

THE INFLUENCE OF WINTER FIELD COVER ON SPRING NITROUS OXIDE
EMISSIONS

A Thesis

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ABSTRACT

Agriculture is responsible for 58% of the anthropogenic emissions of nitrous oxide (N₂O), a source of stratospheric ozone depletion and a greenhouse gas that contributes to global climate change. In temperate regions, a majority of this N₂O is emitted during freeze-thaw cycles (FTC) in the spring. Climate change models predict that warming trends in the northeast will result in less snow cover, potentially leading to colder soils that may in turn lead to higher N₂O emissions. A winter soil management strategy is needed to mitigate spring N₂O emissions. In this study, I examined the influence of two winter field covers, snow and winter rye, on soil temperature and nitrogen (N) content and subsequent spring N₂O emissions from a NY corn field over two years. A 2 x 2 factorial of rye (+/-) by snow cover (+/-) was established in a randomized complete block design. Nitrous oxide emissions were measured bi-weekly using a static chamber method. The first season (2006-07) was a cold winter (2309 h below 0°C at 8 cm soil depth), historically typical for the region. The snow removal treatment resulted in colder soils and higher N₂O fluxes (73.3 vs. 57.9 ng N₂O-N cm⁻² h⁻¹). The rye cover had no effect on N₂O emissions. The second season (2007-08) was a much milder winter (1271 h below freezing at 8 cm soil depth), with lower N₂O fluxes overall. Winter rye cover resulted in lower N₂O fluxes (5.9 vs. 33.7 ng N₂O-N cm⁻² h⁻¹), but snow removal had no effect. These results suggest that if winters remain typically cold in the Northeastern U.S., but snowfall is reduced, we may expect higher N₂O emissions, with winter rye cover unlikely to mitigate this. If, however, less snow cover is due to warmer temperatures as predicted, we may be trending towards lower spring N₂O emissions where winter rye cover cropping may be a useful mitigation tool. The field experiment showed that temperature buffering created by an insulating soil cover during the winter may lead to

lower N₂O emissions in the spring. Insulation may result in higher minimum soil temperatures, shorter freeze duration, fewer FTC, and slower rates of freezing and thawing. One of these temperature variables, slower thawing, was examined by measuring N₂O fluxes in a laboratory-simulated FTC. Slower thawing led to higher N₂O emissions (1200 vs. 750 ng N₂O-N cm⁻² h⁻¹). This suggests that slower thawing is not the mechanism responsible for lower N₂O emissions observed in agricultural fields with soil cover. Rather, one of the other variables mentioned may be more important. It is unclear if high spring emissions result from a decrease in the efficiency of N₂O reduction to N₂. A laboratory-simulated FTC was also used to investigate the ratio of N₂O to total gaseous N emitted (rN₂O) during periods of high N₂O emissions. Results showed that rN₂O decreased (0.64 → 0.0) over time after thawing. This suggests that a lack of reduction of N₂O to N₂ may contribute to high N₂O emissions measured during soil thawing. Gaining an understanding of why N₂O emissions are high during spring thawing and how these emissions are affected by snow cover, rye cover cropping and the rate of soil thawing will aid researchers and land owners in designing useful N₂O emission mitigation strategies.

BIOGRAPHICAL SKETCH

Ranae was born near Radcliffe, Iowa, to Rand and Tammy Faaborg, and has three brothers, Tyler, Tanner, and Trey, and one sister, Riley. She attended Hubbard-Radcliffe elementary, middle, and high schools until going to college at the University of Minnesota – Morris. During her summers, Ranae worked in the Natural Resource Ecology and Management Department at Iowa State University, Agro-Soyuz in Majskoye, Ukraine, and the ARS North Central Soil Conservation Laboratory in Morris, MN. Ranae graduated from UM-Morris in 2006 with a B.A. in Biology and married Kevin Dietzel the following summer. She began her Master's work at Cornell in fall of 2006.

Мы не перестанем исследовать и конец всего нашего исследования придет тогда, когда мы очутимся на том же месте где начали и познаем это место впервые Т.С.Элиот.

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CHAPTER 1

INTRODUCTION

Humans live outside of the basic needs required by most other species. As such, we do not participate in the usual system of checks and balances that equilibrate natural ecosystems. In this chapter, I review global biogeochemical processes, how humans have altered these processes, and the consequences of these actions. From there, I focus on nitrous oxide (N_2O) as an important greenhouse gas. I discuss strategies that can be used to reduce N_2O emissions from agricultural systems and review the scientific understanding needed to make these strategies more effective. Finally, I present the hypotheses that were tested in the work reported here.

Global Biogeochemical Carbon and Nitrogen Cycling

Our planet is, for the most part, a closed system. With the exception of meteors and rockets, matter does not enter or exit this system and can be neither created nor destroyed (Schlesinger 1997). Elements within this system can change state, transforming between solid, liquid, and gas and moving from pool to pool as various functions are served. To maintain balance, each element needs to return to its original state to create a cycle. Elemental cycles often have key transformations that are carried out by living organisms.

Carbon (C)

Carbon is the main elemental component of biota. Energy from the sun is captured by photosynthetic organisms, creating organic C compounds that fuel chemosynthetic heterotrophs. As these heterotrophs metabolize organic C compounds, C is released back into the atmosphere in the form of CO_2 . Under very

reducing conditions where other electron acceptors are limiting and in the presence of methanogens, C compounds can also be reduced to methane (CH₄). Carbon that is incorporated into molecules too complex to be metabolized is subjected to longer term decay (sometimes thousands of years) by weathering or incorporated into mineral structures (Horwath 2007).

