NITROGEN GAS FLUXES IN NORTHEASTERN FORESTS: THE END OF THE LINE FOR REACTIVE NITROGEN?

by Madhura Vinayak Kulkarni

This thesis/dissertation document has been electronically approved by the following individuals:

Yavitt,Joseph B (Chairperson)

Goodale,Christine L (Minor Member)

Lassoie,James Philip (Field Appointed Minor Member)

NITROGEN GAS FLUXES IN NORTHEASTERN FORESTS: THE END OF THE LINE FOR REACTIVE NITROGEN?

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Madhura Vinayak Kulkarni

August 2010

© 2010 Madhura Vinayak Kulkarni

NITROGEN GAS FLUXES IN NORTHEASTERN FORESTS:

THE END OF THE LINE FOR REACTIVE NITROGEN?

Madhura Vinayak Kulkarni, Ph. D.

Cornell University 2010

A long history of mystery surrounds the fate of nitrogen (N) deposited in northeastern forests. Budgets of N inputs, outputs and storages reveal a great deal of "missing" N on the output and storage side. Losses in leaching to groundwater and via streams do not account for the gap. Gaseous emissions from soils were discounted as the answer to this mystery after publication of studies that found low rates of nitrous oxide (N_2O) and nitric oxide (NO) production. The fate of this missing N is of concern because fixed or "reactive" nitrogen (Nr)—all forms other than gaseous, inert N_2—can transform and travel through system after system, polluting soils, air, and waters along the way in what is called the nitrogen cascade.

In a series of studies on northeastern forests, I found that much of this missing N is lost as N_2, effectively cutting off the cascade of Nr. I also measured N_2O fluxes and our measurements corroborate previous observations of low rates. Moreover, relationships between fluxes and some potential controlling variables (e.g. N richness, moisture) were strong, allowing for spatially explicit extrapolation models. Uncertainties in our estimates of N gas loss remain, but improved methods for measuring and extrapolating fluxes promise to reduce these uncertainties. Still, evidence from these studies is strong that large portions of N inputs to northeastern forests are transformed into inert N_2 and removed from the cascade of polluting Nr.

BIOGRAPHICAL SKETCH

Madhura Kulkarni grew up in Cincinnati, Ohio. She left Ohio to attend Duke University in North Carolina. There, she desired to pursue studies that would allow her to further her interest in biology but also explore other disciplines. At first, she thought she might pursue the intersection of the various sciences at the molecular level, but then realized that she found the wider field of environmental studies more interdisciplinary and more interesting. After spending time at the Bermuda Biological Station for Research, the Duke Marine Laboratory, and the Costa Rican site of the School for Field Studies, she completed her double majors in Biology, and Environmental Science and Policy, with a concentration in Marine Science. At the Duke Marine Laboratory, she conducted a study on the feeding habits of harbor porpoises in the Mid-Atlantic Bight with Andrew Reed.

During a year off from academics after college, Madhura worked at an environmental consulting firm for a few months. She also volunteered with a social and environmental NGO in India, the Cincinnati Natural History Museum, and the City of Cincinnati's Office of Environmental Management. Finally, she taught Marine Invertebrate Zoology to high school students at the Duke Marine Laboratory before beginning graduate studies.

Under the guidance of Bill Currie at the University of Maryland's Appalachian Laboratory, she studied wetlands at a reclaimed coal mine in Frostburg, Maryland. Her M.S. thesis compared the denitrification potential of this wetland with a natural wetland as it related to environmental history and found that over time, such wetlands would develop differently but still possess the potential to remove large portions of

nitrogen from water flowing through via denitrification.

Her interest in denitrification continued in her Ph.D. studies at Cornell, where she studied denitrification rates at the Hubbard Brook Experimental Forest and the wider White Mountains with Peter Groffman (Cary Institute of Ecosystem Studies), and Joseph Yavitt (Cornell). Although her studies were firmly in the realm of the basic sciences, she explored science policy as well. In 2006, she lobbied the United States government on behalf of the biological science community with other representatives of the American Institute of Biological Sciences. She also visited the New York state Legislature to lobby on behalf of Cornell University. Following graduation, Madhura is pursuing her interest in the intersection of science and policy by working for the Clean Air Task Force on the climate effects of using forest resources for biological energy production.

I dedicate this work to my sons, Ojas and Rohan.

ACKNOWLEDGMENTS

First, I'd like to thank my committee members: Peter Groffman, Joe Yavitt, Christy Goodale, and Jim Lassoie. Christy and Jim have been especially helpful in guiding and encouraging me in my exploration of science beyond basic research. Joe has supported me in my research and student life from near and far. And most of all, Peter has been available, insightful and patient with me throughout my Ph.D.

Thanks also to Bill Currie, who introduced me to biogeochemistry, and helped lead me to and prepare me for Cornell University and this Ph.D. program.

Lisa Martel, Stephanie Juice and Dave Lewis helped to collect and analyze much of the data presented in this dissertation. They also served as excellent companions during many hours in the field and lab. Terry Schwarz and Francoise Vermeylen provided technical assistance with hydrological and statistical models, respectively.

Mary Martin, Rod Venterea, and Paul Schwarz shared their data on site characteristics, including information on foliar nitrogen, soils, and vegetation. The employees of Stantec, Inc. in Topsham, ME shared their workspace and advice with me in the final days before my defense. David Harris and colleagues at the University of California-Davis analyzed my isotope samples.

My friends and colleagues in the Natural Resources Department and the Biogeochemistry and Environmental Biocomplexity Program were always there for me as sounding boards, from near and far. The Hubbard Brook research community was also very helpful as I explored and analyzed Hubbard Brook Experimental Forest.

Funding for this work was provided by the National Science Foundation's (NSF) excellent Integrative Graduate Education and Research Traineeship Program (DGE 0221658). NSF also funded this work through grants DEB 0614158 (Ecosystem Studies) and DEB 0423259 (Hubbard Brook LTER). This work was also supported by a NASA Earth Systems Science Graduate Felowship (07-Earth07R-0021) and Northeastern States Research Cooperative (Grant # 02- CA-11242343-105). Finally, small grants from the Cornell Center for the Environment and the NSF Biogeochemistry and Environmental Biocomplexity IGERT Program provided key funding for laboratory analyses.

Finally, I reserve my biggest thanks for my family. My parents, grandparents, aunts and uncles have always supported my in every way possible, and especially by setting an example of excellence in education. My sister and brother have also assisted in many ways, even editing dissertation chapters. My children have shared me with my other great responsibility – this dissertation – and provided motivation for making the most of myself. Lastly, my husband, Todd, has been my inspiration throughout my years as a graduate student.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF BOXES

CHAPTER 1

SOLVING THE GLOBAL NITROGEN PROBLEM: IT'S A GAS![1]

1.1 ABSTRACT

Anthropogenic acceleration of global nitrogen cycling has doubled "reactive" nitrogen levels, degrading air and water quality and affecting human health. Denitrification is the primary process by which reactive nitrogen is recycled to inert N_2. Unfortunately, past attempts to estimate denitrification at scales relevant to pollution and health problems have been associated with great uncertainties arising mainly from great variability in rates, difficulty measuring N_2 fluxes over background levels, and multiple reactants and products which are involved in other nitrogen cycling processes. New approaches to quantifying broad-scale denitrification address some of these issues. Novel techniques allow detection of small changes in N_2 levels arising from denitrification. Recent models creatively identify "hot spots" of denitrification in the landscape. New remote sensing products improve inputs to models of denitrification. These developments, and others, hold promise for advancing our understanding of denitrification and its potential to mitigate the environmental impacts of reactive nitrogen.

[1]This chapter is adapted from a manuscript of the same title by Madhura V. Kulkarni, Peter M. Groffman, and Joseph B. Yavitt. My original contributions include conducting the literature review, writing and revising the paper, and preparing all figures.

1.2 INTRODUCTION

Human activities are accelerating the global nitrogen cycle, releasing ever-increasing amounts of reactive nitrogen (Nr)– all nitrogen species other than dinitrogen (N_2) – into the environment (Galloway and Cowling 2002). Excess Nr has been linked to degradation of air, soil, and water quality in many areas (Box 1-1, Figure 1-1). But nitrogen fixation, the creation of Nr, can be reversed. Denitrification is the process by which Nr, in the forms of nitrate (NO_3^-), nitrite (NO_2^-), nitric oxide (NO), and nitrous oxide (N_2O), is sequentially reduced to N_2; it occurs in soils, sediments, and waters all over the world (Figure 1-2). High levels of NO_3^- and NO_2^- in water can cause algal blooms, which may be toxic to other organisms and/or deplete oxygen from water bodies. Nitric oxide is a precursor to ozone and is therefore an air pollutant. Nitrous oxide is a greenhouse gas in the lower atmosphere and destroys the ozone layer in the stratosphere (Paul and Clark 1996). As the primary mechanism converting Nr to inert N_2, denitrification could be the "solution" to the global nitrogen pollution problem.

Box 1-1. *Excess reactive nitrogen as a pollutant*

Increasing loads of reactive nitrogen are linked to pollution in air, soil and water. Nitric oxide is a component of smog and a precursor to tropospheric ozone, which is blamed for problems ranging from reduced visibility in parks to respiratory health issues. Nitrous oxide also pollutes our air as a greenhouse gas and destroyer of the stratospheric ozone layer (Galloway and Cowling 2002). Elevated nitrogen deposition onto the landscape can acidify soils and lakes as well as push low nitrogen systems to "nitrogen saturation"; forests in some high nitrogen deposition areas appear to be experiencing a decline due to these conditions (Matson et al. 2002). Downstream, rivers and coastal waters experiencing high nitrogen loads may suffer from eutrophication, a cascade of effects that often leads to harmful algal blooms and low oxygen conditions that can kill the flora and fauna of large areas (Howarth and Marino 2006). The Gulf of Mexico experiences such a problem when high nitrogen inputs from the Mississippi River produce a "dead zone" where fisheries and other populations suffer from depleted oxygen (Mitsch et al. 2001).

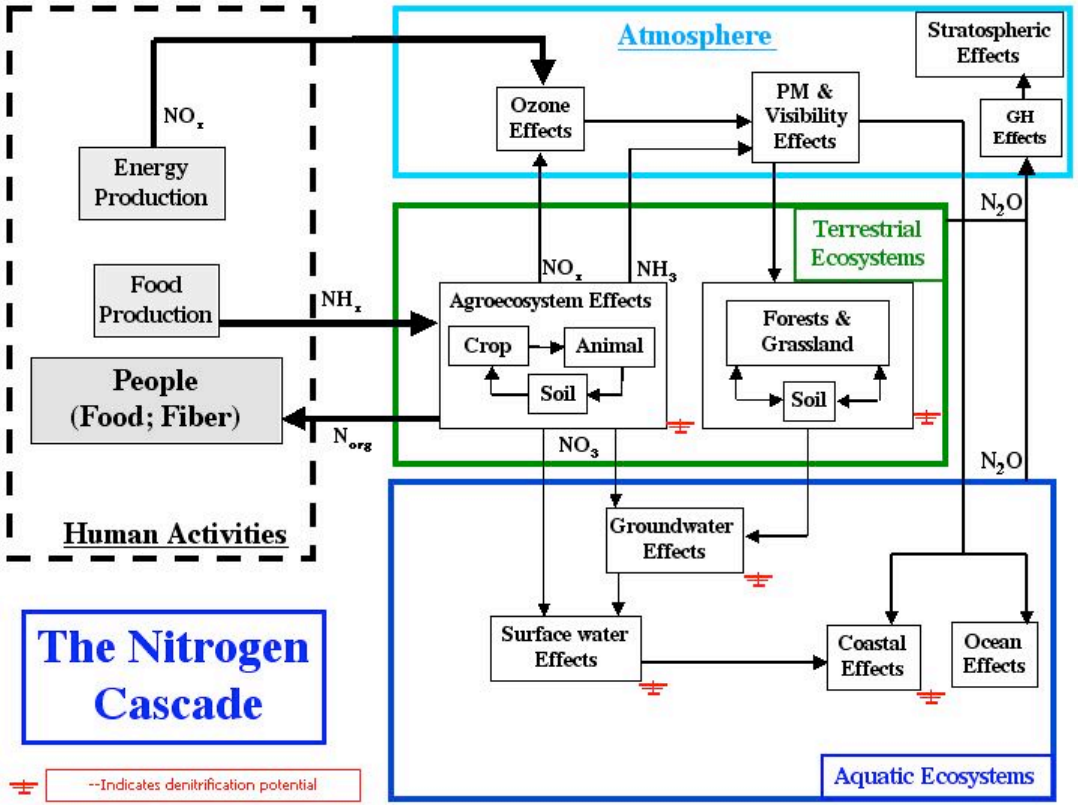


Figure 1-1. The Nitrogen Cascade. (From Galloway et al. 2003).

Scientists have studied denitrification for a long time and numerous reviews have been published (Nõmmik 1956, Firestone 1982, Tiedje 1988). Despite this history, the state of knowledge on denitrification lags behind that of most other parts of the nitrogen cycle (Figure 1-2). This lag arises largely from difficulty in quantifying denitrification accurately. This difficulty, in turn, arises mainly from two characteristics of the process: 1) denitrification rates exhibit very high spatial and temporal variability, and 2) denitrification involves several nitrogen species as reactants and products, all of which are also reactants and products of other parts of the nitrogen cycle, which complicates complete accounting of fluxes (Groffman et al. 2006). More fundamentally, denitrification is a process that occurs at the scale of microns but has important effects at the scale of meters, kilometers, and larger (Figure 1-3). The scaling of biogeochemical process rates such as denitrification is one of the great current challenges in environmental science (Miller et al. 2004). The International Nitrogen Initiative (INI, www.initrogen.org), an effort dedicated to combating global nitrogen pollution problems, has identified denitrification as a major cross-cutting theme and critical area of research (Erisman 2004) and has co-sponsored (along with the U.S. National Science Foundation [NSF]) a denitrification Research Coordination Network (RCN) to address issues related to research in denitrification (www.denitrification.org). Here, we describe why denitrification is such a challenging process to study and highlight some promising advances that suggest that our understanding and estimates of this process at scales relevant to landscape, regional, and global questions are likely to improve in the near future.

1.3 CONTROLS ON DENITRIFICATION

To fully appreciate the dynamics of denitrification, we must understand its controls and how they interact. For this reason, the first step in assessing patterns of

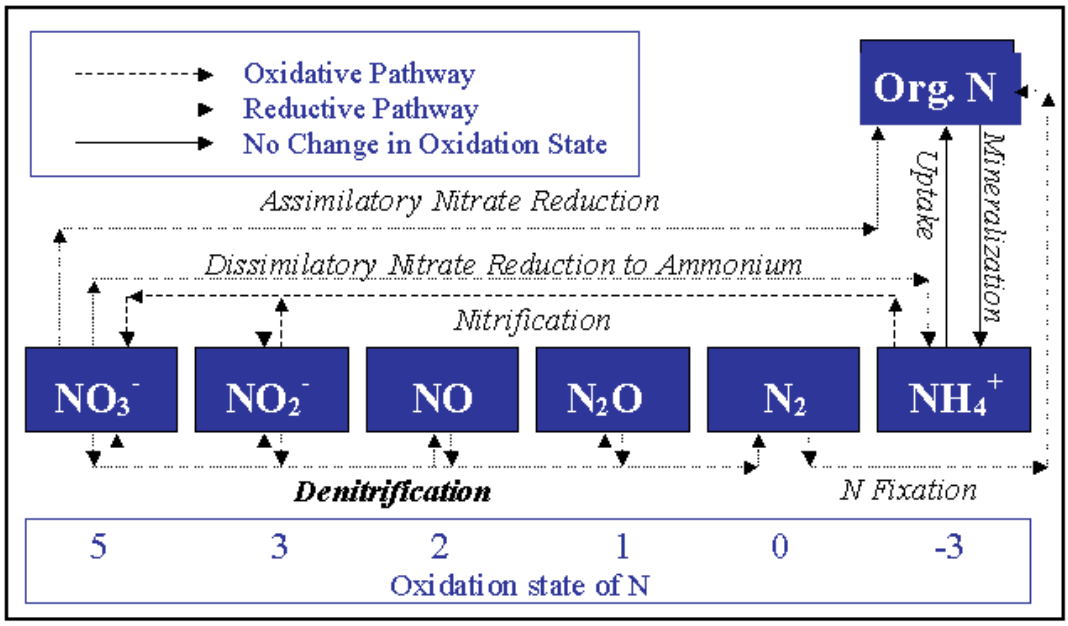


Figure 1-2. The Nitrogen Cycle. (Adapted from Fenchel and Blackburn 1979).

Figure 1-3. Denitrification is a process that is often measured at the scale of square centimeters over minutes to days (a), but important to understand over much larger areas and time spans (b). Photo (a) shows me taking N_2/N_2O samples from a gas flux chamber at the Hubbard Brook Experimental Forest for a study of denitrification rates. Photo (b) shows the Hubbard Brook valley as seen from the top of the watershed (Photo courtesy of the U.S. Forest Service). Methods to measure denitrification directly at the scale of this watershed are not available so scientists must estimate rates for such areas using models or extrapolation of measurements made at relatively fine scales.

denitrification in a given area is to examine the distribution of levels of its controls so that one can "map" variability in denitrification rates. Denitrification is a microbial process that requires anoxic conditions since almost all denitrifying microbes are facultative anaerobic bacteria, i.e. they preferentially use O_2 over NO_3^- as a terminal electron acceptor. So under aerobic conditions, they will reduce oxygen instead of nitrate, precluding denitrification. Denitrifying microbes are diverse and ubiquitous. Some fungi and chemoautorophic bacteria have been known to denitrify nitrate (Laughlin and Stevens 2002), but most denitrifiers are heterotrophic bacteria, requiring a carbon source as a reductant. Denitrification, therefore, has three main requirements: 1) available nitrate (or intermediate forms of oxidized nitrogen such as NO_2^- and NO), 2) anoxic conditions, and 3) a source of organic carbon. Since it is a biotic process, two other environmental factors also exert control over rates of denitrification: 4) pH and 5) temperature (Paul and Clark 1996).

Heterogeneity in the levels of controlling factors over landscapes (and waterscapes) in space and time induces the great heterogeneity observed in denitrification rates. For example, Parsons et al. (1991) found that coefficients of variations for 12 replicate samples taken from within 5 m-radius plots typically ranged from 100 - 150%. Temporal variation was also high; rates were undetectable much of the year, but peaked in the winter and spring, ranging between 12 and 70 g N ha^{-1} d^{-1}. Parkin et al. (1987) found so much spatial variability in an agricultural soil that they determined that soil samples of 10 - 15 kg of would be required to accurately estimate denitrification rates.

A promising approach to dealing with the extreme variability in denitrification is to focus on the occurrence of "hot spots" and "hot moments" of denitrification, defined

as relatively small areas or brief periods of time when all the conditions are optimal for denitrification, producing elevated denitrification rates (McClain et al. 2003). The hot spot/moment concept is scale dependent, so when investigating N cycling at scales relevant to nitrogen pollution problems, a hot spot may be as large as a subcontinent of an entire planet (e.g. India as a hot spot of N fixation on earth) and a hot moment may be a period of several decades (eg. the 20th century in the history of N fixation on earth) (Galloway and Cowling 2001). The hot spot idea originated with Parkin (1987), who found that 85% of the denitrification in a 98 g soil core was maintained by a tiny fragment of decomposing plant detritus. More recently, the hot spot concept has been applied to whole landscapes and regions. It is even being applied in streams, where organic debris dams appear to function as hot spots of denitrification (Bernhardt et al. 2003, Groffman et al. 2005). New hydro-ecological models that depict the flow of water and nutrients across the landscape in a high-resolution, spatially explicit format, are a promising tool for applying this concept at broad scales. For example, Band et al. (2001) and Hong et al. (2005) have identified potential hot spots of nitrogen cycling with the Regional Hydro-Ecological Simulation System (RHESSys), and Simple Nitrogen Cycle (SINIC) hydro-ecological models respectively. However, neither group had data on denitrification rates to validate their model predictions. Hot moments may occur in response to rapid changes in the soil environment. For example, in a study of European agricultural soils, Priemé and Christensen (2001) found hot moments of denitrification occurring after wetting dry soils and thawing frozen soils. Studies hoping to account for temporal variability in flashy systems (those with short periods of intense activity) must account for hot moments, perhaps by increasing the number of measurements during precipitation events and spring thaws.

A more traditional approach to research design is to stratify sampling along spatial and temporal gradients of controls on denitrification. In such an experimental design, the objective is to capture the full variability of denitrification rates within an area to serve as a basis for extrapolations (Groffman 1991, Figure 1-4). Measurement locations may be blocked or stratified over the study site and period according to gradients in important controlling factors that operate at the scale of study. However, because the five proximal controls listed above can be difficult to quantify directly over broad scales, it is useful to consider distal controls that regulate the proximal controls at broader spatial and temporal scales. For example, plant community composition and canopy chemistry are landscape-scale controllers, or indicators, of nitrogen and carbon dynamics, which are field- or ecosystem-scale controllers of the flow of oxygen, nitrate and carbon to denitrifying cells at the micro-scale (Figure 1-4).

The potential to combine new ideas about hot spots, with more traditional ideas of spatial and temporal stratification of landscapes and regions (Figures 1-3 & 1-4), in the context of eco-hydrological models suggests that we are on the threshold of developing powerful new experimental designs for denitrification studies at scales relevant to global nitrogen pollution problems.

1.4 MEASURING DENITRIFICATION RATES

Recently, researchers around the world have collaborated to produce estimates of denitrification for the USA (Howarth et al. 2002), Europe (van Egmond et al. 2002), and Asia (Zheng et al. 2002). All of these studies, however, estimated denitrification by difference, taking measurements of all other nitrogen fluxes and pools and assuming the missing part of the budget to be denitrification. Calculating denitrification by difference combines the errors of all other measurements and

Spatial Controls on Denitrification		Temporal Controls on Denitrification	
oxygen, nitrate, carbon	ORGANISM ↓	SECONDS-MINUTES ↓	substrate availability, physical disruption
organic matter, physical disruptions	MICROSITE ↓	HOURS-DAYS ↓	moisture, diel variability, weather events
water, nitrification, decomposition	FIELD ↓	MONTHS-SEASONS ↓	temperature and precipitation regimes
soil type, land use	LANDSCAPE ↓	YEARS-DECADES ↓	drought/flood years, el niño/la niña
community structure, geography	REGIONAL ↓	CENTURIES	glacial/interglacial periods
biome type, climate	GLOBAL		

Figure 1-4. Factors controlling denitrification at different scales of investigation ordered from finest scale (top), to broadest (bottom). Another way to conceptualize these controls is as ordered from proximal to distal, meaning that the controls at the top of figure operate directly on the reduction of nitrogen species in a denitrifying organism, whereas those at the bottom affect the proximal controls, and therefore indirectly control denitrification rates (Adapted from Groffman 1991).

assumes that the budget is complete. This method, therefore, gives estimates of denitrification with great associated uncertainties.

Many broad-scale studies of nitrogen budgets indicate that denitrification is likely an important nitrogen sink (e.g. Howarth et al. 2002, van Egmond et al. 2002, Zheng et al. 2002, van Breemen et al. 2002). But is it really? For denitrification to serve as an Nr sink, the nitrogen species must be converted to N_2. Other species in the denitrification sequence are reactive and can be pollutants. Since NO and N_2O may comprise a large portion of denitrification products, we must know how denitrification products are proportioned to know how well denitrification serves as an Nr sink. Again, however, we are faced with a lack of adequate methodology. Very few studies have addressed all three gaseous products of denitrification, mainly because available methods are not capable of simultaneously measuring them. Most methods only examine one product, usually N_2O (Groffman et al. 2006). Dinitrogen emissions are especially difficult to measure over background levels since N_2 makes up 78% of the atmosphere, making atmospheric contamination a troublesome problem (Schlesinger 1997).

Currently, options for measuring denitrification rates that account for N_2 emissions are limited. They include N_2:Ar ratio, recirculating gas flow-core (RGFC), and ^{15}N isotope methods. All three are useful in their own right – N_2:Ar ratios for aquatic samples, RGFC for time series data with simultaneous measurement of N_2O and N_2, and ^{15}N for tracing N through transformations – but they all also have limitations. None are currently used for simultaneous measurement of NO, N_2O, and N_2; the N_2:Ar ratio methods gives *net* denitrification rates (denitrification minus N_2 fixation); the RGFC method does not give *in situ* rates; and the ^{15}N method requires some

modification of the system prior to measurement (i.e. adding tracer, usually dissolved in water). The fact that NO and N_2O are byproducts of nitrification also complicates measurements of denitrification by all methods.

New developments in methods for measuring denitrification are improving our ability to measure denitrification, especially N_2. For example, Böhlke et al. (2002) have employed naturally occurring gradients in stable isotopes of nitrogen and oxygen to quantify denitrification along groundwater flowpaths, and the N_2:Ar method as implemented using membrane inlet mass spectrometry is now widely used in marine systems (Kana et al. 1994, Groffman et al. 2006). Additionally, the Lotic Intersite Nitrogen Experiment's effort to survey nitrogen dynamics of streams nationwide employs ^{15}N-tracers to examine denitrification (Mulholland et al. 2004, *http://www.biol.vt.edu/faculty/webster/linx)*. Butterbach-Bahl et al. (2002) used an RGFC system to measure N_2 and N_2O emissions simultaneously with high accuracy for forest soils in Germany. They determined that these emissions were equivalent to 30 - 50% of the high rates of atmospheric N deposition at these sites. Methods for measuring denitrification continue to improve. Better measurement methods combined with better experimental design (see above) and scaling techniques (see below), will lead to progress on broad-scale denitrification estimates. The new RCN on denitrification grew out of a 2004 workshop on measuring denitrification and coordinated another workshop in 2006 on modeling denitrification. They are currently planning other network activities to promote information exchange on measuring and modeling denitrification.

1.5 EXTRAPOLATING DENITRIFICATION RATES

Current methods of measuring denitrification do not allow for direct estimation of denitrification at broad scales, therefore, point measurements must be extrapolated to achieve these estimates (except when calculating by difference). Accurate extrapolation of denitrification rates cannot be achieved by simply multiplying fine-scale measurements by the area of the study region. The high variability of rates in time and space, including hot spots and hot moments, would lead to erroneous estimates. More robust extrapolation requires linking knowledge of controls to measurements of denitrification. In other words, it requires linking "pattern to process" or "structure to function". These catch phrases of ecology may seem trite but they are the key to better broad-scale estimates of heterogeneous phenomena (Paerl and Steppe 2003). If we know which factor is controlling denitrification in certain areas, and how levels of that factor are distributed, we can efficiently predict denitrification rates. But this statement simplifies the exercise. The key connection between the "pattern" and the "process" or the "structure" and the "function" is a quantitative understanding of the relationship between the two.

A simple quantitative approach to link controls to denitrification rates is developing statistical regression relationships between denitrification and single predictors. At the opposite extreme, denitrification can be predicted within the context of a complex process model. Developing useful models anywhere in this spectrum is a great challenge. Determining which parameters and variables are important, assessing how and when they act and interact, measuring their levels, and quantifying the relationship between their levels and denitrification rates is not a trivial task. While highly detailed models have the capacity to depict the complex controls on denitrification,

making a model more mechanistic is only helpful up to a point. The great effort, amount of data, and computing ability required often do not pay off in heavily parameterized models (Pace 2003, DeAngelis and Mooij 2003).

Process models, which attempt to get at the mechanisms of the relationships between controls and denitrification rates, hold great promise for balancing simplicity and complexity in depiction of denitrification. Numerous carbon and nitrogen cycle process models exist, but many lack an explicit denitrification component (e.g. PnET-CN, Aber and Driscoll 1997). In other models e.g., NASA-CASA (Potter et al. 1996), RHESSys (Band et al. 1993, 2001), SINIC (Hong et al. 2005), and DNDC (Li et al. 2000), algorithms that depict overall ecosystem C and N processes and soil and climate drivers generate inputs to a denitrification submodel. However, the ability of these models to accurately simulate denitrification, especially N_2 flux, has not been tested at broad scales. Boyer et al. (2006) recently published a review of models that address denitrification rates at regional scales; they conclude that there is a great need for advances in modeling *and* measurement to promote further development and testing of models.

To strike the right balance between accuracy and expediency, as determined by model complexity, one must consider the amount and distribution of the available data on denitrification rates and controlling variables, as well as computing capabilities. Furthermore, controlling phenomena that manifest at the scale of the study site and period should drive the model. Conceptual models, like that proposed by Brumme et al. (1999) to examine controls on N_2O flux, offer insight into hierarchies of control as they operate at differing scales. In this model, the authors have arranged controls into a hierarchy in which long-term change in state variables exerts top down control on

N_2O flux and generates site variation, while the physical and chemical contexts that change over the short term produce temporal variation in N_2O flux. Another conceptual approach is the "cradle to the grave" approach, in which the various forms of nitrogen are traced as they are transported and transformed in different parts of the landscape (Mosier et al. 1998). This approach has been used by Seitzinger et al. (2006) in a global synthesis of denitrification rates and fits well in the nitrogen cascade framework proposed by Galloway et al. (2003) for examining Nr transport, transformation, and elimination via denitrification (Figure 1-1). The synthesis calculates Nr loss to denitrification at each step along a soil-to-ocean gradient with great uncertainties, but still provides some of the most informed estimates of global scale denitrification available to date. Hydro-ecological models like RHESSys (Band et al. 2001) and SINIC (Hong et al. 2005) also trace nitrogen through transport and transformations. This approach often directs attention to the human-scale processes that control nitrogen transformations, but can also identify finer and broader scale drivers of processes like denitrification. Although the "cradle to the grave" approach and hydro-ecological models are not as explicit about hierarchies of control as the Brumme et al. (1999) model, they examine nitrogen processing in a very dynamic way, emphasizing processes with strong effects on N fluxes and excluding those with minimal effects at the scale of interest to the study. Focusing on the phenomena that operate at the scale of the study, while cutting out minor influences, should make models more elegant and efficient, while maintaining accuracy.

Producing broad-scale estimates of denitrification requires that models be run in spatially and temporally explicit contexts, as in a geographic information system (GIS). High quality data sets of multiple parameters that span large spaces and long time periods are rare. For example, high resolution digital soil maps have been shown

to be capable of depicting riparian hot spots of denitrification at landscape and regional scales (Rosenblatt et al. 2001), but these data are not available for all areas. Long-running research sites, like those in the Long Term Ecological Research network, may be able to provide such data, making these sites well equipped to support efforts to estimate broad-scale rates of denitrification. The Hubbard Brook Ecosystem Study, for example, has data on soils (texture, moisture, etc.), N cycling (mineralization, nitrification, etc.), vegetation (species, foliar N, etc.), and other factors influencing denitrification, some of which are part of a continuous record dating back to 1956 (www.hubbardbrook.org).

Technological advances hold great promise for providing data relevant to denitrification at broad scales. Scientists are now using remote sensing data from airplane flyovers and satellite imagery to measure a vast array of characteristics of the earth's surface, including foliar N levels and soil moisture. Advances in remote sensing, e.g., high spectral resolution ("hyperspectral") and multiangle imaging, are changing the way ecologists collect broad-scale data (Asner et al. 1998). Data from remote sensing imagery could serve as input to a denitrification model or its contextual simulation model. For example, the NASA-CASA model utilizes remotely sensed data to derive its net primary productivity input (Potter et al. 1993). Of particular promise for denitrification is the ability to remotely sense foliar N content (Wessman et al. 1988, Ollinger et al. 2002 Smith et al. 2002) with the Airborne Visible/Infrared Imaging Specrometer (AVIRIS) (Figure 1-5) or other hyperspectral sensors, (e.g. Hyperion, a satellite-borne hyperspectral imager). Because foliar N is tightly linked to patterns of N availability in time and space, these measurements should be strong predictors of denitrification and other processes dependent on N availability at landscape and regional scales.

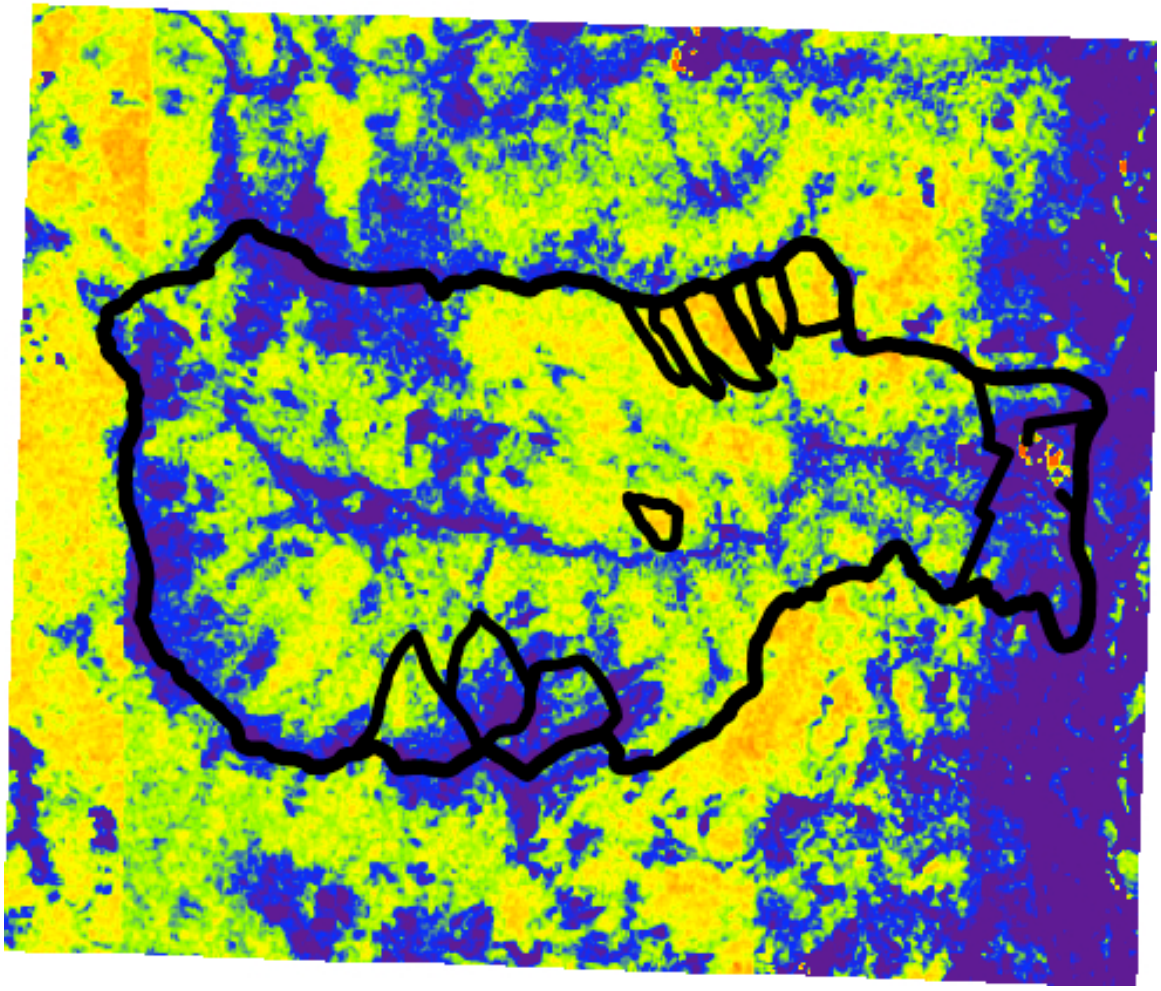


Figure 1-5. Map of canopy foliar nitrogen concentration at the Hubbard Brook watershed (outlined in black). This figure was derived from data presented in Smith et al. (2002), in which nitrogen concentration was determined using reflectance data from NASA's AVIRIS hyperspectral sensor. Note the high nitrogen levels, shown in light colors, in the small experimental watersheds dominated by young vegetation (in the upper right hand corner of the image). Low nitrogen levels, shown in dark colors, dominate in areas with a high concentration of conifers at the highest elevations (on the left side of the image) and along the main stream (across the middle of the watershed).

