

Western University

Scholarship@Western

Digitized Theses

Digitized Special Collections

2008

INTERACTIVE EFFECTS OF WARMING AND ATMOSPHERIC NITROGEN DEPOSITION ON SOIL NITROGEN DYNAMICS IN A TEMPERATE OLD FIELD

Michelle Turner

Follow this and additional works at: <https://ir.lib.uwo.ca/digitizedtheses>

Recommended Citation

Turner, Michelle, "INTERACTIVE EFFECTS OF WARMING AND ATMOSPHERIC NITROGEN DEPOSITION ON SOIL NITROGEN DYNAMICS IN A TEMPERATE OLD FIELD" (2008). *Digitized Theses*. 4850.
<https://ir.lib.uwo.ca/digitizedtheses/4850>

This Thesis is brought to you for free and open access by the Digitized Special Collections at Scholarship@Western. It has been accepted for inclusion in Digitized Theses by an authorized administrator of Scholarship@Western. For more information, please contact wlsadmin@uwo.ca.

INTERACTIVE EFFECTS OF WARMING AND ATMOSPHERIC NITROGEN
DEPOSITION ON SOIL NITROGEN DYNAMICS IN A TEMPERATE OLD FIELD

(Spine Title: Effects of Warming and Nitrogen on Soil Nitrogen Dynamics)

(Thesis Format: Integrated Article)

by

Michelle Turner

Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirement for the degree of
Master of Science

SGPS
The University of Western Ontario
London, Ontario, Canada

© Michelle Turner 2008

Abstract and Keywords

Winter climate plays a critical role in the cycling of nitrogen (N). Soils in temperate regions may be particularly vulnerable to freeze-thaw cycles since they remain close to freezing over much of winter. These systems are also experiencing high rates of atmospheric N deposition and the extent to which these added inputs can be retained has important implications for productivity and plant species composition. Warming treatments were applied year-round or exclusively during winter and were crossed with a N addition treatment. I examined the interactive effects of warming and N deposition on i) net N mineralization and leaching losses using *in situ* resin cores and lysimeters, and ii) the retention of N after the growing season and over winter using ^{15}N added as a pulse during spring melt 2007. Warming treatments did not affect net N mineralization or leaching over winter 2007 or 2008, however warming over winter 2007 increased mineral N availability the following growing season. Changes in soil temperatures in early spring may have important carry over effects on growing season N dynamics. Likewise, warming over winter did not alter ^{15}N retention. Year-round warming increased the recovery of excess ^{15}N in plants to the extent that it equaled the reductions in plant excess ^{15}N recovery caused by nitrogen fertilization. Nitrogen fertilization caused substantial soil ^{15}N losses, which overwhelmed any warming effects on ^{15}N plant recovery. Climate warming may only moderate the effects of N deposition on ecosystem N losses to a limited extent in this system, although more extreme responses to more intense warming cannot be ruled out.

Keywords: Winter, climate, nitrogen, soil, temperate, freeze-thaw, atmospheric deposition, warming, net mineralization, leaching, growing season, ^{15}N .

Co-Authorship Statement

Dr. Hugh Henry will be given co-authorship on any manuscripts published from the data chapters of this thesis. Dr. Henry provided invaluable guidance and direction during the design and implementation of these experiments, as well as during data analysis and interpretation.

Dedication

I dedicate this thesis to my family, whose endless love and support has continually allowed me to move forward, and to Laura, whose strength and courage continues to inspire me.

Acknowledgements

I would like to thank my supervisor, Dr. Hugh Henry, for his continual insight and invaluable guidance throughout my time here. His involvement in my project, and knowing that his door was always open, has been incredibly helpful. I would also like to thank my advisory committee, Dr. Norman Huner and Dr. Ben Rubin, for all of their constructive feedback on my project along the way. I also thank Richard Doucette at the Colorado Stable Isotope Laboratory at Northern Arizona University for analyzing all of my isotope samples. I would also like to express my gratitude to Gena Braun for her work on establishing the project infrastructure. I would also like to thank my lab mates - Germaine, Jennifer, Paul, and Eric; and my friend Kathryn, whose support and friendship throughout these past two years have helped me complete this final chapter in my academic career. I would also like to thank Holly for our endless coffee mornings, which always helped keep me going. I'd also like to extend thanks to my London family, Laura and Brad, for always being there for me and for giving me a place to call home. Finally, I want to thank my family back home for their endless encouragement.

Table of Contents

Certificate of Examination	ii
Abstract and Keywords	iii
Co-authorship Statement	iv
Dedication	v
Acknowledgements	vi
Table of Contents	vii
List of Tables	ix
List of Figures	x
List of Abbreviations	xi
 1. General Introduction	
1.1 Scientific Rationale	1
1.2 Objectives and Hypotheses	13
1.3 Thesis Organization	14
1.4 References	15
 2. Interactive effects of warming and increased nitrogen deposition on net nitrogen mineralization and leaching losses	
2.1 Introduction	24
2.2 Methods	27
2.2.1 Site description	27
2.2.2 Design of main warming and nitrogen addition experiment	27
2.2.3 Net N mineralization	32
2.2.4 Soil N leaching – lysimeter sampling	37
2.2.5 Statistical Analyses	37
2.3 Results	39
2.3.1 Soil N mineralization cores and resin bags	39
2.3.2 Soil lysimeter N	44
2.4 Discussion	47
2.4.1 Net N mineralization	47
2.4.2 Soil N leaching – lysimeter sampling	50
2.5 Conclusions	51
2.6 References	52
 3. Interactive effects of warming and increased nitrogen deposition on the recovery of ¹⁵N tracers added at spring melt	
3.1 Introduction	59
3.2 Methods	63
3.2.1 Site description	63
3.2.2 Design of main warming and nitrogen addition experiment	63
3.2.3 ¹⁵ N tracer addition	67
3.2.4 ¹⁵ N analyses	70
3.2.5 Statistical analyses	71

3.3 Results	72
3.3.1 Recovery of excess ^{15}N in the fall	72
3.3.2 Over winter changes in excess ^{15}N recovered	75
3.3.3 Total excess ^{15}N recovery in spring	78
3.4 Discussion	81
3.4.1 Recovery of excess ^{15}N in the fall	81
3.4.2 Over winter changes in excess ^{15}N recovered	82
3.4.3 Total excess ^{15}N recovery in spring	83
3.5 Conclusions	84
3.6 References	85
 4. General Discussion & Conclusions	
4.1 Research findings	91
4.2 Potential limitations	93
4.3 Directions for future research	94
4.4 References	96
 Curriculum vitae	100

List of Tables

Table 2.1 Extractable nitrate and ammonium from soil mineralization cores for both pre-incubation (initial) and post-incubation (final) expressed per unit of soil dry weight	43
--	----

List of Figures

Figure 2.1 A) air temperatures, B) snow cover, Ci) ambient soil temperatures at 1 cm depth and Cii) effect of warming treatments on soil temperature for the duration of the experiment. Sampling periods for mineralization resin cores, nitrogen addition timelines and lysimeter sample collections are also indicated	30
Figure 2.2 Experimental design showing 1 of 10 blocks	32
Figure 2.3 Design of mineralization cores	36
Figure 2.4 A) changes in soil extractable inorganic N and B) resin-captured inorganic N from soil N mineralization cores incubated over i) winter 2007, ii) summer 2007 and iii) winter 2008	42
Figure 2.5 Concentrations of nitrate, ammonium and organic N in lysimeter samples in i) fall 2006, ii) spring 2007 and iii) spring 2008	46
Figure 3.1 A) air temperatures, B) snow cover, Ci) ambient soil temperatures at 1 cm depth and Cii) effect of warming treatments on soil temperature for the duration of the experiment. ¹⁵ N tracer addition and sampling periods are also indicated	66
Figure 3.2 Concept diagram showing how ¹⁵ N tracer was added to plots	69
Figure 3.3 Mean excess ¹⁵ N recovered from experimental plots on 3 November, 2007 in A) shoots of <i>Poa pratensis</i> , B) roots and C) bulk soil.....	74
Figure 3.4 Mean over-winter changes in excess ¹⁵ N recovered from A) shoots of <i>Poa pratensis</i> , B) roots and C) bulk soil	77
Figure 3.5 Mean excess ¹⁵ N recovered from experimental plots on 24 March, 2008 in A) shoots of <i>Poa pratensis</i> , B) roots and C) bulk soil	80

List of Abbreviations

EPA - Environmental Protection Agency

FTC - freeze thaw cycle

IR - infrared radiation

^{14}N - 'light' stable isotope of nitrogen, molecular weight of 14

^{15}N - 'heavy' stable isotope of nitrogen, molecular weight of 15

$^{15}\text{NH}_4\text{Cl}$ - ammonium chloride ^{15}N tracer

K^{15}NO_3 - potassium nitrate ^{15}N tracer

$\delta^{15}\text{N}$ - sample ^{15}N enrichment relative to the natural abundance standard

PTFE – polytetrafluoroethylene (Teflon)

PVC - polyvinyl chloride

TDR - time domain reflectrometry

Chapter 1

General Introduction

1.1 Scientific Rationale

Global Climate Change

Warming of the earth's climate is now indisputable, as is apparent from increases in global average air and ocean temperatures, widespread melting of snow and ice and rising global average sea level (IPCC, 2007a). Research on global climate change has intensified over the past decade because of concerns that changes to climate will have complex and varied effects on terrestrial ecosystem processes (Rustad *et al.*, 2001). Current climate models predict a mean global temperature increase of 1.1 - 6.4 °C over the next century, with the greatest warming occurring on land and at high latitudes (IPCC, 2007a). Projected warming is significant since temperature regulates many important terrestrial biogeochemical processes, such as soil respiration (Raich and Schlesinger, 1992), litter decomposition (Jansson and Berg, 1985; Hobbie, 1996), nitrogen mineralization and nitrification (MacDonald *et al.*, 1995), denitrification (Malhi *et al.*, 1990), fine root dynamics (Pregitzer *et al.*, 2000; Gill and Jackson, 2000; Fitzhugh *et al.*, 2001), and plant nutrient uptake (BassiriRad *et al.*, 2000). The need to understand ecosystem responses to changing temperature has been addressed by a growing number of temperature manipulation experiments. Such experiments have shown that rates of soil respiration increase with warmer temperatures (Peterjohn *et al.*, 1993, 1994; McHale *et al.*, 1998; Rustad and Fernandez, 1998), however the response to warming of other

critical ecosystem processes, such as nitrogen mineralization and plant productivity has been much more variable (Ineson *et al.*, 1998; Jamieson *et al.*, 1998; Verburg *et al.*, 1999; Thompson *et al.*, 2000).

Effects of warming on temperate systems

Warming in temperate regions over the next century is projected to be greatest during the winter, with minimum winter temperatures increasing more than average temperatures. Snow season length will decrease and snow cover is projected to contract (IPCC, 2007b). For example, current climate models predict that southern Ontario will experience both warming by 3.0 - 4.0 °C and a decrease in precipitation (1-10 %) during winter by 2050 (Canadian Centre for Climate Modeling and Analysis, 2006). In temperate systems, warmer temperatures may also contribute to an earlier spring, an overall longer growing season, and in shifts in plant species (IPCC, 2007a). However, most studies examining ecosystem dynamics over winter have been conducted in the Arctic or in Alpine systems (e.g. Clein and Schimel, 1995; Schimel and Chapin, 1996; Schimel *et al.*, 2004; Schimel and Mikan, 2005; Grogan and Jonasson, 2003, Grogan *et al.*, 2004; Larsen *et al.*, 2002, 2007; Edwards *et al.*, 2006; Buckeridge and Grogan, 2008), both due to the proportionately long winters in these systems and because high latitude regions are predicted to experience the greatest increases in warming, particularly over winter (Plummer *et al.*, 2006; IPCC, 2007a). These studies have confirmed the importance of winter for annual carbon and nutrient cycling, indicated by ongoing microbial respiration and nitrogen mineralization beneath the snowpack (Lipson *et al.*, 2002; Mikan *et al.*, 2002; Grogan and Jonasson, 2003; Schimel *et al.*, 2004; Monson *et*

al., 2006; Miller *et al.*, 2007). The over-winter ecosystem dynamics of temperate systems have received much less attention (Campbell *et al.*, 2005). Although temperate regions are projected to experience less warming by comparison, these systems experience prolonged periods of cold temperatures and snow cover, and may be particularly vulnerable to winter warming since soils remain close to freezing over much of winter (Campbell *et al.*, 2005; Henry, 2008). Over the long-term, responses and tolerances to soil freezing stress may be key regulators of nutrient cycling in temperate ecosystems experiencing a warmer climate. However, the future importance of these over winter processes may be difficult to predict given the potentially complex interactions and feedbacks among climate, productivity and nutrient availability. These over winter processes are important to understand because the dynamics of nitrogen may be particularly important in driving interactions among global change factors (Hungate *et al.*, 2003).

Nitrogen as a limiting element

The lack of access to nitrogen in forms that can be used for growth and reproduction of organisms is one of the major restrictions limiting the abundance of plants and animals (White, 1993). Nitrogen is a key element in many of the compounds found in cells, such as nucleic acids and proteins, and is the mineral nutrient required in the greatest quantities by plants (Epstein, 1965). In contrast to the high nitrogen demand of living organisms, the supply of bioavailable nitrogen is relatively limited. The nitrogen cycle is unique in that it consists of an abundant, yet unavailable pool of N₂ in the atmosphere (Vitousek *et al.*, 1997). While 78% of the atmosphere's volume is nitrogen (Chapin *et al.*, 2002), 99.95% of this volume exists as inert N₂ gas, which is

unavailable to most organisms because of the exceptional strength of the covalent triple bond that holds the two nitrogen atoms together (Galloway *et al.*, 1994). The conversion of this molecular nitrogen into biologically available forms is known as nitrogen fixation and is carried out by free living bacteria and by bacteria in symbiotic associations with plants (Taiz and Zeiger, 1991). Even when biologically fixed, the substantial mobility of nitrogen across ecosystem boundaries through leaching and denitrification often prevents additional nitrogen inputs from resulting in enrichment (Vitousek and Howarth, 1991). In the absence of nitrogen fixation, most nitrogen comes from deposition (Vitousek *et al.*, 2002). Approximately half of the nitrogen entering terrestrial systems is in a plant available inorganic form, while the other half exists in organic form, bound in litter or as particulate matter in soil. Most organic nitrogen is covalently bonded to carbon, meaning that it is chemically stable and requires higher amounts of energy to be released (Vitousek *et al.*, 2002). Once established, nitrogen limitation can set in motion a positive feedback that accentuates this limitation (Vitousek *et al.*, 1982; Pastor and Post, 1986), whereby nitrogen-deficient plants produce litter that decomposes more slowly, resulting in higher levels of microbial immobilization (Melillo *et al.*, 1989). These processes collectively slow the cycling of nitrogen relative to other elements. High nitrogen requirements by organisms combined with its relative biological scarcity has resulted in nitrogen limitation in most terrestrial systems (Vitousek and Howarth, 1991).

Nitrogen cycling in terrestrial systems

Inputs and outputs of nitrogen are a small fraction of the quantity of nutrients that cycle internally, producing relatively closed ecosystems (Chapin *et al.*, 2002). However, human activities have increased these inputs and outputs dramatically (Galloway *et al.*, 2004). Internal cycling processes include the conversion of nutrients from organic to inorganic forms, chemical reactions that alter elements from one ionic form to another, uptake by plants and microorganisms, and the exchange of nutrients on surfaces within the soil matrix (Chapin *et al.*, 2002). The form of nitrogen entering systems determines its ecosystem consequences. Inorganic nitrogen is available for immediate plant and microbial uptake, whereas most organic nitrogen must first be mineralized.

Mineralization is the enzymatic process of conversion of organic forms of nitrogen (e.g. proteins, peptides, and amino acids) to inorganic forms (NH_3 or NH_4^+ and NO_3^-) by microorganisms that use carbon as energy and both carbon and nitrogen to build biomass (Benbi and Richter, 2002). As such, mineralization is always coupled with microbial immobilization. The first step in the process, called ammonification, involves the conversion of organic nitrogen to NH_3 and is carried out by a range of decomposer microorganisms. The subsequent conversion of NH_3 to NO_3^- , termed nitrification, is carried out exclusively by two groups of autotrophic bacteria, *Nitrosomonas* and *Nitrobacter* (Benbi and Richter, 2002; Chapin *et al.*, 2002). As such, net mineralization of nitrogen depends on the balance between microbial immobilization of nitrogen to support microbial growth and the secretion of nitrogen that exceeds microbial growth requirements (Chapin *et al.*, 2002). While recent research has shown that plant uptake of organic nitrogen in the form of amino acids also represents a substantial proportion of

uptake in high latitude nitrogen limited systems, such as the arctic and alpine tundra (Chapin, 1995; Schimel and Chapin, 1996; Henry and Jefferies, 2003), it is still unclear how important this pathway of acquisition is in temperate systems, especially when compared to nitrogen uptake from mineralization (Schimel and Bennett, 2004).

Increasing atmospheric nitrogen deposition

Agricultural and industrial intensification in recent decades has led to dramatic increases in the production of airborne reactive nitrogen (Vitousek *et al.*, 1997; Galloway *et al.*, 2004). As a result, the transfer from the unreactive atmospheric pool to biologically available forms on land has been doubled (Vitousek *et al.*, 1997). Due to an increasing population and enhanced per-capita energy and food consumption, the human contribution to global biological nitrogen fixation will reach 270 Tg (270×10^{12} grams) N yr⁻¹ by 2050, compared to 156 Tg N yr⁻¹ in the 1990s (Galloway *et al.*, 2004). Yet our knowledge on how ecosystems will respond to increasing atmospheric nitrogen deposition remains poor (Asner *et al.*, 2001). These inputs into the global nitrogen cycle are important because added nitrogen can alter the composition and productivity of natural ecosystems substantially (Vitousek *et al.*, 2002).

Temperate ecosystems have experienced the greatest increases in nitrogen inputs from the atmosphere (Asner *et al.*, 2001), with some temperate regions experiencing chronically elevated inputs for decades (Wright and Van Breemen, 1995; Fenn *et al.*, 1998). Such inputs are projected to continue to increase in the future (Galloway *et al.*, 2004). For example, nitrogen deposition rates in parts of southern Canada are projected to increase from 0.5 g N m⁻² yr⁻¹ during the 1990's to 2-5 g N m⁻² yr⁻¹ by 2050 (Galloway *et al.*, 2004). While elevated nitrogen will likely stimulate primary productivity in the

short term (Vitousek and Howarth, 1991), continual inputs may eventually lead to nitrogen saturation, characterized by enhanced nitrogen losses and decreased plant growth (Matson *et al.*, 2002; Asner *et al.*, 2001). Excess nitrogen inputs that lead to higher rates of nitrate leaching may also drive the loss of other elements, further limiting plant growth (Matson *et al.*, 2002). Chronic rates of nitrogen deposition may also lead to decreases in plant productivity through indirect processes such as soil acidification and base cation impoverishment (Aber *et al.*, 1989). The extent to which ecosystems can retain this added nitrogen under future climate scenarios has important implications for net primary productivity and plant species composition (Heunneke *et al.*, 1990; Tilman and Downing, 1994; Vitousek *et al.*, 1997). Furthermore, elevated nitrogen deposition and other global change factors, such as warming, may interact over the coming century to alter ecosystem productivity (Dukes and Mooney, 1999; Matson *et al.*, 2002).

Seasonal variation in interactions between soil nitrogen and warming

Increasing temperatures caused by global climate change have important implications for terrestrial nutrient cycling (Rustad *et al.*, 2001). While warming can have several direct effects on ecosystem productivity (Chapin *et al.*, 2002; Walther, 2003), warming may also alter productivity indirectly by influencing soil nitrogen dynamics (Vitousek *et al.*, 1997; Rustad *et al.*, 2001; Shaw and Harte, 2001; Henry, 2008). Most studies examining the effects of warming on nitrogen mineralization have been done in the context of the plant growing season. If soil moisture is not limiting, warming during this time can increase plant productivity through enhancing photosynthetic rates, and by extending the growing season (Vitousek *et al.*, 1997; Rustad

et al., 2001). Warming may also indirectly increase plant productivity by enhancing nutrient availability due to higher levels of litter decomposition (Rustad *et al.*, 2001) and nitrogen mineralization (Sierra, 1997; De Valpine and Harte, 2001; Rustad *et al.*, 2001). Therefore, a warming induced increase in the internal production of inorganic nitrogen over the growing season could stimulate plant growth and net ecosystem productivity. However, in systems that are already impacted by elevated nitrogen deposition, a warming-induced increase in mineralization could lead to nitrogen saturation and increased leaching losses (Vitousek *et al.*, 1997).

While growing season dynamics are important, seasonal variation in the interactions between soil nitrogen dynamics and warming may change the overall effects of warming on nitrogen availability (Jamieson *et al.*, 1999; Sturm *et al.*, 2005; Aerts *et al.*, 2006; Koch *et al.*, 2007; Henry, 2008). Climate warming during the winter, at a time when plants are largely inactive may alter nitrogen cycling by changing soil freezing dynamics (Groffman *et al.*, 2001; Henry, 2008). Microbial biomass and activity remains high during the winter (Brooks *et al.*, 1998; Schmidt and Lipson, 2004), because of soil particles that continue to have liquid water films around them down to temperatures of -10 °C (Mikan *et al.*, 2002; Schimel *et al.*, 2004). Microbial substrate usage tends to shift above zero from processing nitrogen-poor detritus to nitrogen-rich recycled microbial biomass, causing nitrogen availability for either plant uptake or leaching to be greatest when soils are near zero (Schimel and Mikan, 2005). Since soil freezing is influenced strongly by both air temperature and insulation by the snowpack, winter climate warming may lead to increased soil freezing as a result of reduced snowpack thickness (Groffman *et al.*, 2001; Henry, 2008). Snow removal projects have shown that

reductions in snow cover may lead to increases in soil freezing (Groffman *et al.*, 2001; Decker *et al.*, 2003), resulting in fine root injury (Weih and Karlsson, 2002), stress on soil microbial populations (Groffman *et al.*, 2001; Sulkava and Huhta 2003), and increases in nitrogen leaching (Fitzhugh *et al.*, 2001; Groffman *et al.*, 2001). Similarly, soil under shallow or inconsistent snow cover tends to export higher levels of nitrate (Brooks *et al.*, 1998).

