Svalbard, we considered this area to be at the extreme low end of the N-deposition gradient (Holmgren et al. 2010).

Lakes in southern Norway occupy watersheds that are undeveloped with the exception of sheep grazing and occasional seasonal cottages. Exposed rock, grass, and scattered trees, including Norway spruce (*Picea abies*), characterized the catchments. At Svalbard, the catchments were predominantly occupied by talus; sparse vegetation included mosses and lichens. In all areas, the sampled lakes were generally small and deep, with surface area < 20 ha and maximum depth greater > 5 m (Fig. 8).

Field sampling and sample analyses

Each lake was sampled once using an inflatable boat (Table 10). The depth of the lake was measured with a hand-held echo sounder. Water temperature and O_2 concentration at the sampling depth were measured with a YSI model 85 temperature-oxygen probe (YSI, Yellow Springs, Ohio, USA). Surface sediments were collected using a LaMotte dredge from a water depth of approximately 10 m or at the maximum lake depth if

the lake was < 10 m. Sediments were stored in plastic bags at 4ºC in dark conditions until analysis. Total carbon (C) and N contents of dried sediments were measured with a Flash EA 1112 elemental analyzer (Thermo Finnigan, Milan, Italy). Total phosphorus (P) content of combusted sediments was measured colorimetrically following extraction with 0.5 M hydrochloric acid using the acid-molybdate technique (Lukkari et al. 2007). Sediment water content was determined as mass loss after drying subsamples at 105°C for 48 h and organic matter (OM) content was determined as mass loss on ignition at 550°C for 4 h.

Water was collected from just above the sediments using a submersible pump. All lake water samples were filtered with Pall A/E glass fiber filters and frozen until analysis of dissolved nutrient species. Water was analyzed for DOC on a Shimadzu TOC 5000 (Shimadzu Corporation, Kyoto, Japan). Nitrate plus nitrite (hereafter: NO_3^-) concentration was determined on a Metrohm 761 Compact ion chromatograph (Metrohm, Riverview, Florida, USA).

Incubation procedure

For each lake, 10 g of sediment were combined with 40 mL of incubation media and a Teflon magnetic stir bar in 120-mL serum bottles. The incubation medium was composed of 2 mM NO_3^- (as KNO_3) and 5 mM glutamate that was adjusted to a pH of 7.1 with 0.1 M sodium hydroxide. Bottles were capped with butyl-rubber septa and an aluminum

crimp seal. To remove background gases in the sediment slurry and headspace, bottles were evacuated and filled with helium (He) for five cycles while constantly stirred. The bottles were incubated in an automated system described in detail by Molstad et al. (2007). Briefly, incubation bottles were held in a water bath at a temperature of 12°C and stirred continuously throughout the experiment. Headspace gas was collected by an autosampler attached to a peristaltic pump and analyzed by an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with electron-capture and thermal-conductivity detectors to determine concentrations of O_2, CO_2, N_2O, and N_2. A Teledyne 200E Chemiluminesence NO/NO_x analyzer (Teledyne Technologies, Thousand Oaks, CA, USA) was used to determine the NO concentration. Each bottle was sampled 20 times over the course of 64-h incubations. Gas removed for sampling was replaced with an equal volume of He. While the replacement of sampled headspace gas with He maintained a constant pressure in the bottles, this procedure resulted in a ~3% dilution of headspace gas. To correct for dilution, the measured gas concentrations were adjusted based on bottles that contained only incubation medium in a He-filled headspace, with a spike of neon gas. Neon is inert and found in extremely low concentrations in ambient air, making it an appropriate indicator of sampling-related dilution. Corrections for the leakage of N_2 and O_2 though tubing into the bottle headspace were determined based

on the accumulation of these gases in bottles that contained incubation medium in a He-filled headspace. Published gas solubility constants for O_2, CO_2, NO, N_2O, and N_2 were used to determine the total amount of gas in the gas and liquid phases (Wilhelm et al. 1977).

Data analysis was conducted on total N denitrified, cumulative net production of NO and N_2O, and cumulative production of N_2 gas over the incubation period for each lake. Total N denitrified was determined as the sum of NO, N_2O, and N_2 (μmol) at the end of the incubation period (at 64 h). The net cumulative production of each gas was determined by measuring the area below the curve using SigmaPlot 10 (Systat Software, Inc., San Jose, CA, USA). Total N denitrified and cumulative net NO, N_2O, and N_2 produced are reported on the basis of sediment dry mass.

Statistics

We used a multivariate approach to identify relationships between the sampled lakes with respect to NO, N_2O, and N_2 production. Principal components analysis (PCA) was applied to the correlation matrix of total N denitrified and the net cumulative production of NO, N_2O, and N_2 gases. Components with an eigenvalue > 1 were retained in the analysis. In interpreting the factor pattern, a variable was said to load on a given component if the factor loading was > 0.5 or < -0.5. Multiple-linear regression analysis was used to determine the importance of DOC, NO_3^-

and, sediment nutrients in predicting the principal components, the cumulative production of gases, and total N denitrified.

Student's t-tests were used to compare water and sediment nutrients, principal components, cumulative production of gases, and total N denitrified between deposition regions. When necessary, response and predictor variables were transformed to improve normality. Statistical analyses were performed in JMP 8.0.1 (SAS Institute, Inc., Cary, NC, USA) with α = 0.05.

RESULTS

Sediment and water characteristics

Lakes were unproductive and the hypolimnion of each lake was oxic. Lake water temperatures averaged 12°C at the sediment collection depth from which sediments were collected, except for the Svalbard lakes, which were 4°C. Nitrate was significantly greater in high deposition lakes compared to low deposition lakes, at 11.4 ($\pm$ 1.7 SE) and 0.8 ($\pm$ 0.1 SE) μmol N L^{-1}, respectively (Table 10). The concentration of DOC did not differ between deposition regions (P > 0.05) and averaged 138 ($\pm$ 9.7 SE) μmol L^{-1} across all lakes. There were also no differences in sediment OM, C, N, or P contents nor in ratios of C:N, C:P, or N:P between N deposition regions (P > 0.05, Table 10).

Denitrification dynamics

The production of NO, N_2O, and N_2 through the course of the incubations followed a similar pattern (Table 11). There was initial and generally sequential accumulation of NO and N_2O, which decreased when these intermediates were reduced to N_2, followed by accumulation of N_2. Production of N_2 typically occurred 20 - 24 h after the start of the incubation but was later than 40 h for several lakes. These patterns are illustrated for one lake, Skjævelandvatnet, in Fig. 9. There was one exception, Åsdalstjørna, which showed no N_2 production during the 64-h incubation. Subsequent analysis of the headspace of the Åsdalstjørna incubation bottle at 240 h detected N_2; however, the exact time that N_2 production commenced is not known.

The total amount of N denitrified varied widely among the lakes, between 1.3 and 138.6 μmol N g^{-1}. Across all lakes the average total N denitrified was 22.8 μmol N ($\pm$ 6 SE). Neither total N denitrified nor the cumulative net production of NO, N_2O, or N_2 by sediments differed significantly between N deposition regions. Initial concentrations of O_2 in the bottles that were not removed by He-flushing were 400-700 ppm, which remained stable or decreased during the incubation period. Exponential production of CO_2 was observed in the incubations. Cumulative production of CO_2 did not differ between sediments in either deposition region.

Total N denitrified and cumulative N_2O and N_2 production were not related to water or sediment nutrients. Net cumulative NO production was positively related to sediment OM (R^2 = 0.15, P = 0.04, log cumulative NO production = 3.92 * OM – 0.67). The PCA of NO, N_2O, and N_2 production and total N denitrified found that 77% of the variance of these variables was explained by two components (Table 12). Component 1 was associated with N_2O production and total N denitrified and Component 2 was associated with N_2 and NO production. Neither component differed between the high and low N deposition regions (Table 11, Fig. 10). Regression analysis revealed that component 1 was not predicted by DOC, NO_3^- or any of the measured sediment resources. Component 2 was positively related to sediment OM (Fig. 11, R^2 = 0.28, P = 0.01, Component 2 = 7.21 * OM – 1.26).

DISCUSSION

The dynamics of NO, N_2O, and N_2 production during denitrification are often explored in laboratory cultures or in cultures extracted from environmental samples. Here, we investigated denitrification dynamics of bacterial communities in sediments of 29 lakes that were located across a gradient of atmospheric N deposition. We controlled for O_2, temperature, and initial pH and supplied NO_3^- and oC in non-limiting concentrations to reveal differences in the performance of active sediment communities in terms of the production of N gases during denitrification. Principal

components analysis suggest that lake sediments differ in their efficiency of converting the intermediate products to N_2 as well as in the total amount of N that is denitrified. Variables that differentiated the performance of the sampled sediment communities were cumulative net N_2O production and total N denitrified, which loaded on component 1. There was no evidence that chronic N loading has altered the behavior of sediment denitrifying communities.

Nitric oxide production was the only response variable that was related to any of the identified predictor variables. Sediment OM positively related to cumulative net NO production and principal component 2, which was related to NO production. There is evidence that humic compounds in lake sediments can reduce the activity of enzymes (Håkanson and Jansson 2002). Humic compounds have large surface areas and charged sites that attract and hold a variety of molecules (Brady and Weil 2002). Thus, even though the sediment slurries were constantly stirred, OM could have delayed diffusion NO to the active of sites of enzymes and allowed NO to accumulate. Indeed, Åsdalstjørna presented a unique regulation pattern with respect to NO across all lakes. Net cumulative NO production for this lake was 40 times greater than the average of the other lakes and at the end of the incubation, there was no detectable N_2. We expected such elevated NO concentrations would have been toxic for the sediment microbial community (Zumft, 1997). At some point between 64 and 240 h,

however, NO was consumed and production of N_2O and N_2 occurred. The OM content of Åsdalstjørna sediments, 49% of dry mass, was greatest of all lakes, which averaged 16% (excluding Åsdalstjørna). It is not clear, however, why such an effect of OM would occur for NO and not the other gases. These results illustrate the challenge of identifying the factors that are most influential for the functional performance of a microbial community.

Actual denitrification rates will not only depend on the underlying bacterial community, but also on environmental conditions such as temperature, pH, and concentrations of O_2, oC, and NO_3^- (Wallenstein et al. 2006). Here, we compared of responses between sediments by adding oC and NO_3^- in excess of background concentrations. Thus, incubation conditions are not representative of *in situ* conditions. To this end, our assays reflect potential denitrification rather than actual denitrification rates. Prior work with sediments of many of the same lakes found that denitrification was significantly greater in lakes from high N deposition regions relative to low deposition regions due to elevated NO_3^- concentrations in lake water (McCrackin and Elser 2010). Experimental evidence suggests that patterns of denitrification and N_2O production and consumption are related to microbial community composition (Balser and Firestone 2005). For example, Cavigelli and Robertson (2000, 2001) found the denitrification rate and N_2O flux to differ between two soil

109

microbial communities that differed taxonomically, even under controlled conditions. The application of molecular techniques would be useful to further characterize the denitrifying communities in order to relate taxonomic differences to functional performance.

The sediments of lakes we sampled were likely oxic at the sediment-water interface because all of the sampled lakes are unproductive and we found no evidence of anoxia in the hypolimnion. Thus, the anoxic conditions that we created during the incubations were likely typical of deeper sediments where the bulk of denitrification occurs. Exponential CO_2 production that we observed for the incubations suggests that there was growth of the microbial communities as a whole during the incubation (Colores et al. 1996). Within the sediment of each lake, however, we do not know whether all members of the microbial community responded consistently to the incubation conditions. It is also not known how the sediment microbial community varies within lakes.

It is also interesting that the functional performance of sediments from the Arctic lakes, Blokkvatnet and Hajeren, are comparable to that of lakes in southern Norway. Denitrifying communities are structured by long-term environmental conditions (Wallenstein et al. 2006), which differ between the regions we sampled. One obvious environmental difference is temperature, which averages -5.8°C at Ny Ålesund (Birks et al. 2004) and between 0.5 and 5°C in southern Norway (http://www.worldclim.org/).

The temperature regime affects the timing and duration of ice cover, and thus, the delivery of atmospheric N deposition to the lake as well as the length of the growing season, which is important in terms of the quantity and quality of oC available to heterotrophic bacteria (Wetzel 2001, Adrian et al. 2009). Perhaps the differences in climate between temperate-boreal biome in southern Norway and the Arctic are not great enough to result in changes in the functional performance of the community as, say, the contrast between tropical and Arctic biomes. Additionally, we only sampled two lakes in Svalbard, so the responses we observed may not be representative of other Arctic lakes.

In summary, certain microbial assemblages may be inherently more effective than others in removing N via denitrification, regardless of background N inputs. Chronic N loading in the high-deposition region of Norway (10-20 kg N ha^{-1} y^{-1}) does not appear to have altered the dynamics of denitrifying communities in lake sediments. This finding is important because atmospheric N loading is expected to increase 30% in Norway by 2100 and similar increases are expected in other areas depending on precipitation patterns (Galloway et al. 2004, Hole and Engardt 2008). Differences in denitrification performance between sediment communities could have important implications for mitigating the effects of N loading in lakes. Lakes with more efficient denitrifying communities could be strong sinks for N and reduce exports to

downstream ecosystems. We were not able to explicitly determine the factors that influence NO, N_2O, and N_2 dynamics and further investigation of the denitrifying community composition may allow us to better understand the observed denitrification dynamics.

ACKNOWLEDGEMENTS

LITERATURE CITED

Adrian, R., C. M. O'Reilly, H. Zagarese, S. B. Baines, D. O. Hessen, W. Keller, D. M. Livingstone, R. Sommaruga, D. Straile, E. Van Donk, G. A. Weyhenmeyer, and M. Winder. 2009. Lakes as sentinels of climate change. Limnology and Oceanography **54**:2283-2297.

Balser, T. C. and M. K. Firestone. 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry **73**:395-415.

Bergstrom, A. K., P. Blomqvist, and M. Jansson. 2005. Effects of atmospheric nitrogen deposition on nutrient limitation and phytoplankton biomass in unproductive Swedish lakes. Limnology and Oceanography **50**:987-994.

Betlach, M. R. and J. M. Tiedje. 1981. Kinetic explanation for accumulation of nitrite, nitric-oxide, and nitrous-oxide during bacterial denitrification. Applied and Environmental Microbiology **42**:1074-1084.

Birks, H. J. B., V. J. Jones, and N. L. Rose. 2004. Recent environmental change and atmospheric contamination on Svalbard as recorded in lake sediments. Journal of Paleolimnology **31**.

Brady, N. C. and R. R. Weil. 2002. The Nature and Properties of Soils. Prentice Hall, Upper Saddle River, NJ, USA.

Cavigelli, M. A. and G. P. Robertson. 2000. The functional significance of denitrifier community composition in a terrestrial ecosystem. Ecology **81**:1402-1414.

Cavigelli, M. A. and G. P. Robertson. 2001. Role of denitrifier diversity in rates of nitrous oxide consumption in a terrestrial ecosystem. Soil Biology & Biochemistry **33**:297-310.

Colores, G. M., S. K. Schmidt, and M. C. Fisk. 1996. Estimating the biomass of microbial functional groups using rates of growth-related soil respiration. Soil Biology & Biochemistry **28**:1569-1577.

Desnues, C., V. D. Michotey, A. Wieland, C. Zhizang, A. Fourcans, R. Duran, and P. C. Bonin. 2007. Seasonal and diel distributions of denitrifying and bacterial communities in a hypersaline microbial mat (Camargue, France). Water Research **41**:3407-3419.

Elser, J. J., T. Andersen, J. S. Baron, A. K. Bergstrom, M. Jansson, M. Kyle, K. R. Nydick, L. Steger, and D. O. Hessen. 2009. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science **326**:835-837.

Firestone, M. K., R. B. Firestone, and J. M. Tiedje. 1980. Nitrous oxide from soil denitrification: factors controlling its biological production. Science **208**:749-751.

Firestone, M. K., M. S. Smith, R. B. Firestone, and J. M. Tiedje. 1979. Influence of nitrate, nitrite, and oxygen on the composition of the gaseous products of denitrification in soil. Soil Science Society of America Journal **43**:1140-1144.

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

Håkanson, L. and M. Jansson. 2002. Principles of Lake Sedimentology. Blackburn Press, Caldwell, NJ, USA.

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

Hessen, D. O., T. Andersen, S. Larsen, B. L. Skjelkvale, and H. A. de Wit. 2009. Nitrogen deposition, catchment productivity, and climate as determinants of lake stoichiometry. Limnology and Oceanography **54**:2520-2528.

Hole, L. and M. Engardt. 2008. Climate change impact on atmospheric nitrogen deposition in northwestern Europe: A model study. Ambio **37**:9-17.

Holmgren, S. U., C. Bigler, O. Ingolfsson, and A. H. Wolfe. 2010. The Holocene–Anthropocene transition in lakes of western Spitsbergen, Svalbard (Norwegian High Arctic): climate change and nitrogen deposition. Journal of Paleolimnology **43**:393-412.

Klein, H. and A. Benedictow. 2006. Transboundary air pollution by main pollutants (S, N, O_3) and PM: Norway.*in* Norwegian Meteorological Institute, editor. Programme for Monitoring and Evaluation of the Long-range Transmission of Air Pollutants in Europe.

Knowles, R. 1982. Denitrification. Microbiological Reviews **46**:43-70.

Lukkari, K., H. Hartikainen, and M. Leivuori. 2007. Fractionation of sediment phosphorus revisited. I: Fractionation steps and their biogeochemical basis. Limnology and Oceanography: Methods **5**:433-444.

McCrackin, M. L. and J. J. Elser. 2010. Atmospheric nitrogen deposition alters denitrification and nitrous oxide production in lake sediments. Ecology **91**:528-539.

McCrackin, M. L. and J. J. Elser. In revision. Denitrification and microbial communities in high elevation lakes receiving atmospheric nitrogen deposition. Biogeochemistry.

Molstad, L., P. Dorsch, and L. R. Bakken. 2007. Robotized incubation system for monitoring gases (O2, NO, N2O, N2) in denitrifying cultures. Journal of Microbiological Methods **71**:202-211.

Morley, N., E. M. Baggs, P. Dorsch, and L. Bakken. 2008. Production of NO, N2O and N2 by extracted soil bacteria, regulation by NO2- and O2 concentrations. Fems Microbiology Ecology **65**:102-112.

Rodionov, D. A., I. L. Dubchak, A. P. Arkin, E. J. Alm, and M. S. Gelfand. 2005. Dissimilatory metabolism of nitrogen oxides in bacteria: Comparative reconstruction of transcriptional networks. Plos Computational Biology **1**:415-431.

Santoro, A. E., A. B. Boehm, and C. A. Francis. 2006. Denitrifier community composition along a nitrate and salinity gradient in a coastal aquifer. Applied and Environmental Microbiology **72**:2102-2109.

Saunders, D. L. and J. Kalff. 2001. Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia **443**:205-212.

Tørseth, K. and A. Semb. 1998. Deposition of nitrogen and other major inorganic compounds in Norway, 1992-1996. Environmental Pollution **102**:299-304.

Wallenstein, M. D., D. D. Myrold, M. K. Firestone, and M. Voytek. 2006. Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. Ecological Applications **16**:2143-2152.

Wetzel, R. G. 2001. Limnology: Lake and River Ecosystems. Academic Press, San Diego, CA, USA.

Wilhelm, E., E. Battino, and R. J. Wilcock. 1977. Low-pressure solubility of gases in liquid water. Chemical Reviews:219-262.

Wolfe, A. P., C. A. Cooke, and W. O. Hobbs. 2006. Are current rates of atmospheric nitrogen deposition influencing lakes in the eastern Canadian arctic? Arctic, Antarctic, and Alpine Research **38**:465-476.

Table 10. Average values (and standard error, s.e.) for various water and sediment parameters for the study lakes.

High-deposition lakes

Sample Date	Region	Lake	Hypolimnetic Water		Sediment			
			DOC µmol/L	NO_3^- µmol/L	Total C mmol g^{-1}	Total N mmol g^{-1}	Total P mmol g^{-1}	Organic Matter
7/5/2009	Egersund	Brynesland	129.7	18.8	8.7	0.5	0.17	0.17
6/12/2009	Egersund	Helleren	107.4	10.6	12.2	0.6	0.18	0.22
6/12/2009	Egersund	Holmavatnet	90.1	7.9	16.3	0.7	0.06	0.21
7/4/2009	Egersund	Krokavatnet	100.2	9.7	15.3	0.8	0.10	0.21
7/4/2009	Egersund	Kydlandsvatnet	46.7	6.3	12.6	1.0	0.21	0.15
6/23/2009	Egersund	Indre Kydlandsvatnet	99.8	10.0	11.2	0.6	0.04	0.19
6/12/2009	Egersund	Ytre Kydlandsvatnet	122.6	21.2	9.9	0.5	0.16	0.17
6/12/2009	Egersund	Netlandsvatnet	128.6	10.3	11.1	0.6	0.05	0.18
7/5/2009	Egersund	Nordra Furevatnet	189.1	6.4	11.8	0.6	0.12	0.20
7/4/2009	Egersund	Nordravatnet	122.4	9.1	10.0	0.4	0.08	0.19
7/3/2009	Egersund	Revsvatnet	226.6	9.7	17.8	0.7	0.12	0.23
6/13/2009	Egersund	Saglandsvatnet	131.5	26.4	5.6	0.3	0.06	0.12
6/13/2009	Egersund	Skjævelandvatnet	179.9	9.1	11.2	0.6	0.17	0.20
7/3/2009	Egersund	Ulsvatnet	94.8	3.8	14.5	0.7	0.11	0.21
		Mean	126.4	11.4	12.0	0.6	0.12	0.19
		s.e.	12.7	1.7	0.9	0.0	0.02	0.01

Table 10, continued. Non-significant differences between regions indicated by n.s.

Low-deposition lakes

Sample Date	Region	Lake	Hypolimnetic Water		Sediment			
			DOC µmol/L	NO$_3^-$ µmol/L	Total C mmol g^{-1}	Total N mmol g^{-1}	Total P mmol g^{-1}	Organic Matter
7/16/2009	Atna	Åsdalstjørna	131.5	1.5	27.5	0.9	0.12	0.49
8/5/2009	Svalbard	Blokkvatnet	76.3	1.5	8.7	0.5	0.17	0.03
7/14/2009	Atna	Bolvatnet	70.8	0.6	11.1	0.6	0.10	0.20
6/24/2009	Lillehammer	Djupen	215.7	0.6	8.2	0.3	0.16	0.16
7/14/2009	Atna	Flaksjøen	108.3	1.0	10.8	0.6	0.12	0.15
8/2/2009	Svalbard	Hajeren	114.5	0.7	1.7	0.1	0.02	0.05
7/15/2009	Atna	Hamntjønna	172.5	0.6	8.1	0.5	0.15	0.17
6/23/2009	Lillehammer	Hornsjoen	144.4	0.7	14.0	0.9	0.09	0.18
7/14/2009	Atna	Langrumpa	159.3	1.3	10.9	0.6	0.02	0.10
7/13/2009	Atna	Langtønna	98.7	0.8	4.1	0.4	0.03	0.19
6/23/2009	Lillehammer	Mellsjoen	149.1	0.5	16.8	0.7	0.01	0.22
7/15/2009	Atna	Muvatnet	126.9	0.4	8.7	0.4	0.04	0.14
6/24/2009	Lillehammer	Ner-Åst	277.0	0.4	14.2	0.8	0.24	0.13
6/13/2009	Lillehammer	Reinsvatnet	189.6	0.7	4.7	0.5	0.10	0.12
7/15/2009	Atna	Settningen	191.6	0.4	3.1	0.2	0.01	0.06
		Mean	148.4	0.8	10.2	0.5	0.09	0.16
		s.e.	14.7	0.1	1.7	0.1	0.02	0.03
		high vs. low deposition	high = low	high > low	high = low	high = low	high = low	high = low
		P	n.s.	< 0.0001	n.s.	n.s.	n.s.	n.s.

Table 11. Incubation results for each lake. Cumulative production of gases is for the 64 h incubation period. Total denitrified N is the sum of N_2, N_2O, and NO at time = 64 h. Principal components are based on net cumulative production of each gas and total N denitrified.

| High-deposition lakes | Cumulative production (64 h) | | | Total N denitrified | | |
	N_2 μmol	N_2O μmol	NO μmol	μmol	PCA 1	PCA 2
Brynesland	18.2	4.7	1.9	7.2	-0.753	0.135
Helleren	69.9	8.2	3.6	12.2	-0.557	0.073
Holmavatnet	17.1	36.4	1	4.4	-0.682	0.193
Indre Kydlandsvatnet	38.5	2.6	2.6	36	-0.077	0.039
Krokavatnet	13.5	11.9	2.7	3.8	-0.805	0.217
Kydlandsvatnet	415.9	8.8	0.7	8.7	-0.186	-1.020
Netlandsvatnet	66.5	0.3	0.3	2.8	5.217	1.561
Nordra Furevatnet	2.5	16	4.5	1.9	-0.844	0.352
Nordravatnet	125	14.8	0.2	8.6	-0.539	-0.218
Revsvatnet	213	5.2	3.6	26.5	-0.055	-0.366
Saglandsvatnet	166.4	3	0.3	15	-0.390	-0.377
Skjævelandsvatnet	1025.5	3	5.2	65.9	1.891	-2.644
Ulsvatnet	33.1	6.2	3.3	6	-0.756	0.169
Ytre Kydlandsvatnet	795.4	1.5	0.2	68.6	1.646	-2.261
Mean	214.3	8.8	2.2	19.1	-0.753	0.135
s.e.	88.2	2.6	0.5	6.2	-0.557	0.073

Table 11, continued. Non-significant differences between regions indicated by n.s.

Low-deposition lakes	Cumulative production (64 h)			Total N		
	N$_2$ μmol	N$_2$O μmol	NO μmol	denitrified μmol	PCA 1	PCA 2
Brynesland	18.2	4.7	1.9	7.2	0.209	4.001
Helleren	69.9	8.2	3.6	12.2	-0.745	-0.069
Holmavatnet	17.1	36.4	1	4.4	0.743	-0.297
Indre Kydlandsvatnet	38.5	2.6	2.6	36	-0.922	0.148
Krokavatnet	13.5	11.9	2.7	3.8	2.646	0.715
Kydlandsvatnet	415.9	8.8	0.7	8.7	-0.446	-0.248
Netlandsvatnet	66.5	0.3	0.3	2.8	1.225	-0.822
Nordra Furevatnet	2.5	16	4.5	1.9	-0.727	0.016
Nordravatnet	125	14.8	0.2	8.6	-0.399	0.039
Revsvatnet	213	5.2	3.6	26.5	-0.698	0.194
Saglandsvatnet	166.4	3	0.3	15	-0.912	0.173
Skjævelandvatnet	1025.5	3	5.2	65.9	-0.781	0.085
Ulsvatnet	33.1	6.2	3.3	6	-0.810	-0.078
Ytre Kydlandsvatnet	795.4	1.5	0.2	68.6	-0.762	0.248
Mean	214.3	8.8	2.2	19.1	-0.731	0.044
s.e.	88.2	2.6	0.5	6.2	-0.207	0.277
high vs. low deposition	high = low	high = low	high = low	high = low	high = low	high = low
P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 12. Principal components analysis of total N denitrified and net

cumulative production of NO, N_2O, and N_2 gases. Values in bold are

considered to load on the given component.

	Component 1	Component 2
Factor Loadings:		
N_2	0.31	**-0.66**
N_2O	**0.62**	0.41
NO	0.00	**0.62**
Total N denitrified	**0.72**	-0.07
Eigenvalue	1.87	1.15
Fraction of variance explained	46.7%	30.1%
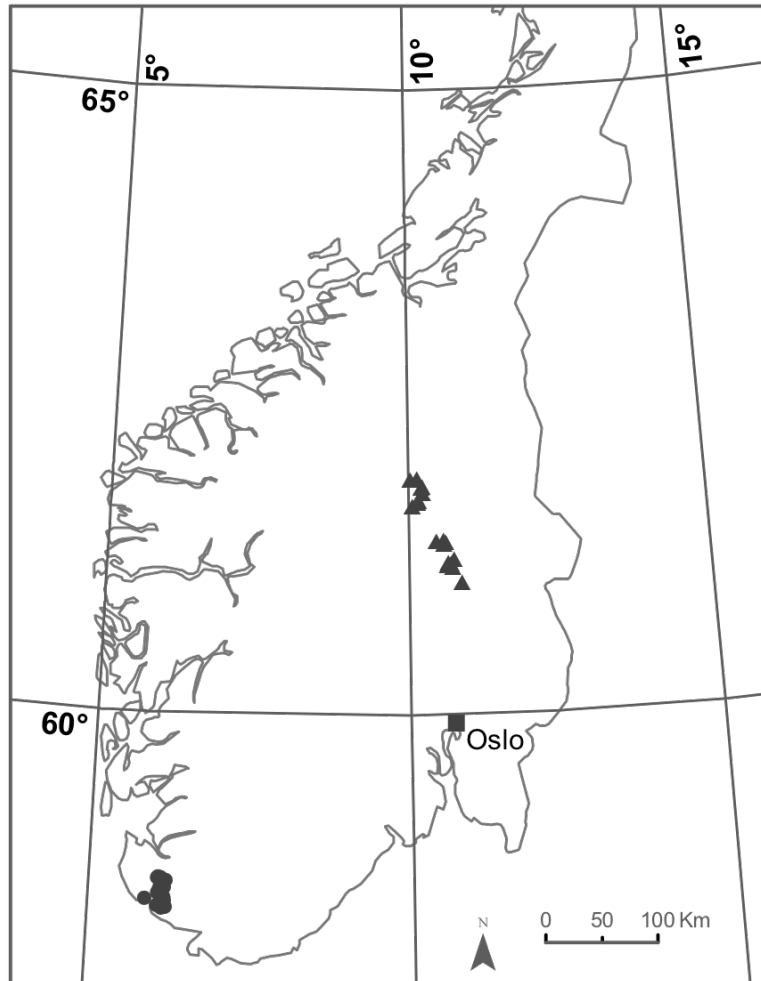
Figure 8. Location of study sites in Norway. Fieldwork was conducted in
two regions that encompass a gradient of atmospheric nitrogen
deposition. Nitrogen deposition is greatest in extreme southwestern
Norway, with rates of 10-20 kg N ha^{-1} y^{-1}, and decreases to less than 4 kg
N ha^{-1} y^{-1} in northeastern areas (Hole and Tørseth 2002). Circles denote
lakes sampled in high-deposition areas and lakes sampled in low-
deposition areas are denoted by triangles.
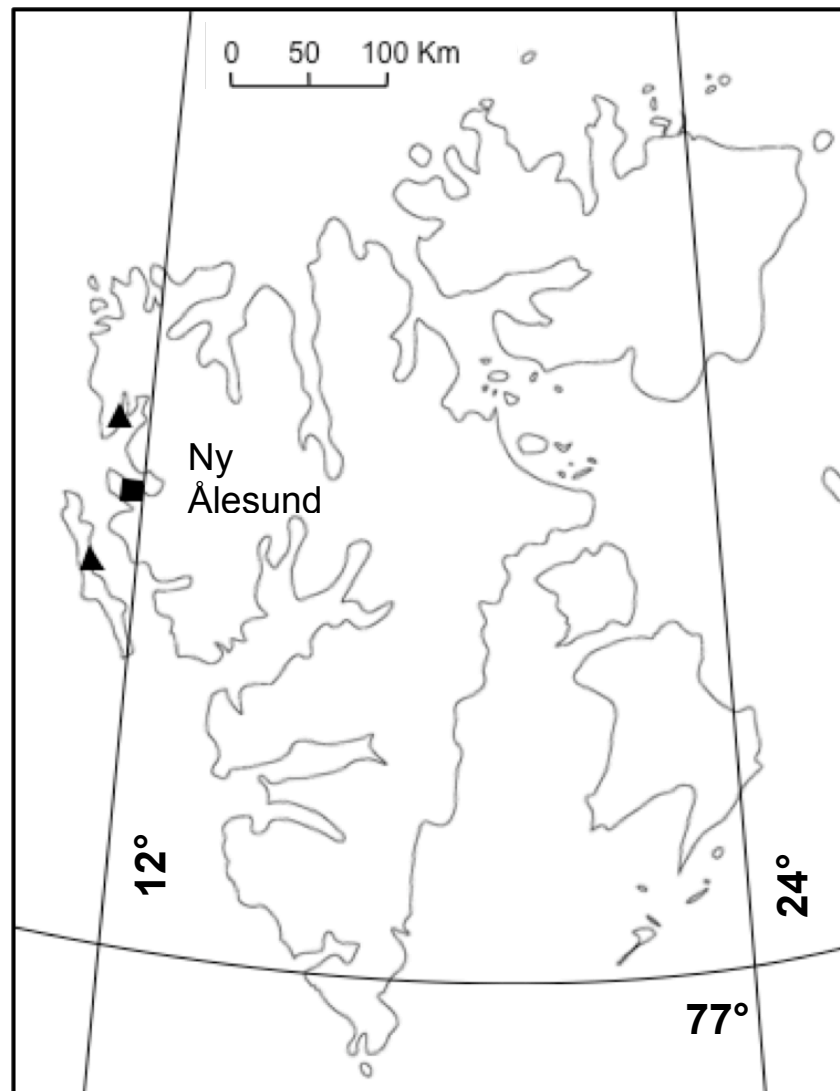
Figure 8, continued. Location of study sites in Svalbard archipelago.