Remotely sensed data also offer the promise of greater temporal resolution of broad-scale spatially explicit data. Regular flyovers by satellites make more frequent collection of images possible. These images do, however, demand skillful processing and ground-truthing for extraction of data, somewhat limiting their utility. Furthermore, their spatial resolution must be adequate for discerning areas of variability in the characteristic being examined. With careful treatment, however, remote sensing images provide information on potential controlling variables that cannot, currently, be gathered any other way.

Spatially and/or temporally explicit data sets may also be generated from models, e.g. nitrification may be modeled as a subroutine in a larger nitrogen cycling model to generate nitrate availability for a denitrification process. Models like TOPMODEL have been extensively used to generate hydrologic data on variables such as soil moisture, a powerful driver of denitrification rates that can be readily modeled with high resolution climate and soil data (Beven 1997). Climate and soil information are more frequently and extensively collected than direct measurements of soils moisture.

1.6 THE IMPORTANCE OF SCALE

Scale is often viewed as a unifying theme in ecology. It is certainly at the crux of the issue of how denitrification plays into the world's nitrogen pollution problem. While we have a good understanding of denitrification at the molecular, microbial, and daily scales (Figures 1-3 & 1-4), the nitrogen pollution problems we face are manifest at much broader scales, as emphasized in the report of a recent INI workshop on nitrogen policy (Barbut and Ramakrishna 2006). If denitrification is the only way to reverse nitrogen fixation, we must develop a better understanding of it at the appropriate scales, which range from field to global, and year to century scales.

While most large-area estimates of denitrification have been produced by difference, these estimates are limited by their inability to adjust to changing conditions, e.g. increasing reactive nitrogen levels; denitrification rates may very well respond in a non-linear fashion to changes in various controlling factors. Some studies have applied many of the promising new techniques discussed above to produce more robust estimates. For example, Butterbach-Bahl et al. (2004) combined extensive field measurements of N_2O and NO with nitrogen process models (PnET-DNDC, Li et al. 2000) and a detailed GIS database to produce regional-scale estimates of fluxes for Saxony in Germany. These analyses allowed them to address questions about the importance of soil emissions relative to atmospheric deposition and industrial emissions in a heavily human-dominated region. Donner et al. (2004) used terrestrial and aquatic process models with detailed climate data to simulate patterns of denitrification in the Mississippi Basin, allowing them to address questions relevant to hypoxia in the Gulf of Mexico (Mitsch et al. 2001). These studies are real examples that show that our ability to asses the importance of denitrification to nitrogen pollution questions is improving.

1.7 CONCLUSIONS

As the world faces a growing problem of excess Nr polluting the planet, scientists are seeking to reduce N fixation and better understand the fates of Nr. As the primary mechanism for converting Nr to inert N_2, denitrification could, in a way, be the "solution" to the global N pollution problem. The current state of knowledge on denitrification, however, is not sufficient to estimate denitrification rates at what might be considered *broad* scales (i.e. greater than field scale) without a great deal of uncertainty. There is a clear need to develop better estimates of denitrification at

scales relevant to N pollution issues so that we may address them with greater confidence.

Recent advances in methods for measuring denitrification, approaches to experimental design, and biogeochemical modeling offer hope for progress in deriving broad-scale estimates of denitrification. Methods are improving, even for measuring N_2, in both terrestrial and aquatic systems. More studies are being carried out at landscape and regional scales, leading to improved understanding of how controls of a micro-scale process are expressed at the scales relevant to N pollution problems. New models are able to depict denitrification as it is regulated by C and N cycle processes, and these models can be run at landscape and regional scales using high resolution GIS and remote sensing techniques. So new *measurement* methods allow for better estimates of denitrification rates, but not necessarily at broader spatial or temporal scales. New *extrapolation* methods bridge the gap between the scales of measurements and questions about nitrogen pollution. Most promising, these new tools are being applied in important contexts, e.g., the Gulf of Mexico, where denitrification may play a critical role in regulating the nature and extent of the effects of N pollution on water and air quality. As our understanding of denitrification increases, we will have a stronger basis for evaluating how to solve N pollution problems, not just through source reduction, but also by protection and/or promotion of denitrification hot spots.

1.8 DISSERTATION OVERVIEW

The research presented in this dissertation aims to characterize the importance of denitrification as a fate of Nr in northeastern forests. To that end, this first chapter was designed to explain the reasons for pursuing this research, describe the issues that must be addressed, and suggest ideas to do so.

Chapter 2 presents two methods for measuring denitrification and the results of tests we conducted to evaluate their usefulness. One of those methods, the ^{15}N failed to give robust flux rates because of problems with measurements of soil nitrate pool enrichment. However, we were able to use the rest of the data collected for this method to calculate percent recovery of applied ^{15}N as an assessment of short-term transformation of wet N deposition via denitrification. These results are analyzed in Chapter 3.

Chapter 4 presents emissions of N_2 and N_2O, as measured using a direct flux method, across three sites in White Mountain National Forest (WMNF), including Hubbard Brook Experimental Forest. Patterns in emissions, within and among sites, and their relationships to other variables are analyzed and used to model valley-wide fluxes of gaseous N.

Chapter 5 reviews and synthesizes the contents of the first four chapters by assembling the conclusions of each one and reinforcing the common thread that denitrification to N_2 is an important fate of Nr in WMNF. It then speculates about directions for research that may follow and implications of the results presented here.

WORKS CITED

Aber JD and Driscoll CT. 1997. Effects of land use, climate variation, and N deposition on N cycling and C storage in northern hardwood forests. Global Biogeochemical Cycles 11(4): 639-648.

Asner GP, Braswell BH, Schimel DS, and Wessman CA. 1998. Ecological needs from multiangle remote sensing data. Remote Sensing of Environment 63: 155-165.

Band LE, Patterson P, Nemani R, and Running SW. 1993. Forest ecosystem processes at the watershed scale: incorporating hillslope hydrology. Forest Meteorology 63: 93-126.

Band LE, Tague C, and Groffman PM. 2001. Forest ecosystem processes at the watershed scale: hydrological and ecological controls of nitrogen export. Hydrological Processes 15: 2013-2028.

Barbut M and Ramakrishna K. 2006. UNEP-WHRC Nitrogen Policy Workshop, Paris 8-10 March 2006, Chairs' Summary of Proceedings and Next Steps. Available at http://initrogen.org/nitrogen_policy_wksp.0.html.

Bernhardt ES, Likens GE, Buso DC, and Driscoll CT. 2003. In-stream uptake dampens effects of major forest disturbance on watershed nitrogen export. Proceedings of the National Academy of Sciences of the United States of America 100(18): 10304-10308.

Beven KJ (Ed.). 1997. Distributed Modelling in Hydrology. Applications of TOPMODEL, Wiley, Chichester.

Böhlke JK, Wanty R, Tuttle M, Delin G, and Landon M. 2002. Denitrification in the recharge area and discharge area of a transient agricultural nitrate plume in a glacial outwash sand aquifer, Minnesota. Water Resources Research 38(7): Art. No. 1105.

Boyer EW, Alexander RB, Parton WJ, Li C, Butterbach-Bahl K, Donner SD, Skaggs W, and Del Grosso SJ. 2006. Modeling denitrification in terrestrial and aquatic ecosystems at regional scales. Ecological Applications 16(6): 2123-2142.

Brumme R, Borken W, and Finke S. 1999. Hierarchical control on nitrous oxide emission in forest ecosystems. Global Biogeochemical Cycles 13(4): 1137-1148.

Butterbach-Bahl K, Willibald G, and Papen H. 2002. Soil core method for direct simulataneous determination of N2 and N2O emissions from forest soils. Plant and Soil 240(1): 105-116.

Butterbach-Bahl K, Kesik M, Miehle P, Papen H, and Li C. 2004. Quantifying the regional source strength of N-trace gases across agricultural and forest ecosystems with process based models. Plant and Soil 260(1-2): 311-329.

DeAngelis DL and Mooij WM. 2003. Ch. 5: In praise of mechanistically rich models. In: Canham CD, Cole JJ, Lauenroth WK, (Eds.) Models in Ecosystem Science. Princeton University Press, Princeton. 63-82.

Donner SD, Kucharik CJ, and Foley JA. 2004. Impact of changing land use practices on nitrate export by the Mississippi River. Global Biogeochemical Cycles 18(1): Art. No. GB1028.

Erisman JW. 2004. The Nanjing Declaration on management of reactive nitrogen. *BioScience* 54: 286-287.

Fenchel T and Blackburn TH. 1979. Bacteria and mineral cycling. Academic Press, London. 225 p.

Firestone MK. 1982. Biological denitrification. Pp.289-326. In: FJ Stevenson, (Ed.) Nitrogen in Agricultural Soils. American Society of Agronomy, Madison.

Galloway JN and Cowling EB. 2002. Reactive nitrogen and the world: 200 years of change. Ambio 31(2): 64-71.

Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, and Cosby BJ. 2003. The nitrogen cascade. Bioscience 53(4): 341-356.

Groffman PM. 1991. Ecology of nitrification and denitrification in soil evaluated at scales relevant to atmospheric chemistry. In: Whitman WB, Rodgers J (Eds.) Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides and Halomethanes. American Society of Microbiology, Washington, DC. 201-217.

Groffman PM, Dorsey AM, and Mayer PM. 2005. Nitrogen processing within geomorphic features in urban streams. Journal of the North American Benthological Society 24(3): 613-625.

Groffman PM, Altabet MA, Bohlke JK, Butterbach-Bahl K, David MB, Firestone MK,Giblin AE, Kana TM, Nielsen LP, and Voytek MA. 2006. Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecological Applications 16(6): 2091-2122.

Hong, BG, Strawderman RL, Swaney DP, and Weinstein DA. 2005. Bayesian estimation of input parameters of a nitrogen cycle model applied to a forested reference watershed, Hubbard Brook Watershed Six. Water Resources Research 41(3): Art. No. W03007.

Howarth RW, Boyer EW, Pabich WJ, and Galloway JN. 2002. Nitrogen use in the United States from 1961-2000 and potential future trends. Ambio 31(2): 88-96.

Howarth RW and Marino R. 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. Limnology and Oceanography 51(1): 364-376.

Kana TM, Darkangelo C, Hunt MD Oldham JB, Bennett GE, and Cornwell JC. 1994. Membrane inlet mass-spectrometer for rapid high-precision determination of N-2, O-2, and Ar in environmental water samples. Analytical Chemistry 66(23): 4166-4170.

Li CS, Aber J, Stange F, Butterbach-Bahl K, and Papern H. 2000. A process-oriented model of N2O and NO emissions from forest soils: 1. Model development. Journal of Geophysical Research-Atmospheres 105(D4): 4369-4384.

Matson P, Lohse KA, and Hall SJ. 2002. The globalization of nitrogen deposition: Consequences for terrestrial ecosystems. Ambio 31(2): 113-119.

McClain ME, Boyer EW, Dent CL, Gergel SE, Grimm NB, Groffman PM, Hart SC, Harvey JW, Johnston CA, Mayorga E, McDowell WH, and Pinay G. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6: 301-312.

Miller JR, Turner MG, Smithwick EAH, Dent CL, and Stanley EH. 2004. Spatial extrapolation: the science of predicting ecological patterns and processes. Bioscience 54: 310-320.

Mitsch WJ, Day Jr. JW, Gilliam JW, Groffman PM, Hey DL, Randall GW, and Wang N. 2001. Reducing nitrogen loading to the Gulf of Mexico from the Mississippi River basin: strategies to counter a persistent ecological problem. BioScience 51: 373-388.

Mosier A, Kroeze C, Nevison C, Oenema O, Seitzinger S, and van Cleemput O. 1998. Closing the global atmospheric N2O budget: Nitrous Oxide emissions through the agricultural nitrogen cycle. Nutrient Cycling in Agroecosystems 52: 225-248.

Mulholland PJ, Valett HM, Webster JR, Thomas SA, Cooper LW, Hamilton SK, and Peterson BJ. 2004. Stream denitrification and total nitrogen uptake rates measured using a field N-15 tracer addition approach. Limnology and Oceanography 49(3): 809-820.

Nõmmik, H. 1956. Investigations on denitrification in soils. Acta Agriculturae Scandinavica 6: 195–228

Pace ML. 2003. The utility of simple models in ecosystem science. In: Canham CD, Cole JJ, and Lauenroth WK (Eds.) Models in Ecosystem Science. Princeton University Press, Princeton. 49-62.

Paerl HW and Steppe TF. 2003. Scaling up: the next challenge in environmental microbiology. Environmental Microbiology 5(11): 1025-1038.

Parkin TB. 1987. Soil microsites as a source of denitrification variability. Soil Science Society of America Journal 51: 1194–1199.

Parkin TB, Starr JL, and Meisinger JJ. 1987. Influence of sample size on measurement of soil denitrification. Soil Science Society of America Journal 51: 1492-1501.

Parsons LL, Murray RE, and Smith M. 1991. Soil denitrification dynamics: Spatial and temporal variations of enzyme activity, populations, and nitrogen gas loss. Soil Science Society of America Journal 55: 90-95.

Paul EA and Clark FE. 1996. Soil Microbiology and Biochemistry. Academic Press, San Diego, CA.

Potter CS, Randerson JT, Field CB, Matson PA, Vitousek PM, Mooney HA, and Klooster SA. 1993. Terrestrial ecosystem production – A process model based on global satellite and surface data. Global Biogeochemical Cycles 7(4): 811-841.

Potter CS, Matson PA, Vitousek PM, and Davidson EA. 1996. Process modeling of controls on nitrogen trace gas emissions from soils worldwide. Journal of Geophysical Reasearch – Atmospheres 101(D1): 1361-1377.

Priemé A, and Christensen S. 2001. Natural perturbations, drying-wetting and freezing-thawing cycles, and the emission of nitrous oxide, carbon dioxide and methane from farmed, organic soils. Soil Biology and Biochemistry 33: 2083-2091.

Ollinger SV, Smith ML, Martin ME, Hallett RA, Goodale CL, and Aber JD. 2002. Regional variation in foliar chemistry and N cycling among forests of diverse history and composition. Ecology 83(2): 339-355.

Rosenblatt AE, Gold AJ, Stolt MH, Groffman PM, and Kellogg DG. 2001. Identifying riparian sinks for watershed nitrate using soil surveys. Journal of Environmental Quality 30: 1596-1604.

Schlesinger WH. 1997. Biogeochemistry: An Analysis of Global Change. Academic Press, San Diego, CA. 588 pp.

Seitzinger SP, Harrison JA, Böhlke JK, Bouwman AF, Lowrance R, Peterson B, Tobias C, and van Drecht G. 2006. Denitrification across landscapes and waterscapes: A synthesis. Ecological Applications 16(6): 2064-2090.

Smith ML, Ollinger SV, Martin ME, Aber JD, Hallett RA, and Goodale CL. 2002. Direct estimation of aboveground forest productivity through hyperspectral remote sensing of canopy nitrogen. Ecological Applications 12: 1286-1302.

Tiedje JM. 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Zehnder, AJB (Ed). Biology of anaerobic microorganisms. John Wiley and Sons, New York, New York, USA. 170-244.

van Breemen N, Boyer EW, Goodale CL, Jaworski NA, Paustian K, Seitzinger SP, Lajtha K, Mayer B, van Dam D, Howarth RW, Nadelhoffer KJ, Eve M, and Billen G. 2002. Where did all the nitrogen go? Fate of nitrogen inputs to large watersheds in the northeastern USA. Biogeochemistry 57(1): 267-293.

van Egmond K, Bresser T, and Bouwman L. 2002. The European nitrogen case. Ambio 31(2): 72-78.

Wallenstein MD, Myrold DD, Firestone M, and Voytek M. 2006. Environmental controls on denitrification rates: Insights from molecular methods. Ecological Applications 16(6): 2143-2152.

Wessman CA, Aber JD, Peterson DL, and Melillo JM. 1988. Remote sensing of canopy chemistry and nitrogen cycling in temperate forest ecosystems. Nature 335(6186): 154-156.

Zheng X, Fu C, Xu X, Yan, X, Huang Y, Han S, Hu F, and Chen G. 2002. The Asian nitrogen cycle case study. Ambio 31(2): 79-87.

CHAPTER 2

DIRECT FLUX AND ^{15}N TRACER METHODS FOR MEASURING DENITRIFICATION IN FOREST SOILS

2.1 ABSTRACT

Estimates of denitrification are one of the key uncertainties in the terrestrial nitrogen cycle, primarily because reliable measurements of this process – especially the production of its terminal product (N_2) – are difficult to obtain. I evaluated the ability of gas-flow soil core and ^{15}N tracer methods to provide reliable estimates of denitrification in forest soils. My objectives were to 1) describe and present typical results from new gas-flow soil core and *in situ* ^{15}N tracer methods for measuring denitrification, 2) discuss factors that affect the relevance of these methods to actual *in situ* denitrification and 3) compare denitrification estimates produced by the two methods for a series of sites in a northern hardwood forest ecosystem. Both methods were able to measure accumulations of N_2 over relatively short (2-5 hour) incubations of either unamended or tracer-amended intact soils. Rates measured by the direct flux soil core method were very sensitive to the O_2 concentration in the recirculation gas, decreasing with increasing O_2 levels ($p=0.001$ for N_2 and $p=0.02$ for N_2O). Incubation O_2 concentrations should therefore be closely paired with field oxygen levels. Rates measured by the *in situ* ^{15}N tracer method were very sensitive to the enrichment of the NO_3^- pool undergoing denitrification, limiting the applicability of this method for quantifying denitrification in N-poor ecosystems. While its ability to provide accurate estimates of denitrification was limited, the ^{15}N tracer method provided estimates of the short-term abiotic and biotic transformations of atmospheric N deposition N to gas. Results from these methods suggest that denitrification and N_2:N_2O ratios are

higher than previously thought in the northern hardwood forest and that short-term abiotic and biotic transformations of atmospheric N deposition to gas are significant in this ecosystem.

2.2 INTRODUCTION

In recent years, interest in understanding the balance between dinitrogen (N_2) and all other forms in regional to global nitrogen cycles has been increasing due to massive anthropogenic alterations of the global N cycle (Kulkarni et al. 2008). These other forms of nitrogen are considered biologically, chemically, or radiatively reactive and are therefore grouped into the category called reactive nitrogen (Nr). Reactive nitrogen is necessary for such beneficial activities as growing crops, but can also pollute soil, air and water as it moves through what Galloway and Cowling (2001) call the "nitrogen cascade". The fate of Nr, especially to denitrification (the reduction of oxidized forms of inorganic N to N_2O and N_2), has therefore recently been listed as one of the most "vexing questions" about the global nitrogen cycle (Galloway et al. 2008). The main reason that this question remains so vexing after over a century of studies on the nitrogen cycle is that reliable measurements of denitrification, especially the transformation of Nr to N_2 rather than other reactive species, remain elusive (Davidson and Seitzinger 2006, Groffman et al. 2006a).

The dearth of data on N_2 fluxes may be causing scientists to underestimate total denitrification. Several studies of regional to continental scale nitrogen budgets indicate that denitrification may be a more important sink for soil (and aquatic) nitrogen than previously thought (Howarth et al. 2002, van Breemen et al. 2002, van Egmond et al. 2002, Zheng et al. 2002). Furthermore, nitrogen inputs from nitrogen fixation and deposition may also be widely underestimated (Russow et al. 2001,

Fulweiler et al. 2007, He et al. 2007). New methods to quantify N deposition using ^{15}N dilution have found that bulk deposition as measured using precipitation collectors accounted for only 24-45% of total N deposition (He et al 2007). Bormann et al. (1993, 2002) measured unexplained nitrogen accumulations of 40-150 kg N ha^{-1} y^{-1} in "sandbox" mesocosms at the Hubbard Brook Experimental Forest (HBEF), my study site, which may have arisen from nitrogen fixation. Whatever the source, this accumulation represents an input to the system that is 1-2 orders of magnitude higher than current measurements of atmospheric deposition, suggesting that the pool of available reactive nitrogen for denitrification could be much greater than expected based solely on currently measured deposition inputs. If inputs from N fixation and deposition are greater than current measurements indicate, the denitrification rates – as calculated by difference in N budgets – reported in most broad scale studies of the nitrogen cycle (Howarth et al. 2002, van Breemen et al. 2002, van Egmond et al. 2002, Zheng et al. 2002) must also be greater.

Classical denitrification is the reduction of nitrate (NO_3^-) to dinitrogen in the following sequence: NO_3^- ➔ NO_2^- ➔ NO ➔ N_2O ➔ N_2. Nitrate and nitrite (NO_2^-) are aqueous species that can cause excessive nutrient enrichment of water bodies ("eutrophication"). Nitric oxide (NO) and nitrous oxide (N_2O) are both gaseous air pollutants: NO precipitates tropospheric ozone production and N_2O is a greenhouse gas that also destroys stratospheric ozone. Because of the various potential detrimental effects of these nitrogen species, it is important to understand the partitioning of denitrification end products (Galloway et al. 2008, Kulkarni et al. 2008).

Methods that address multiple products of denitrification are few, especially those for use in soils. N_2O fluxes from soils have been extensively studied, especially in agricultural systems, but measurements of N_2 fluxes are uncommon (Groffman et al. 2006a, Stehfest and Bouwman 2006). Two categories of methods that can address both species exist: direct flux and ^{15}N stable isotope tracer methods. Among the direct flux methods is the long-used, but increasingly unpopular acetylene block method, which exploits acetylene (C_2H_2) inhibition of the reduction of N_2O to N_2. Since N_2O is much more easily measured than N_2 because of the very high atmospheric background levels of N_2, total N fluxes from denitrification (with the possible exception of NO production) can be readily measured on a gas chromatograph (GC) fitted with an electron capture detector (ECD). Unfortunately, acetylene also blocks nitrification, which transforms ammonium (NH_4^+) into the NO_3^- that is needed for denitrification. Therefore, denitrification as measured by this method only measures denitrification that is not coupled to nitrification. Furthermore, the acetylene itself may be consumed by soil microbes in long (greater than several days) incubations (Yeomans and Beauchamp 1982). Finally, the acetylene method doesn't allow for quantification of denitrification as a reactive nitrogen sink (i.e. how much ends up as N_2).

Gas-flow soil core and ^{15}N stable isotope tracer methods allow for measurements of denitrification that are not encumbered by the limitations of the acetylene block method. Gas-flow soil core systems connect containers for soil cores to GCs via gas-tight tubing and fittings. The GCs are equipped with detectors that can analyze both N_2 and N_2O. The incubations are run after the cores' headspaces have been replaced with an N_2- and N_2O-free atmosphere and accumulations of these two gases in the core containers are measured over time. By using sensitive detectors and high quality stainless steel tubing and fittings, gas-flow soil core systems allow for precise

measurements of N_2 and N_2O at very low levels (Swerts et al. 1995, Scholefield et al. 1997a, Butterbach-Bahl et al. 2002). This method allows for measurement of denitrification fluxes without modification or destruction of the soil core. It does, however, necessitate removing the soil from the field and is very time-intensive (Groffman et al. 2006a).

^{15}N tracer methods have the great advantage of providing *in situ* measurements that can be run over considerably shorter time-periods than gas flow soil core incubation methods. These methods require addition of $^{15}NO_3^-$ to the soil as the stable isotope tracer, along with water to carry the label into the soil. Such additions can artificially increase denitrification, but tracer level N additions (~5% enrichment of the extractable N pool) are not thought to have a significant effect on rates. A major challenge is achieving and assessing uniform distribution of the labeled nitrogen in the soil (Knowles and Blackburn 1992). Accurate calculation of denitrification rates requires measuring the true enrichment of the pool of NO_3^- that is actively being reduced in denitrification. Siegel et al. (1982) and Spott and Stange (2007) have developed precise ways to determine this enrichment using a mass spectrometer to measure isotopic ratios of evolved gases. Mulvaney and VandenHeuvel (1988) and Mulvaney (1988) showed that such methods provide accurate estimates of N gas evolution from denitrification without uniform distribution of labeled nitrate. These methods, however, require enriching the NO_3^- pool well beyond tracer levels. Another impediment to using this method is cost; analysis of enriched gases and soil extracts on an isotope ratio mass spectrometer (IRMS), as well as buying ^{15}N-labeled NO_3^- in the first place, are expensive.

Assessments of how various methods of measuring denitrification compare with one another – especially *in situ* vs. *in vitro* methods – are rare (Parkin et al. 1985, Myrold 1990, Payne 1991, Groffman et al. 2006a). Denitrification rates from gas-flow soil core methods and ^{15}N tracer methods, in particular, have not been compared in the literature on the same soils sampled at the same time. Comparisons can be tricky since, in most cases, results from different methods must be interpreted differently. For example, the ^{15}N tracer method gives N_2O flux rates that arise only from denitrification whereas the gas-flow soil core method gives a total N_2O flux (from denitrification and nitrification). The physical conditions of the two methods also differ, i.e. extracted cores versus *in situ* chambers (Ryden et al. 1987). There is danger of bias in large-scale extrapolations of rates when use of any one method in an ecosystem type predominates (e.g. acetylene block in terrestrial systems). However, since denitrification methods tend to be either time- or resource-intensive (or both), researchers usually cannot afford to apply two methods in the same study. Given the variety of methods used within and among systems, however, knowledge of how rates measured using different methods compare with each other is critical to our understanding of N gas fluxes across studies and systems.

In this study, I compared gas-flow soil core and ^{15}N tracer methods to measure denitrification in forest soils. My objectives were to 1) describe and present typical results from new gas-flow soil core and *in situ* ^{15}N tracer methods for measuring denitrification, 2) discuss factors that affect the relevance of these methods to actual *in situ* denitrification and 3) compare denitrification estimates produced by the two methods in a northern hardwood forest ecosystem.

2.3 METHODS

2.3.1 Study Site and Sampling Regime

The HBEF is located in a 3160 ha watershed in the White Mountain National Forest, New Hampshire, USA. (43º 56' N, 71º 45' W). Vegetation at the HBEF is northern hardwood forest dominated by yellow birch (*Betula alleghaniensis)*, American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*) and red maple (*Acer rubrum*) with red spruce (*Picea rubens)* and balsam fir (*Abies balsamea)* more common at higher elevations. The forest was selectively cut in the 1880s and 1910s, and some of the older stands were damaged by a hurricane in 1938. Soils are shallow (75 – 100 cm), acidic (pH 3.9) Typic Haplorthods developed from unsorted basal tills (USDA 1996).

I sampled in 16 circular 0.05 ha plots, a subset of those used by Venterea et al. (2003) in a study of NO_3^- and nitrification patterns across the landscape at HBEF. Soil samples for direct flux measurements were taken from 8 of these 16 plots and *in situ* ^{15}N measurements were made at all 16 plots monthly from May through October of 2005. Samples for the direct flux measurements were taken at the same time and immediately adjacent to the chambers where ^{15}N flux measurements were made.

2.3.2 Direct Flux Method

My direct flux gas-flow soil core system was modeled after that of Swerts et al. (1995) and is similar to that of Butterbach-Bahl et al. (2002). In my system, soil samples were loaded in stainless steel tubes, held in place with polyester wool packing, and sealed at the ends with raised-middle O-rings and handcuff-style brackets (Swagelok, Crawford Fitting Co., Solon, OH). The tubes were enclosed in a plexiglass box that was flushed with high-purity helium gas and were connected with stainless steel tubing and fittings (Swagelok, Crawford Fitting Co., Solon, OH) to two GCs

(Shimadzu GC8A, Kyoto, Japan). One GC was fitted with an ECD for measuring N_2O and CO_2 the other with a thermal conductivity detector (TCD) for measuring N_2 and O_2. CO_2 and O_2 evolution rates were monitored for quality control but are not reported here.

Samples were collected as a set of cores from near each gas chamber used for the ^{15}N method in 8 plots. The samples were taken from the forest floor (combined Oi, Oe, and Oa horizons) and the number of cores taken was determined by the depth of the organic layer. In most cases, 2 cores sufficed to make up the 15-25 cm of core needed for the sample. Samples were refrigerated for up to 10 days before they were analyzed.

Once samples were loaded into the stainless steel tubes, a 95% He and 5% O_2 mixture (HelOx) was flushed through the soil cores for at least one hour to replace the tube headspace with HelOx and remove all N_2 and N_2O. Testing with cores perfused with an inert tracer gas (SF_6) demonstrated that one hour was adequate to purge N_2 from these soils, which were very porous (bulk density from 0.1 to 0.3 g/cm^3) and not water-logged. Headspace gas was mixed and sampled by displacement from the tubes by the addition of approximately 40-50 ml HelOx (the exact amount was calculated from the gas transfer rate and time for each injection) at 0, 1, 3, and 5 hours. Leak rates were determined to be below detection limits by running incubations with empty cores. *In vitro* flux rates were calculated by regression of N_2-N or N_2O-N accumulation over time. These flux rates were divided by the total ground surface area of the soil samples taken in the field. This area was the number of cores taken for the sample multiplied by 12.6 cm^2, the cross-sectional area of the corer. Rates were also adjusted for temperature using a Q_{10} factor of 2 (Scholefield et al. 1997b).

A separate experiment was conducted to evaluate the effects of incubation oxygen concentration on denitrification rate. Four cores were incubated under headspace O_2 concentrations of 1%, 5%, 10% and 20% (balance He) over consecutive days. They were incubated under 5% O_2 every other day to monitor any changes in denitrification rate over the course of the experiment. Samples were refrigerated in between incubations.

2.3.3 ^{15}N Tracer Method

This technique involves spraying ^{15}N-labeled NO_3^- onto the soil, and monitoring ^{15}N - N_2O and N_2 accumulation in gas sampling chambers (287-mm diameter (ID) by 40-mm high polyvinyl chloride (PVC)). The bottom portion of the chamber, or "collar", was imbedded in the soil at least 2 days before the first sampling. Solutions of 99 atom percent $K^{15}NO_3^-$ were applied to the soil within the collars with a spray bottle to bring the enrichment up to approximately 5% of the existing KCl-extractable NO_3^- pool (based on data collected by Venterea et al. 2003). The area inside the collar was then sprinkled with 0.25 cm of water to wash the label into the soil. A duplicate collar was placed next to the original collar and treated the same way with $^{15}NO_3^-$ solution and water. A soil sample was taken from this collar for measurement of NO_3^- pool enrichment. Meanwhile, the original collar was capped to enclose the chamber and, after approximately 10 minutes, a 9 ml gas sample was taken from the headspace ("Time 0" sample). At 1 and 2 hours after this sampling, similar Time 1 and Time 2 samples were taken from the headspace. Field ambient air samples were taken at the beginning of the incubations from air near the chambers. Soil samples were brought back to the laboratory and extracted using 2 M KCl. The extracts were then analyzed for nitrate concentration on a Lachat autoanalyzer and diffused onto glass fiber filter

disks using the method developed by Stark and Hart (1996). Filter disks and gas samples taken in the field were sent to the University of California-Davis Stable Isotope Facility for analysis. The disks were analyzed for NO_3^- pool enrichment and the gas samples for N_2 and N_2O concentrations and enrichments.

I calculated N_2-N and N_2O-N fluxes as follows:

$R_{sam} = ((\delta N_{sam}/1000) + 1) * R_{std}$

Where: R_{sam} = isotope ratio of gas sample, δN_{sam} = the ^{15}N enrichment of a gas sample, and R_{std} = $^{15}N/^{14}N$ ratio of the standard (atmospheric N_2)

$F_{sam} = R_{sam}/(R_{sam} + 1)$

Where: F_{sam} = the fraction of N in the sample as ^{15}N

$mol^{15}N = F_{sam} * molN$

Where: $mol^{15}N$ = moles of N_2-^{15}N or N_2O-^{15}N in the sample, and molN = moles of total N_2-N or N_2O-N in the sample

$T_{sam} = molN/Vol_{sam}$

Where: T_{sam} = total concentration of N_2-N or N_2O-N in the sample in mol N cc^{-1},

and Vol_{sam} = the volume of the sample = 9 cc

$^{15}N_{sam} = F_{sam} * T_{sam}$

Where: $^{15}N_{sam}$ = concentration of N_2-^{15}N or N_2O-^{15}N in the sample in mol ^{15}N cc^{-1}

$^{15}N_{hsm} = {}^{15}N_{sam} * Vol_{chamber} / Area_{chamber}$

Where: $^{15}N_{hsm}$ = amount of ^{15}N in the chamber headspace in mol ^{15}N m^{-2}, $Vol_{chamber}$ = volume of the gas chamber in m^3, and $Area_{chamber}$ = cross-sectional area of the gas chamber

$^{15}N_{hsg} = {}^{15}N_{hsm} * 14.006$ g N $mol^{-1} * 2$ mol N mol^{-1} N_2 or $N_2O * 0.001$ mg g^{-1}

Where: $^{15}N_{hsg}$ = amount of ^{15}N in the chamber headspace in mg ^{15}N m^{-2}

The change in mg ^{15}N over the 2 hour sampling period was calculated by regression of $^{15}N_{hsg}$ against time for the samples taken at times 0, 1 and 2 hours. The slope of this regression was called Nslope and N_2-N and N_2O-N fluxes were then calculated as: $Nflux = Nslope / X_N$

Where: Nflux = N_2-N or N_2O-N flux in mg N m^2 h^{-1}, Nslope = slope of the regression between $^{15}N_{hsg}$ and time in mg N m^2 h^{-1}, and X_N = enrichment of the NO_3^- source pool as a unitless fraction.