Studies examining cold season nutrient cycling have found nitrogen mineralization to be strongly sensitive to snow timing and depth. Areas with earlier and deeper snow accumulation have higher levels of soil nitrogen and greater mineralization rates due to warmer soil temperatures (Brooks *et al.*, 1996, 1998; Brooks and Williams, 1999; Schimel *et al.*, 2004). While it is recognized that soil freeze-thaw events may have important effects on nitrogen cycling, these events have not been well characterized due to the complexity of the soil ecosystem and to the multiple effects that freezing has on different biological, physical, and chemical variables (Groffman *et al.*, 2001). Many studies have suggested that freeze-thaw cycles result in microbial lysis (DeLuca *et al.*, 1992; Clein and Schimel, 1995; Schimel and Clein, 1996) and in subsequent bursts of nitrogen mineralization, as the growth and activity of the surviving microbes are stimulated by the input of substrate (Schimel and Clein, 1996; Groffman *et al.*, 2001). However, this mineralization flush upon freeze-thaw may be short-lived and tends to decrease with each successive cycle (Schimel and Clein, 1996; Skogland *et al.*, 1988; Herrmann and Witter, 2002). For example, while root mortality caused by colder soils represented a significant input of labile nitrogen to the soil microbial community, no significant increase in rates of nitrogen mineralization and nitrification were detected in

response (Groffman *et al.*, 2001). In other studies, nitrogen mineralization showed no consistent patterns below zero (Clein and Schimel, 1995; Miller *et al.*, 2007). It is likely that the response of mineralization to warming and to freeze-thaw events associated with colder soils will vary with soil temperature, the size of the microbial biomass, and other soil conditions (Edwards and Cresser, 1992).

The potential for warming to increase winter mineralization, however, has important implications for plant nutrition. Some plant roots maintain a ready uptake potential under the snowpack (Bilbrough *et al.*, 2000), enabling increased winter nitrogen retention. Nitrogen released at snowmelt is further available for plant uptake, although this pool of available nitrogen present at the end of winter is also vulnerable to leaching. Thus, increased cold-season mineralization could lead to either more or less plant-available nitrogen depending on the sensitive balance among soil flushing, plant activity, and soil microbial processes (Schimel and Mikan, 2005).

Climate warming field experiments over winter

A variety of methods have been used to study soil warming, including buried heated wires (Peterjohn *et al.*, 1994), heated soil surface tubes (Hillier *et al.*, 1994) and overhead heaters (Nijs *et al.*, 1996). Of these techniques, overhead heaters are the most appropriate because they mimic natural warming by heating soil from above (Shen and Harte, 2000). However, studies using overhead heaters have typically only applied warming year round (Harte *et al.*, 1995), without further isolating the effects of winter warming. Further to this, snow removal experiments exploring the effects of winter climate change have only isolated the effects of snow depth on soil dynamics (Groffman

et al., 2001; Fitzhugh *et al.*, 2001) without further incorporating natural warming or the interactive effects of nitrogen deposition.

In situ field measurements of net N mineralization

The net accumulation of inorganic N in the absence of plant roots provides a good index of N availability to plants. Numerous methods are available for measuring net nitrogen mineralization under field conditions. However, obtaining quantitative estimates of net N mineralization in the field has been complicated by soil disturbance caused by sieving soils prior to incubation (Binkley and Hart, 1989) and experimental artifacts associated with intact soil cores, such as root severing and subsequent decomposition (Hart *et al.*, 1989). The buried bag method involves incubating soil in buried polyethylene bags, which remain permeable to gases, but impermeable to liquids (Gordon *et al.*, 1987). However, this method only integrates onsite soil water dynamics if soil water content at the beginning of the incubation is representative of conditions throughout the entire incubation. The covered-cylinder method was developed as a more durable alternative to the buried bag method (Adams *et al.*, 1989). While the open-bottom cylinder increased aeration, it created problems in terms of mineral N loss and allowed root growth into the cylinders (Subler *et al.*, 1995). Another method, involving intact soil cores coupled with ion exchange resins (Distefano and Gholz, 1986), allows water content to fluctuate while preventing the loss of NH_4^+ and NO_3^- from the confined soil. This *in situ* mineralization resin core method has long been considered the best technique for measuring net nitrogen mineralization. Ion exchange resins absorb ions from soil solution similarly to diffusive ion uptake by plant roots while field soil

conditions, such as temperature and moisture, are closely simulated within the core (Hart and Binkley, 1985; Distefano and Gholz, 1986; Lajtha, 1988; Lundell, 1989; Binkley *et al.*, 1992; Giblin *et al.*, 1994; Kolberg *et al.*, 1997; Hanselman *et al.*, 2004). This lessens inaccuracies associated with soil disturbance (Raison *et al.*, 1997; Cabrera and Kissel, 1988; Sierra, 1992, 1996). Furthermore, this method also provides the advantage of integrating leaching losses over time.

The use of ^{15}N as a tracer in ecosystems

Isotopes are atoms of the same element that differ in atomic mass due to differences in the number of neutrons contained in the atoms' nuclei. The use of stable N isotopes of masses 14 and 15 as tracers is based on the fact that ^{14}N and ^{15}N occur naturally in an almost constant ratio of $\sim 272:1$ ($^{14}\text{N}/^{15}\text{N}$). This means that approximately 0.336% of naturally occurring N is ^{15}N . Stable isotope tracer techniques rely on adding compounds that are artificially enriched in the rare (heavy) isotope of the element of interest. By adding substrates that are $\sim 100\%$ ^{15}N enriched, the fate of ^{15}N in a system can be traced through the subsequent change in N-isotope ratios, relative to control samples (Hauck, 1982). Tracking the fate of added ^{15}N can provide insights as to how warming and increased nitrogen deposition may affect soil nitrogen dynamics over the growing season and over winter. Although we have an understanding of the increasing amounts of reactive nitrogen being deposited, along with knowledge of its regional dispersion, we have a poor understanding of its ultimate fate. This question is critical to answer for it currently limits our ability to determine the rate of nitrogen accumulation in our systems (Galloway *et al.*, 2004).

1.2 Objectives and Hypotheses

The overall objective of my thesis was to investigate the interactive effects of climate warming and increased atmospheric nitrogen deposition on temperate ecosystem soil nitrogen dynamics. Emphasis was placed on nitrogen dynamics over winter since the effects of climate warming are projected to be the most pronounced during the winter for temperate systems and changes in soil nitrogen dynamics in response to warming remain unclear. My overall objective was divided into the following sections.

Objective 1: To determine the interactive effects of climate warming and nitrogen deposition on net nitrogen mineralization and leaching losses.

I used mineralization resin cores to estimate net nitrogen mineralization and leaching in response to warming and nitrogen treatments in the fall, winter, and over the growing season. I also used soil lysimeters to measure nitrogen being leached from the system. My hypothesis was that warming would increase net nitrogen mineralization over all seasons, potentially leading to increased nitrogen leaching losses over winter. Furthermore, I predicted that winter warming would increase nitrogen leaching in nitrogen-addition plots, partially offsetting nitrogen enrichment.

Objective 2: To determine the interactive effects of climate warming and nitrogen deposition on the recovery of ^{15}N added at spring melt, both at the end of the growing season and over the following winter.

I added ^{15}N -labeled ammonium and nitrate to the soil surface during spring melt to examine the interactive effects of warming and nitrogen deposition on the partitioning of the added nitrogen among aboveground biomass, roots, and soil. The ^{15}N enrichment

in each ecosystem pool was estimated at the end of the growing season and again after winter at the beginning of the following spring. I hypothesized that increased freeze-thaw cycling in the warmed plots in early spring would reduce their retention of the ^{15}N pulse, although increased total plant nitrogen uptake in plots also warmed over summer might increase uptake of the remaining ^{15}N tracer. I also hypothesized that the recovery of ^{15}N from added ammonium would exceed that of nitrate. In terms of nitrogen retention over winter, I hypothesized that an increased frequency of freeze-thaw cycles would enhance ^{15}N losses from warmed plots, particularly if they became nitrogen saturated as a result of nitrogen fertilization.

1.3 Thesis Organization

This thesis has been written in the integrated article format and consists of two manuscripts. This first chapter included relevant background information and an overview of the research experiment in the context of my research hypotheses. The first manuscript (Chapter 2) addresses my first research objective by presenting the findings of the *in-situ* nitrogen mineralization resin core experiment that examined net nitrogen mineralization and nitrogen leaching. The second manuscript (Chapter 3) addresses my second research objective by presenting the results of an experiment where a ^{15}N isotope was used as a tracer to examine the fate of added nitrogen at the end of the growing season and after winter. Both experiments investigated the interactive effects of warming and increased atmospheric nitrogen deposition, with an emphasis placed on winter ecology. The general discussion and conclusion section (Chapter 4) connects the results of the two experiments together and provides insights into future research directions.

1.4 References

- Aber J.D., Nadelhoffer K.J., Steudler P., Melillo J.M., 1989. Nitrogen saturation in northern forest ecosystems. *Bioscience* **39**(6): 378-386.
- Adams M.A., Polglase P.J., Attiwill P.M., Weston C.J., 1989. *In situ* studies of nitrogen mineralization and uptake in forest soils: some comments on methodology. *Soil Biology & Biochemistry* **21**: 423-429.
- Aerts R., Cornelissen J.H.C., Dorrepaal E., 2006. Plant performance in a warmer world: general responses of plants from cold, northern biomes and the importance of winter and spring events. *Plant Ecology* **182**: 65-77.
- Asner G.P., Townsend A.R., Riley W.J., Matson P.A., Neff J.C., Cleveland C., 2001. Physical and biogeochemical controls over terrestrial ecosystem responses to nitrogen deposition. *Biogeochemistry* **54**: 1-39.
- BassiriRad H., 2000. Kinetics of nutrient uptake by roots: responses to global change. *New Phytologist* **147**: 155-169.
- Benbi D., and Richter J., 2002. A critical review of some approaches to modeling nitrogen mineralization. *Biology & Fertility of Soils* **35**:168-183.
- Bilbrough C., Welker J., Bowman W., 2000. Early spring nitrogen uptake by snow-covered plants: a comparison of arctic and alpine plant function under the snowpack. *Arctic, Alpine and Antarctic Research* **32**: 404-411.
- Binkley D., Bell R., Sollins P., 1992. Comparison of methods for estimating soil nitrogen transformations in adjacent conifer and alder-conifer forests. *Canadian Journal of Forest Research* **22**: 858-863.
- Binkley D., and Harte S.C., 1989. The components of nitrogen availability assessments in forest soils. *Soil Science* **10**: 57-112.
- Brooks P.D., and Williams M.W., 1999. Snowpack controls on nitrogen cycling and export in seasonally snow-covered catchments. *Hydrological Processes* **13**: 2177-2190.
- Brooks P.D., Williams M.W., Schmidt S.K., 1996. Microbial activity under alpine snowpacks, Niwot Ridge, Colorado. *Biogeochemistry* **32**: 93-113.
- Brooks P.D., Williams M.W., Schmidt S.K., 1998. Inorganic nitrogen and microbial biomass dynamics before and during spring snowmelt. *Biogeochemistry* **43**: 1-15.
- Buckeridge K., and Grogan P., 2008. Deepened snow alters soil microbial nutrient limitations in arctic birch hummock tundra. *Applied Soil Ecology* **39**: 210-222.

Cabrera M.L., and Kissel D.E., 1988. Potentially mineralizable nitrogen in disturbed and undisturbed soil samples. *Soil Science Society of America Journal* **52**: 1010–1015.

Campbell J.L., Mitchell M.J., Groffman P.M., Christenson L.M., Hardy J.P., 2005. Winter in northeastern North America: a critical period for ecological processes. *Frontiers in Ecology & the Environment* **3**(6): 314–322.

Canadian Centre for Climate Modeling and Analysis, 2006. Cited online: <http://atlas.nrcan.gc.ca/site/english/maps/climatechange/scenarios/nationalwintertemp2050/1>

Chapin, F.S. III, Matson P.A., Mooney H.A., 2002. Principles of terrestrial ecosystem ecology. Springer, New York, N.Y., U.S.A. pp. 197-223.

Clein J.S., and Schimel J.P., 1995. Microbial activity of tundra and taiga soils at subzero temperatures. *Soil Biology & Biochemistry* **27**: 1231–1234.

De Valpine P., and Harte J., 2001. Plant responses to experimental warming in a montane meadow. *Ecology* **82**(3): 637-648.

Distefano J.F., and Gholz H.L., 1986. A proposed use of ion-exchange resins to measure nitrogen mineralization and nitrification in intact soil cores. *Communications in Soil Science and Plant Analysis* **17**: 989–998.

Decker K.L.M., Wang D., Waite C., Sherbatskoy T., 2003. Snow removal and ambient air temperature effects of forest soil temperatures in northern Vermont. *Soil Science Society of America Journal* **67**: 1234–42.

Deluca T.H., Keeney D.R., McCarty G.W., 1992. Effect of freeze–thaw events on mineralization of soil-nitrogen. *Biology & Fertility of Soils* **14**: 116–120.

Dukes J.S., and Mooney H.A., 1999. Does global change increase the success of invaders? *Trends in Ecology and Evolution* **14**(4): 135-139.

Edwards A.C., and Cresser M.S., 1992. Freezing and its effect on chemical and biological properties of soil. *Soil Science*. **18**: 59–79.

Edwards K.A., McCulloch J., Kershaw P.G., Jefferies R.L., 2006. Soil microbial and nutrient dynamics in a wet Arctic sedge meadow in late winter and early spring. *Soil Biology & Biochemistry* **38**: 2843–2851.

Epstein E., 1965. Mineral metabolism. *Plant Biochemistry*. (eds J. Bonner and J.E. Varner), pp.438-466, Academic Press, New York.

Fenn M.E., Poth M.A., Aber J.D., Baron J.S., Bormann B.T., Johnson D.W., Lemly A.D., McNulty S.G., Ryan D.F., Stottlemyer R., 1998. Nitrogen excess in North American

ecosystems: predisposing factors, ecosystem responses, and management strategies. *Ecological Applications* **8**: 706–733.

Fitzhugh R.D., Driscoll C.T., Groffman P.M., Tierney G.L., Fahey T.J., Hardy J.P., 2001. Effects of soil freezing, disturbance on soil solution nitrogen, phosphorus, and carbon chemistry in a northern hardwood ecosystem. *Biogeochemistry* **56**: 215–238.

Galloway J.N., Dentener F.J., Capone D.G., Boyer E.W., Howarth E.W., Seitzinger S.P., Asner G.P., Cleveland C.C., Green P.A., Holland E.A., Karl D.M., Michaels A.F., Porter J.H., Townsend A.R., Vorosmarty C.J., 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* **70**: 152–226.

Giblin A. E., Laundre J. A., Nadelhoffer K. J., Shaver G. R., 1994. Measuring nutrient availability in arctic soils using synthetic ion exchange resins: A field test. *Soil Science Society of America Journal* **58**: 1154–1162.

Gill R.A., and Jackson R.B., 2000. Global patterns of root turnover for terrestrial ecosystems. *New Phytologist* **147**: 13–31.

Gordon A., Tallas M., Van Cleve K., 1987. Soil incubations in polyethylene bags: effect of bag thickness and temperature on nitrogen transformations and CO permeability. *Canadian Journal of Soil Science* **67**: 65–75.

Groffman P.M., Driscoll C.T., Fahey T.J., Hardy J.P., Fitzhugh R.D., Tierney G.L., 2001. Colder soils in a warmer world: a snow manipulation study in a northern hardwood forest ecosystem. *Biogeochemistry* **56**: 135–150.

Grogan P., and Jonasson S., 2003. Controls on annual nitrogen cycling in the understory of a subarctic birch forest. *Ecology* **84**(1): 202–218.

Grogan P., Michelsen A., Ambus P., Jonasson S., 2004. Freeze-thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. *Soil Biology & Biochemistry* **36**: 641–654.

Hanselman T.A., Graetz D.A., Obreza T.A., 2004. A comparison of *in situ* methods for measuring net nitrogen mineralization rates of organic soil amendments. *Journal of Environmental Quality* **33**: 1098–1105.

Hart S.C., and Firestone M.K., 1989. Evaluation of three *in situ* soil nitrogen availability assays. *Canadian Journal of Forest Research* **19**: 185–191.

Hart S. C., and Binkley D., 1985. Correlations among indices of forest soil nutrient availability in fertilized and unfertilized loblolly pine plantations. *Plant and Soil* **85**: 11–21.

Harte J., Torn M.S., Chang F.R., Feifarek B., Kinzig A.P., Shaw R., Shen K., 1995. Global warming and soil microclimate: results from a meadow-warming experiment. *Ecological Applications* **5**: 132–150.

Hauck R.D., 1982. Nitrogen – Isotope-ratio analysis. pp. 735-779. In A.L. Page et al., (ed.) *Methods of soil analysis. Part 2*. 2nd ed. Agron Monogr. 9. ASA and SSSA, Madison, WI.

Henry H.A.L., 2008. Climate change and soil freezing dynamics: historical trends and projected changes. *Climatic Change*. **87**: 421–434.

Henry H.A.L., and Jefferies B., 2003. Interactions in the uptake of amino acids, ammonium and nitrate ions in the Arctic salt-marsh *Puccinellia phryganodes*. *Plant Cell & Environment* **26**(3): 419-428.

Herrmann A., and Witter E., 2002. Sources of C and N contributing to the flush in mineralization upon freeze–thaw cycles in soils. *Soil Biology & Biochemistry* **34**: 1495–1505.

Hillier S.H., Sutton F., Grime J.P., 1994. A new technique for the experimental manipulation of temperature in plant communities. *Functional Ecology* **8**: 755–762.

Hobbie S.E., 1996. Temperature and plant species control over litter decomposition in Alaskan tundra. *Ecological Monographs* **66**: 503–522.

Huenneke L.F., Hamburg S.P., Koide R., Mooney H.A., Vitousek P.M., 1990. Effects of soil resources on plant invasion and community structure in a California serpentine grassland. *Ecology* **71**: 478-491.

Hungate B.A., Dukes J.S., Shaw M.R., Luo Y.Q., Field C.B., 2003. Nitrogen & Climate Change Science **302**(5650): 1512-1513.

Ineson P., Benham D.G., Poskitt J., Harrison A.F., Taylor K., Woods C., 1998. Effects of climate change on nitrogen dynamics in upland soils. A soil warming study. *Global Change Biology* **4**: 153–161.

Intergovernmental Panel on Climate Change (IPCC), 2007a. Climate Change 2007 Synthesis Report: Summary for Policymakers (based on the assessment carried out by the three Working Groups of IPCC). pp 1-73.

Intergovernmental Panel on Climate Change (IPCC), 2007b. The physical science basis – Chapter 11: Regional climate projections. Cited online: <http://www.ipcc.ch/pdf/assessment-report/ar4/wg1/ar4-wg1-chapter11.pdf>

Jamieson N., Barraclough D., Unkovich M., Monaghan R., 1998. Soil N dynamics in a natural calcareous grassland under a changing climate. *Biology & Fertility of Soils* **27**: 267–273.

Jamieson N., Monaghan R., Barraclough D., 1999. Seasonal trends of gross N mineralization in a natural calcareous grassland. *Global Change Biology* **5**(4): 423-431.

Jansson P.E., and Berg B., 1985. Temporal variation of litter decomposition in relation to simulated soil climate. Long-term decomposition in a Scots pine forest. *Canadian Journal of Botany* **63**: 1008–1016.

Koch O., Tscherko D., Kandeler E., 2007. Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. *Global Biogeochemical Cycles* **21**(4): GB4017.

Kolberg R.L., Rouppet B., Westfall D.G., Peterson G.A., 1997. Evaluation of an *in situ* net soil nitrogen mineralization method in dryland agroecosystems. *Soil Science Society of America Journal* **61**: 504–508.

Lajtha K., Klein M., 1988. The effect of varying nitrogen and phosphorus availability on nutrient use by *Larrea-tridentata*, a desert evergreen shrub. *Oecologia* **75**(3): 348-353.

Larsen K.S., Grogan P., Jonasson S., Michelsen A., 2007. Respiration and microbial dynamics in two subarctic ecosystems during winter and spring thaw: effects of increased snow depth. *Arctic, Antarctic and Alpine Research* **39**(2): 268–276.

Larsen K. S., Jonasson S., Michelsen A., 2002: Repeated freeze-thaw cycles and their effects on biological processes in two arctic ecosystem types. *Applied Soil Ecology* **21**: 187–195.

Lipson D.A., Schadt C.W., Schmidt S.K., 2002. Changes in microbial community structure and function following snowmelt in an alpine soil. *Microbial Ecology* **43**: 307–314.

Lundell Y., 1989. *In situ* ion exchange resin bags to estimate forest site quality. *Plant and Soil* **119**: 186–190.

MacDonald N.W., Zak D.R., Pregitzer K.S., 1995. Temperature effects on kinetics of microbial respiration and net nitrogen and sulfur mineralization. *Soil Science Society of America Journal* **59**: 233–240.

Malhi S.S., McGrill W.B., Nyborg N., 1990. Nitrate losses in soils: effects of temperature, moisture, and substrate concentration. *Soil Biology & Biochemistry* **22**: 917–927.

Matson P.A., Lohse K.A., Hall S.J., 2002. The Globalization of Nitrogen Deposition: Consequences for Terrestrial Ecosystems. *Ambio* **31**(2): 113-119.

McHale P.J., Mitchell M.J., Bowles F.P., 1998. Soil warming in a northern hardwood forest: trace gas fluxes and leaf litter decomposition. *Canadian Journal of Forest Research* **28**: 1365–1372.

Melillo J.M., Aber J.D., Linkins A.E., Ricca A., Fry B., Nadelhoffer K., 1989. Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. *Plant and Soil* **115**: 189-198.

Mikan C.J., Schimel J.P., Doyle A.P., 2002. Temperature controls of microbial respiration in arctic tundra soils above and below freezing. *Soil Biology & Biochemistry* **34**: 1785–1795.

Miller A.E., Schimel J.P., Sickman J.O., Meixner T., Doyle A.P., Melack J.M., 2007. Mineralization responses at near-zero temperatures in three alpine soils. *Biogeochemistry* **84**: 233–245.

Monson R.K., Lipson D.L., Burns S.P., Turnipseed A.A., Delany A.C., Williams M.W., Schmidt S.K., 2006. Winter forest soil respiration controlled by climate and microbial community composition. *Nature* **439**: 711–714.

Nijs I., Kocketbergh F., Teughels H., Blum H., Hendrey G., Impens I., 1996. Free air temperature increase (FATI): a new tool to study global warming effects on plants in the field. *Plant, Cell and Environment* **19**: 495–502.

Pastor J., and Post W.M., 1986. Influence of Climate, soil moisture, and succession on forest carbon and nitrogen cycles. *Biogeochemistry* **2**(1): 3-27.

Peterjohn W.T., Melillo J.M., Bowles S.T., 1993. Soil warming and trace gas fluxes: experimental design and preliminary flux results. *Oecologia* **93**: 18–24.