Most of the Earth's C is found in sedimentary rocks in the form of organic compounds (1.56×10^{22} g C) and carbonate (6.5×10^{22} g C). Extractable fossil fuels are estimated at 4×10^{18} g C and active pools near the Earth's surface contain about 40×10^{18} g C. The biggest of these active pools is the ocean (3.8×10^{19} g C), which contains about 56 times as much C as the atmosphere. The atmosphere, in turn, contains more C than all living vegetation, but less than is contained in soils (Figure 1.1) (Schlesinger 1997).

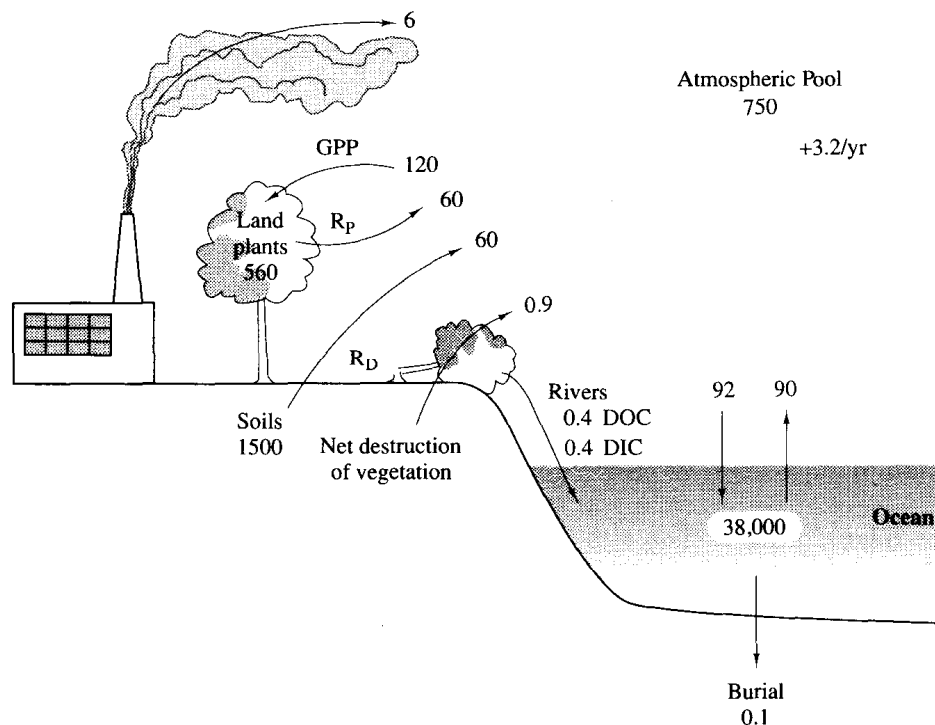


Figure 1.1 Present day global carbon cycle. All pools are expressed in 10^{15} g C and annual fluxes in units of 10^{15} g C y^{-1} , averaged for the 1980s (from Schlesinger 1997).

Carbon in the atmosphere has a mean residence time of 5 y before it is captured by plants during photosynthesis, bacteria during chemosynthesis or exchanged into the ocean. Once incorporated into vegetation, most fixed organic C will eventually be used by other organisms for growth and metabolism (Horwath 2007). The time scale for this varies, but two-thirds of the Earth's vegetation is seasonal, meaning C may remain in plants and/or their residues a matter of months before being decomposed. Decomposition converts all but the most recalcitrant organic C back to CO₂ in the atmosphere. Carbon dioxide absorbed by the ocean has a mean residence time of ~11 y in surface waters, but ~350 y if mixed into the deep ocean sediments (Schlesinger 1997).

Nitrogen (N)

Nitrogen is also an element important to and influenced by the biota. In many systems, N is the element limiting biological production. Nitrogen can be part of assimilatory processes as it is used to build nucleic acids and proteins. It can also be used in dissimilatory processes as a terminal electron acceptor (Robertson and Groffman 2007). The terrestrial nitrogen cycle is complex and N takes many forms.

Most nitrogen is biologically unavailable as it is in the form of nitrogen gas (N₂). Nitrogen gas consists of two N atoms joined by a triple bond which requires high amounts of energy to break. In nature, this bond can be broken and N can be "fixed" either by lightning or N₂ fixing bacteria or archaea (diazotrophs) (Purves et al. 2001).

When N is fixed biologically, it becomes incorporated into biomass either by the diazotroph responsible for the fixation or a plant having a symbiotic relationship with the diazotroph. Thus N fixed from the atmosphere to NH₄⁺ is rapidly converted