Note that I assumed a fractionation factor (α) of 1.000 because the effects of fractionation are thought to be insignificant in well drained soils where the reaction is limited by diffusion of substrate to the reaction site rather than by the reaction rate itself (Groffman et al. 2006a, Högberg 1997).

2.4 RESULTS

2.4.1 Direct Flux Method

Incubations of soils for the gas-flow soil-core ("direct flux") method showed steadily increasing accumulations of N_2, although accumulation rates were generally slightly

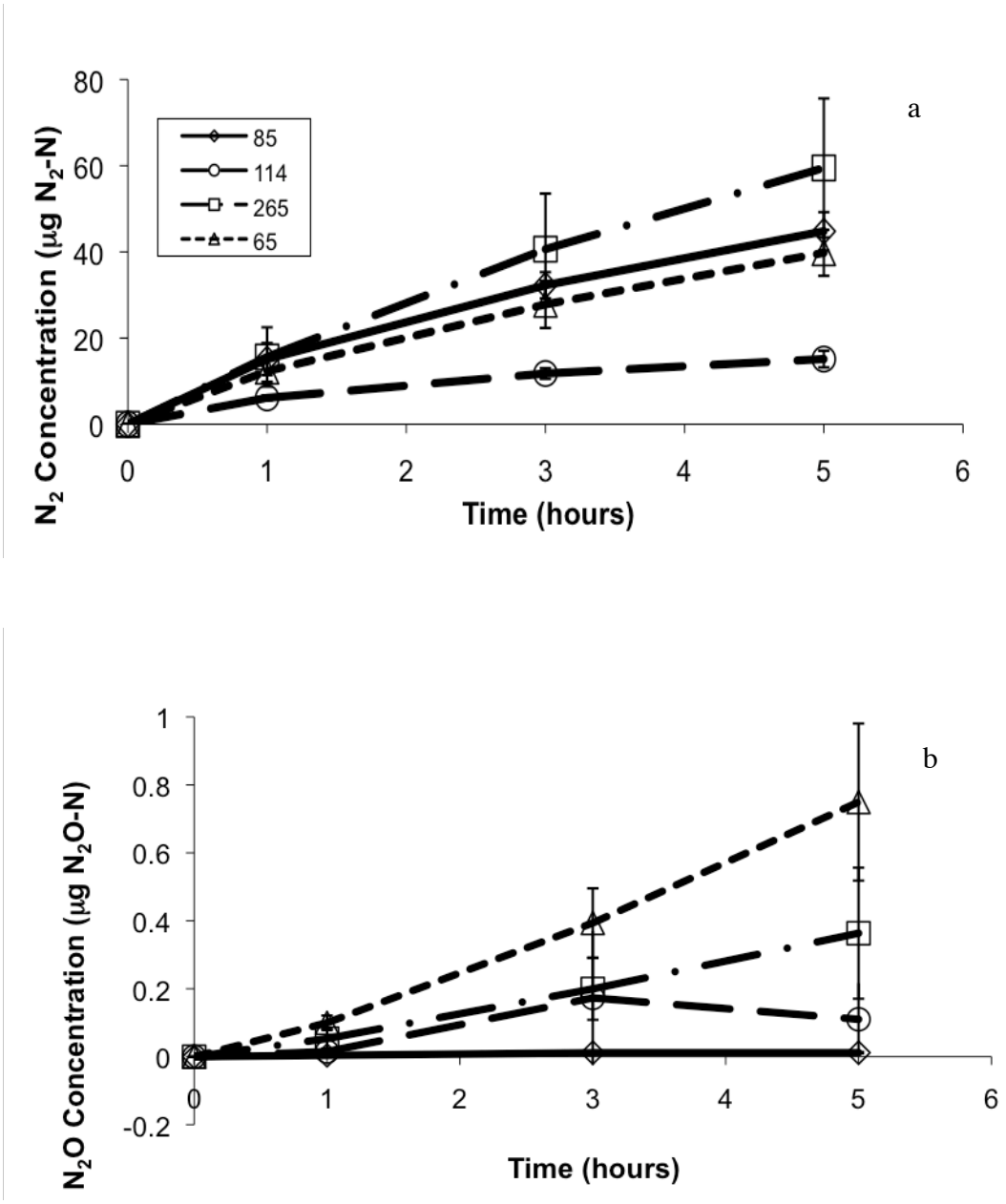


Figure 2-1. Typical accumulations of N_2 (a) and N_2O (b) in direct flux soil core method incubations. Values are means (standard deviation) of three core incubations.

higher in the first hour than later in the incubations, possibly due to the final degassing of dissolved N_2 in pore water (Figure 2-1a). I therefore calculated flux rates using regressions of the 1-, 3-, and 5-hour accumulations (excluding Time 0 measurements). N_2O accumulations did not display increased flux rates in the first hour, nor were they always steady over the course of five hours (Figure 2-1b). In some cores, accumulations decreased toward the end of the incubations (e.g. plot 114, Figure 2-1b), likely due to the reduction of headspace N_2O to N_2 by denitrifying bacteria.

Rates of both N_2 and N_2O flux were highly sensitive to the O_2 concentration of the recirculating air stream (Figure 2-2). N flux rates decreased exponentially with increasing O_2 concentrations from 0 to 5 to 10%. Above 10% O_2, rates of both N_2 and N_2O production were negligible. My routine procedure was to incubate cores at 5% O_2.

Detection limits for the direct flux method depend on the sensitivity of the gas chromatograph, the headspace of the gas flow system and the surface area and density of the soil sample. These concerns are much more critical for N_2 than for N_2O, which can be detected with very high sensitivity and has a low atmospheric background. For N_2, my gas chromatograph can detect a change of 7.5 ppmv N_2. Given a system volume of approximately 250 ml, soil surface area of approximately 25 cm^2 and soil bulk density of 0.3 g cm^3 (typical for organic forest soil horizons) I was able to detect fluxes as low as 0.04 kg N ha^{-1} d^{-1} (175 μg N m^{-2} h^{-1}) or 11.6 μg N kg^{-1} h^{-1}. I was able to detect much lower fluxes of N_2O, as low as 0.050 g N ha^{-1} d^{-1} (0.21 μg N m^{-2} h^{-1}).

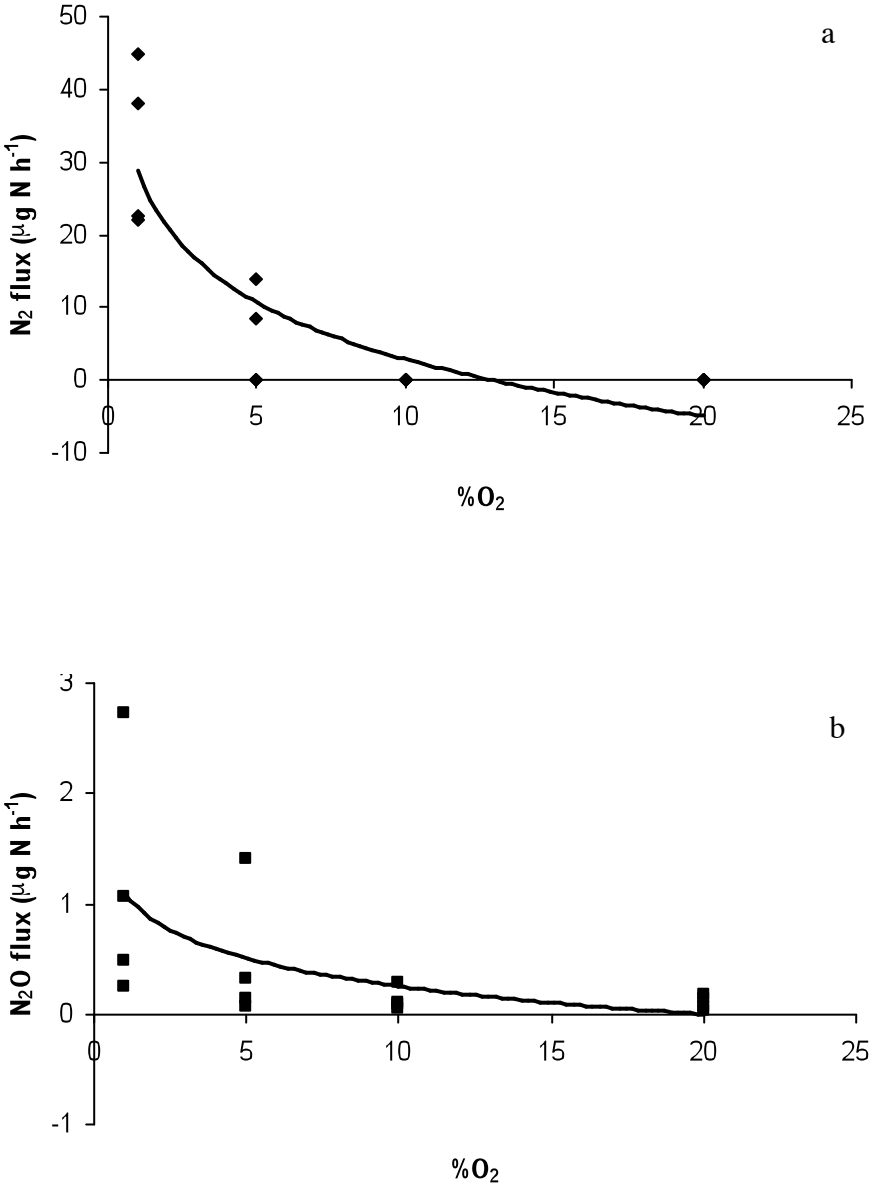


Figure 2-2. N_2 (a) and N_2O (b) fluxes versus the O_2 concentration of the recirculating gas in the direct flux system. $R^2 = 0.55$, $p = 0.001$ for the N_2 curve and $R^2 = 0.38$, $p = 0.02$ for the N_2O curve.

2.4.2 ^{15}N Tracer Method

Several different $^{15}N_2$ and $^{15}N_2O$ accumulation patterns emerged in the incubations for the isotope tracer method. The simplest pattern was a linear increase from ambient levels of ^{15}N, indicating biological denitrification of the ^{15}N that I added (Figures 2-3b and 2-4b). The most complex patterns I observed involved Time 0 values above ambient (Figures 2-3a, 2-3c, 2-4a, 2-4c). I considered the source of this initial burst of ^{15}N production – occurring in the approximately 10 minutes between capping of the chambers and taking the first (Time 0) gas sample – to be chemodenitrification, induced by the addition of NO_3^- and water. In some cases, headspace ^{15}N decreased after the initial burst (Figure 2-3c). When this occurred with N_2, I assumed a zero biological N_2 flux rate. For N_2O, I calculated decreases in enrichment as negative fluxes, or biological consumption of N_2O (Figure 2-4a). In many cases, I observed increases in headspace ^{15}N after the initial burst suggesting that both abiotic and biotic production were occurring (Figures 2-3a and 2-4c).

Quantifying detection limits for the ^{15}N method is challenging given the low concentrations of ^{15}N that I observed in the headspace of my chambers. In many cases, the changes that I observed over the course of the incubation are within the error range of the mass spectrometer and/or the variation in ambient atmospheric ^{15}N levels. I therefore examined changes in headspace ^{15}N concentration on a chamber-by-chamber basis for evidence of detectable flux. I assumed that if the R^2 of the regression of the headspace ^{15}N concentration versus time was greater than 0.95, then I could calculate a rate of biological denitrification. The smallest such slope observed in my N_2 data was 0.79 mg ^{15}N m^{-2} h^{-1}, which yields a minimum detectable rate of 2.3 mg N_2-N m^{-2} h^{-1} (or 540 g N_2-N ha^{-1} d^{-1}) using the estimated enrichment (3.5 atom %)

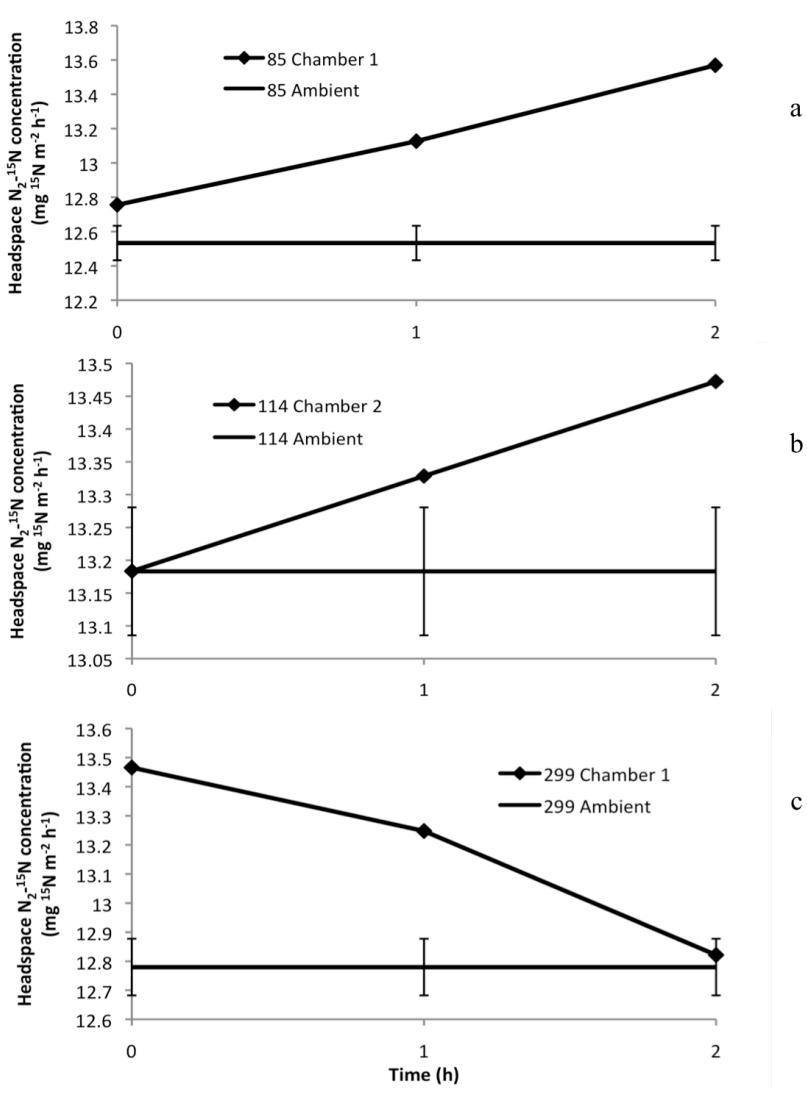


Figure 2-3. Accumulations of ^{15}N in N_2 in ^{15}N tracer method incubations for 3 sample chambers [in plot numbers 85 (a), 114 (b), and 299(c)] in July 2005 illustrating different patterns of abiotic and biotic production. Lines with error bars (standard deviations, n = 2) represent the ^{15}N concentration of ambient air outside the incubation chambers; those without error bars show headspace ^{15}N concentration within a chamber.

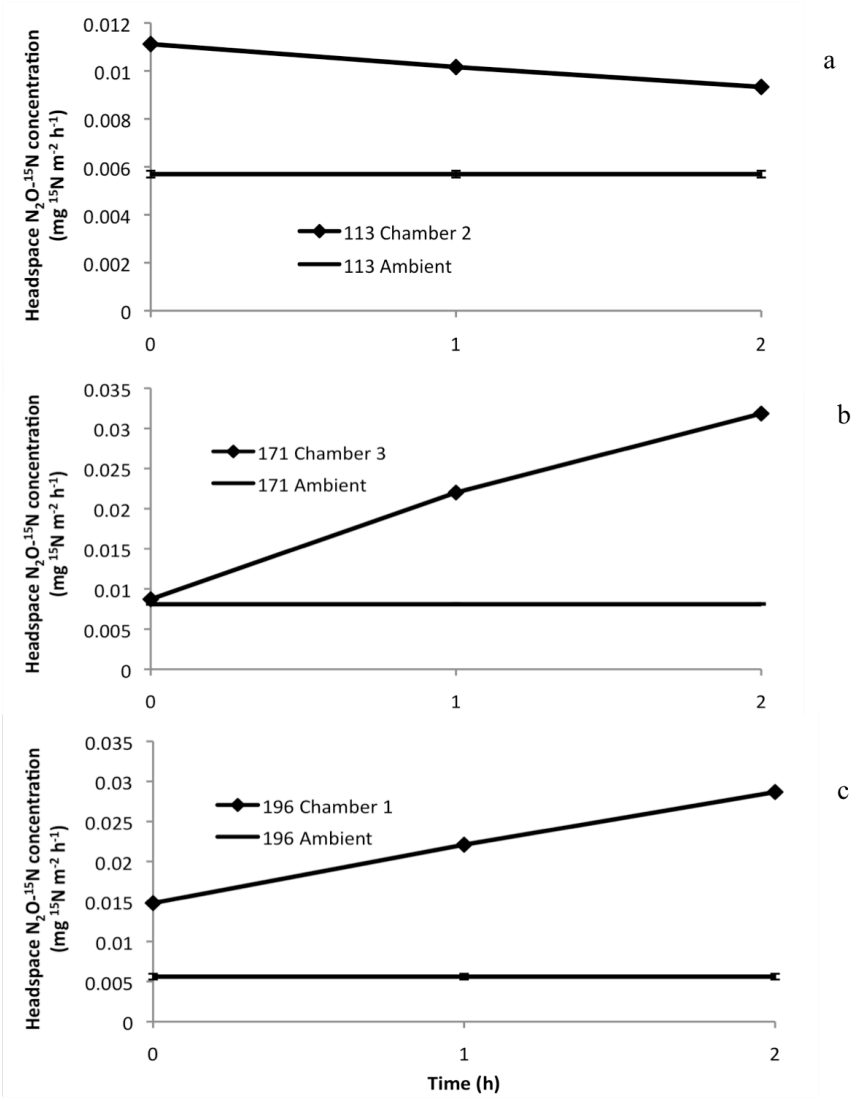


Figure 2-4. Accumulation of ^{15}N in N_2O in ^{15}N tracer method incubations for 3 sample chambers [in plot numbers 113 (a), 171 (b), and 196(c)] in July 2005 illustrating different patterns of abiotic and biotic production. Lines with error bars (standard deviations, n = 2) represent the ^{15}N concentration of ambient air outside the incubation chambers; those without error bars show headspace ^{15}N concentration within a chamber.

for $X_{N.}$ (See below for explanation of estimated vs. diffusion enrichments.) The significant slope with the smallest absolute value observed in my N_2O data was 0.13 μg ^{15}N m^{-2} h^{-1}, which yields a minimum detectable rate of 2.6 μg N_2O-N m^{-2} h^{-1} (or 0.63 g N_2O-N ha^{-1} d^{-1}) using the estimated enrichment (4.9 atom %) for X_N. Using diffusion enrichments (X_N = 5.0 atom % and 1.8 atom % for N_2 and N_2O, respectively) in these calculations yields minimum detection limits of 1.6 mg N_2-N m^{-2} h^{-1} (or 380 g N_2-N ha^{-1} d^{-1}) for N_2 and 7.1 μg N_2O-N m^{-2} h^{-1} (or 1.7 g N_2O-N ha^{-1} d^{-1}) for N_2O. Note that X_N varies by N-species because minimum slopes for N_2 and N_2O occurred in different plots, which had slightly different applications of ^{15}N.

Abiotic production was calculated as the difference between the Time 0 ^{15}N concentration and the field ambient ^{15}N concentration. I set a minimum detection limit of twice the standard deviation of ambient ^{15}N concentration of the atmosphere outside my field chambers. As these standard deviations varied across plots and sampling dates, so did detection limits. Overall, 6% of N_2 fluxes and 17% of N_2O fluxes exceeded detection limits. The average detectable abiotic burst was 0.24 mg N m^{-2} (standard deviation = 0.29 mg N m^{-2}) for N_2 and 1.6 μg N m^{-2} (SD = 2.2 μg N m^{-2}) for N_2O.

Calculating denitrification rates from accumulation of $^{15}N_2$ in the headspace in field chambers requires knowing the ^{15}N enrichment of the pool of NO_3^- undergoing denitrification. While my $^{15}NO_3^-$ additions were calculated to create a 5% enrichment in the NO_3^- pool, measurements of NO_3^- source pool enrichment were often much lower than expected. This was likely due to my soil sampling procedure, in which the entire organic layer was sampled and homogenized prior to NO_3^- extraction. This sampling likely included un-labeled soil, which led to low estimates of NO_3^- source

pool enrichment and overestimation of denitrification rate. I therefore calculated denitrification rates two ways using two different estimates of NO_3^- source pool enrichment (X_N). "Estimated enrichment" refers to the enrichment expected from uniform distribution of the applied $^{15}NO_3^-$ (~3-6%) over the entire forest floor and "diffusion enrichment" is the enrichment actually measured in my homogenized,

extracted and diffused soil water samples. Recovery of added ^{15}N in the headspace ranged from 0% to 98%. Recovery in abiotic pulses ranged from 0% to 67% of added ^{15}N and recovery in biological fluxes ranged from 0% to 98%. Note that the abiotic and biotic minima and maxima do not sum up to the total percent recovery range because these extremes did not occur in the same chambers.

Denitrification rates ranged up to 2900 g N ha^{-1} d^{-1} as calculated using estimated enrichments and up to 17000 g N ha^{-1} d^{-1} using diffusion enrichments ($N_2O + N_2$). The relationship between individual N_2 flux rates as calculated using estimated vs. diffusion enrichments was positive (slope = 3.6) and significant, but not highly explanatory ($p=0.018$, $R^2 = 0.32$) (Figure 2-5).

2.4.3 Method Comparison

Relationships between individual N_2 flux rates measured using the direct flux method and each of the two tracer methods were not significant, with negative slopes and R^2 values less than 0.1 (Figure 2-6a). When the data were averaged over all plots but kept separated by month, the relationships remained slightly negative, weak, and not significant ($R^2 < 0.05$, $p > 0.7$) (Figure 2-6b). However, when the data were averaged over all months but kept separated by plot, the relationships became positive and slightly stronger ($R^2 = 0.09$ for direct flux vs. diffusion enrichment and $R^2 = 0.37$ for

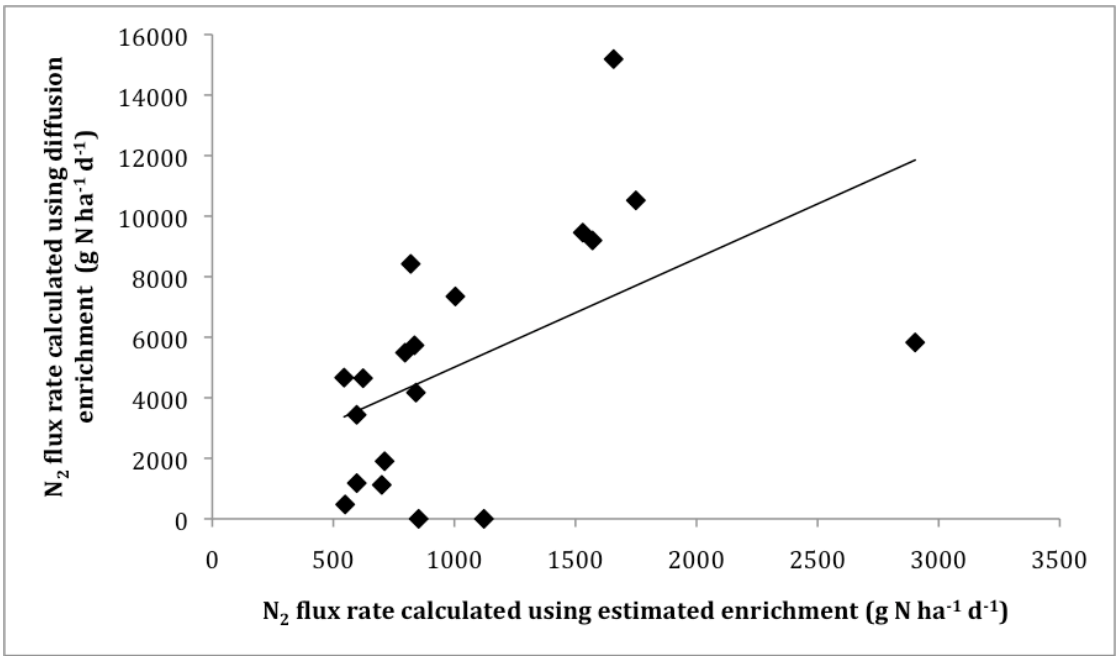
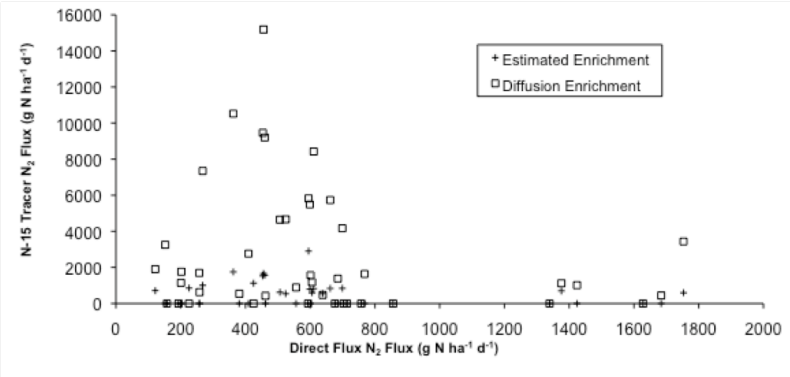
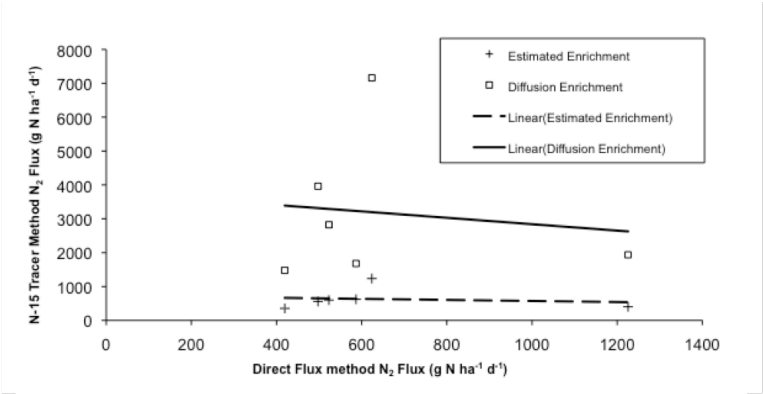


Figure 2-5. N_2 flux rates measured by the ^{15}N tracer method calculated using "estimated enrichments" versus rates calculated using "diffusion enrichment" values for ^{15}N enrichment of the NO_3^- pool being denitrified (regression line: $R^2 = 0.32$, $p = 0.18$).

a



b



c

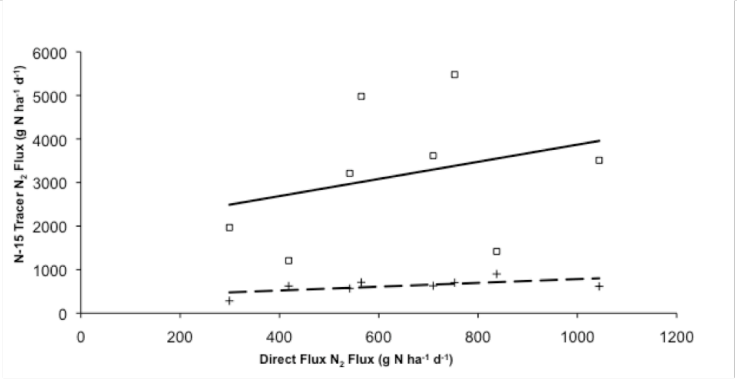


Figure 2-6. Relationships between N_2 flux rates measured by the ^{15}N tracer and direct flux soil core methods at different temporal scales of aggregation. Plots show all values measured at monthly samplings from May through October, 2005 (a), mean values averaged over all plots by month of sampling (b), and mean values averaged over all months by plot (c). ^{15}N tracer method rates were calculated using both estimated and diffusion values for ^{15}N enrichment of the NO_3^- pool being denitrified. None of the relationships are statistically significant.

direct flux vs. estimated enrichment) although they were not significant (Figure 2-6c). When averaged over both plot and month sampled, estimates of denitrification from the direct flux method and estimated enrichment calculation of the ^{15}N tracer method were close for both N_2 and N_2O fluxes. The direct flux method did not agree well with the diffusion enrichment calculations of the ^{15}N tracer method, which were much higher (Table 2-1). The N_2:N_2O ratios were, therefore, comparable for the direct flux and estimated enrichment methods, but lower than those obtained using the direct flux method. N_2:N_2O ratios calculated for each method and all scales were, however, very high, indicating that denitrification is proceeding to its endpoint of N_2 production with minimal losses to N_2O fluxes (Table 2-1).

2.4.4 Extrapolating Rates

Producing seasonal estimates of denitrification from the point measurements made by both methods requires an approach to temporal extrapolation. Given the strong control of flux rate by O_2 (Figure 2-2), I produced seasonal estimates of flux by focusing on rainfall events as drivers of low O_2 levels in soils. For the direct flux method, I incubated cores at 5% O_2, a relatively low level given recent data that suggest that oxygen concentrations in the soil pore space in the Hubbard Brook valley generally range from 10-17% (pers. comm. Colin Fuss). Temporal extrapolation therefore requires estimates of how many days of the season soil oxygen concentrations fall to 5%. A scenario assuming that a rain event of at least 2 cm pushes O_2 concentrations down to 5% for one day yields 38 days of denitrification (the number of days with > 2 cm rain in 2005) and a seasonal N_2 flux of approximately 8 kg N ha^{-1} (vs. 100 kg N ha^{-1} if measured rates were extrapolated over 184 days, Table 2-1). Increasing the threshold to 3 cm rain gives a seasonal flux of approximately 4 kg N ha^{-1}. These scenarios are conservative as they do not account for the possibility

Table 2-1. Seasonal biotic N gas fluxes (kg N ha^{-1} $season^{-1}$) and N_2:N_2O as measured by the direct flux soil core and ^{15}N tracer methods with different assumptions. For the direct flux method, estimates were produced by extrapolating mean rates over all sampling dates and plots either by 184 days or by the number of days affected by either 2 or 3 cm rainfall events. Estimates for the ^{15}N tracer method were computed using both measured and estimated values for ^{15}N enrichment of the NO_3^- pool being denitrified. Mean rates over all sampling dates were extrapolated either by 184 days or by the number of days affected by a 0.25 cm rainfall event (equivalent to the amount of water added with the ^{15}N tracer).

Method	N_2 Flux	N_2O Flux	N_2: N_2O
Direct Flux:			
184 day season	100	1.4	73
2 cm threshold (15 days)	8	0.11	73
3 cm threshold (7 days)	4	0.054	73
^{15}N Tracer, Estimated Enrichment Calculation			
184 day season	110	1.3	85
0.25 cm threshold (59 days)	36	0.42	85
^{15}N Tracer, Diffusion Enrichment Calculation			
184 day season	570	2.7	210
0.25 cm threshold (59 days)	180	0.87	210

of rates elevated over that which you would expect at 5% oxygen. During very heavy rain events that produce prolonged high moisture conditions, one might expect soil O_2 levels to dip below 5%, and the oxygen curves show higher N fluxes at 1% oxygen than at 5%. There may thus be large peaks in N fluxes that my scenarios do not account for.

The ^{15}N tracer method is not as strongly affected by distortion of soil oxygen levels as the direct flux method. However, some stimulation of rates likely resulted from the addition of 0.25 cm of water for distribution of the label. I therefore calculated seasonal total N flux rates for the ^{15}N tracer method using both the estimated and diffusion enrichments and a threshold of 0.25 cm rain for extrapolation. For the May through October 2005 season, these calculations yielded 36 kg N ha^{-1} and 180 kg N ha^{-1} for the estimated and diffusion enrichments respectively (Table 2-1).

2.5 DISCUSSION

2.5.1 Direct Flux Method

Results of the data collected using the direct flux method generally showed steadily increasing accumulations of N gases in cores and, therefore, relatively steady flux rates. In some cores, I saw decreasing N_2O levels in the later parts of incubations, indicating consumption of N_2O. N_2 flux rates, however, did not seem to increase correspondingly, suggesting that headspace N_2O was not limiting N_2 flux rates (Figure 2-1). Overall, the accumulation profiles I observed allowed for measurement of high and low rates of denitrification to both N_2 and N_2O. My N_2 detection limits were comparable to those of other studies; i.e. my detection limit was 175 μg N m^{-2} h^{-1} for N_2 flux vs. 10 μg N m^{-2} h^{-1} (Butterbach-Bahl et al. 2002) or 11.6 μg N kg^{-1} h^{-1} vs. 90

μg N_2-N kg^{-1} measurement interval $^{-1}$ (Swerts et al. 1995). N_2O flux detection limits were similar among studies at 0.21 μg N m^{-2} h^{-1} (present study), <1 μg m^{-2} h^{-1} (Butterbach-Bahl et al. 2002), and 0.04 μg N_2O-N kg^{-1} measurement $interval^{-1}$ (Swerts et al. 1995). Detection limits for N_2 can be improved using more sensitive detectors or larger soil samples. Most importantly, this method is sensitive enough to address questions about the importance of N_2 flux in the context of natural ecosystem mass balance and atmospheric deposition questions.

The high N_2 flux rates and N_2:N_2O ratios that I observed contribute to the body of new knowledge that is changing the old paradigm that denitrification rates in upland forest soils are very low and dominated by N_2O fluxes (Table 2-1) (Bowden 1986, Davidson et al. 1990). My results suggest that given sufficiently low oxygen levels (5%, see discussion below) northern hardwood forests are capable of supporting significant amounts of denitrification and N_2 production. Yavitt et al. (1995) demonstrated that low-oxygen conditions may be more persistent in northeastern hardwood forests than previously thought. These conditions likely predominate in microsites in the soil that provide otherwise favorable conditions for denitrification as well (available source of oxidized nitrogen, organic matter, and appropriate pH and temperature). For this reason, soil structure must be maintained as well as possible in studies of denitrification rate, a clear advantage of intact core-based gas-flow systems (Parkin et al. 1984, Scholfield et al. 1997b, Butterbach-Bahl et al. 2002). However, it can also be argued that simply removing the soil core from the field is a major disturbance to soil structure and further testing of the validity of these extracted core methods is warranted, e.g., by comparing N_2O and CO_2 flux rates in extracted cores and *in situ* field chambers. It is interesting to note that my seasonal estimates of N_2O flux (Table 1) are very similar to seasonal estimates for HBEF derived from *in situ* field chambers

(Groffman et al. 2006b) suggesting that the extracted core method does not fundamentally alter soil physical conditions and flux rates.