Peterjohn W.T., Melillo J.M., Steudler P.A., Newkirk K.M., Bowles S.T., Aber J.D., 1994. Responses of trace gas fluxes and N availability to experimentally elevated soil temperatures. *Ecological Applications* **4**: 617–625.

Plummer D.A., Caya D., Frigon A., Cote H., Giguere M., Paquin D., Biner S., Harvey R., de Elia R., 2006. Climate and climate change over North America as simulated by the Canadian Regional Climate Model. *Journal Climate* **19**: 3112–3132.

Pregitzer K.S., King J.S., Burton A.J., Brown S.E., 2000. Responses of tree fine roots to temperature. *New Phytologist* **147**: 10–115.

Raich J.W., and Schlesinger W.H., 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus* **44**: 81–89.

Raison R.J., Connell M.J., Khanna P.K., 1987. Methodology for studying fluxes of soil mineral-N *in situ*. *Soil Biology & Biochemistry* **19**: 521–530.

Rustad L.E., Campbell J.L., Marion G.M., Norby R.J., Mitchell M.J., Hartley A.E., Cornelissen J.H.C., Gurevitch J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* **126**: 543–562.

Rustad L.E., and Fernandez I.J., 1998. Experimental soil warming effects on CO₂ and CH₄ flux from a low elevation spruce fir forest soil in Maine, USA. *Global Change Biology* **4**: 597–607.

Schimel J.P., and Bennett J., 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* **85**: 591–602.

Schimel J.P., Bilbrough C., Welker J.A., 2004. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. *Soil Biology & Biochemistry* **36**: 217–27.

Schimel J.P., and Chapin F.S., 1996. Tundra plant uptake of amino acid and NH₄ nitrogen *in situ*: plants compete well for amino acid N. *Ecology* **77**: 2142–2147.

Schimel J.P., and Clein J.S., 1996. Microbial response to freeze-thaw cycles in tundra and taiga soils. *Soil Biology & Biochemistry* **28**: 1061–1066.

Schimel J.P., and Mikan C., 2005. Changing microbial substrate use in Arctic tundra soils through a freeze-thaw cycle. *Soil Biology & Biochemistry* **37**(8): 1411–1418.

Schmidt S.K., and Lipson D.A., 2004. Microbial growth under the snow: implications for nutrient and allelochemical availability in temperate soils. *Plant and Soil* **259**: 1–7.

Shaw M.R., and Harte J., 2001. Response of nitrogen cycling to simulated climate change: differential responses along a subalpine ecotone. *Global Change Biology* **7**(2): 193–210.

Shen K.P., and Harte J., 2000. Ecosystem climate manipulations. In: Sala, O.E., Jackson, R.B., Mooney, H.A., Howarth, R.W. (Eds.), *Methods in Ecosystem Science*. Springer, New York, pp. 353–369.

Sierra J., 1992. Relationship between mineral N content and N mineralization rate in disturbed soil samples incubated under field and laboratory conditions. *Australian Journal of Soil Research* **30**: 477–492.

Sierra J., 1996. Nitrogen mineralization and its error of estimation under field conditions related to the light-fraction soil organic matter. *Australian Journal of Soil Research* **34**(5): 755–767.

Sierra J., 1997. Temperature and soil moisture dependence of N mineralization in intact soil cores. *Soil Biology & Biochemistry* **29**: 1557–1563.

Skogland T., Lomeland S., Goksoyr J., 1988. Respiratory burst after freezing and thawing of soil: experiments with soil bacteria. *Soil Biology & Biochemistry* **20**: 851–856.

Sturm M., Schimel J., Michaelson G., Welker J.M., Oberbauer S.F., Liston G.E., Fahnestock J., Romanovsky V.E., 2005. Winter biological processes could help convert arctic tundra to shrubland. *Bioscience* **55**(1): 17–26.

Subler S., Parmelee R.W., Allen M.F., 1995. Comparison of buried bag and PVC core methods for *in situ* measurement of nitrogen mineralization rates in an agricultural soil. *Communications in Soil Science* **26**: 2369–2381.

Sulkava P., Huhta V., 2003. Effects of hard frost and freeze–thaw cycles on decomposer communities and N mineralisation in boreal forest soil. *Applied Soil Ecology* **22**: 225–239.

Taiz L., and Zeiger E., 1991. *Plant Physiology*. pp. 292–314. Benjamin/Cummings Publishing Company Inc. Redwood City, California US.

Thompson K., Masters G.J., Grime J.P., Brown V.K., Hillier S.H., Clarke I.P., Askew A.P., Corker D., Kieley J.P., 2000. Predicting the response of limestone grassland to climate change. *Aspects Applied Biology* **58**: 329–336.

Tilman D., Downing J.A., 1994. Biodiversity and stability in grasslands. *Nature* **367**: 363–365.

Verburg P.S.J., Van Loon W.K.P., Lukewille A., 1999. The Climex soil-heating experiment: soil response after 2 years of treatment. *Biology & Fertility of Soils* **28**: 271–276.

Vitousek P.M., 1982. Nutrient cycling and nutrient use efficiency. *American Naturalist* **119**(4): 553–572.

Vitousek P.M., Aber J., Howarth R.W., Likens G.E., Matson P.A., Schindler D.W., Schlesinger W.H., Tilman G.D., 1997. Human alteration of the global nitrogen cycle: causes and consequences. *Ecological Applications* **7**: 737–750.

Vitousek P.M., Hättenschwiler S., Olander L., Allison S., 2002. Nitrogen and Nature. *Ambio* **31**(2): 97–101.

Vitousek P.M., and Howarth R.W., 1991. Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* **13**: 87–115.

Walther G.R., 2003. Plants in a warmer world. *Perspectives Plant Ecology* **6**: 169–185.

Weih M., and Karlsson P.S., 2002. Low winter soil temperature affects summertime nutrient uptake capacity and growth rate of mountain birch seedlings in the subarctic, Swedish Lapland. *Arctic, Antarctic and Alpine Research* **34**(4): 434-439.

White T.C.R. 1993. *The Inadequate Environment - Nitrogen and the abundance of animals*. Springer-Verlag, New York.

Wright R., and Van Breemen N., 1995. The nitrex project: an introduction. *Forest Ecology and Management* **71**: 1-6.

Chapter 2

Interactive effects of warming and increased nitrogen deposition on net nitrogen mineralization and leaching losses

2.1 Introduction

In addition to the direct effects of climate warming on ecosystem productivity (Chapin *et al.*, 2002; Walther, 2003), climate warming may alter productivity indirectly by influencing soil nitrogen (N) dynamics (Vitousek *et al.*, 1997; Rustad *et al.*, 2001; Shaw and Harte, 2001; Henry, 2008). Increased rates of N mineralization driven by climate warming may increase plant N availability (Sierra, 1997; De Valpine and Harte, 2001; Rustad *et al.*, 2001). Coupled with an extended growing season, a warming-induced increase in N mineralization could stimulate plant growth in N limited systems, provided water is not limiting (Vitousek *et al.*, 1997). Most studies of N mineralization in the context of warming have taken place over the plant growing season, whereas seasonal variation in interactions between soil N dynamics and warming may change the overall effects of warming on N availability (Jamieson *et al.*, 1999; Sturm *et al.*, 2005; Aerts *et al.*, 2006; Koch *et al.*, 2007; Henry, 2008). Over winter, microbial activity and N cycling continue at temperatures below freezing (Brooks *et al.*, 1998; Herrmann *et al.*, 2002; Lipson *et al.*, 2002; Mikan *et al.*, 2002; Schmidt and Lipson, 2004; Monson *et al.*, 2006; Miller *et al.*, 2007), therefore winter climate can play a critical role in the retention or loss of biologically important nutrients (Schimel *et al.*, 1996, 2004; Kielland *et al.*, 2006). Warming over winter, combined with microbial carbon limitation at this time, can increase N mineralization rates (Rustad *et al.*, 2001; Mikan *et al.*, 2002; Aerts *et al.*,

2006). In addition, an increased frequency of soil freeze-thaw cycles (FTC) over winter (Groffman *et al.*, 2001; IPCC, 2007; Henry, 2008) can promote the physical disruption of soil aggregates, and the lysis of microbial cells and fine roots, leading to increased soluble N concentrations in soil (Schimel and Clein 1996; Groffman *et al.*, 2001; Tierney *et al.*, 2001; Matzner and Borken, 2008), although the effects of FTC on soil N release are minimal in some systems (Lipson and Monson, 1998; Lipson *et al.*, 2000; Nielsen *et al.*, 2001; Herrmann *et al.*, 2002; Grogan *et al.*, 2004). Plant N uptake largely diminishes over winter due to root dormancy and cold temperatures (Cumbus and Nye, 1982; Macduff *et al.*, 1987; Clarkson *et al.*, 1992; Laine *et al.*, 1994). Therefore, in the absence of substantial N immobilization by soil microorganisms over winter, soluble N that accumulates in soil over this period may be lost as leachate or as trace gases during spring melt (Hobbie and Chapin, 1996; Vitousek *et al.*, 2002).

Many studies of soil N dynamics over winter have been conducted in the arctic or in alpine regions (e.g. Clein *et al.*, 1995; Schimel *et al.*, 1996, 2003, 2004, 2005; Grogan *et al.*, 2003, 2004; Larsen *et al.*, 2002, 2007; Edwards *et al.*, 2006; Buckeridge and Grogan, 2008), which are projected to experience a large degree of climate warming over the next century (Plummer *et al.*, 2006; IPCC, 2007). The over-winter N dynamics of northern temperate systems have received less attention (Campbell *et al.*, 2005).

Although temperate systems are expected to experience less severe warming over winter, many northern temperate ecosystems experience several months of freezing temperatures over winter, and the soils in these systems may be particularly vulnerable to increases in the frequency of FTC with warming since they remain close to the freezing point over much of winter (Henry, 2008). These systems are also experiencing increasingly high

rates of N deposition (Asner *et al.*, 2001; Galloway *et al.*, 2004), and the extent to which these systems can retain this added nitrogen under future climate scenarios has important implications for primary productivity and plant species composition (Heunneke *et al.*, 1990; Tilman and Downing, 1994; Vitousek *et al.*, 1997).

In this study, I examined the interactive effects of climate warming and N deposition on N mineralization and N leaching losses in a temperate old field. Warming treatments were applied either year-round or exclusively during the winter using overhead infrared heaters, and these treatments were crossed with a N addition treatment in a factorial design. While the all-year warming treatment was applied to simulate projected warming scenarios for this region, the winter-only warming treatment was designed to isolate the effects of winter warming from the year-round effect on soil N dynamics. I hypothesized that warming would increase net N mineralization over all seasons, potentially leading to increased N leaching losses over winter. I also predicted that winter warming would increase N leaching in N-addition plots, partially offsetting N enrichment.

2.2 Methods

2.2.1 Site description

My field research was conducted in a former agricultural field at the Agriculture Canada Southern Crop Protection and Food Research Centre, in London Ontario (43° 01' 46" N, 81° 12' 52" W). The site has not been ploughed, fertilized, or mowed for over 20 years. The soil is classified as "well to imperfect drained silt loam glacial till" (Hagerty and Kingston, 1992), and pH is approximately 7.5 (Terry Bell, *unpublished data*). Mean annual temperature in this region was 6.3 °C and mean annual precipitation was 818.3 mm for the period of experimentation (Environment Canada, National Climate Data and Information Archive). Ambient air temperature and snow cover over the duration of the collection periods are displayed in Figure 2.1 A, B. The vegetation at the site is dominated by the perennial grasses *Poa pratensis* (L.) and *Bromus inermis* (L.), while the forb *Cirsium arvense* (L.) and the legume *Lotus corniculatus* (L.) are also common, but patchy. The forbs *Asclepias syriaca* (L.), *Aster ericoides* (L.), *Solidago altissima* (L.) and the mosses *Fissidens taxifolius* Hedw. and *Brachythecium salebrosum* (Web and Mohr) BSG are also present at lower density.

2.2.2 Design of main warming and nitrogen addition experiment

The field manipulations consist of three warming treatments (warming all year, warming only in winter {from late fall after plant senescence until spring melt}, and control) factorially crossed with two N treatments (added N and control), in a block design (n=10) (Figure 2.2). Plots are heated by single 150 W ceramic infrared heaters (Zoo-Med Laboratories, San Luis Obispo, CA, USA), suspended 50 cm above the plot

centers. These heaters simulate solar heating without giving off photosynthetically active radiation (Harte *et al.*, 1995). The temperature in warmed plots is raised (or lowered, depending on season) by between 1 to 4 °C 1 cm below the soil surface (Figure 2.1 Ci, ii). In fertilized plots, a pulse of aqueous ammonium nitrate is added in the early spring at a rate of 2 g m⁻² y⁻¹ and ammonium nitrate is also added at a rate of 4 g m⁻² y⁻¹ in late spring in the form of slow release pellets (Florikan ESA, Sarasota, FL, USA; see Figure 2.1 for addition dates). These addition rates are designed to simulate projected increases in atmospheric deposition over the next century (Galloway *et al.*, 2004). Warming, which began in November 2006, is monitored by underground temperature probes (107-BAM, Campbell Scientific Canada Corp., Edmonton, AB, Canada) at 1 and 5 cm depth (only data for 1 cm depth is presented). These data are used to quantify the frequency of freeze-thaw cycles and the temperature difference between warmed and ambient plots is shown in Figure 2.1 Ci, ii. Soil moisture probes (CS616-L TDR probes, Campbell Scientific Canada Corp., Edmonton, AB, Canada) also record integrated measurements from depths of 0-15 cm and 0-30 cm. All soil temperature and moisture data are recorded year-round, on an hourly basis.

Figure 2.1 Measurements of **A)** daily air temperatures, **B)** daily snow cover at the London airport, **Ci)** ambient soil temperatures at 1 cm depth and **Cii)** the effect of yr-round and winter warming treatments on soil temperature at 1 cm depth for the duration of the experiment (when warming treatment lines are above or below the line crossing at zero, this represents the degrees C by which they are warmer or colder than the ambient plots). Black arrows on **A)** indicate placement (down arrow) and removal (up arrow) of mineralization resin cores and white arrows on **B)** indicate N additions. Ammonium nitrate was added in liquid form during spring melt periods and as slow-release pellets before the summer for a total of $6 \text{ g N m}^2 \text{ y}^{-1}$. The * symbols on **A)** represent lysimeter sample collections. Lysimeter samples were not collected during freezing temperatures in the winter and during dry conditions in the summer. Samples were not collected during fall 2007 because soil conditions were too dry.

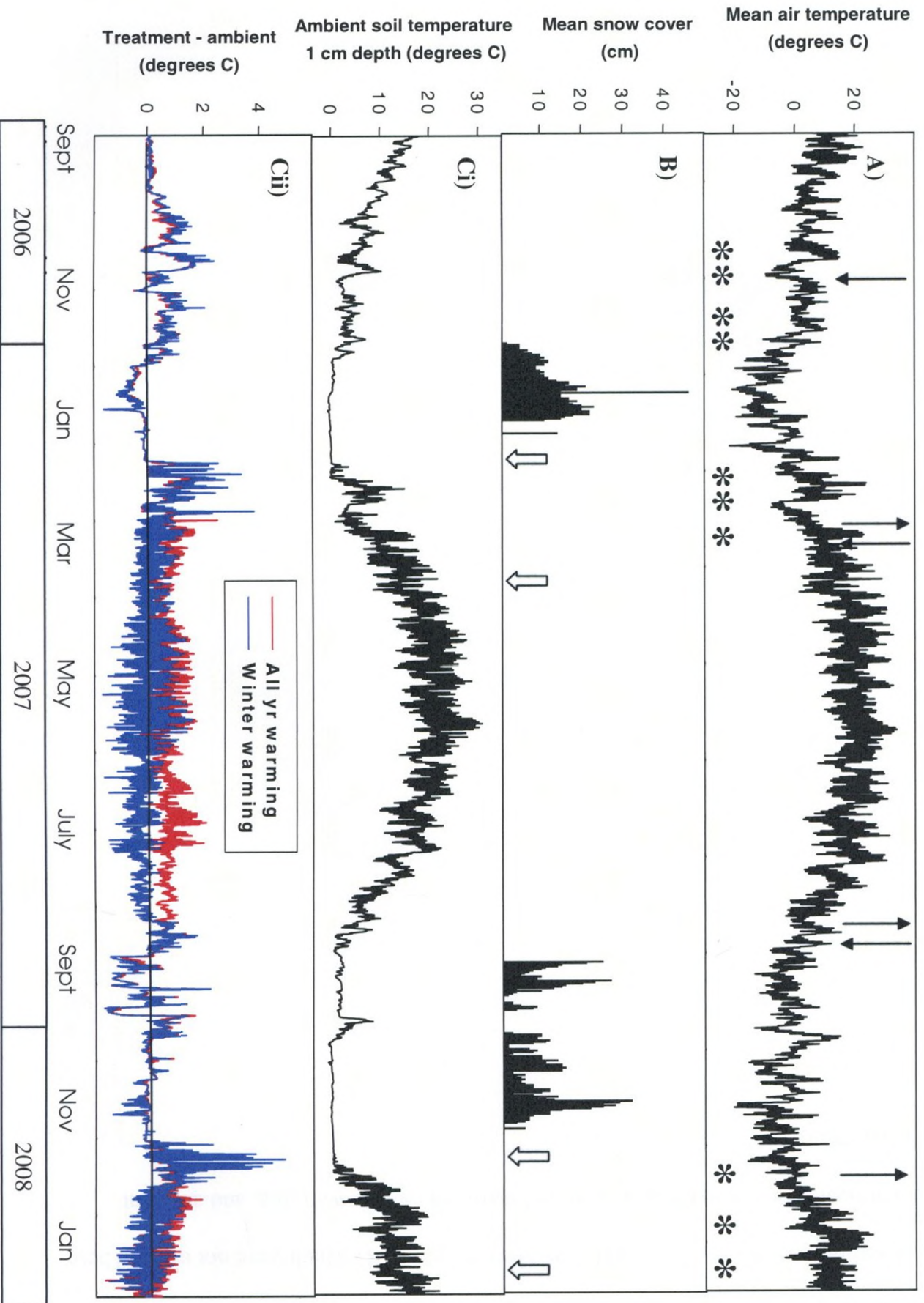
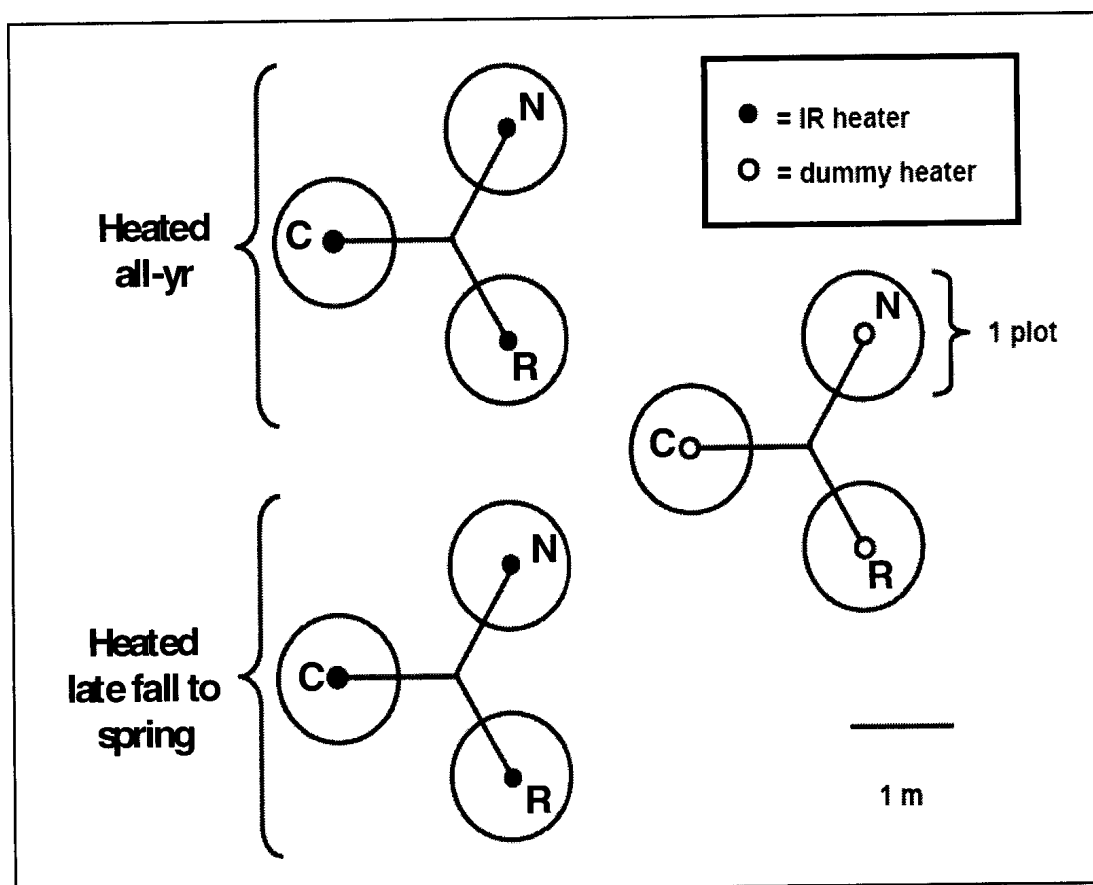


Figure 2.2 Experimental design showing 1 of 10 blocks. Plots consist of those receiving N additions (N), control plots (C), and reserve plots (R) which were not used. These treatments are crossed with year-round warming, winter warming, and ambient temperatures.