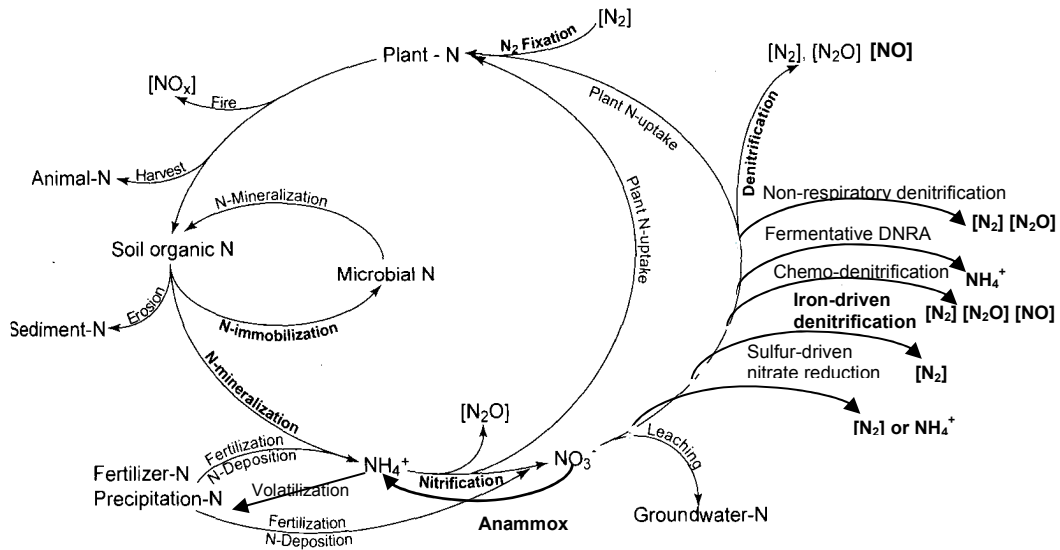


Figure 1.2 A representation of the major N pools in the N cycle (gases are bracketed) (from Robertson and Groffman 2007) combined with pathways of NO_3^- removal as described by Burgin and Hamilton (2007).

to an organic form by enzymes, such as glutamine synthetase, to form amino acids. The transformation of organic N to inorganic N is called mineralization (Figure 1.2). Nitrogen mineralization occurs as microorganisms release N as a by-product of consumption of organic materials (Robertson and Groffman 2007). Microorganisms consume organic materials primarily to gain energy and carbon to support their growth, but also need N for nucleic acids, proteins, and cell walls. If the amount of C available in the organic material exceeds the amount of N needed for growth, N will not be released as a by-product, but instead immobilized in biomass (Figure 1.2). Nitrogen that was already available in the environment may also be immobilized under high C conditions (Brady and Weil 2002).

Nitrogen that is mineralized first takes the form of ammonia (NH_3) and its ionized form ammonium (NH_4^+). Oxidation of these forms of nitrogen leads to nitrite (NO_2^-) and then nitrate (NO_3^-). This oxidation is called nitrification (Figure 1.2) and is responsible for most of the NO_3^- found in the soil. During nitrification, some

intermediary N compounds take gaseous forms, such as N_2O , that can be lost to the atmosphere during the process (Robertson and Groffman 2007).

Once N takes the negatively charged form of NO_3^- , it becomes available to different organisms and is easily mobilized by water (Brady and Weil 2002). Nitrate has an oxidation state of +5 and is used as a terminal electron acceptor in a number of biological pathways (Figure 1.2). Denitrification is anaerobic microbial respiration in which NO_3^- is sequentially reduced to N_2 , returning N to the atmosphere (Robertson and Groffman 2007).

The following processes also remove NO_3^- from the N cycle, but their mechanisms, importance, and ubiquity are poorly understood. Non-respiratory denitrification is similar to respiratory denitrification, but does not enhance growth and occurs in aerobic environments (Robertson and Groffman 2007). Nitrate can also be transformed back to NH_4^+ by dissimilatory nitrate reduction to ammonia (DNRA). DNRA can be either a fermentative process or performed by chemolithoautotrophs when coupled to the reduction of sulfur (S) (Burgin and Hamilton 2007). Reduction of nitrate can also be coupled with the oxidation of methane or to iron (Fe) cycling. When NO_3^- is reduced in the beginning of many of these pathways, the first product is NO_2^- . Nitrite can then be combined with NH_4^+ , producing N_2 in a process known as anaerobic ammonium oxidation (anammox). Since the NO_2^- originally comes from NO_3^- , this is also considered a form of nitrate removal (Burgin and Hamilton 2007).

The largest pool of N is in the atmosphere (3.9×10^{21} g N) followed by smaller amounts in terrestrial biomass (3.5×10^{15} g N) and soil organic matter ($95\text{-}140 \times 10^{15}$ g N). Despite large annual fluxes, inorganic pools of N in the soil are very small due to rapid biological uptake. Rivers carry about 36×10^{12} g N to the ocean annually, while groundwater may receive about 11×10^{12} g N every year. In addition to N

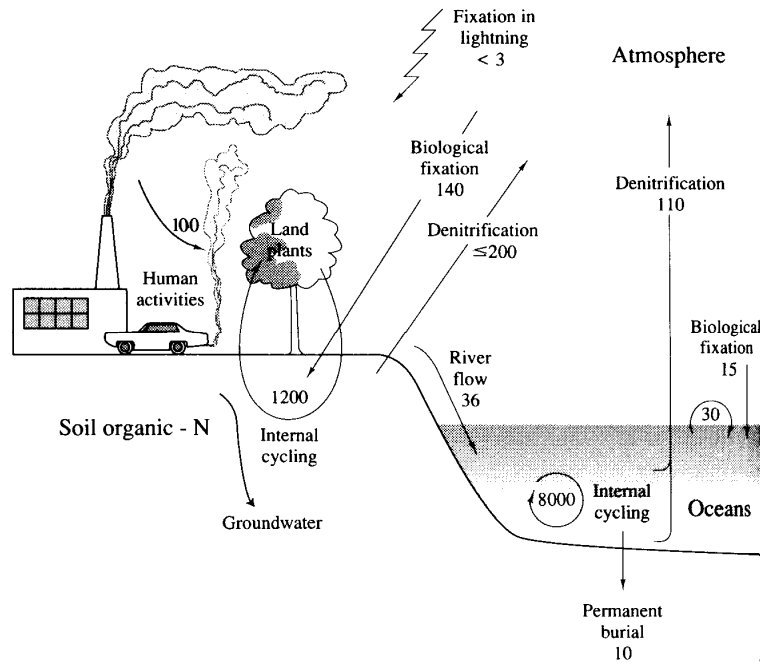


Figure 1.3 The global nitrogen cycle. Each flux is shown in units of 10^{12} g N y^{-1} (from Schlesinger 1997).

received from rivers, the ocean takes in 30×10^{12} g N from precipitation and 15×10^{12} g N by biological N_2 -fixation (Figure 1.3) (Schlesinger 1997).