The key to linking flux measured in the laboratory using the soil core incubation system with field N flux rates appears to be soil oxygen levels. I observed strong control of denitrification rate by O_2, with rates declining exponentially from 0 to 5 to 10 to 20% O_2. Limited field measurements of O_2 in lysimeter water indicate concentrations at the HBEF ranging from 10-17% O_2 (pers. comm. Colin Fuss). These concentrations, however, were measured in soil water that had accumulated in lysimeters over time and therefore do not reflect the short term variation in O_2 concentration that would be expected to result from weather-related wetting and drying cycles. As such, I can speculate that O_2 concentrations of less than 10% and even 5% occur during wetting events (Sierra and Renault 1998). My scenarios posit two possible thresholds for depressing O_2 concentrations to 5% – 2 cm and 3 cm of rain in a day – and produce estimates of seasonal N flux of 8 and 4 kg N ha^{-1} $season^{-1}$ (Table 2-1). These scenarios allow us to estimate a range of nitrogen gas losses from HBEF as well as to hypothesize about the effects of weather and climate on broad scale nitrogen cycling in this area. There is, however, a clear need to verify these scenarios with field measurements of soil oxygen responses to rainfall events. Developing relationships between precipitation and O_2 concentrations in soil and/or using continuous soil O_2 data to drive simple models linking denitrification and soil oxygen levels are promising approaches for temporal extrapolation of point measurements of denitrification to seasonal scales.

2.5.2 ^{15}N Tracer Method

The ^{15}N tracer method allows *in situ* determination of denitrification with minimal disturbance of soil structure (arising from collar installation). It does, however, require physical (increasing moisture) and chemical (increasing NO_3^- and ^{15}N concentrations) modification of the soil. Increasing moisture could be taken to simulate rain events, following which one would expect pulses of denitrification. Such pulses likely result from both biotic and abiotic denitrification. I saw quick bursts of N_2 and N_2O in the ten minutes between enclosure of the gas sampling chamber and the removal of my Time 0 gas sample. These bursts may arise from rapid chemodenitrification of the applied tracer, which might be expected after the wetting of acidic soils (Clough et al. 2001, Davidson 1992). Further testing of the mechanisms producing these rapid N gas losses is warranted.

My ^{15}N method required increasing soil nitrate pools as well as moisture, however, the chemical modification of the soil required for tracer level additions is minimal. Such small increases in the enrichment of the soil NO_3^- pool could result in undetectable enrichment of the N_2 pool over background levels in the headspace. Indeed, most of my chambers did not have sufficient ^{15}N-labelled gas fluxes to overcome detection limits. However, in many chambers I found small but clear increases in this pool, allowing for calculation of N_2 fluxes (Figure 2-2).

The minimum detection limits (MDL) for biological N_2 flux are disappointingly high and raise doubts about the utility of this method for measuring denitrification rates in unfertilized soil. Lower MDLs could be realized by adding sampling points in the incubation, which would increase the number of regression points and therefore the likelihood of achieving high R^2 values for lower slopes. Alternatively, increasing the

enrichment of the nitrate pool would result in larger slopes without increasing MDL rates (because X_N would be higher) and could also make the method more sensitive. This option, however, requires fertilizer-level additions of ^{15}N.

The most problematic aspect of the ^{15}N tracer method was determination of the enrichment of the source pool (NO_3^-). I directly measured the enrichment of this pool using extraction and diffusion of soil NO_3^-. However, it is impossible to sample just the volume of soil enriched by the tracer addition and as a result, I probably sampled a large volume of unlabeled soil in many cores. This sampling produced measurements of enrichment that were much lower than expected and that likely did not reflect the enrichment of actual denitrifying sites in the soil. Use of these data in denitrification rate calculations resulted in unrealistically high N flux rates. There is a clear need for improving estimates of pool enrichment for the ^{15}N tracer method. Siegel et al. (1982) and Spott and Stange (2007) described methods, based on original research by Hauck et al. (1958) and Hauck and Bouldin (1961), that use the signatures of $^{30}N_2$:$^{28}N_2$ and $^{29}N_2$:$^{28}N_2$ in headspace gases to back calculate the exact enrichment of the NO_3^- pool actively undergoing denitrification. Mulvaney (1988) showed that these methods are robust in the face of isotopic heterogeneity in the enrichment of the nitrate source pool undergoing denitrification. Unfortunately, $^{30}N_2$ detection limits on mass spectrometers are currently not low enough to allow use of these calculations on incubations with tracer level enrichments. Spott and Stange (2007) stipulate that their calculations depend on enrichment of the NO_2^- and NO_3^- pools to at least 10% and 20% respectively, well above tracer levels. Improvements in mass spectrometer sensitivity would greatly facilitate use of *in situ* ^{15}N tracer techniques for measuring denitrification.

At tracer levels, ^{15}N methods may be useful assays of the instantaneous fate of atmospheric N. The tracer was added in an amount of water equal to a 0.25 cm rainfall event and recovery of this tracer in the headspace of the chambers provides an estimate of the short-term abiotic and biotic transformations of atmospheric N to gas. Recoveries in the N_2 pool were 1-2 orders of magnitude higher than those in the N_2O pool. Given that I recovered up to 98% of the tracer in the three-hour incubations, these transformations may be a significant fate of atmospheric N, important regulators of the effects of deposition on ecosystem N cycles, and key endpoints in the nitrogen cascade of northeastern forests.

An interesting pattern emerged when I compared the results of each method using increasingly aggregated data. Individual flux rates for separate plots and dates compared reasonably well between the two ^{15}N tracer calculation methods, but the relationship was not 1:1 as expected; rather, it was ~4:1 (diffusion enrichment: estimated enrichment) (Figure 2-5). When these data were compared with direct flux data, no relationship was found (Figure 2-6a). Similarly, when they were aggregated by plot, but kept separated by month, no relationship was found (Figure 2-6b), however, when they were aggregated by month and kept separated by plot, a relationship began to emerge, especially between the direct flux and estimated enrichment calculation of the ^{15}N tracer method (Figure 2-6c). Finally, when the data were aggregated by both month and plot, the direct flux and estimated enrichment rates were very close for N_2 (100 vs. 110 kg N ha^{-1} $season^{-1}$) and nearly identical for N_2O (1.4 vs. 1.3 kg N ha^{-1} y^{-1}). The diffusion enrichment calculation of the ^{15}N tracer method resulted in much higher fluxes (Table 2-1). These data suggest that while microsite variability complicates comparison of the two methods (Parkin et al. 1985),

the direct flux and estimated enrichment calculations of the ^{15}N tracer method produce similar estimates of denitrification at relatively large spatial and temporal scales.

2.5.3 The Importance of Denitrification in Terrestrial Ecosystems

Most of the seasonal estimates of flux that I produced (Table 2-1) are high given what we know about nitrogen mass balances at HBEF and in the northeastern U.S. Firstly, Van Breeman et al. (2002) calculated nitrogen losses from watersheds of the northeastern U.S., and estimated that ~2.3 kg N ha^{-1} y^{-1} is denitrified in the forests of the Merrimack River watershed (of which the HBEF is a part). To reconcile my results with this number, HBEF would have to be a hot spot in the forested parts of the Merrimack watershed. Secondly, the HBEF is estimated to experience a bulk nitrogen deposition rate of approximately 6-8 kg N ha^{-1} y^{-1} (Dittman et al. 2007). The estimates produced by the 2 and 3 cm threshold scenarios using the direct flux method fall squarely within this range. If, however, any of my other estimates of denitrification are in the right order of magnitude, HBEF would be losing much more nitrogen every year than it gains. This notion, however, assumes that nitrogen fixation at Hubbard Brook is negligible, a difficult assumption to make.

Roskoski (1980) measured N fixation rates of approximately 2 kg N ha^{-1} y^{-1} in woody litter at HBEF. Bormann et al. (1993) studied the nitrogen budgets of "sandboxes" established near the HBEF and found very large unexplained accumulations of nitrogen. Bormann et al. (2002) revisited these data and estimated the accumulations to range from approximately 40 to 150 kg N ha^{-1} y^{-1}. Furthermore, new methods of quantifying N deposition have found that methods currently used at Hubbard Brook may be vastly underestimating total N deposition, accounting for only 24-45% of total wet and dry deposition (He et al. 2007). Other studies at HBEF have found nitrogen

exports to be lower than expected in light of inputs. Goodale et al. (2003) found decreased nitrate in stream export despite two decades of chronic N deposition and forest maturation, and Judd et al. (2007) saw stream nitrate exports that were 1-2 orders of magnitude lower than nitrate production in soils. If similar phenomena of unexplained N accumulation and retention, or of underestimated N deposition are affecting Hubbard Brook's nitrogen mass balance, the direct flux and estimated enrichment estimates of denitrification fall well within the expected range of nitrogen loss from the system. My direct flux method – with 2 and 3 cm scenarios – gives estimates of denitrification that fit with our current understanding of nitrogen inputs, although they exceed earlier estimates at HBEF.

Thus, I hypothesize that nitrogen gas losses from HBEF soils, especially of N_2, are greater than previous estimates. I propose further study of denitrification at this site using methods that measure both N_2 and N_2O (e.g. my gas-flow soil core technique) linked to data on soil oxygen conditions to further elucidate patterns of N gas loss in northeastern forests.

2.5.4 Conclusions

Two new methods suggest that denitrification is more important than previously thought in the northern hardwood forest at Hubbard Brook. Rates appear to be higher than previous estimates and, in most of my scenarios, equal to or higher than atmospheric deposition to the site. This suggests that either deposition or N fixation may be higher than previously thought at this location.

Both methods consistently measured very high N_2:N_2O ratios across all months and plots sampled. These soils could, therefore be returning a large amount of reactive N

to the atmospheric N_2 pool. Rates measured by the direct flux soil core method are very sensitive to the O_2 concentration in the recirculation gas. These concentrations must be keyed to actual soil O_2 levels. Developing relationships between precipitation events and soil oxygen concentrations and linking these relationships to continuous soil O_2 measurements shows promise as a temporal extrapolation tool for denitrification rates.

Results from the *in situ* ^{15}N tracer method suggest that bursts of chemodenitrification may occur when water and NO_3^- are added to these soils. The mechanisms of this response and relevance of these results to atmospheric deposition during actual rainfall events warrant further investigation. Rates measured by the *in situ* ^{15}N tracer method are very sensitive to the enrichment of the NO_3^- pool undergoing denitrification. Since this enrichment is difficult to measure in tracer-level addition experiments, the usefulness of the ^{15}N tracer method is dependent on development of highly sensitive mass spectrometers capable of measuring the ^{28}N, ^{29}N and ^{30} masses in N_2 as described by Siegel et al. (1982) and Spott and Stange (2007).

The ^{15}N tracer method provides estimates of the short-term abiotic and biotic transformations of atmospheric N deposition N to gas. Given that I recovered up to 98% of the tracer in the two hour incubations, these transformations may be an important fate for N deposition in northeastern forest soils.

WORKS CITED

Bormann BT, Bormann FH, Bowden WB, Pierce RS, Hamburg SP, Wang D, Snyder MC, Li CY, and Ingersoll RC. 1993. Rapid N_2 fixation in pines, alder, and locust: evidence from the sandbox ecosystem study. Ecology 74: 581–98.

Bormann BT, Keller CK, Wang D, and Bormann FH. 2002. Lessons from the sandbox: Is unexplained nitrogen real? Ecosystems 5: 727-733.

Bowden WB. 1986. Gaseous nitrogen emissions from undisturbed terrestrial ecosystems: An assessment of their impacts on local and global nitrogen budgets. Biogeochemistry 2: 249-279.

Butterbach-Bahl K, Willibald G, and Papen H. 2002. Soil core method for direct simultaneous determination of N_2 and N_2O emissions from forest soils. Plant and Soil 240: 105–116.

Clough TJ, Stevens RJ, Laughlin RJ, Sherlock RR, and Cameron KC. 2001. Transformations of inorganic-N in soil leachate under differing storage conditions. Soil Biology and Biochemistry 33: 1473-1480.

Davidson EA. 1992. Sources of nitric oxide and nitrous oxide following wetting of dry soil. Soil Science Society of America Journal 56: 95-102.

Davidson EA and Seitzinger S. 2006. The enigma of progress in denitrification research. Ecological Applications 16: 2057-2063.

Davidson EA, Myrold DD, and Groffman PM. 1990. Denitrification in temperate forest ecosystems. In: Gessel SP, Lacate DS, Weetman GF, and Powers RF, (Eds.), Proceedings of the Seventh North American Forest Soils Conference. University of British Columbia, Faculty of Forestry Publication, Vancouver. 196-220.

Dittman JA, Driscoll CT, Groffman PM, and Fahey TM. 2007. Dynamics of nitrogen and dissolved organic carbon at the Hubbard Brook Experimental Forest. Ecology 88: 1153-1166.

Fulweiler, RW, Nixon SW, Buckley BA, and Granger SL. 2007. Reversal of the net dinitrogen gas flux in coastal marine sediments. Nature 448: 180-182.

Galloway JN, and Cowling EB. 2002. Reactive nitrogen and the world: 200 years of change. Ambio 31: 64-71.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney, JR, Martinelli LA, Seitzinger SP, and Sutton MA. 2008. Transformations of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 320: 889-892.

Groffman PM, Altabet MA, Böhlke JK, Butterbach-Bahl K, David MB, Firestone MK, Giblin AE, Kana TM, Nielsen LP, and Voytek MA. 2006a. Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecological Applications 16: 2091-2122.

Groffman PM, Fisk MC, Driscoll CT, Likens GE, Fahey TJ, Eagar C, and Pardo LH.

2006b. Calcium additions and microbial nitrogen cycle processes in a northern hardwood forest. Ecosystems 9: 1289-1305.

Hauck RD and Bouldin DR. 1961. Distribution of isotopic nitrogen in nitrogen gas during denitrification. Nature 191: 871-872.

Hauck RD, Melsted SW, and Yankwich PE. 1958. Use of N-isotope distribution in nitrogen gas in the study of denitrification. Soil Science 86: 287–291.

He C-E, Liu X, Fangmeier A, and Zhang F. 2007. Quantifying the total airborne nitrogen input into agroecosystems in the North China Plain. Agriculture, Ecosystems and Environment 121: 395–400.

Högberg P. 1997. ^{15}N natural abundance in soil-plant systems. New Phytologist 137: 179-203.

Howarth RW and Marino R. 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: evolving views over three decades. Limnology and Oceanography 51: 364–76.

Howarth RW, Boyer EW, Pabich WJ, and Galloway JN. 2002. Nitrogen use in the United States from 1961-2000 and potential future trends. Ambio 31: 8-96.

Knowles R and Blackburn TH. 1992. Nitrogen Isotope Techniques. Academic Press, London.

Mulvaney RL. 1988. Evaluation of nitrogen-15 tracer techniques for direct measurement of denitrification in soil: III. Laboratory studies. Soil Science Society of America Journal 52: 1327-1332.

Mulvaney RL and Vanden Heuvel RM. 1988. Evaluation of nitrogen-15 tracer techniques for direct measurement of denitrification in soil: IV. Field Studies. Soil Science Society of America Journal 52: 1332-1337.

Myrold DD. 1990. Measuring denitrification in soils using ^{15}N techniques. In: Revsbeck NP and Sørensen J (Eds.), Denitrification in Soil and Sediment. Plenum Press, New York, NY. 181-198.

Parkin TB, Kaspar HF, Sexstone AJ, and Tiedje JM. 1984. A gas-flow soil core method to measure field denitrification rates. Soil Biology and Biochemistry 16: 323-330.

Parkin TB, Sexstone AJ, and Tiedje JM. 1985. Comparison of field denitrification rates determined by acetylene-based soil core and nitrogen-15 methods. Soil Science Society of America Journal 49: 94-99.

Payne WJ. 1991. A review of methods for field measurements of denitrification. Forest Ecology and Management 44: 5-14.

Russow R, Boehme F, and Neue H-U. 2001. A new approach to determine the total airborne N input into the soil/plant system using 15N isotope dilution (ITNI): results for agricultural areas in Central Germany. Science World 1: 255–260.

Ryden JC, Skinner JH, and Nixon DJ. 1987. Soil core incubation system for the field measurement of denitrification using acetylene-inhibition. Soil Biology and Biochemistry 19: 753-757

Scholefield D, Hawkins JMB, and Jackson SM. 1997a. Development of a Helium atmosphere soil incubation technique for direct measurement of nitrous oxide and dinitrogen fluxes during denitrification. Soil Biology and Biochemistry 29: 1345-1352.

Scholefield D, Hawkins JMB, and Jackson SM. 1997b. Use of a flowing helium atmosphere incubation technique to measure the effects of denitrification controls applied to intact cores of a clay soil. Soil Biology and Biochemistry 29: 1337–1344.

Siegel RS, Hauck RD, and Kurtz LT. 1982. Determination of $^{30}N_2$ and application to measurement of N_2 evolution during denitrification. Soil Science Society of America Journal 46: 68-74.

Sierra J and Renault P. 1998. Temporal Pattern of Oxygen Concentration in a Hydromorphic Soil. Soil Science Society of America Journal 62: 1398-1405.

Spott O and Stange CF. 2007. A new mathematical approach for calculating the contribution of anammox, denitrification and atmosphere to an N_2 mixture based on a ^{15}N tracer technique. Rapid Communications in Mass Spectrometry 21: 2398–2406.

Stark JM and Hart SC. 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Science Society of America Journal 60: 1846-1855.

Stehfest E and Bouwman L. 2006. N_2O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. Nutrient Cycling in Agroecosystems 74: 207-228

Swerts M, Uytterhoeven G, Merckx R, and Vlassak K. 1995. Semicontinuous measurement of soil atmosphere gases with gas-flow soil core method. Soil Science Society of America Journal 59: 1336-1342.

USDA. 1996. Hubbard Brook ecosystem study: Site description and research activities. USDA Forest Service Publication No. NE-INF-96–96R, 2nd edition USDA Forest Service, Northeastern Forest Experiment Station, Newtown Square, PA.

van Breemen N, Boyer EW, Goodale CL, Jaworski NA, Paustian K, Seitzinger SP, Lajtha K, Mayer B, van Dam D, Howarth RW, Nadelhoffer KJ, Eve M, and Billen G. 2002. Where did all the nitrogen go? Fate of nitrogen inputs to large watersheds in the northeastern USA. Biogeochemistry 57: 267-293.

van Egmond K, Bresser T, and Bouwman L. 2002. The European nitrogen case. Ambio 31: 72-78.

Venterea RT, Lovett GM, Groffman PM, and Schwarz PA. 2003. Landscape patterns of soil nitrate production in a northern hardwood-conifer forest. Soil Science Society

of America Journal 67: 527-539.

Yavitt JB, Fahey TJ, and Simmons JA. 1995. Methane and carbon-dioxide dynamics in a northern hardwood ecosystem. Soil Science Society of America Journal 59: 769-804.

Yeomans J and Beauchamp E. 1982. Acetylene as a possible substrate in the denitrification process. Canadian Journal of Soil Science 62: 139-144.

Zheng X, Fu C, Xu X, Yan X, Huang Y, Han S, Hu F, and Chen G. 2002. The Asian nitrogen cycle case study. Ambio 31: 79-87.

CHAPTER 3

RECOVERY OF WET NITROGEN DEPOSITION IN N_2 AND N_2O FROM TEMPERATE FOREST SOILS

3.1 ABSTRACT

The fate of reactive nitrogen (Nr) has been a long-standing mystery in science, the resolution of which becomes more urgent as humans continue to increase N fixation. The northeastern USA is a hot spot of Nr inputs via atmospheric deposition; however, the fate of nitrogen (N) in this area is not well understood. I simulated wet N deposition events in *in situ* gas chambers set up on the soils of 3 parts of the White Mountain National Forest in New Hampshire, USA. I used ^{15}N-labelled NO_3^- and traced its recovery in N_2 and N_2O pools over 2-hour incubations. I observed high recoveries of ^{15}N in these pools, especially N_2 (up to 98% of applied ^{15}N). Some of the recoveries were observed in the first gas sampling of the incubation, indicating that a pulse of gas loss had occurred in the ~10 minutes between capping of the chamber and removal of the first gas sample. I attributed these accumulations to abiotic processes and those occurring more slowly over the 2-hour incubation to biotic processes, but expect that some overlap occurred. Overall, recoveries in abiotic fluxes averaged 28% and 0.34% for N_2 and N_2O respectively and those in biotic fluxes averaged 39% and 3.2% for N_2 and N_2O respectively. My conservative detection limits and widely distributed data set constrain my ability to draw strong conclusions about spatial and temporal patterns in recoveries, however, my results suggest that quick turnover of deposited N via denitrification could be an important output in northeastern N budgets. Furthermore, most of this N is likely lost as inert N_2 rather than the greenhouse gas, N_2O. I also observed unusual patterns in the natural abundance of ^{15}N in N_2 and N_2O.

Both varied from the expected value of ~0 ‰, and displayed similar patterns across sites. These findings, as well as those of my ^{15}N recoveries warrant further investigation.

3.2 Introduction

Humans have more than doubled the global rate of nitrogen (N) fixation in the last 100 years and the fate of this anthropogenic reactive N (Nr) is not well understood (Galloway et al. 2003). Galloway et al. (2008) pose the "vexing question" of the fate of anthropogenic Nr as a priority for future research and Schlesinger's (2009) synthesis of the literature on this topic reveals that estimated sinks for Nr do not account for all of the 150 Tg y^{-1} of extra Nr fixed by humans. One possible fate is denitrification, the reduction of oxidized N to successively more reduced N species, culminating in inert dinitrogen (N_2). Denitrification to the endpoint of N_2 is a desirable fate for Nr since the other species involved – nitrate (NO_3^-), nitrite (NO_2^-), nitric oxide (NO), and nitrous oxide (N_2O) – are Nr species with various potential polluting effects on soil, air and water (Galloway et al. 2003).

The northeastern USA is a hot spot for elevated Nr deposition from upwind anthropogenic N fixation (Driscoll et al. 2003). Van Breeman et al. (2002), however were not able to close the budgets of many northeastern watersheds and concluded by attributed the fate of this missing 37% of inputs to loss via denitrification. Historically, denitrification has not been thought to be an important sink for this Nr, especially via soils in this largely forested region. Since denitrification is an anaerobic process, the well-aerated soils of an upland forest were not expected to foster high rates of denitrification. Further, measurements of N_2O in the 1980s and 1990s at a few northeastern sites showed minimal N gas loss from forest soils in the northeast (Keller

et al. 1983, Bowden and Bormann 1986, Bowden et al. 1991). Gas losses as N_2, however, were not measured. In the last decade, the importance of microsite variability, hot spots, and hot moments in promoting processes like denitrification, especially to N_2, has emerged as a focus for research on nitrogen cycling (Groffman et al. 2009, Kulkarni et al. 2008, McClain et al. 2003). The juxtaposition of aerated soil pores -- where mineralization and nitrification occur, producing nitrate -- with the anaerobic interiors of soil aggregates makes for ideal conditions for denitrification. Further, the temporal juxtaposition of rain-induced anoxia and enhanced diffusion of dissolved nitrogen with generally dry conditions promotes the production of nitrate (in between rain events) and consumption of nitrate via denitrification (during and after rain events). The rain itself also provides an added nitrate source for denitrifiers. As such, the potential for immediate transformation of atmospheric wet N deposition into N gases is great.

Several studies have shown soils responding to wetting events with pulses of N gas emissions (Clough et al. 2006, Davidson 1992, Goodroad and Keeney 1984, Groffman and Tiedje 1988). Other studies have found positive correlations between N deposition and denitrification (Butterbach-Bahl et al. 1998, Hall and Matson 1999, Papen and Butterbach-Bahl 1999, Pilegaard et al. 2006). Most of these studies, however, focus on emissions of the trace gases NO and N_2O. The response of N_2 production to wetting and N deposition is less well understood, in part because it is simply much more difficult to measure. Zak et al. (2004) and Tietema et al. (1998) discount the importance of denitrification, especially to N_2, as a sink for N deposition in studies of temperate forests in Michigan and Europe, respectively, but neither study directly measured N_2 production. Recent advances in techniques for measuring N_2 have enabled direct quantification of N_2 emissions from soils without the addition of

^{15}N. Using these direct techniques, Butterbach-Bahl et al. (2002) and Dannenmann et al. (2008) have detected large quantities of N_2 emissions from temperate forest soils -- up to 50% of N deposition -- illustrating denitrification as a potentially important sink for Nr in such systems.

Most of the research to date has focused on biological production of N gases, but abiotic processes may also result in emissions. Chemodenitrification can produce NO, NO_2, N_2O or N_2. Davidson (1992) attributed some of the N gas fluxes he observed following wetting to abiotic processes and McCalley and Sparks (2009) recently showed that abiotic gaseous emissions are primarily responsible for nitrogen loss from Mojave Desert soils. In forest soils, the organic layer may be more prone to abiotic N gas loss since low pH and the presence of lignin, humic acids, fulvic acids, and phenolic compounds have been shown to facilitate chemodenitrification (Knowles 1981, Mørkved et al. 2007, Stevenson et al. 1970). Stevenson et al. (1970) saw large emissions of NO and N_2, with small amounts of N_2O from agricultural soils dosed with NO_2^- solution. Mørkved et al. (2007) demonstrated an immediate burst of chemodenitrification upon addition of NO_2^- solution to autoclaved soil, recovering 10% - 51% of the added N in NO emissions; recoveries were higher in the more acidic soils. Other recent studies have also focused on NO and other N oxides as a product of abiotic processes (McCalley and Sparks 2009, Schindlebacher et al. 2004, Venterea et al. 2005); examinations of abiotic N_2 production are rare.

Questions of how temperate forest soils respond to wet deposition remain: What portion of added N is returned to the atmosphere in gaseous form? What types of gases are produced? How are these gases produced? I investigated the response of temperate forest soils in New Hampshire, USA to artificial wet deposition events.

Specifically, I examined *in situ* recoveries of added $^{15}NO_3^-$ in gaseous form ($^{15}N_2O$ and $^{15}N_2$) and distinguished abiotic versus biotic production of these gases. My objective was to determine if gaseous loss is a significant immediate fate for atmospheric N as it is deposited on forest soils.

3.3 METHODS

3.3.1 Study Sites

The Hubbard Brook Experimental Forest (HBEF) is located in the southern part of White Mountain National Forest (WMNF), New Hampshire, USA (43° 56' N, 71° 45' W) and covers 3160 ha. It is an extensively studied Long Term Ecological Research (LTER) site dominated by mixed hardwood forest with sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and yellow birch (*Betula allegheniensis*) dominating the lower and mid-slopes, and spruce-fir (*Picea rubens* and *Abies balsamea*) forest at higher elevations. Commercial logging operations at HBEF ended in 1915-1917 although much of the forest has been re-growing since before the cessation of logging (see www.hubbardbrook.org for more information about this site). Some logging took place in the mid to late 20th century for scientific purposes in a series of experimental watersheds at HBEF (Likens and Bormann 1995), but these were not included in the present study. Plots within HBEF were chosen to represent a variety of both moisture and N richness levels using existing maps of topgraphic index and foliar N.

Sites were also located at Lafayette Brook (LF) and Mount Bickford (MB) in the Franconia Notch area of WMNF, about 20 miles north of HBEF. LF is an old growth forest tract on the north slope of Mt. Lafayette with no evidence of past harvest. The New Hampshire National Heritage Inventory (Sperduto and Engstrom 1993) indicates

that the site contains old-aged northern hardwoods on the lower slopes and transitions to spruce and yellow birch at higher elevations, with some spruce trees reaching 76 cm diameter at breast height. MB is an adjacent site that is known to have burned in 1903. It has not been harvested since. Goodale and Aber (2001) showed that LF had very high nitrification and stream water N loss rates compared with several other sites in the region while MB exhibited the opposite pattern.

3.3.2 Sampling Regime and Analysis

In 2005, I sampled 16 circular 0.05 ha plots at the HBEF that had previously been studied by Schwarz et al. (2003) and Venterea et al. (2003b) for factors controlling variability in trees species abundance and nitrification, respectively. In 2006, I sampled six plots at the HBEF (three of the eight plots that were sampled in 2005 plus three new plots) and 6 plots each at LF and MB. Each plot was sampled monthly during the growing season (May through October in 2005 and June through September in 2006). A total of 504 flux measurements were made. Two samples of ambient air were taken at each plot every time fluxes were measured, for a total of 336 ambient air analyses.

Gas sampling chambers (287-mm diameter (ID) by 40-mm high polyvinyl chloride) were used in combination with ^{15}N-labeled NO_3^-. The bottom portion of the chamber, or "collar", was imbedded in the soil at least 2 days before the first sampling. Solutions of 99 atom percent $K^{15}NO_3^-$ were applied to the soil within the collars with a spray bottle to bring the enrichment up to approximately 5% of the existing NO_3^- pool (based on data collected by Venterea et al. 2003). The area inside the collar was then sprinkled with 0.25 cm of water to wash the label into the soil, simulating rainfall.

Rates of wet N deposition – calculated from month-averaged precipitation samples collected over my sampling period – ranged from 0.2 - 4 mg N m^{-2} in a 0.25 cm rain event (data available at www.hubbardbrook.org). ^{15}N application rates ranged from approximately 1 - 30 mg N m^{-2}. Following application of the $^{15}NO_3^-$ and water, the collar was capped to enclose the chamber and, after approximately 10 minutes, a 9 cc gas sample was taken from the headspace ("Time 0" sample). At 1 and 2 hours after this sampling, similar Time 1 and Time 2 samples were taken from the headspace. Natural abundance air samples were taken at the beginning of the incubations from air near the chambers. Gas samples were analyzed for N_2 and N_2O concentrations and enrichments at the University of California-Davis Stable Isotope Facility.

I calculated N_2-N and N_2O-N fluxes as follows:

$R_{sam} = ((\delta N_{sam}/1000) + 1) * R_{std}$

Where: R_{sam} = isotope ratio of gas sample, δN_{sam} = the ^{15}N enrichment of a gas sample, and R_{std} = $^{15}N/^{14}N$ ratio of the standard (atmospheric N_2)

$F_{sam} = R_{sam}/(R_{sam} + 1)$

Where: F_{sam} = the fraction of N in the sample as ^{15}N

$mol^{15}N = F_{sam} * molN$

Where: $mol^{15}N$ = moles of N_2-^{15}N or N_2O-^{15}N in the sample, and molN = moles of total N_2-N or N_2O-N in the sample

$T_{sam} = molN/Vol_{sam}$

Where: T_{sam} = total concentration of N_2-N or N_2O-N in the sample in mol N cc^{-1},

and Vol_{sam} = the volume of the sample = 9 cc

$^{15}N_{sam} = F_{sam} * T_{sam}$

Where: $^{15}N_{sam}$ = concentration of N_2-^{15}N or N_2O-^{15}N in the sample in mol ^{15}N cc^{-1}

$^{15}N_{hsm} = {}^{15}N_{sam} * Vol_{chamber} / Area_{chamber}$

Where: $^{15}N_{hsm}$ = amount of ^{15}N in the chamber headspace in mol ^{15}N m^{-2}, $Vol_{chamber}$ = volume of the gas chamber in m^3, and $Area_{chamber}$ = cross-sectional area of the gas chamber

$^{15}N_{hsg} = {}^{15}N_{hsm}$ *14.006 g N mol^{-1}* 2 mol N mol^{-1} N_2 or N_2O * 0.001 mg g^{-1}

Where: $^{15}N_{hsg}$ = amount of ^{15}N in the chamber headspace in mg ^{15}N m^{-2}

Note that I assumed a fractionation factor (a) of 1.000 because the effects of fractionation are thought to be insignificant in well drained soils where the reaction is limited by diffusion of substrate to the reaction site rather than by the reaction rate itself (Groffman et al. 2006, Högberg 1997).

The change in ^{15}N over the 2 hour sampling period was calculated by regression of $^{15}N_{hsg}$ against time for the samples taken at times 0, 1 and 2 hours. If the slope had an $R^2 > 0.95$, the chamber was considered to have a detectable biotic N gas flux. Percent recovery in biotic flux was calculated as the quotient of the milligrams of N_2-^{15}N or N_2O-^{15}N accumulated in the headspace between Time 0 and Time 2 (including that

removed in gas samples) relative to the milligrams of NO_3-^{15}N applied in the tracer solution.

If the Time 0 sample had ^{15}N concentrations elevated over ambient levels, it was considered to be a product of an instantaneous burst of abiotic denitrification upon application of the tracer solution. The detection limit for this abiotic burst was set at 2 standard deviations above ambient ^{15}N levels. Percent recoveries in abiotic fluxes were calculated as the quotient of the milligrams of N_2-^{15}N or N_2O-^{15}N in the headspace at Time 0 relative to the milligrams of NO_3-^{15}N applied in the tracer solution.

3.3.3 Statistics

For statistical analyses (conducted using SAS statistical software), data were grouped into 4 site/year combinations: HBEF 2005, HBEF 2006, LF 2006, and MB 2006. Mixed models evaluated variability in natural abundance values and percent recoveries among and within site/year combinations by accounting for the complicated nature of the sampling design including: repeated measures (at each plot over a season), the combination of fixed and random (plot and replicate within plot) effects, and the combination of class (sampling month, plot, replicate) and continuous (percent recovery) variables.

3.4 RESULTS

3.4.1 Percent Recoveries

Detection limits were conservative so the number of measurements that produced significant fluxes was small. Among the abiotic flux data, 9% of the incubations produced significant N_2 fluxes and 40% of the incubations produced significant N_2O

fluxes. Among biotic flux data, 9% of incubations produced significant N_2 fluxes and 21% of incubations produced significant N_2O fluxes. Because this small number of data points was distributed across many plots and months, my statistical models lacked the power to discern differences between sites and sample dates.