2.2.3 Net N mineralization

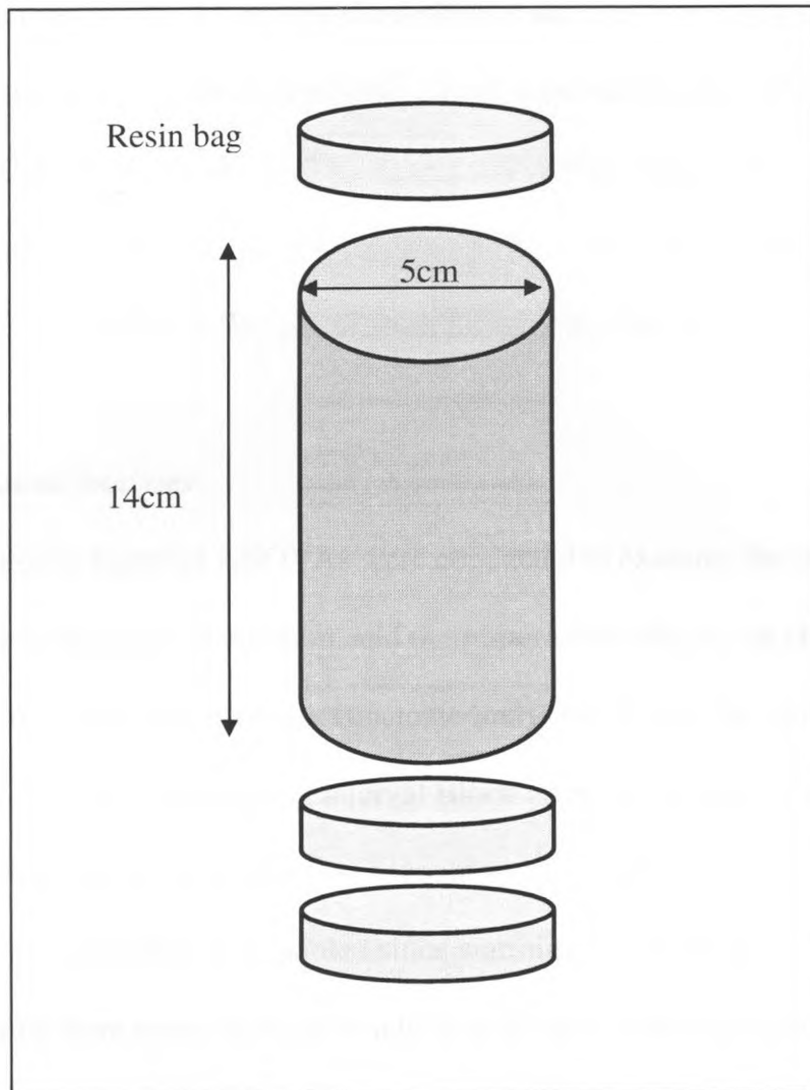
I used *in situ* soil cores, encased in 4.3 cm diameter × 15 cm deep PVC tubes, to quantify net N mineralization and N leaching through surface soils in response to the warming and N treatments. I placed one resin bag on top of each core to deionize deposition from above and placed two resin bags on the bottom, with the upper one capturing ions leaching from the soil core and the lower one deionizing water entering the tube from below (Figure 2.3). In each bag, I combined approximately 2.5 g of a sulfonic acid-based cation resin (HCR-W2, H⁺ form) with 2.5 g of a trimethylbenzyl ammonium-based anion resin (21 K, Cl⁻ form) (Dow Chemical Company, Calgary, Alberta). I based these combinations on wet exchange capacities (1.3eq/l anion to 1.8eq/l for cation) and activated the resins by soaking them in 5 M NaCl overnight to remove the H⁺ by loading them with Na⁺ (Hanselman, *et al.*, 2004; Szillery *et al.*, 2006). Reference soil samples were collected before each core incubation to quantify “time zero” extractable ammonium and nitrate.

I placed one soil core in each of the 60 plots on 27 November 2006 to quantify net N mineralization over winter. I removed these cores on 18 April 2007, following spring melt. After removal, I analyzed the cores, rebuilt them using fresh soil from within the treated plot area, then returned them to the plots within 48 hours to measure net N mineralization during the growing season. I collected and replaced the cores again on 15 November 2007 and collected them for the final time on 24 March 2008 to assess N mineralization over the following winter (Figure 2.1 A).

I extracted a 15 g subsample of soil from each core using 75 ml of 2 M KCl for one hour, then filtered the extracts through pre-leached Whatman No. 1 filter paper and

froze them. I later analyzed the extracts colorimetrically for nitrate and ammonium using a Westco SmartChem 140 discrete auto-analyzer (EPA Methods 1213N-0405C and 1376N-0405C, Westco Scientific Instruments Inc). I discarded both outer resin bags and extracted the inner-bottom resin bag using 2 M KCl for 30 min followed by the same method of analysis as stated above for soil. The post-incubation extractable ammonium and nitrate from the soil and inner bottom resin bag minus the pre-incubation extractable ammonium and nitrate from the soil was used to estimate net N mineralized (Weaver *et al.*, 1994; Hanselman, *et al.*, 2004).

Figure 2.3 Design of mineralization cores. Outer resin bags deionize throughput; extractable NH_4^+ and NO_3^- from the soil and inner-bottom resin bag, along pre-incubation soil N quantities, are used to estimate net N mineralization.



2.2.4 Soil N leaching - lysimeter sampling

PTFE-quartz suction lysimeters (Prenart Equipment ApS, Frederiksberg, Denmark) were also installed in each plot, at a depth of 50 cm, to measure N leaching losses below the rooting zone in response to the treatments (sample collection dates indicated in Fig. 2.1 A). I analyzed the lysimeter samples colorimetrically for nitrate and ammonium using the methods described above. I estimated total soluble N by persulfate oxidation (Cabrera and Beare, 1993). I used a persulfate reagent to convert all N in my samples to nitrate, followed by analysis for nitrate. I determined total soluble organic N by subtracting ammonium and nitrate from the total soluble N.

2.2.5 Statistical Analyses

Two-way factorial ANOVAs were conducted to examine the main effects of warming treatments and N addition, and their interactive effects, on changes in soil extractable inorganic N and resin extractable inorganic N, and the sum of the two (net N mineralization) for each sampling interval. Block number was included in the ANOVA model as a random factor to take into account variation among blocks. For winter 2007, both warming treatments were pooled since warming started in late fall 2006 and the nitrogen terms were removed since N addition did not commence until spring 2007. Values were log-transformed to improve normality and homogeneity of variance. Tukey's HSD tests were used to resolve differences among treatment combinations following a significant ANOVA result ($P < 0.05$).

For the lysimeter data, three-way factorial ANOVAs were conducted to examine the effects of warming, N addition and sampling date on concentrations of nitrate,

ammonium, soluble organic N and total organic N within each of the three main sampling periods. Block and plot number were also included as random factors. Values were log-transformed to improve normality and homogeneity of variance. Tukey's HSD tests were used to resolve differences among treatment combinations following a significant ANOVA result ($P < 0.05$). All statistical analyses were conducted using JMP 4.0 (SAS Institute Inc.).

2.3 Results

2.3.1 Soil N mineralization cores and resin bags

Overall, treatment effects on both changes in soil extractable inorganic N (Fig. 2.4 A) and resin extractable inorganic N (Fig. 2.4 B) from the soil mineralization cores varied both seasonally and among years. While warming significantly reduced increases in soil extractable inorganic N over winter in 2007 ($P_{\text{warm}}=0.049$; Fig 2.4 A-i), only winter warming significantly decreased (under ambient N) or increased (under added N) soil extractable inorganic N in 2008 ($P_{\text{warm}*N}=0.033$; Fig. 2.4 A-iii). Soil extractable inorganic N also increased significantly over summer in response to the winter warming treatment in 2007 ($P_{\text{warm}}<0.001$; Fig 2.4 A-ii). The relative contributions of nitrate and ammonium to changes in soil extractable inorganic N also varied seasonally (Table 2.1). The accumulation of ammonium was responsible for the overall increase in soil inorganic N over winter in 2007, yet elevated inorganic N in the warmed plots prior to the incubations was responsible for the significant treatment effect on changes in soil extractable inorganic N displayed in Fig 2.4 A-i (Table 2.1). While ammonium also accumulated in the soil in general over winter in 2008, decreases in nitrate and large increases in ammonium over the incubation period were responsible, respectively, for the significant decrease (under ambient N) and increase (under N addition) in soil extractable inorganic N displayed in response to winter warming in Fig. 2.4 A-iii (Table 2.1). The increases in soil extractable inorganic N over summer in winter warmed plots, displayed in Fig. 2.4 A-ii, were explained primarily by increased nitrate (Table 2.1).

Inorganic N extracted from resin bags positioned at the bottoms of the soil mineralization cores was substantially higher over summer than in the two winters (Fig.

2.4 B). Winter warming approximately doubled the inorganic N captured by the resin bags over summer ($P_{\text{warm}} < 0.018$; Fig 2.4 B-ii). While there were no significant warming effects over winter 2007 (Fig. 2.4 B-i), warming over winter 2008 increased resin extractable inorganic N significantly ($P_{\text{warm}} = 0.004$; Fig 2.4 B-iii). Nitrate accounted for 94%, 99% and 86% of the total inorganic extracted from the resins incubated over winter 2007, summer 2007 and winter 2008, respectively (data not shown).

When changes in soil extractable inorganic N and resin extractable inorganic N were combined to obtain estimates of net N mineralization (e.g. the sum of a given bar in Fig. 2.4 A with the bar directly below it in Fig. 2.4 B), there were no significant treatment effects over winter 2007 ($P_{\text{warm}} = 0.17$) or winter 2008 ($P_{\text{warm}} = 0.11$, $P_{\text{N}} = 0.32$ and $P_{\text{warm} \times \text{N}} = 0.17$). However, net N mineralization in the winter warmed plots was significantly higher than in the other treatments over summer 2007 ($P_{\text{warm}} = 0.007$).

Figure 2.4 A) changes in soil extractable inorganic N and **B)** resin-captured inorganic N from soil N mineralization cores incubated over i) winter 2007 (27 Nov. - 18 Apr.), ii) summer 2007 (28 Apr. - 15 Nov.) and iii) winter 2008 (22 Nov. - 24 Mar.). Means and standard errors are presented, with different lower case letters indicating significant differences among treatments within a date ($P < 0.05$, Tukey's HSD test). $N = 60$, with data pooled across treatments only when significant differences were not present.

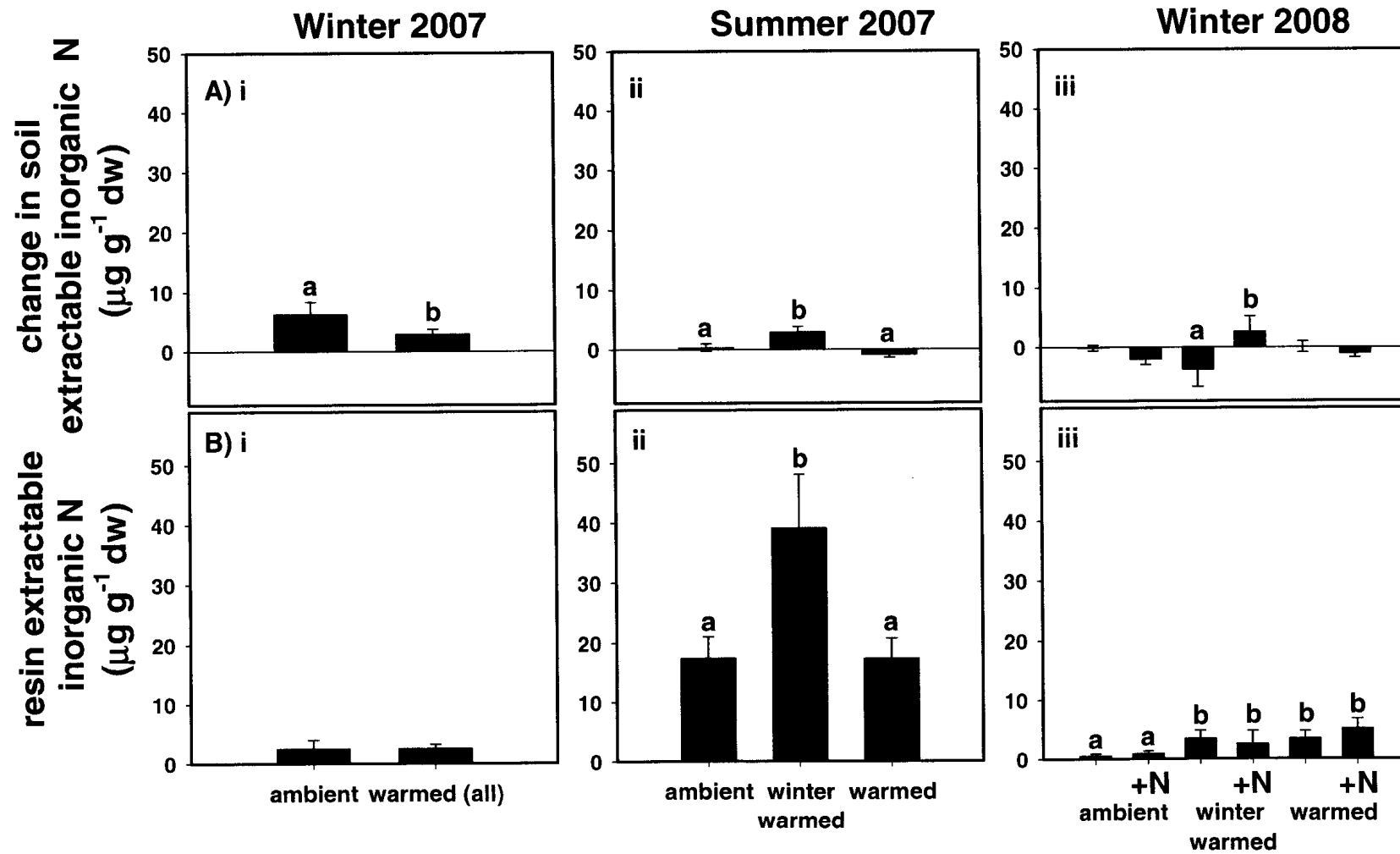


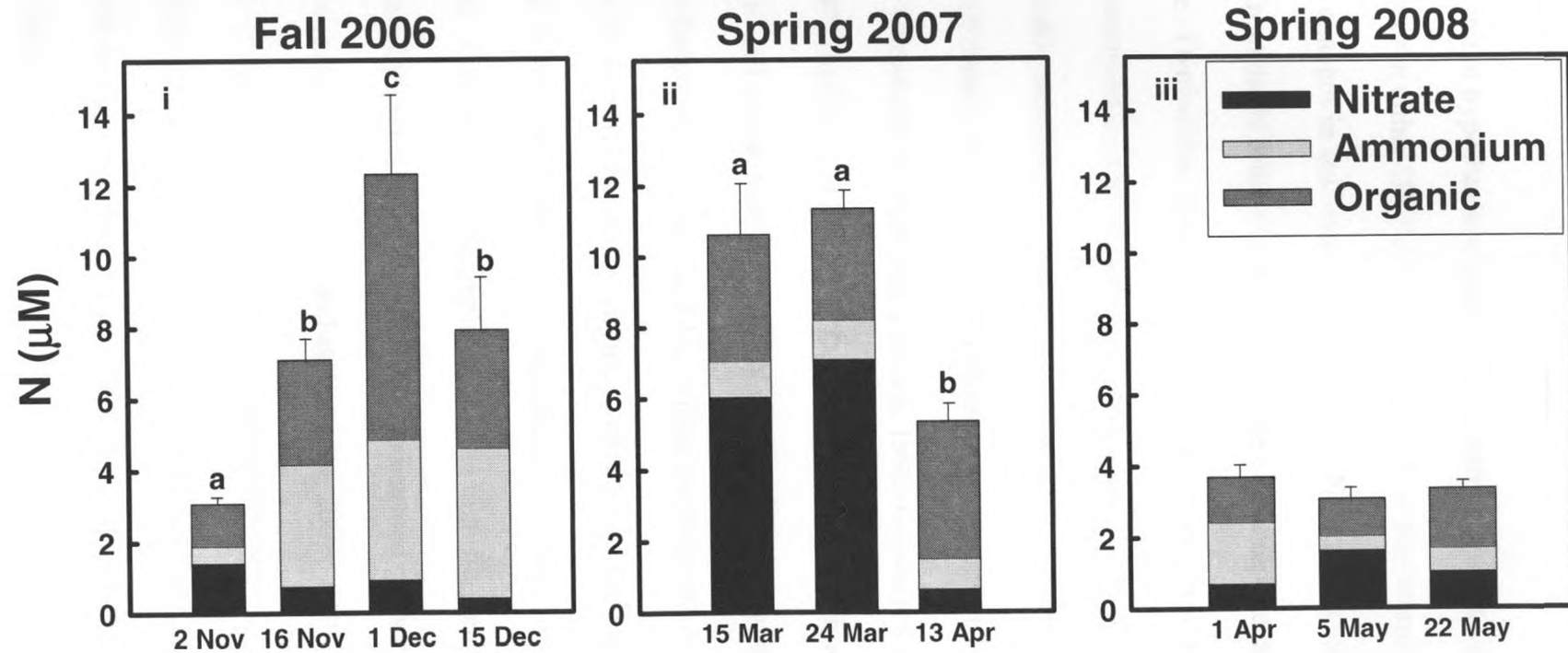
Table 2.1 Extractable nitrate and ammonium from soil mineralization cores both pre-incubation (initial) and post-incubation (final), expressed per unit of soil dry weight. Cores were incubated over winter 2007 (22 Nov. - 18 Apr), summer 2007 (28 Apr. - 16 Nov.) and winter 2008 (22 Nov. - 31 Mar.). Standard errors for each mean are provided in parentheses. Different lower case letters indicate significant differences among treatments within a date (Tukey's HSD test, $P < 0.05$). Data were only pooled across treatments (indicated by 'all') if differences among groups were not significant.

	Treatment		n	Initial NO ₃ ⁻		Final NO ₃ ⁻		Initial NH ₄ ⁺		Final NH ₄ ⁺	
	Warming	Nitrogen		(μg g ⁻¹ dw)		(μg g ⁻¹ dw)		(μg g ⁻¹ dw)		(μg g ⁻¹ dw)	
Winter 2007	ambient	all	20	0.56	(0.05) ^a	0.78	(0.09)	0.78	(0.09) ^a	6.87	(2.01)
	warmed (all)	all	40	0.87	(0.11) ^b	1.27	(0.33)	1.13	(0.09) ^b	3.59	(0.78)
Summer 2007	ambient	all	20	0.75	(0.05)	2.14	(0.69) ^a	1.63	(0.28) ^a	0.49	(0.07)
	winter warmed	all	20	0.66	(0.04)	4.58	(0.90) ^b	1.67	(0.16) ^{ab}	0.71	(0.22)
	warmed	all	20	0.80	(0.08)	1.55	(0.23) ^{ab}	2.20	(0.29) ^b	0.52	(0.10)
Winter 2008	ambient	ambient	10	2.11	(0.61)	0.89	(0.09) ^a	0.59	(0.18)	1.65	(0.59) ^{ab}
		NH ₄ NO ₃	10	3.26	(0.91)	0.93	(0.09) ^a	0.61	(0.25)	0.88	(0.15) ^a
	winter warmed	ambient	10	6.10	(2.52)	1.75	(0.63) ^b	0.55	(0.08)	1.09	(0.21) ^{ab}
		NH ₄ NO ₃	10	3.78	(1.07)	3.01	(1.22) ^b	0.52	(0.12)	3.89	(1.07) ^b
	warmed	ambient	10	1.83	(0.57)	1.13	(0.25) ^{ab}	0.67	(0.16)	1.40	(0.47) ^{ab}
		NH ₄ NO ₃	10	2.84	(0.68)	1.13	(0.18) ^{ab}	1.10	(0.24)	1.73	(0.38) ^{ab}

2.3.2 *Soil lysimeter N*

There were no significant treatment effects on nitrate, ammonium, soluble organic N or total soluble N collected from soil lysimeters in fall 2006, spring 2007 or spring 2008. However, there were significant differences in total soluble N among seasons and among sampling dates within seasons (Fig. 2.5). Total soluble N increased across the first three sampling dates in fall 2007, explained primarily by increased soluble organic N and ammonium (Fig. 2.5 i). It decreased across sampling dates in spring 2007, explained primarily by decreased nitrate (Fig. 2.5 ii). Total soluble N concentrations were also lower in spring 2008 than in spring 2007 (Fig. 2.5 ii,iii).

Figure 2.5 Concentrations of nitrate (black bars), ammonium (light gray bars) and organic N (dark gray bars) in solution collected from 50 cm deep soil lysimeters in i) fall 2006, ii) spring 2007 and iii) spring 2008, pooled across all treatments (n=60). Error bars denote standard error for the mean total soluble N (the sum of nitrate, ammonium and organic N), and different lower case letters indicate differences among dates in total soluble N within a season ($P < 0.05$, Tukey's HSD).



2.4 Discussion

2.4.1 Net N mineralization

Contrary to my initial hypothesis, experimental warming did not increase net N mineralization over winter in either 2007 or 2008. However, winter warming did significantly alter both changes in soil extractable inorganic N and resin extractable inorganic N collected from the N mineralization cores, the two components of the N mineralization estimate. Over winter 2007, decreased temperatures in warmed plots, which occurred as a consequence of increased snow melt, coincided with diminished increases in soil extractable inorganic N relative to ambient temperature plots. This result is consistent with overall decreases in net N mineralization in response to colder soil temperatures observed elsewhere (Emmer and Tietema, 1990; Goncalves and Caryle, 1994; Reich *et al.*, 1997; Groffman *et al.*, 2001; Decker *et al.*, 2003; Schimel *et al.*, 2004). Furthermore, changes in soil extractable inorganic N were higher over winter 2007 than in 2008, which reflects the unusually warm and late winter experienced in 2007. Over winter 2008, there was no overall effect of warming on changes in soil extractable inorganic N, and unlike winter 2007, there was a significant effect of warming on resin extractable inorganic N. The latter may be explained by the high frequency of FTC experienced over winter 2008 relative to 2007 (~5 FTC in warmed plots in comparison to ~2 mild FTC in ambient plots during winter and early spring 2007; >10 FTC in warmed plots in comparison to ~3 mild FTC in ambient plots during winter and early spring 2008), as FTC may increase N leaching losses due to microbial lysis (Yanai *et al.*, 2004; Larsen *et al.*, 2002), root damage (Fitzhugh *et al.*, 2001) or the disruption of soil aggregates (Six *et al.*, 2004).

When reference samples were collected at time zero for the net N mineralization cores, it was unexpected that there was no increase in extractable inorganic N in samples collected from N addition plots. This lack of N enrichment in the soluble N pool could be attributed either to rapid uptake by plants or to losses from the system following application of the ammonium nitrate. By multiplying the increase in plant tissue % N in N addition plots (0.5% - see Chapter 3) by mean plant biomass in these plots (682 g m^{-2} - Jennifer Hutchison, *unpublished data*), I estimate that approximately 3 g m^{-2} (half of the added N) was taken up by plants. Concentrations of N in leachate collected in the soil lysimeters were not significantly higher in N addition plots than in ambient N plots. N trace gas losses may contribute substantially to ecosystem N losses and can be particularly high over winter or in early spring (Groffman *et al.*, 2001; Grogan *et al.*, 2004; Matzner and Borken, 2008). However, N trace gas losses were not accounted for in my estimates of net N mineralization.