Role of C and N in Climate Forcing

Carbon and N are crucial to biota on the Earth's surface, but their global cycles are also significant to the functioning of the planet itself. Although CO_2 and reactive forms of N have very small concentrations in the atmosphere, they play big roles in atmospheric processes.

The greenhouse effect

Carbon and N are major players in the global process known as the greenhouse effect. The sun radiates energy at very short wavelengths (visible to near visible, e.g., ultra-violet) to the Earth, a third of which is reflected off of the Earth's atmosphere back into space. The rest proceeds to the Earth's surface where it is

absorbed and used for energy by the planet's photoautotrophs. To balance this energy exchange, the Earth must radiate the same amount of energy back to space. The Earth is much colder than the sun, so this is done in longer wavelengths in the infrared part of the spectrum. These longer waves are absorbed by the atmosphere and reradiated back to the land and oceans (Forster et al. 2007).

Constituents of the atmosphere determine how much of the sun's energy penetrates the atmosphere and how much is radiated back to the Earth. Dinitrogen and oxygen (O₂) make up 78% and 21% of the atmosphere, respectively, but have very little greenhouse effect. Water vapor is the most effective greenhouse gas (GHG), followed by CO₂, CH₄, and N₂O (Forster et al. 2007). Increasing concentrations of these gases in the atmosphere lead to more of an effect, which can encourage more water vapor. Concentrations of these gases in the atmosphere depend upon changes in the C and N cycles.

Ozone depletion

The production of ozone (O₃) in the stratosphere accounts for most of the absorption of ultraviolet (UV) sunlight ($h\nu$) at wavelengths of 180-240 nm in the reaction $(O_2 + h\nu) + 2O_2 \rightarrow O + O + 2O_2 \rightarrow 2O_3$. Absorption of wavelengths between 200 and 320 nm destroys O₃, warms the stratosphere and blocks UVB rays. Ozone can also be destroyed in the stratosphere by reactions with nitric oxide (NO). This NO comes from N₂O, which mixes up from the troposphere. Nitrous oxide is destroyed through photolysis in the equation $N_2O \rightarrow N_2 + O(^1D)$ and then further reacts with the O(^1D) in the reaction $N_2O + O(^1D) \rightarrow 2NO$ (Schlesinger 1997). Ozone depletion resulting from this equation does not warm the stratosphere and upsets the balance between O₃ creation and destruction, leading to increases in UV at the earth's surface.

Anthropogenic Influences on C and N Cycling

Carbon

Humans have influenced the C cycle by extracting deeply buried C in the form of fossil fuels and releasing it to the atmosphere through combustion used for energy production. While this C took thousands of years to accumulate, much of it has been released over the last hundred years since industrialization. Before the invention of internal combustion engines at the turn of the 20th century and resulting industrialization, the atmospheric CO₂ concentration was 280 ppm. By 2005, it increased to 379 ppm. Previous to this increase, the largest increase was by 20 ppm and this occurred over an 8000 year period (Denman et al. 2007). In addition to CO₂ increases from fossil fuel emissions, humans have changed land use to decrease the amount of C stored in soils and vegetation. Agriculture has resulted in losses in soil organic matter and deforestation to create cropland. Two-thirds of the change in CO₂ concentration comes from fossil fuel emissions and one-third comes from land use change. Only 50% of anthropogenic emissions are removed through exchange with vegetation and the ocean each year. This upset in the balance of the C cycle is of concern due to the status of CO₂ as a GHG. Increases in CO₂ concentrations have led to a 20% increase in radiative forcing since 1995 (Forster et al. 2007).

Methane emissions have also been increased by anthropogenic activity. Pre-industrial CH₄ emissions are estimated to have been at 200-250 Tg CH₄ y⁻¹, while current emissions are 582 Tg CH₄ y⁻¹. More than 60% of these emissions are from human influences in the form of rice agriculture, livestock husbandry, landfills, fossil fuel and biomass burning, and waste treatment. Methane is responsible for 18% of current radiative forcing (not including forcing from water vapor) but in recent years its atmospheric concentration has remained stable for reasons not understood (Denman et al. 2007).

Nitrogen

Problems have been created in the C cycle by increasing its concentration in the atmosphere. However, humans have negatively influenced the N cycle by removing N from the atmosphere and transforming it into a form that is biologically available, commonly referred to as reactive N (rN) (Galloway et al. 2003). Increases in rN have come from fixation of N through the Haber-Bosch process, an industrial process that combines N_2 and hydrogen (H) under high heat and pressure to produce NH_3 . This process is now responsible for fixing 160 Tg N y^{-1} , more than is produced by natural biological nitrogen fixation on land (110 Tg N y^{-1}) or in the ocean (140 Tg N y^{-1}) (Gruber 2008). While the Haber-Bosch process was invented to create various N compounds for use in explosives used during the first World War, it was quickly adapted as a way to produce fertilizer for agriculture to support a growing population (Gruber 2008). In addition to N fixed by the Haber-Bosch process, agriculture has also increased the amount of legumes present on this planet, greatly increasing the amounts of biological N fixation from 15 Tg N y^{-1} in 1860 to 33 Tg N y^{-1} in 2000 (Galloway et al. 2003). Included in this increase in rN is an increase in N_2O .