Triangles denote sampled low-deposition lakes.
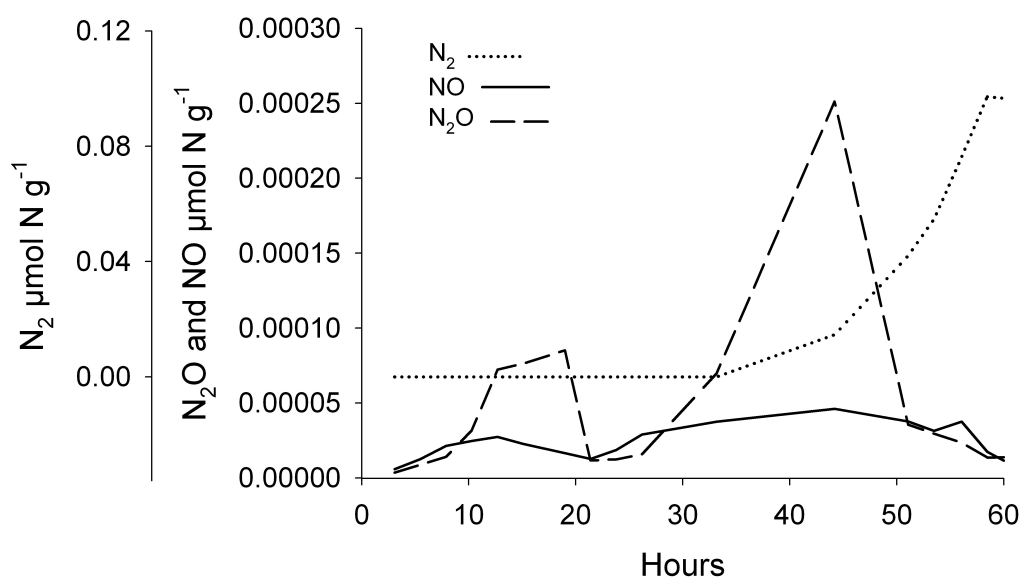
Figure 9. The accumulation of NO, N_2O, and N_2 for Skjævelandvatnet sediment over the course of the incubations. Gaseous production for the other lakes generally followed a similar pattern.
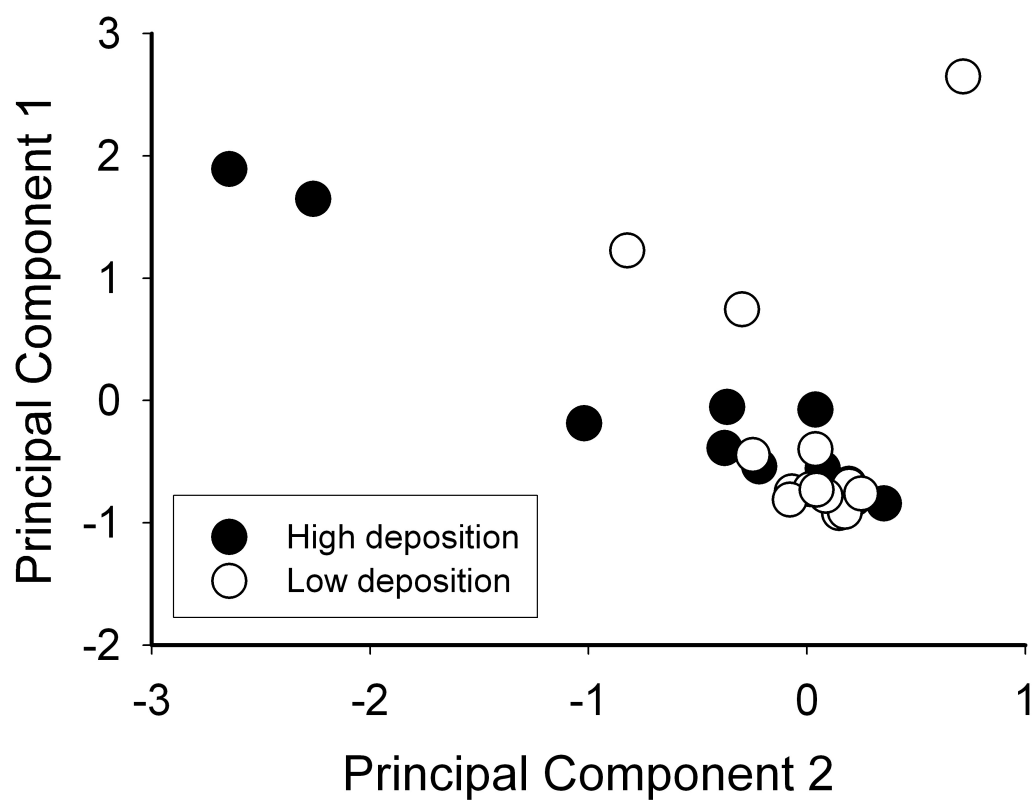
Figure 10. Principal components analysis of total N denitrified and the cumulative net production of NO, N_2O, and N_2 gases.
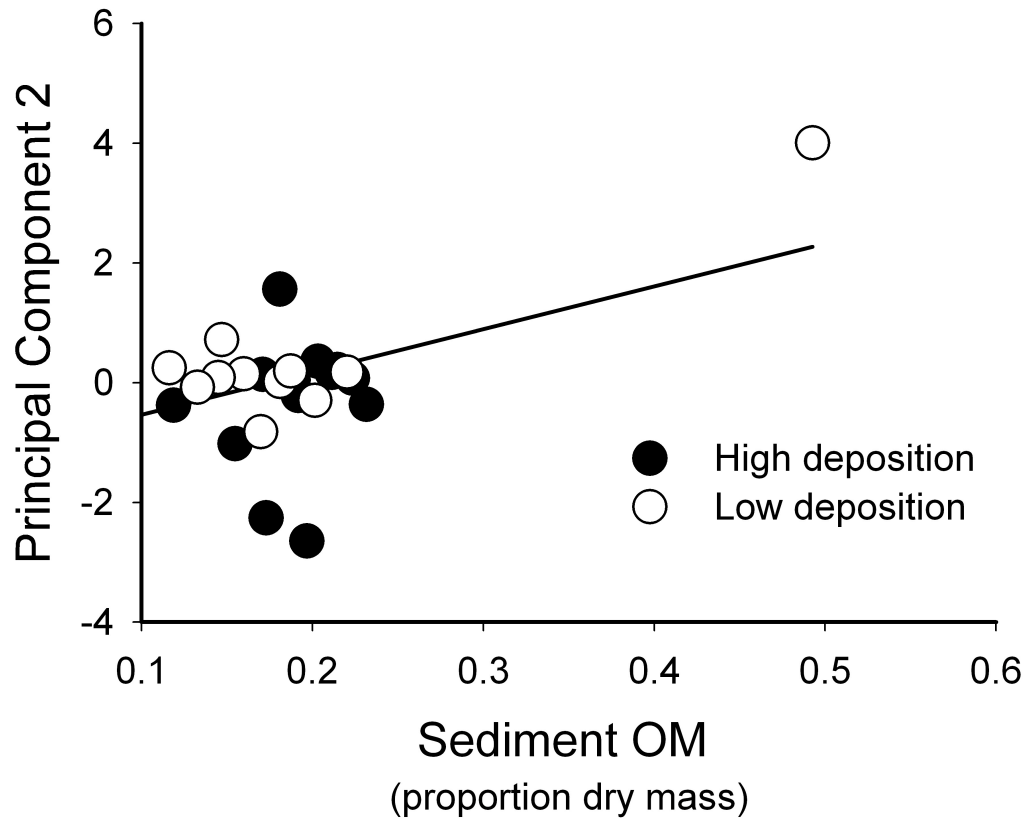
Figure 11. Relationship between principal component 2 and sediment OM

content (R^2 = 0.28, P = 0.01, Component 2 = 7.21 * OM – 1.26).

CHAPTER 5

Pelagic and Sediment Bacterial Responses to Resource Enrichment in

Lakes Receiving Atmospheric Nitrogen Deposition (Norway)

ABSTRACT

Reactive nitrogen (N) compounds are transported long distances by the atmosphere and deposited onto watersheds and lakes even in remote, unpopulated areas. Global rates of N deposition are expected to increase due to various human activities. The effects of such N inputs on bacteria in the water column and sediments of lakes are not well understood. We examined metabolic responses of pelagic and sediment bacteria to organic carbon (oC), nitrate (NO_3), and phosphorus (P) enrichment. We also measured nitrification potential in sediments. Sampled lakes were located in southern Norway and received either high (10-20 kg N ha^{-1} y^{-1}) or low (<4 kg N ha^{-1} y^{-1}) inputs of N deposition. The surface water of high-deposition lakes had greater concentrations of NO_3^- and total dissolved N (TDN) than low-deposition lakes, while the reverse was true for dissolved organic carbon (DOC). Total dissolved phosphorus (TDP) did not differ between regions. Results of 2-day bioassays indicated that growth of bacterioplankton was co-limited by oC, N, P, regardless of N deposition rate. For sediments, there were no differences in organic matter (OM) and total carbon (C), N, and P contents between deposition regions. Sediment respiration responded most strongly to additions of oC, while denitrification

responded to NO_3^- additions. These responses did not differ between regions. Nitrification potential was significantly greater high-deposition lakes. Our observation of enhanced nitrification by autotrophic microbes, however, is of particular concern as it may contribute to accumulation of NO_3^- in surface waters.

INTRODUCTION

Human activities, such as agriculture and fossil fuel combustion, have dramatically altered the balance of nitrogen (N) in the environment and resulted in shifts in the trophic status of aquatic ecosystems (Conley et al. 2009). Reactive N species (such as NO_3^- and ammonium, NH_4^+) are of particular concern as they may be transported by the atmosphere and deposited to lakes and watersheds in unpopulated areas. Indeed, N deposition to lakes has been found to alter water chemistry and algal community composition high-elevation and Arctic lakes (Baron et al. 2000, Wolfe et al. 2006). The effects of N deposition on bacteria in lakes are not well documented. Nitrogen deposition may alter bacterial processes in lakes and potentially explain phenomenon such as accumulation of NO_3^- in surface water (Taylor and Townsend 2010).

It is important to examine the responses of bacterioplankton to N deposition because bacteria play an important role in trophic dynamics and resource cycling in aquatic ecosystems, representing an average of ~20% of phytoplankton production in lakes (Cole et al. 1988). This role is

128

greater in oligotrophic lakes because higher bacterial biomass relative to phytoplankton biomass is observed in low-productivity lakes compared to eutrophic lakes (Cotner and Biddanda 2002). In terrestrial ecosystems, chronic N loading has changed the composition of soil microbial communities and influenced rates of microbial respiration and N cycling (Lovett and Rueth 1999, Bowden et al. 2004, Wallenstein et al. 2006). However, the effects of N deposition on bacterial processes in lakes are not as well studied as those in terrestrial ecosystems.

We conducted resource enrichment experiments with pelagic and benthic bacteria from lakes located across a gradient of atmospheric N deposition in southern Norway. We examine both pelagic and benthic habitats because climate and seasonal processes differentially affect these environments, and these differences may modulate the effects of N deposition. For example, the pelagic zone is subject to diurnal changes in light, primary production, and grazing, as well as strong seasonal shifts in temperature and mixing due to the presence of ice cover. Except for seasonal mixing and sedimentation, profundal benthic zones of temperate-boreal lakes experience more stable conditions.

Beyond relatively direct effects on N availability, atmospheric deposition may also affect bacteria indirectly by inducing shifts in productivity and nutrient limitation in the catchment and water column of lakes, which in turn will influence the organic matter that is available in the

water or sediments, and thus, the activities of bacterial communities.

Recent studies have found that N deposition has increased concentrations

of chlorophyll and dissolved inorganic N (DIN: NO_3^- + NH_4) and increased

ratios of chlorophyll:P and seston C:P in lakes (Bergstrom and Jansson

2006, Elser et al. 2009b). Atmospheric N deposition has also shifted

nutrient limitation of phytoplankton from N to P and there is emerging

evidence that this limitation extends to zooplankton as well (Elser et al.

2009a, Elser et al. 2010). While the availability of oC has traditionally been

considered the main factor limiting the growth heterotrophic pelagic

bacteria, a number of studies have found that availability of N and P also

stimulates bacterial production (Elser et al. 1995, Wetzel 2001, Cotner and

Biddanda 2002). Additionally, experimental evidence suggests that

respiration of heterotrophic microbes may be P limited (Cleveland et al.

2002, Corstanje et al. 2007). Co-limitation of bacterial growth by oC and N

and/or P has also been observed in oligotrophic lakes (Carlsson and

Caron 2001, Bertoni et al. 2008). To evaluate effects of N deposition on

resource limitation of microbial processes, we measured changes in the

abundance of bacterioplankton during short-term bioassays in response to

additions of organic oC, N, and P. For sediment bacteria, we measured

two forms of respiration, oxic respiration that produces carbon dioxide

(CO_2) production and denitrification (respiration in sub-oxic conditions

130

where NO_3^- is reduced to N_2 gas), in response to experimental additions of oC, N and P.

Because of deposition-induced differences in resource availability in the water column, we expected greater bacterial growth in response to oC and P or only to P in high-deposition lakes and greater response to oC and N or only to N in low-deposition lakes (Figure 12). Previous work found no differences in OM, C, N, or P content of lake sediments across a gradient of N deposition (McCrackin and Elser 2010). Measurement of bulk resources, however, may not reveal differences in the bioavailability or quality of these resources in sediments. If N deposition-related resource limitation observed for phytoplankton and bacterioplankton extends to the sediments, we would expect evidence of P limitation of respiration in high-deposition lakes and N limitation in low-deposition lakes.

Lastly, increased N loading is often associated with increased nitrification in soils and streams; however, similar studies in lakes are lacking (Hanson et al. 1994, Strauss et al. 2002, Gundersen et al. 2006). Nitrification is the oxidation of NH_4^+ to NO_3^- by chemoautotrophic bacteria and archaea. We measured the potential rate of nitrification in sediments and expected the rate to be greater in high-deposition lakes because of elevated atmospheric inputs of NH_4^+ deposition (Tørseth and Semb 1998).

METHODS

Study sites

During summer 2007 and 2009, we sampled lakes located at the high and low ends of a gradient of N deposition that spans over 400 km across southern Norway. Norway receives atmospheric pollution from industrial regions of Europe and total (wet and dry) N deposition rates are 10-20 kg N ha^{-1} y^{-1} in southwest regions of the country, decreasing to less than 4 kg N ha^{-1} y^{-1} in east-central areas (Tørseth and Semb 1998, Klein and Benedictow 2006). Lakes near Egersund (58.35° latitude, 6.04° longitude), in southwestern Norway, are considered to be in the in the high-deposition region, while lakes near Lillehammer (61.07° latitude, 10.27° longitude) and Atna (61.52° latitude, 10.27° longitude) are considered to be in the low-deposition region.

Samples for bacterioplankton and sediment analyses were collected in 2007 and 2009, respectively, from the lakes at the ends of the N deposition gradient. The lakes occupy watersheds that are generally unpopulated and sparsely vegetated, making them well suited to evaluate the influence of atmospheric N deposition without confounding influences of variable land use history and vegetation type. The sampled lakes were generally small and deep, with surface area < 20 ha and maximum depth > 5 m. Mean annual precipitation is approximately 1,700 mm in the high-deposition area and 700 mm in the low-deposition area

(http://biogeo.berkeley.edu/worldclim1_4/grid/curprec_30s_esri). Mean elevation of the sampled lakes was 230 and 993 m above sea level in regions receiving high and low levels of atmospheric deposition, respectively.

Field sampling and laboratory procedures

In both years, each lake was visited once and fieldwork was staggered so that sampling of lakes in high- and low-deposition regions was not strongly influenced by seasonal effects. Of the 20 and 28 lakes sampled in 2007 and 2009, respectively, 13 lakes were sampled in both years (Fig.12, Tables 13 and 14). Each lake was sampled using an inflatable boat. Lake depth was measured with a hand-held echo sounder. Water was collected from two depths using a battery-powered submersible pump. For bacterioplankton, water samples were collected at a depth of 1 - 1.5 m. For sediments, water was sampled just above the sediments at 10 m depth or at the maximum lake depth if < 10 m.

Water was filtered with 1-µm polycarbonate filters (Whatman, Piscataway, New Jersey, USA) to remove algae, flagellates, and other protozoa, allowing us to assay bacterioplankton growth rate without grazing losses. Three replicate 10-mL samples were amended with one of four different incubation media: 50 μmol/L organic carbon (as dextrose and acetate), 7.5 μmol/L N (as NH_4NO_3) 0.5 μmol/L P (as KH_2PO_4), or combined medium that included all three resources (hereafter: oCNP) at

133

the concentrations just described. Bacterial bioassays were incubated in 20-mL bottles for two days at lake surface temperature (about 15°C). Samples were filtered and flash frozen in liquid N_2 and held at -80°C until analysis. Bacterial abundance was determined as the concentration of DNA, which correlates well with bacterial abundance based on epifluorescence counts (Tranvik 1997). DNA was extracted with Triton X-100 (Dow Chemical Co., Midland, MI, USA) and stained with PicoGreen (Invitrogen Corp., Carlsbad CA, USA), a sensitive fluorescent nucleic acid stain for quantifying double-stranded DNA. Stained bacterial samples were read on a Turner BioSystems Model TD-700 fluorometer (Promega Corporation, Sunnyvale, CA, USA) at 480 nm excitation and 525 nm emission.

Chemical analysis of dissolved nutrients in lake water followed the same protocol in both sampling years. All water samples were filtered with Pall A/E glass fiber filters (Pall Corporation, Port Washington, NY, USA) and frozen until analysis of dissolved nutrient species. Water was analyzed for DOC and total dissolved N (TDN) on a Shimadzu TOC 5000 (Shimadzu Corporation, Kyoto, Japan). Nitrate plus nitrite (hereafter: NO_3^-) concentrations were determined on a Metrohm 761 Compact Ion Chromatograph (Metrohm, Riverview, Florida, USA) in 2007 (Elser et al. 2009a) and on a Lachat Quick Chem 8000 autoanalyzer (Hach Corporation, Loveland, Colorado, USA) in 2009. Nitrate concentrations for

lakes sampled in 2007 were reported in Elser et al. (2009a). Ammonium

was not measured, but is generally low across Norwegian lakes (Hessen

et al. 2009). Concentrations of total dissolved P (TDP) were determined

via colorimetric analysis using the ammonium-molybdate method following

persulfate oxidation (APHA 2005). In 2007, surface water filtered onto

Whatman GF/C glass fiber filters that were analyzed for chlorophyll by

extraction with 100% methanol followed fluorometric analysis using a

Turner BioSystems Model TD-700 fluorometer (Promega Corporation,

Sunnyvale, CA, USA).

In 2009, surface sediments were collected from 14 lakes at each end

of the N deposition gradient. Sediments were collected using a LaMotte

dredge from a water depth of approximately 10 m or at the maximum lake

depth if the lake was < 10 m. Sediments were stored in plastic bags at 4°C

in dark conditions until analysis. We conducted a resource enrichment

experiment to investigate CO_2 respiration and denitrification responses to

additions of oC, NO_3^-, or P. We measured metabolic processes rather than

growth because bacterial DNA extractions were not feasible given the

large number of samples. Three replicate 50-g subsamples of sediment

from each lake were slurried with 40-mL of one of five different media. The

incubation media were: distilled water as a control, 8.3 mmol L^{-1} oC (as

glucose), 6.7 mmol L^{-1} N (as KNO_3^-), 0.4 mmol L^{-1} P (as KH_2PO_4) or a

combined medium that included all three resources (hereafter: oCNP). We

flushed the incubations with N_2 gas to reduce levels oxygen (O_2) to ~10%

of headspace volume. Acetylene was added to block nitrification and the

reduction of nitrous oxide (N_2O) to N_2 (Yoshinari and Knowles 1976). We

collected initial and final samples at the end of 48-h incubation at 12°C in

dark conditions, both after vigorous shaking. Bottles were not shaken

during the two-day incubation. Gas samples were analyzed for CO_2, N_2O,

and O_2 on an Agilent 7890A Gas Chromatograph (Agilent Technologies,

Santa Clara, CA, USA) with electron-capture and thermal conductivity

detectors. Gas samples for one lake, Muvatnet, were damaged during

transport and could not be analyzed. Rates of respiration and

denitrification were determined as the production of CO_2 and N_2O,

respectively, on the basis of dry sediment mass. Exponential production of

CO_2 results from dividing microbial populations during growth (Colores et

al. 1996). Because there were only two sampling points for our

incubations, we were unable to determine an exponential CO_2 response

curve, however it is likely that CO_2 produced over the incubation period

results from bacterial growth.

Nitrification potential assays were conducted using the shaken-slurry

method (Hart et al. 1994). For each lake, 10 g of wet sediment was

combined in a 250-mL Erlenmeyer flask with 100 mL of media containing

50 mmol L^{-1} NH_4^+ (as $(NH_4)_2SO_4$) in a phosphate-buffered solution of 1 M

monopotassium phosphate (KH_2PO_4) and 1 M dipotassium hydrogen

136

phosphate (K_2HPO_4) that was adjusted to a pH of 7.0. Sediment slurries were shaken for 48 h at 17°C. At four times during the incubation period, a 15-mL aliquot of the sediment slurry was removed from the flask and centrifuged for 10 minutes at 3000 rpm. The liquid portions of the extracts were frozen until analysis for NO_3^- on a Bran+Luebbe autoanalyzer (SPX Corporation, Charlotte, North Carolina, USA). The potential nitrification rate was calculated as the linear slope of NO_3^- concentration in the sediment extracts over the 48-h incubation period.

Total C and N content of dried sediments were measured with a Flash EA 1112 Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy). Total P content of combusted sediment was measured colorimetrically following extraction with 0.5 M hydrochloric acid using the acid-molybdate technique (Lukkari et al. 2007). Sediment water content was determined as mass loss after drying subsamples at 105°C for 48 h and organic matter (OM) content was determined as mass loss on ignition at 550°C for 4 h.

Statistical analysis

Averages of all sediment and water parameters in replicate samples (n = 3) were calculated for each lake and compared between deposition regions using t tests. Results of bacterioplankton and sediment enrichment experiments were analyzed by deposition region and individually by lake. Responses for the enrichment treatments (n = 3) were

averaged for each lake and analyzed by analysis of variance (ANOVA) to evaluate the main effects of resource enrichment treatment (control, oC, N, P, and oCNP) and N deposition level (high and low). Post-hoc analyses were performed using Tukey's HSD.

For individual lakes, responses were analyzed by ANOVA (generally df = 3, 15) to evaluate the effects of resource enrichment treatment (control, oC, N, P, and oCNP). Each lake's response was classified into several categories depending on the ANOVA results. If the ANOVA was not significant, the response was classified as "no resource limitation." If one of the single enrichments (oC, N, or P) was not statistically different from the combined oCNP treatment and if both of these treatments were significantly greater than the control and other treatments, then the response was classified as "single resource limitation (X)", where X is oC, N, or P. For example, if $Z_{oCNP} = Z_{oC} > Z_N = Z_P = Z_{control}$, where Z is one of the responses (final bacterial DNA concentration, CO_2 flux, or N_2O flux) with the treatment denoted in the subscript, then the experiment would be classified as "single resource limitation (oC)". For other significant responses, the we assessed the nature of the interactive effect by calculating a parameter R similar to that described by Elser and colleagues (2009b): $R = (Z_{oCNP} - Z_{control})/((Z_{oC} - Z_{control}) + (Z_N - Z_{control}) + Z_P - Z_{control}))$. When R > 1, the oCNP interaction was classified as super-additive and when R < 1, the interaction was classified as sub-additive.

Experiments classified as super-additive were further divided into categories based on the results of individual t tests that contrasted single enrichment treatments and control. If there were no significant differences such that $Z_{control} = Z_P$, $Z_{control} = Z_N$, $Z_{control} = Z_{oC}$, then the experiment was classified as "strict co-limitation (oCNP)." If two or all of the single enrichment treatments were greater than the control, then the experiment was classified as "synergistic co-limitation". Experiments where $R < 1$ were further classified by pair-wise comparisons of the treatments (oC, N, P, oCNP) with the control, also determined by t tests. If all treatments were greater than the control, the response was classified as "constrained". This indicates that one or more resources limited the response variable, but that the response was less than expected if all resources were added simultaneously. If one or more of the contrasts was not significant, the experiment was classified as "antagonistic." Experiments classified as "single limitation (X)" or "sequential co-limitation (X)" were considered evidence of a primary limitation by resource X (oC, N or P). The analyses of individual lakes treat sediment and water sub-samples as replicates, although they are not statistically independent. Hence, these results should be interpreted with caution because of pseudoreplication. Nonetheless, we believe these analyses provide insight into variability of bacterial responses between lakes that may not be

observed in the comparison of aggregate lake responses between N deposition regions.

Multiple-linear regression was used to identify relationships among predictor variables and the initial bacterial DNA concentration, rates of CO_2 and N_2O production in the control treatment (as an estimate of background rates), and the potential nitrification rate. Predictor variables for bacterial DNA concentration were pH, water concentrations of NO_3^-, DOC, chlorophyll, and the ratios of $DOC:NO_3^-$ and TDN:TDP. For sediment processes, predictor variables were sediment OM, C, N, and P contents, and concentrations of NO_3^- and DOC. Models were selected by considering all subsets on the basis of adjusted R_2 and AIC. We evaluated multi-colinearity and selected final models for which tolerance values were >0.5 for all predictor variables. When necessary, response and predictor variables were transformed to improve normality. Statistical analyses were performed using the software JMP 8.0.1 (SAS Institute, Inc., Cary, NC, USA) with α = 0.05.

RESULTS

Bacterioplankton

The lakes differed considerably in terms various water chemistry parameters (Table 13). Lakes in the high-deposition region had lower pH than low-deposition lakes (6.1 vs. 6.7). Consistent with the oligotrophic nature of the lakes, epilimnetic concentrations of DOC and chlorophyll

were generally low, averaging 202 ($\pm$ 33.4 SE) μmol/L C and 4 ($\pm$ 1.1 SE) μg L^{-1} across all lakes, respectively. Average concentrations of DOC and chlorophyll were lower in high-deposition lakes compared to those in the low-deposition region, at 127 versus 297 μmol L^{-1} C and 2.2 versus 6.5 μg L^{-1} chlorophyll, respectively. The concentration of NO$_3^-$ averaged 22.7 and 0.13 μmol L^{-1} in lakes in receiving elevated and low levels of atmospheric N, respectively. Total dissolved P did not differ between regions, averaging 0.24 ($\pm$ 0.1 SE) μmol/L P across all lakes. The ratio of TDN:TDP was greater in high-deposition lakes than in low deposition lakes (240 vs. 40.2) while the reverse was true for DOC:NO$_3^-$ (7.1 vs. 7,159). The ratio of DOC:TDN:TDP was 1320:240:1 and 1693:40:1 for high- and low-deposition lakes, respectively.

Background concentrations of bacterial DNA did not differ between deposition regions, averaging 0.52 ($\pm$ 0.06 SE) μg mL^{-1} across all lakes. Regression analysis of the aggregated lake data found that the initial bacterial DNA concentration in high-deposition lakes was positively related to the ratio of DOC:NO$_3^-$ (R^2 = 0.40, P = 0.049, log bacterial DNA = 0.27 * [DOC/NO$_3^-$] – 0.06). The bacterial DNA concentration for low-deposition lakes was not related to any of the predictor variables (P > 0.05). The results of ANOVA indicate that the DNA concentration was significantly greater in the oCNP treatment compared to control, oC, N, and P and that there was no statistical difference between the control and the oC, N, and

P treatments (Fig. 13). There was also no significant effect of deposition region on overall bacterial DNA concentration and no interaction of the deposition region with the enrichment treatments.

The analyses of individual lakes found that bacterial DNA concentrations were greatest in response to the oCNP treatment, except for three lakes where there was no significant treatment effect (Table 15). The dynamics of co-limitation by oC, N, and P were more varied: one lake showed single nutrient limitation, 14 lakes showed co-limitation, and 6 lakes showed constrained or antagonistic resource limitation. Atmospheric N deposition did not influence the frequency of these response categories.

Sediment bacteria

The hypolimnetic concentration of NO_3^- was greater in high-deposition compared to low deposition lakes (11.4 v 0.8 μmol L^{-1} N, Table 14) but there was no difference in the concentration of DOC, which averaged 141.6 ($\pm$ 9.7 SE) μmol L^{-1} C across all lakes. There were also no differences in sediment C, N, or P contents nor in ratios of C:N, C:P, or N:P between N deposition regions (Table 14). Sediment organic matter content did not differ between regions and averaged 18% ($\pm$ 1.4 SE) for all lakes.

For aggregated lake data, ANOVA revealed that there was no significant effect of N region for either sediment CO_2 or N_2O production ($P > 0.05$, Figs. 14A and B). Fluxes of CO_2 in the oCNP treatment did not

differ between deposition region, while N_2O fluxes were significantly greater in the high-deposition region (P = 0.0003). Fluxes of CO_2 and N_2O in the oCNP treatment were positively related to the NO_3^- concentration, with R^2 of 0.33 and 0.39, respectively (P = 0.001, log CO_{2oCNP} = 0.41 * log $[NO_3^-]$ + 1.22; P = 0.0004, log N_2O_{oCNP} = 0.51 * log $[NO_3^-]$ + 0.29). Across all lakes combined, the flux of CO_2 was greatest in the oC and oCNP treatments, which did not differ statistically (Fig. 14A). Responses in the N and P treatments did not differ from the control. Average CO_2 flux for the oCNP treatment was 19 (+ 3 SE) μmol C kg^{-1} h^{-1}. Background CO_2 production (control) was positively related to sediment OM content (R^2 = 0.24, P = 0.01, log CO_2 flux = 4.42 * OM + 0.10). Denitrification was greatest in the N and oCNP treatments, which were not different (Fig. 14B). There were no differences in responses between the control and the oC and P treatments. The N_2O flux in the oCNP treatment was 5.4 ($\pm$ 0.7 SE) μmol N kg^{-1} h^{-1} for all lakes combined. The production of N_2O in the control treatment was best predicted by sediment OM (R^2 = 0.27, P = 0.007, log N_2O flux = 5.5 * OM – 1.98).

Similar to the bacterioplankton bioassays, analysis of individual experiments found that sediment enrichment responses varied between lakes. For CO_2 production, 7 lakes showed no limitation, 8 lakes showed single resource limitation, 4 lakes showed co-limitation, and 8 lakes showed constrained or antagonistic responses to resource enrichment. In

the case of denitrification, one lake showed no limitation, 11 lakes showed single resource limitation, 13 lakes showed co-limitation, and 2 lakes showed constrained or antagonistic responses to resource enrichment. The frequency of these responses did not differ between N deposition regions.

Nitrification potential was significantly greater in sediments of high-deposition lakes compared to low deposition lakes, at 156 ($\pm$ 24 SE) and 61 ($\pm$ 20 SE) μmol N kg^{-1} h^{-1}, respectively (Fig. 15). Across all lakes, nitrification potential averaged 108 ($\pm$ 18 SE) μmol N kg^{-1} h^{-1} was best predicted by NO_3^- concentration in the hypolimnion (R^2 = 0.35, P = 0.001, log nitrification potential = 0.43 * log [NO_3^-] + 1.66).

DISCUSSION

Pelagic and benthic bacterial responses to resource enrichment

Contrary to our expectations and previous results for phytoplankton (Bergstrom and Jansson 2006, Elser et al. 2009a), there were no deposition-induced differences in resource limitation of bacterioplankton. Rather, we observed co-limitation of growth by oC, N, and P across all lakes. These results suggest that in these lakes resources available to bacteria are stoichiometrically balanced relative to bacterial growth needs. The experimental design was not fully factorial, so we could make only limited assessments on the interactive effects of oC, N, and P. Our analysis of individual lakes suggests that the nature of resource limitation

144

varies among lakes but not between deposition regions. The majority of lakes were co-limited by a combination of resources or, in the case of four lakes, sequentially by P. Only one lake was limited by a single resource, oC. The remaining lakes showed no resource limitation or sub-additive responses to resource enrichment. Our results are surprising because the availability of dissolved resources is markedly different between deposition regions. The concentration of NO_3^- and TDN:TDP ratio were significantly greater in high-deposition lakes, while the DOC concentration was greater in low- deposition lakes. Nevertheless, available supplies of these major resources appear to be sufficient to satisfy microbial demands during the 2-d bioassay experiments.