Overall, net N mineralization over summer was much higher than over winter. The most striking result of the net N mineralization experiment was the large effect of winter warming relative to ambient and year-round warming on resin extractable inorganic N over summer. This result was unexpected, and could not be explained directly by warming since the heating in the winter warmed plots corresponded with that of the year round warming plots over winter and the ambient plots over summer. Likewise, there were no significant differences among winter warmed plots and the other plots with respect to plant biomass or species composition in 2007 (Jennifer Hutchison, *unpublished data*). Instead, net N mineralization in the winter warmed plots may have increased as a result of the extended cool period that occurred in these plots following

spring melt when their heaters were turned off (e.g. winter warmed plots thawed early but then, unlike the year round heated plots, ceased to be warmed). A possible artifact of the winter warming treatment was that soil mineralization may have increased after thaw at a time when soils were still too cool for substantial plant N uptake (Henry and Jefferies 2003; Castle *et al.*, 2006). However, I collected soil for the summer net N mineralization cores on 20 April, nine days after the heaters were turned off in the winter warmed plots, and extractable inorganic N was not elevated in the winter warmed plots at this time. Likewise, while soil protease activity may increase in response to soil warming in early spring (Rejsek *et al.*, 2008), soluble organic N was not elevated in winter warmed plots in early spring (Terry Bell, *unpublished data*). Given that the restructuring of microbial communities can introduce or accelerate biogeochemical processes (Schimel and Gullledge, 1998; Scherer-Lorenzen *et al.*, 2003; Schmidt and Lipson, 2004), carryover effects of this prolonged cool period on the soil microbial community in winter warmed plots might explain increased inorganic N captured by the resin over summer. Alternatively, mosses present at the soil surface may have been damaged by freeze-thaw activity during the early spring in winter warmed plots. This moss-derived N may have leached through the mineralization cores at a later date, although the tolerance of mosses to freezing is often high (Sakai, 1961; Rutten and Santarius, 1992; Nagao *et al.*, 2006). In addition, mosses *Fissidens taxifolius* and *Brachythecium salebrosum* were not dominant species and were unevenly distributed throughout the site.

Ultimately, although the mechanism behind the winter warming effect remain unclear, this response demonstrates how changes in soil temperatures in early spring can have important carry over effects on N dynamics over the plant growing season.

However, the implications of the observed increase in resin N over summer for soil N losses in winter warmed plots are unclear, particularly since this increased mineral N may have been either leached through the soil column or captured over summer by live plant roots, which were absent from the net N mineralization cores.

2.4.2 Soil N leaching - lysimeter sampling

As discussed above, the result that low concentrations of soluble N were present in lysimeter samples collected at 50 cm depth, even in N addition plots, indicates that the bulk of N deposition in these plots is either intercepted by plant roots, microbes and soil at more shallow depths, or lost to the atmosphere in the form of N trace gases. In addition, contrary to my initial hypothesis, it did not appear that winter warming enhanced N leaching losses in this system. While it is possible that large but transient pulses of N may have been missed in the intervals between lysimeter sampling events, such pulses are often expected to occur at spring thaw (Lipson *et al.*, 2000, 2002; Grogan and Jonasson, 2003; Schmidt and Lipson, 2004), and we did not detect an N pulse at this time in either year. Furthermore, soil water penetration to 50 cm depth over both summer and fall of 2007 was insufficient for the collection of lysimeter samples.

The seasonal trends in lysimeter sample soluble N concentrations across all treatments appeared to correspond with plant root activity. For example, decreasing root N uptake in late fall is consistent with higher N leaching losses (MacDuff *et al.*, 1987; Chapin *et al.*, 2002; Laine *et al.*, 2004), and the proportional increase in soluble organic N over the fall may be driven by plant senescence and the loss of soluble compounds from plant litter (Aber and Melillo, 1980; Melillo *et al.*, 1989). Similarly, increased root

activity upon soil warming in spring (Henry and Jefferies 2003; Castle *et al.*, 2006) explains the decrease in soluble N concentration by late spring 2007. Decreased nitrate leaching as spring progressed was further consistent with increased plant uptake (Scherer-Lorenzen *et al.*, 2003). Following a winter with high snow fall, soluble nitrate concentrations in lysimeter samples were very low throughout spring 2008. Therefore, the low lysimeter N concentrations in spring 2008 likely represent a dilution effect.

2.5 Conclusions

Overall, my results reveal that warming does not increase net N mineralization over winter because of colder soil temperatures and an increased frequency of FTC. In addition, the moderate FTC caused by warming over winter were not severe enough to increase N leaching losses from the system. Conversely, warming during the onset of spring may increase mineral N availability for plants during the growing season. However, this increase in mineral N is also susceptible to loss if it occurs before increases in plant N uptake levels.

2.6 References

- Aber J.D., and Melillo J.M., 1980. Litter decomposition: Measuring relative contribution of organic matter and nitrogen to forest soils. *Canadian Journal of Botany* **58**: 416-421.
- Aerts R., Cornelissen J.H.C., Dorrepaal E., 2006. Plant performance in a warmer world: general responses of plants from cold, northern biomes and the importance of winter and spring events. *Plant Ecology* **182**: 65-77.
- Asner G.P., Townsend A.R., Riley W.J., Matson P.A., Neff J.C., Cleveland C., 2001. Physical and biogeochemical controls over terrestrial ecosystem responses to nitrogen deposition. *Biogeochemistry* **54**: 1-39.
- Brooks P.D., Williams M.W., Schmidt S.K., 1998. Inorganic nitrogen and microbial biomass dynamics before and during spring snowmelt. *Biogeochemistry* **43**: 1-15.
- Buckeridge K., and Grogan P., 2008. Deepened snow alters soil microbial nutrient limitations in arctic birch hummock tundra. *Applied Soil Ecology* **39**: 210-222.
- Cabrera, M.L., and Beare M.H., 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Science Society of America Journal* **57**: 1007-1012.
- Campbell J.L., Mitchell M.J., Groffman P.M., Christenson L.M., Hardy J.P., 2005. Winter in northeastern North America: a critical period for ecological processes. *Frontiers of Ecology & the Environment* **3**(6): 314-322.
- Campbell Scientific Canada Corp., 2007. 107-BAM underground temperature probes, and CS616-L TDR soil moisture probes. Edmonton, AB, Canada. Cite online: <http://www.campbellsci.ca/>
- Castle M.L., Crush J.R., Rowarth J.S., 2006. The effect of root and shoot temperature of 8 degrees C or 24 degrees C on the uptake and distribution of nitrogen in white clover (*Trifolium repens* L.). *Australian Journal of Agricultural Research* **57** (5): 577-581.
- Chapin, F.S. III, Matson P.A., Mooney H.A., 2002. Principles of terrestrial ecosystem ecology. Springer, New York, N.Y., U.S.A. pp. 197-223.
- Clarkson D.T., Jones L.H.P., Purves J.V., 1992. Absorption of nitrate and ammonium ions by *LoHum perenne* from flowing solution cultures at low root temperatures. *Plant, Cell and Environment* **15**: 99-106.
- Clein J.S., and Schimel J.P., 1995. Microbial activity of tundra and taiga soils at subzero temperatures. *Soil Biology & Biochemistry* **27**: 1231-1234.

Cumby I.P., Nye P.H., 1982. Root zone temperature effects on growth and nitrate absorption in rape (*Brassica napus* cv. Emerald). *Journal of Experimental Botany* **33**: 1138-1146.

Decker K.L.M., Wang D., Waite C., and Sherbatskoy T., 2003. Snow removal and ambient air temperature effects of forest soil temperatures in northern Vermont. *Soil Science Society of America Journal* **67**: 1234-1242.

De Valpine P., and Harte J., 2001. Plant responses to experimental warming in a montane meadow. *Ecology* **82**(3): 637-648.

Dow Chemical Company., 2007. Dowex Ion Exchange Resins. Calgary AB Canada. Properties Table cited online: <http://www.serva.de/products/knowledge/071119.shtml>

Edwards K.A., McCulloch J., Kershaw P.G., Jefferies R.L., 2006. Soil microbial and nutrient dynamics in a wet Arctic sedge meadow in late winter and early spring. *Soil Biology & Biochemistry* **38**: 2843-2851.

Emmer I.M., and Tietema A., 1990. Temperature-dependent nitrogen transformation in acid oak-beech forest litter in the Netherlands. *Plant and Soil* **122**: 193-196.

Environment Canada, 2008. National Climate Data and Information Archive cited online: http://www.climate.weatheroffice.ec.gc.ca/advanceSearch/searchHistoricData_e.html?timeframe=1&Prov=XX&StationID=99999&Year=2008&Month=8&Day=20&errMessage

Fitzhugh R.D., Driscoll C.T., Groffman P.M., Tierney G.L., Fahey T.J., Hardy J.P., 2001. Effects of soil freezing, disturbance on soil solution nitrogen, phosphorus, and carbon chemistry in a northern hardwood ecosystem. *Biogeochemistry* **56**: 215-238.

Florikan ESA., 2007. Fertilizer: slow release ammonium nitrate pellets. Sarasota, FL, USA.

Galloway J.N., Dentener F.J., Capone D.G., Boyer E.W., Howarth E.W., Seitzinger S.P., Asner G.P., Cleveland C.C., Green P.A., Holland E.A., Karl D.M., Michaels A.F., Porter J.H., Townsend A.R., Vorosmarty C.J., 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* **70**: 152-226.

Goncalves J.L.M., and Caryle J.C., 1994. Modeling the influence of moisture and temperature on net nitrogen mineralization in a forested sandy soil. *Soil Biology and Biochemistry* **26**: 1557-1564.

Groffman P.M., Driscoll C.T., Fahey T.J., Hardy J.P., Fitzhugh R.D., Tierney G.L., 2001. Colder soils in a warmer world: A snow manipulation study in a northern hardwood forest ecosystem. *Biogeochemistry* **56**: 135-150.

Grogan P., and Jonasson S., 2003. Controls on annual nitrogen cycling in the understory of a subarctic birch forest. *Ecology* **84**(1): 202-218.

Grogan P., Michelsen A., Ambus P., Jonasson S., 2004. Freezethaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. *Soil Biology & Biochemistry* **36**: 641–654.

Hagerty T.P., and Kingston M.S., 1992. *The Soils of Middlesex County*. Ontario Ministry of Agriculture and Food.

Hanselman T.A., Graetz D.A., Obreza T.A., 2004. A comparison of *in situ* methods for measuring net nitrogen mineralization rates of organic soil amendments. *Journal of Environmental Quality* **33**: 1098-1105.

Harte J., Torn M.S., Chang F.R., Feifarek B., Kinzig A.P., Shaw R., Shen K., 1995. Global warming and soil microclimate – results from a meadow warming experiment. *Ecological Applications* **5**(1): 132-150.

Henry H.A.L., 2008. Climate change and soil freezing dynamics: historical trends and projected changes. *Climatic Change* **87**: 421–434.

Henry H.A.L., and Jefferies B., 2003. Interactions in the uptake of amino acids, ammonium and nitrate ions in the Arctic salt-marsh *Puccinellia phryganodes*. *Plant Cell & Environment* **26**(3): 419-428.

Herrmann A., and Witter E., 2002. Sources of C and N contributing to the flush in mineralization upon freeze–thaw cycles in soils. *Soil Biology & Biochemistry* **34**: 1495–1505.

Heunneke L.F., Hamburg S.P., Koide R., Mooney H.A., Vitousek P.M., 1990. Effects of Soil Resources on Plant Invasion and Community Structure in Californian Serpentine Grassland. *Ecology* **71** (2): 478-491.

Hobbie S.E., and Chapin III F.S., 1996. Winter regulation of tundra litter carbon and nitrogen dynamics. *Biogeochemistry* **35**: 327–338.

Intergovernmental Panel on Climate Change (IPCC), 2007. Contribution of working group I to the fourth assessment report of the IPCC: The physical science basis – Chapter 11: Regional climate projections. Cited online: <http://www.ipcc.ch/pdf/assessment-report/ar4/wg1/ar4-wg1-chapter11.pdf>

Jamieson N., Monaghan R., Barraclough D., 1999. Seasonal trends of gross N mineralization in a natural calcareous grassland. *Global Change Biology* **5**: 423-431.

Kielland K., Olson K., Ruess R.W., Boone R.D., 2006. Contribution of winter processes to soil nitrogen flux in taiga forest ecosystems *Biogeochemistry* **81**: 349–360.

- Koch O., Tschierko D., Kandeler E., 2007. Temperature sensitivity of microbial respiration, nitrogen mineralization and potential soil enzyme activities in organic alpine soils. *Global Biogeochemical Cycles* **21**(4): 233-245.
- Laine P., Bigot J., Ourry A., Boucaud J., 1994. Effects of low temperature on nitrate uptake, and xylem and phloem flows of nitrogen, in *Secale cereale* L., and *Brassica napus* L. *New Phytologist* **127**: 675-683.
- Larsen K. S., Jonasson S., Michelsen A., 2002. Repeated freeze-thaw cycles and their effects on biological processes in two arctic ecosystem types. *Applied Soil Ecology*, **21**: 187-195.
- Larsen K.S., Grogan P., Jonasson S., Michelsen A., 2007. Respiration and microbial dynamics in two subarctic ecosystems during winter and spring thaw: effects of increased snow depth. *Arctic, Antarctic and Alpine Research* **39**(2): 268-276.
- Lipson D.A., and Monson R.K., 1998. Plant-microbe competition for soil amino acids in the alpine tundra: effects of freeze thaw and dry-rewet events. *Oecologia* **113**: 406-414.
- Lipson D.A., Schmidt S.K., Monson R.K., 2000. Carbon availability and temperature control the post-snowmelt decline in alpine soil microbial biomass. *Soil Biology & Biochemistry* **32**: 441-448.
- Lipson D.A., Schadt C.W., Schmidt S.K., 2002. Changes in microbial community structure and function following snowmelt in an alpine soil. *Microbial Ecology* **43**: 307-314.
- Macduff H., Hopper M., Wild A., Trim F.E., 1987. Comparison of the effects of root temperature on nitrate and ammonium nutrition of oilseed rape in flowing solution culture. I. Growth and uptake of nitrogen. *Journal of Experimental Botany* **38**: 1104-1120.
- Matzner E., and Borken W., 2008. Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review *European Journal of Soil Science* **59**: 274-284.
- Melillo J.M., Aber J.D., Linkins A.E., Ricca A., Fry B., Nadelhoffer K., 1989. Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. *Plant and Soil* **115**: 189-198.
- Mikan C.J., Schimel J.P., Doyle A.P., 2002. Temperature controls of microbial respiration in arctic tundra soils above and below freezing. *Soil Biology & Biochemistry* **34**: 1785-1795.
- Miller A.E., Schimel J.P., Sickman J.O., Meixner T., Doyle A.P., Melack J.M., 2007. Mineralization responses at near-zero temperatures in three alpine soils. *Biogeochemistry* **84**: 233-245.

Monson R.K., Lipson D.L., Burns S.P., Turnipseed A.A., Delany A.C., Williams M.W., Schmidt S.K., 2006. Winter forest soil respiration controlled by climate and microbial community composition. *Nature* **439**: 711–714.

Nagao M., Oku K., Minami A., Mizuno K., Sakurai M., Arakawa K., Fujikawa S., Takezawa D., 2006. Accumulation of the androse in association with development of freezing tolerance in the moss *Physcomitrella patens*. *Phytochemistry* **67**: 702–709.

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

Plummer D.A., Caya D., Frigon A., Cote H., Giguere M., Paquin D., Biner S., Harvey R., de Elia R., 2006. Climate and climate change over North America as simulated by the Canadian Regional Climate Model. *Journal of Climate* **19**: 3112–3132.

Prenart Equipment ApS., 2007. Soil water samplers. Frederiksberg, Denmark. Product information cited online: <http://www.prenart.dk/sampler.php>

Reich P.B., Grigal D.F., Aber J.D., Gower S.T., 1997. Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. *Ecology* **78**: 335–347.

Rejsek K., Formanek P., Pavelka M., 2008. Estimation of protease activity in soils at low temperatures by casein amendment and with substitution of buffer by demineralized water Estimation of protease activity in soils at low temperatures by casein amendment and with substitution of buffer by demineralized water. *Amino Acids* **35** (2): 411–417.

Rustad L.E., Campbell J.L., Marion G.M., Norby R.J., Mitchell M.J., Hartley A.E., Cornelissen J.H.C., Gurevitch J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* **126**: 543–562.

Rutten D., and Santarius K.A., 1992. Relationship between frost tolerance and sugar concentration of various bryophytes in summer and winter. *Oecologia* **91**: 260–265.

Sakai A., 1961. Studies on the frost-hardiness of woody plants. The causal relation between sugar content and frost-hardiness. *Low Temperature Science. Plant Physiology* **21**: 1–40.

SAS Institute Inc. 2000. JMP Version 4.0.

Scherer-Lorenzen M., Palmborg C., Prinz A., Schulz E.D., 2003. The role of plant diversity and composition for nitrate leaching in grasslands. *Ecology* **84**(6): 1539–1552.

Schimel J.P., and Clein J.S., 1996. Microbial response to freeze-thaw cycles in tundra and taiga soils. *Soil Biology & Biochemistry* **28**: 1061–1066.

Schimel J.P., Bilbrough C., Welker J.M., 2003. The effect of increased snow depth on microbial activity and nitrogen mineralization in two Arctic tundra communities. *Soil Biology & Biochemistry* **36**: 217–227.

Schimel J.P., Bilbrough C., Welker J.A., 2004. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. *Soil Biology & Biochemistry* **36**: 217–27.

Schimel J.P., and Gulledge J., 1998. Microbial community structure and global trace gases. *Global Change Biology* **4**: 745–758.

Schimel J.P., and Mikan C., 2005. Changing microbial substrate use in Arctic tundra soils through a freeze-thaw cycle. *Soil Biology & Biochemistry* **37**: 1411–1418.

Schmidt S.K., and Lipson, D.A., 2004. Microbial growth under the snow: implications for nutrient and allelochemical availability in temperate soils. *Plant and Soil* **259**: 1–7.

Shaw M.R., and Harte J., 2001. Response of nitrogen cycling to simulated climate change: differential responses along a subalpine ecotone. *Global Change Biology* **7**(2): 193–210.

Sierra J., 1997. Temperature and soil moisture dependence of N mineralization in intact soil cores. *Soil Biology & Biochemistry* **29**: 1557–1563.

Six J., Bossuyt H., Degryse S, Denef K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil & Tillage Research* **79**: 7–31.

Sturm M., Schimel J., Michaelson G., Welker J.M., Oberbauer S.F., Liston G.E., Fahnestock J., Romanovsky V.E., 2005. Winter biological processes could help convert arctic tundra to shrubland. *Bioscience* **55**(1): 17–26.

Szillery J.E., Fernandez I.J., Norton S.A., Rustad L.E., White A.S., 2006. Using ion exchange resins to study soil response to experimental watershed acidification. *Environmental Monitoring and Assessment* **116**: 383–398.

Tierney G.L., Fahey T.J., Groffman P.M., Hardy J.P., Fitzhugh R.D., Driscoll C.T., 2001. Soil freezing alters fine root dynamics in a northern hardwood forest. *Biogeochemistry* **56**: 175–190.

Tilman D., and Downing J.A., 1994. Biodiversity and stability in grasslands. *Nature* **367**: 363–365.

Vitousek P.M., Aber J., Howarth R.W., Likens G.E., Matson P.A., Schindler D.W., Schlesinger W.H., Tilman G.D., 1997. Human alteration of the global nitrogen cycle: causes and consequences. *Ecological Applications* **7**: 737–750.

Vitousek, P.M., Cassman K., Cleveland C., Crews T., Field C.B., Grimm N.B., Howarth R.W., Marino R., Martinelli L., Rastetter E.B., Sprent J.I., 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* **57/58**: 1–45.

Vitousek P.M., Hättenschwiler S., Olander L., Allison S., 2002. Nitrogen and Nature. *Ambio* **31**(2): 97-101.

Walther G.R., 2003. Plants in a warmer world. *Perspectives Plant Ecology* **6**: 169–185.

Weaver R., Angle S., Bottomley P., Bezdicsek D., Smith S., Tabatabai A., Wollum A., 1994. SSSA: Book Series 5 - Methods of soil analysis. Part 2: Microbiological and biogeochemical properties. Soil Science Society of America Inc.

Westco, Scientific Instruments Inc., 2007. SmartChem 140 Method # 1213N-0405C for Ammonia; Method # 1381N-0405C for Nitrate.

Yanai Y., Toyota K., Okazaki M., 2004. Effects of successive soil freeze–thaw cycles on soil microbial biomass and organic matter decomposition potential of soils. *Soil Science & Plant Nutrition* **50**: 821–829.

Zoo-Med Laboratories, 2006. Ceramic infrared heaters. San Luis Obispo, CA, USA.

Chapter 3

Interactive effects of warming and increased nitrogen deposition on the recovery of ^{15}N tracers added at spring melt

3.1 Introduction

During the last century, fossil fuel emissions and agricultural activity have increased atmospheric reactive nitrogen deposition dramatically in temperate regions, and in North America, many temperate ecosystems are predicted to experience rates of atmospheric N deposition as high as 2 to 5 g m⁻² above pre-industrial rates over the coming century (Galloway *et al.*, 2004). Since most temperate systems are N limited, N deposition can increase productivity and biomass accumulation in the short term (Vitousek and Howarth, 1991); however, in addition to increasing N availability, N deposition has increased the mobility of N (Vitousek *et al.*, 1997) and it can alter the rates and pathways of N cycling and loss (Aber *et al.*, 1995).

The extent to which increased nitrogen inputs will drive changes in plant productivity and species composition over the next century will depend on how N deposition interacts with other influential global change factors, such as climate warming, to affect the N retention of ecosystems (Zavaleta *et al.*, 2003; Majdi and Ohrvik, 2004). By increasing annual production and extending the time over which roots remain highly active, warming may decrease soil N losses (Kasper and Bland, 1992; Chapin and Shaver, 1996; BassiriRad, 2000; Pregitzer *et al.*, 2000; Walker *et al.*, 2006). However, changes to winter climate may also strongly influence ecosystem N retention (Schimel and Clein, 1996, Schimel *et al.*, 2004; Kielland *et al.*, 2006). Warming over winter may increase

soil N mineralization (Miller *et al.*, 2007) although soils may still remain cold enough to restrict plant root activity (Henry and Jefferies 2003; Castle *et al.*, 2006). Reduced snow cover may also increase the frequency and intensity of soil freeze thaw cycles (Mellander *et al.* 2007; Henry, 2008), which can disrupt soil aggregates, lyse microbial cells and damage fine roots (Schimel and Clein 1996; Groffman *et al.*, 2001; Tierney *et al.*, 2001; Matzner and Borken, 2008). Both of these mechanisms contribute to an accumulation of soluble N in soil over winter, which could increase soil N leaching and trace gas losses during mid-winter melts or at spring thaw (Hobbie and Chapin, 1996; Schimel and Clein, 1996; Ludwig *et al.*, 2004). Although a large proportion of soil N dynamics research over winter has been restricted to arctic and alpine systems (Campbell *et al.*, 2005), there is evidence for over winter processes affecting annual N budgets in temperate forest systems (Fitzhugh *et al.*, 2001) and agricultural systems (Cookson *et al.*, 2001; Miltner *et al.*, 2004). The N dynamics of temperate systems may be highly sensitive to climate change over winter because their soils often remain close to freezing throughout this season (Henry, 2008).