Consequences of Changes in C and N Cycling

According to the recent report by the IPCC (Forster et al. 2007), increases in CO_2 , CH_4 , and N_2O in the atmosphere and the resulting increase in radiative forcing has led to changes in the planet's climate. Foremost among these is an average global warming of 0.74°C over the last 100 years. Direct effects of this climate change are rising sea levels (3.1 mm y^{-1}), decreases in snow and ice cover, melting glaciers, increasing ground instability in permafrost regions, increasing drought since the 1970's, more frequent heat waves, earlier timing of spring events, northward shifts in plant and animal populations, increasing heat-related mortality and changes in

infectious disease vectors in the Northern Hemisphere (Rosenzweig et al. 2007). As climate change continues to occur at the current rate, these changes will intensify, leading to a predicted decrease of 30% in species diversity, limited food resources due to agricultural failure, and loss of land to rising sea levels (Schneider et al. 2007).

Increases in rN also have far reaching consequences (Vitousek et al. 1997). Reactive N is dispersed widely by global transport in the hydrosphere and atmosphere and accumulates in the environment because its creation is now greater than its removal through denitrification (Gruber and Galloway 2008). This has led to many changes including decreases in tropospheric ozone, increases in vegetative productivity that alter previous ecosystem dynamics, decreases in biodiversity in natural ecosystems, acidification in lakes and streams, eutrophication in coastal areas, and increased N₂O emissions which further increase radiative forcing (Vitousek 1997).

Nitrous Oxide

Carbon and N play crucial roles in the Earth's functioning and anthropogenic influences on C and N cycles have led to detrimental climate and environmental change. To design effective strategies to mitigate negative anthropogenic influences, we must first be able to identify and understand their sources. Carbon dioxide comes mainly from increases in fossil fuel use and it is understood that using less fossil fuels will result in lower CO₂ emissions. However, stopping the use of all industrial and personal machines powered by fossil fuels is socially, economically, and politically unfeasible. Instead, ways need to be sought to power these machines without fossil fuels. In the same way, we know that agriculture is responsible for a majority of the N₂O produced over the last 50 years (Smith et al. 2007), but it is unrealistic to plan to return to agricultural practices as they were 100 years ago. The human population has grown exponentially and its continued growth will most likely require continued,

large-scale, N-intensive agriculture. Our challenge is to find management strategies that will allow sufficient food production while decreasing the effect this production has on associated ecosystems. As engineers design cars that emit less CO₂, we as agronomists have an obligation to design agricultural systems that emit less N₂O. Testing N₂O mitigation strategies is one of the aims of the work reported herein.

As mentioned previously, N₂O plays a role in climate forcing both as a greenhouse gas and as a source of NO leading to stratospheric ozone depletion. Atmospheric concentrations of N₂O have increased 16% since the pre-industrial era, rising from 270 ppb to 319 ppb with an annual growth rate of about 0.3%. Total annual emissions are estimated at 20.6 Tg N y⁻¹, with 38% of these coming from anthropogenic sources (Denman et al. 2007). Of the anthropogenic emissions, 58% come from agriculture and the IPCC points to enhanced microbial N₂O production in expanding and fertilized agricultural lands as the primary source of increased post-industrial N₂O emissions (Smith et al. 2007). Logging has been estimated to increase N₂O emissions by 30-330% in forested systems, depending on conditions. Coastal N₂O emissions are responsible for approximately 0.2 Tg N y⁻¹ of annual emissions and river and estuaries for 1.5 Tg N y⁻¹. The biggest natural sources of N₂O are from vegetated soils (6.6 Tg N y⁻¹), followed by ocean emissions (3.8 Tg N y⁻¹). On a microscale, N₂O has many sources, although it is most commonly a by-product of microbially mediated N transformations. Denitrification, non-respiratory denitrification, nitrification, DNRA, and anammox all use N₂O as an intermediate and may release it as a by-product. Denitrification follows the sequence:



Nitrification is less understood, but probably proceeds as shown in Figure 1.4 (Robertson and Groffman 2007). DNRA and anammox are even less well

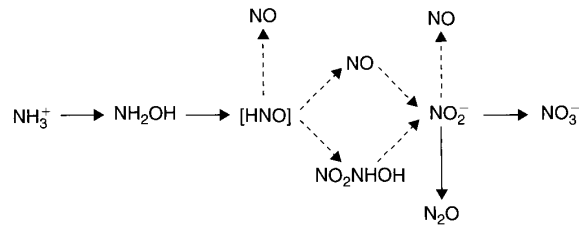


Figure 1.4 Autotrophic nitrification pathways including pathway for gas loss. Broken lines indicate unconfirmed pathways (from Robertson and Groffman 2007).

understood as N_2O sources and no pathways have been described for how N_2O is produced. It is possible that N_2O is also a by-product during other NO_3^- removal pathways, such as iron-driven denitrification and sulfur-driven nitrate reduction. The relative importance of these processes to N_2O emissions is unknown, although their prevalence under different conditions has been hypothesized as shown in Figure 1.5 (Burgin and Hamilton 2007).