The lack of deposition-induced changes to resource limitation in pelagic bacteria is consistent with the results of our sediment assays. We found no evidence that N deposition has altered the resource status of sediment CO_2 production or denitrification in either aggregated or individual lake analyses. In the case of the former, sediments responded most strongly to additions of oC and NO_3^- in terms of rates of respiration and denitrification, respectively, regardless of N deposition level. As observed for bacterioplankton assays, there were a variety of responses for experiments of individual lakes. Respiration in sediments from less than half of the lakes (11) was limited by oC while respiration in sediments from seven lakes was not resource limited. Interestingly, eight lakes

145

showed sub-additive responses (constrained or antagonistic) to resource enrichment. The constrained response could occur if resources were immobilized in the oCNP treatment rather than used to support respiration. In this case, "the sum of the parts is *less* than the whole." Antagonistic responses could result if "excess oC" was respired in response to oC enrichment, resulting in greater respiration rates in this treatment compared to the oCNP treatment. Indeed, for experiments with antagonistic responses, additional pairwise comparisons revealed that the oC treatment was greater than oCNP, N, P, and control. It is unclear why respiration responses to resource enrichment results varied between experiments. There could be differences in the bioavailability of sediment resources or in the composition of microbial community.

Denitrification in all but four of lakes was limited or sequentially co-limited by NO_3^-. Our findings are consistent with a large body of research that finds NO_3^- concentration to be an important predictor of denitrification (Seitzinger et al. 2006). Interestingly, our previous work found no deposition-induced differences in potential denitrification (in response to between non-limiting concentrations of oC, N, P, (McCrackin and Elser 2010). Here we found that N_2O production in the oCNP treatment was greatest in the high-deposition region. It is unclear why the results differ between these experiments because many of the same lakes were sampled in both years. The major methodological difference was the

146

length of the assays, which were 2 d in present study compared to 4 h in the earlier studies. A longer incubation period could allow for greater contact time between NO_3^- and the active sites of enzymes and reveal differences in the denitrifying community between regions.

Factors influencing resource limitation

We found that atmospheric N deposition has not altered the nutrient status of bacterioplankton. Further, there was no difference in the initial abundance of bacterioplankton between deposition regions and abundances were not strongly related to concentrations of chlorophyll or DOC as has been observed in other studies (Cole et al. 1988, Simon et al. 1992). These findings are interesting because chronic N loading has shifted the nutrient status of phytoplankton and zooplankton (Bergstrom and Jansson 2006, Elser et al. 2009a, Elser et al. 2010). The lack of deposition-induced differences in bacterioplankton responses to nutrient enrichment is consistent with a similar study conducted in Colorado lakes across a gradient of N deposition (M. Kyle, unpublished data). There was potential for pelagic bacteria to show strong responses to unbalanced resources in the water column because the C:N:P of bacterial biomass is thought to be relatively fixed compared to that of phytoplankton (Sterner and Elser 2002). Experimental evidence suggests that bacteria have some flexibility to alter their cellular element composition in response to substrate C:N:P ratios (Tezuka 1990, Chrzanowski et al. 1996), which

147

could explain the results we observed. Additionally, bacterial strains with different elemental composition will have different resource requirements (Makino et al. 2003). Hence, one possible explanation for apparent co-limitation of bacterioplankton growth by oC, N, and P in both deposition regions is shifts in the composition of the bacterial communities. Chronic differences in lake nutrients could result in different assemblages between lakes and these assemblages may have nutrient requirements that are consistent with the available resources (Fisher et al. 2000, Makino and Cotner 2004). Our assays do not reflect seasonal changes in the bacterioplankton community composition, however, so our results may not be representative of responses in other periods. Alternatively, it is possible that factors such as grazing or viral lysis have a stronger influence on bacterial communities than available nutrients (Cotner and Biddanda 2002). Our findings suggest that community-level responses are often more difficult to interpret than those of pure cultures. Regardless of anthropogenic N inputs, however, pelagic bacteria are growing below their potential rate in unproductive Norwegian lakes and are constrained by a suite of key resources.

It is important to understand the factors the influence respiration and denitrification in sediments, because of the role these processes play in nutrient cycling. Contrary to our expectations, we found no evidence that N deposition has altered the nutrient status of sediment CO_2

148

production or denitrification, because there were no differences between deposition regions in responses to the treatments. While sediment OM was nearly 20% of dry mass, respiration responded strongly to additions of glucose, perhaps because OM in sediments is not labile. Denitrification responded most strongly to additions of NO_3^-, suggesting that background concentrations in the sediments were low. Our assays were amended with acetylene, which would have inhibited nitrification. Therefore we do not know the extent to which nitrification and denitrification are coupled in sediments of the sampled lakes.

Recent studies have suggested that denitrification becomes oC limited when concentrations of NO_3^- are elevated relative to oC (Weyhenmeyer and Jeppesen 2009, Taylor and Townsend 2010). Ratios of DOC:NO_3^- were lower in high-deposition lakes compared to low-deposition lakes (11 vs. 199, Table 14). Hence, limitation of denitrification by oC might explain why NO_3^- accumulates in the surface waters of certain ecosystems. It is not clear, however, that water column ratios of DOC:NO_3^- are indicative of conditions in the sediments, where most denitrification occurs. The denitrification rate in the N and oCNP treatments did not differ, suggesting that there is adequate C available to support denitrification even when NO_3^- was supplied at concentrations that were two orders of magnitude above background. Indeed, a study of NO_3^--amended estuarine sediments found that denitrification exhausts oC after

149

1,600 hours of incubation (Abell et al. 2009). Sediments of the sampled lakes have significant denitrification potential and our data suggest that the actual denitrification rate will be constrained primarily by processes that regulate NO_3^- diffusion to denitrifiers.

Sediment nitrification

Nitrification potential was greater in high-deposition lakes than in low- deposition lakes. Thus, chronic N loading appears to have altered the functional performance of the nitrifying community. Nitrification occurs in two steps, the oxidation of NH_4^+ to nitrite and of nitrite to NO_3^-. These steps are mediated by two different groups of microorganisms: NH_4^+-oxidizers and nitrite-oxidizers. Ammonium oxidation is believed to be the rate-limiting step in nitrification (Kowalchuk and Stephen 2001). A positive relationship between abundance of NH_4^+-oxidizers and the potential nitrification rate has been observed in fertilized agricultural fields (Chu et al. 2007, Chu et al. 2008). In lakes, the abundance of $NH4^+$-oxidizers has been found to be positively related to trophic status (Whitby et al. 2001). Our assays measured NO_3^- production from added NH_4^+ and, as a result, did not reveal whether one or both groups or microbes were affected by N deposition. Further work is required to understand how the nitrifying community and *in situ* nitrification rate have been altered by N deposition in lake sediments.

Notably, the potential nitrification rate across all lakes was about 20 times the maximum denitrification rate we measured. The interpretation of this relationship is difficult because the nitrification and denitrification rates are potential rates and not indicative of *in situ* N-cycling rates. The extent to which nitrification and denitrification are coupled in lake sediments is influenced by competition for NH_4^+ with plankton and diffusion of NO_3^- into sediments from the water column (Sundbäck et al. 2004, Seitzinger et al. 2006). For the lakes we sampled, however, it appears that sediment nitrification more than meets potential NO_3^- requirements of sediment denitrifiers. Excess NO_3^- could diffuse from the sediments and possibly explain the accumulation of NO_3^- in surface waters (Finlay et al. 2007), such as we observed in high-deposition lakes.

We do not know the source of NH_4^+ to sediment nitrifiers for our study lakes. In southern Norway, oxidized and reduced forms of N are 56% and 44%, respectively, of N deposition (Tørseth and Semb 1998). Thus, it is possible that considerable NH_4^+ is directly available to sediments as a substrate for nitrification, especially in high-deposition lakes. Another potential source of NH_4+ is the dissimilatory reduction of NO_3^- (DNRA). This pathway is s believed to occur in environments with abundant labile oC relative to NO_3^- (Burgin and Hamilton 2007). There are few studies of DNRA in lakes, but this process would be unlikely in sediments of high-deposition lakes because of elevated background NO_3^-

concentrations. Alternatively, N deposition reduces soil C:N ratios, enhancing microbial N mineralization and potentially the supply of NH_4^+ from the catchment to the lake (Aber et al. 1998, Baron et al. 2000). Nitrifiers are typically poor competitors for NH_4^+ relative to periphyton (Sundbäck and Miles 2000). If needs of autotrophs are met, which would indicate N saturation or growth limitation by another nutrient, then NH_4^+ may be more available for nitrification in sediments. Indeed, this seems possible given the evidence that N deposition has increased the prevalence of phytoplankton nutrient limitation by P (Elser et al. 2009a). Increased access to NH_4^+ could allow populations of NH_4^+-oxidizers to proliferate and explain the potential nitrification rates that we observed.

This study suggests that resource limitation of heterotrophic bacterial processes is not responsive to atmospheric N deposition in either pelagic or profundal environments. While these responses are not strictly comparable for the two habitats, growth and respiration are often tightly linked in bacterial communities (Russell and Cook 1995). The specific mechanisms responsible for our observations need further research. Nitrogen deposition may have altered the composition of the bacterioplankton community and the abundance of nitrifying microorganisms. Elevated nitrification rates are of particular concern because of the potential to exacerbate NO_3^- accumulation in high-deposition lakes.

ACKNOWLEDGEMENTS

LITERATURE CITED

Abell, J., A. M. Laverman, and P. Van Cappellen. 2009. Bioavailability of organic matter in a freshwater estuarine sediment: long-term degradation experiments with and without nitrate supply. Biogeochemistry **94**:13-28.

Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forests; hypotheses revised. Bioscience **48**:921-934.

APHA. 2005. Standard methods for the examination of water and wastewater, 21st edition. American Public Health Association, Washington, D.C.

Baron, J. S., H. M. Rueth, A. M. Wolfe, K. R. Nydick, E. J. Allstott, J. T. Minear, and B. Moraska. 2000. Ecosystem responses to nitrogen deposition in the Colorado Front Range. Ecosystems:352-368.

Bergstrom, A. K. and M. Jansson. 2006. Atmospheric nitrogen deposition has caused nitrogen enrichment and eutrophication of lakes in the northern hemisphere. Global Change Biology **12**:635-643.

Bertoni, R., C. Callieri, E. Balseiro, and B. Modenutti. 2008. Susceptibility of bacterioplankton to nutrient enrichment of oligotrophic and ultraoligotrophic lake waters. Journal of Limnology **67**:120-127.

Bowden, R. D., E. Davidson, K. Savage, C. Arabia, and P. Steudler. 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. Forest Ecology and Management **196**:43-56.

Burgin, A. J. and S. K. Hamilton. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Frontiers in Ecology and the Environment **5**:89-96.

Carlsson, P. and D. A. Caron. 2001. Seasonal variation of phosphorus limitation of bacterial growth in a small lake. Limnology and Oceanography **46**:108-120.

Chrzanowski, T. H., M. Kyle, J. J. Elser, and R. W. Sterner. 1996. Element ratios and growth dynamics of bacteria in an oligotrophic Canadian shield lake. Aquatic Microbial Ecology **11**:119-125.

Chu, H. Y., T. Fujii, S. Morimoto, X. G. Lin, and K. Yagi. 2008. Population size and specific nitrification potential of soil ammonia-oxidizing bacteria under long-term fertilizer management. Soil Biology & Biochemistry **40**:1960-1963.

Chu, H. Y., T. Fujii, S. Morimoto, X. G. Lin, K. Yagi, J. L. Hu, and J. B. Zhang. 2007. Community structure of ammonia-oxidizing bacteria under long-term application of mineral fertilizer and organic manure in a sandy loam soil. Applied and Environmental Microbiology **73**:485-491.

Cleveland, C. C., A. R. Townsend, and S. K. Schmidt. 2002. Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. Ecosystems **5**:680-691.

Cole, J., S. Findlay, and M. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Marine Ecology-Progress Series **43**:1-13.

Colores, G. M., S. K. Schmidt, and M. C. Fisk. 1996. Estimating the biomass of microbial functional groups using rates of growth-related soil respiration. Soil Biology & Biochemistry **28**:1569-1577.

Conley, D. J., H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, K. E. Havens, C. Lancelot, and G. E. Likens. 2009. Controlling Eutrophication: Nitrogen and Phosphorus. Science **323**:1014-1015.

Corstanje, R., K. R. Reddy, and K. M. Portier. 2007. Soil microbial ecophysiology of a wetland recovering from phosphorus eutrophication. Wetlands **27**:1046-1055.

Cotner, J. B. and B. A. Biddanda. 2002. Small players, large role: Microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems **5**:105-121.

Elser, J. J., T. Andersen, J. S. Baron, A. K. Bergstrom, M. Jansson, M. Kyle, K. R. Nydick, L. Steger, and D. O. Hessen. 2009a. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science **326**:835-837.

Elser, J. J., M. Kyle, L. Steger, K. R. Nydick, and J. S. Baron. 2009b. Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. Ecology **90**:3062-3073.

Elser, J. J., A. Peace, M. Kyle, M. Wojewodzic, M. L. McCrackin, T. Andersen, and D. O. Hessen. 2010. Atmospheric nitrogen deposition is associated with elevated phosphorus limitation of lake zooplankton. Ecology Letters **13**:1256-1261.

Elser, J. J., L. B. Stabler, and R. P. Hassett. 1995. Nutrient limitation of bacterial growth and rates of bacterivory in lakes and oceans - a comparative study. Aquatic Microbial Ecology **9**:105-110.

Finlay, J. C., R. W. Sterner, and S. Kumar. 2007. Isotopic evidence for in-lake production of accumulating nitrate in Lake Superior. Ecological Applications **17**:2323-2332.

Fisher, M. M., J. L. Klug, G. Lauster, M. Newton, and E. W. Triplett. 2000. Effects of resource and trophic interactions on freshwater bacterioplankton diversity. Microbial Ecology **40**:125-138.

Gundersen, P., I. K. Schmidt, and K. Raulund-Rasmussen. 2006. Leaching of nitrate from temperate forests - effects of air pollution and forest management. Environmental Reviews **14**:1-57.

Hanson, G. C., P. M. Groffman, and A. J. Gold. 1994. Symptoms of nitrogen saturation in a riparian wetland. Ecological Applications **4**:750-756.

Hart, S. C., J. M. Stark, E. A. Davidson, and M. K. Firestone. 1994. Nitrogen mineralization, immobilization, and nitrification. Methods of Soil Analysis, Part 2, Microbial and Biochemical Properties. Soil Science Socity of America, Madison, WI.

Hessen, D. O., T. Andersen, S. Larsen, B. L. Skjelkvale, and H. A. de Wit. 2009. Nitrogen deposition, catchment productivity, and climate as determinants of lake stoichiometry. Limnology and Oceanography **54**:2520-2528.

Klein, H. and A. Benedictow. 2006. Transboundary air pollution by main pollutants (S, N, O_3) and PM: Norway.*in* Norwegian Meteorological Institute, editor. Programme for Monitoring and Evaluation of the Long-range Transmission of Air Pollutants in Europe.

Kowalchuk, G. A. and J. R. Stephen. 2001. Ammonia-oxidizing bacteria: A model for molecular microbial ecology. Annual Review of Microbiology **55**:485-529.

Lovett, G. M. and H. M. Rueth. 1999. Soil nitrogen transformations in beech and maple stands along a nitrogen deposition gradient Ecological Applications **9**:1330-1344.

Lukkari, K., H. Hartikainen, and M. Leivuori. 2007. Fractionation of sediment phosphorus revisited. I: Fractionation steps and their biogeochemical basis. Limnology and Oceanography: Methods **5**:433-444.

Makino, W. and J. B. Cotner. 2004. Elemental stoichiometry of a heterotrophic bacterial community in a freshwater lake: implications for growth- and resource-dependent variations. Aquatic Microbial Ecology **34**:33-41.

Makino, W., J. B. Cotner, R. W. Sterner, and J. J. Elser. 2003. Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C : N : P stoichiometry. Functional Ecology **17**:121-130.

McCrackin, M. L. and J. J. Elser. 2010. Atmospheric nitrogen deposition alters denitrification and nitrous oxide production in lake sediments. Ecology **91**:528-539.

Russell, J. B. and G. M. Cook. 1995. Energetics of bacterial-growth: balance of anabolic and catabolic reactions. Microbiological Reviews **59**:48-62.

Seitzinger, S. P., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, and C. Tobias. 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecological Applications **16**:2064-2090.

Simon, M., B. C. Cho, and F. Azam. 1992. Significance of bacterial biomass in lakes and the ocean: comparison to phytoplankton biomass and biogeochemical implications. Marine Ecology-Progress Series **86**:103-110.

Sterner, R. W. and J. J. Elser. 2002. Ecological Stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton, NJ, USA.

Strauss, E. A., N. L. Mitchell, and G. A. Lamberti. 2002. Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH. Canadian Journal of Fisheries and Aquatic Sciences **59**:554-563.

Sundbäck, K., F. Linares, F. Larson, and A. Wulff. 2004. Benthic nitrogen fluxes along a depth gradient in a microtidal fjord: The role of denitrification and microphytobenthos. Limnology and Oceanography **49**:1095-1107.

Sundbäck, K. and A. Miles. 2000. Balance between nitrification and microalgal incorporation of nitrogen in microtidal sediments, NE Kattegat. Aquatic Microbial Ecology **22**:291-300.

Taylor, P. G. and A. R. Townsend. 2010. Stoichiometric control of organic carbon-nitrate relationships from soils to the sea. Nature **464**:1178-1181.

Tezuka, Y. 1990. Bacterial regeneration of ammonium and phosphorus as affected by the carbon : nitrogen : phosphorus ratio of organic substrate. Microbial Ecology **19**:227-238.

Tørseth, K. and A. Semb. 1998. Deposition of nitrogen and other major inorganic compounds in Norway, 1992-1996. Environmental Pollution **102**:299-304.

Tranvik, L. J. 1997. Rapid fluorometric assay of bacterial density in lake water and seawater. Limnology and Oceanography **42**:1629-1634.

Wallenstein, M. D., S. McNulty, I. J. Fernandez, J. Boggs, and W. H. Schlesinger. 2006. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. Forest Ecology and Management **222**:459-468.

Wetzel, R. G. 2001. Limnology: Lake and River Ecosystems. Academic Press, San Diego, CA, USA.

Weyhenmeyer, G. A. and E. Jeppesen. 2009. Nitrogen deposition induced changes in DOC:NO_3-N ratios determine the efficiency of nitrate removal from freshwaters. Global Change Biology:doi: 10.1111/j.1365-2486.2009.02100.x.

Whitby, C. B., J. R. Saunders, R. W. Pickup, and A. J. McCarthy. 2001. A comparison of ammonia-oxidiser populations in eutrophic and oligotrophic basins of a large freshwater lake. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology **79**:179-188.

Wolfe, A. P., C. A. Cooke, and W. O. Hobbs. 2006. Are current rates of atmospheric nitrogen deposition influencing lakes in the eastern Canadian arctic? Arctic, Antarctic, and Alpine Research **38**:465-476.

Yoshinari, T. and R. Knowles. 1976. Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochemical and Biophysical Research Communications **69**:705-710.

Table 13. Average values (and standard error, s.e.) for various water parameters for the bacterioplankton study.

High-deposition Lakes

					Surface Water		
Sample Date	Lake	pH	Chl µg/L	DOC µmol/L	DOC:NO_3^- (molar)	TDN µmol/L	TDP µmol/L
7/28/07	Berse	6.3	3.8	164	11.3	18.0	0.09
7/29/07	Brynesland	6.7	4.6	165	3.6	43.7	0.13
8/1/07	Eigevatnet	6.4	6.1	113	3.2	32.6	0.08
7/22/07	Eptelandsvatnet	5.5	1.3	105	7.7	15.4	0.08
8/1/07	Glypstadvatnet	6.0	2.2	90	2.7	43.1	2.17
6/28/07	Holmavatnet	5.5	1.1	67	5.2	14.0	0.03
7/26/07	Mossingtjorna	6.0	0.0	329	11.8	32.1	0.18
6/25/07	Nordravatnet	6.5	1.9	85	5.0	16.9	0.09
6/21/07	Revsvatnet	6.6	2.6	159	14.1	32.2	0.15
7/25/07	Saglandsvatnet	6.7	1.5	73	1.9	34.8	0.16
6/19/07	Skineldvatnet	5.2	0.9	101	13.0	10.3	0.06
7/28/07	Snosvatnet	5.1	0.7	79	5.2	16.3	0.06
Mean		6.0	2.2	127	7.1	25.8	0.27
s.e.		0.1	0.4	16.6	1.0	2.7	0.14

161

Table 13, continued. Results of statistical test indicate if there is a significant difference between deposition regions.

Nonsignificant results indicated by n.s.

Low-deposition Lakes

					Surface Water		
Sample Date	Lake	pH	Chl µg/L	DOC µmol/L	DOC:NO$_3^-$ (molar)	TDN µmol/L	TDP µmol/L
8/12/07	Atnasjoen	6.4	8.0	127.0	114	4.2	0.07
8/13/07	Bolvatnet	6.3	3.6	421.4	10,534	7.9	0.19
8/14/07	Flaksjoen	6.8	5.9	289.2	7,231	7.4	0.13
7/11/07	Goppollvatnet	6.8	2.8	147.2	3,680	5.2	0.13
8/8/07	Hamntjonna	6.2	2.7	210.5	5,262	5.5	0.28
8/17/07	Langrumpa	7.0	3.7	447.7	11,193	8.1	0.20
8/8/07	Ljosvatnet	6.9	2.3	151.5	3,788	4.2	0.16
8/9/07	Musvoltjonna	6.5	2.6	387.7	9,692	7.5	0.16
8/18/07	Muvatnet	7.5	3.6	207.9	5,196	5.9	0.13
7/8/07	Ner-Åst	6.7	4.7	332.5	8,312	7.1	0.18
7/10/07	Nevelvatnet	6.5	10.3	218.6	5,466	5.5	0.16
7/8/07	Vasjoen	6.7	18.7	373.3	9,331	8.7	0.20
Mean		6.7	5.7	276.2	6,650	6.4	0.17
s.e.		0.1	1.1	26.8	780	0.4	0.01
high vs low deposition		high < low	high < low	high < low	high < low	high > low	high = low
P		< 0.004	< 0.004	< 0.0006	0.006	< 0.0001	n.s.

162

Table 14. Average values (and standard error, s.e.) for various water and sediment parameters for the sediment bacteria study.

High-deposition Lakes			Hypolimnetic Water		Sediment			
Sample Date	Region	Lake	DOC µmol/L	NO$_3^-$ µmol/L	Total C mmol g^{-1}	Total N mmol g^{-1}	Total P mmol g^{-1}	Sediment OM
7/5/2009	Egersund	Brynesland	129.7	18.8	8.7	0.5	0.17	0.17
6/12/2009	Egersund	Helleren	107.4	10.6	12.2	0.6	0.18	0.22
6/12/2009	Egersund	Holmavatnet	90.1	7.9	16.3	0.7	0.06	0.21
6/23/2009	Egersund	Krokavatnet Indre	100.2	9.7	15.3	0.8	0.10	0.21
7/4/2009	Egersund	Kydlandsvtn Ytre	99.8	10.0	11.2	0.6	0.04	0.19
7/4/2009	Egersund	Kydlandsvtn	122.6	21.2	9.9	0.5	0.16	0.17
6/12/2009	Egersund	Kydlandsvtn	46.7	6.3	12.6	1.0	0.21	0.15
7/5/2009	Egersund	N. Furevnt	189.1	6.4	11.8	0.6	0.12	0.20
7/4/2009	Egersund	Netlandsvtn	128.6	10.3	11.1	0.6	0.05	0.18
7/3/2009	Egersund	Nordravatnet	122.4	9.1	10.0	0.4	0.08	0.19
6/13/2009	Egersund	Revsvatnet	226.6	9.7	17.8	0.7	0.12	0.23
6/13/2009	Egersund	Saglandsvavtn	131.5	26.4	5.6	0.3	0.06	0.12
7/3/2009	Egersund	Skjævelandvtn	179.9	9.1	11.2	0.6	0.17	0.20
6/12/2009	Egersund	Ulsvatnet	94.8	3.8	14.5	0.7	0.11	0.21
Mean			126.4	11.4	12.0	0.6	0.12	0.19
s.e.			12.7	1.7	0.9	0.0	0.02	0.01

Table 14, continued. Non-significant differences between regions indicated by n.s.

Low-deposition Lakes			Hypolimnetic Water		Sediment			
Sample Date	Region	Lake	DOC µmol/L	NO$_3^-$ µmol/L	Total C mmol g^{-1}	Total N mmol g^{-1}	Total P mmol g^{-1}	Sediment OM
7/16/2010	Atna	Åsdalstjørna	131.5	1.5	27.5	0.9	0.12	0.49
7/14/2009	Atna	Bolvatnet	70.8	0.6	11.1	0.6	0.10	0.20
6/24/2009	Lillehammer	Djupen	215.7	0.6	8.2	0.3	0.16	0.16
7/14/2009	Atna	Flaksjøen	108.3	1.0	10.8	0.6	0.12	0.15
7/15/2009	Atna	Hamntjønna	172.5	0.6	8.1	0.5	0.15	0.17
6/23/2009	Lillehammer	Hornsjoen	144.4	0.7	14.0	0.9	0.09	0.18
7/14/2009	Atna	Langrumpa	159.3	1.3	10.9	0.6	0.02	0.19
7/13/2009	Atna	Langjtønna	98.7	0.8	4.1	0.4	0.03	0.10
6/23/2009	Lillehammer	Mellsjoen	149.1	0.5	16.8	0.7	0.01	0.22
7/15/2009	Atna	Muvatnet	126.9	0.4	8.7	0.4	0.04	0.14
6/24/2009	Lillehammer	Ner-Åst	277.0	0.4	14.2	0.8	0.24	0.13
6/22/2009	Lillehammer	Nevelvatnet	159.4	1.4	8.0	0.6	0.15	0.14
6/13/2009	Lillehammer	Reinsvatnet	189.6	0.7	4.7	0.5	0.10	0.12
7/15/2009	Atna	Settningen	191.6	0.4	3.1	0.2	0.01	0.06
Mean			156.8	0.8	10.7	0.6	0.10	0.17
s.e.			14.5	0.1	1.7	0.1	0.02	0.03
high vs. low deposition			high = low	high > low <	high = low	high = low	high = low	high = low
P			n.s.	0.0001	n.s.	n.s.	n.s.	n.s.

164

Table 15. Responses of bacterial DNA concentrations to experimental

resource enrichment for the study lakes.

Lake	ANOVA P value	Result
High-deposition Lakes		
Berse	0.0145	Antagonistic
Brynesland	0.0066	Sequential co-limitation (P)
Eigevatnet	0.0001	Constrained
Eptelandsvatnet	0.0001	Synergistic co-limitation
Glypstadvatnet	0.1843	No limitation
Holmavatnet	0.1558	No limitation
Mossingtjorna	0.0001	Synergistic co-limitation
Nordravatnet	0.0452	Antagonistic
Revsvatnet	0.0006	Synergistic co-limitation
Saglandsvatnet	0.0002	Strict co-limitation (oCNP)
Skineldvatnet	0.0001	Synergistic co-limitation
Snosvatnet	0.0001	Sequential co-limitation (P)

Lake	ANOVA P value	Result
Low-deposition Lakes		
Atnasjoen	0.0001	Sequential co-limitation (P)
Bolvatnet	0.001	Constrained
Flaksjoen	0.0001	Synergistic co-limitation
Goppollvatnet	0.0004	Strict co-limitation (oCNP)
Hamntjonna	0.0003	Sequential co-limitation (P)
Langrumpa	0.0001	Constrained
Ljosvatnet	0.1253	No limitation
Musvoltjonna	0.0002	Single limitation (oC)
Muvatnet	0.001	Antagonistic
Ner-Åst	0.0005	Strict co-limitation (oCNP)
Nevelvatnet	0.0001	Synergistic co-limitation
Vasjoen	0.0001	Synergistic co-limitation

Table 16. Sediment responses to experimental resource enrichment for the study lakes.

Lake	CO$_2$ sediment flux		N$_2$O sediment flux	
	ANOVA P value	Result	ANOVA P value	Result
High-deposition Lakes				
Brynesland	0.0001	Sequential co-limitation (oC)	0.0001	Sequential co-limitation (N)
Helleren	0.0008	Constrained	0.0001	Sequential co-limitation (N)
Holmavatnet	0.2486	No resource limitation	0.0001	Single limitation (N)
Krokavatnet Indre	0.0077	Single limitation (oC)	0.0001	Single limitation (N)
Kydlandsvatnet Ytre	0.0001	Constrained	0.0001	Sequential co-limitation (N)
Kydlandsvatnet	0.0001	Single limitation (oC)	0.0001	Single limitation (N)
Kydlandsvatnet Nordra	0.7449	No resource limitation	0.0001	Sequential co-limitation (N)
Furevatnet	0.0001	Single limitation (oC)	0.0001	Sequential co-limitation (N)
Netlandsvatnet	0.0001	Constrained	0.0001	Single limitation (N)
Nordravatnet	0.0001	Single limitation (oC)	0.0001	Sequential co-limitation (N)
Revsvatnet	0.0118	Constrained	0.0004	Antagonistic
Saglandsvatnet	0.0001	Sequential co-limitation (oC)	0.0001	Sequential co-limitation (N)
Skjævelandvatnet	0.0002	Sequential co-limitation (oC)	0.0001	Sequential co-limitation (N)
Ulsvatnet	0.0001	Single limitation (oC)	0.0001	Sequential co-limitation (N)

166

Table 16, continued.