While some plant species accomplish substantial N uptake over winter (Andresen and Michelsen, 2005), the N sink capacity for plants and roots during the winter is relatively unknown (Grogan and Jonasson, 2003), and uptake of nutrient pulses by plants may be more important than uptake under steady state conditions over winter (Jonasson and Chapin, 1991). Spring thaw, in particular, may be a critical time for N retention, with the release of atmospheric nitrogen deposition that accumulates over winter in the snow pack and soil (Williams *et al.*, 1996). Although cold-adapted plants can exploit N pulses following snow melt in early spring (Bilbrough and Caldwell, 1997; Tye *et al.*,

2005), the ability of plants to exploit N pulses during spring melt can vary widely among systems (Bilbrough *et al.*, 2000), and in some systems there may be an asynchrony between spring melt and the production of active root biomass (Sickman *et al.*, 2001; Lafreniere and Sharp, 2005). When the latter occurs, the profile of N forms present in early spring pulses may be critical for determining its persistence. For example, nitrate is the dominant component in atmospheric N deposition in many temperate systems such as those in eastern North America, particularly over winter when agricultural activities decrease (Sickles and Shadwick, 2007), yet nitrate is particularly susceptible to losses through leaching and volatilization to the atmosphere (Benbi and Richter, 2002).

In this study, I examined the interactive effects of simulated climate warming and N deposition on the recoveries of ^{15}N -labeled ammonium and ^{15}N -labeled nitrate tracers added as pulses to temperate old field plots following spring thaw. In addition to the year round warming treatment, a winter only warming treatment was applied to a subset of plots to explore the contribution of this component of climate warming to the overall warming effect. I quantified the recovery of the added label in plant shoots, roots and soil at both the end of the growing season following ^{15}N addition and again after snow melt the next spring. The first sampling was performed to estimate treatment differences in the retention of early spring N pulses in the context of the following plant growing season. I hypothesized that increased freeze-thaw cycling in the warmed plots would reduce their retention of the ^{15}N pulse, although increased total plant N uptake in plots also warmed over summer might increase uptake of the remaining ^{15}N tracer. I also hypothesized that the recovery of ^{15}N from added ammonium would exceed that of nitrate because of the mobility of the NO_3^- ion within the soil exchange complex. I then

used the end of season ^{15}N enrichment values in combination with those measured the following spring to explore treatment differences in the retention of plant and soil N over winter. I hypothesized that an increased frequency of freeze-thaw cycles over the winter would enhance ^{15}N losses from warmed plots, particularly if they became N saturated as a result of N fertilization.

3. 2 Methods

3.2.1 Site description

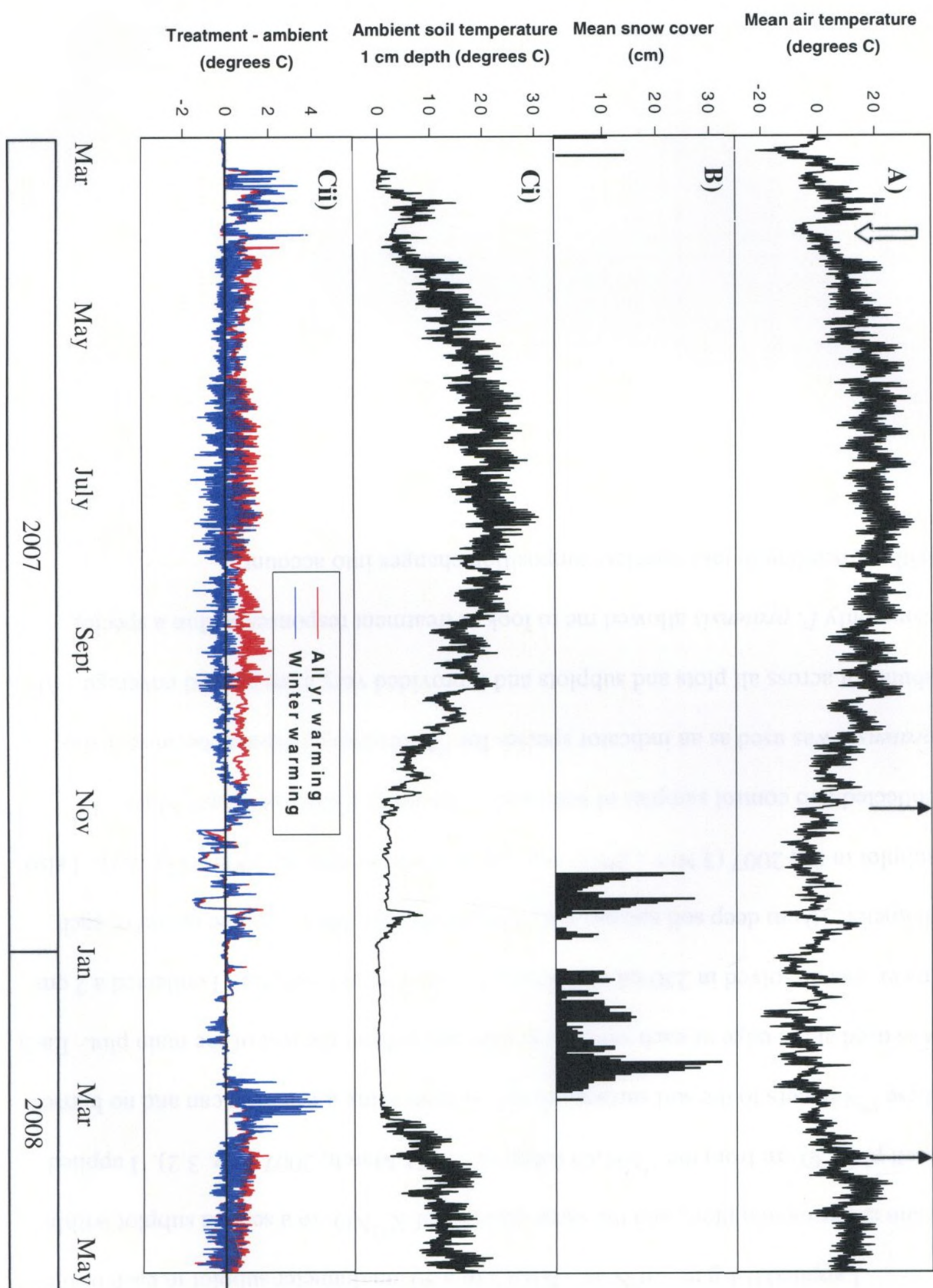
My field research was conducted in a former agricultural field at the Agriculture Canada Southern Crop Protection and Food Research Centre, in London Ontario (43° 01' 46" N, 81° 12' 52" W). The site has not been ploughed, fertilized, or mowed for over 20 years. The soil is classified as "well to imperfect drained silt loam glacial till" (Hagerty and Kingston, 1992), and pH is approximately 7.5 (Terry Bell, *unpublished data*). Mean annual temperature in this region was 6.3 °C and mean annual precipitation was 818.3 mm for the period of experimentation (Environment Canada, National Climate Data and Information Archive). Ambient air temperature and snow cover over the duration of the collection periods are displayed in Figure 3.1 A, B. The vegetation at the site is dominated by the perennial grasses *Poa pratensis* (L.) and *Bromus inermis* (L.), while the forb *Cirsium arvense* (L.) and the legume *Lotus corniculatus* (L.) are also common, but patchy. The forbs *Asclepias syriaca* (L.), *Aster ericoides* (L.), *Solidago altissima* (L.) and the mosses *Fissidens taxifolius* Hedw. and *Brachythecium salebrosum* (Web and Mohr) BSG are also present at lower density.

3.2.2 Design of main warming and nitrogen addition experiment

The field manipulations consist of three warming treatments (warming all year, warming only in winter {from late fall after plant senescence until spring melt}, and control) factorially crossed with two N treatments (added N and control), in a block design (n=10) (Figure 2.2). Plots are heated by single 150 W ceramic infrared heaters (Zoo-Med Laboratories, San Luis Obispo, CA, USA), suspended 50 cm above the plot centers. These heaters simulate solar heating without giving off photosynthetically active

radiation (Harte *et al.*, 1995). The temperature in warmed plots is raised (or lowered, depending on season) by between 1 to 4 °C 1 cm below the soil surface (Figure 3.1 Ci, ii). In fertilized plots, a pulse of aqueous ammonium nitrate is added in the early spring at a rate of 2 g m⁻² y⁻¹ and ammonium nitrate is also added at a rate of 4 g m⁻² y⁻¹ in late spring in the form of slow release pellets (Florikan ESA, Sarasota, FL, USA; see Figure 2.1 for addition dates). These addition rates are designed to simulate projected increases in atmospheric deposition over the next century (Galloway *et al.*, 2004). Warming, which began in November 2006, is monitored by underground temperature probes (107-BAM, Campbell Scientific Canada Corp., Edmonton, AB, Canada) at 1 cm depth and 5 cm depth (only data for 1 cm depth is presented). These data are used to quantify the frequency of freeze-thaw cycles and the temperature difference between warmed and ambient plots is shown in Figure 3.1 Ci, ii. Soil moisture probes (CS616-L TDR probes, Campbell Scientific Canada Corp., Edmonton, AB, Canada) also record integrated measurements from depths of 0-15 cm and 0-30 cm. All temperature and moisture data are recorded year-round, on an hourly basis.

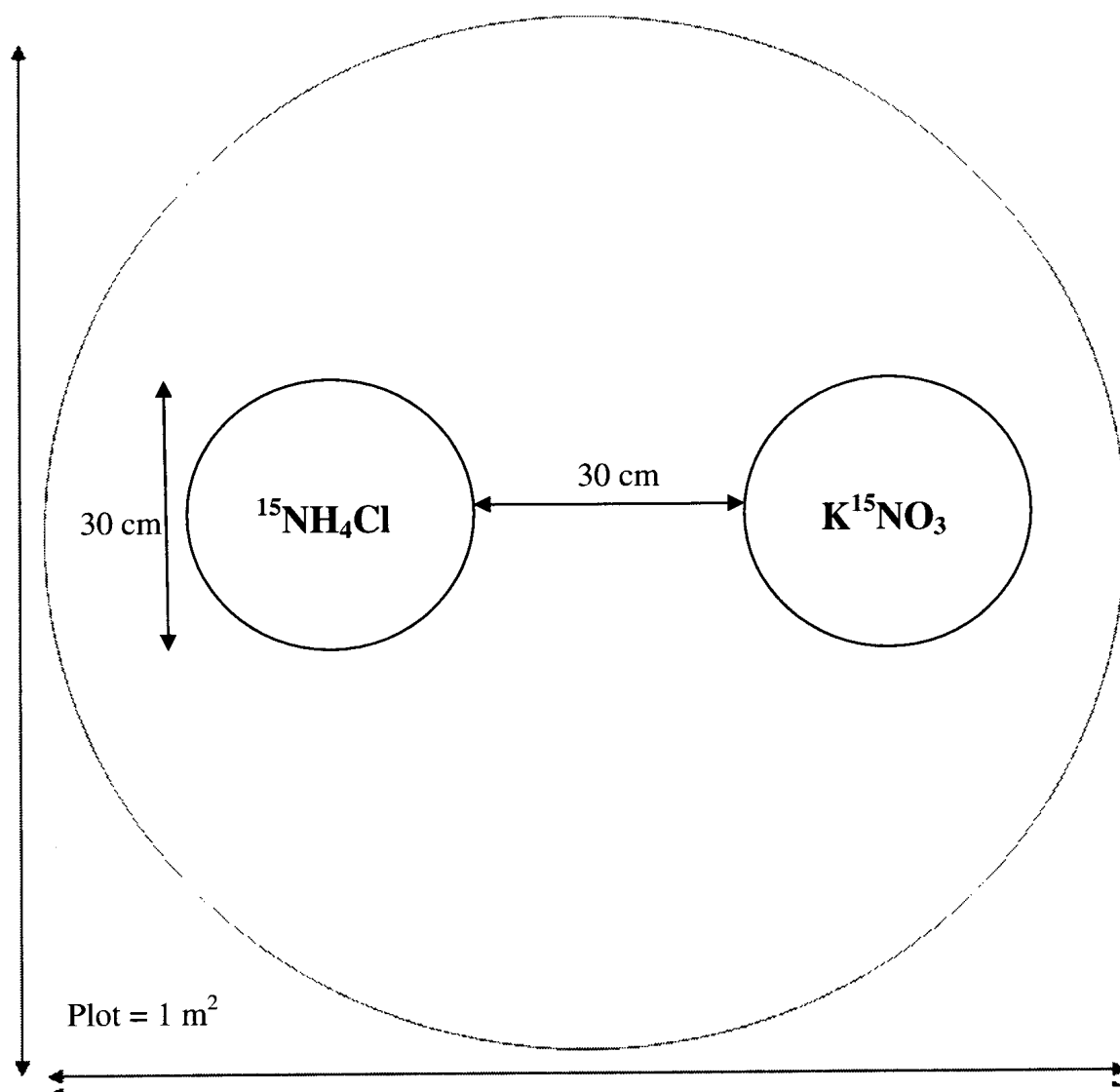
Figure 3.1 Measurements of **A)** daily air temperatures and **B)** daily snow cover at the London airport, **Ci)** ambient soil temperatures at 1 cm depth and **Cii)** the effect of yr-round and winter warming treatments on soil temperature at 1 cm depth for the duration of the experiment (when warming treatment lines are above or below the line crossing at zero, this represents the degrees C by which they are warmer or colder than the ambient plots). The white arrow indicates when the ^{15}N tracer was added and black arrows indicate when sampling occurred. The center of all $^{15}\text{NH}_4\text{Cl}$ and K^{15}NO_3 subplots were marked with pegs and during collections in each enriched subplot, one soil sample was taken at 10 cm depth and approximately two shoots of *Poa pratensis* (L.) were collected at random from the shoot base. Two controls were taken from each block to control for the N treatment.



3.2.3 ^{15}N tracer addition

I applied 0.1 g m^{-2} of N as $^{15}\text{NH}_4\text{Cl}$ to a 30 cm diameter subplot in each of the main experimental plots, and the same quantity of K^{15}NO_3 to a second subplot within each plot (30 cm from the $^{15}\text{NH}_4\text{Cl}$ subplots) on 21 March, 2007 (Fig. 3.2). I applied these ^{15}N tracers to the soil surface of each subplot using a watering can and no barrier was used at the edge of each subplots to partition it from the rest of the main plot. Each tracer was dissolved in 250 ml of deionized water for each subplot. I collected a 2 cm diameter, 10 cm deep soil sample and 2 *Poa pratensis* tillers from the center of each subplot in fall 2007 (3 Nov., 2007) and spring 2008 (24 March, 2008) (Fig. 3.1). I also collected two control samples of soils and *P. pratensis* tillers from each block. *P. pratensis* was used as an indicator species for ^{15}N recovery in shoots because it was abundant across all plots and subplots and it provided very even ground coverage. Also, using only *P. pratensis* allowed me to look at treatment responses within a species without needing to take species composition changes into account.

Figure 3.2 Approximately 0.1 g of N per m⁻² of ¹⁵NH₄Cl and K¹⁵NO₃ was added by using a watering can to distribute 250 ml ¹⁵N-enriched solution per respective 30 cm diameter circular subplots of the main experimental plots during spring thaw on 21 March 2007.



3.2.4 ^{15}N analyses

Within 48 h of collection I separated roots (from all plant species) from each soil sample, rinsed with them with 0.5 mM CaCl_2 and dried them at 60 °C for 24 h. *P. pratensis* tillers were dried for 72 h at 60 °C and ground to a fine powder using a ball mill (Spex Certiprep Geno/grinder 2000, Metuchen, NJ, U.S.A). Approximately 4 mg of dried, ground root and shoot material were placed into 4 × 6 mm tin capsules, while approximately 12 mg of dried soil was placed into 9 × 10 mm tin capsules. Samples were analyzed for ^{15}N content using mass spectrometry, performed by the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University using a NC 2100 Elemental Analyzer interfaced with a Finnigan Delta Plus XL isotope ratio mass spectrometer. The ^{15}N isotope composition was expressed in standard notation ($\delta^{15}\text{N}$) in parts per thousand relative to atmospheric N_2 , where:

$$\delta^{15}\text{N}_{\text{sample}} = [({}^{15}\text{N}/{}^{14}\text{N})_{\text{sample}} / ({}^{15}\text{N}/{}^{14}\text{N})_{\text{standard}} - 1] \times 1000.$$

I then solved for $^{15}\text{N}/{}^{14}\text{N}_{\text{sample}}$ to calculate percent ^{15}N content in plant tissue and soil collected from treatments and control plots. I then subtracted percent $^{15}\text{N}_{\text{control}}$ from percent $^{15}\text{N}_{\text{treatment}}$ for shoots, roots and soil in each block and multiplied these differences by total N in the respective pools to estimate excess ^{15}N . The end of growing season and early spring biomass data from the main experiment, required for the calculation of the N pool sizes for roots and *P. pratensis* shoots, were provided by Jennifer Hutchison (*unpublished data*).

3.2.5 Statistical analyses

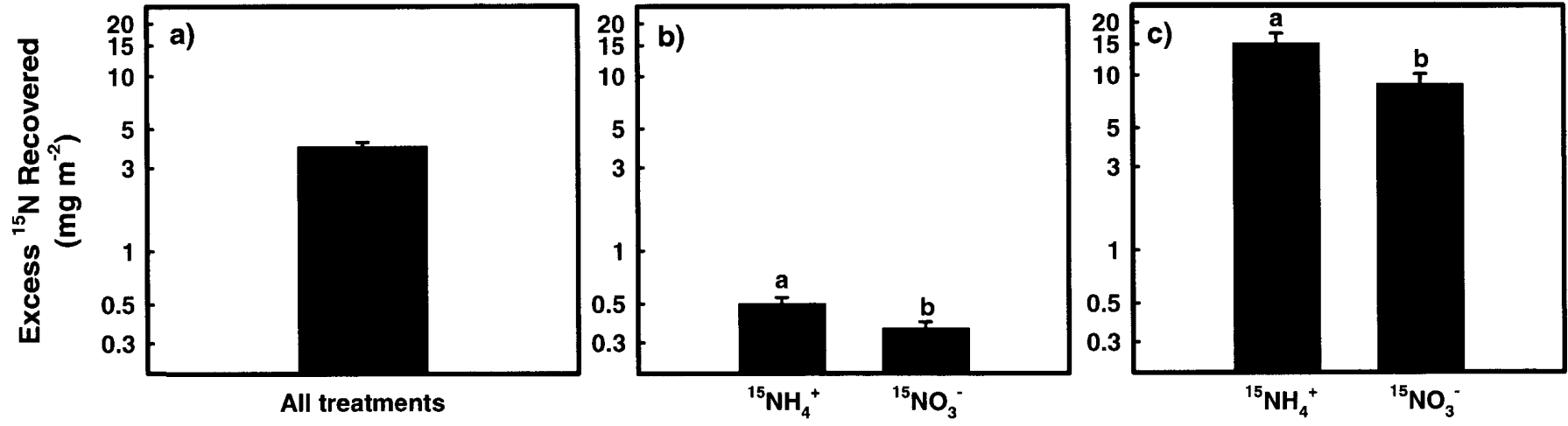
Three-way factorial ANOVAs were conducted to examine the main effects of warming treatments, N addition and ^{15}N tracer type, and their interactive effects, on the recovery of excess ^{15}N in *P. pratensis* shoots, roots (all species) and soil at the end of the plant growing season and the following spring. The over winter changes in ^{15}N were also examined. Block number was also included in the ANOVA models as a random factor, and the dependent variables were log-transformed to improve normality and homogeneity of variance. Tukey's HSD tests were used to resolve differences among treatment combinations following a significant ANOVA result ($P < 0.05$). All statistical analyses were conducted using JMP 4.0 (SAS Institute Inc.).

3.3 Results

3.3.1 Recovery of excess ^{15}N in the fall

In the fall, subsequent to the addition of ~ 100 mg N per square meter of $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ during the previous spring thaw, approximately 9-15% of the ^{15}N label (in excess of naturally occurring ^{15}N) was recovered in the bulk soil, 4% in the shoots of *P. pratensis* and 0.5% in the roots (all species) (Fig. 3.3). There were no significant differences among treatments in excess ^{15}N recovered in shoots of *P. pratensis* (Fig. 3.3 A). While warming and nitrogen fertilization also had no significant effects on ^{15}N recovery in roots or bulk soil, almost 50% more excess ^{15}N was recovered from roots in $^{15}\text{NH}_4^+$ addition plots than in $^{15}\text{NO}_3^-$ addition plots ($P=0.004$; Fig. 3.3 B), and excess ^{15}N recovery in bulk soil was also double in $^{15}\text{NH}_4^+$ addition plots than in $^{15}\text{NO}_3^-$ addition plots ($P=0.003$; Fig. 3.3 C).