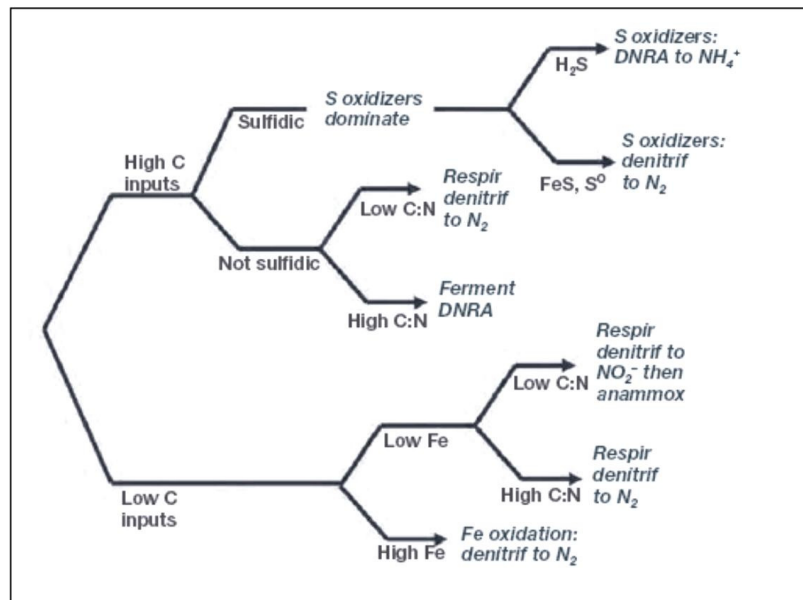


Figure 1.5 Hypothesized controls on pathways of NO_3^- removal under different environmental conditions (from Burgin and Hamilton 2007).

Denitrification

Respiratory denitrification will be the primary source of N_2O discussed in this thesis because it is widely believed to be the dominant source of N_2O in the environment studied in my work. Denitrifying bacteria obtain their energy through oxidative phosphorylation, requiring a terminal electron acceptor (TEA) (Zumft 1997). Oxygen (O_2) is the preferred TEA, but in its absence NO_3^- can be used by many microorganisms. Carbon is also required as a substrate for this process. Nitrate goes through a series of reductions and intermediary compounds with the final product of complete reduction being N_2 gas. During these reductions, N takes many forms, some of which are not further reduced. This is the case for intermediaries such as NO and N_2O (Robertson and Groffman 2007). Nitrogen transformations during denitrification are catalyzed by a number of enzymes as illustrated in Figure 1.6.

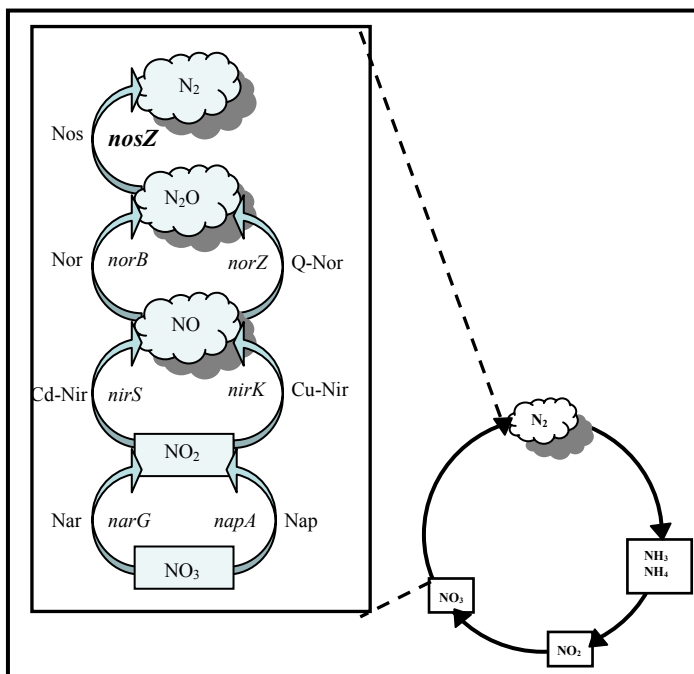


Figure 1.6 Denitrification pathway as part of a simplified N cycle. Enzyme names appear to the outside of each step in the pathway. Names for the corresponding genes appear on the inside of each step in the pathway.

Nitrate is initially reduced to nitrite by nitrate reductase, of which there are two different types, Nar and Nap. Both enzymes are molybdenum (Mo) dependent and possess the cofactor molybdopterin. However, Nar is a membrane-bound enzyme, while Nap is periplasmic and can also be expressed under aerobic conditions (Throback 2006). Denitrifying bacteria possess either one or both of these enzymes, but the enzymes are not limited solely to denitrifiers (Carter et al. 1995, Roussel-Delif et al. 2005).

In the next step, nitrite is transformed from a soluble form to a gaseous state. This action is specific to denitrifiers and is catalyzed by nitrite reductase (Nir) in the form of copper (Cu)-Nir or cadmium (Cd)-Nir. Cu-Nir is a copper nitrite reductase and Cd-Nir is a cytochrome Cd1 nitrite reductase (Zumft 1997). The enzymes are equivalent and while no bacteria have been found to possess the genes for both enzymes, some have been described that have two copies of *nirS*, the gene encoding Cd-Nir (Etchebere and Tiedje 2005). In such cases, one copy is constitutively expressed and the other positively regulated by nitrite concentration.