	CO$_2$ sediment flux		N$_2$O sediment flux	
Lake	ANOVA P value	Result	ANOVA P value	Result
Low-deposition Lakes				
Åsdalstjørna	0.2501	No resource limitation	0.0717	No resource limitation
Bolvatnet	0.0009	Single limitation (oC)	0.0001	Sequential co-limitation (N)
Djupen	0.0001	Single limitation (oC)	0.0001	Single limitation (N)
Flaksjøen	0.2295	No resource limitation	0.0001	Sequential co-limitation (N)
Hamntjønna	0.0874	No resource limitation	0.0245	Strict co-limitation (oCNP)
Hornsjoen	0.1129	No resource limitation	0.0001	Single limitation (N)
Langrumpa	0.0001	Single limitation (oC)	0.0001	Single limitation (N)
Langjtønna	0.0001	Antagonistic	0.0015	Sequential co-limitation (N)
Mellsjoen	0.0094	Antagonistic	0.0001	Constrained
Ner-Åst	0.0001	Antagonistic	0.0001	Single limitation (N)
Nevelvatnet	0.007	Strict co-limitation (oCNP)	0.0001	Single limitation (N)
Reinsvatnet	0.0001	Constrained	0.0001	Single limitation (N)
Settningen	0.4873	No resource limitation	0.0073	Single limitation (N)

167
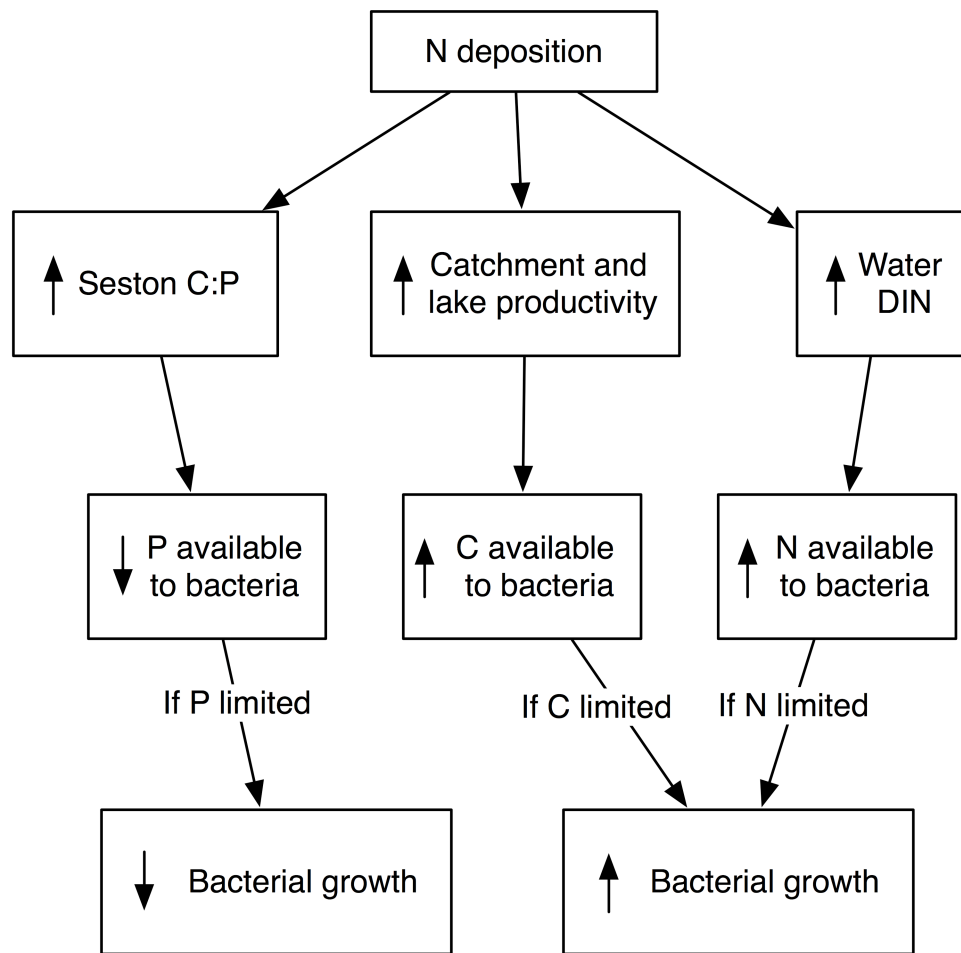
Figure 12. Potential effects of atmospheric N deposition on bacterial

nutrient limitation (after Treseder 2008).
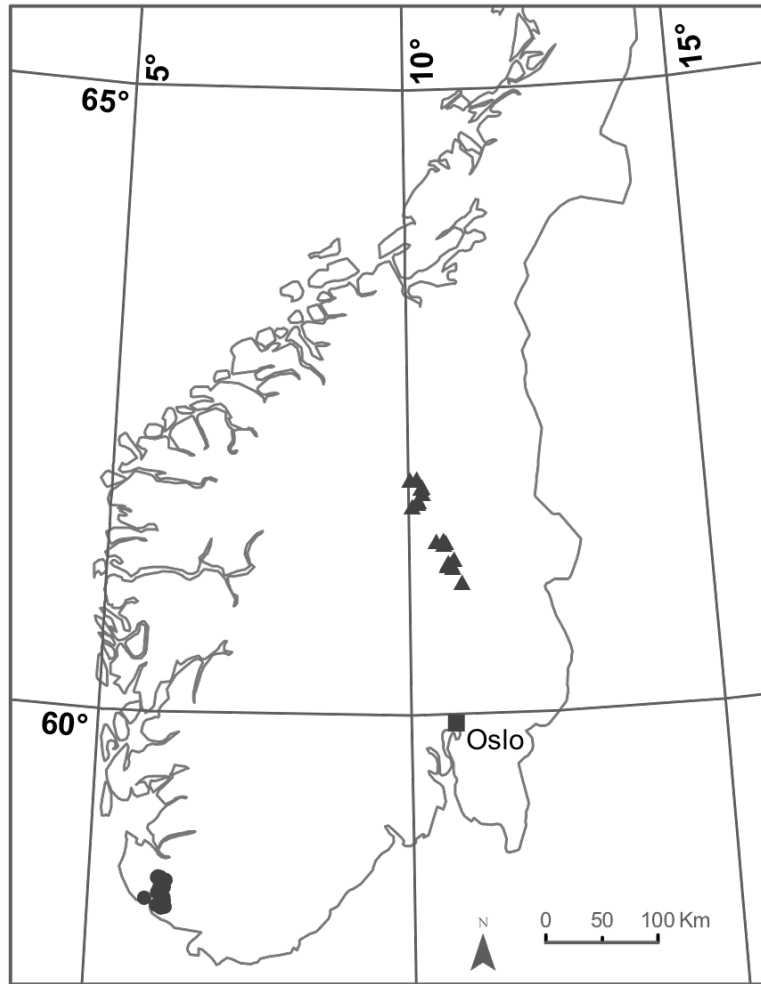
Figure 13. Location of study sites in Norway. Fieldwork was conducted in two regions that encompass a gradient of atmospheric nitrogen deposition. Nitrogen deposition is greatest in extreme southwestern Norway, with rates of 10-20 kg N ha^{-1} y^{-1}, and decreases to less than 4 kg N ha^{-1} y^{-1} in northeastern areas (Hole and Tørseth 2002). Circles denote lakes sampled in high deposition areas and lakes sampled in low deposition areas are denoted by triangles.

169
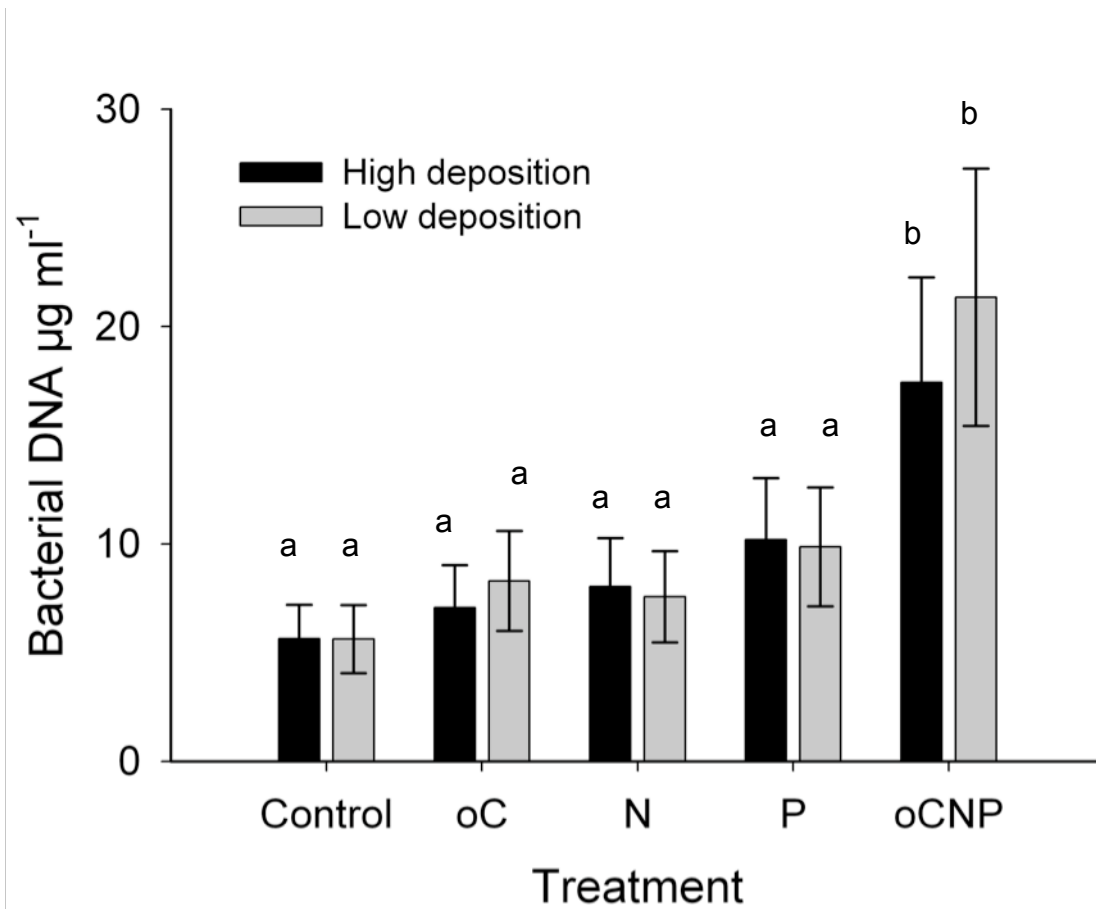
Figure 14: Bacterial DNA responses to resource enrichment experiment.

Bars are means ($\pm$ SE) for N = 10 lakes in each deposition region. Letters

above bars indicate significant differences (P < 0.05).

Figure 15. Sediment (A) CO_2 and (B) N_2O fluxes in response to experimental resource enrichment. Bars are means ($\pm$ SE) for lakes in each deposition region. Letters above bars indicate significant differences ($P < 0.05$).

Figure 16. Comparison of nitrification potential between regions. Bars are means ($\pm$ SE) for lakes in each deposition region. Letters above bars indicate significant differences ($P < 0.05$).

CHAPTER 6

Greenhouse Gas Dynamics In Lakes Receiving
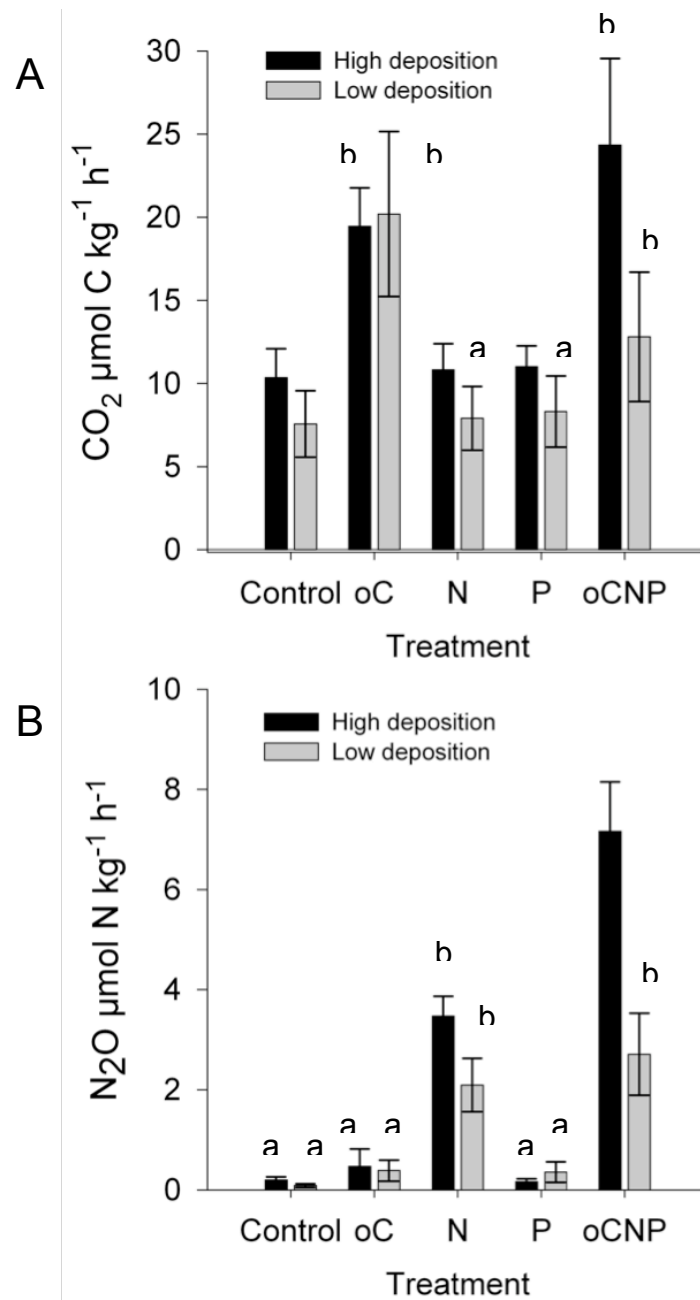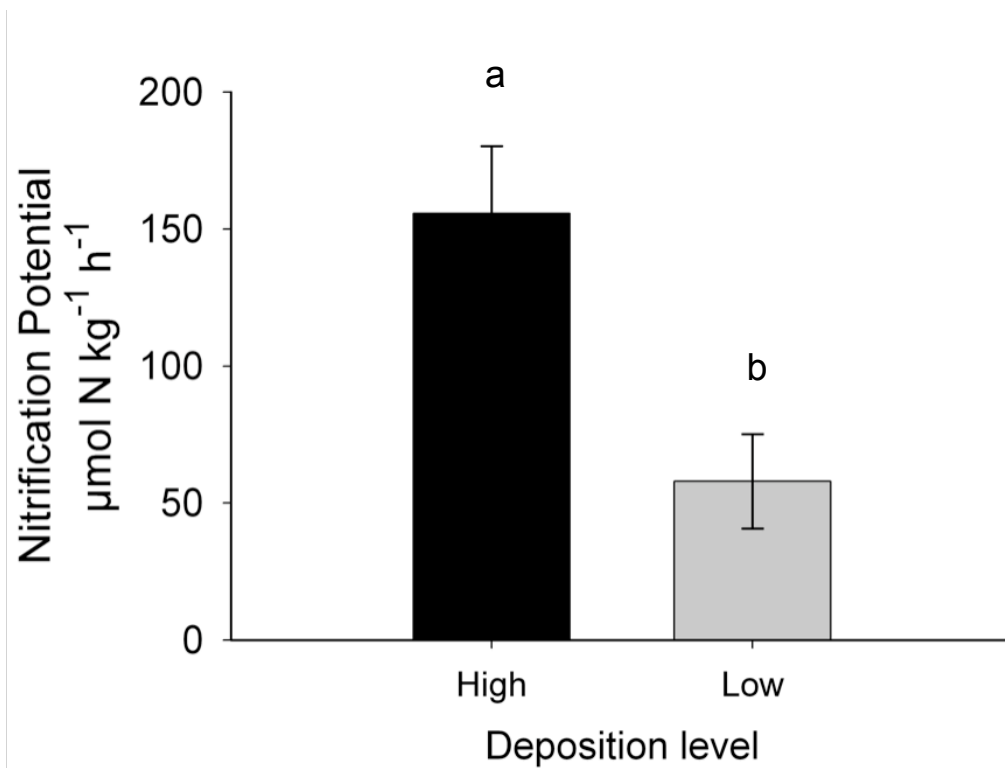
Atmospheric Nitrogen Deposition (Colorado, USA)

ABSTRACT

Anthropogenic nitrogen (N) inputs have been found to influence emissions of greenhouse gases from a variety of ecosystems; however, the effects of N loading on greenhouse gas dynamics in lakes are not well documented. We measured concentrations of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) in 26 lakes in the Colorado Rocky Mountains (USA) receiving elevated (>6 kg N ha^{-1} yr^{-1}) or low (<2 kg N ha^{-1} y^{-1}) levels of atmospheric N deposition. The mean CO_2 concentration in surface waters was 26.9 μmol L^{-1} and did not differ between deposition regions. The CH_4 concentration was greater in low-deposition lakes (167 nmol L^{-1}) compared to high-deposition lakes (48 nmol L^{-1}), while the opposite was true for N_2O. The concentration of N_2O in surface water averaged 29 nmol L^{-1} in high-deposition lakes compared to 22 nmol L^{-1} in low-deposition lakes. Nitrous oxide is of particular interest because it is more potent than CO_2 as a greenhouse gas and because of its role in the destruction of stratospheric ozone. To understand the potential magnitude of lake N_2O production related to atmospheric N deposition, we applied the methodology of Intergovernmental Panel on Climate Change to available datasets. We estimated global N_2O emissions from lakes to be

7–371 Gg N y^{-1} for 1993, increasing to 11–705 Gg N y^{-1} in 2050. The 1993 estimates represent 0.5–25% of emissions from rivers and estuaries, suggesting that further research is required to better quantify emission rates from lentic ecosystems.

INTRODUCTION

Lakes play an important role in the regulation of climate though the exchange of heat, water, carbon (C), and greenhouse gases with the surrounding terrestrial ecosystem and atmosphere (Williamson et al. 2009). Both CO_2 and CH_4 fluxes result from microbial mineralization of organic matter imported from the watershed or produced in the lake. Nitrous oxide is produced as an intermediate product of denitrification, the microbial reduction of nitrate (NO_3^-) to N_2 gas, and of nitrification, the microbial oxidation of ammonium to NO_3^- (Knowles 1982, Wrage et al. 2001). Per molecule, CH_4 and N_2O are 25 and 298 times more potent, respectively, than CO_2 in terms of global warming potential (Forster et al. 2007). Previous work has shown that production of greenhouse gases has been enhanced by N loading in a variety of ecosystems including forests, grasslands, agricultural fields and streams (Beaulieu et al. 2008, Liu and Greaver 2009), but data for lentic ecosystems are lacking.

Elevated N nitrogen inputs may accelerate rates of C cycling and thus result in increased emissions of CO_2 and CH_4 from lakes (Tranvik et al. 2009). Nitrogen loading also increases microbial N_2O production

174

through enhanced nitrification and denitrification in soils and sediments (Seitzinger and Nixon 1985, Ullah and Zinati 2006). Indeed, ninety percent of N_2O production by rivers and estuaries (1.5 Tg N y^{-1}) is estimated to be derived from anthropogenic N loading (Kroeze et al. 2005). The current atmospheric concentration of N_2O is 319 ppb and is increasing 0.3% per year due to human activities (Forster et al. 2007, Nevison et al. 2007). Increases in the production of N_2O are of particular concern because, in addition to being a potent greenhouse gas, N_2O is currently considered the single most important ozone-depleting substance (Ravishankara et al. 2009).

As fertilizer runs off from agricultural soils and is transported through groundwater, streams, and rivers to the oceans, a fraction of N leaves the ecosystems as N_2O. Such emissions are considered indirect, and under methodology of the Intergovernmental Panel on Climate Change (IPCC), are estimated using factors that are applied to N loading rates. There are separate emission factors for groundwater, river, and estuarine ecosystems based on the ratio of N_2O to NO_3^- in water (IPCC 2006). Nitrous oxide emissions from lakes are not included in greenhouse gas inventories, even with respect to indirect emissions relating to agriculture. Further, global N_2O emissions from lakes have not been quantified even though lakes cover the same surface area as rivers and have the potential for substantial N_2O production due to their longer water

175

residence times. The few studies that have investigated N_2O dynamics in lakes have suggested that lakes are not significant sources of N_2O (Mengis et al. 1997, Huttunen et al. 2003). Consequently, the rate of N_2O emission from lakes is poorly constrained.

Here we report on concentrations of CO_2, CH_4, and N_2O in alpine and subalpine lakes in the Colorado Rocky Mountains receiving elevated or low rates of atmospheric N deposition. We also measured production of these gases by sediments from these lakes. Atmospheric deposition is the dominant source of N to oligotrophic high-elevation lakes that are otherwise not subject to human perturbations, such as land use change, wastewater, and direct runoff from agricultural fields (Burns 2004). Catchments of lakes receiving chronic N deposition have been diagnosed as showing signs of advanced N saturation, meaning that available N exceeds biological demand (Burns 2004). Hence, we expected a positive relationship between the N deposition rate and concentrations of dissolved greenhouse gases and sediment gas fluxes because of increased microbial activity. Such increased microbial activity would include enhanced respiration, nitrification, denitrification, and methanogenesis. Heterotrophic microorganisms could be fueled by dissolved organic carbon (DOC) as an energy source because N deposition can increase concentrations of DOC in lake water through increased primary production

in N-deficient catchments and lakes (Tranvik et al. 2009, Weyhenmeyer and Jeppesen 2009).

METHODS

Study site

We sampled 26 lakes located in the Rocky Mountains of Colorado, USA between June and August 2008 (Fig. 17). Selected lakes were within 6 km of a trailhead. The eastern slopes of the Rocky Mountains near Niwot Ridge Long Term Ecological Research site (NWT) and the Loch Vale Watershed Research site (LVW) receive atmospheric N deposition from fossil fuel combustion and agricultural sources (Nanus et al. 2003, Burns 2004). The rate of inorganic N (NO_3^- + NH_4^+) deposition has increased over the past 20 years to 6-8 kg ha^{-1} y^{-1} (Fig. 17; data from the National Atmospheric Deposition Program, nadp.sws.uiuc.edu). Lakes in central and western Colorado near the Rocky Mountain Biological Laboratory (RMBL, Gothic, CO) and the Mountain Studies Institute (MSI, Silverton, CO) receive <2 kg ha^{-1} y^{-1} atmospheric inputs of N. Lakes near NWT and LVW are considered to be in the high-deposition region and lakes near RMBL and MSI are considered to be in the low-deposition region.

Mean annual temperatures at high elevations are less than 2°C and the lakes are generally ice-covered between November and June (Baron et al. 2000). Englemann spruce and subalpine fir forests are found below

177

tree line (~ 3,300 m) and alpine tundra is found above tree line. With the

exception of Estes, the sampled lakes were small, generally ~ 0.1 km^2 and

the watersheds were unpopulated. The watersheds occupy geologically

diverse bedrock (Kent and Porter 1980). Precambrian-age granite, gneiss,

and schist dominate the underlying geology, in the vicinity of Rocky

Mountain National Park. Near RMBL the geologic parent materials include

Mesozoic sedimentary rocks and Paleozoic metamorphics and intrusives.

Surrounding the MSI, the San Juan Mountains are characterized by

extensive volcanic deposits and felsic gneisses and granites of the

Uncompahgre formation. Other studies performed simultaneously in these

areas have found that concentrations of particulate and dissolved N and

phytoplankton nutrient limitation in these lakes are significantly influenced

by the level of atmospheric N deposition (Elser et al. 2009a, Elser et al.

2009b)

Field sampling and laboratory procedures

Thirteen lakes in each N deposition region were visited once during

summer 2008 (Table 17). Fieldwork was staggered so that sampling of

lakes in high- and low-deposition regions was not strongly skewed by

date. High-deposition lakes were sampled in late June and late July 2008

and low-deposition lakes were sampled in early July and early August

2008.

Lakes were sampled from an inflatable boat. The depth of each lake was measured using a portable echo sounder. Water samples were collected from just above the sediments using a battery-powered submersible pump fitted with tubing to take in water at 0.5 – 10 m. Water samples were filtered with Pall A/E glass fiber filters (Pall Corporation, Port Washington, NY, USA) and frozen until analysis for DOC on a Shimadzu TOC 5000. Nitrate plus nitrite (hereafter: NO_3^-) concentration was measured on a Lachat Quick Chem 8000 Autoanalyzer. Data on DOC and NO_3^- concentrations for a subset of lakes were previously reported by McCrackin and Elser (In revision).

Surface sediments were collected using a LaMotte dredge from a water depth of approximately 10 m or at the maximum lake depth if the lake was < 10 m. The dredge collected sediments from an area of 221 cm^2 to a depth of ~7 cm. We were not able to collect sediment from all lakes because rocks, debris, or macrophytes prevented the dredge from operating properly (Table 17). Sediments were returned to the laboratory and processed within 24 hours of collection. For each lake, three analytical replicate 100-g subsamples of homogenized sediments were slurried with 80-mL of lake water collected from just above the sediments. Bottles were purged of oxygen with nitrogen gas (N_2). After vigorous shaking, we collected 10-mL samples from the headspace volume (about 550 mL) at the beginning of the incubations (0 h) and at 4 h. Incubations

were conducted at 4°C in the dark. Gas samples were analyzed for CO_2, CH_4, and N_2O on a Varian CP-3800 gas chromatograph (Agilent Technologies, Santa Clara, CA, US) equipped with an electron capture detector, a thermal conductivity detector, and a flame ionization detector. Sediment flux rate was determined as the accumulation of each gas over the incubation period expressed in terms of dry sediment mass that was converted to an areal basis using the sediment bulk density for each lake (Garcia-Ruiz et al. 1998, Rudaz et al. 1999). Background denitrification rates were measured using the acetylene inhibition method and reported separately (McCrackin and Elser In revision).

For measurement of dissolved trace gasses in the water column, 750-mL glass serum bottles were filled using a battery-powered submersible pump fitted with tubing to take in water just below the surface to above the sediment at 10 m depth, or the maximum lake depth if less than 10 m. The temperature and dissolved oxygen content of the water at the sampling depth was measured with a YSI model 85 temperature-oxygen probe (YSI, Yellow Springs, Ohio, USA). At least one bottle volume was allowed to overflow prior to introducing a 60-mL headspace of ambient air. Bottles were sealed with a screw cap fitted with gray-butyl stopper and shaken vigorously for 1 minute (Mengis et al. 1997, Cole and Caraco 1998). Immediately after shaking, a 20-mL gas sample was collected from the headspace with a syringe and injected into a 10-mL

serum vial. Three replicates were collected at each sampling depth per lake. Prior to the injection of the environmental sample the serum vials were flushed with helium, sealed with gray butyl stopper and aluminum crimp top, and evacuated. Gas samples were analyzed for CO_2, CH_4, and N_2O on the Varian CP-3800 gas chromatograph as previously discussed.

The percent saturation of CO_2, CH_4, and N_2O in water samples was calculated as % saturation = ($[gas]_{measured}$ /$[gas]_{saturated}$) * 100, where $[gas]_{measured}$ is the measured concentration of each gas adjusted for the introduction of trace gases in the ambient air headspace during equilibration, where $[gas]_{saturated}$ is the saturated concentration of each gas reflecting the atmospheric concentration of each gas and the solubility constant of each gas at the measured water temperature (Weiss 1974, Weiss and Price 1980, Wiesenburg and Guinasso 2002).

Sediment water content was determined as mass loss after drying subsamples at 105°C for 48 h and organic matter (OM) content was determined as mass loss on ignition at 550°C for 4 h. Total C and N content of dried sediments were measured with a PerkinElmer CHN elemental analyzer (PerkinElmer, Inc., Waltham, MA, USA). Total phosphorus (P) content of combusted sediment was measured colorimetrically following extraction with 0.5 M hydrochloric acid using the acid molybdate technique (Lukkari et al. 2007). Results of sediment

analyses have been reported separately and are not included here in detail (McCrackin and Elser In revision).

Statistical analysis

We performed t tests to compare sediment CO_2 and CH_4 fluxes, concentrations of dissolved NO_3^-, DOC, CO_2, CH_4, and N_2O between high- and low-deposition regions. We used the Wilcoxon signed-rank test to compare concentrations of dissolved CO_2, CH_4, and N_2O between the lake surface and above the sediment. Multiple-linear regression was used to identify relationships among predictor variables and gas flux rates from slurry incubations and surface water concentrations of dissolved gases. For sediment fluxes, predictor variables included concentrations of NO_3^- and DOC, sediment OM, sediment C, N, and P content, and ratios of sediment C:N, C:P, and N:P. Predictor variables for dissolved CO_2, CH_4, and N_2O in surface water were DOC and NO_3^- concentrations, and lake elevation as a proxy for climate (e.g. annual temperatures, length of winter). Models were selected by considering all subsets on the basis of adjusted R^2 and AIC. We evaluated multi-colinearity and selected final models for which tolerance values were >0.5 for all predictor variables. When necessary, response and predictor variables were transformed to improve normality. All statistical tests were performed using JMP (SAS Institute, Inc., Version 8.0.1) with $\alpha = 0.05$.

RESULTS

Greenhouse gases in lake water

The mean concentrations of NO_3^- and dissolved N_2O were significantly greater in high-deposition lakes relative to low-deposition lakes (Tables 17 and 18). In contrast, average concentrations of DOC, CO_2, and CH_4 were greater in low deposition lakes than high-deposition lakes. The surface water concentration of CO_2 was positively related to concentrations of DOC and NO_3^- and CH_4 was positively related to DOC but negatively related to NO_3^- (Table 19). The concentration of N_2O was positively related to NO_3^- (Fig. 18). There was no correlation between sampling dates and measured concentrations of DOC and dissolved trace gases ($R^2 < 0.04$, $P > 0.3$) and minimal correlation for NO_3^- ($R^2 = 0.15$, $P = 0.05$). Across all lakes, mean concentrations of dissolved CO_2 and CH_4 were greater in water just above the sediment compared to surface water ($P < 0.001$), while mean concentrations of dissolved N_2O did not differ between depths ($P > 0.05$). The surface waters of lakes were supersaturated with CO_2, CH_4, and N_2O relative to the atmosphere. For CO_2, the degree of saturation did not differ between deposition regions ($P > 0.05$). Across all lakes, the mean CO_2 saturation was 190% (SE $\pm$ 14%). The mean saturation of CH_4 in surface water was 1,930% (SE $\pm$ 100%) and 8,280% (SE $\pm$ 2,630%) in high- and low-deposition lakes, respectively, a significant difference ($P = 0.01$). The mean saturation of

183

N_2O was greater in high-deposition lakes compared to low-deposition

lakes (P = 0.02), with values of 156% (SE $\pm$ 5%) and 138% (SE $\pm$ 5%),

respectively. Lakes in the low-deposition region were generally thermally

stratified whereas lakes in the high-deposition region were not. All lakes

were oxic at the depth where sediments were collected.

Sediment greenhouse gas production

Sediment characteristics were reported previously in detail

(McCrackin and Elser In revision). Briefly, there were no significant

differences in sediment OM, total C, N, and P content, or ratios of C:N,

C:P, and N:P between lakes in high- and low-deposition regions (P >

0.05). Sediment C, N, and P contents averaged 8.2 mmol g^{-1}, 0.6 mmol g^{-1}, and 0.1 mmol g^{-1}, respectively across all lakes.

Sediment production of CO_2 under anoxic conditions averaged 0.16

mmol C m^{-2} d^{-1} and 0.32 mmol C m^{-2} d^{-1} for high- and low-deposition

lakes, respectively, a significant difference (Table 20). Methane fluxes

were greater in sediments from low deposition lakes, averaging 0.8 μmol

C m^{-2} h^{-1}, compared to high-deposition lakes, which averaged 2.2 μmol C

m^{-2} h^{-1}. Across all lakes, CO_2 and CH_4 sediment fluxes were negatively

related to NO_3^- concentrations (Table 19). Nitrous oxide production in

sediments was only observed for four of the sampled lakes and averaged

2.3 ($\pm$ 1.6 SE) μmol N m^{-2} h^{-1} for these lakes. The sediment N_2O flux did

not differ between deposition regions was not predicted by any of the identified variables.

DISCUSSION

Carbon mineralization in sediments

Contrary to our expectations, we found no evidence that atmospheric N deposition has enhanced C cycling rates in lake sediments. Rather, sediment CO_2 production was greater in low-deposition lakes compared to high-deposition lakes. Across all lakes, C released by sediment CO_2 production was the two orders of magnitude greater than that released through CH_4 production. Further, there was no difference in sediment OM and C content of bulk sediments between regions, but the concentration of DOC was greater in low deposition lakes compared to high-deposition lakes. The higher concentration of DOC in low N deposition lakes is consistent with greater sediment C mineralization rates in the low N deposition region compared to the high-deposition region. However, we expected the opposite result because of the fertilization effect that N deposition could have on primary production in the catchments and water column. Indeed, previous fieldwork at many of the same lakes found that concentrations of chlorophyll and seston C were significantly greater in high- deposition lakes (Elser et al. 2009a). Such differences between studies could reflect seasonal or year-to-year differences in catchment or lake productivity. It is also possible that growth

of heterotrophic bacterioplankton in low deposition lakes are N limited, allowing for DOC to accumulate in the water column (Taylor and Townsend 2010). Lastly, at current rates of atmospheric N loading, catchment-specific properties for the sampled lakes might have a greater influence on lake DOC concentrations than does N deposition, as has been observed in other regions (Hessen et al. 2009).

Methane may represent 20-60% of total C mineralization in lake sediments (Bastviken et al. 2008). In our sediment slurry incubations, however, CH_4 production represented only an average of 7% of total C mineralization. In fact, CH_4 production by sediments for the sampled lakes was two orders of magnitude less than that reported for a eutrophic lake and at the low end of that reported for boreal lakes (Liikanen et al. 2002, Algesten et al. 2005, Bastviken et al. 2008). The low rate of CH_4 production we observed in sediment could be partially explained by temperature, as we conducted our incubations at 4°C, which is colder than temperatures for other studies. Methane production in peat soils is strongly sensitive to temperature, with Q_{10} values of 5.3 to 16 (Dunfield et al. 1993) and similar results could occur in sediments. It is also possible that our slurry incubations were not completely anoxic. During denitrification, ratios of $CO_2:N_2$ production are 1.5 – 6 (Groffman et al. 2006), but for our incubations the average ratio of $CO_2:N_2O$ production (as a proxy for $CO_2:N_2$) was significantly greater at 95 ($\pm$ 26 SE). This

186

suggests that there was available oxygen for respiration. Methanogenesis is sensitive to reduction-oxidation potential and the presence of strong oxidants, such as oxygen or NO_3^-, will suppress CH_4 production (Le Mer and Roger 2001). We also observed a negative relationship between water NO_3^- concentrations and CH_4 production (Table 20). This might explain why CH_4 production was greater in low-deposition lakes compared to high-deposition lakes. Further, published data for CH_4 production in sediments are often collected from eutrophic lakes and lakes surrounded by peatlands or bogs, which would likely have anoxic conditions in the sediments and, thus, be more favorable for CH_4 production (Huttunen et al. 2003). Lastly, the generally low CH_4 fluxes we observed could also be result from poor quality OM in sediments.