I recovered up to 98% of added $^{15}NO_3^-$ in the headspace of the chambers. N_2 recoveries (5-98%) tended to be higher than N_2O recoveries (0-65%) and biotic flux recoveries (0-98%) tended to be higher than abiotic flux (0-68%) recoveries (Figure 3-1). Average recoveries in abiotic fluxes were 28% and 0.34% for N_2 and N_2O respectively and average recoveries in biotic fluxes were 39% and 3.2% for N_2 and N_2O respectively. Within all site/year combinations, the statistical model lacked the power and/or sample size to discern a difference (or lack thereof) between N_2 and N_2O recoveries in either the abiotic or biotic flux pools (Figure 3-1a-d, f-g). The one exception was the HBEF 2005 biotic flux model, which did not show a significant difference between recoveries of ^{15}N in the two species (Figure 3-1e). Similarly, analyses to discern differences in recovery among sites lacked power and/or sample size in all cases except recovery in the N_2 pool resulting from biotic N flux, which was not significant (Figure 3-1).

Statistical analyses of changes in N_2 recoveries within a season returned results in all cases except those for abiotic flux at MB in 2006 and biotic flux at HBEF in 2006. Similarly for N_2O, results were returned in all analyses except those of biotic recoveries in 2006 at HBEF and LF. Recoveries changed detectably over the season in abiotic N_2 flux at HB in 2005 ($p=0.049$) and LF in 2006 ($p=0.0046$) but not in other site/year combinations. At HBEF, the pattern fluctuated across the season, neither trending upward nor downward consistently. At LF, recoveries appeared to increase

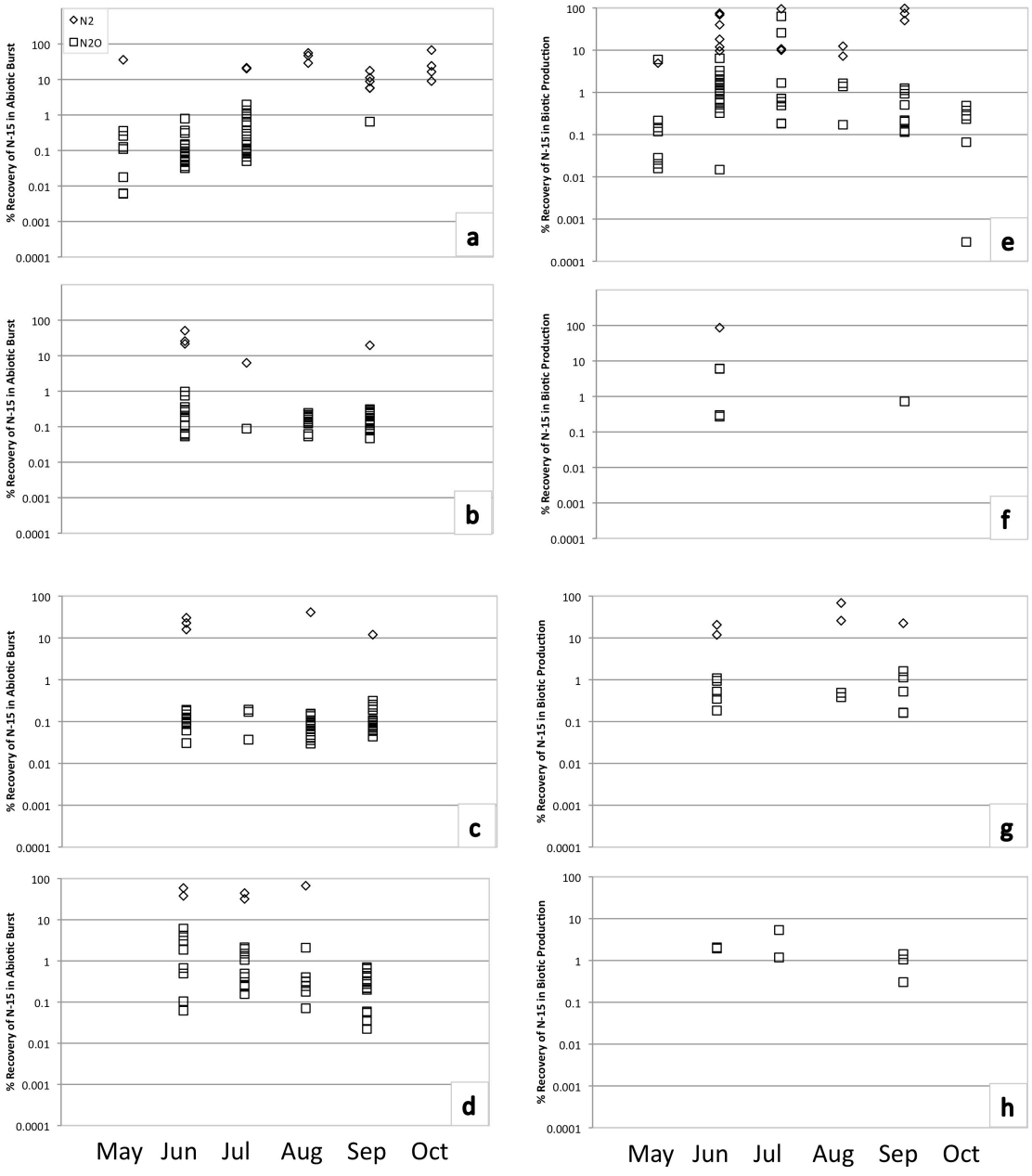


Figure 3-1. Percent recoveries of ^{15}N in N_2 and N_2O gases via abiotic (a-d) and biotic (e-h) production at Hubbard Brook in 2005 (a & e), Hubbard Brook in 2006 (b & f), Lafayette Brook (c & g), and Mount Bickford (d & h) sites. Recoveries are shown by chamber for May through October in 2005 and June though September in 2006 data. Note that percent recoveries are graphed on a logarithmic scale.

from June to August, then decrease in September; however, this data set contained only 5 points. Recoveries in abiotic N_2O flux also increased detectably over the early season at HB in 2005 ($p=0.035$) before they became virtually undetectable, but no change was detected in other site/year combinations. Changes in ^{15}N recovery in the N_2 or N_2O biotic flux pool were not detectable in any site/year combination (Figure 3-1).

3.4.2 Natural Abundance Values

Variation in natural abundance values of ^{15}N in both N_2 and N_2O was high across plots and over the season. N_2 enrichments differed significantly by month ($p<0.0001$) for all site/year combinations except MB 2006 ($p=0.75$). In 2005, they increased from spring to summer then leveled out. In 2006, they decreased from June to July and then increased again at HB and LF. N_2 enrichments also differed significantly by plot for HBEF 2006 ($p=0.0054$) and LF 2006 ($p=0.0077$) (Figure 3-2a-d).

N_2O ambient ^{15}N enrichments differed significantly by month in all site/year combinations ($p<0.01$ in all cases) but did not differ by plot in any of them. In HBEF 2005, enrichments increased in both magnitude and variability in the summer and fall over May and June levels. HBEF 2006 and LF 2006 displayed a dramatic increase in the magnitude and variability of enrichments in July over June levels, and then a sharp decrease in both in August and September. MB 2006 displayed a similar pattern except that June enrichments were greater in magnitude and variability than July levels (Figure 2 e-g).

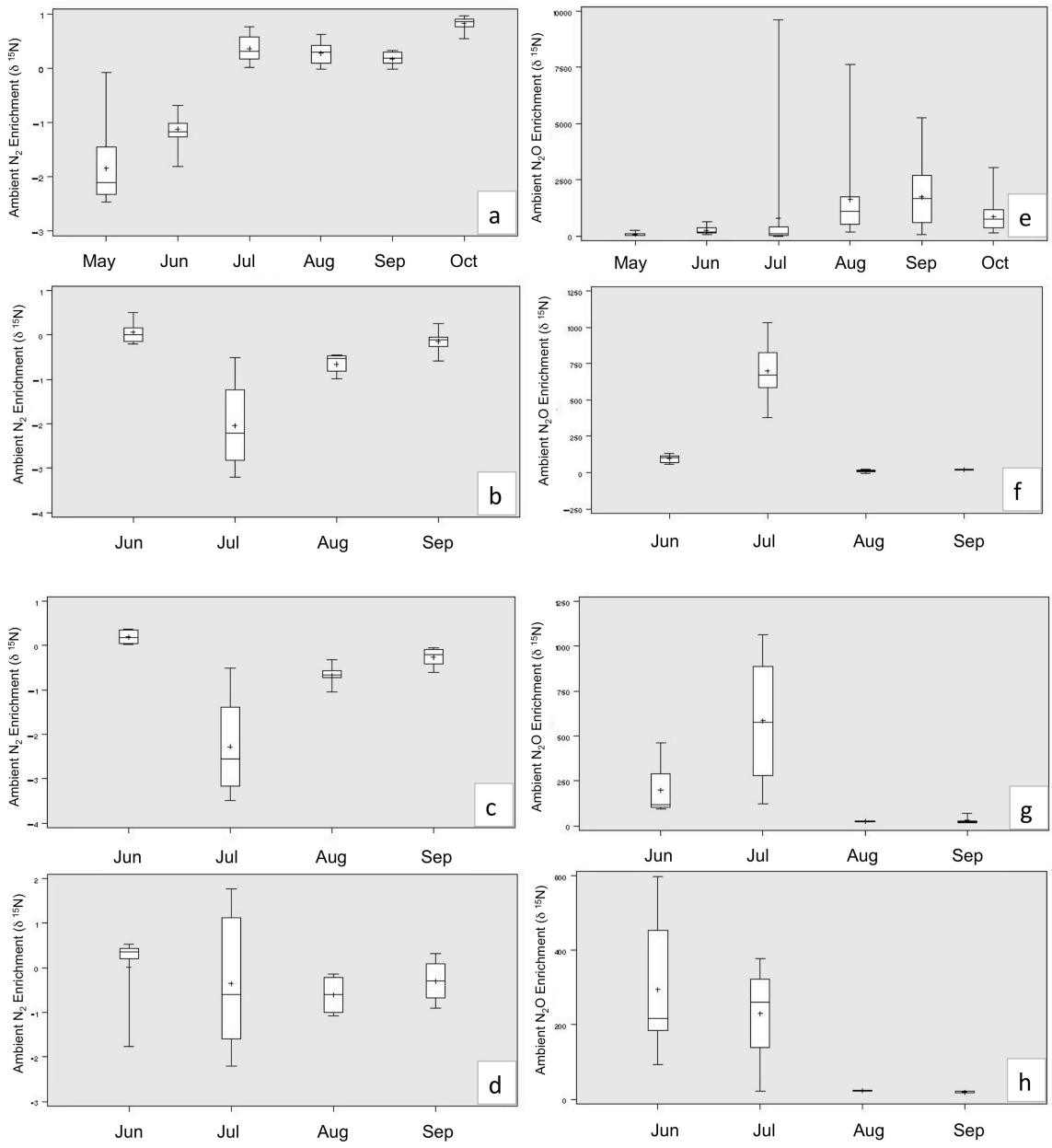


Figure 3-2. ^{15}N enrichments of N_2 and N_2O in natural abundance air samples at Hubbard Brook in 2005 (a & e), Hubbard Brook in 2006 (b & f), Lafayette Brook (c & g), and Mount Bickford (d & h) sites. Plot enrichments are summarized by month for May through October in 2005 and June though September in 2006 data.

Natural abundance values of ^{15}N in N_2 ($p<0.0001$) and N_2O ($p=0.009$) also differed by site/year combination. Overall, N_2 enrichments ranged from -3.5‰ to 1.7‰. N_2O enrichments ranged from -8.4‰ to 9600‰ (Figure 3-2).

3.5 DISCUSSION

3.5.1 Patterns in ^{15}N Recoveries

I recovered large portions – up to 98% – of the deposited $^{15}NO_3$-N in N gases in many chambers, indicating that loss of Nr to the atmosphere could be an important fate of wet N deposition to forest soils in the northeastern U.S. Recoveries in the N_2 pool were much higher than those in the N_2O pool, with N_2 recoveries averaging 2 orders of magnitude higher than N_2O recoveries in abiotic fluxes and one order of magnitude higher in biotic fluxes. For N_2O, biotic recoveries averaged higher than abiotic (Figure 3-1). It is important to note that 80% of the incubations did not produce fluxes in excess of detection limits. However, since my detection limits were conservative, and the sensitivity of the mass spectrometer analysis is not exceptional, I expect that our analysis overlooks a good deal of real gaseous N loss from the study systems. My results suggest that northeastern forest soils are capable of processing wet N deposition on time scales of seconds and minutes to hours and returning this N to the atmosphere. Further, gaseous N losses appear to be dominated by N_2 rather than N_2O, making these soils an Nr sink. Low recoveries in the N_2O pool are consistent with earlier observations (Keller et al. 1983, Bowden and Bormann 1986, Bowden et al. 1991) and indicate that short-term turnover of wet N deposition in these forests does not result in large emissions of this greenhouse gas.

Because my sample set was both restricted by conservative detection limits and distributed across a variety of plots, sites, and sampling times, my statistical analyses

were lacking in power to draw many conclusions about spatial and temporal patterns in recoveries of N gases; i.e. in most cases, the analyses failed to give any result, not just a negative one. The HBEF 2005 sample set was much larger than the other site/year combinations with 16 sites sampled 6 times, as opposed to 6 sites sampled 4 times for HBEF 2006, LF 2006, and MB 2006. As such, I was able to detect more patterns here than in the other sets (Figure 3-1).

Percent recoveries in abiotic fluxes of both N_2 and N_2O changed significantly at HBEF in 2005, with an increase in N_2O over the early season and a more complicated vacillating pattern in N_2 recoveries (Figure 3-1a, b). The N_2O pattern could be a result of increasing temperatures since abiotic processes are temperature dependent (Venterea et al. 2005, McCalley and Sparks 2009). Patterns may have also corresponded with changes in the type of organic matter available. Lignin, phenolics and humic substances have been shown to facilitate abiotic N_2O and, especially, N_2 production, by providing a substrate for nitrosation (Stevenson 1970), i.e., the addition of a nitroso group (–N=O) to an organic molecule. Nitroso compounds then undergo further reactions to form NO, N_2O, and N_2. If this mechanism is important at HBEF, fresh inputs of organic matter from senescence of vegetation at the end of the season may have induced the uptick seen in October 2005 recovery of ^{15}N in abiotic N_2 fluxes. Recovery of ^{15}N in abiotic N_2 fluxes was also significantly different among months at LF in 2006, however, since this analysis included only 5 data points, I can not draw any conclusions from it (Figure 3-1c).

Lack of data also confounded my efforts to discern differences among sites. The only analysis that returned results was a comparison of ^{15}N recoveries in biotic N_2 fluxes and it showed no difference among site/year combinations (Figure 3-1e-f). Again,

however, the data set for all of 2006 (including 3 sites/year combinations) contained only 6 points, so I could not draw any conclusions from it.

One consideration in interpreting the biotic flux results is that in some cases where the recovery in abiotic fluxes was high, the nitrogen pool available for biological denitrification may have been too depleted in ^{15}N to show a significant increasing trend. Rather, the enriched N_2 or N_2O pool may have displayed decreasing enrichment as the N gas pulse stimulated by wetting (not just the N deposition) may have used up the tracer and continued to denitrify oxidized ^{14}N. Thus, I would observe a decline in enrichment of the headspace N gases while N gas emissions continue. Therefore, although precipitation events may stimulate more N gas emissions than are indicated by my results, the present study focuses on the fate of N deposited during that event.

I attributed the burst of N gas emissions immediately after the simulated wet deposition events to abiotic processes because I assumed that biotic production at this time scale would be slowed by need to first synthesize denitrifying enzymes. However, as Davidson (1992) points out, pre-existing stocks of enzymes can allow some amount of biological denitrification to take place in rapid response to wetting. Conversely, some of the emissions taking place during the two-hour incubation may have arisen from abiotic processes. Several studies of abiotic N gas production have shown emissions to continue on this time scale (Davidson 1992, McCalley and Sparks 2009, Mørkved et al. 2007). I therefore conclude that although I have termed emissions on the two time scales of seconds-minutes and minutes-hours after perturbation as "abiotic" and "biotic" (respectively) in origin and believe these to be the dominant mechanisms at these time scales, I expect that there is some overlap in the mechanisms operating at both time scales.

3.5.2 Patterns in Natural Abundance Values

I observed unexpected patterns in natural abundance values of both N_2 and N_2O (Figure 2). Not only did enrichments deviate from 0 (quite dramatically in the case of N_2O), they also varied significantly over time. Seasonal patterns N_2 enrichments were similar in 2006 at HBEF and LF and, to a lesser extent, MB. The same was true for N_2O enrichments. However, 2005 enrichments at HBEF followed different patterns. This result suggests that variability in enrichments was driven by either a cross-site variable (e.g. weather) or an error in some part of the sample handling process (e.g. leakage). Since these samples were of ambient air, I did not expect leakage of air into or out of the sample vials to induce great changes in the sample enrichment. If leakage had been a problem, it should have decreased variability, bringing enrichments closer to 0. N_2O enrichments, especially, deviated from expected range of approximately 0-10‰ by up to 3 orders of magnitude (Kim and Craig 1993, Rock et al. 2007, Yoshida and Matsuo 1983). These extreme observations warrant further investigation.

3.5.3 Conclusions

In keeping with others' observations of a quick N gas loss following wetting events (Clough et al. 2006, Davidson 1992, Goodroad and Keeney 1984, Groffman and Tiedje 1988), simulated rainfall induced N gas losses in many of the incubations. Because I applied conservative detection limits, I believe this type of gas loss occurred in more incubations than shown in the data presented here and that quick (within 2 hours) processing of N deposited in precipitation events could be an important fate of Nr reaching northeastern forests. Furthermore, recovery of this deposited N as N_2 dominated N_2O recoveries, making denitrification in these soils a "sink" for Nr.

Although lack of data and statistical power limit my ability to clearly quantify the magnitude of this gas loss, it appears that fast turnover of N deposition via denitrification could remove a large proportion of N inputs soon after they arrive, shunting available N away from processes that retain N within the forest system (e.g. uptake by vegetation or burial in soil) and those that convey it downstream (e.g. leaching to groundwater or streams). These losses are likely occurring via both abiotic and biotic processes, but the exact time scales of the mechanisms operating require further investigation. Unusual patterns of ^{15}N enrichment in natural abundance samples also emerged in the data. ^{15}N concentrations in ambient air samples of both N_2 and N_2O varied from the expected range, rather dramatically in the case of N_2O. These patterns warrant further investigation.

WORKS CITED

Butterbach-Bahl K, Gasche R, Huber, CH, Kreutzer K, and Papen H. 1998. Impact of N-input by wet deposition on N-trace gas fluxes and CH_4-oxidation in spruce forest ecosystems of the temperate zone in Europe. Atmospheric Environment 32: 559–564.

Butterbach-Bahl K, Willibald G, and Papen H. 2002. Soil core method for direct simultaneous determination of N_2 and N_2O emissions from forest soils. Plant and Soil 240: 105-116.

Bowden RD, Melillo JM, Steudler PA, and Aber JD. 1991. Effects of nitrogen additions on annual nitrous-oxide fluxes from temperate forest soils in the northeastern United States. Journal of Geophysical Research-Atmospheres 96: 9321-9328.

Bowden WB and Bormann FH. 1986. Transport and loss of nitrous oxide in soil water after forest clearcutting. Science 233: 867-869.

Clough TJ, Kelliher FM, Wang YP, and Sherlock RR. 2006. Diffusion of ^{15}N-labelled N_2O into soil columns: a promising method to examine the fate of NO in subsoils. Soil Biology and Biochemistry 38: 1462-1468.

Clough TJ, Rolston DE, Stevens RJ, and Laughlin RJ. 2003. N_2 and N_2O gas fluxes, soil gas pressures, and wbulition events following irrigation of $^{15}NO_3^-$-labelled subsoils. Australian Journal of Soil Research 41: 401-420.

Dannenmann M, Butterbach-Bahl K, Gasche R, Willibald G, and Papen H. 2008.

Dinitrogen emissions and the N2:N2O emission ratio of a Rendzic Leptosol as influenced by pH and forest thinning. Soil Biology and Biochemistry 40: 2317-2323.

Davidson EA. 1992. Sources of nitric oxide and nitrous oxide following wetting of dry soil. Soil Science Society of America Journal 56: 95-102.

Driscoll CT, Whitall D, Aber J, Boyer E, Castro M, Cronan C, Goodale CL, Groffman PM, Hopkinson C, Lambert K, Lawrence G, and Ollinger S. 2003. Nitrogen pollution in the northeastern United States: Sources, effects, and management options. Bioscience 53: 357-374.

Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, and Cosby BJ. 2003. The nitrogen cascade. BioScience 53: 341–56.

Galloway JN, Aber JD, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, and Sutton MA. 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 320: 889-892.

Goodale CL andAber JD. 2001. The long term effects of land-use history on nitrogen cycling in northern hardwood forests. Ecological Applications 11(1): 253-267.

Groffman PM, Altabet MA, Bohlke JK, Butterbach-Bahl K, David MB, Firestone MK, Giblin AE, Kana TM, Nielsen LP, and Voytek MA. 2006a. Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecological Applications 16: 2091-2122.

Hall SJ and Matson PA. 1999. Nitrogen oxide emissions after nitrogen additions in tropical forests. Nature 400: 152-155.

Högberg P. 1997. ^{15}N natural abundance in soil-plant systems. New Phytologist 137: 179-203.

Keller M, Goreau TJ, Wofsy SC, Kaplan WA, and McElroy MB. 1983. Production of nitrous oxide and consumption of methane by forest soils. Geophysical Research Letters 10: 1156-1159.

Kim KY and Craig H. 1993. Nitrogen-15 and oxygen-18 characteristics of nitrous oxide: A global perspective. Science 262: 1855-1857.

Knowles R. 1981. Denitrification. In: Paul EA and Ladd J (Ed.), Soil biochemistry, vol. 5. Marcel Dekker Inc., New York. 323- 369.

McCalley CK and Sparks JP. 2009. Abiotic gas formation drives nitrogen loss from a desert ecosystem. Science 326: 837-840.

Mørkved PT, Dörsch P, and Bakken LR. 2007. The N_2O product ratio of nitrification and its dependence on long-term changes in soil pH. Soil Biology & Biochemistry 39: 2048–2057.

Papen H and Butterbach-Bahl K. 1999. A 3-year contiuous record of nitrogen trace gas fluxes from untreated and limed soil of a N-saturated sprice and beech forest ecosystem in Germany 1. N_2O emissions. Journal of Geophysical Research 104(D15):

18487-18503.

Pilegaard K, Skiba U, Ambus P, Beier C, Bruggemann N, Butterbach-Bahl K, Dick J, Dorsey J, Duyzer J, Gallagher M, Gasche R, Horvath L, Kitzler B, Leip A, Pihlatie MK, Rosenkranz P, Seufert G, Vesala T, Westrate H, and Zechmeister-Boltenstern S. 2006. Factors controlling regional differences in forest soil emissions of nitrogen oxides (NO and N_2O). Biogeosciences 3: 651–661.

Rock L, Ellert BH, Mayer B, and Norman AL. 2007. Isotopic composition of tropospheric and soil N2O from successive depths of agricultural plots with contrasting crops and nitrogen amendments. Journal of Geophysical research 112: D18303.

Schindlebacher A, Zechmeister-Boltenstern S, and Butterbach-Bahl K. 2004. Effects of soil moisture and temperature on NO, NO_2, and N_2O emissions from European forest soils. Journal of Geophysical Research 109: D17302.

Schlesinger WH. 2009. On the fate of anthropogenic nitrogen. Proceeding of the National Academies of Sciences 106(1): 203-208.

Sperduto DD and Engstrom B. 1993. An ecological inventory of the White Mountain National Forest, New Hampshire: second year interim report. NH Natural Heritage Inventory, Department of Resources and Economic Development, Concord, New Hampshire, USA.

Stevenson FJ, Harrison RM, Wetselaar R, and Leeper RA. 1970. Nitrosation of soil

organic matter: III. Nature of gases produced by reaction of nitrite with lignins, humic substances, and phenolic constituents under neutral and slightly acidic conditions. Soil Science Society of America Proceedings 34: 430-435.

van Breemen N, Boyer EW, Goodale CL, Jaworski NA, Paustian K, Seitzinger SP, Lajtha K, Mayer B, van Dam D, Howarth RW, Nadelhoffer KJ, Eve M, and Billen G. 2002. Where did all the nitrogen go?: fate of nitrogen inputs to large watersheds in the northeastern USA. Biogeochemistry 57: 267-293.

Venterea RT, Rolston DE, and Cardon ZG. 2005. Effects of soil moisture, physical, and chemical characteristics on abiotic nitric oxide production. Nutrient Cycling in Agroecosystems 72: 27–40.

Yoshida N and Matsuo S. 1983. Nitrogen isotope ratio of atmospheric N_2O as a key to the global cycle of N_2O. Geochemical Journal. 17: 231-239.

CHAPTER 4

LANDSCAPE AND REGIONAL PATTERNS IN NITROGEN GAS FLUXES IN NORTHERN HARDWOOD FORESTS

4.1 ABSTRACT

The importance of nitrogen gas fluxes in the nitrogen budgets of northern hardwood forests is not well known, although previous estimates of low nitrous oxide (N_2O) fluxes have led to a widespread assumption that these fluxes are negligible. I measured growing season denitrification rates (N_2 + N_2O fluxes) at three sites in White Mountain National Forest, NH, USA to assess both the magnitude and patterns of N gas fluxes in this region. The Hubbard Brook Experimental Forest (HBEF) was logged in the early 20th century as is typical for the region. Mount Bickford (MB) was burned in the early 20th century and represents a low nutrient status condition for the region. Lafayette Brook (LF) is an old-growth forest and is a relatively N-rich site. I found high N_2 fluxes, low N_2O fluxes, and high N_2:N_2O ratios at all sites, with best estimates of ~3-4 kg N ha^{-1} season^{-1} for N_2 and ~0.005-0.05 kg N ha^{-1} season^{-1}, for N_2O fluxes. N flux rates exhibited considerable spatial variability both within and among sites, as well as temporal variability within a season and among seasons (HBEF was sampled for 2 years). MB exhibited the highest N fluxes, LF the lowest, and HBEF produced intermediate fluxes in both years. Indicators of N richness (e.g. net nitrification, C:N ratio, and soil ammonium concentrations) were the strongest predictors of N gas fluxes suggesting that these fluxes are controlled by N limitation in this region.

Regressions between flux and topographic index (TI) and foliar N (FN), static indicators of moisture and N richness respectively, were used to create maps of seasonal N flux for HBEF for 2005 and 2006. Because the relationship between FN and N flux was positive in 2005 and negative in 2006, the maps are quite different in their N flux patterns and suggest that denitrification models and extrapolation tools for northeastern forests still require significant development.

4.2 INTRODUCTION

Nitrogen transformations involving gases are a poorly understood part of the nitrogen cycle, especially in terrestrial systems. Among those transformations, denitrification – the sequential reduction of nitrogen in the forms of nitrate (NO_3^-), nitrite (NO_2^-), nitric oxide (NO), and nitrous oxide (N_2O), to dinitrogen (N_2) -- may be the largest "missing" (i.e. not directly measured) flux in nitrogen budgets (Allison 1955, van Breemen et al. 2002). At broad scales, especially, denitrification rates are rarely measured and are generally calculated by difference in budgets (Kulkarni et al. 2008). The lack of reliable denitrification estimates is an important constraint on efforts to assess and mitigate nitrogen pollution problems at landscape and regional scales (Schlesinger 2009, Galloway et al. 2003).

A major challenge in evaluating denitrification at ecosystem, landscape and regional scales is that current methods to measure denitrification do not directly address rates at scales broader than the plot level (Groffman et al. 2006a). For estimates of denitrification rates at the ecosystem scale and broader, we must extrapolate fine-scale measurements. This extrapolation can be very problematic since denitrification rates are known to be highly heterogeneous in time and space over every scale (Parkin 1987). They frequently exhibit "hot moment" and "hot spot" behavior, where

relatively brief time periods and small areas account for a high percentage of activity (Parkin 1987, McClain et al. 2003). There is a great need to advance scaling methods from simple multiplication (by time and space) to using variability in controlling factors to scale up rates in a more informed manner (Groffman et al. 2009a). We must, however, first find robust relationships between controlling factors and denitrification rates and also have broad scale data available for those controlling factors in order to pursue such extrapolations (Kulkarni et al. 2008, Miller et al. 2004).

Extrapolating rates of microbial processes like denitrification is currently one of the most pressing challenges in environmental science. Good information on constraints and mechanisms, or "top-down" and "bottom-up" controls, as they relate to these processes is the key to scaling rates (Pearl and Steppe 2003). Denitrification is performed by a variety of ubiquitous microbes that are facultative anaerobic heterotrophs, i.e. they preferentially reduce oxygen over nitrogen oxides (Teidje et al. 1984). Most denitrifiers are heterotrophs, requiring a source of available organic carbon as a reductant. Thus, the three primary controls on denitrification are available nitrate (or other intermediate species), anaerobic conditions, and organic carbon. Furthermore, since it is a biological process, temperature and pH may also regulate denitrification rates (Paul and Clark 1996). While information on these five proximal controls can explain a significant percentage of denitrification activity at fine scales, these controls are difficult to quantify directly at broad scales. Brumme et al. (1999) suggest that it can be helpful to conceptualize a hierarchy of controls. In a study of N_2O emissions, they designated factors that affect long term patterns in emissions as higher order controls and those that affect short-term dynamics as lower order controls. These higher and lower order controls explained patterns in their data at different scales.

Patterns in denitrification rates over ecosystem scales and broader may be better discerned with the help of distal controls (Groffman et al. 1988, Wallenstein et al. 2006), i.e. higher order variables that reflect patterns in one or more proximal controls at broader spatial or temporal scales. For example, soil type aggregates information on soil moisture and carbon status and can be viewed as a proximal controller of denitrification at the landscape scale (Groffman and Tiedje 1989). Climate zone integrates long-term patterns in moisture and temperature and can be used to predict regional or global patterns in denitrification (Houlton et al. 2006). The challenge is to identify and measure distal controls at the scale of interest for a particular study (Robertson 1989).

New advances in remote sensing and geographic information systems (GIS) have improved the availability of information on distal controllers of denitrification at landscape and regional scales. Ollinger et. al (2002) found that foliar N (FN), mapped using hyperspectral data from an airborne sensor, was strongly related to soil nitrification rates, a proximal controller of soil nitrate supply. Landscape patterns in soil moisture can be portrayed with topographic index (TI), a GIS-based product derived from digital elevation models that identifies wet areas in a landscape (Beven and Kirkby 1997, Whelan and Gandolfi 2002). These new tools suggest that it is possible to assemble information on distal controllers that can be used extrapolate or predict denitrification rates at larger scales.

Nitrogen cycling at all scales, but especially landscape to regional scales, has been studied more in the northeastern United States than most other areas, largely as a result of elevated acid deposition in this region (Driscoll et al. 2003). Still, the enigma of

"missing nitrogen" remains (Allison 1955, van Breemen et al. 2002). Several calculations of nitrogen budgets in the northeast have shown large imbalances between inputs and outputs. The missing outputs are generally assumed to consist of a combination of storage in soils and vegation and N gas fluxes out of the system (Howarth et al. 1996, Castro et al. 2000, van Breemen et al. 2002, Goodale et al. 2002). As forests in the northeast age, however, nitrogen storage rates may be slowing down. Surprisingly, even as nitrogen inputs have remained elevated in the northeast, stream nitrogen outputs are declining, therefore increasing the budget imbalance (Martin et al. 2000, Goodale et al. 2003).

Gaseous loss of nitrogen has been discounted as a major output in budgets for northeastern forests for many years based on results from studies that found low N_2O fluxes at the Hubbard Brook Experimental Forest (HBEF) in New Hampshire and Harvard Forest in Massachusetts (Bowden 1986, Davidson et al. 1990, Keller et al. 1983, Bowden et al. 1990, 1991, 1993, Venterea et al 2003a). Fluxes of other nitrogenous gases were largely ignored, in part because of the difficulty in measuring them (Groffman et al. 2006a). Denitrification, however, may stop at any point in the reduction sequence, potentially producing NO, N_2O, N_2, or any combination of these three gases. The absolute and relative amounts of the gases produced are important not only in terms of understanding the nitrogen cycle and budgets, but also in the broader context of nitrogen pollution and management. As the primary mechanism for converting fixed or reactive nitrogen (Nr) into inert N_2, denitrification could be the most desirable "sink" for potentially polluting forms of Nr (Kulkarni et al. 2008). Nitrate and nitrite can cause eutrophication and harmful algal blooms, NO is a precursor to tropospheric ozone, and N_2O is a powerful greenhouse gas that also contributes to the destruction of stratospheric ozone (Galloway et al. 2003).

Unfortunately, methods that address all three gaseous products of denitrification simultaneously are not well developed. Even those that address only two products are new so historic data are generally limited to only one end product at a time, or two combined (N_2O plus N_2), in the case of acetylene-block techniques (Groffman et al. 2006a).

In this study, a direct flux method that measures both N_2O and N_2 fluxes was used to assess the magnitude and controls of denitrification in northern hardwood forests of the White Mountains of New Hampshire, USA. These two gases likely comprise the bulk of denitrification products in this region as NO fluxes have been found to be low in unfertilized northeastern forests (Venterea et al. 2004). The method was first used to survey denitrification rates at HBEF across plots with varying moisture and N richness in the growing season of 2005. These measurements were continued in 2006 and also expanded to two nearby sites in the White Mountains that were chosen to compare well with HBEF in terms of geography and climate but contrast in environmental history. One, Lafayette Brook (LF), is an old growth forest, while the other, Mount Bickford (MB) burned completely in the early 20th century and is considered a relatively nutrient poor forest. Goodale et al. (2000) found LF and MB – as well as other old growth and recently burned forests in the White Mountains -- to display highly contrasting levels of nitrogen storage and export in streams. Furthermore, they found medium-aged logged forests like HBEF to display rates of N storage and export similar to burned forests, although they did not include HBEF in their study. Specifc objectives were to 1) quantify patterns in denitrification rates across gradients in controlling variables (moisture, nitrogen) within the landscape at HBEF, 2) explore regional patterns in denitrification driven by ecosystem disturbance history and 3) develop algorithms for extrapolation of results to the landscape and

regional scale. I hypothesized that denitrification rates would vary with N richness and soil moisture within and between sites and that it would be possible to use remote sensing-derived estimates of foliar N and spatially explicit maps of moisture indices as tools for scaling plot measurements of N gas flux to larger areas.