The reduction of NO is then catalyzed by nitric oxide reductase (Nor). Nor activity is closely coupled with Cd-Nir, but not Cu-Nir. There are two different types of Nor. cNor receives electrons from cytochrome c and qNor receives electrons from a quinol pool. While the gene that codes for cNor is unique to denitrifiers, a gene coding for qNor is also found in other bacteria (Throback 2006).

Nos, the enzyme that catalyzes nitrous oxide reduction, is a periplasmic homodimeric protein that is lacking in some denitrifiers. This enzyme seems to be the most sensitive to changes in the immediate environment (Firestone et al. 1980, Holtan-Hartwig 2002). It should be recognized that the absence or dysfunction of Nos prevents N₂O from being reduced to N₂.

Agricultural N₂O mitigation

Agriculture contributes a large proportion of N₂O emitted, but by definition is a practice we have some control over. As such, many methods aimed to mitigate N₂O emissions have been proposed and investigated including crop rotations, changes in fertilizer regimes, cover cropping, and tillage. Crop rotations can reduce N₂O emissions (Halvorson et al. 2008) with the lowest emissions occurring during legume use (Pennock et al. 2004, Gregorich et al. 2007, Dusenbury et al. 2008) and the highest emissions occurring following fertilizer demanding crops, such as corn (*Zea mays*) and wheat (*Triticum aestivum* L.) (Dusenbury et al. 2008, Drury et al. 2007).

Fertilizer plays an important role because it determines the availability of NO₃⁻ during denitrification and NH₄⁺ during nitrification. Peaks in N₂O emissions are typically seen after application of N fertilizers (Tan et al. 2008, Dusenbury et al. 2008, Halvorson et al. 2008, Phillips et al. 2007, Burton et al. 2007). Many authors call for better N use efficiency through more synchronous timing of N applications with crop N uptake (Burton et al. 2007, Gregorich et al. 2007, Phillips 2008), more appropriate application rates (Smith et al. 1997, Mosier et al. 1998) and more precise placement (Komatsuzaki and Ohta 2007).

Cover cropping can have mixed effects on N₂O emissions, with some studies finding no effect on annual N₂O emissions (Parkin and Kaspar 2006) and lowered N₂O fluxes in others (Parkin et al. 2006). Cover crops may lower N₂O emissions by limiting available NO₃⁻. Winter rye (*Secale cereale* L.) has been found to be an especially effective N scavenger (Shipley et al. 1992, Guillard 1995, Strock et al. 2004, Rich 2007). Cover crops may also contribute to N₂O emissions by promoting denitrification. Root respiration and microbial respiration stimulated by root exudates have been shown to reduce O₂ levels sufficiently enough to induce anaerobic

environments and, subsequently, denitrification (Smith and Tiedje 1979, Haider et al. 1986, Klemedtsson et al. 1987, Prade and Trolldenier 1988).

Tillage is by far the most researched management practice affecting N₂O emissions. This is because reduced tillage also has great potential to mitigate CO₂ emissions by sequestering C. The IPCC (Smith et al. 2007) estimates that agriculture could mitigate 5,500-6,000 Mt of CO₂ equivalents (CO₂-e) y⁻¹ by 2030 and that 89% of this would be accomplished through soil C sequestration. However, the estimates of the ability of no-till to sequester C range from -0.73 to 1.8 t CO₂ ha⁻¹ y⁻¹ (Smith et al. 2007). Regardless of this uncertainty, the Chicago Climate Exchange uses the numbers 0.3 to 0.88 t CO₂ ha⁻¹ y⁻¹ (depending upon region) to determine payment for no-till carbon credits (CCE 2008).

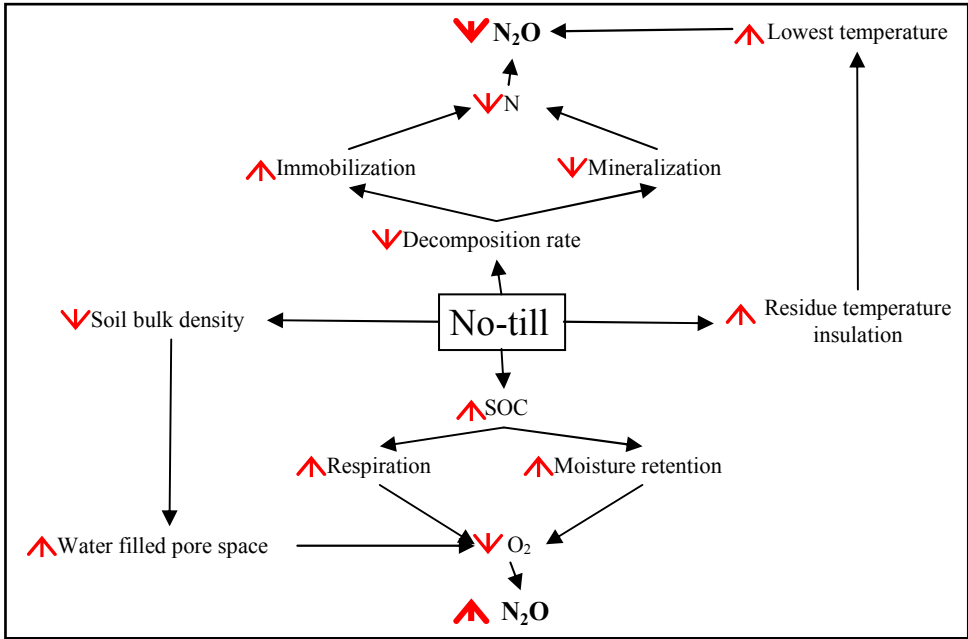


Figure 1.7 Possible effects of no-till practices on N₂O emissions.