Greenhouse gas emissions from lakes

Lake gas emissions not only depend on the concentration gradient between the lake and the atmosphere, but also on the gas exchange coefficient (Wanninkhof et al. 1987). We did not measure gas exchange rates for the sampled lakes, so we cannot determine gas emissions. However, the surface waters were supersaturated with CO_2, CH_4, and N_2O, suggesting that the lakes are sources of these gases to the atmosphere. The surface water concentrations of CO_2 are three orders of magnitude greater than that of CH_4 or N_2O; thus, CO_2 is likely the dominant greenhouse gas emitted from the sampled lakes. The dynamics

of CO_2 in the surface water do not appear to be influenced by atmospheric N inputs. We did observe a positive relationship between surface water concentrations of CO_2 and DOC and NO_3^- (Table 19), although there was no significant relationship with either predictor variable when considered individually. This is interesting because we found NO_3^- to be negatively related to sediment CO_2 production and because other studies have found strong positive relationships between CO_2 concentrations and DOC (Sobek et al. 2003). It is possible that respiration by bacterioplankton and sediment bacteria respond differently to N loading, thus, it would be useful to further investigate the extent to which bacteria in sediments and pelagic areas contribute to CO_2 in surface water of the sampled lakes. Overall, however, our data are consistent with the well-documented finding that most lakes are heterotrophic and sources of CO_2 to the atmosphere (Cole et al. 1994, Tranvik et al. 2009).

There are two major pathways for CH_4 emissions from lakes, diffusion and ebullition (bubble flux) of gas produced in the sediments. Ebullition may account for 20 – 70% of CH_4 emissions from sediments, especially in shallow, eutrophic lakes (Bastviken et al. 2008, Juutinen et al. 2009). Our approach for measuring CH_4 concentrations only reflects diffusive sediment fluxes and are at the very low end of the range reported for lakes using similar methods (Bastviken et al. 2004). While there is a general lack of CH_4-related data for high-elevation lakes, Smith and Lewis

(1992) sampled five lakes in the Colorado Front Range, including one of the lakes we visited (Long Lake). The dissolved CH_4 concentration we observed for Long Lake is within the range they reported. Overall, evidence presented here suggests that N deposition has reduced sediment CH_4 production and concentrations of dissolved CH_4. Methanogenesis is not a significant C mineralization pathway or source of greenhouse gas in the sampled lakes, independent of atmospheric N inputs.

Our study lakes were generally supersaturated with N_2O and concentrations were comparable to limited reports for other lakes (Table 21). These data suggest that atmospheric N deposition has increased concentrations of dissolved N_2O and potentially emissions of N_2O from lakes, consistent with studies of boreal lakes and other aquatic ecosystems (Seitzinger et al. 1984, Liikanen et al. 2003, Beaulieu et al. 2008). In lakes, N_2O is produced by denitrification in sediments and by nitrification in sediments and the water column (Mengis et al. 1997, Wrage et al. 2001). For the lakes we sampled, the source of N_2O in the sampled lakes is unclear. We expected a correlation between sediment N_2O production and lake water concentrations of N_2O. Denitrification-related N_2O production, however, was only observed in sediments of four lakes. We do not know why there were no N_2O fluxes for the majority of sediments we sampled. Similar assays conducted with sediments from

189

lakes in Norway found significantly greater N_2O fluxes in lakes that receive elevated levels of N deposition (McCrackin and Elser 2010). If our assays were not completely anoxic, rates denitrification and related N_2O production could have been repressed. The sediments are still a possible source of N_2O production because experiments conducted simultaneously found considerable denitrification capacity under NO_3^- enrichment (McCrackin and Elser In revision). Alternatively, nitrification could produce N_2O as has been observed in soils (Bateman and Baggs 2005). Assays conducted with sediments from Norwegian lakes in high-deposition regions showed significantly greater rates of nitrification potential (in response to non-limiting concentrations of ammonium) than those in low deposition regions (M. McCrackin, unpublished data). Further investigation is required to determine the source of dissolved N_2O observed in the water column.

We do not know how concentrations of dissolved CO_2, CH_4, and N_2O in the sampled lakes vary seasonally. These alpine and subalpine lakes are covered with ice for more than half of the year. Gases that accumulate in the hypolimnion during periods with ice cover might be released during spring. Thus, concentrations of dissolved gases that we measured in summer months may be greater than average annual concentrations. While CH_4 and N_2O are more potent than CO_2 in terms of radiative forcing, the low concentrations of these trace gases relative to

CO_2 indicate that lake greenhouse gas dynamics are dominated by CO_2. The relatively small surface area of the sampled lakes suggests that they do not contribute disproportionately to such dynamics in the Colorado Rocky Mountains. Given that N_2O plays a significant role in the depletion of stratospheric ozone, however, potential N_2O emissions from lakes, and the effects of N deposition on them, deserve further consideration.

Under IPCC guidelines for determining greenhouse gas inventories, indirect N_2O emissions from aquatic ecosystems are estimated for groundwater, rivers, and estuaries. These emissions are estimated using emission factors that are determined based on the ratio of dissolved N_2O to NO_3^-. While lakes are not currently included in greenhouse gas inventories, we followed the IPCC methodology and found that for the lakes we sampled, the $N_2O:NO_3^-$ ratio averaged 0.01 with a range of 0.001 – 0.07 (Table 21). These values are somewhat larger than the current IPCC emission factor for aquatic ecosystems of 0.0075 with a range of 0.0005 to 0.025. Clarifying the emission factor is important, but the dynamics of N_2O in lakes are not well documented. We surveyed the scientific literature and, where concentrations of N_2O and NO_3^- were reported, determined the $N_2O:NO_3^-$ ratio to be between 0 and 0.12 (Table 22). This broad range indicates that a single $N_2O:NO_3^-$ ratio cannot be generalized across all lakes. Further, values for the $N_2O:NO_3^-$ ratio at the high end of this range suggest there is potential for N_2O emissions from

191

lakes subject to elevated N loading. Indeed, Wang et al., (2006) reported

N_2O production of 300 µmol N m^{-2} d^{-1} in pelagic areas of a hyper-eutrophic

lake, which is an order of magnitude greater than maximum emission

rates reported for agricultural fields (Bouwman and Boumans 2002).

Global N_2O emissions from lakes receiving N deposition

Our data suggest a possibility that lakes could be an

underappreciated component of global N_2O cycling, especially given

elevated atmospheric N inputs. Here we attempt to quantify this possibility.

Globally, the largest source of anthropogenic N_2O emissions is the

enhanced conversion of N fertilizer by microorganisms in agricultural soils

(Forster et al. 2007). Emissions from aquatic ecosystems, however, are

also significant. Kroeze et al. (2005) estimate N_2O emissions by rivers and

estuaries is 1,256 and 251 Gg N y^{-1}, respectively, which represents

approximately 15% of total anthropogenic N_2O emissions (Denmen et al.

2007). We used different approaches to quantify global N_2O emissions

from lakes resulting from atmospheric N deposition. First, we estimated

global N loading to lakes via atmospheric N deposition using published

datasets of N deposition rates (for 1993 and 2050) and of small lakes,

large lakes, and reservoirs >1 km^2 (Lehner and Döll 2004, Dentener 2006)

as, atmospheric N deposited to lake surface N kg y^{-1} = N deposition rate

kg ha^{-1} y^{-1} * lake surface area (ha).

Total estimated N input directly to lakes surfaces is 1.1 and 2.1 Tg
N y^{-1} for 1993 and 2050, respectively. Based on our analysis, over 90% of
the global surface area of lakes (2.6 million km^2) is subject to atmospheric
N deposition. To account for watershed inputs of N to the lakes, we
assumed the catchment area was 25 times greater than that of the lake
and that 50% of all N deposited to the watershed is subsequently leached
to the lake (Sjoeng et al. 2007, Harrison et al. 2009). Based on this, inputs
of N from the watershed were estimated as 13.7 Tg N y^{-1} for 1993 and
26.1 Tg N y^{-1} for 2050. The extent to which lakes are subject to N
deposition is of interest not only because of the potential for increased
N_2O emissions but also because of the documented effects of N on lake
stoichiometry and food web dynamics (Elser et al. 2009a, Hessen et al.
2009). Even lakes that are not directly influenced by human activity are at
risk from atmospherically delivered pollution, which is of particular concern
because N deposition rates are expected to increase globally in the next
few decades, driven by energy demands and agricultural activities
(Dentener 2006).

Second, we estimated N_2O production as in Kroeze et al. (2005),
which assumes that such emissions result from nitrification and
denitrification of inorganic N inputs as, N_2O-N kg y^{-1} = (Nitrification +
Denitrification) * EF.

This approach is described in detail by Seitzinger and Kroeze (1998). Briefly, it is assumed that 50% of N inputs are denitrified and that the nitrification rate exceeds the denitrification rate by 20%. The emission factor, EF, is 0.3% of denitrification and nitrification except where N loading rates exceed 10 kg N ha^{-1} y^{-1}, where the EF is 3%. Using this method, we estimated N_2O emissions to be 146 Gg N y^{-1} in 1993, increasing to 445 Gg N y^{-1} in 2050. The 1993 emissions represent about 10% of N_2O emitted from rivers and estuaries as estimated by Kroeze et al. (2005), although our estimates are not strictly comparable because we do not consider all sources of N inputs to lakes, such as run-off from agriculture fields or waste water from treatment plants.

We also estimated N_2O emissions using IPCC methodology for indirect greenhouse emissions from aquatic ecosystems resulting from run-off from agricultural systems. This approach does not specifically address atmospheric N deposition to lakes, but in our opinion is most appropriate under current IPCC guidelines. Here, N_2O emissions are determined as N_2O-N kg y^{-1} = atmospheric deposition to lake kg N y^{-1} * EF_5, where atmospheric N deposition is calculated as previously described and EF_5 is the overall emission factor for aquatic ecosystems, with a default value of 0.0075 and an uncertainty range of 0.0005 – 0.025 (Table 22). The emission factors for runoff were based on the observed ratio of N_2O to NO_3^- in groundwater, streams, rivers, and estuaries (IPCC 2006).

The resulting estimates for 1993 emissions were 105 Gg N y^{-1} with a range of 7 to 371 Gg N y^{-1}, representing 0.5% to 25% of N$_2$O emissions from rivers and estuaries as estimated by Kroeze et al. (2005). Forecasted 2050 emissions increase to 198 Gg N y^{-1} with an uncertainty range of 14 to 705 Gg N y^{-1} (Fig. 19). The bulk of the emissions are from lakes in North and South America. Emissions calculated using the approach of Kroze et al (2005) fall within the ranges determined using the IPCC methodology. It is not clear that emission factors developed for groundwater, rivers, and estuaries are appropriate for lakes given the wide range of N$_2$O:NO$_3^-$ ratios we found for lakes (Table 21). Overall increases in estimates between 1993 and 2050 results from increased N deposition rates. In any case, our high-level estimates suggest that lakes should be considered in global inventories of N$_2$O emissions from aquatic ecosystems. Further analysis is needed to constrain emission factors for lakes and to quantify N$_2$O emissions resulting from all sources of N to lakes.

ACKNOWLEDGMENTS

195

LITERATURE CITED

Algesten, G., S. Sobek, A. K. Bergstrom, A. Jonsson, L. J. Tranvik, and M. Jansson. 2005. Contribution of sediment respiration to summer CO2 emission from low productive boreal and subarctic lakes. Microbial Ecology **50**:529-535.

Baron, J. S., H. M. Rueth, A. M. Wolfe, K. R. Nydick, E. J. Allstott, J. T. Minear, and B. Moraska. 2000. Ecosystem responses to nitrogen deposition in the Colorado Front Range. Ecosystems:352-368.

Bastviken, D., J. Cole, M. Pace, and L. Tranvik. 2004. Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. Global Biogeochemical Cycles **18**:GB4009, doi:4010.1029/2004GB002238.

Bastviken, D., J. J. Cole, M. L. Pace, and M. C. Van de Bogert. 2008. Fates of methane from different lake habitats: Connecting whole-lake budgets and CH4 emissions. Journal of Geophysical Research-Biogeosciences **113**:G02024, doi:02010.01029/02007JG000608.

Bateman, E. J. and E. M. Baggs. 2005. Contributions of nitrification and denitrification to N2O emissions from soils at different water-filled pore space. Biology and Fertility of Soils **41**:379-388.

Beaulieu, J. J., C. P. Arango, S. K. Hamilton, and J. L. Tank. 2008. The production and emission of nitrous oxide from headwater streams in the Midwestern United States. Global Change Biology **14**:878-894.

Bouwman, A. F. and L. M. Boumans. 2002. Emissions of N2O and NO from fertilized fields: Summary of available measurement data. Global Biogeochemical Cycles **16**:1058, doi:1010.1029/2001GB001811.

Burns, D. A. 2004. The effects of atmospheric nitrogen deposition in the Rocky Mountains of Colorado and southern Wyoming, USA - a critical review. Environmental Pollution **127**:257-269.

Cole, J. J. and N. F. Caraco. 1998. Atmospheric exchange of carbon dioxide in a low-wind oligotrophic lake measured by the addition of SF6. Limnology and Oceanography **43**:647-656.

Cole, J. J., N. F. Caraco, G. W. Kling, and T. K. Kratz. 1994. Carbon dioxide supersaturation in the surface waters of lakes. Science **265**:1568-1570.

Denmen, K. L., G. Brasseur, A. Chidthaisong, P. Ciais, P. M. Cox, R. E. Dickinson, D. Hauglustaine, C. Heinze, E. A. Holland, D. Jacob, U. Lohmann, S. Ramachandran, P. L. da Silva Dias, S. C. Wofsy, and X. Zhang. 2007. Couplings between changes in the climate system and biogeochemistry.*in* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, USA.

Dentener, F. J. 2006. Global maps of atmospheric nitrogen deposition, 1860, 1993, amd 2050. Data set. Available on-line [http://www.daac.ornl.gov] from Oak Ridge National Laboratory Distributed Active Archive Center, Oak Ridge, Tennessee, USA.

Dunfield, P., R. knowles, R. Dumont, and T. R. Moore. 1993. Methane production and consumption in temperate and subarctic peat soils: Response to temperature and pH. Soil Biology and Biochemistry **25**:321-326.

Elser, J. J., T. Andersen, J. S. Baron, A. K. Bergstrom, M. Jansson, M. Kyle, K. R. Nydick, L. Steger, and D. O. Hessen. 2009a. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science **326**:835-837.

Elser, J. J., M. Kyle, L. Steger, K. R. Nydick, and J. S. Baron. 2009b. Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. Ecology **90**:3062-3073.

Forster, P., V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D. W. Fahey, J. Haywood, J. Lean, D. C. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, M. Schulz, and R. Van Dorland. 2007. Changes in atmospheric constituents and in radiative forcing.*in* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Climate Change 2007: the Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK; New York, NY, USA.

Garcia-Ruiz, R., S. N. Pattinson, and B. A. Whitton. 1998. Denitrification in river sediments: relationship between process rate and properties of water and sediment. Freshwater Biology **39**:467-476.

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

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

Hessen, D. O., T. Andersen, S. Larsen, B. L. Skjelkvale, and H. A. de Wit. 2009. Nitrogen deposition, catchment productivity, and climate as determinants of lake stoichiometry. Limnology and Oceanography **54**:2520-2528.

Huttunen, J. T., J. Alm, A. Liikanen, S. Juutinen, T. Larmola, T. Hammar, J. Silvola, and P. J. Martikainen. 2003. Fluxes of methane, carbon dioxide and nitrous oxide in boreal lakes and potential anthropogenic effects on the aquatic greenhouse gas emissions. Chemosphere **52**:609-621.

IPCC. 2006. Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme.*in* H. S. Eggleston, L. Buendia, K. Miwa, T. Ngara, and K. Tanabe, editors. IGES, Hayama, Japan.

Juutinen, S., M. Rantakari, P. Kortelainen, J. T. Huttunen, T. Larmola, J. Alm, J. Silvola, and P. J. Martikainen. 2009. Methane dynamics in different boreal lake types. Biogeosciences **6**:209-223.

Kent, H. C. and K. W. Porter, editors. 1980. Colorado Geology. Rocky Mountain Association of Geologists, Denver.

Knowles, R. 1982. Denitrification. Microbiological Reviews **46**:43-70.

Kroeze, C., E. Dumont, and S. Seitzinger. 2005. New estimates of global emissions of N_2O from rivers and estuaries. Environmental Sciences **2**:159-165.

Le Mer, J. and P. Roger. 2001. Production, oxidation, emission and consumption of methane by soil: a review. European Journal of Soil Biology **37**:25-50.

Lehner, B. and P. Döll. 2004. Development and validation of a global database of lakes, reservoirs, and wetlands. Journal of Hydrology **296**:1-22.

Liikanen, A., L. Flojt, and P. J. Martikainen. 2002. Gas dynamics in eutrophic lake sediments affected by oxygen, nitrogen, and sulfate. Journal of Environmental Quality **31**:338-350.

Liikanen, A., E. Ratilainen, S. Saarnio, J. Alm, P. J. Martikainen, and J. Silvola. 2003. Greenhouse gas dynamics in boreal, littoral sediments under raised CO2 and nitrogen supply. Freshwater Biology **48**:500-511.

Liu, L. and T. L. Greaver. 2009. A review of nitrogen enrichment effects on three biogenic GHGs: the CO_2 sink may be largely offset by stimulated N_2O and CH_4 emissions. Ecology Letters **12**:1103-1117.

Lukkari, K., H. Hartikainen, and M. Leivuori. 2007. Fractionation of sediment phosphorus revisited. I: Fractionation steps and their biogeochemical basis. Limnology and Oceanography: Methods **5**:433-444.

McCrackin, M. L. and J. J. Elser. 2010. Atmospheric nitrogen deposition alters denitrification and nitrous oxide production in lake sediments. Ecology **91**:528-539.

McCrackin, M. L. and J. J. Elser. In revision. Denitrification and microbial communities in high elevation lakes receiving atmospheric nitrogen deposition. Biogeochemistry.

Mengis, M., R. Gachter, and B. Wehrli. 1997. Sources and sinks of nitrous oxide (N_2O) in deep lakes. Biogeochemistry **38**:281-301.

Nanus, L., D. H. Campbell, G. P. Ingersoll, D. W. Clow, and M. A. Mast. 2003. Atmospheric deposition maps for the Rocky Mountains. Atmospheric Environment **37**:4881-4892.

Nevison, C. D., N. M. Mahowald, R. F. Weiss, and R. G. Prinn. 2007. Interannual and seasonal variability in atmospheric N_2O. Global Biogeochemical Cycles **21**:GB3017, doi:3010.1029/2006GB002755.

Ravishankara, A. R., J. S. Daniel, and R. W. Portmann. 2009. Nitrous oxide: the dominant ozone-depleting substance emitted in the 21st century. Science **326**:123-125.

Rudaz, A. O., E. Walti, G. Kyburz, P. Lehmann, and J. Fuhrer. 1999. Temporal variation in N_2O and N_2 fluxes from a permanent pasture in Switzerland in relation to management, soil water content and soil temperature. Agriculture Ecosystems & Environment **73**:83-91.

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

Seitzinger, S. P. and S. W. Nixon. 1985. Eutrophication and the rate of denitrification and N_2O production in coastal marine sediments. Limnology and Oceanography **30**:1332-1339.

Seitzinger, S. P., S. W. Nixon, and M. E. Q. Pilson. 1984. Denitrification and nitrous oxide production in a coastal marine ecosystem. Limnology and Oceanography **29**:73-83.

Sjoeng, A. M. S., O. Kaste, K. Torseth, and J. Mulder. 2007. Nitrogen leaching from small upland headwater catchments in southwestern Norway. Water Air and Soil Pollution **179**:323-340.

Smith, L. K. and W. M. Lewis. 1992. Seasonality of methane emissions from five lakes and associated wetlands of the Colorado Rockies. Global Biogeochemical Cycles **6**:323-338.

Sobek, S., G. Algesten, A. K. Bergstrom, M. Jansson, and L. J. Tranvik. 2003. The catchment and climate regulation of pCO(2) in boreal lakes. Global Change Biology **9**:630-641.

Taylor, P. G. and A. R. Townsend. 2010. Stoichiometric control of organic carbon-nitrate relationships from soils to the sea. Nature **464**:1178-1181.

Tranvik, L. J., J. A. Downing, J. B. Cotner, S. A. Loiselle, R. G. Striegl, T. J. Ballatore, P. Dillon, K. Finlay, K. Fortino, L. B. Knoll, P. L. Kortelainen, T. Kutser, S. Larsen, I. Laurion, D. M. Leech, S. L. McCallister, D. M. McKnight, J. M. Melack, E. Overholt, J. A. Porter, Y. Prairie, W. H. Renwick, F. Roland, B. S. Sherman, D. W. Schindler, S. Sobek, A. Tremblay, M. J. Vanni, A. M. Verschoor, E. von Wachenfeldt, and G. A. Weyhenmeyer. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. Limnology and Oceanography **54**:2298-2314.

Ullah, S. and G. M. Zinati. 2006. Denitrification and nitrous oxide emissions from riparian forest soils exposed to prolonged nitrogen runoff. Biogeochemistry **81**:253-267.

Wang, H. J., W. D. Wang, C. Q. Yin, Y. C. Wang, and J. W. Lu. 2006. Littoral zones as the "hotspots" of nitrous oxide (N_2O) emission in a hyper-eutrophic lake in China. Atmospheric Environment **40**:5522-5527.

Wanninkhof, R., J. R. Ledwell, W. S. Broecker, and M. Hamilton. 1987. Gas-exchange on Mono Lake and Crowley Lake, California. Journal of Geophysical Research-Oceans **92**:14567-14580.

Weiss, R. F. 1974. Carbon dioxide in water and seawater: the solubility of a non-ideal gas. Marine Chemistry **2**:203-215.

Weiss, R. F. and B. A. Price. 1980. Nitrous-oxide solubility in water and seawater. Marine Chemistry **8**:347-359.

Weyhenmeyer, G. A. and E. Jeppesen. 2009. Nitrogen deposition induced changes in $DOC:NO_3$-N ratios determine the efficiency of nitrate removal from freshwaters. Global Change Biology:doi: 10.1111/j.1365-2486.2009.02100.x.

Wiesenburg, D. A. and N. L. Guinasso. 2002. Equilibrium solubilities of methane, carbon monoxide, and hydrogen in water and sea water. Journal of Chemical and Engineering Data **24**:356-360.

Williamson, C. E., J. E. Saros, W. F. Vincent, and J. P. Smol. 2009. Lakes and reservoirs as sentinels, integrators, and regulators of climate change. Limnology and Oceanography **54**.

Wrage, N., G. L. Velthof, M. L. van Beusichem, and O. Oenema. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology & Biochemistry **33**:1723-1732.

Table 17: Study lakes by N deposition level and average values for DOC and NO_3^-.

	Sample date	Elevation (m)	Sediment collected	Lake depth (m)	DOC mM	NO_3^- µM
High-deposition Lakes						
Albion	7/1/08	3,345	no	12	0.26	6.5
Blue	7/23/08	3,398	no	8	0.52	20.5
Brainard	6/26/08	3,154	yes	3	0.27	12.6
Dream	7/4/08	3,032	yes	4	0.28	16.6
Emerald	7/3/08	3,082	no	10	0.49	20.2
Estes	6/29/08	2,277	yes	>20	0.45	6.4
Green Lake 1	7/25/08	3,421	yes	9	0.33	9.5
Green Lake 2	7/25/08	3,416	yes	5	0.36	11.1
Green Lake 3	7/25/08	3,467	no	10	0.43	15.6
Haiyaha	7/28/08	3,117	no	10	0.14	19.1
Isabelle	6/30/08	3,314	yes	40	0.39	19.3
Long	7/5/08	3,219	yes	3	0.72	12.4
Mitchell	7/23/08	3,280	yes	1.5	0.62	10.8
				Mean	0.40	13.9
				s.e.	0.05	1.4
Low-deposition Lakes						
Andrews	7/11/08	3,284	yes	6	1.44	3.4
Clear	7/10/08	3,633	yes	>25	0.53	6.3
Dollar	8/6/08	3,059	yes	5	0.60	0.4
Emerald	8/7/08	3,175	yes	18	0.86	18.5
Haviland	7/16/08	2,472	no	3	0.77	9.0
Highland Mary	7/15/08	3,708	no	30	2.39	2.9
Irwin	8/7/08	3,148	yes	5	0.35	0.5
Little Molas	7/8/08	3,329	yes	6	0.84	4.1
Lost	8/6/08	3,010	yes	10	0.18	3.3
Lost Slough	8/13/08	2,939	yes	4	0.29	0.3
Potato	7/18/08	2,983	yes	17	1.47	11.4
Pothole #2	8/12/08	2,482	no	3	0.77	0.4
Spring Creek	8/12/08	3,040	yes	8	1.45	0.3
				mean	0.92	4.7
				s.e.	0.18	1.6

Table 18. Average (and standard error, s.e.) concentrations and saturation % for greenhouse gases in surface waters of the study lakes.

Lake	CO$_2$ uM	CH$_4$ nM	N$_2$O nM	CO$_2$ Saturation	CH$_4$ Saturation	N$_2$O Saturation
High-deposition Lakes						
Albion	15.6	13	26	113%	603%	158%
Blue	50.6	79	33	287%	3,074%	158%
Brainard	33.5	23	33	219%	969%	178%
Dream	32.7	23	29	210%	961%	151%
Emerald	29.2	16	31	174%	632%	152%
Estes	29.5	48	28	184%	1,906%	145%
Green Lake 1	12.5	34	23	97%	1,664%	145%
Green Lake 2	15.2	5	21	126%	244%	142%
Green Lake 3	19.6	5	24	153%	219%	143%
Haiyaha	19.4	6	28	127%	236%	146%
Isabelle	56.8	344	45	327%	13,045%	209%
Long	29.4	20	27	210%	905%	157%
Mitchell	25.6	15	25	183%	674%	149%
Mean	28.4	48	29	185%	1,933%	156%
s.e.	3.8	26	2	19%	887%	5%

205

Table 18, continued. The results of a statistical test comparing these variables between deposition regions are shown.

Non-significant differences indicated by n.s.

Lake	CO_2 uM	CH_4 nM	N_2O nM	CO_2 Saturation	CH_4 Saturation	N_2O Saturation
Low-deposition Lakes						
Andrews	35.0	649	22	304%	34,765%	162%
Clear	28.3	7	33	177%	282%	165%
Dollar	21.3	183	24	193%	10,118%	179%
Emerald	25.8	14	22	207%	674%	145%
Haviland	20.9	8	24	305%	8,247%	123%
Highland Mary	36.4	161	16	170%	352%	113%
Irwin	19.0	87	20	152%	4,328%	131%
Little Molas	29.3	206	19	254%	11,033%	137%
Lost	22.7	43	24	167%	1,993%	144%
Lost Slough	8.9	61	19	79%	3,287%	138%
Potato	24.6	441	25	157%	18,184%	131%
Pothole #2	16.4	159	16	138%	8,106%	112%
Spring Creek	41.7	151	18	327%	6,222%	119%
Mean	25.4	167	22	202%	8,276%	138%
s.e.	2.6	54	1	21%	2,738%	6%
high vs. low deposition	high = low	high < low	high > low	high = low	high < low	high > low
P	n.s.	0.02	0.003	n.s.	0.01	0.03

Table 19: Comparison of multiple-linear regression models for estimates of sediment gas fluxes and dissolved greenhouse gas concentrations. R^2 denotes goodness-of-fit values adjusted for the number of parameters in the model. No significant model indicated by n.s.

Response variable	Predictor variables	R^2	P	Equation
Sediment CO_2 flux ($mmol\ C\ m^{-2}\ h^{-1}$)	$[NO_3^-]$	0.38	< 0.01	Log sediment CO_2 flux = -0.34 * log $[NO_3^-]$ + 2.62
Sediment CH_4 flux ($\mu mol\ C\ m^{-2}\ h^{-1}$)	$[NO_3^-]$	0.28	0.03	Log sediment CH_4 flux = -0.64 * log $[NO_3^-]$ + 1.05
Sediment N_2O flux ($\mu mol\ N\ m^{-2}\ h^{-1}$)	n.s.	n.s	n.s	n.s.
$[CO_2]$ surface	$[NO_3^-]$, $[DOC]$	0.25	0.01	Log CO_2 emission = 0.12 * log $[NO_3^-]$ + 0.28 * log $[DOC]$ + 0.53
$[CH_4]$ surface	$[NO_3^-]$, $[DOC]$	0.35	0.003	Log CH_4 emission = – 0.38 * log $[NO_3^-]$ + 0.95 * log $[DOC]$ – 0.69
$[N_2O]$ surface	$[NO_3^-]$	0.43	<0.001	Log N_2O emission = 0.11 * $[NO_3^-]$ + 1.31

Table 20. Average (and standard error, s.e.) sediment greenhouse gas
fluxes for the study lakes and results of statistical test comparing
deposition regions. Nonsignificant results indicated by n.s.

	CO_2 flux $\mu mol\ C\ m^{-2}\ h^{-1}$	CH_4 flux $\mu mol\ C\ m^{-2}\ h^{-1}$	N_2O flux $\mu mol\ N\ m^{-2}\ h^{-1}$
High-deposition Lakes			
Brainard	103.0	3.6	0.8
Dream	117.4	1.0	6.4
Estes	273.7	2.5	0.0
Green Lake 1	233.0	5.0	0.0
Green Lake 3	101.3	0.2	0.0
Isabelle	145.6	1.2	0.0
Long	200.0	1.1	1.1
Mitchell	134.0	2.9	0.0
Mean	163.5	2.2	0.6
s.e.	26.3	0.7	0.4
Low-deposition lakes			
Andrews	197.3	7.2	0.0
Clear	463.9	5.6	0.0
Dollar	333.0	5.6	0.0
H Mary	312.0	11.6	0.0
Little Molas	546.6	7.4	0.0
Lost	223.5	19.1	0.0
Lost Slough	206.8	9.4	0.0
Potato	177.1	4.5	1.0
Spring Creek Reservoir	408.2	1.6	0.0
Mean	318.7	8.0	0.1
s.e.	53.6	2.1	0.1
high vs. low deposition	high < low	high < low	high = low
P	0.004	0.003	n.s.

Table 21. Comparison of dissolved N_2O and sediment N_2O fluxes among different studies.