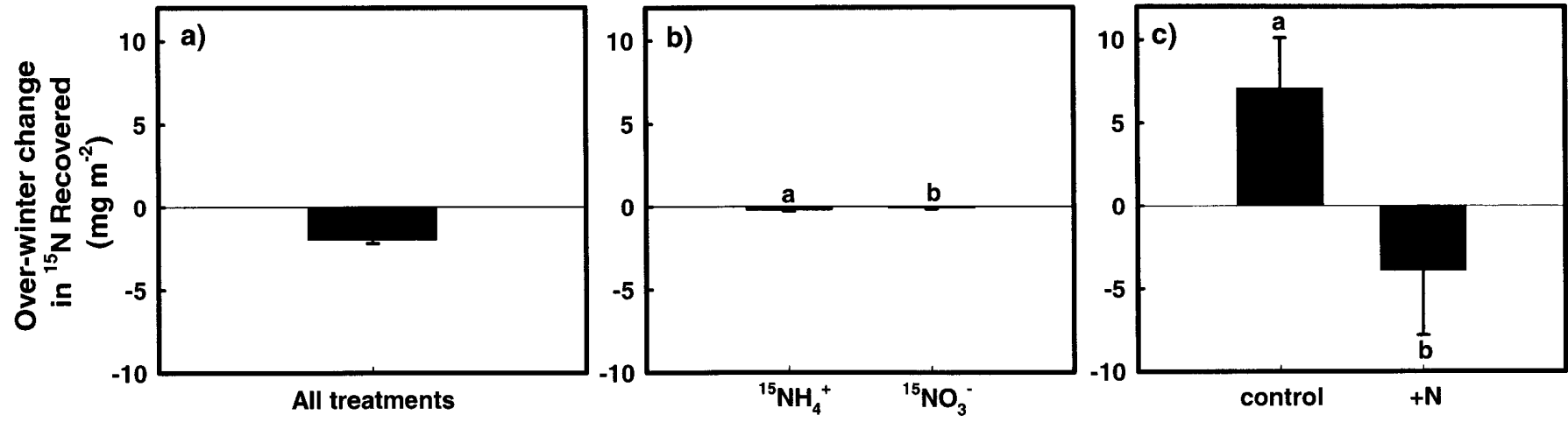
Figure 3.3 Mean excess ^{15}N recovered from experimental plots on 3 November, 2007 in **A)** shoots of *Poa pratensis*, **B)** roots (all species) and **C)** bulk soil, with the latter two sampled from the soil surface to a depth of 10 cm. The ^{15}N tracers were added on 21 March, 2007 during spring thaw. Bars were pooled over treatments that were not significantly different ($P > 0.05$, 3-factor ANOVA), and different lower case letters above bars are used to indicate significant treatments differences within each panel ($P < 0.05$, Tukey's HSD test). Error bars represent standard error ($n=120$), and y-axes are plotted on a \log_{10} scale. $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ on the x-axis refer to the ammonium and nitrate tracer addition treatments, respectively.



3.3.2 Over winter changes in excess ^{15}N recovered

Over winter, approximately half of the excess ^{15}N present in the fall was lost from shoots of *P. pratensis*, although the amount lost did not differ among treatments (Fig. 3.4 A). For roots, excess ^{15}N losses from $^{15}\text{NH}_4^+$ addition plots were more than double losses from $^{15}\text{NO}_3^-$ addition plots ($P=0.033$), although the total quantity of ^{15}N lost from roots was low (Fig. 3.4 B). Nitrogen fertilization had a dramatic effect on excess ^{15}N retention in bulk soil over winter, with a large net gain in excess ^{15}N in control plots and a net decrease in nitrogen fertilized plots ($P=0.032$; Fig. 3.4 C).

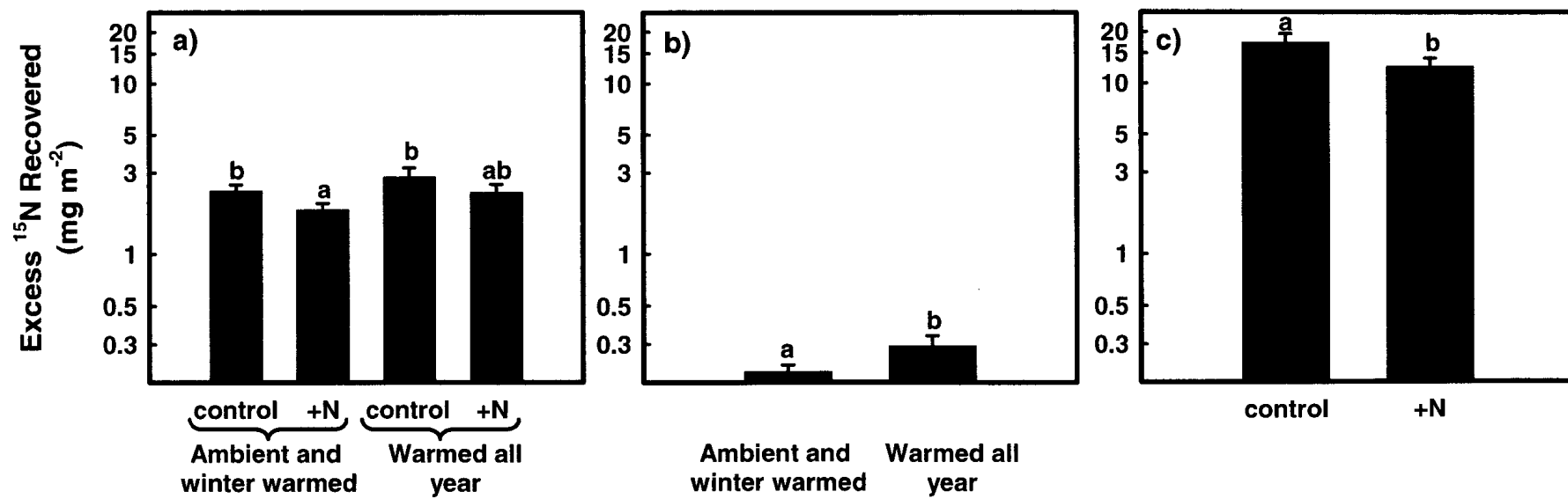
Figure 3.4 Mean over-winter changes (from 3 Nov., 2007 to March 24, 2008) in excess ^{15}N recovered from **A**) shoots of *Poa pratensis*, **B**) roots (all species) and **C**) bulk soil, with the latter two sampled from the soil surface to a depth of 10 cm. The ^{15}N tracers were added on 21 March, 2007 during spring thaw. Bars were pooled over treatments that were not significantly different ($P>0.05$, 3-factor ANOVA), and different lower case letters above bars are used to indicate significant treatments differences within each panel ($P<0.05$, Tukey's HSD test). Error bars represent standard error ($n=120$). $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ on the x-axis refer to the ammonium and nitrate tracer addition treatments, respectively, whereas control and +N refer to the levels of the (unlabeled) ammonium nitrate fertilization treatment.



3.3.3 Total excess ^{15}N recovered in spring

In the early spring, a year after the addition of the ^{15}N -labelled substrates, excess ^{15}N recovery in *P. pratensis* shoots was decreased by the N fertilization treatment ($P=0.011$) and increased by year round warming ($P=0.022$) (Fig. 3.5 A). Excess ^{15}N in roots was also higher in plots warmed year round than in the ambient or winter-warmed plots ($P=0.0069$, Fig. 3.5 B). The recovery of excess ^{15}N in bulk soil was lower in N fertilized plots than in control plots ($P=0.041$; Fig. 3.5 C). Significant treatment effects on both shoot and root ^{15}N reflected differences in ^{15}N -enrichment, as there were no significant effects of warming or N addition on *P. pratensis* shoot litter or roots among plots in spring 2008 (Jennifer Hutchison, *unpublished data*).

Figure 3.5 Mean excess ^{15}N recovered from experimental plots on 24 March, 2008 in **A)** shoots of *Poa pratensis*, **B)** roots (all species) and **C)** bulk soil, with the latter two sampled from the soil surface to a depth of 10 cm. The ^{15}N tracers were added on 21 March, 2007 during spring thaw. Bars were pooled over treatments that were not significantly different ($P>0.05$, 3-factor ANOVA), and different lower case letters above bars are used to indicate significant treatments differences within each panel ($P<0.05$, Tukey's HSD test). Error bars represent standard error ($n=120$), and y-axes are plotted on a \log_{10} scale. Control and +N refer to the levels of the (unlabeled) ammonium nitrate fertilization treatment, whereas ambient, winter warmed and warmed all year refer to levels of the warming treatment.



3.4 Discussion

3.4.1 Recovery of excess ^{15}N in the fall

There were no effects of warming on the fall recovery and partitioning of ^{15}N added at spring melt. This result is consistent with the soil freezing dynamics observed in early spring, in that the plots melted out synchronously, and the rapid soil warming following snow melt overwhelmed the soil warming treatment effects. Therefore, although my prediction of increased N losses in warmed plots was not realized, the intact snow pack present in late winter followed by the rapid onset of warm temperatures in spring in 2007 overwhelmed the warming treatments and prevented differences in soil freezing dynamics among plots at this time. In addition, although I predicted that warming and N had the potential to increase plant uptake of any remaining ^{15}N over summer by increasing overall plant biomass and N uptake, it was an unusually dry summer, and there were no biomass or plant species responses to the experimental treatments. However, N and warming treatments increased plant biomass in the following, wetter summer (Jennifer Hutchison, *unpublished data*).

My results also suggest that there were no carryover effects from warming over the previous winter. In some cases root damage caused by FTC over the winter has been implicated in reduced N retention over the following growing season (Fitzhugh *et al.*, 2001; Groffman *et al.*, 2001; Matzner and Borken, 2008), although there was no evidence for that in my data since winter warming had no effect on growing season shoot and root N retention. However, my experimental design did not address the possible effects of warming on the accumulation or losses of N deposition over winter. I added an equivalent amount of N to each treatment in the early spring, whereas less N deposition

may be available at spring melt in warmer winters as a result of losses through runoff, leaching and denitrification (Brooks and William, 1999; Teepe *et al.*, 2001). Although warming over winter didn't reduce over winter N retention in my exp. (see 3.4.2), my experiment only examined the retention of ^{15}N already incorporated into plants, microbes and soil, and did not examine the fate of N deposited on snow surface or soil over winter.

As predicted, more ^{15}N from added ammonium at spring melt was recovered than that of nitrate, which is more mobile in the soil and susceptible to denitrification losses (Ryden *et al.*, 1984; Scherer-Lorenzen *et al.*, 2003; Allred, 2007). However, the ratio of nitrate to ammonium in atmospheric deposition in this region is approximately 2:1 on an annual basis, with ammonium deposition declining over winter due to reduced agricultural activity (Sickles and Shadwick, 2007). Therefore, differences in retention among ammonium and nitrate over the short term of one growing season are likely offset to an approximately equivalent amount by differences in their deposition rates. In total, I recovered approximately 20% of the $100 \text{ mg m}^{-2} \text{ }^{15}\text{N}$ added to the $^{15}\text{NH}_4$ addition plots in spring. However, this estimate does not account for the ^{15}N in non- *P. pratensis* shoots or from roots and the soil below 10 cm depth.

3.4.2 Over winter changes in excess ^{15}N recovered

Despite clear effects of warming on the soil freezing dynamics over the winter (>10 FTC in warmed plots in comparison with ~3 mild FTC in ambient plots throughout winter and early spring) there were no increased ^{15}N losses from warmed plots over winter. These results are consistent with evidence that suggests that freeze-thaw cycle amplitudes must be relatively extreme to promote soil and litter N losses (Isard and

Schaetzl, 1998; Deluca *et al.*, 1992; Nielsen *et al.*, 2001; Grogan *et al.*, 2004; Joseph and Henry, 2008). The large ^{15}N losses in N fertilized plots suggest net nitrogen mineralization occurred under N saturated conditions (Aber *et al.*, 1998; Fenn *et al.*, 1998; Matson *et al.*, 2002), whereas net ^{15}N increases in the soil in ambient N plots likely reflect the retention of ^{15}N lost from plant tissue decomposition over winter under conditions of N limitation (Berg and Staaf, 1981; Schmidt and Lipson, 2004). Although the quantity of ^{15}N lost from *P. pratensis* shoots and roots from all species was not equal to the ^{15}N gained by soil in the ambient N plots, *P. pratensis* shoot biomass as a proportion of the total shoot biomass was relatively low over the summer of 2007 (~20% of total shoot biomass, as compared with approx 50% of total shoot biomass in 2008; Jennifer Hutchison, *unpublished data*), and the excess shoot ^{15}N of the other plant species was likely the additional source of ^{15}N transferred to the soil over winter. The significantly greater root ^{15}N losses from $^{15}\text{NH}_4$ addition plots than from $^{15}\text{NO}_3$ addition plots over winter were relatively small in magnitude and resulted from the greater ^{15}N enrichment in the $^{15}\text{NH}_4$ addition plots at the start of the winter.

3.4.3 Total excess ^{15}N recovered in spring

The decreased recovery of soil ^{15}N in N fertilized plots in spring was consistent with the high ^{15}N losses from these plots over winter. However, the significant warming and nitrogen fertilization effects on excess ^{15}N recovered in plant shoots and roots could not be extrapolated from the significant fall and over winter effects. Decreased excess ^{15}N recovery in plant shoots from N fertilized plots could be explained by the increased soil ^{15}N losses from these plots, yet the significant increase in excess ^{15}N recovered in

plant shoots and roots from plots warmed year round largely offset this N effect. Given that there was no significant effect of winter warming alone on excess ^{15}N recovery in plants, the lengthening of the plant growing season (An *et al.*, 2005; Wan *et al.*, 2005) or increased soil microbial immobilization in the fall (Jaeger *et al.*, 1999; Bardgett *et al.*, 2005) likely explains the increased excess ^{15}N recovery from plants in the year round warmed plots. The strong effect of ^{15}N addition type noted in the fall for soil was not apparent in spring, possibly due to transfer of ^{15}N from the plant pool (where there was no effect of ^{15}N addition type) over winter into the soil pool.

3.5 Conclusions

Overall, contrary to my hypothesis, my results indicate that warming-induced changes to soil temperature and freezing dynamics over winter do not alter ^{15}N retention in this old field system. Instead, one year after ^{15}N addition, year round warming increased the recovery of the added ^{15}N in plants to the extent that it equaled the reductions in plant excess ^{15}N recovery caused by nitrogen fertilization. Nevertheless, even accounting for the excess ^{15}N likely present in the shoots of plants other than *P. pratensis*, the overall effect of nitrogen fertilization on soil ^{15}N losses largely overwhelmed any effects on ^{15}N recovery in plant tissue. This result suggests that climate warming may only modulate the effects of atmospheric nitrogen deposition on ecosystem nitrogen losses to a limited extent in this system, although more extreme responses to more intense warming cannot be ruled out.

3.6 References

- Aber J.D., Magill A., McNulty S.G., Boone R.D., Nadelhoffer K.J., Downs M., Hallett R., 1995. Forest biogeochemistry and primary production altered by nitrogen saturation. *Water Air & Soil Pollution* **85**(3): 1665-1670.
- Aber J.D., McDowell W., Nadelhoffer K., Magill A., Berntson G., Kamakea M., McNulty S.G., Currie W., Rustad L., Fernandez I., 1998. Nitrogen saturation in temperate forest ecosystems: Hypotheses revisited. *Bioscience* **48**: 921-933.
- Allred B.J., 2007. Effects of nitrate concentration and ionic strength on nitrate anion exclusion under unsaturated flow conditions. *Soil Science* **172**(11): 842-860.
- An Y., Wan S., Zhou X., Subedar A.A., Wallace L.L., Luo Y., 2005. Plant nitrogen concentration, use efficiency, and contents in a tallgrass prairie ecosystem under experimental warming. *Global Change Biology* **11**: 1733-1744.
- Andresen L.C., and Michelsen A., 2005. Off-season uptake of nitrogen in temperate heath vegetation. *Oecologia* **144**: 585-597.
- Bardgett R.D., Bowman W.D., Kaufmann R., Schmidt S.K., 2005. Linking aboveground and belowground communities: a temporal approach. *Trends in Ecology & Evolution* **20**: 634-640.
- BassiriRad H., 2000. Kinetics of nutrient uptake by roots: responses to global change. *New Phytologist* **147**: 155-169.
- Benbi D., and Richter J., 2002. A critical review of some approaches to modeling nitrogen mineralization. *Biology & Fertility of Soils* **35**: 168-183.
- Berg B., and Staaf H., 1981. Leaching, accumulation and release of nitrogen in decomposing forest litter. *Terrestrial Nitrogen Cycles* Eds. F.E. Clark and T. Rosswall. pp 163-178. *Ecological Bulletin*, Volume 33, Stockholm, Sweden.
- Bilbrough C., Caldwell M., 1997. Exploitation of springtime ephemeral N pulses by six Great Basin plant species. *Ecology* **78**(1): 231-243.
- Bilbrough C., Welker J., Bowman W., 2000. Early spring nitrogen uptake by snow-covered plants: a comparison of arctic and alpine plant function under the snowpack. *Arctic, Alpine and Antarctic Research* **32**: 404-411.
- Brooks P.D., and William M.W., 1999. Snowpack controls on nitrogen cycling and export in seasonally snow covered catchments. *Hydrological Processes* **13**(14-15): 2177-2190.

Campbell J.L., Mitchell M.J., Groffman P.M., Christenson L.M., Hardy J.P., 2005. Winter in northeastern North America: a critical period for ecological processes. *Frontiers in Ecology & the Environment* **3**(6): 314–322.

Campbell Scientific Canada Corp., 2007. 107-BAM underground temperature probes and CS616-L TDR soil moisture probes. Edmonton, AB, Canada. Cite online: <http://www.campbellsci.ca/>

Castle M.L., Crush J.R., Rowarth J.S., 2006. The effect of root and shoot temperature of 8 degrees C or 24 degrees C on the uptake and distribution of nitrogen in white clover (*Trifolium repens* L.). *Australian Journal of Agriculture Research* **57**(5): 577-581.

Chapin, F.S. III, Matson P.A., Mooney H.A., 2002. Principles of terrestrial ecosystem ecology. Springer, New York, N.Y., U.S.A. pp. 151-223.

Chapin F.S. III., Shaver G.R., 1996. Physiological and growth responses of arctic plants to a field experiment simulating climatic change. *Ecology* **77**: 822–840.

Cookson W.R., Rowarth J.S., Cameron K.C., 2001. The fate of autumn-, late winter- and spring-applied nitrogen fertilizer in a perennial ryegrass (*Lolium perenne* L.) seed crop on a silt loam soil in Canterbury, New Zealand. *Agriculture, Ecosystems & Environment* **84**: 67-77.

Deluca T.H., Keeney D.R., McCarty G.W., 1992. Effect of freeze–thaw events on mineralization of soil-nitrogen. *Biology and Fertility of Soils* **14**: 116–120.

Environment Canada, 2008. National Climate Data and Information Archive cited online: http://www.climate.weatheroffice.ec.gc.ca/advanceSearch/searchHistoricData_e.html?timeframe=1&Prov=XX&StationID=99999&Year=2008&Month=8&Day=20&errMessage

Fenn M.E., Poth M.A., Aber J.D., Baron J.S., Bormann B.T., Johnson D.W., Lemly A.D., McNulty S.G., Ryan D.F., Stottlemyer R., 1998. Nitrogen excess in North American ecosystems: predisposing factors, ecosystem responses, and management strategies. *Ecological Applications* **8**: 706–733.

Fitzhugh R.D., Driscoll C.T., Groffman P.M., Tierney G.L., Fahey T.J., Hardy J.P., 2001. Effects of soil freezing, disturbance on soil solution nitrogen, phosphorus, and carbon chemistry in a northern hardwood ecosystem. *Biogeochemistry* **56**: 215–238.

Florikan ESA., 2007. Fertilizer: slow release ammonium nitrate pellets. Sarasota, FL, USA.

Galloway J.N., Dentener F.J., Capone D.G., Boyer E.W., Howarth E.W., Seitzinger S.P., Asner G.P., Cleveland C.C., Green P.A., Holland E.A., Karl D.M., Michaels A.F., Porter

J.H., Townsend A.R., Vorosmarty C.J., 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* **70**: 152-226.

Geibe C.E., Danielsson R., Van Hees P.A.W., Lundstron U.S., 2006. Comparison of soil solution chemistry sampled by centrifugation, two types of suction lysimeters and zero-tension lysimeters. *Applied Geochemistry* **21**: 2196-2111.

Groffman P.M., Driscoll C.T., Fahey T.J., Hardy J.P., Fitzhugh R.D., Tierney G.L., 2001. Colder soils in a warmer world: A snow manipulation study in a northern hardwood forest ecosystem. *Biogeochemistry* **56**: 135–150.

Grogan P., and Jonasson S., 2003. Controls on annual nitrogen cycling in the understory of a subarctic birch forest. *Ecology* **84**(1): 202-281.

Grogan P., Michelsen A., Ambus P., Jonasson S., 2004. Freezethaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. *Soil Biology & Biochemistry* **36**: 641–654.

Hagerty, T.P., Kingston, M.S., 1992. The Soils of Middlesex County. Ontario Ministry of Agriculture and Food.

Harte J., Torn M.S., Chang F.R., Feifarek B., Kinzig A.P., Shaw R., Shen K., 1995. Global warming and soil microclimate: results from a meadow-warming experiment. *Ecological Applications* **5**: 132–150.

Henry H.A.L., 2008. Climate change and soil freezing dynamics: historical trends and projected changes. *Climatic Change* **87**: 421–434.

Henry H.A.L., and Jefferies B., 2003. Interactions in the uptake of amino acids, ammonium and nitrate ions in the Arctic salt-marsh *Puccinellia phryganodes*. *Plant Cell & Environment* **26**(3): 419-428.

Hobbie S. E., and Chapin III F. S., 1996. Winter regulation of tundra litter carbon and nitrogen dynamics. *Biogeochemistry* **35**: 327–338.

Isard S.A., Schaetzl R.J., 1998. Effects of winter weather conditions on soil freezing in southern Michigan. *Physical Geography* **19**: 71-94.

Jaeger C.H., Monson R.K., Fisk M.C., Schmidt S.K., 1999. Seasonal partitioning of nitrogen by plants and soil microorganisms in an alpine ecosystem. *Ecology* **80**: 1883–1891.

Jonasson S., and Chapin F.S., 1991. Seasonal uptake and allocation of phosphorus in *Eriophorum-vaginatum* L. measured by labeling with P-32. *New Phytologist* **118**(2): 349-357.

Joseph G., and Henry H.A.L., 2008a. Retention of surface nitrate additions in a temperate old field: implications for atmospheric nitrogen deposition over winter. Submitted to Plant and Soil.

Joseph G., and Henry H.A.L., 2008b. Soil nitrogen leaching losses in response to freeze-thaw cycles and pulsed warming in a temperate old field. Soil Biology & Biochemistry **40**(7): 1947-1953.

Kasper T.C., and Bland W.L., 1992. Soil temperature and root growth. Soil Science **154**: 290-299.

Kielland K., Olson K., Ruess R.W., Boone R.D., 2006. Contribution of winter processes to soil nitrogen flux in taiga forest ecosystems Biogeochemistry **81**: 349–360.

Lafreniere M.J., and Sharp M.J., 2005. A comparison of solute fluxes and sources from glacial and non-glacial catchments over contrasting melt seasons. Hydrological Processes **19**(15): 2991-3012.

Laine P., Bigot J., Ourry A., Boucaud J., 1994. Effects of low temperature on nitrate uptake, and xylem and phloem flows of nitrogen, in *Secale cereale* L, and *Brassica napus* L. New Phytologist **127**: 675-683.

Ludwig B., Wolf I., Teepe R., 2004. Contribution of nitrification and denitrification to the emission of N₂O in a freeze-thaw event in an agricultural soil. Journal of Plant Nutrition & Soil Science **167**(6): 678-684.

Majdi H., and Ohrvik J., 2004. Interactive effects of soil warming and fertilization on root production, mortality, and longevity in Norway spruce stand in Northern Sweden. Global Change Biology **10**: 182–188.