4.3 METHODS

4.3.1 Study Sites

HBEF is located in the southern part of White Mountain National Forest (WMNF) (43° 56' N, 71° 45' W) and covers 3160 ha. It is an extensively studied Long Term Ecological Research (LTER) site dominated by mixed hardwood forest with sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and yellow birch (*Betula allegheniensis*) dominating the lower and mid-slopes, and spruce-fir (*Picea rubens* and *Abies balsamea*) forest at higher elevations. Commercial logging operations at HBEF ended in 1915-1917 although much of the forest has been re-growing since before the cessation of logging (see www.hubbardbrook.org for more information about this site). Some logging took place in the mid to late 20th century for scientific purposes in a series of experimental watersheds at HBEF (Likens and Bormann 1995), but these were not included in the present study. Plots within HBEF were chosen to represent a variety of both moisture and N richness levels using existing maps of TI and FN (discussed below).

The LF and MB sites are located approximately 20 miles north of HBEF in the Franconia Notch area of WMNF. LF is an old growth forest tract on the north slope of Mt. Lafayette that is not known to have ever been cut. The New Hampshire National Heritage Inventory (Sperduto and Engstrom 1993) indicates that the site contains old-aged northern hardwoods on the lower slopes and transitions to spruce and yellow

birch at higher elevations, with some spruce trees reaching 76 cm diameter at breast height. MB is a nearby site that is known to have burned in 1903. It has not been harvested since. Goodale and Aber (2001) showed that LF had very high nitrification and stream water N loss rates compared with several other sites in the region while MB exhibited the opposite pattern.

4.3.2 Sampling Design

In 2005, I sampled eight circular 0.05 ha plots at HBEF that had previously been studied by Schwarz et al. (2003) and Venterea et al. (2003b) for factors controlling variability in trees species abundance and nitrification, respectively. In 2006, I sampled six plots at HBEF (two of the eight plots that were sampled in 2005 plus four new plots) and 6 plots each at LF and MB.

Each plot was sampled monthly during the growing season (May through October) although logistical constraints and instrument problems eliminated the May 2006 sampling for all sites and the June 2006 sampling for HBEF only. The sampling consisted of taking 3 replicate sets of soil cores from each plot. The cores were stored, as intact as possible, in zip-top plastic bags under refrigeration for no more than 10 days until analysis. A set of cores consisted of a sufficient number of organic layer (combined Oi, Oe, and Oa horizons) cores to fill six to twelve inches of an incubation tube of slightly larger diameter than the cores. The number of cores required, generally two to four, was recorded and denitrification rates were corrected for the total surface area represented by the number of cores used in the sample. In October 2006, extra soil samples were taken from all plots for analysis of total carbon, total nitrogen, potential net N mineralization and potential net nitrification.

4.3.3 N Gas Fluxes

Our direct flux gas-flow soil core system for measuring denitrification rates was modeled after that of Swerts et al. (1995) and is similar to that of Butterbach-Bahl et al. (2002). In my system, soil samples were loaded into stainless steel tubes, held in place with polyester wool packing, and sealed at the ends with raised-middle O-rings and handcuff-style brackets (Swagelok, Crawford Fitting Co., Solon, OH). The tubes were enclosed in a plexiglass box that was flushed with high-purity helium gas and were connected with stainless steel tubing and fittings (Swagelok, Crawford Fitting Co., Solon, OH) to two gas chromatographs (GCs, Shimadzu GC8A, Kyoto, Japan). One GC was fitted with an electron capture detector (ECD) for measuring N_2O and CO_2 and the other had a thermal conductivity detector (TCD) for measuring N_2 and O_2.

Once samples were loaded into the stainless steel tubes, a 95% He and 5% O_2 mixture (HelOx) was flushed through the soil cores for at least one hour to replace the tube headspace with HelOx and remove all N_2 and N_2O. Testing with cores perfused with an inert tracer gas (SF_6) demonstrated that one hour was adequate to purge N_2 from these soils, which were very porous (bulk density from 0.1 to 0.3 g/cm^3) and not water-logged. Headspace gas was mixed and sampled by displacement from the tubes by the addition of approximately 40-50 ml HelOx (the exact amount was calculated from the gas transfer rate and time for each injection) at 0, 1, 3, and 5 hours. *In vitro* flux rates were calculated by regression of N_2-N or N_2O-N accumulation over time. These flux rates were further divided by the total ground surface area of the soil samples taken in the field. This area was the number of cores taken for the sample multiplied by 12.6 cm^2, the cross-sectional area of the corer. Rates were also adjusted for field temperatures using a Q_{10} factor of 2 (Scholefield et al. 1997) and soil temperature data available at www.hubbardbrook.org. The denitrification rate, or total

N flux, was calculated as the sum of N_2 and N_2O fluxes. It is important to note, however, that both the N_2 and N_2O fluxes are net fluxes; i.e. the former is comprised of N_2 produced by denitrification minus any N_2 consumed by nitrogen fixation and the latter is comprised of N_2O produced by both denitrification and nitrification minus any N_2O consumed by denitrification. For simplicity, I use the terms N_2 flux, N_2O flux and denitrification rate as described above in the rest of the paper.

To produce seasonal estimates of denitrification, I used rainfall events as a tool to extrapolate point measurements to longer time periods as the O_2 concentrations in my incubations (5%) were lower than mean field conditions (~10-17%, pers. comm. Colin Fuss). I therefore assumed that rain events of either 2 or 3 cm were necessary to induce 24 hours of soil conditions similar to my laboratory incubations, i.e. 5% O_2. During the growing season in 2005, there were thus 15 days of denitrification using a 2 cm threshold and 7 days using a 3 cm threshold. In 2006, there were 17 days of denitrification using a 2 cm threshold and 7 days using a 3 cm threshold. Seasonal rates were then calculated by extrapolating *in vitro* rates over the appropriate number of days for each scenario.

4.3.4 Other Soil Measurements

Gravimetric soil moisture was determined for each replicate analyzed for denitrification by weighing soil subsamples before and after drying for at least 24 hours at 60° F (McInnes et al. 1994). In October 2006, 3 samples from each site were dried, ground and analyzed for total N and C content (Carlo Erba CNS combustion analyzer) (Nelson and Sommers 1994). Other samples from this date were used for determination of potential net N mineralization and nitrification rates within 3 days of sampling (Robertson et al. 1999). These samples were sieved (8 mm), homogenized

and incubated for 10 days at room temperature in mason jars with lightly fastened lids. Subsamples were extracted with 2 M KCl and analyzed colorimetrically (Lachat Quickchem 8100 Flow Injection Analyzer) for inorganic N at the beginning and end of the incubation. Potential net nitrification rate was calculated from the change in NO_2^--N + NO_3^--N over the course of the incubation and net N mineralization rates were calculated from the change in NH_4^+-N + NO_2^--N + NO_3^--N over the course of the incubation.

4.3.5 Soil and Site Data From Other Sources

A map of topographic index (TI) for HBEF was created using a 10 m digital elevation model for HBEF available at www.hubbardbrook.org. A map of soil topographic index (STI) was created from the TI map and soil surveys from the USDA Natural Resources Conservation Survey. TI and STI are moisture indices that approximate relative moisture conditions over an area using the following formulas: TI = ln (a/tan β) and STI = ln (a/T_0 tan β) where a = upslope area draining through a point per unit contour length, β = local slope angle, and T_0 = soil transmissivity at saturation (Ambroise et al. 1996, Wang et al. 2005). A map of percent FN was derived from hyperspectral images taken by NASA's Airborne Visible/Infrared Imaging Spectrometer (AVIRIS) (Martin et al. 2008). Data on precipitation and temperature were obtained from www.hubbardbrook.org. Data on a number of attributes of each of the HBEF sampling plots (elevation, aspect, tree species basal areas, soil organic matter content, pH) were taken from Venterea et al. (2003b) and Schwarz et al. (2003).

4.3.6 Statistics and Modeling

In the following analyses (conducted using SAS statistical software), data were generally grouped into 4 site/year combinations: HBEF 2005, HBEF 2006, LF 2006, and MB 2006. Mixed models evaluated variability in N fluxes over a season by accounting for the complicated nature of the sampling design including: repeated measures (at each plot over a season), the combination of fixed and random (plot and replicate within plot) effects, and the combination of class (sampling month, plot, replicate) and continuous (N fluxes) variables. I performed ANOVAs with Tukey's tests to assess differences in N fluxes and site characteristics (e.g. soil moisture, C:N and mineralization rate) among the 4 groups.

To analyze the relationships between site characteristics and N fluxes within each site grouping, I performed two statistical analyses. The first was a simple Pearson correlation analysis and the second was a more rigorous mixed model with autoregressive structure, similar to that used in analysis of temporal changes in N fluxes (see above). The mixed model was very conservative in attributing variation in the dependent variables (N fluxes) to individual independent variables. Mixed models were also used to assess relationships between site characteristics and N fluxes across sites. Site characteristics evaluated in these models included soil moisture, net N mineralization, net nitrification, and soil C:N ratio.

Simple regressions of static data by plot, i.e. data collected only once or aggregated over a season, were used to evaluate relationships between total N fluxes and the three variables for which I had spatially explicit data across the HBEF watershed: TI, STI and FN. Multiple regressions using TI and FN were also performed to develop models for extrapolating total N fluxes across the HBEF watershed using both 2005 and 2006

data. This extrapolation was performed in a geographic information system (GIS) using Manifold GIS software.

4.4 RESULTS

4.4.1 Patterns in N fluxes

N_2 fluxes varied widely across site, plot and time (Figure 4-1, Table 4-1). In 2005 at HBEF, rates ranged from ~0.2-0.8 kg N_2-N ha^{-1} d^{-1} in the early season, but the range widened as the season progressed, with some plots dropping off to negligible rates and others increasing to near 2 kg N_2-N ha^{-1} d^{-1}. Overall rates did not show a significant change over the season. In 2006, rates appeared to decrease from mid-season to late-season at HBEF and MB, with a slight, late-season uptick at some plots in MB, although only the changes at MB were significant in a mixed model ($p=0.0004$). Temporal patterns were less clear and not significant at LF, although August had the lowest overall N_2 flux rates (Figure 4-1).

N_2O fluxes were consistently much lower than N_2 fluxes across all sites, plots, and times (Figures 4-1, 4-2). In 2005 at HBEF, N_2O fluxes stayed below 0.015 kg N_2O-N ha^{-1} d^{-1} but different plots appeared to follow different patterns across the season ($p=0.048$ in overall mixed model), with 2 plots experiencing peaks in June and October. HBEF N_2O fluxes generally decreased in 2006 from 2005 levels, although the set of plots studied was different in the two years. Of the two plots that were sampled in both years, one exhibited similar rates for both years (Plot #265), while the other (#338) exhibited a decline of about 30% from 2005 to 2006 (Table 4-1). In 2006, all plots showed decreasing rates of N_2O flux ever the course of the season, with some plots experiencing more dramatic declines than others ($p=0.0033$). Similar patterns held for nearly all plots at both LF and MB, with some LF plots exhibiting

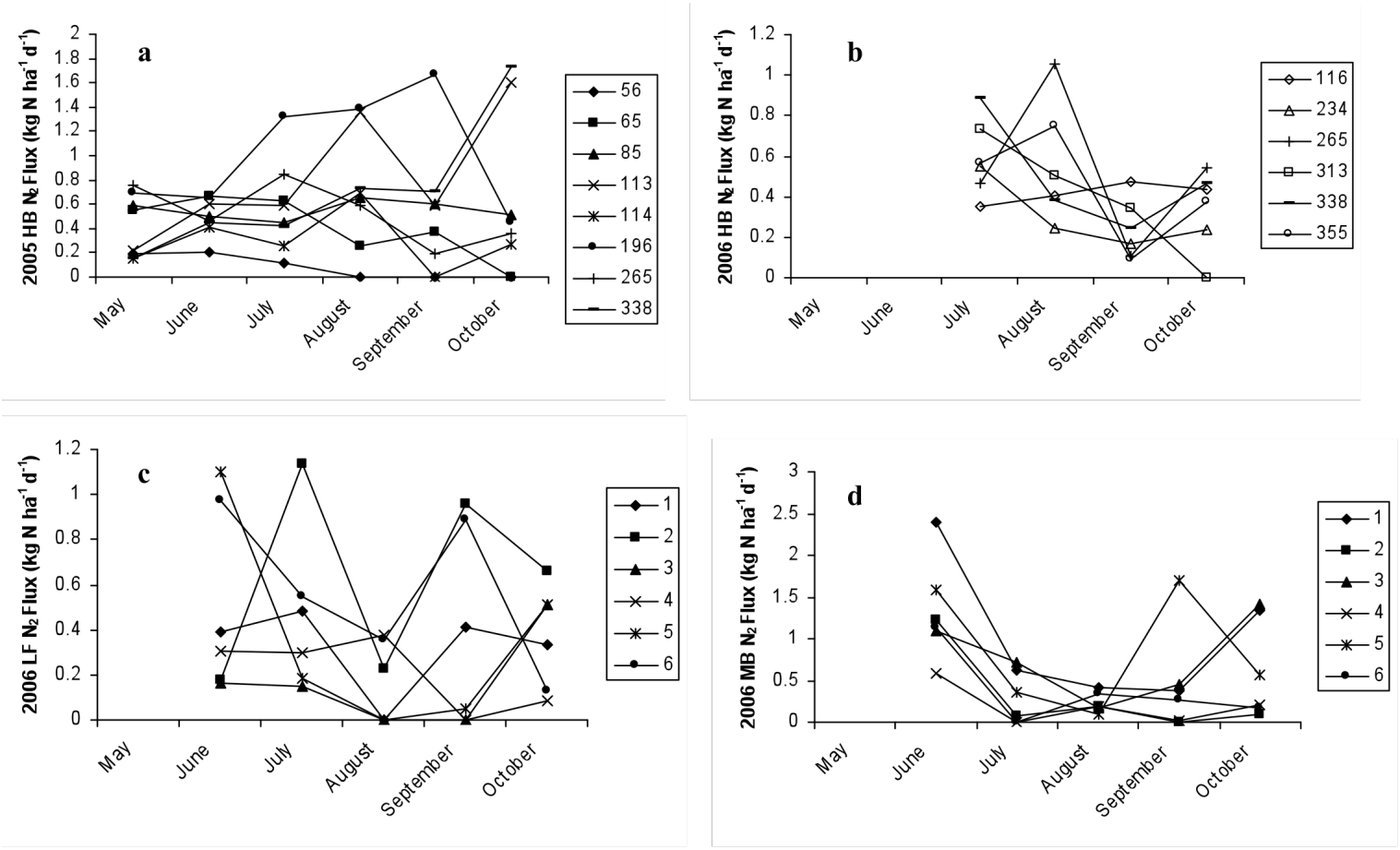


Figure 4-1. N_2 fluxes over the season for Hubbard Brook in 2005 (a), Hubbard Brook in 2006 (b), Lafayette in 2006 (c), and Mount Bickford in 2006 (d). Site abbreviations: HB=Hubbard Brook Experimental Forest, LF=Lafayette Brook, MB=Mount Bickford.

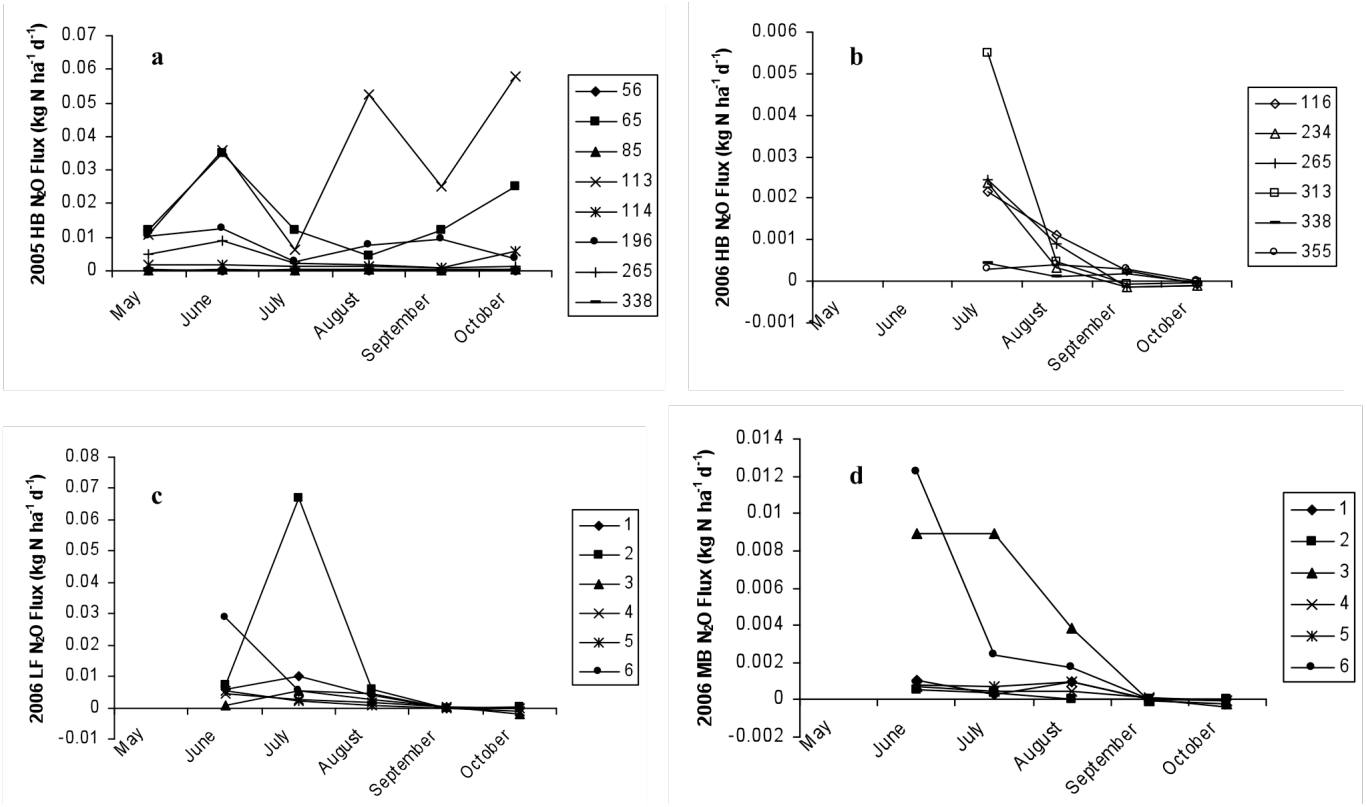


Figure 4-2. N_2O fluxes over the season for Hubbard Brook in 2005 (a), Hubbard Brook in 2006 (b), Lafayette in 2006 (c), and Mount Bickford in 2006 (d). Site abbreviations: HB=Hubbard Brook Experimental Forest, LF=Lafayette Brook, MB=Mount Bickford.

Table 4-1. Nitrogen fluxes for each Hubbard Brook plot by year, extrapolated temporally using 3 precipitation thresholds. Topographic index (TI), soil topographic index (STI) and percent foliar N (%FN) are also shown for each plot.

	Nitrogen flux (kg N ha^{-1} $season^{-1}$) with no precipitation threshold				Nitrogen flux (kg N ha^{-1} $season^{-1}$) with precipitation threshold of 2 cm				Nitrogen flux (kg N ha^{-1} $season^{-1}$) with precipitation threshold of 3 cm				TI	STI	%FN
	2005		2006		2005		2006		2005		2006				
Plot	N_2	N_2O	N_2	N_2O	N_2	N_2O	N_2	N_2O	N_2	N_2O	N_2	N_2O			
56	16	ND			1	ND			1	ND			4.1	5.3	2.16
65	75	3.0646			6	0.2498			3	0.1166			-3.9	10.4	1.84
85	101	0.0539			8	0.0044			4	0.0020			2.8	5.9	2.02
113	151	5.7337			12	0.4674			6	0.2181			6.0	4.5	2.13
114	54	0.3952			4	0.0322			2	0.0150			3.2	3.5	1.57
116			76	0.1586			7	0.0147			3	0.0060	3.5	11.3	2.06
196	187	1.3624			15	0.1111			7	0.0518			3.5	6.0	2.43
234			55	0.1133			5	0.0105			2	0.0043	-3.9	5.9	2.31
265	97	0.6063	99	0.1414	8	0.0494	9	0.0131	4	0.0231	4	0.0054	6.4	6.3	1.59
313			72	0.2681			7	0.0248			3	0.0102	3.0	-4.4	2.14
338	127	0.0336	90	0.0336	10	0.0027	8	0.0031	5	0.0013	3	0.0013	4.3	5.0	1.99
355			81	0.0444			8	0.0041			3	0.0017	4.9	3.7	1.97

somewhat higher rates in the early season than the other two sites, although the seasonal pattern was only significant at MB (p=0.0004) (Figure 4-2).

Estimates of potential annual N_2 flux (i.e. where rates measured in the laboratory at 5% O2 were simply extrapolated over 180 days) ranged from 69-108 kg N ha^{-1} y^{-1} (Table 4-2). Rates among the four site groupings (HBEF 2005, HBEF 2006, LF 2006, and MB 2006) were significantly different (p=0.0088) in an overall ANOVA with the highest rates occurring at MB (the low N richness site), the lowest at LF (the high N richness site) and both years of HBEF data falling in between. In pairwise comparisons, only MB and LF N_2 fluxes were significantly different.

N_2O fluxes were smaller than N_2 fluxes by 3-4 orders of magnitude at all sites, and the pattern among sites was different than that of N_2 fluxes with LF having the highest and HBEF having the lowest fluxes in 2006 (Table 4-2). Differences among the four site groupings for N_2O flux were significant in an overall ANOVA (p<0.0001) with more significant pairwise differences: HBEF 2005, HBEF 2006 and MB 2006 were all significantly different from each other, although none were different from LF 2006. Since total N fluxes were comprised almost entirely of N_2 fluxes, the same pattern held among site groupings for total N flux as N_2 fluxes (p=0.0088). In contrast, the ratio of N_2 fluxes to N_2O fluxes (N_2:N_2O), did not differ among site groupings (p=0.14), (Table 4-3).

Seasonal estimates of denitrification produced by assuming that denitrification only occurs after rainfall events of 2 or 3 cm were much lower than potential rates produced by multiplying rates measured in the laboratory at 5% O_2 over the full season (Table 4-2). The 2 and 3 cm scenarios resulted in a multiplication of my average daily plot

Table 4-2. Nitrogen fluxes (with standard deviations) for Hubbard Brook (HB), Lafayette (LF), and Mount Bickford (MB) sites by year, extrapolated temporally using 3 precipitation thresholds.

Year	Site	Nitrogen flux (kg N ha^{-1} $season^{-1}$) with no precipitation threshold		Nitrogen flux (kg N ha^{-1} $season^{-1}$) with precipitation threshold of 2 cm		Nitrogen flux (kg N ha^{-1} $season^{-1}$) with precipitation threshold of 3 cm	
		N_2	N_2O	N_2	N_2O	N_2	N_2O
2005	HB	101 ± 80	1.4 ± 2.5	8 ± 6	0.11 ± 0.21	4 ± 3	0.054 ± 0.096
2006	HB	79 ± 46	0.13 ± 0.24	7 ± 4	0.012 ± 0.022	3 ± 2	0.0048 ± 0.0089
2006	LF	69 ± 62	0.98 ± 2.4	6 ± 6	0.091 ± 0.22	3 ± 2	0.037 ± 0.089
2006	MB	108 ± 112	0.27 ± 0.56	10 ± 10	0.025 ± 0.052	4 ± 4	0.010 ± 0.021

Table 4-3. Soil characteristics by plot and site. N fluxes, calculated at the 3 cm rain threshold, and moisture are averaged over the season; the others were only measured (in triplicate) once per plot. An asterisk (*) indicates significant differences among site/year combinations in the soil characteristic. Site abbreviations: HB=Hubbard Brook Experimental Forest, LF=Lafayette Brook, MB=Mount Bickford.

Year	Site	Plot	N_2 Flux* (kg N ha^{-1} $season^{-1}$)	N_2O Flux* (kg N ha^{-1} $season^{-1}$)	Total N Flux* (kg N ha^{-1} $season^{-1}$)	N_2-N: N_2O-N	Moisture (g H_2O g^{-1} dry soil)	Soil N* (%)	Soil C* (%)	C:N*	Ammonium** (g NH_4^+-N g^{-1} dry soil)	Nitrate** (g NO_3^--N g^{-1} dry soil)	Net Mineralization* (mg N g^{-1} dry soil d^{-1})	Net Nitrification* (mg N g^{-1} dry soil d^{-1})
2005	HB	56	0.60	0.00032	0.60	1900	243	1.63	49.7	30.6	82.4	0.824	8.8	1.48
2005	HB	65	2.9	0.12	3.0	25	94.2	1.61	29.4	18.4	54.0	14.8	21.6	9.49
2005	HB	85	3.8	0.0021	3.8	1900	276	2.25	48.5	21.5	193	0.965	14.5	0.137
2005	HB	113	5.7	0.22	6.0	26	246	2.31	44.4	19.4	71.5	27.6	24.0	15.0
2005	HB	114	2.0	0.015	2.1	140	195	2.36	47.8	20.4	170	1.90	-6.2	-0.361
2005	HB	196	7.1	0.052	7.2	140	145	1.58	29.8	18.9	201	26.8	14.9	8.48
2005	HB	265	3.7	0.023	3.7	160	178	2.31	43.2	18.7	94.6	16.8	15.2	5.22
2005	HB	338	4.8	0.0021	4.8	2400	160	1.81	50.4	27.9	198	8.87	7.6	-0.0319
2006	HB	116	2.9	0.0060	2.9	480	171	1.73	31.9	18.4	86.4	8.46	22.0	7.52
2006	HB	234	2.1	0.0043	2.1	480	247	2.38	44.3	18.6	200	9.19	15.1	3.53
2006	HB	265	3.8	0.0054	3.8	700	198	2.31	43.2	18.7	215	9.39	15.2	5.22
2006	HB	313	2.7	0.010	2.8	270	199	1.76	31.4	17.8	107	9.54	10.9	0.854
2006	HB	338	3.4	0.0013	3.4	2700	119	1.81	50.4	27.9	215	0.424	7.6	-0.0319
2006	HB	355	3.1	0.0017	3.1	1800	201	2.34	49.8	21.3	210	0.521	10.7	-0.0269
2006	LF	1	2.2	0.027	2.3	82	99	1.13	20.5	18.2	117	24.3	20.8	10.4
2006	LF	2	4.4	0.11	4.5	39	182	1.86	35.9	19.3	695	173	20.0	10.5
2006	LF	3	1.1	0.0096	1.1	120	164	1.65	30.2	18.3	950	215	17.5	2.27
2006	LF	4	1.5	0.012	1.5	130	197	2.32	47.5	20.4	118	10.5	20.8	6.24
2006	LF	5	2.6	0.0095	2.6	270	133	2.00	38.5	19.3	62.7	8.05	26.0	3.54
2006	LF	6	4.0	0.054	4.1	74	175	1.77	42.7	24.3	164	1.62	14.7	4.94
2006	MB	1	7.1	0.0032	7.2	2200	270	1.99	46.1	23.2	51.7	0.879	8.2	0.243
2006	MB	2	2.2	0.0012	2.2	1800	228	1.86	51.0	27.5	56.0	0.897	5.8	-0.0187
2006	MB	3	5.3	0.030	5.4	180	177	1.84	40.1	22.0	94.5	14.3	13.7	3.77
2006	MB	4	1.4	0.0023	1.4	620	103	1.50	30.7	20.4	46.9	3.26	8.4	1.36
2006	MB	5	6.0	0.0034	6.0	1800	725	1.83	40.5	22.1	87.8	0.520	8.8	0.281
2006	MB	6	2.6	0.022	2.7	120	207	1.76	34.6	19.7	84.1	14.2	10.6	4.81

Table 4-3 continued.

Year	Site	Plot	N_2 Flux* (kg N ha^{-1} $season^{-1}$)	N_2O Flux* (kg N ha^{-1} $season^{-1}$)	Total N Flux* (kg N ha^{-1} $season^{-1}$)	N_2-N: N_2O-N	Moisture (g H_2O g^{-1} dry soil)	Soil N* (%)	Soil C* (%)	C:N*	Ammonium** (g NH_4^+-N g^{-1} dry soil)	Nitrate** (g NO_3^--N g^{-1} dry soil)	Net Mineralization* (mg N g^{-1} dry soil d^{-1})	Net Nitrification* (mg N g^{-1} dry soil d^{-1})
2005	**HB**	**Mean**	**3.8**	**0.1**	**3.9**	**820**	**192**	**1.99**	**42.9**	**22.0**	**103**	**16.2**	**12.6**	**4.93**
2006	**HB**	**Mean**	**3.0**	**0.0**	**3.0**	**1100**	**189**	**2.05**	**41.8**	**20.5**	**76.4**	**13.3**	**13.6**	**2.85**
2006	**LF**	**Mean**	**2.6**	**0.0**	**2.7**	**120**	**158**	**1.78**	**35.9**	**20.0**	**120**	**31.1**	**20.0**	**6.30**
2006	**MB**	**Mean**	**4.1**	**0.0**	**4.1**	**1100**	**285**	**1.80**	**40.5**	**22.5**	**51.7**	**8.9**	**9.3**	**1.75**

rates by 15 and 7 days respectively for 2005 data and 14 and 7 days respectively for 2006 data.

4.4.2 Patterns in Site Characteristics

Soil moisture did not differ among sites ($p=0.11$). Soil C, N and C:N differed among sites ($p<0.0001$, $p<0.0001$, $p=0.0029$, respectively), with the first two being significantly different among HBEF, LF, and MB and the last showing significant differences only between LF and each of the other two sites. MB had the highest C:N and LF the lowest, with HBEF displaying intermediate values. Net mineralization and nitrification also differed among sites ($p<0.0001$ for overall ANOVAs of both). Both processes differed significantly between all pairings of site groups except the two sets of plots used in 2005 and 2006 at HBEF. Both were highest at LF, lowest at MB and intermediate at HBEF. NH_4^+ and NO_3^- levels also differed significantly among sites ($p=0.034$ and $p=0.041$ respectively) but the only significant pairwise comparison was that LF had more NH_4^+ than MB (Table 4-3).

4.4.3 Relationships Among Site Characteristics and N Fluxes by Site and Year

I analyzed relationships between N fluxes (N_2, N_2O, N_2:N_2O) and site characteristics two different ways: using correlation and mixed models. The correlations highlighted many possible relationships among these variables while the mixed models were more conservative and identified only a few significant relationships. Note that there were 32 possible site characteristics for HBEF and only 8 for LF and MB. Site characteristics that were not available for the analysis for LF and MB included elevation, aspect, topographic indices, foliar N and tree species abundances.

Many site characteristics were correlated with N fluxes at Hubbard Brook in 2005, however only a handful of these relationships were significant in the mixed model analysis. There were highly significant ($p < 0.001$) correlations between N_2 flux and soil nitrate, eastness, red spruce basal area, and soil pH. All of these correlations were positive except for red spruce basal area. Other correlations were significant at higher alpha levels. None of these relationships, however, was significant in the mixed model analysis (Table 4-4).

N_2O fluxes were correlated with more site characteristics than N_2 fluxes; the strongest relationships were with soil nitrate, net nitrification, elevation, conifer basal area, yellow birch basal area, balsam fir basal area, red maple basal area, soil pH soil organic matter content, and soil C:N ($p<0.001$ for all relationships). Of these relationships, nitrate, nitrification, elevation, yellow birch basal area, and pH had positive correlation coefficients with N_2O flux and the rest were negative. Net nitrification was the only independent variable that was found to have a significant relationship with N_2O flux in a mixed model analysis (Table 4-4).

As with N_2O fluxes, the N_2:N_2O ratio at HBEF in 2005 was correlated with many independent variables including soil nitrate, net nitrification, C:N, elevation, conifer basal area, red spruce basal area, striped maple basal area, red maple basal area, eastern hemlock basal area, soil pH, and soil organic matter content ($p<0.001$ for all relationships). All of the correlation coefficients for these relationships were negative except for soil C:N, conifer basal area, red spruce basal area, eastern hemlock basal area, and soil organic matter content. Of these, net nitrification ($p<0.05$), elevation ($p<0.01$), conifer basal area ($p<0.001$), and red maple basal area ($p<0.01$) also had significant relationships with the N_2:N_2O in a mixed model analysis (Table 4-4).

Table 4-4. Relationships between explanatory and response variables as determined by correlation and mixed models for Hubbard Brook (2005 and 2006), Lafayette Brook (2006) and Mount Bickford (2006) sites. The number of asterisks indicates the level of significance where x: not significant, *: significant at α=0.05, **: significant at α=0.01, ***: significant at α=0.001. Site abbreviations: HB=Hubbard Brook Experimental Forest, LF=Lafayette Brook, MB=Mount Bickford.

		Response	Explanatory	Correlation		Mixed Model	
Year	Site	Variable	Variable	Coefficient	Significance	Coefficient	Significance
2005	HB	N_2	NO_3^-	0.202	***		x
			Net Nitrification	-0.172	*		x
			Foliar N	0.227	**		x
			Eastness	0.317	***		x
			Conifer Basal Area	-0.215	**		x
			Sugar Maple Basal Area	0.179	*		x
			Yellow Birch Basal Area	0.191	*		x
			Red Spruce Basal Area	-0.324	***		x
			Soil pH	0.349	***		x
			Soil Organic Matter	-0.497	**		x
			C:N	-0.221	**		x
		N_2O	Net Mineralization	0.231	**		x
			Net Nitrification	0.586	***	0.443	**
			NO_3^-	0.289	***		x
			Elevation	0.399	***		x
			Total Basal Area	0.271	**		x
			Conifer Basal Area	-0.353	***		x
			Sugar Maple Basal Area	0.167	*		x
			Yellow Birch Basal Area	0.457	***		x
			Red Spruce Basal Area	-0.238	**		x
			Balsam Fir Basal Area	-0.227	***		x
			Paper Birch Basal Area	-0.252	**		x
			Striped Maple Basal Area	0.19	**		x
			Red Maple Basal Area	-0.262	***		x
			Eastern Hemlock Basal Area	-0.184	*		x
			Soil pH	0.387	***		x
			Soil Organic Matter	-0.497	***		x
			%C	-0.225	**		x
			C:N	-0.308	***		x

Table 4-4 continued.