Dissolved N_2O, nmol N

	Mean	Range	% Saturation	Study
26 lakes, CO, USA	26.4	11 - 58	112 - 208%	This study
Taihu Lake, China		25 - 62	80 - 689%	Wang et al. 2009
Lake Baldegg, Switzerland		10 - 120		Mengis et al. 1996
15 lakes, Switzerland	43.9	14 - 152	99 - 798%	Mengis et al. 1997

N_2O sediment fluxes, μmol N m^{-2} h^{-1}

	Mean	Range	Study
17 lakes, CO, USA	0.4	0.0 - 5.6	This study
32 lakes, Norway	0.5	0.0 - 6.8	McCrackin and Elser 2010
Lake Kevätön, Finland		0.4 - 7.1	Liikannen et al. 2003
Lake Kevätön, Finland		0 - 1.3	Liikanen et al. 2002
Humber Estuary, UK		0.2 - 25.2	Barnes and Owens 1998
Narragansett Bay, RI, USA		0.0 - 0.9	Seitzinger et al. 1983

Table 22: Comparison of lake N_2O emission factors (determined as [N_2O/NO_3^-]).

| | Emission Factor | | |
	Mean	Range	Study
26 lakes, CO, USA	0.01	0.001 - 0.07	This study
Taihu Lake, China	0.002	0.0003 - 0.02	Wang et al. 2009
Greifensee, Switzerland	0.002	0 - 0.017	Mengis et al. 1997
Lago di Lugano, Switzerland	0.0015	0 - 0.009	Mengis et al. 1997
Lake Baldegg, Switzerland	0.016	0.001 - 0.12	Mengis et al. 1996
Lake Huron, USA	0.0011	0.001 - 0.0012	Lemon and Lemon 1981
Lake Ontario, USA	0.001	0.003 - 0.0006	Lemon and Lemon 1981
Cayuga Lake, USA	0.0003	0.0002 - 0.0006	Lemon and Lemon 1981
	Default value	Uncertainty Range	
Indirect N_2O Emission Factor (EF_5)	0.0075	0.0005 - 0.025	IPCC 2006
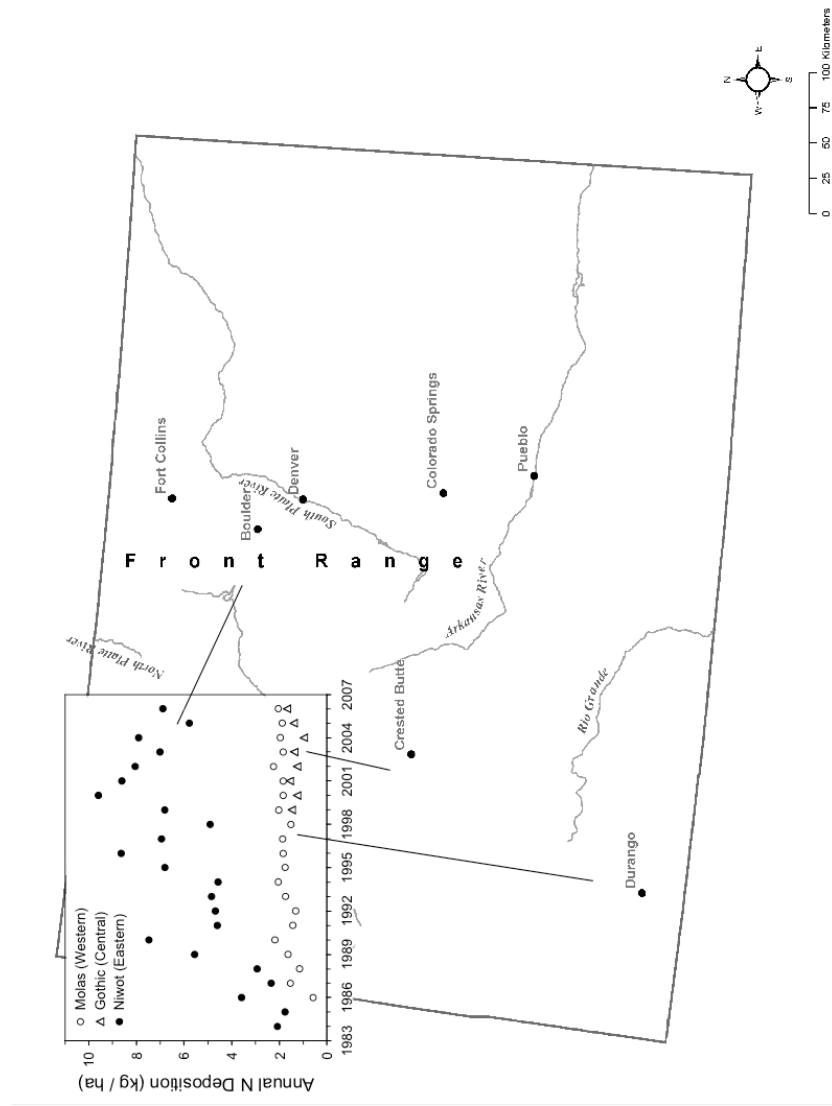
Figure 17. Locations of study sites in Colorado, USA. The inset figure shows the annual deposition (wet) of inorganic

N at monitoring stations close to each of the three sampling regions.
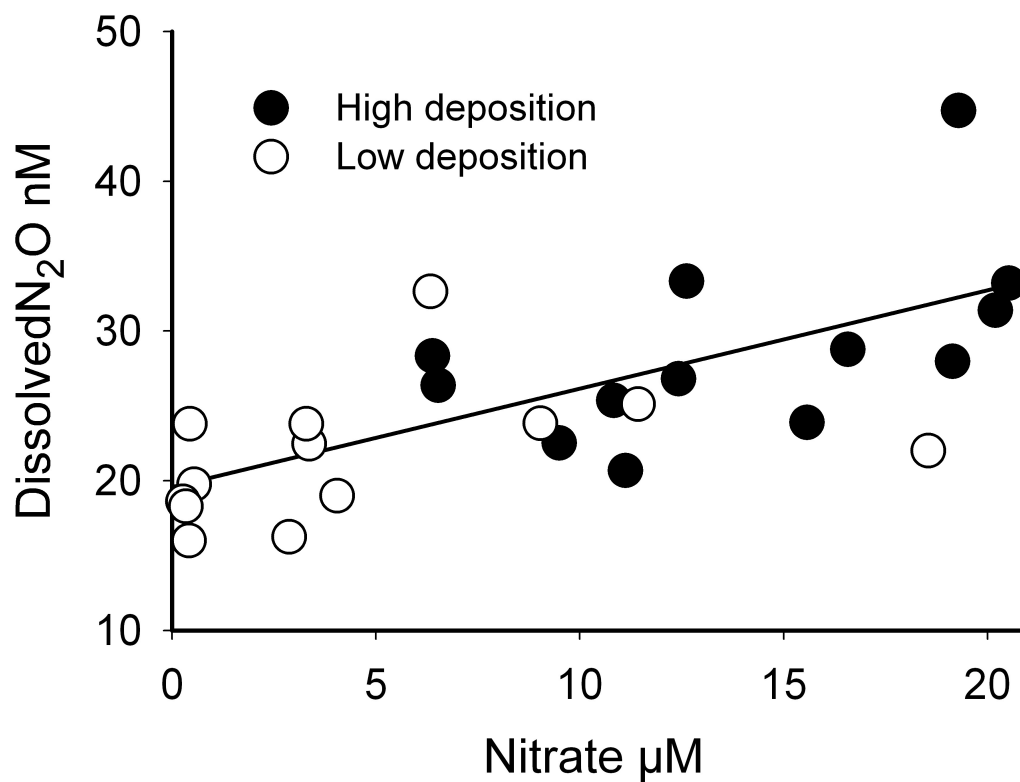
Figure 18: Relationship between dissolved N_2O and NO_3^-. Regression line

is shown for all lakes, R^2 = 0.43, P < 0.001.
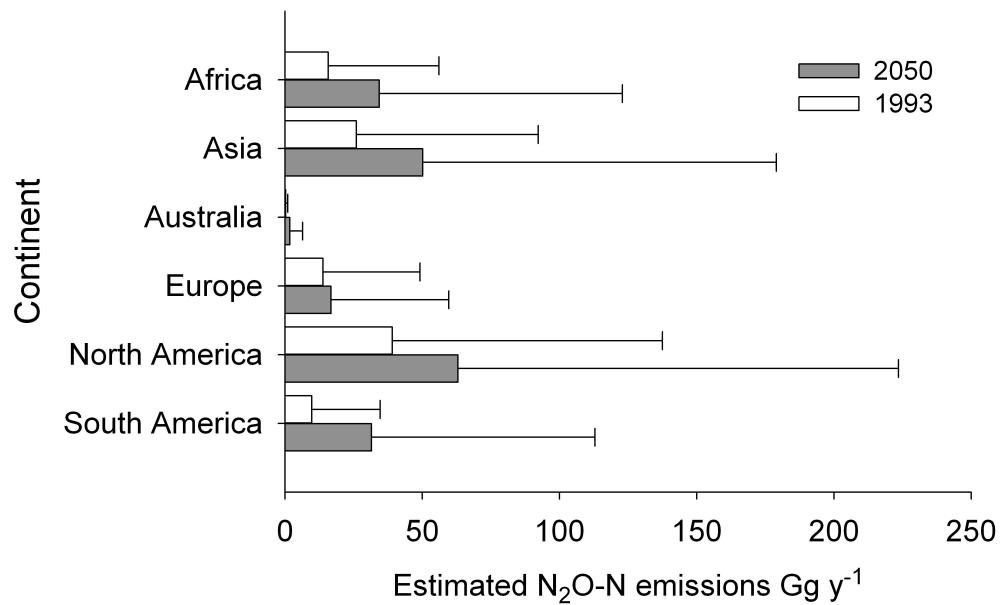
Figure 19. Estimated emissions of N_2O (Gg N y^{-1}) from lakes receiving

atmospheric N deposition by continent. Bars are mean emissions based

on the IPCC default value emission factor (0.0075). Error bars show the

upper IPCC uncertainty range for emissions (emission factor of 0.025).

CHAPTER 7

Summary

Even in remote or unpopulated areas, lakes are subject to
anthropogenic influences, via atmospheric N (N) deposition. Indeed, N
that is transported and deposited by the atmosphere can be the dominant
source of "new" N to many watersheds (Sickman et al. 2002), and can
decrease water quality and alter in phytoplankton and fish species
communities (Driscoll et al. 2003, Fenn et al. 2003). Fates of N in lakes
are biological assimilation, burial in sediments, or denitrification. Of these,
denitrification is the primary and only permanent mechanism by which N is
removed (Saunders and Kalff 2001). Global rates of N deposition are
expected to increase because human population growth will drive demand
for food and energy (Galloway et al. 2004). Most investigations into the
ecological effects of N loading have been conducted in terrestrial
ecosystems and the water column of lakes. Yet there are interactions
between the terrestrial portion of the watershed, the pelagic zone of lakes,
and lake sediments and N loading may influence these interactions.

This research reported here contributes to the body of scientific
knowledge in several ways. First, we extend previous investigations into
the ecological effects of N deposition from terrestrial ecosystems and the
pelagic habitats of lakes into the benthos. We conducted our investigation
on lakes located at the ends of two gradients of N deposition, in Colorado

and Norway. These gradients encompass different geological substrata, land use-histories, climates, and sources and patterns of atmospheric N, hence, making them useful to understand how generalizable the findings are. Second, we conducted experiments to understand the capacity of sediment denitrification to respond to predicted increases in N loading. Third, we scaled up our findings to understand the role of lakes in removing N via denitrification and the magnitude of N deposition-induced nitrous oxide (N_2O) emissions from lakes. Nitrous oxide emissions have been quantified from a variety of terrestrial and aquatic ecosystems, but these estimates have not included lakes.

Summary of findings

Denitrification

We contrasted sediment and water resources between N deposition regions to assess the magnitude of deposition-induced changes to the environment in which denitrification occurs. There was no evidence of changes in key sediment resources between N deposition regions in either Norway or Colorado. Total carbon (C), N, phosphorus (P) and organic matter (OM) did not differ between high- and low-deposition regions (Table 22, Chapters 2 and 3). Total C, N, P, and OM contents averaged 10.3 mmol g^{-1}, 0.75 mmol g^{-1}, 0.1 mmol g^{-1}, and 20%, respectively, across all lakes. In both Colorado and Norway, lakes receiving elevated N deposition had significantly greater concentrations of

215

nitrate (NO_3^-) compared to lakes in low-deposition regions (15 times greater, Table 22, Chapters 2 and 3). The opposite was true for dissolved organic carbon (DOC) concentrations, which were two times greater in low-deposition lakes compared to high-deposition lakes due to Norwegian lakes; DOC did not differ between deposition regions for lakes in Colorado.

When sediments were amended with ambient concentrations of NO_3^- in lake water, the denitrification rate was greater in high-deposition lakes relative to low-deposition lakes (Table 22, Chapter 2). For lakes in both regions, there was a strong positive relationship between denitrification and water NO_3^- (Fig. 20). These results are consistent with other studies that have found NO_3^- to be an important predictor of denitrification (Seitzinger et al. 2006, Mulholland et al. 2008).

We estimated the fraction of N that may be removed from all sampled lakes as $R_N = 1 - \exp(-V_f / (\bar{z}/\tau))$, where V_f is the piston velocity for N (m y^{-1}) in lake sediments, $\bar{z}$ (m) is the mean lake depth, and τ (y) is the water residence time (Kelly et al. 1987, Harrison et al. 2009). The piston velocity was determined using the denitrification rate and NO_3^- concentration for each lake as $V_f =$ denitrification rate/$[NO_3^-]$. The water residence time was assumed to be 0.083 y (1 month) or 1 y, which is in the ranges of those reported for lakes in areas where we sampled. The average fraction removed was bounded with a 95% confidence interval

(CI) based on the standard error of R_N to reflect uncertainty and variability of the estimates.

Overall, denitrification in the sampled lakes may remove 13% (6-19% CI) of N inputs assuming a one-month water residence time and 39% (28-51% CI) assuming a one-year water residence time. Lakes in high-deposition regions are estimated to remove 16% (9-23% CI) and 64% (49-78% CI) of N loading if the water residence time is one month and one year, respectively. Low-deposition lakes may remove an average of 10% (1-20% CI) of N inputs if the water residence time is short (1 month) compared to 15% (2-28% CI) if the water residence time is one year. While these estimates are approximate and do not reflect seasonal and inter-annual changes in key factors such as NO_3^- concentrations and temperature, they are consistent with a growing body of evidence that lakes are important sinks for N (Seitzinger et al. 2006, Harrison et al. 2009).

While high-deposition lakes have the potential to remove more NO_3^- than low-deposition lakes, we evaluated the efficiency of NO_3^- removal by plotting V_f (piston velocity, calculated as described above) and NO_3^- for lakes in each deposition region. While there was no relationship between V_f and NO_3^- concentrations for Colorado lakes, we found a strong negative relationship between V_f and NO_3^- for Norwegian lakes (Fig. 21, $R^2 = 0.53$), indicating that NO_3^- removal by denitrification decreases with increasing

NO_3^- concentration, consistent with observations in streams (Mulholland et al. 2008).

Given the role of denitrification as a mechanism for N removal, we investigated the effects of chronic N loading on denitrification performance, the abundance of sediment denitrifiers, and resource limitation of denitrification. First, incubations that were enriched with non-limiting concentrations of oC, NO_3^-, and P (denitrification potential assays) revealed that lake sediments possess considerable denitrification capacity, regardless of region or N deposition level (Table 22, Fig. 22, Chapters 2 and 3). Results of dose-response experiments with sediments of Colorado lakes (Chapter 3) further suggest that sediments can remove N inputs through enhanced denitrification without secondary limitation by oC or P. Indeed, under Michaelis-Menton kinetics, the half-saturation constant of denitrification was ~35 times greater than background NO_3^- concentrations. Overall, our findings suggest that sediment denitrifiers will be able to respond to predicted increases in N deposition rates, provided that they have access to NO_3^- itself.

Second, we quantified the abundance of sediment bacteria capable of NO_3^- and nitrite reduction (together as denitrifiers) in Colorado lakes (Chapter 3). There was no difference in abundances between lakes receiving elevated or low rates of N deposition. We compared our findings to those determined with similar methods and found that the abundance of

lake sediment denitrifiers to be within the ranges reported for desert, riparian, and prairie ecosystems, but less than that for agricultural soils. Denitrifiers have flexible metabolic pathways and use electron acceptors other than NO_3^-, perhaps allowing them to develop similar abundance in a wide range of ecosystems.

Third, we conducted resource enrichment experiments with lake sediments (Chapter 5). We measured metabolic responses of bacteria to enrichment with oC, NO_3, and P. Results of 48-h bioassays indicated that sediments, respiration (CO_2 production) responded most strongly to additions of oC, while denitrification was limited by NO_3^-. These responses varied between individual lakes, but overall were the same both high- and low-deposition lakes. We also contrasted the rate of potential nitrification (in response to non-limiting concentrations of ammonium) between deposition regions in Norway. Nitrification potential was significantly greater high-deposition lakes, suggesting that this process may be a source of NO_3^- to denitrification and contribute to elevated concentrations of NO_3^- in surface waters.

Greenhouse gas dynamics

In addition to increasing denitrification rates, we found that N deposition has influenced the production of N_2O during denitrification. There was a positive relationship between sediment N_2O fluxes and NO_3^-. In addition, there was a positive relationship between surface water

concentrations of dissolved N_2O and NO_3^- for lakes sampled in Colorado.

Nitrous oxide is produced not only by denitrification but also by

nitrification. Elevated rates of nitrification potential in sediments of high-

deposition lakes suggest that dissolved N_2O observed in the water column

could result from nitrification. Regardless of the source(s) of N_2O, our

evidence suggests that N deposition has enhanced microbial N-cycling

and N_2O concentrations in the water column, which could lead to

increased N_2O emissions from lake surfaces. Because of the role that N_2O

plays in the destruction of stratospheric ozone, we estimated the global

magnitude of N lake N_2O emissions. We applied the methodology of the

Intergovernmental Panel on Climate Change (IPCC) to spatially explicit

datasets of N deposition and lakes surface area (Chapter 6). Nitrous oxide

emissions were estimated as N_2O-N kg y^{-1} = atmospheric deposition to

lake kg N y^{-1} * EF_5, where EF_5 is the IPCC's overall emission factor for

aquatic ecosystems, with a default value of 0.0075 and an uncertainty

range of 0.0005 – 0.025 (IPCC 2006). Such estimated emissions are 7–

371 Gg N y^{-1} for 1993, increasing to 11–705 Gg N y^{-1} in 2050. The 1993

estimates represent 0.5–25% of emissions from rivers and estuaries,

suggesting lakes could be a source of N_2O, especially if all sources of N to

lakes are considered. Further research is required to better quantify

emission rates from lentic ecosystems.

In addition to N_2O, we investigated dynamics of carbon dioxide

(CO_2) and methane (CH_4, Chapter 6). In assays amended with lake water,

rates of CO_2 and CH_4 production were greater in sediments of lakes from

low-deposition lakes relative to high-deposition lakes, the opposite of N_2O

production. Concentrations of dissolved CO_2 did not differ between

deposition regions and CH_4 was greater in the surface waters of lakes in

the low-deposition region. Overall, CO_2 was the dominant greenhouse gas

in the sampled lakes; both surface water concentrations and sediment

fluxes were two orders of magnitude greater than those for CH_4 and N_2O.

Future directions

There was strong correlative evidence that N deposition has

increased NO_3^- concentrations, however, the sources of NO_3^- in lake water

are not clear. Analysis of stable isotopes of NO_3^- in lake water,

precipitation, and catchment run-off would be useful for understanding the

extent to which NO_3^- results from nitrification or originates from

anthropogenic sources. It would also be interesting to further investigate

the extent to which nitrification and denitrification are coupled and the role

of nitrification as a source of N_2O in the water column. Lastly, future

investigations could study sedimentation rates and the activities of

enzymes involved in OM mineralization to determine the fate of

deposition-enhanced primary production in the water column and

sediments.

Cross-habitat comparison

Critical processes and perturbations, such as nutrient cycling and nutrient enrichment, occur in both terrestrial and aquatic habitats. Understanding the extent to which ecosystem processes in soils and lakes respond similarly to atmospheric N deposition may improve our overall understanding of ecosystem functioning. Asymmetry in citations patterns of aquatic and terrestrial ecologists, however, suggests that terrestrial ecologists tend to ignore the literature of aquatic ecologists (Menge et al. 2009). This is surprising because lentic ecosystems, such as lakes, reservoirs, and ponds, are embedded in larger terrestrial ecosystems. Perhaps there is a perception that the influence of terrestrial ecosystems on lakes is unidirectional through "downhill" inputs of water, sediment, and nutrients. Lakes affect local climate through the exchange of water, heat, and greenhouse gases with the surrounding watershed (Krinner 2003, Tranvik et al. 2009). Such reciprocal influences of lakes on terrestrial ecosystems may be less appreciated or less relevant to those who study land-based habitats. In order to gain insights from cross-habitat comparisons, we summarized the effects of atmospheric N deposition between soils and lakes (Table 23).

C cycling

Nitrogen limitation of primary production in terrestrial ecosystems is widespread, as is co-limitation by N and P (Elser et al. 2007, LeBauer and

222

Treseder 2008). Atmospheric N deposition has been found to relieve N limitation, increasing primary production and C storage in temperate and boreal forests ecosystems (Magnani et al. 2007, Liu and Greaver 2009). In these forests, incremental C sequestration due to deposition is predominantly stored in above-ground biomass compared to soils (Townsend et al. 1996). Soil C is influenced by increased litter fall resulting from deposition-induced enhancement of net primary production and by slowed microbial decomposition of OM (De Vries et al. 2006). Decomposition is influenced by rates of microbial respiration and activity of extracellular enzymes that degrade humus. The responses of these processes to N enrichment vary both in magnitude and direction depending on ecosystem type. For example, N fertilization repressed soil respiration in certain temperate forests (e.g., Bowden et al. 2004, Burton et al. 2004) but increased soil respiration in others (e.g., Gallardo and Schlesinger 1994, Waldrop et al. 2004). Similarly, NO_3^- fertilization decreased the synthesis of lignin-degrading enzymes by certain types of soil fungi in sugar-maple forests, but increased it in oak forests (Grandy et al. 2008, Zak et al. 2008).

It is not clear how relevant terrestrial processes are as models for understanding N deposition effects on lake sediment OM. Except for shallow areas dominated by macrophytes, sediments generally lack the root-microbe interactions that are present in soils. Also, the environmental

conditions (moisture and temperature regime, oxygen concentrations)

differ greatly between sediments and soils. Lastly, the magnitude and

nature (e.g. litter versus dissolved forms) of terrestrially derived C inputs to

sediments deserve further consideration to fully compare responses of

sediment and soil processes to N enrichment.

In low productivity lakes, there is evidence that phytoplankton

communities experience N limitation (Elser et al. 2009), similar to many

terrestrial ecosystems (Elser et al. 2007). Consequently, N deposition has

increased concentrations of chlorophyll by a factor of ~3 and seston

(particulate C) by factor of 1.5 in the sampled lakes (Elser et al. 2009). To

the extent that N deposition increases primary production in the catchment

and water column, we would expect DOC concentrations to be elevated in

high-deposition lakes. Indeed, other studies found a positive relationship

between N deposition and DOC (Weyhenmeyer and Jeppesen 2009).

Surprisingly, the opposite was true for lakes we sampled in Norway; the

concentration of DOC in low-deposition lakes was two times greater than

that in high-deposition lakes (Table 22). There was no difference in DOC

between deposition regions for Colorado lakes. Dissolved organic carbon

is an important energy source for heterotrophic bacteria (Wetzel 2001)

and thus, enhanced bacterial activity could deplete DOC in the water

column of high-deposition lakes if bacteria are N limited. However, we

found no evidence that N deposition has influenced either the abundance

or nutrient status of bacterioplankton. For lakes in Norway, DOC concentrations were positively related to the proportion of bogs in the catchment (R^2 = 0.44, P < 0.0001). We do not know which factors influence DOC in Colorado lakes. Regardless, it appears that N deposition does not have a strong effect on DOC concentrations, in contrast to chlorophyll and seston C concentrations. There is potential for increases in seston C to increase sedimentation rates and, thus, sediment C content. We found no difference in bulk sediment C or OM contents between high- and low-deposition lakes. This finding was based on analyses of the homogenized sediments that were collected to a depth of 7 cm with a dredge. It is possible that finer-scale analysis of intact sediment cores would reveal differences in patterns of C accumulation between deposition regions.

In soils, N fertilization has been found to enhance CH_4 production by nearly 100% and reduce CH_4 oxidation (consumption) by 38% (Liu and Greaver 2009). The net effect of N on CH_4 emissions, however, may depend on a variety of factors, such as the type of N applied (NO_3^- or NH_4), soil water status, and whether methanotrophic bacteria are N limited (Bodelier and Laanbroek 2004). Interestingly, we observed a negative relationship between water NO_3^- concentrations and both sediment CH_4 production and surface water concentrations of CH_4. While we did not investigate the nutrient status of methanotrophs, it seems unlikely that N

225

limited the growth of these bacteria in high-deposition lake sediments because of chronic N inputs. Alternatively, it is possible that the presence of a strong oxidizer, such as NO_3^-, suppressed CH_4 production (Le Mer and Roger 2001). While other studies have focused on reservoirs as a source of CH_4 because of land use changes (St. Louis et al. 2000), further work is required to understand the effects of N enrichment on CH_4 dynamics in lentic ecosystems.

For sediments, we found that sediment respiration (CO_2 production) in assays that were amended with lake water was greater in low-deposition lakes compared to high-deposition lakes. There was a strong negative relationship between NO_3^- concentrations and CO_2 production, which suggests that N deposition may repress sediment bacterial respiration. In contrast, there was a strong positive effect of N deposition on denitrification rates, which also produces CO_2 (in ratios of 1.5 – 6 CO_2:N_2 (Groffman et al. 2006). Thus, the impact of N deposition on sediment CO_2 production may depend on the balance of oxic respiration and denitrification, although further investigation is needed. While microbial respiration and biomass are strongly related in soils (Treseder 2008), we found no relationship between the abundance of denitrifying bacteria and denitrification rates. In addition, there was no difference in the abundance of denitrifying bacteria between lakes in high- and low-deposition regions. Overall, it is not clear how sediment respiration

contributes to overall lake respiration. Concentrations of CO_2 in surface

water were the same across high- and low-deposition lakes, suggesting

that benthic and pelagic respiration, as a whole, has not been influenced

by N deposition. Nitrogen deposition appears to have the strongest effect

on concentrations of particulate C in water column and but not total

sediment C. Further research should investigate deposition-induced

changes in lake C cycling at broader spatial and temporal scales.

N cycling

Nitrogen enrichment of soils by atmospheric deposition has been

well documented (Fenn et al. 2003). Increased N assimilation by

vegetation results in increased soil N content though litter fall and

decomposition (McNulty et al. 1991, Baron et al. 2000). Similar processes

in lakes could influence the N content of water and sediments. N

deposition has increased total N, primarily as NO_3^-, decreased ratios of

seston C:N but not increased seston N concentrations (Elser et al. 2009,

Hessen et al. 2009, Weyhenmeyer and Jeppesen 2009). We did not

measure dissolved organic N (DON) but other studies have found it to be

more strongly related to catchment properties (e.g. elevation or tree cover)

than to N deposition (Ito et al. 2005, Hessen et al. 2009). Concentrations

of dissolved N_2O were greater in high-deposition lakes relative to low-

deposition lakes and positively related to NO_3^- concentrations. Analyses of

bulk sediments found no deposition-induced changes in sediment N

content or the ratio of C:N. Thus, N deposition has increased the total N content of lakes, but this effect appears to be limited to the water column.

Enhanced nitrification and denitrification as a result of fertilization has been well established in studies of agricultural ecosystems (Bouwman et al. 2005, Schlesinger 2009). Elevated nitrification and denitrification have also been observed in natural terrestrial ecosystems subject to N loading (Backman and Klemedtsson 2003, Seitzinger et al. 2006). In lake sediments, we found that denitrification was significantly greater in high-deposition relative to low-deposition rates and a strong, positive relationship between denitrification and hypolimnetic NO_3^- concentrations (Fig. 20).

Sediment nitrification rates, when given a non-limiting supply of ammonium (NH_4^+), were significantly greater in lakes from high-deposition regions compared to low-deposition regions. Such potential nitrification rates are not indicative of actual nitrification rates, which will be influenced by pH and concentrations of oC, oxygen, and NH_4^+ (Rysgaard et al. 1994, Strauss et al. 2002). A review of the literature found relatively few studies of nitrification in lakes compared to other ecosystems and even fewer studies of the effects of N loading on lake nitrification. This would be an interesting direction for future studies. Given the role that nitrification plays in accumulation of NO_3^- in Lake Superior (McManus et al. 2003, Finlay et al. 2007), for example, it is possible that this process contributes to low

ratios of DOC:NO_3^- observed in different aquatic ecosystems

(Weyhenmeyer and Jeppesen 2009, Taylor and Townsend 2010).

Conclusion

Both soils and sediments are composed of degraded OM, living

organisms, mineral particles, and water (Hedges and Oades 1997).

Despite such similarities, there are marked differences between these

substrata in terms of their physical properties, sources of materials and

resources, and rates of nutrient cycling. The research presented here

identified key contrasts and similarities between soil and sediment

responses to N deposition. Unlike soils, there was no evidence of

deposition-related changes to C or N contents of surface sediments.

Rather, such effects were more visible in the overlying lake water. As far

as similarities, N deposition does not appear to have influenced the

abundance of bacteria capable of NO_3^- and nitrite reduction, consistent

with studies that found no N deposition-induced reductions in soil bacteria.

In terms of bacterial processes, N loading increases rates of denitrification

and related N_2O production in both soils and sediments.

As land based N inputs are transported from soils to, lakes and

streams, and ultimately, to the ocean, denitrification plays an important

role in reducing N loading and eutrophication in downstream ecosystems

(Seitzinger et al. 2006). We found that lakes could be important

biogeochemical sinks for N along the land-to-ocean pathway. Sediments

possess considerable capacity to remove NO_3^- by denitrification, when

NO_3^- is available. Diffusion of NO_3^- to the active sites of denitrifying

enzymes will limit the role of NO_3^- removal by sediments. Consequently,

there is potential for managed lake ecosystems, such as reservoirs, to

retain more N than unmanaged lakes (Harrison et al. 2009) because of

differences in morphology and water residence time. Lentic water bodies

that are shallow or have long residence times may remove more NO_3^- than

those that are deep or have short residence times because of greater

contact between the water column and sediments (Kelly et al. 1987,

Weyhenmeyer et al. 2007). There may be trade-offs, however, between

NO_3^- removal and increased greenhouse gas production. Indeed, elevated

CO_2 and CH_4 emissions have been observed in reservoirs (St. Louis et al.

2000) and increased N_2O emissions are possible, as well. This research

provided a better understanding of the capacity of denitrification in lakes

that are subject to chronic N loading and the related dynamics of

greenhouse gases, primarily N_2O.

LITERATURE CITED

Backman, J. S. K. and A. K. Klemedtsson. 2003. Increased nitrification in acid coniferous forest soil due to high nitrogen deposition and liming. Scandinavian Journal of Forest Research **18**:514-524.

Baron, J. S., H. M. Rueth, A. M. Wolfe, K. R. Nydick, E. J. Allstott, J. T. Minear, and B. Moraska. 2000. Ecosystem responses to nitrogen deposition in the Colorado Front Range. Ecosystems:352-368.

Bodelier, P. L. E. and H. J. Laanbroek. 2004. Nitrogen as a regulatory factor of methane oxidation in soils and sediments. Fems Microbiology Ecology **47**:265-277.

Bouwman, A. F., G. Van Drecht, and K. W. Van der Hoek. 2005. Global and regional surface nitrogen balances in intensive agricultural production systems for the period 1970-2030. Pedosphere **15**:137-155.

Bowden, R. D., E. Davidson, K. Savage, C. Arabia, and P. Steudler. 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. Forest Ecology and Management **196**:43-56.

Burton, A. J., K. S. Pregitzer, J. N. Crawford, G. P. Zogg, and D. R. Zak. 2004. Simulated chronic NO3- deposition reduces soil respiration in northern hardwood forests. Global Change Biology **10**:1080-1091.

De Vries, W., G. J. Reinds, P. Gundersen, and H. Sterba. 2006. The impact of nitrogen deposition on carbon sequestration in European forests and forest soils. Global Change Biology **12**:1151-1173.

Driscoll, C. T., D. Whitall, J. Aber, E. Boyer, M. Castro, C. Cronan, C. L. Goodale, P. Groffman, C. Hopkinson, K. Lambert, G. Lawrence, and S. Ollinger. 2003. Nitrogen pollution in the northeastern United States: Sources, effects, and management options. Bioscience **53**:357-374.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters **10**:1-8.

Elser, J. J., M. Kyle, L. Steger, K. R. Nydick, and J. S. Baron. 2009. Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. Ecology **90**:3062-3073.

Fenn, M. E., J. S. Baron, E. B. Allen, H. M. Rueth, K. R. Nydick, L. Geiser, W. D. Bowman, J. O. Sickman, T. Meixner, D. W. Johnson, and P. Neitlich. 2003. Ecological effects of nitrogen deposition in the western United States. Bioscience **53**:404-420.

Finlay, J. C., R. W. Sterner, and S. Kumar. 2007. Isotopic evidence for in-lake production of accumulating nitrate in Lake Superior. Ecological Applications **17**:2323-2332.

Gallardo, A. and W. H. Schlesinger. 1994. Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biology & Biochemistry **26**:1409-1415.

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

Grandy, A. S., R. L. Sinsabaugh, J. C. Neff, M. Stursova, and D. R. Zak. 2008. Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. Biogeochemistry **91**:37-49.

Groffman, P. M., M. A. Altabet, J. K. Bohlke, K. Butterbach-Bahl, M. B. David, M. K. Firestone, A. E. Giblin, T. M. Kana, L. P. Nielsen, and M. A. Voytek. 2006. Methods for measuring denitrification: Diverse

approaches to a difficult problem. Ecological Applications **16**:2091-2122.

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

Hedges, J. I. and J. M. Oades. 1997. Comparative organic geochemistries of soils and marine sediments. Organic Geochemistry **27**:319-361.

Hessen, D. O., T. Andersen, S. Larsen, B. L. Skjelkvale, and H. A. de Wit. 2009. Nitrogen deposition, catchment productivity, and climate as determinants of lake stoichiometry. Limnology and Oceanography **54**:2520-2528.

IPCC. 2006. Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme.*in* H. S. Eggleston, L. Buendia, K. Miwa, T. Ngara, and K. Tanabe, editors. IGES, Hayama, Japan.

Ito, M., M. J. Mitchell, C. T. Driscoll, and K. M. Roy. 2005. Nitrogen input–output budgets for lake-containing watersheds in the Adirondack region of New York Biogeochemistry **72**:283-314.