Matson P., Lohse K.A., Hall S.J., 2002. The Globalization of Nitrogen Deposition: Consequences for Terrestrial Ecosystems. Ambio **31**(2): 113-119.

Matzner E., and Borken W., 2008. Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review European Journal of Soil Science **59**: 274–284.

Melillo J.M., Aber J.D., Linkins A.E., Ricca A., Fry B., Nadelhoffer K., 1989. Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. Plant and Soil **115**: 189-198.

Mellander P.E., Lofvenius M.O., Laudon H., 2007. Climate change impact on snow and soil temperature in boreal Scots pine stands. Climatic Change **85**(1-2): 179-193.

Miller A.E., Schimel J.P., Sickman J.O., Meixner T., Doyle A.P., Melack J.M., 2007. Mineralization responses at near-zero temperatures in three alpine soils. Biogeochemistry **84**: 233–245.

Miltner E.D., Stahnke G.K., Johnston W.K., Golob C.T., 2004. Late fall and winter nitrogen fertilization of turfgrass in two pacific northwest climates. *Hortscience* **39**(7): 1745-1749.

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

Nissinen A., Kareinen T., Tanskanen N., Ilvesniemi H., 2000. Apparent cation exchange equilibria and aluminium solubility in solution obtained from two acidic forest soils by centrifuge drainage method and suction lysimeters. *Water, Air, & Soil Pollution* **119**: 23-43.

Pregitzer K.S., King J.S., Burton A.J., Brown S.E., 2000. Responses of tree fine roots to temperature. *New Phytologist* **147**: 105–115.

Ryden J. C., Ball P.R., Garwood E.A., 1984. Nitrate leaching from grassland. *Nature* **311**: 51-53.

SAS Institute Inc. 2000. JMP Version 4.0.

Scherer-Lorenzen M., Palmborg C., Prinz A., Schulz E.D., 2003. The role of plant diversity and composition for nitrate leaching in grasslands. *Ecology* **84**(6): 1539-1552.

Schimel J.P., Bilbrough C., Welker J.A., 2004. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. *Soil Biology & Biochemistry* **36**: 217–27.

Schimel J.P., and Clein J.S., 1996. Microbial response to freeze-thaw cycles in tundra and taiga soils. *Soil Biology & Biochemistry* **28**: 1061–1066.

Schmidt S.K., Lipson D.A., 2004. Microbial growth under the snow: implications for nutrient and allelochemical availability in temperate soils. *Plant Soil* **259**: 1–7.

Sickman J.O., Leydecker A., Melack J.M., 2001. Nitrogen mass balances and abiotic controls on N retention and yield in high-elevation catchments of the Sierra Nevada, California, United States. *Water Resources Research* **37**(5): 1445-1461.

Sickles J.E., and Shadwick D.S., 2007. Seasonal and regional air quality and atmospheric deposition in the eastern United States. *Journal of Geophysical Research – Atmospheres* **112** (D17): D17302.

Spex Certiprep Geno/grinder, 2000, Metuchen, NJ, U.S.A.

Teepe R., Brumme R., Beese F., 2001. Nitrous oxide emissions from soil during freezing and thawing periods. *Soil Biology & Biochemistry* **33**: 1269–1275.

Tierney G.L., Fahey T.J., Groffman P.M., Hardy J.P., Fitzhugh R.D., Driscoll C.T., 2001. Soil freezing alters fine root dynamics in a northern hardwood forest. *Biogeochemistry* **56**: 175–190.

Tye A.M., Young S.D., Crout N.M.J., West H.M., Stapleton L.M., Poulton P.R., Laybourn-Parry J., 2005. The fate of N-15 added to high Arctic tundra to mimic increased inputs of atmospheric nitrogen released from a melting snowpack. *Global Change Biology* **11**(10): 1640-1654.

Vitousek P.M., Aber J., Howarth R.W., Likens G.E., Matson P.A., Schindler D.W., Schlesinger W.H., Tilman G.D., 1997. Human alteration of the global nitrogen cycle: causes and consequences. *Ecological Applications* **7**: 737–750.

Vitousek P.M., and Howarth R.W., 1991. Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* **13**: 87–115.

Walker M.D., Wahren C.H., Hollister R.D., Henry G.H.R., Ahlquist L.E., Alatalo J.M., Bret-Harte M.S., Calef M.P., Callaghan T.V., Carroll A.B., 2006. Plant community responses to experimental warming across the tundra biome. *Proceedings of the National Academy of Sciences of the USA* **103**: 1342–1346.

Wan S.Q., Hui D.F., Wallace L., Luo L.Q., 2005. Direct and indirect effects of experimental warming on ecosystem carbon processes in a tallgrass prairie. *Global Biogeochemical Cycles* **19**(2): GB2014, doi: 10.1029/2004GB002315.

Williams M.W., Brooks P.D., Mosier A., Tonnessen K.A., 1996. Mineral N transformations in and under seasonal snow in a high elevation catchment, Rocky Mountains, USA. *Water Resources Research* **32**: 3175–3185.

Zavaleta E.S., Shaw R.M., Chiariello N.R., Mooney H.A., Field C.B., 2003. Additive effects of simulated climate changes, elevated CO₂, and nitrogen deposition on grassland diversity. *Proceedings of the National Academy of Sciences of the USA* **100**(13): 7650-7654.

Zoo-Med Laboratories, 2006. Ceramic infrared heaters. San Luis Obispo, CA, USA.

Chapter 4

General Discussion & Conclusions

4.1 Research findings

Contrary to my hypothesis, winter warming had no significant effect on winter soil N dynamics. This result, however, supports the idea that freeze-thaw cycle amplitudes may need to be relatively extreme to promote soil and litter N losses (Isard and Schaetzl, 1998; Deluca *et al.*, 1992; Nielsen *et al.*, 2001; Joseph and Henry, 2008). Studies imposing more moderate cycles have demonstrated little or no effect of FTC on soil N dynamics (Herrmann and Witter 2002; Grogan *et al.*, 2004). This may be due to high freeze-thaw tolerance of the soil microbial community, which has been observed in alpine systems (Lipson and Monson 1998; Lipson *et al.*, 2000). While the effects of FTC appear to increase with colder soil temperatures, a threshold temperature has not yet been defined (Matzner *et al.*, 2008). Another possibility is that since the effects of FTC tend to be short lived (e.g. 40 days, Hermann *et al.*, 2002) and dissipate over each successive cycle (Skogland *et al.*, 1998; Schimel and Clein, 1996; Hermann *et al.*, 2002) mild to moderate FTC effects may not have been distinguished since net N mineralization was estimated cumulatively for winter. Overall, the key finding of my *in situ* soil core experiment, as shown through elevated resin N, is that while winter warming may not alter soil N dynamics over winter, it might have important carry over effects on soil N dynamics during the growing season. Furthermore, the absence of strong warming effects in the winter and over the growing season suggests the possibility that the effect

of warming is non-linear, in that a threshold temperature may need to be reached before significant effects on soil N can occur.

While year-round warming contributed to small increases in N retention in shoots and roots in the spring, winter warming also had no effect on N dynamics in my ^{15}N experiment. The key finding of my tracer study is that changes in climate may be less important than the direct effect of increasing N on soil N dynamics. Nitrogen inputs drove substantial soil N leaching losses over winter, which reflects elevated nitrogen mineralization under conditions of N saturation (Magill *et al.*, 1996; Fenn *et al.*, 1997; Aber *et al.*, 1998; McDowell *et al.*, 1998; Matson *et al.*, 2002). Despite enhanced N mineralization, N saturation and leaching can result in large decreases in productivity due to cation losses and nutrient imbalances (Emmett *et al.*, 1995; Currie *et al.*, 1996). In addition to the direct effects of N deposition on soil N, indirect effects caused by N-induced changes in plant species composition are also important, as they influence ecosystem function (Jackson *et al.*, 2002; Hooper *et al.*, 2005; Wardle and Zackrisson, 2005), and can modify the impact of further N deposition (Bardgett *et al.*, 1999; Reich *et al.*, 2001; Craine *et al.*, 2003; Dijkstra *et al.*, 2004). For example, compositional changes can result in the loss of key functional species or can increase productivity and accelerate N mineralization rates (Van Vuuren *et al.*, 1992).

Overall, since climate change research has focused mostly on arctic and alpine regions, temperate ecosystems in North America have been largely overlooked (Campbell *et al.*, 2005). As such, there is a substantial shortage of experimental data on the effects of climate change on soil freezing dynamics and associated changes in soil physical and biological processes (Henry, 2008). Alongside these oversights,

uncertainties about the effects of climate warming and increasing levels of atmospheric N deposition are highlighting the need for a more thorough understanding of temperate ecosystem responses. My experimental results contribute to our knowledge on soil freezing dynamics and confirm the paradox that warmer winter air temperatures are likely to result in colder, more variable soil temperatures and increases in FTC. This highlights the need for more research to be done in temperate systems during the winter and also raises questions about the effects of warming during seasonal transitions. Aside from climate change, my research also has a strong temporal aspect and contributes to general knowledge on seasonal N mineralization and retention in temperate systems. My ^{15}N tracer study in particular also emphasizes the importance of looking at interactive vs. single factor responses.

4.2 Potential limitations

Natural field conditions may have limited treatment effects. For example, there were no effects of warming or nitrogen addition on aboveground biomass or relative species abundance during the growing season in 2007 (Hutchison, *unpublished data*). Summer 2007 was unusually dry and therefore water likely limited plant responses to treatments. However, significant changes in both species composition and biomass occurred over summer 2008, which was substantially wetter than the previous summer. During this growing season, plots receiving warming and N had twice as much biomass in comparison to ambient plots. Warming also resulted in significant changes in plant species composition (Hutchison, *unpublished data*). Such changes in plant biomass and species composition can indirectly alter affect N dynamics through altering root and litter soil inputs, which may in turn alter the composition and function of soil microbial

communities. Had such changes occurred over the growing season in 2007, treatment effects on soil N dynamics may have been more pronounced in experimental results.

In many temperate ecosystems, rates of atmospheric nitrogen deposition remain high over winter despite decreased agricultural activity over this season. Nitrogen released from the snowpack over warmer winters through runoff, leaching, or denitrification (Brooks and William, 1999; Teepe *et al.*, 2001) may mean less N is released during spring melt. In this experiment, however, ^{15}N was added once to the soil surface during the spring as a pulse. While the uptake of nutrient pulses by plants may be more important than uptake under steady state conditions over winter (Jonasson and Chapin, 1991; Jonasson *et al.*, 1999), the ability of plants to exploit nitrogen pulses during spring melt can vary widely among systems (Bilbrough *et al.*, 2000). Studies that have shown high plant uptake at spring melt (Andresen and Michelsen, 2005) also used less mobile forms of nitrogen than nitrate and injected the labeled substrate directly into the soil. Surface additions may be more vulnerable to runoff or denitrification under the water-saturated soil conditions in early spring (Muller *et al.*, 2002).

4.3 Directions for future research

Extreme events may be more important to soil N dynamics than mean temperature changes. For example, unusually late or early frosts, in the absence of a protective snow cover, may have large effects on soil N. Hard frost events have been shown to decrease microbial biomass (Herrmann and Witter, 2002; Sulkava and Huhta, 2003; Dorsch *et al.*, 2004; Hentschel *et al.*, 2008), trigger N leaching (Boutin and Robitaille, 1995; Brooks *et al.*, 1998) and increase N_2O emissions (Flessa *et al.*, 1995; Sylvasalo *et al.*, 2004). While studies have shown that microbes can recover from severe frost (Stenberg *et al.*, 1998;

Pesaro *et al.*, 2003), the effects of hard frost on slow growing microbial communities (e.g. nitrifiers) need more attention in future work (Matzner and Borken, 2008). While substantial changes in the timing and length of the growing season may increase productivity, changes to plant phenology caused by warming can also increase the vulnerability of plants to early or late season frosts (Norby *et al.*, 2003).

Increasing CO₂ will also have substantial direct effects on plant growth and important indirect effects on soil N dynamics. These effects, however, remain uncertain given that a system's response to elevated CO₂ is a combination of various plant component responses and soil feedbacks that operate on multiple time scales (Rastetter *et al.*, 1997). For example, in the short term, elevated CO₂ may increase photosynthetic rates, though in the long term altered litter chemistry may affect soil processes and soil N cycling that will feedback on the initial plant growth response (Medlyn and McMurtrie, 2004). Furthermore, plants grown under elevated CO₂ have been shown to have lower freeze tolerance, making even the most freeze-tolerant species vulnerable to extreme temperature events, such as frost damage at warmer freezing temperatures (Repo *et al.*, 1996; Lutze *et al.*, 1998; Barker *et al.*, 2005). Therefore, increasing atmospheric CO₂ introduces another important factor that will interact with warming and increasing N deposition to further alter soil N dynamics.

4.4 References

- Aber J.D., McDowell W., Nadelhoffer K., Magill A., Berntson G., Kamakea M., McNulty S.G., Currie W., Rustad L., Fernandez I., 1998. Nitrogen saturation in temperate forest ecosystems: Hypotheses revisited. *Bioscience* **48**: 921–933.
- Andresen L.C., and Michelsen A., 2005. Off-season uptake of nitrogen in temperate heath vegetation. *Oecologia* **144**: 585–597.
- Bardgett R.D., Mawdsley J.L., Edwards S., Hobbs P.J., Rodwell J.S., Davies W.J., 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. *Functional Ecology* **13**: 650–660.
- Barker D.H., Loveys B.R., Egerton J.J.G., Gorton H., Williams W.E., Ball M.C., 2005. CO₂ enrichment predisposes foliage of a eucalypt to freezing injury and reduces spring growth. *Plant, Cell and Environment* **28**: 1506–1515.
- Bilbrough C.J., Welker J.M., Bowman W.D., 2000. Early spring nitrogen uptake by snow covered plants: A comparison of arctic and alpine plant function under the snowpack. *Arctic, Antarctic & Alpine Research* **32**: 404–411.
- Bilbrough C.J., and Caldwell M.M., 1997. Exploitation of springtime ephemeral N pulses by six Great Basin plant species. *Ecology* **78**: 231–243.
- Boutin R., and Robitaille G., 1995. Increased soil nitrate losses under mature sugar maple trees affected by experimentally induced deep frost. *Canadian Journal of Forest Research* **25**: 588–602.
- Brooks P.D., and William M.W., 1999. Snowpack controls on nitrogen cycling and export in seasonally snow covered catchments. *Hydrological Processes* **13**(14–15): 2177–2190.
- Campbell J.L., Mitchell M.J., Groffman P.M., Christenson L.M., Hardy J.P., 2005. Winter in northeastern North America: a critical period for ecological processes. *Frontiers in Ecology & the Environment* **3**(6): 314–322.
- Craine J.M., Reich P.B., Tilman D., Ellsworth D., Fargione J., Knops J., 2003. The role of plant species in biomass production and response to elevated CO₂ and N. *Ecology Letters* **6**: 623–630.
- Cui M., and Caldwell M.M., 1997. Growth and nitrogen uptake by *Agropyron desertorum* and *Pseudoroegneria spicata* when exposed to nitrate pulses of different duration. *Australian Journal of Plant Physiology* **24**: 637–642.
- Currie W., Aber J.D., McDowell W.H., Boone R.D., Magill A.H., 1996. Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. *Biogeochemistry* **35**: 471–505.

- Deluca T.H., Keeney D.R., McCarty G.W., 1992. Effect of freeze-thaw events on mineralization of soil-nitrogen. *Biology and Fertility of Soils* **14**: 116–120.
- Dijkstra F.A., Hobbie S.E., Knops J.M.H., Reich P.B., 2004. Nitrogen deposition and plant species interact to influence soil carbon stabilization. *Ecology Letters* **7**: 1192–1198.
- Dorsch P., Palojarvi A., Mommertz S., 2004. Overwinter greenhouse gas fluxes in two contrasting agricultural habitats. *Nutrient Cycling in Agroecosystems* **70**: 117–133.
- Emmett B.A., Brittain A., Hughes S., Gorres J., Kennedy V., Norris D., Rafarel R., Reynolds B., Stevens P.A., 1995. Nitrogen additions at Aber forest, Wales. I. Response of throughfall and soil water chemistry. *Forest Ecology & Management* **71**: 45–60.
- Fenn M.E., Poth M.A., Aber J.D., Baron J.S., Bormann B.T., Johnson D.W., Lemly A.D., McNulty S.G., Ryan D.F., Stottlemyer R., 1998. Nitrogen excess in north American ecosystems: predisposing factors, ecosystem responses, and management strategies. *Ecological Applications* **8**(3): 706–733.
- Flessa H., Dorsch P., Beese F., 1995. Seasonal variation of N₂O and CH₄ fluxes in differently managed arable soils in southern Germany. *Journal of Geophysical Research* **100**: 23115–23124.
- Grogan P., Michelsen A., Ambus P., Jonasson S., 2004. Freezethaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. *Soil Biology & Biochemistry* **36**: 641–654.
- Henry H.A.L., 2008. Climate change and soil freezing dynamics: historical trends and projected changes. *Climatic Change* **87**: 421–434.
- Hentschel K., Borken W., Matzner E., 2008. Leaching losses of nitrogen and dissolved organic matter following repeated frost/ thaw events in a forest soil. *Journal of Plant Nutrition and Soil Science*, in press.
- Herrmann A., and Witter E., 2002. Sources of C and N contributing to the flush in mineralization upon freeze-thaw cycles in soils. *Soil Biology & Biochemistry* **34**: 1495–1505.
- Hooper D.U., Chapin F.S., Ewel J.J., Hector A., Inchausti P., Lavorel S., 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* **75**: 3–35.
- Isard S.A., and Schaetzl R.J., 1998. Effects of winter weather conditions on soil freezing in southern Michigan. *Physical Geography* **19**: 71–94.
- Jackson R.B., Banner J.L., Jobaggy E.G., Pockman W.T., Wall D.H., 2002. Ecosystem carbon loss with woody plant invasion of grasslands. *Nature* **418**: 623–626.

Jonasson S., and Chapin F.S., 1991. Seasonal uptake and allocation of phosphorus in *Eriophorum vaginatum* L. measured by labeling with ^{32}P . *New Phytologist* **118**: 349-357.

Jonasson S., Michelsen A., Schmidt I.K., 1999. Coupling of nutrient cycling and carbon dynamics in the Arctic, integration of soil microbial and plant processes. *Applied Soil Ecology* **11**: 135-146.

Joseph G., and Henry H.A.L., 2008. Soil nitrogen leaching losses in response to freeze-thaw cycles and pulsed warming in a temperate old field. *Soil Biology & Biochemistry* **40**(7): 1947-1953.

Lutze J.L., Roden J.S., Holly C., Wolfe J., Egerton J.J.G., Ball M.C., 1998. Elevated atmospheric CO_2 promotes frost damage in evergreen tree seedlings. *Plant, Cell & Environment* **21**: 631-635.

Magill A.H., Downs M.R., Nadelhoffer K.J., Hallett R.A., Aber J.D., 1996. Forest ecosystem response to four years of chronic nitrate and sulfate additions at Bear Brooks Watershed, Maine, USA. *Forest Ecology & Management* **84**: 29-37.

Matson P., Lohse K.A., Hall S.J., 2002. The Globalization of Nitrogen Deposition: Consequences for Terrestrial Ecosystems. *Ambio* **31**(2): 113-119.

Matzner E., and Borken W., 2008. Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review, *European Journal of Soil Science* **59**: 274-284.

McDowell W., Currie W. S., Aber J.D., Yano Y., 1998. Effects of chronic nitrogen amendments on production of dissolved organic carbon and nitrogen in forest soils. *Water, Air, and Soil Pollution* **105**: 175-182.

Medlyn B.E., and McMurtrie R.E., 2004. Effects of CO_2 on plants at different timescales. In: *A History of Atmospheric CO_2 and Its Effects on Plants, Animals, and Ecosystems* (eds Ehleringer JR, Cerling TE, Dearing MD), pp. 441-465. Springer Science, New York.

Muller C., Martin M., Stevens R.J., Laughlin R.J., Kammann C., Ottow J.C.G., Jager H.J., 2002. Processes leading to N_2O emissions in grassland soil during freezing and thawing. *Soil Biology & Biochemistry* **34**: 1325-1331.

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

Norby R.J., Hartz-Rubin J.S., Verbrugge M.J., 2003. Phenological responses in maple to experimental atmospheric warming and CO₂ enrichment. *Global Change Biology* **9**: 1792–1801.

Rastetter E.B., Agren G.I., Shaver G.R., 1997. Responses of N-limited ecosystems to increased CO₂: a balanced-nutrition, coupledelement-cycles model. *Ecological Applications* **7**: 444–460.

Reich P.B., Knops J., Tilman D., Craine J., Ellsworth D., Tjoelker M., 2001. Plant diversity enhances ecosystem responses to elevated CO₂ and nitrogen deposition. *Nature* **410**: 809–812.

Repo T., Hanninen H., Kellomaki S., 1996. The effects of long term elevation of air temperature and CO₂ on the frost hardiness of Scots pine. *Plant, Cell & Environment* **19**: 209–216.

Skogland T., Lomeland S., Goksøyr J., 1988. Respiratory burst after freezing and thawing of soil: experiments with soil bacteria. *Soil Biology & Biochemistry* **20**: 851–856.

Sulkava P., and Huhta V., 2003. Effects of hard frost and freeze-thaw cycles on decomposer communities and N mineralisation in boreal forest soil. *Applied Soil Ecology* **22**: 225–239.

Sylvasalo E., Regina K., Pihlatie M., Esala M., 2004. Emissions of nitrous oxide from boreal agricultural clay and loamy sand soils. *Nutrient Cycling in Agroecosystems* **69**: 155–165.

Teepe R., Brumme R., Beese F., 2001. Nitrous oxide emissions from soil during freezing and thawing periods. *Soil Biology & Biochemistry* **33**: 1269–1275.
Tilman D., Downing J.A., 1994. Biodiversity and stability in grasslands. *Nature* **367**: 363–365.

Tye A.M., Young S.D., Crout N.M.J., West H.M., Stapleton L.M., Poulton P.R., Laybourn-Parry J., 2005. The fate of N-15 added to high Arctic tundra to mimic increased inputs of atmospheric nitrogen released from melting snowpack. *Global Change Biology* **11**: 1640–1654.

Van Vuuren M.M.I., Aerts R., Berendse F., De Visser W., 1992. Nitrogen mineralization in heathland ecosystems dominated by different plant species. *Biogeochemistry* **16**: 151–166.

Wardle D.A., and Zackrisson O., 2005. Effects of species and functional group loss on island ecosystem properties. *Nature* **435**: 806–810.