Year	Site	Response Variable	Explanatory Variable	Correlation Coefficient	Correlation Significance	Mixed Model Coefficient	Mixed Model Significance
		N_2:N_2O	NO_3^-	-0.22	***		x
			Net Nitrification	-0.424	***	-148	*
			%C	0.243	**		x
			C:N	0.489	***		x
			Southness	0.184	*		x
			Elevation	-0.529	***	-11.1	**
			Conifer Basal Area	0.549	***	168	***
			Sugar Maple Basal Area	-0.193	*		x
			Yellow Birch Basal Area	-0.221	*		x
			Red Spruce Basal Area	0.31	***		x
			Paper Birch Basal Area	0.243	**		x
			Striped Maple Basal Area	-0.316	***		x
			Red Maple Basal Area	0.48	***	362	**
			Eastern Hemlock Basal Area	0.394	***		x
			Water Holding Capacity	-0.293	**		x
			Soil pH	-0.446	***		x
			Soil Organic Matter	0.373	***		x
2006	HB	N_2O	NH_4^+	0.666	***	0.00343	***
2006	LF	N_2	Moisture	0.3	*		x
		N_2:N_2O	C:N	-0.299	*	-209	*
2006	MB	N_2	NH_4^+	0.444	***		x
			%N	0.283	*		x
		N_2O	NO_3^-	0.257	*		x
			NH_4^+	0.45	***		x
			Net Mineralization	0.325	**		x
			Net Nitrification	0.466	***	0.293	***
		N_2:N_2O	%C	0.285	*		x
			C:N	0.424	**	8760	**

In 2006, many fewer significant correlations were found compared to 2005. For HBEF sites, the only significant relationship was between soil ammonium levels and N_2O flux. It was negative and highly significant ($p<0.001$) in both correlation and mixed model analysis. Similarly, for the 2006 LF data, only two relationships were found by correlation analysis and one in the mixed model analysis ($0.01<p<0.05$ for all relationships). Moisture was positively correlated with N_2 flux and C:N ratios were negatively correlated with N_2:N_2O. Of these only the latter remained significant in the mixed model analysis (Table 4-4).

More relationships were found among site characteristics and N fluxes at MB than at the other sites in 2006. N_2 fluxes were correlated with ammonium levels ($p<0.0001$) and soil total N ($p=0.021$). N_2O fluxes were correlated with nitrate ($p=0.021$), ammonium ($p<0.0001$), net mineralization ($p=0.0033$) and net nitrification ($p<0.0001$). N_2:N_2O ratios were correlated with soil carbon ($p=0.033$), and C:N ($p=0.0011$). All of these correlations were positive. Only two of these relationships were significant in mixed model analysis: net nitrification vs. N_2O flux ($p=0.0008$) and C:N ratio vs. N_2:N_2O ($p=0.0016$) (Table 4-4).

A common thread among many of the mixed models was that they attributed a great deal of variability in N fluxes and ratios to changes over the season (Figures 4-1, 4-2). Seasonal variation thus reduced the number of significant relationships between site characteristics and N fluxes in mixed model analysis.

4.4.4 Site Characteristics and N Gas Flux Relationships Across Sites

None of the mixed models assessing relationships between total N flux and site characteristics were significant. These included soil moisture vs. N_2 flux, soil

moisture vs. N_2O flux, net mineralization vs. total N flux, net nitrification vs. total N flux, and C:N vs. total N flux.

4.4.5 Topographic Indices, Foliar N and N Gas Fluxes at Hubbard Brook

Simple regressions between topographic index (TI), soil topographic index (STI), and foliar N (FN) as independent variables and total N fluxes for 2005 and 2006 as dependent variables yielded few strong relationships. Only TI vs. total 2006 N flux R^2=0.84, p=0.0097) and foliar N vs. total 2006 N flux (R^2=0.86, p=0.0082) were significant. The relationship between FN and 2005 N flux would have been significant but for one point, plot #56 (R^2=0.80, p=0.0063), which fell well off the regression line. The FN vs. N gas flux relationship was positive for 2005 and negative for 2006 (Figure 4-3).

Multiple regression with both TI and FN as predictors of total seasonal N flux resulted in a significant model for 2006 data (R^2=0.93, p=0.018) and a non-significant one for 2005 (R^2=0.28, p=0.43). The regression for 2005 run without plot #56, the possible outlier, was highly significant (R^2=0.92, p=0.0066). I used all three regressions to create hypothetical maps of seasonal N flux for 2005 and 2006 using the 3 cm threshold data; only the results of the significant models are shown here (Figure 4-4). Since the relationship between FN and N flux was positive in 2005 and negative in 2006, the maps are quite different in their N flux patterns. Notably, the deep valleys and high elevation spruce-fir areas have high N flux rates on the 2006 map, but low rates on the 2005 map.

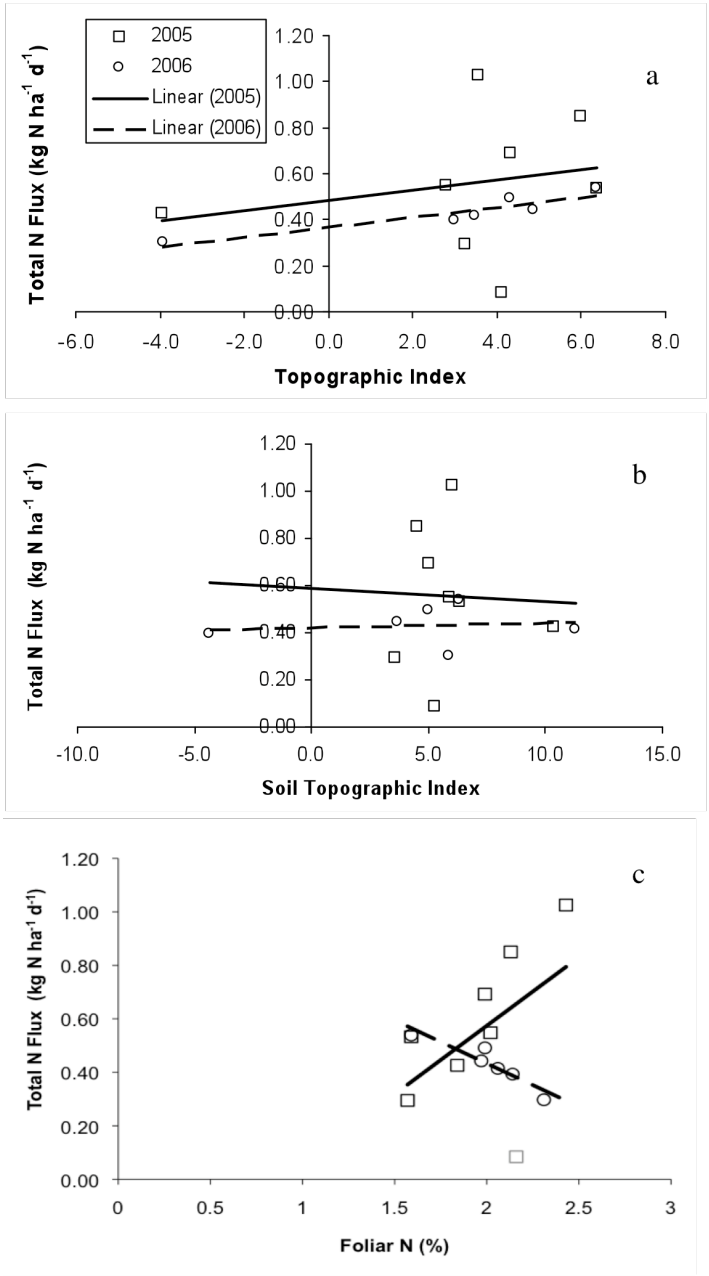


Figure 4-3. Hubbard Brook total N fluxes by year as a function of topographic index (a) (R^2=0.054, p=0.58 for 2005; R^2=0.84, p=0.0097 for 2006), soil topographic index (b) (R^2=0.0014, p=0.93 for 2005; R^2=0.015, p=0.81 for 2006), and percent foliar nitrogen (c) (R^2=0.25, p=0.21 for 2005; R^2=0.86, p=0.0082 for 2006). The regression between 2005 total N flux and % foliar N was also run excluding the point representing plot #56, a possible outlier; it is marked with a grey square and located near the x-axis (R^2=0.80, p=0.0063); this regression line is shown in the figure.

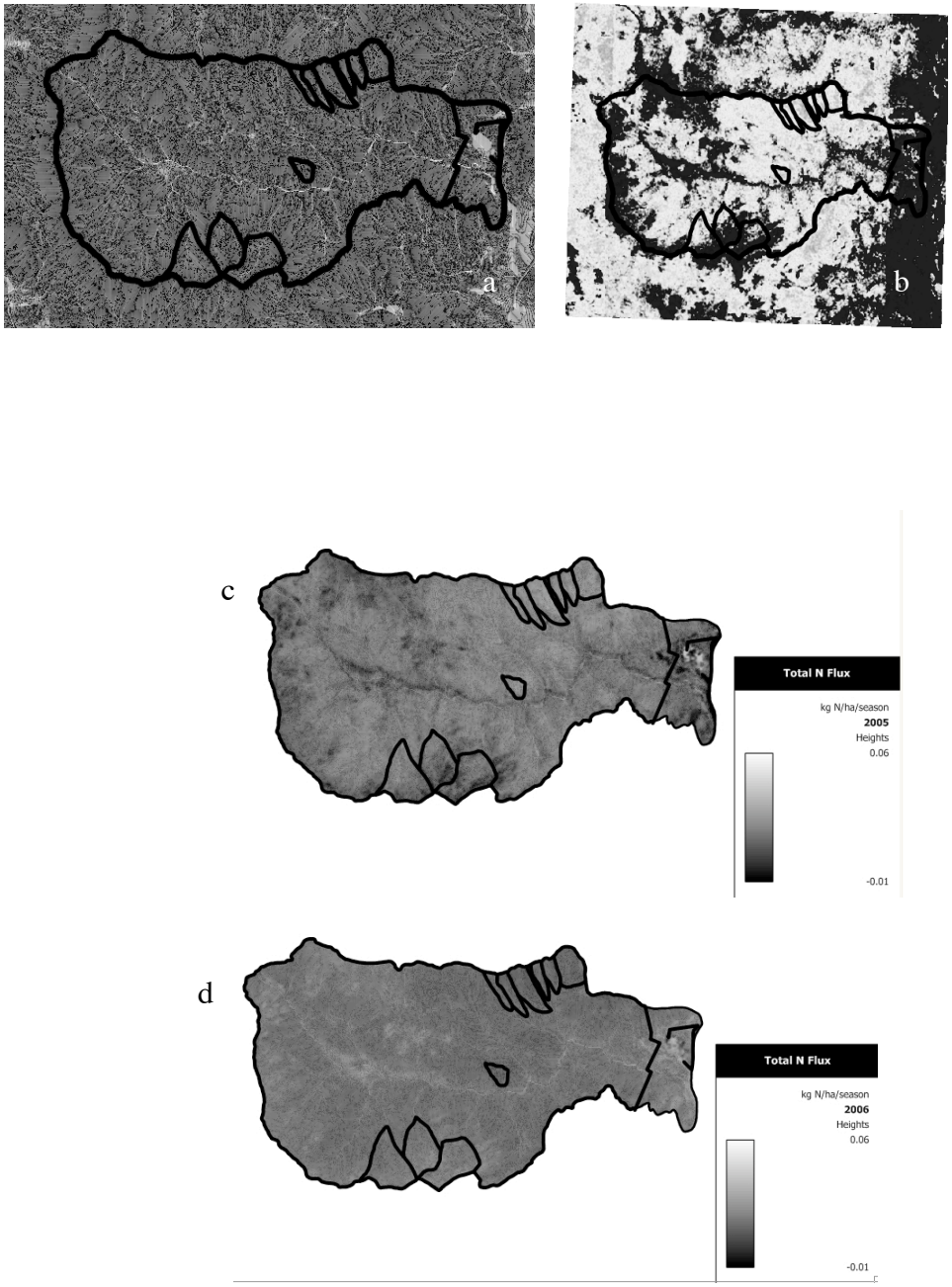


Figure 4-4. Percent foliar N (a), topographic index (b), 2005 N gas flux (c) and 2006 N gas flux (d) for the Hubbard Brook valley. N fluxes (3 cm rain threshold) were estimated from multiple regression models with foliar N and topographic index as independent variables.

4.5 DISCUSSION

4.5.1 Patterns in N Gas Fluxes

Nitrogen gas fluxes in the White Mountain northern hardwood forests at Hubbard Brook, Lafayette Brook, and Mount Bickford appear to be substantial components of the nitrogen cycle, more so than would have been expected in light of some previous measurements (Bowden 1986, Davidson et al. 1990) (Table 4-1). The most conservative estimates – using a 3 cm rain threshold for extrapolating rates across a season – result in N losses via N_2 and N_2O that account for about half of N deposition to the area, which is approximately 6-8 kg N ha^{-1} y^{-1} (Likens and Bormann 1995) (Table 4-2). Previous studies of N gas fluxes in the region have focused on N_2O fluxes, which have been low (Bowden 1986, Bowden et al. 1990, Venterea et al. 2004). Here, N_2:N_2O ratios in total fluxes ranged from the 10s to the 1000s and N_2 fluxes dominated total fluxes at all sites and sampling dates (Table 4-3). The historical lack of data on N_2 fluxes has clearly led us to underestimate the importance of denitrification in nitrogen budgets of this region. Furthermore, the fact that N_2:N_2O ratios are so high indicates these areas could be important sinks for reactive N in the region, turning potentially polluting oxidized N into inert N_2 (Galloway et al. 2003).

Although data on N_2 fluxes and N_2:N_2O ratios are lacking for the northeastern US, studies in other regions have found results similar to mine. Dannenmann et al. (2008) measured N gas fluxes in forests in southern Germany ranging from 161-1071 μg N m^{-2} h^{-1} (or 0.039-0.25 kg N ha^{-1} d^{-1}) and N_2O fluxes ranging from 2-57 μg N m^{-2} h^{-1} (or 0.0005-0.014 kg N ha^{-1} d^{-1}). The resulting N_2:N_2O ratios they calculated varied from 21 to 220, a range similar to the data presented here. Butterbach-Bahl et al. (2002) measured N_2 flux rates of up to 7 kg N ha^{-1} y^{-1} at a spruce site and 12 kg N ha^{-1} y^{-1} in another German forest with high N deposition rates (annual wet deposition is ~20 kg

N ha^{-1} y^{-1}). Their N_2:N_2O ratios ranged from 0.2-100 and averaged ~2. Closer to my sites, Bowden et al. (1993) measured N_2O fluxes of about 0.23 kg N ha^{-1} y^{-1} at Harvard Forest in Massachussetts. In an earlier study at the same site, Bowden et al. (1991) measured N_2O fluxes of 0.041 – 0.26 g N ha^{-1} d^{-1}. Groffman et al. (2006b) found that N_2O fluxes at HBEF remained well below 1 ng N cm^{-2} h^{-1} (0.002 kg N ha^{-1} d^{-1}) for most of the year. All these studies suggest that while N_2O fluxes are low, N_2 fluxes are significant, and that N_2:N_2O ratios are high for temperate hardwood and conifer forests.

4.5.2 Controlling factors at Hubbard Brook

Analysis of relationships between gas fluxes and ancillary variables from two years of data at HBEF suggests that these fluxes are tightly linked to patterns of ecosystem N cycling, albeit with some complexities. Data from HBEF in 2005 showed significant correlations between N_2 fluxes and several indices of ecosystem N cycling, e.g., soil NO_3^- levels, foliar N and soil C:N ratio. Many of these relationships were weak however, likely because the small number of plots sampled detracted from the power of the mixed model analysis. Correlations between N_2O fluxes and explanatory variables were also plentiful and mostly in the expected direction. Furthermore, the relationship between net nitrification and N_2O flux was robust enough to withstand mixed model analysis (Table 4-4).

Since nitrification is a source of NO_3^-, a substrate for denitrification, as well a direct source of N_2O, one might expect it to be the strongest predictor of N_2O fluxes. Relationships between nitrification and FN, which can be remotely sensed may be a useful tool for extrapolating measured N gas flux rates to larger areas. Ollinger et al. (2002) found a strong relationship between nitrification and foliar N in White

Mountain forests and have used remotely sensed FN to drive models of forest ecosystem processes (Ollinger and Smith 2005, Ollinger et al. 2008). Further development of these models to include N gas fluxes would be useful for landscape and regional scale analyses of these fluxes.

There is great interest in understanding the factors controlling N_2:N_2O ratios and using this understanding to develop approaches for estimating and scaling total N gas fluxes. Many explanatory variables correlated well with this ratio and four emerged as significant even under rigorous mixed model analysis for 2005 HBEF data: net nitrification, elevation, conifer basal area and red maple basal area. Interestingly, the relationships were (respectively) negative, negative, positive, and positive. The negative relationship between nitrification and N_2:N_2O could arise as a result of N_2O production associated with nitrification or because higher nitrate availability (as a result of nitrification) allows for preferential production of the earlier products in the denitrification sequence, i.e. N_2O over N_2 (Blackmer and Bremner 1978). This type of link is consistent with the conclusions of Dannenmann (2008) that the high N_2:N_2O ratios they observed at Tuttlingen Research Station as compared with those observed by Butterbach-Bahl et al. (2002) at Höglwald Forest arose from the dramatic differences in the N richness (arising from deposition) at the two German sites.

Two other explanatory variables also behaved as expected at HBEF in 2005. High elevation is associated with spruce-fir dominated vegetation, which is associated with acidic soils, and fewer hardwoods (such as red maple), which are associated with less acidic soils. Low pH has been found to decrease N_2:N_2O ratios arising from denitrification, perhaps by inhibiting the activity of nitrous oxide reductase (Knowles 1982), so this ratio's negative relationship with elevation and positive relationship

with red maple indicate that pH may play a role in producing these patterns (Parkin et al. 1985, Focht 1974). In contrast, I found conifer basal area to be positively related to N_2:N_2O, which would negate the idea that low pH leads to lower N_2:N_2O ratios here. The relationships between pH and N fluxes, and, especially, vegetation type and N fluxes, are not thoroughly understood, however. Butterbach-Bahl et al. (2002) also found that N_2:N_2O ratios were significantly higher at their spruce site than at their hardwood (beech) site. All three of these studies (Butterbach-Bahl et al. 2002, Dannenman et al. 2008 and the present study) used direct flux methods of measuring N_2O so conclusions about N_2:N_2O could be confounded by the fact that these methods measured N_2O production from both nitrification and denitrification. Further studies on the mechanisms of N_2O production in soils of differing pH and under different vegetation types may shed light on the complexities of these relationships.

While the N_2:N_2O ratios I present here are indicative of warm season dynamics, patterns and relationships could change when cold season N fluxes are considered. Groffman et al. (2006b) have shown N_2O fluxes continuing through the winter at HBEF. I do not know how much denitrification proceeds to N_2 -- or even N_2O since the N_2O fluxes measured by Groffman et al. (2006b) may well be a result of nitrification – during these months. If denitrification to N_2 is negligible in the cold season, then annual N_2:N_2O ratios may be lower than what I report here. However, I have no reason to expect that N_2:N_2O ratios are low in winter. Moreover, because N_2O fluxes are relatively low in the winter and spring (0.0016 – 0.0038 kg N ha^{-1} d^{-1}), annual ratios are likely to remain well above 1.

There were marked differences between data collected in 2005 and 2006 at HBEF. It is important to note that except for plots 338 and 265, different plots were sampled in

different years (Table 4-1). Therefore, differences between 2005 and 2006 data were due both to true annual variation as well as to differences among the plots sampled. Plot #338 exhibited N_2 fluxes that were 30% lower in 2006 than in 2005 (Table 1), but annual N_2O fluxes at this site were exactly the same for both years. Plot 265 had very similar fluxes in both years. These data suggest that most of the differences between 2005 and 2006 were due to spatial, rather than true temporal variation.

The most striking difference between the 2005 and 2006 data from HBEF was the lack of relationships between explanatory variables and N fluxes in 2006 (Table 4). The only significant correlation and mixed model relationship was between N_2O flux and soil NH_4^+ levels. While it's not very surprising that ammonium concentrations should be related to N_2O fluxes, especially if nitrification is the source of most of the N_2O, it is surprising that this was the *only* relationship to emerge. Annual variations in N flux are to be expected with varying conditions across years; in fact, Groffman et al. (2001, 2009) found similarly marked, and unexplained inter-annual variability in *in situ* net N mineralization and nitrification at HBEF. These variations in fluxes and controls are even more likely considering the different sets of plots used in the two years. Such vast differences in the relationships (or lack thereof) between N fluxes and explanatory variables complicate the development of robust models or scaling algorithms.

4.5.3 Controlling factors across the White Mountain Region

In addition to within-site variability among years at HBEF, I observed cross-site differences in N flux-explanatory variable relationships within one year (Table 4-4). Although I didn't have nearly as many potential explanatory variables for the LF and MB sites as I did for HBEF, some relationships did emerge. At LF, N_2 fluxes were

positively correlated with moisture (but not significant in the mixed model analysis), and N_2:N_2O ratios were negatively associated with C:N ratios in both correlations and mixed model analysis. The latter relationship is surprising since one might expect that higher N availability (as indicated by low C:N) would favor the earlier products of denitrification (Blackmer and Bremner 1978). The connections between C:N, N richness, NO_3^- availability and N_2:N_2O ratios are complex however, and not thoroughly understood (Lovett et al. 2002).

At MB in 2006, several correlations between N fluxes and explanatory variables were found and all relationships were in the expected direction (Table 4). Of these, however, only two emerged as significant in mixed model analysis: net nitrification vs. N_2O flux and C:N vs. N_2:N_2O. The relationship between nitrification and N_2O flux was positive, but not as steep as that in the 2005 HBEF data. This difference is not surprising given that N_2O fluxes were considerably lower at MB in 2006 than at HBEF in 2005 (Table 2). The relationship between C:N and N_2:N_2O ratios here was both opposite in sign and much larger in magnitude than that found at LF (Table 4-4).

The environmental history of the sites may be the cause of the different relationships between N gas fluxes and C:N ratios. LF is an old growth forest while MB was burned in the last century. C:N ratios were higher at MB than LF, indicating that the younger, burned forest is more nitrogen limited than the older forest. However, how these differing environmental histories and N limitations could produce opposing relationships between C:N and N_2:N_2O is unclear (Table 4-3).

Cross site relationships between C:N and N_2:N_2O followed the expected trend, although it was not a significant one. HBEF, a logged forest of similar age to MB, had

intermediate C:N ratios and intermediate N_2:N_2O ratios as compared to the high C:N ratios and high N_2:N_2O of MB and low C:N ratios and low N_2:N_2O of LF. Goodale and Aber (2001) compared old growth, burned, and logged forests (the latter two being similar in age to my sites) and found a similar relationship between site type and C:N ratios, as well as a negative relationship between forest floor C:N ratio and nitrification rate and a positive relationship between nitrification and stream water N loss. It is logical that gaseous N losses follow a similar pattern as stream water N loss, increasing with increasing nitrification and decreasing with increasing C:N.

Total gaseous N losses were lower at LF than at the two younger forests (HBEF and MB), contrary to Vitousek and Reiners' (1975) hypothesis that as forests mature past a certain point, they are more prone to "leak" limiting nutrients like nitrogen. Like Goodale and Aber (2001), their focus was on nutrient export in stream water, but they mention that gaseous loss via denitrification is another possible mechanism for N loss from ecosystems. Stream water export has long been presumed to be the primary mode of nitrogen loss from northeastern forests, however even my most conservative estimates of total N gas fluxes are 1.5 - 2 times higher than the highest stream water N losses (approximately 2 kg N ha^{-1} y^{-1}) recorded by Goodale and Aber (2001). Interestingly, the Goodale and Aber site with the greatest stream water N losses was LF; MB had the lowest stream water N losses (approximately 0.1 kg N ha^{-1} y^{-1}) of all of their sites (HBEF was not included in their study). These results suggest that 1) denitrifying microbes may compete with hydrological export processes for nitrate at these sites and 2) denitrification may be serving as a relatively large sink for N, both in terms of eliminating a large proportion of available N from a forest and in the larger context of converting reactive NO_3^--N to inert N_2.

Interestingly, stream water N export has decreased in the WMNF region over the last several decades (Martin et al. 2000, Goodale et al. 2003. Therefore, my N gas flux measurements from 2005-2006 (present study) may not be directly comparable to stream water N losses measured in 1996-1997 (Goodale and Aber 2001). That said, decreases in stream water N losses over this time period would only amplify the divergence between the hydrologic and gaseous N losses discussed above. Interactions between hydrology and N richness then become more important in controlling the mechanisms and patterns of ecosystem N loss, i.e. stream water and gaseous exports may respond differently to variation in ecosystem N richness such that at N-rich sites, hydrologic processes compete better with denitrification for available nitrate than at N-poor sites.

We found no relationship between soil moisture and N gas fluxes, however, the spatial and temporal resolution, as well as the limited scope of my sampling scheme restricted my ability to adequately address these relationships. In these landscapes, hot spots for denitrification probably occurred on the order of hectares (e.g. a "Beaver Pond" wetland located in the middle of the HBEF valley) to microns (e.g. in soil aggregates). My experimental design did not include any plots that were saturated at any point, nor were my measurement methods precise enough to evaluate variations in flux at the soil aggregate level. Furthermore, logistics prevented us from planning sampling events around hydrologic events (e.g. rain events, flooding, drought) with high temporal resolution.

4.5.4 Spatially Explicit Extrapolations

I scaled results from my sampling plots to the entire HBEF valley using regressions of total annual N flux (with 3 cm rain threshold) as predicted by topographic index and

foliar N (Figure 4-4). I performed these extrapolations separately based on 2005 and 2006 data. The 2005 data showed increasing N gas fluxes with increasing TI and FN as expected and, when one possible outlier point was excluded, resulted in a strong relationship. The 2006 data showed a surprising negative relationship between N gas fluxes and FN. The 2006 pattern is difficult to explain in the context of FN-nitrification-denitrification associations, but consistent with the idea that at N-rich sites, hydrologic processes compete better with denitrification for available nitrate than at N-poor sites. As such, the two resulting maps of denitrification rates offer alternate hypotheses about patterns in denitrification rates across the HBEF valley that could be tested in future work: whereas the 2005 map shows the lowest rates occurring in the deep valleys and high elevation conifer forest, the 2006 map shows the highest rates occurring in these areas. Spatially explicit extrapolations such as these could be very useful in advancing our understanding of the roles of different parts of the landscape as potential sinks for reactive or available N.

4.5.5 Conclusions

Quantifying N gas fluxes and the mechanistic relationships that produce spatial and temporal patterns in these fluxes is a difficult task. However, this study shows that task is important in northeastern forests for several reasons: 1) total N gas fluxes are most likely much higher than previously surmised from old estimates of N_2O fluxes, 2) N_2:N_2O ratios are consistently very high in these forest systems, making them a sink for reactive N and potentially important nitrogen pollution control "filters" in this region, and 3) patterns in N gas fluxes and their relationships with explanatory factors vary highly from site to site and year to year, making higher resolution and broader scale evaluations of these fluxes and patterns more critical.

I observed potentially promising relationships between remote sensing and GIS-based estimates of N richness and soil wetness that could be powerful tools for extrapolation and modeling of measured denitrification rates. Variability was very high spatially among plots within sites and temporally within and among seasons, however, so the data lacked the spatial and temporal resolution required to extrapolate accurately to seasonal time periods over the study landscapes and region. Future studies of N gas fluxes should, therefore, be sure to include steeper and broader gradients in factors such as moisture and N richness as well as higher resolution data to the extent that resources allow.

Capturing the dynamics of spatial "hot spots" and temporal "hot moments" of denitrification is an especially challenging but important hurdle in scaling N gas fluxes (Groffman et al. 2009b, Tague 2009). In the absence of a method to measure N gas fluxes directly at very broad spatial and temporal scales, modeling is required to extrapolate finer scale measurements to the broader context. Modeling, in turn, requires knowledge of the mechanisms underlying gaseous N loss, or at least of relationships with predictor variables for which appropriately scaled information is available (Kulkarni et al. 2008).

I conclude that WMNF forests appear to be providing the ecosystem service of mitigating the harmful effects of Nr inputs to the region by denitrifying it to N_2. The exact magnitude of this service is variable (in time and space) and uncertain. New developments in measurement and extrapolation techniques show promise for reducing uncertainties, but with current technology an immense effort would be required to collect sufficient data for truly robust assessments at broad spatial and temporal scales.

WORKS CITED

Allison FE. 1955. The enigma of soil nitrogen balance sheets. Advances in Agronomy 7: 213-250.

Ambroise B, Beven KJ, and Freer J. 1996. Toward a generalization of the TOPMODEL concepts: topographic indices of hydrological similarity. Water Resources Research 32(7): 2135-2145.

Beven KJ and Kirkby MJ. 1997. A physically-based variable contributing area model of basin hydrology. Hydrological Sciences Bulletin 24: 4369-4382.

Bowden RD, Steudler P, Melillo J, and Aber J. 1990. Annual nitrous oxide fluxes from temperate forest soils in the northeastern United States. Journal of Geophysical Research – Atmospheres 95(D9): 13997-14005.

Bowden RD, Melillo J, Steudler P, and Aber J. 1991. Effects of nitrogen additions on annual nitrous oxide fluxes from temperate forest soils in the northeastern United States. Journal of Geophysical Research – Atmospheres 96(D5): 9321-9328.

Bowden RD, Castro MS, Melillo JM, Steudler PA, Aber JD. 1993. Fluxes of greenhouse gases between soils and the atmosphere in a temperate forest following a simulated hurricane blowdown. Biogeochemistry 21: 61-71.

Bowden RD, Rullo G, Stevens GR, and Steudler PA. 2000. Soil fluxes of carbon dioxide, nitrous oxide, and methane at a productive temperate deciduous forest. Journal of Environmental Quality 29: 268-276.

Bowden WB. 1986. Gaseous nitrogen emissions from undisturbed terrestrial ecosystems: an assessment of their impact on local and global nitrogen budgets. Biogeochemistry 2: 249-279.

Butterbach-Bahl K, Willibald G, and Papen H. 2002. Soil core method for direct simulataneous determination of N2 and N2O emissions from forest soils. Plant and Soil 240(1): 105-116.

Castro MS, Driscoll CT, Jordan TE, Reay WG, Boynton WR, Seitzinger SP, Styles RV, and Cable JE. 2000. Contribution of atmospheric deposition to the total nitrogen loads to thirty-four estuaries on the Atlantic and Gulf coasts of the United States. In: Valigura RM, Castro MS, Greening H, Meyers T, Paerl H, Turner RE, (Eds.), An Assessment of Nitrogen Loads to United States Estuaries with an Atmospheric Perspective. American Geophysical Union, Washington, D.C. 77-106

Dannenmann M, Butterbach-Bahl K, Gasche R, Willibald, and Papen H. 2008. Dinitrogen emissions and the N_2:N_2O emission ratio of a Rendzic Leptosol as influenced by pH and forest thinning. Soil Biology & Biochemistry 40: 2317–2323.

Davidson EA, Myrold DD, and Groffman PM. 1990. Denitrification in temperate

forest ecosystems. In: Gessel SP, Lacate DS, Weetman GF, and Powers RF, (Eds.), Proceedings of the Seventh North American Forest Soils Conference. University of British Columbia, Faculty of Forestry Vancouver. 196-220.

Dittman JA, Driscoll CT, Groffman PM, and Fahey TM. 2007. Dynamics of nitrogen and dissolved organic carbon at the Hubbard Brook Experimental Forest. Ecology 88: 1153-1166.

Driscoll CT, Whitall D, Aber J, Boyer E, Castro M, Cronan C, Goodale CL, Groffman PM, Hopkinson C, Lambert K, Lawrence G, and Ollinger S. 2003. Nitrogen pollution in the northeastern United States: Sources, effects, and management options. Bioscience 53: 357-374.

Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, and Cosby BJ. 2003. The nitrogen cascade. BioScience 53: 341–56.

Goodale CL and Aber JD. 2001. The long term effects of land-use history on nitrogen cycling in northern hardwood forests. Ecological Applications 11(1): 253-267.

Goodale CL, Lajtha K, Nadelhoffer KJ, Boyer EW, and Jaworski NA. 2002. Forest nitrogen sinks in large eastern U.S. watersheds: estimates from forest inventory and an ecosystem model. Biogeochemistry 57/58: 239-266.

Goodale CL, Aber JD, and Vitousek PM. 2003. An unexpected nitrate decline in New Hampshire streams. Ecosystems 6: 75-86.

Groffman PM, Davidson EA, and Seitzinger S. 2009a. New Approaches to modeling denitrification. Biogeochemistry 93(1-2): 1-5

Groffman PM, Butterbach-Bahl K, Fulweiler RW, Gold AJ, Morse JL, Stander EK, Tague C, Tonitto C, Vidon P. 2009b. Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. Biogeochemistry 93(1-2): 49-77.

Groffman PM, Altabet MA, Bohlke JK, Butterbach-Bahl K, David MB, Firestone MK,Giblin AE, Kana TM, Nielsen LP, and Voytek MA. 2006a. Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecological Applications 16(6): 2091-2122.

Groffman PM, Fisk MC, Driscoll CT, Likens GE, Fahey TJ, Eagar C, and Pardo LH. 2006b. Calcium additions and microbial nitrogen processes in a northern hardwood forest. Ecosystems 9: 1289-1305.

Groffman PM, Tiedje JM, Robertson GP, and Christensen S. 1988. Denitrification at different temporal and geographic scales: proximal and distal controls. In: Wilson JR, (Ed.), Advances in N Cycling in Agricultural Ecosystems. Communications in. Agricultural Burning. International, Wallingford, U.K. 174-192.

Groffman PM and Tiedje JM. 1989. Denitrification in north temperate forest soils: Relationships between denitrification and environmental factors at the landscape scale. Soil Biology & Biochemistry 21: 621-626.

Groffman PM, Hardy JT, Fisk MC, Fahey JT, and Driscoll CT. 2009. Climate variation and soil carbon and nitrogen cycle processes in a northern hardwood forest. Ecosystems 12: 927-943.

Houlton BZ, Sigman DM, and Hedin LO. 2006. Isotopic evidence for large gaseous nitrogen losses from tropical rainforests. Proceedings of the National Academy of Sciences 103: 8745-8750.

Keller M, Goreau TJ, Wofsy SC, Kaplan WA, and McElroy MB. 1983. Production of nitrous oxide and consumption of methane by forest soils. Geophysical Research Letters 10: 1156-1159.

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

Likens GE and Bormann FH. 1995. Biogeochemistry of a Forested Ecosystem. Second Edition, Springer-Verlag New York Inc. 159 pp.