Kelly, C. A., J. W. M. Rudd, R. H. Hesslein, D. W. Schindler, C. T. Dillon, C. T. Driscoll, S. A. Gherini, and R. E. Hecky. 1987. Prediction of biological acid neutralization in acid-sensitive lakes. Biogeochemistry **3**:129-140.

Krinner, G. 2003. Impact of lakes and wetlands on boreal climate. Journal of Geophysical Research-Atmospheres **108**:4520, doi, 4510.1029/2002jd002597.

Le Mer, J. and P. Roger. 2001. Production, oxidation, emission and consumption of methane by soil: a review. European Journal of Soil Biology **37**:25-50.

LeBauer, D. S. and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology **89**:371-379.

Liu, L. and T. L. Greaver. 2009. A review of nitrogen enrichment effects on three biogenic GHGs: the CO_2 sink may be largely offset by stimulated N_2O and CH_4 emissions. Ecology Letters **12**:1103-1117.

Magnani, F., M. Mencuccini, M. Borghetti, P. Berbigier, F. Berninger, S. Delzon, A. Grelle, P. Hari, P. G. Jarvis, P. Kolari, A. S. Kowalski, H. Lankreijer, B. E. Law, A. Lindroth, D. Loustau, G. Manca, J. B. Moncrieff, M. Rayment, V. Tedeschi, R. Valentini, and J. Grace. 2007. The human footprint in the carbon cycle of temperate and boreal forests. Nature **447**:848-850.

McManus, J., E. A. Heinen, and M. M. Baehr. 2003. Hypolimnetic oxidation rates in Lake Superior: Role of dissolved organic material on the lake's carbon budget. Limnology and Oceanography **48**:1624-1632.

McNulty, S. G., J. D. Aber, and R. D. Boone. 1991. Spatial changes in forest floor and foliar chemistry of spruce-fir forests across New-England. Biogeochemistry **14**:13-29.

Menge, B. A., F. Chan, S. Dudas, D. Eerkes-Megrano, K. Grorud-Colvert, K. Heiman, M. Hessing-Lewis, A. Iles, R. Milston-Clements, M. Noble, K. Page-Albins, E. Richmond, G. Rilov, J. Rose, J. Tyburczy, L. Vinueza, and P. Zarnetske. 2009. Terrestrial ecologists ignore aquatic literature: Asymmetry in citation breadth in ecological publications and implications for generality and progress in ecology. Journal of Experimental Marine Biology and Ecology **377**:93-100.

Mulholland, P. J., A. M. Helton, G. C. Poole, R. O. Hall, S. K. Hamilton, B. J. Peterson, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, W. K. Dodds, S. E. G. Findlay, S. V. Gregory, N. B. Grimm, S. L. Johnson, W. H. McDowell, J. L. Meyer, H. M. Valett, J. R. Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, L. T. Johnson, B. R. Niederlehner, J. M. O'Brien, J. D. Potter, R. W. Sheibley, D. J. Sobota, and S. M. Thomas. 2008.

Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature **452**:202-206.

Rysgaard, S., N. Risgaard-Petersen, N. P. Sloth, K. Jensen, and L. P. Nielsen. 1994. Oxygen regulation of nitrification and denitrification in sediments. Limnology and Oceanography **39**:1643-1652.

Saunders, D. L. and J. Kalff. 2001. Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia **443**:205-212.

Schlesinger, W. H. 2009. On the fate of anthropogenic nitrogen. PNAS **106**:203-208.

Seitzinger, S. P., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, and C. Tobias. 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecological Applications **16**:2064-2090.

Sickman, J. O., J. M. Melack, and J. L. Stoddard. 2002. Regional analysis of inorganic nitrogen yield and retention in high-elevation ecosystems of the Sierra Nevada and Rocky Mountains. Biogeochemistry **57**:341-374.

St. Louis, V. L., C. A. Kelly, E. Duchemin, J. W. M. Rudd, and D. M. Rosenberg. 2000. Reservoir surfaces as sources of greenhouse gases to the atmopshere: a global estimate. Bioscience **50**:766-775.

Strauss, E. A., N. L. Mitchell, and G. A. Lamberti. 2002. Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH. Canadian Journal of Fisheries and Aquatic Sciences **59**:554-563.

Taylor, P. G. and A. R. Townsend. 2010. Stoichiometric control of organic carbon-nitrate relationships from soils to the sea. Nature **464**:1178-1181.

Townsend, A. R., B. H. Braswell, E. A. Holland, and J. E. Penner. 1996. Spatial and temporal patterns in terrestrial carbon storage due to deposition of fossil fuel nitrogen. Ecological Applications **6**:806-814.

Tranvik, L. J., J. A. Downing, J. B. Cotner, S. A. Loiselle, R. G. Striegl, T. J. Ballatore, P. Dillon, K. Finlay, K. Fortino, L. B. Knoll, P. L. Kortelainen, T. Kutser, S. Larsen, I. Laurion, D. M. Leech, S. L. McCallister, D. M. McKnight, J. M. Melack, E. Overholt, J. A. Porter, Y. Prairie, W. H. Renwick, F. Roland, B. S. Sherman, D. W. Schindler, S. Sobek, A. Tremblay, M. J. Vanni, A. M. Verschoor, E. von Wachenfeldt, and G. A. Weyhenmeyer. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. Limnology and Oceanography **54**:2298-2314.

Treseder, K. K. 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters **11**:1111-1120.

Waldrop, M. P., D. R. Zak, and R. L. Sinsabaugh. 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology & Biochemistry **36**:1443-1451.

Wetzel, R. G. 2001. Limnology: Lake and River Ecosystems. Academic Press, San Diego, CA, USA.

Weyhenmeyer, G. A. and E. Jeppesen. 2009. Nitrogen deposition induced changes in $DOC:NO_3$-N ratios determine the efficiency of nitrate removal from freshwaters. Global Change Biology:doi: 10.1111/j.1365-2486.2009.02100.x.

Weyhenmeyer, G. A., E. Jeppesen, R. Adrian, L. Arvola, T. Blenckner, T. Jankowski, E. Jennings, P. Noges, T. Noges, and D. Straile. 2007. Nitrate-depleted conditions on the increase in shallow northern European lakes. Limnology and Oceanography **52**:1346-1353.

Zak, D. R., W. E. Holmes, A. J. Burton, K. S. Pregitzer, and A. F. Talhelm. 2008. Similated atmopsheric NO_3^- deposition increases soil organic matter by slowing decomposition. Ecological Applications **18**:2016-2027.

Table 23. Effects of atmospheric N deposition on lake water and sediment characteristics. High- and low-deposition lakes are compared by region and overall; non-significant differences indicated by n.s.

Region Deposition Level	Number of lakes		Water			Sediment			
			NO_3 µM	DOC µM	DOC:NO_3^-	Total C mmol g^{-1}	Total N mmol g^{-1}	Total P mmol g^{-1}	OM content
Norway									
High (10-20 kg ha^{-1} y^{-1})	16	Mean	22.8	128.9	7.5	11.9	0.8	0.2	0.3
		s.e.	3.0	13.0	1.2	1.0	0.1	0.0	0.0
Low (<4 kg ha^{-1} y^{-1})	16	Mean	0.1	287.3	6427	11.4	0.7	0.1	0.3
		s.e.	0.0	123.1	3093	1.4	0.1	0.0	0.0
		P	0.0001	0.0005	0.0001	n.s.	n.s.	n.s.	n.s.
Colorado									
High (5-8 kg ha^{-1} y^{-1})	10	Mean	11.3	497.6	63	9.8	0.8	0.1	0.2
		s.e.	1.3	67.0	19	1.8	0.1	0.0	0.0
Low (<2 kg ha^{-1} y^{-1})	10	Mean	3.3	791.8	852	6.5	0.5	0.1	0.1
		s.e.	1.0	157.1	403	1.2	0.1	0.0	0.0
		P	0.0001	n.s.	0.002	n.s.	n.s.	n.s.	n.s.
Total									
High	26	Mean	18.4	270.7	29	9.5	0.8	0.1	0.2
		s.e.	2.2	44.3	9.0	1.1	0.1	0.0	0.0
Low	26	Mean	1.2	481.4	4283	11.1	0.7	0.1	0.2
		s.e.	0.5	78.6	733	0.9	0.1	0.0	0.0
		P	0.0001	0.01	0.0001	n.s.	n.s.	n.s.	n.s.

Table 23, continued.

			Sediment Processes		
Region Deposition Level	Number of lakes		N_2O-N µmol m^{-2} h^{-1}	Denitrification µmol N m^{-2} h^{-1}	Denitrification Potential µmol N kg^{-1} h^{-1}
Norway					
High	16	mean	1.1	41.7	31.4
(10-20 kg ha^{-1} y^{-1})		s.e.	0.4	7.4	7.8
Low	16	mean	0.0	0.2	20.4
(<4 kg ha^{-1} y^{-1})		s.e.	0.0	0.2	5.2
		P	0.0022	0.0001	n.s.
Colorado					
High	10	mean	0.8	7.9	753.6
(5-8 kg ha^{-1} y^{-1})		s.e.	0.7	6.4	188.8
Low	10	mean	0.1	0.5	535.0
(<2 kg ha^{-1} y^{-1})		s.e.	0.1	0.2	134.6
		P	n.s.	n.s.	n.s.
Total					
High	26	mean	1.0	28.7	309.2
		s.e.	0.4	5.9	96.9
Low	26	mean	0.1	0.3	218.3
		s.e.	0.0	0.1	69.1
		P	0.0003	0.0001	n.s.

238

Table 24. Comparison of N deposition effects on soil and lake (sediment and pelagic) habitats.

	Soil	Sediment	Pelagic
Substrate C	Increased soil organic C in N fertilized plots in Scandinavian, NE USA forests (Hyvonen 2008, Zak 2008, DeVries 2006) Increased C storage in forests (Liu 2009).	No effect of N deposition on sediment C content.	N deposition increased chlorophyll, seston C (Elser 2009, Bergstrom 2006). Decreased dissolved organic C (this study) but increased DOC in other studies (Wehenmayer 2009).
Substrate N	Increased soil N (Zak 2008, Fenn 2003, Baron 2000, McNulty 1991). Fertilization effect depended on forest tree species (Lovett 1999).	No effect of N deposition on sediment N content.	N deposition increased dissolved inorganic N, increased seston N (Elser 2009).
Substrate C:N	Decreased soil C:N in high-elevation Colorado ecosystems and NE USA forests receiving N deposition (Baron 2000, Aber 2003, McNulty 1991).	No effect of N deposition on sediment C:N.	N deposition decreased seston C:N.

Table 24, continued.

	Soil	Sediment	Pelagic
Bacterial abundance	Meta-analysis of bacterial-specific studies found no change in biomass. Across all studies microbial biomass declined 15%. (Treseder 2008).	No effect of N deposition on abundance of NO_3^-- and NO_2^--reducing bacteria.	No effect of N deposition on bacterioplankton abundance.
Respiration (CO_2 production)	Meta-analysis found respiration was positively correlated with microbial biomass. If N fertilization decreased biomass, respiration decreased as well. (Treseder 2008). Increased respiration in non-forest ecosystems (Liu 2009).	Negative effect of N deposition on respiration in Colorado lakes sediments (when amended with lake water), no effect of N deposition on Norwegian lake sediments (when amended with de-ionized water).	Not directly, measured, but no effect of N deposition on dissolved CO_2 in surface water.
Nitrification	N fertilization increased net nitrification (Zak 2006, Aber 2003, McNulty 1991). Positive N deposition effect depended on forest tree species (Lovett 1999).	N deposition increased nitrification potential.	Elevated dissolved N_2O concentrations in eutrophic lakes attributed to nitrification (Mengis 1997).

Table 24, continued.

	Soil	Sediment	Pelagic
Denitrification	N loading increased denitrification (Seitzinger 2006, Barton 1999).	N deposition increased denitrification. No effect of N deposition on potential denitrification.	
N_2O production	N fertilization increased N_2O production (Liu 2009, Forster 2007).	N deposition increased sediment N_2O production.	Elevated dissolved N_2O concentrations eutrophic lakes attributed to nitrification (Mengis 1997).
CH_4 production	N fertilization increased CH_4 production (Liu 2009).	N deposition decreased sediment CH_4 production.	

Figure 20. Relationship between denitrification and NO_3^- concentrations in slurries amended with lake water that was collected just above the sediments. This relationship was only significant for Norwegian lakes ($R^2 = 0.86$, P < 0.0001).
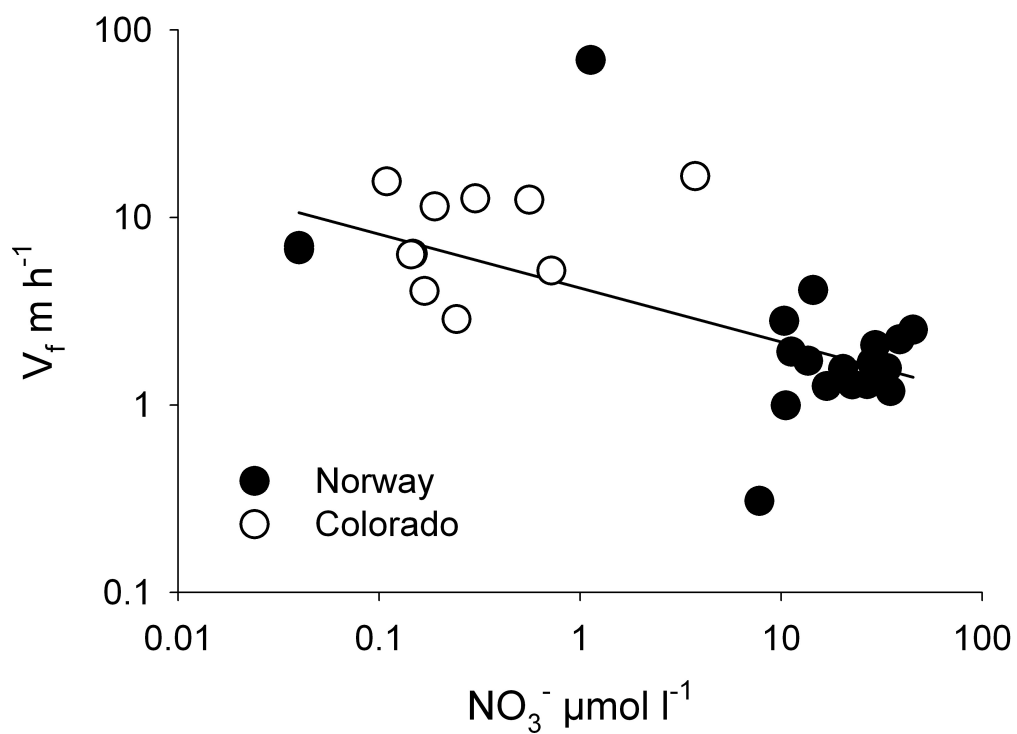
Figure 21. Relationship between piston velocity (estimated as denitrification rate/NO_3^-) and NO_3^- concentrations in slurries amended with lake water that was collected just above the sediments. This relationship was only significant for Norwegian lakes (R^2 = 0.53, P < 0.0004).

Figure 22. Relationship between rate of denitrification potential and NO_3^- concentration in water that was collected just above the sediments.
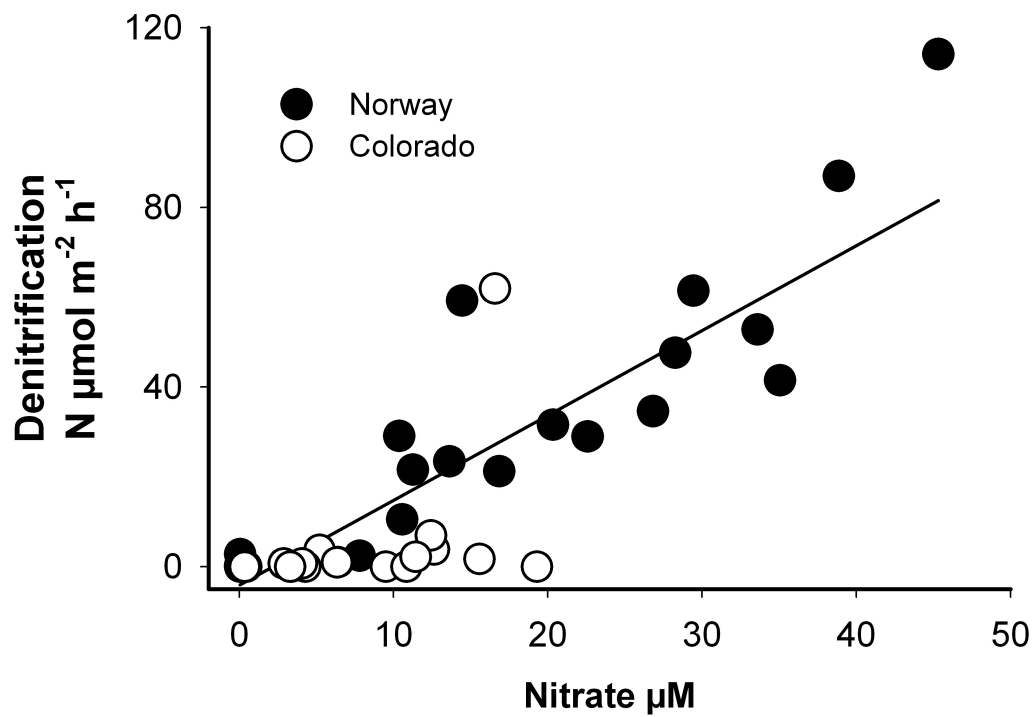
Figure 23. Relationship between sediment N_2O production and NO_3^- concentrations in slurries amended with lake water that was collected just above the sediments. The relationship was only significant for Norwegian lakes ($R^2 = 0.45$, $P < 0.0001$).
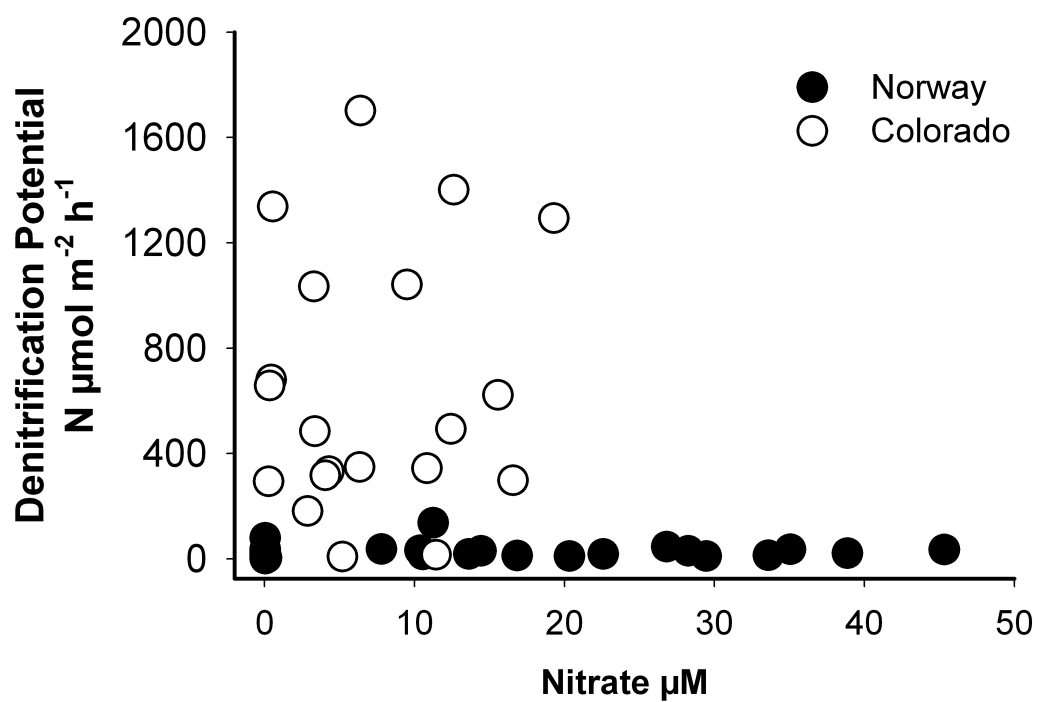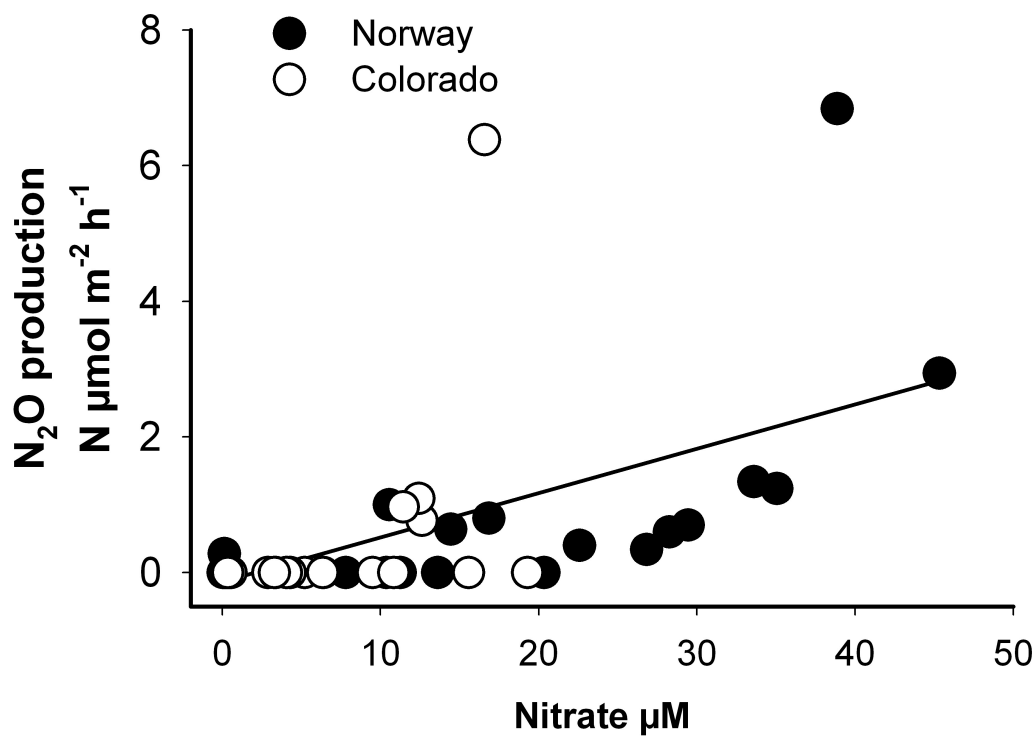
LITERATURE CITED

LITERATURE CITED

Abell, J., A. M. Laverman, and P. Van Cappellen. 2009. Bioavailability of organic matter in a freshwater estuarine sediment: long-term degradation experiments with and without nitrate supply. Biogeochemistry 94:13-28.

Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forests; hypotheses revised. Bioscience 48:921-934.

Adrian, R., C. M. O'Reilly, H. Zagarese, S. B. Baines, D. O. Hessen, W. Keller, D. M. Livingstone, R. Sommaruga, D. Straile, E. Van Donk, G. A. Weyhenmeyer, and M. Winder. 2009. Lakes as sentinels of climate change. Limnology and Oceanography 54:2283-2297.

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

Algesten, G., S. Sobek, A. K. Bergstrom, A. Jonsson, L. J. Tranvik, and M. Jansson. 2005. Contribution of sediment respiration to summer CO_2 emission from low productive boreal and subarctic lakes. Microbial Ecology 50:529-535.

APHA. 2005. Standard methods for the examination of water and wastewater, 21st edition. American Public Health Association, Washington, D.C.

Backman, J. S. K. and A. K. Klemedtsson. 2003. Increased nitrification in acid coniferous forest soil due to high nitrogen deposition and liming. Scandinavian Journal of Forest Research 18:514-524.

Balser, T. C. and M. K. Firestone. 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry 73:395-415.

Baron, J. S. and D. H. Campbell. 1997. Nitrogen fluxes in a high elevation Colorado Rocky Mountain basin. Hydrological Processes 11:783-799.

Baron, J. S., H. M. Rueth, A. M. Wolfe, K. R. Nydick, E. J. Allstott, J. T. Minear, and B. Moraska. 2000. Ecosystem responses to nitrogen deposition in the Colorado Front Range. Ecosystems:352-368.

Bastviken, D., J. Cole, M. Pace, and L. Tranvik. 2004. Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. Global Biogeochemical Cycles 18:GB4009, doi:4010.1029/2004GB002238.

Bastviken, D., J. J. Cole, M. L. Pace, and M. C. Van de Bogert. 2008. Fates of methane from different lake habitats: Connecting whole-lake budgets and CH_4 emissions. Journal of Geophysical Research-Biogeosciences 113:G02024, doi:02010.01029/02007JG000608.

Beaulieu, J. J., C. P. Arango, S. K. Hamilton, and J. L. Tank. 2008. The production and emission of nitrous oxide from headwater streams in the Midwestern United States. Global Change Biology 14:878-894.

Berge, D., E. Fjeld, A. Hindar, and O. Kaste. 1997. Nitrogen retention in two Norwegian watercourses of different trophic status. Ambio 26:282-288.

Bergstrom, A. K., P. Blomqvist, and M. Jansson. 2005. Effects of atmospheric nitrogen deposition on nutrient limitation and phytoplankton biomass in unproductive Swedish lakes. Limnology and Oceanography 50:987-994.

Bergstrom, A. K. and M. Jansson. 2006. Atmospheric nitrogen deposition has caused nitrogen enrichment and eutrophication of lakes in the northern hemisphere. Global Change Biology 12:635-643.

246

Bertoni, R., C. Callieri, E. Balseiro, and B. Modenutti. 2008. Susceptibility of bacterioplankton to nutrient enrichment of oligotrophic and ultraoligotrophic lake waters. Journal of Limnology 67:120-127.

Betlach, M. R. and J. M. Tiedje. 1981. Kinetic explanation for accumulation of nitrite, nitric-oxide, and nitrous-oxide during bacterial denitrification. Applied and Environmental Microbiology 42:1074-1084.

Bernot, M. J. and W. K. Dodds. 2005. Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems 8:442-453.

Birks, H. J. B., V. J. Jones, and N. L. Rose. 2004. Recent environmental change and atmospheric contamination on Svalbard as recorded in lake sediments. Journal of Paleolimnology 31.

Bodelier, P. L. E. and H. J. Laanbroek. 2004. Nitrogen as a regulatory factor of methane oxidation in soils and sediments. Fems Microbiology Ecology 47:265-277.

Bouwman, A. F. and L. M. Boumans. 2002. Emissions of N_2O and NO from fertilized fields: Summary of available measurement data. Global Biogeochemical Cycles 16:1058, doi:1010.1029/2001GB001811.

Bouwman, A. F., G. Van Drecht, and K. W. Van der Hoek. 2005. Global and regional surface nitrogen balances in intensive agricultural production systems for the period 1970-2030. Pedosphere 15:137-155.

Bowden, R. D., E. Davidson, K. Savage, C. Arabia, and P. Steudler. 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. Forest Ecology and Management 196:43-56.

Brady, N. C. and R. R. Weil. 2002. The Nature and Properties of Soils. Prentice Hall, Upper Saddle River, NJ, USA.

Burns, D. A. 2004. The effects of atmospheric nitrogen deposition in the Rocky Mountains of Colorado and southern Wyoming, USA - a critical review. Environmental Pollution 127:257-269.

Burton, A. J., K. S. Pregitzer, J. N. Crawford, G. P. Zogg, and D. R. Zak. 2004. Simulated chronic NO_3- deposition reduces soil respiration in northern hardwood forests. Global Change Biology 10:1080-1091.

Campbell, D. H., C. Kendall, C. C. Y. Chang, S. R. Silva, and K. A. Tonnessen. 2002. Pathways for nitrate release from an alpine watershed: Determination using delta N-15 and delta O-18. Water Resources Research 38:9.

Carlsson, P. and D. A. Caron. 2001. Seasonal variation of phosphorus limitation of bacterial growth in a small lake. Limnology and Oceanography 46:108-120.

Carson, R. T. and R. C. Mitchell. 1993. The value of clean water: the public's willingness-to-pay for boatable, fishable, and swimmable quality water. Water Resources Research 29:2445-2454.

Cavigelli, M. A. and G. P. Robertson. 2000. The functional significance of denitrifier community composition in a terrestrial ecosystem. Ecology 81:1402-1414.

Cavigelli, M. A. and G. P. Robertson. 2001. Role of denitrifier diversity in rates of nitrous oxide consumption in a terrestrial ecosystem. Soil Biology & Biochemistry 33:297-310.

Cetin, M. and A. Erar. 2006. A simulation study on classic and robust variable selection in linear regression. Applied Mathematics and Computation 175:1629-1643.

Chrzanowski, T. H., M. Kyle, J. J. Elser, and R. W. Sterner. 1996. Element ratios and growth dynamics of bacteria in an oligotrophic Canadian shield lake. Aquatic Microbial Ecology 11:119-125.

Chu, H. Y., T. Fujii, S. Morimoto, X. G. Lin, K. Yagi, J. L. Hu, and J. B. Zhang. 2007. Community structure of ammonia-oxidizing bacteria under long-term application of mineral fertilizer and organic manure in a sandy loam soil. Applied and Environmental Microbiology 73:485-491.

Cleveland, C. C., A. R. Townsend, and S. K. Schmidt. 2002. Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. Ecosystems 5:680-691.

Cleveland, C. C. and A. R. Townsend. 2006. Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. Proceedings of the National Academy of Sciences of the United States of America 103:10316-10321.

Clow, D. W., J. O. Sickman, R. G. Striegl, D. P. Krabbenhoft, J. G. Elliott, M. Dornblaser, D. A. Roth, and D. H. Campbell. 2003. Changes in the chemistry of lakes and precipitation in high-elevation national parks in the western United States, 1985-1999. Water Resources Research 39.

Cole, J., S. Findlay, and M. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Marine Ecology-Progress Series 43:1-13.

Cole, J. J., N. F. Caraco, G. W. Kling, and T. K. Kratz. 1994. Carbon dioxide supersaturation in the surface waters of lakes. Science 265:1568-1570.

Cole, J. J. and N. F. Caraco. 1998. Atmospheric exchange of carbon dioxide in a low-wind oligotrophic lake measured by the addition of SF_6. Limnology and Oceanography 43:647-656.

Colores, G. M., S. K. Schmidt, and M. C. Fisk. 1996. Estimating the biomass of microbial functional groups using rates of growth-related soil respiration. Soil Biology & Biochemistry 28:1569-1577.

Conley, D. J., H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, K. E. Havens, C. Lancelot, and G. E. Likens. 2009. Controlling Eutrophication: Nitrogen and Phosphorus. Science 323:1014-1015.

Corstanje, R., K. R. Reddy, and K. M. Portier. 2007. Soil microbial ecophysiology of a wetland recovering from phosphorus eutrophication. Wetlands 27:1046-1055.

Cotner, J. B., J. W. Ammerman, E. R. Peele, and E. Bentzen. 1997. Phosphorus-limited bacterioplankton growth in the Sargasso Sea. Aquatic Microbial Ecology 13:141-149.

Cotner, J. B. and B. A. Biddanda. 2002. Small players, large role: Microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems 5:105-121.

DeForest, J. L., D. R. Zak, K. S. Pregitzer, and A. J. Burton. 2004. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Science Society of America Journal 68:132-138.

Denmen, K. L., G. Brasseur, A. Chidthaisong, P. Ciais, P. M. Cox, R. E. Dickinson, D. Hauglustaine, C. Heinze, E. A. Holland, D. Jacob, U. Lohmann, S. Ramachandran, P. L. da Silva Dias, S. C. Wofsy, and X. Zhang. 2007. Couplings between changes in the climate system and biogeochemistry.in S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, USA.

Dentener, F. J. 2006. Global maps of atmospheric nitrogen deposition, 1860, 1993, amd 2050. Data set. Available on-line [http://www.daac.ornl.gov] from Oak Ridge National Laboratory Distributed Active Archive Center, Oak Ridge, Tennessee, USA.

Desnues, C., V. D. Michotey, A. Wieland, C. Zhizang, A. Fourcans, R. Duran, and P. C. Bonin. 2007. Seasonal and diel distributions of denitrifying and bacterial communities in a hypersaline microbial mat (Camargue, France). Water Research 41:3407-3419.

De Vries, W., G. J. Reinds, P. Gundersen, and H. Sterba. 2006. The impact of nitrogen deposition on carbon sequestration in European forests and forest soils. Global Change Biology 12:1151-1173.

Dodds, W. K., W. W. Bouska, J. L. Eitzmann, T. J. Pilger, K. L. Pitts, A. J. Riley, J. T. Schloesser, and D. J. Thornbrugh. 2009. Eutrophication of U.S. freshwaters: analysis of potential economic damages. Environmental Science & Technology 43:12-19.

Dong, L. F., C. O. Thornton, D. B. Nedwell, and G. J. C. Underwood. 2000. Denitrification in sediments of the River Colne estuary, England. Marine Ecology-Progress Series 203:109-122.

Driscoll, C. T., D. Whitall, J. Aber, E. Boyer, M. Castro, C. Cronan, C. L. Goodale, P. Groffman, C. Hopkinson, K. Lambert, G. Lawrence, and S. Ollinger. 2003. Nitrogen pollution in the northeastern United States: Sources, effects, and management options. Bioscience 53:357-374.

Dunfield, P., R. knowles, R. Dumont, and T. R. Moore. 1993. Methane production and consumption in temperate and subarctic peat soils: Response to temperature and pH. Soil Biology and Biochemistry 25:321-326.

Earl, S. R., H. M. Valett, and J. R. Webster. 2006. Nitrogen saturation in stream ecosystems. Ecology 87:3140-3151.

Elser, J. J., L. B. Stabler, and R. P. Hassett. 1995. Nutrient limitation of bacterial growth and rates of bacterivory in lakes and oceans - a comparative study. Aquatic Microbial Ecology 9:105-110.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10:1-8.

Elser, J. J., M. Kyle, L. Steger, K. R. Nydick, and J. S. Baron. 2009. Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. Ecology 90:3062-3073.

Elser, J. J., T. Andersen, J. S. Baron, A. K. Bergstrom, M. Jansson, M. Kyle, K. R. Nydick, L. Steger, and D. O. Hessen. 2009. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science 326:835-837.

Elser, J. J., A. Peace, M. Kyle, M. Wojewodzic, M. L. McCrackin, T. Andersen, and D. O. Hessen. In press. Atmospheric nitrogen deposition is associated with elevated phosphorus limitation of lake zooplankton. Ecology Letters.

Fenn, M. E., R. Haeuber, G. S. Tonnesen, J. S. Baron, S. Grossman-Clarke, D. Hope, D. A. Jaffe, S. Copeland, L. Geiser, H. M. Rueth, and J. O. Sickman. 2003. Nitrogen emissions, deposition, and monitoring in the western United States. Bioscience 53:391-403.

Fenn, M. E., J. S. Baron, E. B. Allen, H. M. Rueth, K. R. Nydick, L. Geiser, W. D. Bowman, J. O. Sickman, T. Meixner, D. W. Johnson, and P. Neitlich. 2003. Ecological effects of nitrogen deposition in the western United States. Bioscience 53:404-420.

Finlay, J. C., R. W. Sterner, and S. Kumar. 2007. Isotopic evidence for in-lake production of accumulating nitrate in Lake Superior. Ecological Applications 17:2323-2332.

Firestone, M. K. and J. M. Tiedje. 1979. Temporal change in nitrous-oxide and dinitrogen from denitrification following onset of anaerobiosis. Applied and Environmental Microbiology 38:673-679.

Firestone, M. K., M. S. Smith, R. B. Firestone, and J. M. Tiedje. 1979. Influence of nitrate, nitrite, and oxygen on the composition of the gaseous products of denitrification in soil. Soil Science Society of America Journal 43:1140-1144.

Firestone, M. K., R. B. Firestone, and J. M. Tiedje. 1980. Nitrous oxide from soil denitrification: factors controlling its biological production. Science 208:749-751.

Fisher, M. M., J. L. Klug, G. Lauster, M. Newton, and E. W. Triplett. 2000. Effects of resource and trophic interactions on freshwater bacterioplankton diversity. Microbial Ecology 40:125-138.

Forster, P., V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D. W. Fahey, J. Haywood, J. Lean, D. C. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, M. Schulz, and R. Van Dorland. 2007. Changes in atmospheric constituents and in radiative forcing.in S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Climate Change 2007: the Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK; New York, NY, USA.

Gallardo, A. and W. H. Schlesinger. 1994. Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biology & Biochemistry 26:1409-1415.

Galloway, J. N. 1998. The global nitrogen cycle: changes and consequences. Environmental Pollution 201:15-24.

Galloway, J. N., J. D. Aber, J. W. Erisman, S. P. Seitzinger, R. W. Howarth, E. B. Cowling, and B. J. Cosby. 2003. The nitrogen cascade. Bioscience 53:341-356.

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

Garcia-Ruiz, R., S. N. Pattinson, and B. A. Whitton. 1998. Denitrification in river sediments: relationship between process rate and properties of water and sediment. Freshwater Biology 39:467-476.

Gnankambary, Z., U. Stedt, G. Nyberg, V. Hien, and A. Malmer. 2008. Nitrogen and phosphorus limitation of soil microbial respiration in two tropical agroforestry parklands in the south-Sudanese zone of Burkina Faso: The effects of tree canopy and fertilization. Soil Biology & Biochemistry 40:350-359.

Grandy, A. S., R. L. Sinsabaugh, J. C. Neff, M. Stursova, and D. R. Zak. 2008. Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. Biogeochemistry 91:37-49.

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

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

Gundersen, P., I. K. Schmidt, and K. Raulund-Rasmussen. 2006. Leaching of nitrate from temperate forests - effects of air pollution and forest management. Environmental Reviews 14:1-57.

Håkanson L. and M. Jansson. 2002. Principles of Lake Sedimentology. Blackburn Press, Caldwell, NJ, USA.

Hansen, M. R., J. R. DeFries, M. Townshend, M. Carroll, C. Dimiceli, and R. Sohlberg. 2003. Vegetation Continuous Fields MOD44B. 2001 Percent Tree Cover, Collection 3. University of Maryland, College Park, Maryland.

Hanson, G. C., P. M. Groffman, and A. J. Gold. 1994. Symptoms of nitrogen saturation in a riparian wetland. Ecological Applications 4:750-756.

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

Hart, S. C., J. M. Stark, E. A. Davidson, and M. K. Firestone. 1994. Nitrogen mineralization, immobilization, and nitrification. Methods of Soil Analysis, Part 2, Microbial and Biochemical Properties. Soil Science Socity of America, Madison, WI.

Hedges, J. I. and J. M. Oades. 1997. Comparative organic geochemistries of soils and marine sediments. Organic Geochemistry 27:319-361.

Henriksen, A., A. Hindar, D. O. Hessen, and O. Kaste. 1997. Contribution of nitrogen to acidity in the Bjerkreim river in southwestern Norway. Ambio 26:304-311.

Hessen, D. O., T. Andersen, S. Larsen, B. L. Skjelkvale, and H. A. de Wit. 2009. Nitrogen deposition, catchment productivity, and climate as determinants of lake stoichiometry. Limnology and Oceanography 54:2520-2528.

Hole, L. L. and K. Tørseth. 2002. Deposition of major inorganic compounds in Norway 1978-1982 and 1997-2001: status and trends. Norwegian Ministry of the Environment.

Hole, L.L. and M. Engardt. 2008. Climate change impact on atmospheric nitrogen deposition in northwestern Europe: A model study. Ambio 37:9-17.

Holmgren, S. U., C. Bigler, O. Ingolfsson, and A. H. Wolfe. 2010. The Holocene–Anthropocene transition in lakes of western Spitsbergen, Svalbard (Norwegian High Arctic): climate change and nitrogen deposition. Journal of Paleolimnology 43:393-412.

Huttunen, J. T., J. Alm, A. Liikanen, S. Juutinen, T. Larmola, T. Hammar, J. Silvola, and P. J. Martikainen. 2003. Fluxes of methane, carbon dioxide and nitrous oxide in boreal lakes and potential anthropogenic effects on the aquatic greenhouse gas emissions. Chemosphere 52:609-621.

IPCC. 2006. Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme.in H. S. Eggleston, L. Buendia, K. Miwa, T. Ngara, and K. Tanabe, editors. IGES, Hayama, Japan.

IPCC. 2007. Climate Change 2007: The physical basis - summary for policy makers. World Meteorological Organization/United Nations Environmental Programme, Paris.

Ito, M., M. J. Mitchell, C. T. Driscoll, and K. M. Roy. 2005. Nitrogen input–output budgets for lake-containing watersheds in the Adirondack region of New York. Biogeochemistry 72:283-314.

Jensen, H. B., K. S. Jorgensen, and J. Sorensen. 1984. Diurnal-variation of nitrogen cycling in coastal, marine-sediments: nitrous-oxide emission. Marine Biology 83:177-183.

Johnson, R. A. and D. W. Wichern. 2007. Applied Multivariate Statistical Analysis. Pearson Prentice Hall, Upper Saddle River, NJ, USA.

Johnson, S. L., S. Neuer, and F. Garcia-Pichel. 2007. Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands. Environmental Microbiology 9:680-689.

Juutinen, S., M. Rantakari, P. Kortelainen, J. T. Huttunen, T. Larmola, J. Alm, J. Silvola, and P. J. Martikainen. 2009. Methane dynamics in different boreal lake types. Biogeosciences 6:209-223.

Kaste, Ø., J. L. Stoddard, and A. Henriksen. 2003. Implication of lake water residence time on the classification of Norwegian surface water sites into progressive stages of nitrogen saturation. Water Air and Soil Pollution 142:409-424.

Kaste, Ø. and A. Lyche-Solheim. 2005. Influence of moderate phosphate addition on nitrogen retention in an acidic boreal lake. Canadian Journal of Fisheries and Aquatic Sciences 62:312-321.

Kelly, C. A., J. W. M. Rudd, R. H. Hesslein, D. W. Schindler, C. T. Dillon, C. T. Driscoll, S. A. Gherini, and R. E. Hecky. 1987. Prediction of biological acid neutralization in acid-sensitive lakes. Biogeochemistry 3:129-140.

Kent, H. C. and K. W. Porter, editors. 1980. Colorado Geology. Rocky Mountain Association of Geologists, Denver.

Klein, H. and A. Benedictow. 2006. Transboundary air pollution by main pollutants (S, N, O3) and PM: Norway.in Norwegian Meteorological Institute, editor. Programme for Monitoring and Evaluation of the Long-range Transmission of Air Pollutants in Europe.

Knowles, R. 1982. Denitrification. Microbiological Reviews 46:43-70.

Koike, I., A. Hattori, and J. J. Goering. 1978. Controlled ecosystem pollution experiment - effect of mercury on enclosed water columns Marine Science Communications 4:1-12.

Kowalchuk, G. A. and J. R. Stephen. 2001. Ammonia-oxidizing bacteria: A model for molecular microbial ecology. Annual Review of Microbiology 55:485-529.

Krinner, G. 2003. Impact of lakes and wetlands on boreal climate. Journal of Geophysical Research-Atmospheres 108: 4520, doi: 10.1029/2002jd002597.

Kroeze, C., E. Dumont, and S. Seitzinger. 2005. New estimates of global emissions of N_2O from rivers and estuaries. Environmental Sciences 2:159-165.

Laverman, A. M., P. Van Cappellen, D. van Rotterdam-Los, C. Pallud, and J. Abell. 2006. Potential rates and pathways of microbial nitrate reduction in coastal sediments. Fems Microbiology Ecology 58:179-192.

LeBauer, D. S. and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371-379.

Le Mer, J. and P. Roger. 2001. Production, oxidation, emission and consumption of methane by soil: a review. European Journal of Soil Biology 37:25-50.

Lehner, B. and P. Döll. 2004. Development and validation of a global database of lakes, reservoirs, and wetlands. Journal of Hydrology 296:1-22.

Li, L., Z. C. Yu, R. E. Moeller, and G. E. Bebout. 2008. Complex trajectories of aquatic and terrestrial ecosystem shifts caused by multiple human-induced environmental stresses. Geochimica Et Cosmochimica Acta 72:4338-4351.

Liikanen, A., L. Flojt, and P. J. Martikainen. 2002. Gas dynamics in eutrophic lake sediments affected by oxygen, nitrogen, and sulfate. Journal of Environmental Quality 31:338-350.

Liikanen, A., E. Ratilainen, S. Saarnio, J. Alm, P. J. Martikainen, and J. Silvola. 2003. Greenhouse gas dynamics in boreal, littoral sediments under raised CO_2 and nitrogen supply. Freshwater Biology 48:500-511.

Liu, L. and T. L. Greaver. 2009. A review of nitrogen enrichment effects on three biogenic GHGs: the CO_2 sink may be largely offset by stimulated N_2O and CH_4 emissions. Ecology Letters 12:1103-1117.

Lovett, G. M. and H. M. Rueth. 1999. Soil nitrogen transformations in beech and maple stands along a nitrogen deposition gradient Ecological Applications 9:1330-1344.

Lukkari, K., H. Hartikainen, and M. Leivuori. 2007. Fractionation of sediment phosphorus revisited. I: Fractionation steps and their biogeochemical basis. Limnology and Oceanography: Methods 5:433-444.

Magnani, F., M. Mencuccini, M. Borghetti, P. Berbigier, F. Berninger, S. Delzon, A. Grelle, P. Hari, P. G. Jarvis, P. Kolari, A. S. Kowalski, H. Lankreijer, B. E. Law, A. Lindroth, D. Loustau, G. Manca, J. B. Moncrieff, M. Rayment, V. Tedeschi, R. Valentini, and J. Grace. 2007. The human footprint in the carbon cycle of temperate and boreal forests. Nature 447:848-850.

Makino, W., J. B. Cotner, R. W. Sterner, and J. J. Elser. 2003. Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C : N : P stoichiometry. Functional Ecology 17:121-130.

Makino, W. and J. B. Cotner. 2004. Elemental stoichiometry of a heterotrophic bacterial community in a freshwater lake: implications for growth- and resource-dependent variations. Aquatic Microbial Ecology 34:33-41.

McCrackin, M. L. and J. J. Elser. 2010. Atmospheric nitrogen deposition alters denitrification and nitrous oxide production in lake sediments. Ecology 91:528-539.

McCrackin, M. L. and J. J. Elser. In review. Denitrification and microbial communities in high elevation lakes receiving atmospheric nitrogen deposition. Biogeochemistry.

McManus, J., E. A. Heinen, and M. M. Baehr. 2003. Hypolimnetic oxidation rates in Lake Superior: Role of dissolved organic material on the lake's carbon budget. Limnology and Oceanography 48:1624-1632.

McNulty, S. G., J. D. Aber, and R. D. Boone. 1991. Spatial changes in forest floor and foliar chemistry of spruce-fir forests across New-England. Biogeochemistry 14:13-29.

Menge, B. A., F. Chan, S. Dudas, D. Eerkes-Megrano, K. Grorud-Colvert, K. Heiman, M. Hessing-Lewis, A. Iles, R. Milston-Clements, M. Noble, K. Page-Albins, E. Richmond, G. Rilov, J. Rose, J. Tyburczy, L. Vinueza, and P. Zarnetske. 2009. Terrestrial ecologists ignore aquatic literature: Asymmetry in citation breadth in ecological publications and implications for generality and progress in ecology. Journal of Experimental Marine Biology and Ecology 377:93-100.

Mengis, M., R. Gachter, and B. Wehrli. 1997. Sources and sinks of nitrous oxide (N_2O) in deep lakes. Biogeochemistry 38:281-301.

Molstad, L., P. Dorsch, and L. R. Bakken. 2007. Robotized incubation system for monitoring gases (O_2, NO, N_2O, N_2) in denitrifying cultures. Journal of Microbiological Methods 71:202-211.

Morley, N., E. M. Baggs, P. Dorsch, and L. Bakken. 2008. Production of NO, N_2O and N_2 by extracted soil bacteria, regulation by NO_2^- and O_2 concentrations. Fems Microbiology Ecology 65:102-112.

Mulholland, P. J., A. M. Helton, G. C. Poole, R. O. Hall, S. K. Hamilton, B. J. Peterson, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, W. K. Dodds, S. E. G. Findlay, S. V. Gregory, N. B. Grimm, S. L. Johnson, W. H. McDowell, J. L. Meyer, H. M. Valett, J. R. Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, L. T. Johnson, B. R. Niederlehner, J. M. O'Brien, J. D. Potter, R. W. Sheibley, D. J. Sobota, and S. M. Thomas. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202-206.

Musselman, R. C. and W. L. Slauson. 2004. Water chemistry of high elevation Colorado wilderness lakes. Biogeochemistry 71:387-414.

Nanus, L., D. H. Campbell, G. P. Ingersoll, D. W. Clow, and M. A. Mast. 2003. Atmospheric deposition maps for the Rocky Mountains. Atmospheric Environment 37:4881-4892.

Nanus, L., M. W. Williams, D. H. Campbell, E. M. Elliott, and C. Kendall. 2008. Evaluating regional patterns in nitrate sources to watersheds in national parks of the Rocky Mountains using nitrate isotopes. Environmental Science & Technology 42:6487-6493.

Nanus, L., M. W. Williams, D. H. Campbell, K. A. Tonnessen, T. Blett, and D. W. Clow. 2009. Assessment of lake sensitivity to acidic deposition in national parks of the Rocky Mountains. Ecological Applications 19:961-973.

Neilson, C. B., P. M. Groffman, S. P. Hamburg, C. T. Driscoll, T. J. Fahey, and J. P. Hardy. 2001. Freezing effects on carbon and nitrogen cycling in northern hardwood forest soils. Soil Science Society of America Journal 65:1723-1730.

Nevison, C. D., N. M. Mahowald, R. F. Weiss, and R. G. Prinn. 2007. Interannual and seasonal variability in atmospheric N_2O. Global Biogeochemical Cycles 21:GB3017, doi:10.1029/2006GB002755.

261

Nishio, T., I. Koike, and A. Hattori. 1983. Estimates of denitrification and nitrification in coastal and estuarine sediments. Applied and Environmental Microbiology 45:444-450.

Oremland, R. S., C. Umberfer, C. W. Culbertson, and R. L. Smith. 1984. Denitrification in San Francisco Bay intertidal sediments. Applied and Environmental Microbiology 47:1106-1112.

Oren, A. and T. H. Blackburn. 1979. Estimation of sediment denitrification rates at insitu nitrate concentrations. Applied and Environmental Microbiology 37:174-176.

Pina-Ochoa, E. and M. Alvarez-Cobelas. 2006. Denitrification in aquatic environments: a cross-system analysis. Biogeochemistry 81:111-130.

Ravishankara, A. R., J. S. Daniel, and R. W. Portmann. 2009. Nitrous oxide: the dominant ozone-depleting substance emitted in the 21st century. Science 326:123-125.

Richardson, W. B., E. A. Strauss, L. A. Bartsch, E. M. Monroe, J. C. Cavanaugh, L. Vingum, and D. M. Soballe. 2004. Denitrification in the Upper Mississippi River: rates, controls, and contribution to nitrate flux. Canadian Journal of Fisheries and Aquatic Sciences 61:1102-1112.

Risgaard-Petersen, N. 2003. Coupled nitrification-denitrification in autotrophic and heterotrophic estuarine sediments: on the influence of benthic microalgae. Limnology and Oceanography 48:93-105.

Rowe, R., R. Todd, and J. Waide. 1977. Microtechnique for most-probable-number analysis. Applied and Environmental Microbiology 33:675-680.

Rudaz, A. O., E. Walti, G. Kyburz, P. Lehmann, and J. Fuhrer. 1999. Temporal variation in N_2O and N_2 fluxes from a permanent pasture in Switzerland in relation to management, soil water content and soil temperature. Agriculture Ecosystems & Environment 73:83-91.

Russell, J. B. and G. M. Cook. 1995. Energetics of bacterial-growth: balance of anabolic and catabolic reactions. Microbiological Reviews 59:48-62.

Rysgaard, S., N. Risgaard-Petersen, N. P. Sloth, K. Jensen, and L. P. Nielsen. 1994. Oxygen regulation of nitrification and denitrification in sediments. Limnology and Oceanography 39:1643-1652.

Santoro, A. E., A. B. Boehm, and C. A. Francis. 2006. Denitrifier community composition along a nitrate and salinity gradient in a coastal aquifer. Applied and Environmental Microbiology 72:2102-2109.

Saunders, D. L. and J. Kalff. 2001. Denitrification rates in the sediments of Lake Memphremagog, Canada-USA. Water Research 35:1897-1904.

Saunders, D. L. and J. Kalff. 2001. Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia 443:205-212.

Schlesinger, W. H. 2009. On the fate of anthropogenic nitrogen. PNAS 106:203-208.

Seitzinger, S. P., S. W. Nixon, and M. E. Q. Pilson. 1984. Denitrification and nitrous oxide production in a coastal marine ecosystem. Limnology and Oceanography 29:73-83.

Seitzinger, S. P. and S. W. Nixon. 1985. Eutrophication and the rate of denitrification and N_2O production in coastal marine sediments. Limnology and Oceanography 30:1332-1339.

Seitzinger, S. P. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. Limnology and Oceanography 33:702-724.

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

Seitzinger, S., C. Kroeze, and R. Styles. 2000. Global distribution of N_2O emissions from aquatic systems: Natural emissions and anthropogenic effects. Chemosphere 2:267-279.

Seitzinger, S. P., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, and C. Tobias. 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecological Applications 16:2064-2090.

Sharma, S., Z. Szele, R. Schilling, J. C. Munch, and M. Schloter. 2006. Influence of freeze-thaw stress on the structure and function of microbial communities and denitrifying populations in soil. Applied and Environmental Microbiology 72:2148-2154.

Sickman, J. O., J. M. Melack, and J. L. Stoddard. 2002. Regional analysis of inorganic nitrogen yield and retention in high-elevation ecosystems of the Sierra Nevada and Rocky Mountains. Biogeochemistry 57:341-374.

Silvennoinen, H., A. Liikanen, J. Torssonen, C. F. Stange, and P. J. Martikainen. 2008. Denitrification and nitrous oxide effluxes in boreal, eutrophic river sediments under increasing nitrate load: a laboratory microcosm study. Biogeochemistry 91:105-116.

Sjoeng, A. M. S., O. Kaste, K. Torseth, and J. Mulder. 2007. Nitrogen leaching from small upland headwater catchments in southwestern Norway. Water Air and Soil Pollution 179:323-340.

Smith, L. K. and W. M. Lewis. 1992. Seasonality of methane emissions from five lakes and associated wetlands of the Colorado Rockies. Global Biogeochemical Cycles 6:323-338.

Smith, M. S. and J. M. Tiedje. 1979. Phases of denitrification following oxygen depletion in soil. Soil Biology & Biochemistry 11:261-267.

Solorzano, L. and J. H. Sharp. 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnol. Oceanogr. 25:754-758.

St. Louis, V. L., C. A. Kelly, E. Duchemin, J. W. M. Rudd, and D. M. Rosenberg. 2000. Reservoir surfaces as sources of greenhouse gases to the atmopshere: a global estimate. Bioscience 50:766-775.

Staley, T. E. and J. B. Griffin. 1981. Simultaneous enumeration of denitrifying and nitrate reducing bacteria by a microtiter most-probable-number (MPN) procedure. Soil Biology & Biochemistry 13:385-388.

Sterner, R. W. and J. J. Elser. 2002. Ecological Stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton, NJ, USA.

Stoddard, J. L., D. S. Jeffries, A. Lukewille, T. A. Clair, P. J. Dillon, C. T. Driscoll, M. Forsius, M. Johannessen, J. S. Kahl, J. H. Kellogg, A. Kemp, J. Mannio, D. T. Monteith, P. S. Murdoch, S. Patrick, A. Rebsdorf, B. L. Skjelkvale, M. P. Stainton, T. Traaen, H. van Dam, K. E. Webster, J. Wieting, and A. Wilander. 1999. Regional trends in aquatic recovery from acidification in North America and Europe. Nature 401:575-578.

Strauss, E. A., N. L. Mitchell, and G. A. Lamberti. 2002. Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH. Canadian Journal of Fisheries and Aquatic Sciences 59:554-563.

Sundareshwar, P. V., J. T. Morris, E. K. Koepfler, and B. Fornwalt. 2003. Phosphorus limitation of coastal ecosystem processes. Science 299.

Sundbäck, K. and A. Miles. 2000. Balance between nitrification and microalgal incorporation of nitrogen in microtidal sediments, NE Kattegat. Aquatic Microbial Ecology 22:291-300.

Sundbäck, K., F. Linares, F. Larson, and A. Wulff. 2004. Benthic nitrogen fluxes along a depth gradient in a microtidal fjord: The role of denitrification and microphytobenthos. Limnology and Oceanography 49:1095-1107.

Taylor, P. G. and A. R. Townsend. 2010. Stoichiometric control of organic carbon-nitrate relationships from soils to the sea. Nature 464:1178-1181.

Tezuka, Y. 1990. Bacterial regeneration of ammonium and phosphorus as affected by the carbon : nitrogen : phosphorus ratio of organic substrate. Microbial Ecology 19:227-238.

Tietema, A. 1998. Microbial carbon and nitrogen dynamics in coniferous forest floor material collected along a European nitrogen deposition gradient. Forest Ecology and Management 101:29-36.

Tørseth, K. and A. Semb. 1998. Deposition of nitrogen and other major inorganic compounds in Norway, 1992-1996. Environmental Pollution 102:299-304.

Townsend, A. R., B. H. Braswell, E. A. Holland, and J. E. Penner. 1996. Spatial and temporal patterns in terrestrial carbon storage due to deposition of fossil fuel nitrogen. Ecological Applications 6:806-814.

Townsend, A. R., R. W. Howarth, F. A. Bazzaz, M. S. Booth, C. C. Cleveland, S. K. Collinge, A. P. Dobson, P. R. Epstein, D. R. Keeney, M. A. Mallin, C. A. Rogers, P. Wayne, and A. H. Wolfe. 2003. Human health effects of a changing global nitrogen cycle. Frontiers in Ecology and the Environment 1:240-246.

Tranvik, L. J. 1997. Rapid fluorometric assay of bacterial density in lake water and seawater. Limnology and Oceanography 42:1629-1634.

Tranvik, L. J., J. A. Downing, J. B. Cotner, S. A. Loiselle, R. G. Striegl, T. J. Ballatore, P. Dillon, K. Finlay, K. Fortino, L. B. Knoll, P. L. Kortelainen, T. Kutser, S. Larsen, I. Laurion, D. M. Leech, S. L. McCallister, D. M. McKnight, J. M. Melack, E. Overholt, J. A. Porter, Y. Prairie, W. H. Renwick, F. Roland, B. S. Sherman, D. W. Schindler, S. Sobek, A. Tremblay, M. J. Vanni, A. M. Verschoor, E. von Wachenfeldt, and G. A. Weyhenmeyer. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. Limnology and Oceanography 54:2298-2314.

Treseder, K. K. 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters 11:1111-1120.

Ullah, S. and G. M. Zinati. 2006. Denitrification and nitrous oxide emissions from riparian forest soils exposed to prolonged nitrogen runoff. Biogeochemistry 81:253-267.

Waldrop, M. P., D. R. Zak, and R. L. Sinsabaugh. 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology & Biochemistry 36:1443-1451.

Wallenstein, M. D., D. D. Myrold, M. K. Firestone, and M. Voytek. 2006. Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. Ecological Applications 16:2143-2152.

Wallenstein, M. D., S. McNulty, I. J. Fernandez, J. Boggs, and W. H. Schlesinger. 2006. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. Forest Ecology and Management 222:459-468.

Wang, H. J., W. D. Wang, C. Q. Yin, Y. C. Wang, and J. W. Lu. 2006. Littoral zones as the "hotspots" of nitrous oxide (N_2O) emission in a hyper-eutrophic lake in China. Atmospheric Environment 40:5522-5527.

Wanninkhof, R., J. R. Ledwell, W. S. Broecker, and M. Hamilton. 1987. Gas-exchange on Mono Lake and Crowley Lake, California. Journal of Geophysical Research-Oceans 92:14567-14580.

Weiss, R. F. 1974. Carbon dioxide in water and seawater: the solubility of a non-ideal gas. Marine Chemistry 2:203-215.

Weiss, R. F. and B. A. Price. 1980. Nitrous oxide solubility in water and seawater. Marine Chemistry 8:347-359.

Wetzel, R. G. 2001. Limnology: Lake and River Ecosystems. Academic Press, San Diego, CA, USA.

Weyhenmeyer, G. A., E. Jeppesen, R. Adrian, L. Arvola, T. Blenckner, T. Jankowski, E. Jennings, P. Noges, T. Noges, and D. Straile. 2007. Nitrate-depleted conditions on the increase in shallow northern European lakes. Limnology and Oceanography 52:1346-1353.

Weyhenmeyer, G. A. and E. Jeppesen. 2009. Nitrogen deposition induced changes in DOC:NO3-N ratios determine the efficiency of nitrate removal from freshwaters. Global Change Biology:doi: 10.1111/j.1365-2486.2009.02100.x.

Whitby, C. B., J. R. Saunders, R. W. Pickup, and A. J. McCarthy. 2001. A comparison of ammonia-oxidiser populations in eutrophic and oligotrophic basins of a large freshwater lake. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 79:179-188.

Wiesenburg, D. A. and N. L. Guinasso. 2002. Equilibrium solubilities of methane, carbon monoxide, and hydrogen in water and sea water. Journal of Chemical and Engineering Data 24:356-360.

Williams, M. W., J. S. Baron, N. Caine, R. Sommerfeld, and R. Sanford. 1996. Nitrogen saturation in the Rocky Mountains. Environmental Science & Technology 30:640-646.

Williamson, C. E., W. K. Dodds, T. K. Kratz, and M. A. Palmer. 2008. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Frontiers in Ecology and the Environment 6:247-253.

Williamson, C. E., J. E. Saros, W. F. Vincent, and J. P. Smol. 2009. Lakes and reservoirs as sentinels, integrators, and regulators of climate change. Limnology and Oceanography 54.

Wilson, M. A. and S. R. Carpenter. 1999. Economic valuation of freshwater ecosystem services in the United States: 1971-1997. Ecological Applications 9:772-783.

Wolfe, A. P., J. S. Baron, and R. J. Cornett. 2001. Anthropogenic nitrogen deposition induces rapid ecological changes in alpine lakes of the Colorado Front Range (USA). Journal of Paleolimnology 25:1-7.

Wolfe, A. P., C. A. Cooke, and W. O. Hobbs. 2006. Are current rates of atmospheric nitrogen deposition influencing lakes in the eastern Canadian arctic? Arctic, Antarctic, and Alpine Research 38:465-476.

Wollheim, W. M. and C. J. Vorosmarty. 2006. Relationship between river size and nutrient removal. Geophysical Research Letters 33:L06410, doi:06410.01029/02006GL025845.

Wollheim, W. M., C. J. Vorosmarty, A. F. Bouwman, P. Green, J. Harrison, E. Linder, B. J. Peterson, S. P. Seitzinger, and J. P. M. Syvitski. 2008. Global N removal by freshwater aquatic systems using a spatially distributed, within-basin approach. Global Biogeochemical Cycles 22:Gb2026, doi:2010.1029/2007GB002963.

Wrage, N., G. L. Velthof, M. L. van Beusichem, and O. Oenema. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology & Biochemistry 33:1723-1732.

Yoshinari, T. and R. Knowles. 1976. Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochemical and Biophysical Research Communications 69:705-710.

Yu, K. W., R. D. DeLaune, and P. Boeckx. 2006. Direct measurement of denitrification activity in a Gulf coast freshwater marsh receiving diverted Mississippi River water. Chemosphere 65:2449-2455.

Zak, D. R., W. E. Holmes, A. J. Burton, K. S. Pregitzer, and A. F. Talhelm. 2008. Similated atmopsheric NO3- deposition increases soil organic matter by slowing decomposition. Ecological Applications 18:2016-2027.

Zumft, W. G. 1997. Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Reviews 61:533-616.

APPENDIX A

PUBLICATIONS CITATIONS

CHAPTER 2: Atmospheric Nitrogen Deposition Influences Denitrification

and Nitrous Oxide Production in Lakes (Norway)

> Citation: McCrackin, M. L. and J. J. Elser. (2010). Ecology.
> 91(2): 528-539

CHAPTER 3: Kinetics of Denitrification and the Abundance of Denitrifying

Bacteria in High-Elevation Lakes Receiving Atmospheric Nitrogen

Deposition (Colorado, USA)

> Citation: McCrackin, M. L. and J. J. Elser. Biogeochemistry. (In
> revision)

CHAPTER 4: Temporal Dynamics of Denitrification in Lake Sediments

(Norway)

> Citation: McCrackin, M. L., P. Dörsch, and J. J. Elser. (In prep.)

CHAPTER 5: Pelagic and Sediment Bacterial Responses to Resource

Enrichment in Lakes Receiving Atmospheric Nitrogen Deposition (Norway)

> Citation: McCrackin, M. L., J. J. Elser, and M. Kyle. (In prep.)

CHAPTER 6: Greenhouse Gas Dynamics in Lakes Receiving Atmospheric

Nitrogen Deposition (Colorado, USA)

> Citation: McCrackin, M. L. and J. J. Elser. Global Biogeochemical
> Cycles. (In review)

 * Co-authors have authorized the use of these chapters in this

dissertation.