Lovett GM, Weathers KC, and Arthur MA. 2002. Control of nitrogen loss from forested watersheds by soil carbon:nitrogen ratio and tree species composition. Ecosystems 5: 712-718

Martin CW, Driscoll CT, and Fahey TJ. 2000. Changes in streamwater chemistry after 20 years from forested watersheds in New Hampshire, USA. Canadian Journal of Forest Research 30: 1206-1213.

McInnes KJ, Weaver RW, and Savage MJ. 1994. Soil water potential. In: Weaver RW, (Ed.), Methods of soil analysis, Part 2 - Microbiological and biochemical properties. Soil Science Society of America, Madison, WI. 53-58.

Miller JR, Turner MG, Smithwick EAH, Dent CL, and Stanley EH. 2004. Spatial extrapolation: the science of predicting ecological patterns and processes. Bioscience 54: 310-320.

Nelson DW and Sommers LE. 1996. Total carbon, organic carbon, and organic matter. In: Sparks DL, (Ed.), Methods of soil analysis, Part 3 - Chemical methods. Soil Science Society of America, Madison, WI. 961-1010.

Ollinger SV and Smith ML. 2005. Net primary production and canopy nitrogen in a temperate forest landscape: An analysis using imaging spectroscopy, modeling and field data. Ecosystems 8: 760-778.

Ollinger SV, Richardson AD, Martin ME, Hollinger DY, Frolking SE, Reich PB, Plourde LC, Katul GG, Munger JW, Oren R, Smith ML, U KTP, Bolstad PV, Cook BD, Day MC, Martin TA, Monson RK, and Schmid HP. 2008. Canopy nitrogen, carbon assimilation, and albedo in temperate and boreal forest relations and potential climate feedbacks. Proceedings of the National Academy of Sciences 105(49): 19336-19341.

Ollinger SV, Smith ML, Martin ME, Hallett RA, Goodale CL, and Aber JD. 2002. Regional variation in foliar chemistry and N cycling among forests of diverse history and composition. Ecology 8833: 339–55.

Parkin TB. 1987. Soil microsites as a source of denitrification variability. Soil Science Society of America Journal 51: 1194–1199.

Pattey E, Strachan IB, Desjardins RL, Edwards GC, Dow D, and MacPherson JI. 2006. Application of a tunable diode laser to the measurement of CH_4 and N_2O fluxes from field to landscape scale using several micrometeorological techniques. Agricultural and Forest Meteorology 136(3-4):222-236.

Paerl HW and Steppe TF. 2003. Scaling up: the next challenge in environmental microbiology. Environmental Microbiology 55: 1025–38.

Prather M, Derwent R, Ehhalt D, Fraser PJ, Sanhueza E and Zhou X. 1995. Other trace gases and atmospheric chemistry. In: Houghton JT, Meiro Filho LG, Callander BA, Harris N, Kattenburg A and Maskell K, (Eds.), Climate Change 1994: Radiative Forcing of Climate Change and an Evaluation of the IPCC IS92 Emission Scenarios. Cambridge University Press, New York. 73-126.

Robertson GP. 1989. Nitrification and denitrification in humid tropical ecosystems. In: Proctor J, (Ed.), Mineral Nutrients in Tropical Forest and Savanna Ecosystems. Blackwell Scientific, Cambridge, Massachusetts, USA. 55-70.

Robertson GP, Wedin D, Groffman PM, Blair JA, Holland EA, Nadelhoffer KA, and Harris D. 1999. Soil carbon and nitrogen availability: Nitrogen mineralization, nitrification and carbon turnover. In: Robertson GP, Bledsoe CS, Coleman DC, and Sollins P, (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York. 258-271.

Scholefield D, Hawkins JMB, and Jackson SM. 1997. Use of a flowing helium atmosphere incubation technique to measure the effects of denitrification controls applied to intact cores of a clay soil. Soil Biology and Biochemistry 29: 1337–1344.

Schlesinger WH. 2009. On the fate of anthropogenic nitrogen. Proceedings of the National Academy of Sciences 106(1): 203-208.

Schwarz PA, Fahey TJ, and McCullough CE. 2003. Factors controlling spatial variation of tree species abundance in a forested landscape. Ecology 84(7): 1862-1878.

Sperduto DD and Engstrom B. 1993. An ecological inventory of the White Mountain National Forest, New Hampshire: second year interim report. NH Natural Heritage Inventory, Department of Resources and Economic Development, Concord, New Hampshire, USA.

van Breemen N, Boyer EW, Goodale CL, Jaworski NA, Paustian K, Seitzinger SP, Lajtha K, Mayer B, van Dam D, Howarth RW, Nadelhoffer KJ, Eve M, and Billen G. 2002. Where did all the nitrogen go?: fate of nitrogen inputs to large watersheds in the northeastern USA. Biogeochemistry 57: 267-293.

Venterea RT, Groffman PM, Verchot LV, Magill AH, Aber JD, and PA Steudler PA. 2003a. Nitrogen oxide gas emissions from temperate forest soils receiving long-term nitrogen inputs. Global Change Biology 9: 346-357.

Venterea RT, Lovett GM, Groffman PM, and Schwarz PA. 2003b. Landscape patterns of net nitrification in a northern hardwood-conifer forest. Soil Science Society of America Journal 67: 527-539.

Venterea RT, Groffman PA, Castro MS, Verchot LV, Fernandez IJ and Adams MB. 2004. Soil emissions of nitric oxide in two forest watersheds subjected to elevated N inputs. Forest Ecology and Management 196: 335-349.

Wallenstein MD, Myrold DD, Firestone M and Voytek M. 2006. Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. Ecological Applications 16: 2143-2152.

Wang J, Endreny TA, and Hassett JM. 2005. A flexible modeling package for topographically based watershed hydrology. Journal of Hydrology 314:78-91.

Whelan MJ and Gandolfi C. 2002. Modeling of spatial controls on denitrification at the landscape scale. Hydrological Processes 16: 1437-1450.

CHAPTER 5

IN SOIL NITROGEN BALANCE SHEETS, 2 + 2 = 5

5.1 THE MISSING NITROGEN

Drastic changes in various element cycles have occurred since the industrial revolution. The carbon cycle gets a lot of attention, but changes in the nitrogen (N) cycle have also emerged as important contributors to the enrichment and detriment of humans and the planet. Through the promotion of artificial and biological nitrogen fixation – i.e., the conversion of inert dinitrogen (N_2) to other forms – and increased combustion of fossil fuels, humans have more than doubled the amount fixed, or "reactive" N (Nr) on earth. Once N is fixed into Nr, it can move through ecosystems, perhaps fertilizing agricultural crops or trees, perhaps warming the atmosphere or giving rise to aquatic blooms of toxic algae. This continuous transformation and transfer of N through ecosystems, with various effects along the way is called the N cascade (Galloway et al. 2003). There is, however, a long-standing history of mystery surrounding the fate of Nr once it disappears from the N cascade.

Scientists often construct input-output budgets of nutrients to assess the importance of various processes in adding, subtracting, and retaining those nutrients in a system. Nitrogen budgets, it seems, almost always come out imbalanced, with missing N from the output side (Kulkarni et al. 2008/Chapter 1). This problem has afflicted N budgets of systems around the world on both large and small scales, and it has done so for decades (Allison 1955, Campbell et al. 2004, van Breeman et al. 2002, Howarth et al. 2002, van Egmond et al. 2002, Zheng et al. 2002). In 1955, F. E. Allison wrote a paper titled *The enigma of soil nitrogen balance sheets* and the issues he described

plague researchers to this day. Somehow, nitrogen is disappearing from these systems undetected. Scientists usually attribute this missing N to gaseous loss, which can be difficult to measure (Groffman et al. 2006a).

Gaseous loss in the form of N_2 is particularly hard to measure against background levels since N_2 comprises 78% of our atmosphere. Loss in the Nr forms of nitric oxide (NO) and nitrous oxide (N_2O) is easier to measure and somewhat better understood than loss as N_2. Interest in these forms has traditionally been great because both are air pollutants. Nitric oxide is a precursor to ozone in the lower atmosphere, which makes it a serious concern since ozone here is implicated in human health issues, atmospheric warming and crop damage (Galloway et al. 2003, Delucchi et al. 1996). N_2O is a greenhouse gas responsible for about 6% of anthropogenic warming and a reactant in the destruction of the stratospheric ozone layer (Davidson 2009).

Yet in many areas, especially the forests of the northeastern USA, measurements of nitrogen gases account for only a small fraction of the N missing from nitrogen budgets (Keller et al. 1983, Bowden and Bormann 1986, Bowden et al. 1991, Groffman et al. 2006b, Venterea et al. 2003). This leaves scientists with a persistent gap in their balance sheets, which tells them, in essence, that 2+2=5. Measured gas losses do not add up to all of the missing N, even when all other types of losses (e.g., export in streams, removal of crops, etc.) and storages are well accounted for. A recent series of studies comprising the Ph.D. dissertation of the author, however, sheds some light on how these gaps might be closed in the forested Northeast of the USA. The results of these studies are synthesized here in the final chapter of the dissertation. The bottom line is that perhaps 2+2=5 after all.

5.2 DENITRIFICATION: SOLVING THE NITROGEN PROBLEM?

In forested systems, gaseous N is primarily produced by two soil processes: nitrification and denitrification. Nitrification can produce NO and N_2O while denitrification can produce NO, N_2O and N_2. Denitrification is, thus, of particular interest as the primary mechanism by which Nr is converted to inert N_2. It is a microbial process by which aqueous N in the forms of nitrate (NO_3^-) and nitrite (NO_2^-) is converted to gaseous forms in the reduction sequence: NO_3^- è NO_2^- è NO è N_2O è N_2. Abiotic denitrification (or "chemodenitrification") also can produce these gases (Paul and Clark 1996).

Most studies of denitrification in forests to date have been limited by problematic methods for measuring gas fluxes and extrapolating them to scales relevant to nitrogen budgets and pollution problems. In the first chapter of the of this dissertation, I argue that recent and continuing advances in techniques for measuring and extrapolating N gas losses should help improve broad scale estimates of denitrification in coming years (Kulkarni et al. 2008/Chapter 1).

The standard method for measuring denitrification for many years was acetylene inhibition, which blocks the reduction of N_2O to N_2 and therefore allow measurement of total N gas losses as buildup of N_2O. (NO fluxes were largely ignored.) But this method has been falling out of favor with scientists interested in the fate of Nr, especially those working with forest soils, where acetylene blocks a major source of the oxidized N required for denitrification, nitrification, and therefore also blocks any denitrification coupled to nitrification. Because of this shortcoming in the predominant method for measuring denitrification in forest soils for many years, many previous studies of N cycling in forests likely underestimate total denitrification and

give no indication of the ratio of N_2:N_2O in these losses. Laboratory methods that can detect N_2 emissions – either by eliminating background N_2, or labeling the emitted N_2 with the stable isotope, ^{15}N – have been gaining ground on acetylene inhibition. These are the gas-flow soil-core and ^{15}N tracer techniques, which promise to help clarify the true rates and products of denitrification in forest soils (Groffman et al. 2006a).

Gas-flow soil-core and ^{15}N tracer methods can only be applied at the scale of centimeters to meters. To get at issues of nitrogen pollution, we must know about denitrification rates at much broader scales. Simply multiplying fine scale measurements by the amount of time and space in question, however, is likely to result in exceedingly erroneous estimates because denitrification rates are highly heterogeneous in time and space. This heterogeneity results from variation in the controls on denitrification: moisture, oxidized nitrogen source, organic carbon source, temperature, and pH. (Note that these controls operate on both biotic and abiotic denitrification, albeit in different ways.) In northeastern forests, moisture levels may for instance, largely determine the variation in denitrification rates across time and space. Moisture, in turn, is affected by topographic position (i.e., hill top vs. valley), soil texture, precipitation patterns, and other factors. Nitrogen richness also probably plays a large role in driving spatial patterns in denitrification rates. Such factors have been shown to interact to create hot spots and hot moments of denitrification, i.e., small areas and brief time periods of high rates that defy extrapolation by multiplication. Mapping patterns in these controlling variables, however, could help find the hot spots and hot moments and therefore allow mapping of patterns in denitrification. But where does information on controlling variables come from?

Remotely-sensed images (from airplane- and satellite-mounted sensors) are increasingly providing scientists with high quality information on water and nitrogen patterns at high spatial, temporal and even spectral resolutions. Hydrologic models have long been employed for describing patterns of water distribution in landscapes. Soil surveys published by the National Resources Conservation Service describe soil types across the entire USA. Most importantly, the burgeoning power of computer hardware and software, e.g., geographic information systems (GIS), allows relatively easy analysis of these large data sets. This last advance is key to developing models that link controls on denitrification with denitrification rates, be they simple regressions or complex process models that track nitrogen atoms through various transformations. Once developed, these models may be applied to more intelligently extrapolate point measurements of denitrification to large areas and long time periods.

The approach of combining newer techniques of measuring denitrification, ones that explicitly quantify N_2 emissions, with newer techniques of extrapolating data holds great promise for illuminating the true role of denitrification in N balance sheets. How might these advances help with the problem of making 2+2 equal 5? The truth is that the mathematical expression "2+2=5" is already correct … for extremely large values of 2.

But it is hard to make sense of it without the proper resolution (i.e., significant digits). New developments in measuring and extrapolating denitrification rates might just be the tools necessary for elucidation of those extra significant digits to help N budgets make sense. Furthermore, if – as current information on NO and N_2O fluxes suggests (Keller et al. 1983, Bowden and Bormann 1986, Bowden et al. 1991, Groffman et al. 2006b, Venterea et al. 2003) – N_2 fluxes dominate N gas fluxes in northeastern forests,

denitrification in these soils may be playing an important role in removing Nr from watersheds and cutting off the cascade of polluting N.

5.3 HOW SHARP ARE THE TOOLS?

Chapter 2 describes tests of the two methods for measuring denitrification rates mentioned above: gas-flow soil-core (a type of direct flux method) and ^{15}N tracer. I tested them on soils from the Hubbard Brook Experimental Forest (HBEF), a very well studied Long Term Ecological Monitoring site of approximately 3000 ha in New Hampshire with numerous sets of ancillary data on potential controlling variables available.

The direct flux (gas-flow soil-core) method is an *in vitro* method that requires removing soil cores from the field and analyzing them in the laboratory. The ^{15}N tracer method is an *in situ* method that allows incubation in the field. I therefore set up gas sampling chambers in the field for the ^{15}N tracer incubations and took soil cores for the direct flux method from areas adjacent to the gas sampling chambers in order to compare results of the two methods.

Both methods measured denitrification rates that were much higher than previously suspected, enough to close the N budget and more. In a recent accounting of inorganic nitrogen inputs and outputs at HBEF, Campbell et al. (2004) estimated a retention rate (i.e., the missing N) of approximately 5-6 kg N ha^{-1} y^{-1} and my estimates of N gas losses were two orders of magnitude higher. Moreover, measurements of N_2 fluxes far exceeded N_2O fluxes, giving N_2:N_2O ratios in the double and triple digits. But the overall rates seemed impossible. How could I be getting 100 kg N ha^{-1} $season^{-1}$ (we measured only spring through fall) out of a system that was only receiving 6-8 kg N

ha^{-1} y^{-1} in inputs (Dittman et al. 2007)? An annual depletion of 90+ kg N ha^{-1} was not likely, so how could I be so wrong?

Here I was presented with the classic problem arising from extrapolation by multiplication. My incubations were hours long over soil surfaces measured in square centimeters, yet I was extrapolating to hectares and seasons simply by multiplying. Clearly I was disregarding heterogeneity in rates over time and space at our own peril. For the purposes of this study, I chose to set aside spatial variability and focus on temporal variability to help me improve my extrapolations.

I learned that I had been incubating our direct flux soil cores at oxygen levels (5%) that were much lower than those predominating in the field (10-17%). Since soil oxygen levels are largely controlled by moisture (higher moisture leads to lower oxygen levels), and my own experiments showed that denitrification was suppressed by increasing oxygen levels (down to nearly zero at 10% oxygen), it followed that my direct flux measurements were reflective only of rates occurring during periods of increased moisture, i.e., during rain events. I therefore extrapolated rates using two scenarios: one that assumed a rain event of at least 2 cm was necessary to depress soil oxygen levels to 5% and another that assumed a rain event of at least 3 cm was necessary. These scenarios isolated hot moments in a season during which N gas fluxes were probably otherwise minimal. Within this construct, N gas losses were extrapolated to 8 and 4 kg N ha^{-1} $season^{-1}$ for the 2 cm and 3 cm thresholds, respectively. Using relatively unsophisticated methods for improving the extrapolations, I achieved ballpark estimates of N gas loss that would fill gaps in N budgets quite nicely.

I used a similar scenario to extrapolate rates as measured using the ^{15}N tracer method. Since I had applied 0.25 cm of water at the beginning of each incubation (to wash in the isotope tracer), I extrapolated the rates only over the number of days experiencing this amount of rain. This extrapolation cut estimates by more than a half, but still left me with an impossible amount of denitrification: ~180 kg N ha^{-1} $season^{-1}$ on average among plots. The problem was in the measurement itself, not just the extrapolation. This technique required knowledge of the enrichment of the soil nitrate pool, the pool I labeled with the isotope tracer. But because of heterogeneity within the soil profile and uncertainty about the depth of labeling, the estimates of enrichment were clearly off. We saw no good way to overcome these issues and therefore found this type of ^{15}N technique inadequate for use in forest soils. (Other types of ^{15}N techniques exist but require isotope applications at fertilization rates much higher than our ~5 atom % tracer level and would artificially elevate rates.)

Although the final estimates are associated with a great deal of uncertainty, they represent considerable progress in knowledge of the role of N gas fluxes in forest nitrogen cycles. Undoubtedly, these fluxes are more important than previous studies limited to NO and N_2O fluxes would lead one to believe. N_2 fluxes were found to dominate over N_2O fluxes in nearly every incubation, across plots, sampling times, and even measurement technique and may well comprise much, if not all, of the missing N in the HBEF N budget. They may well comprise the hidden .4999s in our equation 2+2=5. Two of the tools I explored in this paper allowed me to discern these previously unknown fluxes: the gas-flow soil-core method for measuring fluxes, and the scenarios for extrapolation based on hot moments induced by rain events. As these tools are refined (i.e., measurements and the ability to link measurements to patterns in controlling factors are improved), uncertainty will decrease and the ability to resolve

patterns in N gas emissions and fill in the extra significant digits in N budgets will improve.

5.4 ADD SOME NITROGEN. SEE WHERE IT GOES.

The northeastern USA is a hot spot of atmospheric N deposition, being downwind of many sources, but the fate of this deposition is poorly understood. For this reason, I focused on three areas in the largely forested Northeast for this series of studies: HBEF, a forest logged in the early part of the 20th century, and two sites in the Franconia Notch area of New Hampshire. All three are located within White Mountain National Forest (WMNF). One of the other sites, Mount Bickford, was burned in 1903 and the other, Lafayette, is an old growth forest. I was interested in learning about how these sites respond to N deposition in the short term and how their varying environmental histories might affect their responses.

In Chapter 3, I examined the fate of simulated wet deposition (as $^{15}NO_3^-$) on soils of the three WMNF sites. The focus was on recovery of the applied NO_3^--^{15}N in gaseous forms as N_2-^{15}N and N_2O-^{15}N. It is well known that both wetting events and additions of NO_3^- will stimulate denitrification. But how much N is lost as N_2O, the greenhouse gas, and how much as N_2, is not well known. Moreover, the times scales of the response are not well studied.

I incubated the soils *in situ* in gas sampling chambers for two hours, with the initial gas sample taken approximately 10 minutes after capping. To my surprise, many of the chambers exhibited substantially elevated enrichments (over ambient levels) in this first sampling. I attributed this quick burst of N gas loss to abiotic denitrification, and the slower accumulations over the two-hour incubation to biological processes, which

depend on the synthesis of adequate enzyme stores to take advantage of the applied N. (I expect, however, that there is some overlap in the time scales of these two processes.)

Both abiotic and biotic denitrification produced substantial fluxes of N gases, up to 98% of the applied ^{15}N. On average, 28 and 0.34% of applied ^{15}N in N_2 and N_2O, respectively were recovered, just in abiotic fluxes. Recoveries in biotic fluxes were even higher, averaging 39 and 3.2% for N_2 and N_2O, respectively. Unfortunately, despite making over 500 measurements over two years, the conservative detection limits and widely distributed data set constrained my ability to detect robust spatial and temporal patterns in recoveries. However, these limited observations suggest that denitrification in soils is an important sink for available N in these forests. Moreover, it also appears to be a true sink for *reactive* N, since recoveries of deposited ^{15}N were consistently one to two orders of magnitude higher in inert N_2 than N_2O.

Oddly, the field ambient enrichments were measured deviated from the expected values of ~0 ‰, both for N_2 and especially N_2O. Even in plots heavily fertilized with ^{15}N, published literature values for N_2O enrichments did not exceed ~10 ‰ so I was startled to observe N_2O enrichments in the thousands. Furthermore, I saw consistent temporal patterns across the three sites in the year that all three sites were sampled (2006). These findings have left me perplexed and, along with our ^{15}N recovery findings, suggest the merit of further investigation.

Still, these results clearly point to N gas emissions as an important output in N budgets of northeastern forests. Moreover, this loss is an endpoint in the N cascade, and a quick one. Among the data collected, substantial portions of the simulated wet N

deposition were returned to the atmosphere in gaseous form, mostly as inert N_2 – all within a couple hours of deposition. Thus, denitrification quickly disposed of much the Nr deposited on these soils. The constraints of these data limit the conclusions that can be drawn from them: better measurements are needed for a larger sample size, a larger sample size is required for uncovering spatial and temporal patterns, and knowledge of these patterns is required for extrapolation. Therefore, this analysis does not clarify a great degree of the uncertainty in N budgets, i.e., this work not added many significant digits to the 2+2=5 equation. However, it does point future investigators to denitrification – especially to N_2 – as a mechanism for explaining much of the missing N. Further development of this tool would help scientists better assess turnover of wet N deposition and elucidate finer patterns in N outputs.

5.5 TAKE THE DIRECT APPROACH. GET SOME ANSWERS.

Percent recoveries calculate N gas losses relative to inputs, but I also wanted to learn more about absolute flux rates, which are easier to compare with rates of other N cycle processes. In Chapter 4, I assess and describe N_2 and N_2O fluxes across the three WMNF sites mentioned above (Chapter 3) and evaluate relationships between N gas fluxes and other variables that would allow more accurate extrapolation of rates. To that end, I deployed the direct flux approach (gas-flow soil-core method) tested in Chapter 2, which allowed quantification of absolute fluxes. Using this method, I surveyed N_2 and N_2O fluxes from the HBEF in 2005, and HBEF, LF, and MB in 2006.

The Hubbard Brook watershed is a very well studied forest, with data on environmental variables available from as far back as 1956. One such data set is a digital elevation map, from which I was able to calculate topographic index (TI), a very basic model that uses topographic position and upslope land area to determine

relative moisture levels. Ollinger et al. (2002) developed a map of percent foliar N (FN) across HBEF from remotely sensed imagery. This map is highly useful as a high resolution, spatially explicit data set of relative N richness across the Hubbard Brook valley. Venterea et al. (2003) and Schwarz et al. (2001) conducted valley-wide studies at HBEF of nitrification and tree species distributions, respectively. As part of these studies, they conducted a large battery of measurements relating to soils, vegetation, and nitrogen cycling. Between these data sets and those available as part of the long-term ecological records from the HBEF website (www.hubbardbrook.org), I had access to a very large array of data on factors that potentially affect N gas fluxes. LF and MB are not as well studied, although Goodale and Aber (2001) included them in an examination of nitrogen cycling as related to disturbance history and showed that the old growth forest (LF) had high rates of nitrification and stream water N loss while the burned forest (MB) displayed the opposite pattern.

I observed high N_2 fluxes, low N_2O fluxes, and high N_2:N_2O ratios at all sites, with best estimates of ~3 - 4 kg N ha^{-1} $season^{-1}$ for N_2 and ~0.005 - 0.05 kg N ha^{-1} $season^{-1}$, for N_2O fluxes. Since I believe that the prevailing soil conditions were of higher oxygen concentrations (10 - 17%) than my incubation concentrations (5%), and that the bulk of emissions occurred during episodes of depleted oxygen induced by rain events, I applied a 3 cm rain threshold for temporal extrapolation as described in Chapter 2 to arrive at these best estimates of seasonal fluxes. Among sites, MB exhibited the highest N gas fluxes, LF the lowest, and HBEF produced intermediate fluxes in both years. This gradient ran contrary to the paradigm that the old-growth forests are more "leaky" than younger aggrading forests, i.e., that mature forests experience higher N losses than young forests. However, leakiness could be exhibiting predominately as streamwater export (Goodale and Aber 2001), which, in

turn, could be competing with denitrification for NO_3^- to produce these patterns. The idea that N gas fluxes are controlled by N availability was further supported by the finding that indicators of N richness (e.g., nitrification rates, C:N ratios, and NH_4^+ concentrations) were most strongly related to N gas fluxes.

In order to extrapolate point measurements spatially over the HBEF, I exploited strong relationships between N flux and the two indices of moisture and N richness for which spatially-explicit data were available: TI and FN. Oddly, the relationships between FN and total N gas flux were opposite in direction in 2005 than in 2006, resulting in two very different maps of total seasonal N gas loss across the valley. The deepest valleys and high-elevation coniferous forest exhibited the lowest rates of N gas loss in the 2005 model and the highest rates in the 2006 model. This exercise exposed some of the uncertainty associated with spatial and temporal extrapolation tools: since variability in rates and relationships among variables was high year-to-year, these extrapolation tools are not ready for forecasting N gas losses into the future. Furthermore, since only eight plots were studied in 2005 and six in 2006 at HBEF (a different set of plots each year), the data set of over 200 measurements just at HBEF was not nearly enough to explain variability over two active seasons in a 3000 ha site.

Yet, the results of this study clearly add to the building evidence from Chapters 2 and 3 that denitrification is an important mechanism for N loss from all of these northeastern forests and that N_2 gas losses far outweigh N_2O losses. The direct flux approach I used to measure fluxes allowed spatial and temporal patterns in N gas losses as well as relationships among gas losses and ancillary variables to be discerned. More data points would have helped to elucidate these patterns and relationships more clearly, but the bottom line would most likely remain: a good deal

of Nr inputs to these forests is being lost as inert N_2 and the environmental history of the site affects the rate of turnover.

With this fourth chapter of the dissertation, the question of how 2+2=5 in N budget outputs is at least partially answered. Certainly N_2 fluxes, which had never before been explicitly quantified in WMNF, have been shown by this series of studies to play an important role in filling in gaps in N budgets. These improved measurements and extrapolations of N_2 fluxes have allowed me to elucidate the missing part of our 2+2=5 equation, i.e., the missing N, albeit not with perfect precision. The importance of denitrification to N_2 holds across old-growth, burned, and logged forests in this region. How widely this pattern persists (beyond the WMNF region) remains to be seen. But, as researchers have already found, similarly high N_2 fluxes in European temperate forests (Kreutzer et al. 2009, Dannenman et al. 2008), one might well conjecture widespread loss of Nr to N_2 fluxes in temperate forests, thereby making these forests the end of the line for much of the Nr deposited there.

5.6 2.4999+2.4999=4.9998

More refined modeling approaches are now emerging as solutions to deriving broad scale denitrification estimates. Increasingly sophisticated eco-hydrologic models link water-routing models with nutrient cycles (Groffman et al. 2009); if N gas fluxes were incorporated into such models, they could allow dynamic and spatially-explicit simulation of N gas emissions. But first, more information on N emissions and the relationships among N emissions, water, nitrogen availability, and other factors is necessary to create sub-routines for these models.

Some scientists are working on methods to measure N gas fluxes directly at larger scales with high resolution. Pattey et al. (2006) have combined a tunable diode laser (TDL) for measuring N_2O fluxes with two different micrometeorological platforms (eddy covariance towers and blimps) for field-scale assessments with high temporal resolution. Such broad-scale measurements could help discern both absolute fluxes and relationships with site characteristics, allowing for better scaling to landscape and regional scales. Even these cutting edge techniques, however, could fail to offer a complete picture of the importance of N gases in a budget if N_2 fluxes dominate N_2O fluxes as was found in WMNF. Further developments in measurement and extrapolation techniques, when combined, show promise for extending our knowledge of N gas fluxes in the Northeast as they relate to N budgets and pollution. However, many challenges persist in getting to the point where the gaps in N budgets are completely filled in, i.e., to the point where we can say 2.4999+2.4999=4.9998. With current tools, a massive (perhaps impracticable) effort would be required to collect sufficient data for truly robust assessments at broad spatial and temporal scales.

5.7 WHY BOTHER?

In the end, why does it matter how much N is lost from northeastern forests as gases? Humans are not likely to manage these natural systems to optimize denitrification to N_2. So, although scientists would appreciate being able to tie up N budgets in this region into a neat and complete package, some might argue that it is just an academic exercise. However, I argue that this knowledge is key to understanding and valuing forests. Removing Nr from circulation by converting it to N_2 prevents pollution effects and is, therefore, an important ecosystem service provided by forests to surrounding, downstream, and downwind areas. Since this process takes place in natural forest soils, built up urban areas cannot replicate it. Suburban lawns may or

may not be able to match forests' rates of Nr removal. Furthermore, downstream waters often suffer the effects of excess Nr before they can denitrify it. In light of studies presented in this dissertation, which strongly give evidence for high rates of denitrification to N_2 in forest soils, it can be concluded that developing forested lands to the detriment of their soils is a disservice to humans and the planet in terms of Nr management.

Nr management on the input side of the equation may also be affected by my work. Critical loads establish maximum limits on pollution added to a system, in this case, atmospheric N deposited in WMNF. Knowledge of the system's capacity to process inputs of N deposition is key to setting such limits. Total maximum daily loads (TMDL), a type of critical load tool, are already being effected in the Chesapeake Bay for nitrogen pollution of the estuary (Blankenship 2008), but it appears that none have yet been enacted for N deposition. Maine, however, has used the concept of critical loads for N (as well as sulfur and some other elements) to identify forests sensitive to the acidifying effects of this deposition and resultant cation leaching. According to Macdonald and Miller's (2006) analysis, 36% of Maine's forests are sensitive to increasing acid N and S deposition. Refining this tool with information about the fate of the nitrogen in the deposited nitric acid would allow managers to address the double-edged damage that nitric acid deposition effects on Maine's forests.

5.8 THE BOTTOM LINE

I am excited to report that denitrification appears to be proceeding to its endpoint of N_2 to a degree that removes large portions of potentially polluting N deposited on northeastern forests. Uncertainties in the exact magnitude and variability of this processing remain and merit further investigation. These studies point the way for

future researchers to focus on more intensive and extensive measurements of N gas emissions as well as on developing more refined models that capture the dynamics of moisture and N availability. I also hope that our findings elevate the value of forests in public estimation. In cutting off the N cascade, forests help protect many of earth's resources and human health, In turn, society must assess and preserve their ability to provide this invaluable service.

WORKS CITED

Allison FE. 1955. The enigma of soil nitrogen balance sheets. Advances in Agronomy 7: 213–250.

Blankenship K. 2008. TMDLs are coming, like it or not. Chesapeake Bay Journal, June 2008. (http://www.bayjournal.com/article.cfm?article=3349)

Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, and Cosby BJ. 2003. The nitrogen cascade. BioScience 53: 341–56.

Groffman PM, Altabet MA, Bohlke JK, Butterbach-Bahl K, David MB, Firestone MK,Giblin AE, Kana TM, Nielsen LP, and Voytek MA. 2006a. Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecological Applications 16(6): 2091-2122.

Groffman PM, Fisk MC, Driscoll CT, Likens GE, Fahey TJ, Eagar C, and Pardo LH. 2006b. Calcium additions and microbial nitrogen cycle processes in a northern hardwood forest. Ecosystems 9: 1289-1305.

Goodale CL and Aber JD. 2001. The long term effects of land-use history on nitrogen cycling in northern hardwood forests. Ecological Applications 11(1): 253-267.

Howarth RW, Boyer EW, Pabich WJ, and Galloway JN. 2002. Nitrogen use in the United States from 1961–2000 and potential future trends. Ambio 3311: 88–96.

Kreutzer K, Butterbach-Bahl K, Rennenberg J, and Papen H. 2009. The complete nitrogen cycle of an N-saturated spruce forest ecosystem. Plant Biology 11: 643–649.

Macdonald K and Miller EK. 2006. Assessment of forest sensitivity to nitrogen and sulfur deposition in Maine. Report presented to the Conference of New England Governors and Eastern Canadian Premiers, Forest Mapping Group. (http://www.state.me.us/dep/air/acidrain/ME-Forest-Mapping-Report-2007-01-26.pdf)

Ollinger SV, Smith ML, Martin ME, Hallett RA, Goodale CL, and Aber JD. 2002. Regional variation in foliar chemistry and N cycling among forests of diverse history and composition. Ecology 83: 339-355.

Pattey E, Strachan IB, Desjardins RL, Edwards GC, Dow D, and MacPherson JI. 2006. Application of a tunable diode laser to the measurement of CH_4 and N_2O fluxes from field to landscape scale using several micrometeorological techniques. Agricultural and Forest Meteorology 136(3-4): 222-236.

Paul EA and Clark FE. 1996. Soil microbiology and biochemistry. San Diego, CA: Academic Press.

Schwarz PA, Fahey TJ, and McCullough CE. 2003. Factors controlling spatial variation of tree species abundance in a forested landscape. Ecology 84(7): 1862-1878.

Steinheimer TR, Scoggin KD, and Kramer LA. 1998. Agricultural chemical movement through a field-size watershed in Iowa: Subsurface hydrology and

distribution of nitrate in groundwater. Environmental Science & Technology 32: 1039-1047.

van Breemen N, Boyer EW, Goodale CL, Jaworski NA, Paustian K, Seitzinger SP, Lajtha K, Mayer B, van Dam D, Howarth RW, Nadelhoffer KJ, Eve M, and Billen G. 2002. Where did all the nitrogen go? Fate of nitrogen inputs to large watersheds in the northeastern USA. Biogeochemistry 57(1): 267-293.

van Egmond K, Bresser T, Bouwman L. 2002. The European nitrogen case. Ambio 31(2): 72–78.

Venterea RT, Groffman PM, Verchot LV, Magill AH, Aber JD, and Steudler PA. 2003. Nitrogen oxide gas emissions from temperate forest soils receiving long-term nitrogen inputs. Global Change Biology 9: 346-357.

Zheng X, Fu C, Xu X, Yan X, Huang Y, Han S, Hu F, and Chen G. 2002. The Asian nitrogen cycle case study. Ambio 31(2): 79-87