As carbon trading profits are made from using no-till practices, it has been brought into question whether the goal to increase soil organic matter conflicts directly with the goal to decrease N₂O emissions in agricultural lands (Johnson et al. 2007). There is evidence to support both positive and negative interactions between these two processes (Figure 1.8). There have not yet been any final conclusions and the IPCC notes that no-till effects on N₂O emissions are inconsistent and not well-quantified (Smith et al. 2007). A comparison of 153 cumulative annual N₂O measurements from conventional tillage and no-till across 11 studies illustrates both this inconsistency and the potential for N₂O emissions to offset the 0.3 to 0.88 t CO₂ ha⁻¹ y⁻¹ currently being paid (Figure 1.8).

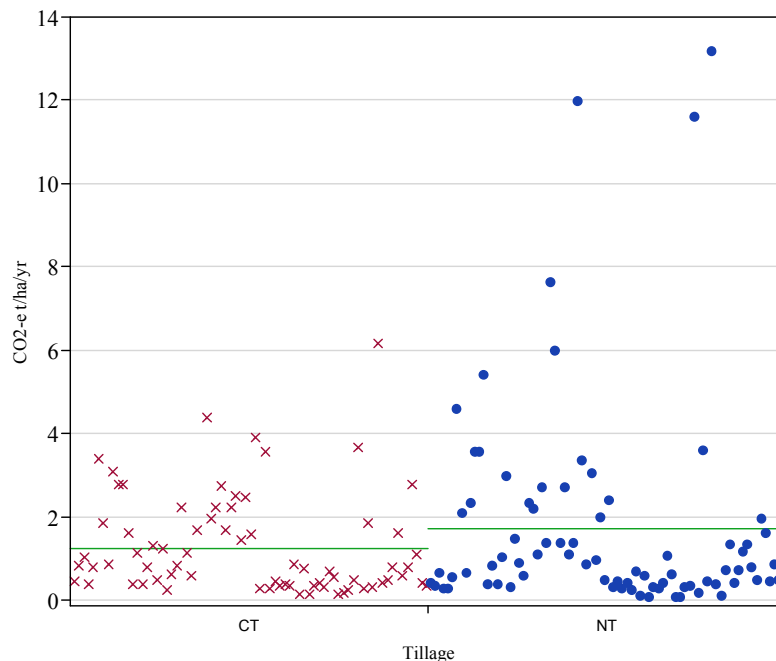


Figure 1.8 Comparison of annual N₂O emissions between conventional tillage (CT) and no-tillage (NT) from 11 studies. Differences between treatments are not significant (Choudhary et al. 2002, Grandy et al. 2006, Parkin and Kasper 2006, Adler et al. 2007, Adviento-Borbe et al. 2007, Gregorich et al. 2007, Malhi and Lemke 2007, Wagner-Riddle et al. 2007, Dusenbury et al. 2008, Elder and Lal 2008).

Tillage and other management effects on N₂O emissions may be better elucidated by focusing on the time of year when the majority of annual N₂O is emitted. Terrestrial N₂O emissions in temperate regions have been found to be relatively high during the fall and spring as soils freeze and thaw. Duxbury et al. (1982) found 66% and 57% of total annual N₂O fluxes in timothy and alfalfa fields occurred during a three week period in the spring. This included 2 days in which 27% of the total annual flux was recorded. Similarly, Rover et al. (1998) found that 70% of emissions occurred during the winter and through the spring thaw in an arable field. Although not always quantified, this phenomenon of N₂O emission peaks in the spring is well documented (Drury et al. 2006, Adviento-Borbe 2007, Beheydt et al. 2008, Dusenbury et al. 2008). Because agriculture contributes so much to anthropogenic N₂O emissions and the majority of these emissions occur during known freezing and thawing periods, it is important to focus on these periods in order to gain a better understanding of this process so that effective management practices can be devised.

Freeze-thaw cycles (FTC)

A soil freeze-thaw cycle is a physical occurrence that results in dramatic changes in soil chemical and biological processes. Soil freezes from the top down and in doing so, draws water to the surface due to the water potential gradient. The process is called freezing induced water redistribution (Dirksen and Miller 1966). As water reaches the freezing front, it creates an ice lens throughout the soil profile. When the surface eventually warms, melting occurs from the top down. Melted ice and snow above the ice lens is blocked and unable to drain. Thus, the soil above the ice lens is supersaturated and anaerobic conditions prevail (Miller 1980).

This anaerobic environment favors denitrification over nitrification for production of N₂O. Morkved et al. (2006) showed that only 4.4% of FTC N₂O

emissions came from nitrification. Ludwig et al. (2004) also found denitrification to be the dominant source of N_2O during thawing. Koponen et al. (2006) found very little evidence of nitrification during thawing as well. In comparing the relationship between nitrification and denitrification at different temperatures, Oquist et al. (2004) discovered that denitrification dominated at low temperatures. The importance of other NO_3^- removal pathways during a freeze-thaw cycle is unknown.

Soil solution chemical changes during FTC further encourage denitrification. As the soil solution freezes, solutes are excluded from the ice and contained in the remaining water films. These films are enriched in nutrients and low in pH (Stahli and Stadler 1997). Microorganisms living in these films have a good supply of alternative electron acceptors to O_2 in an anaerobic environment. Many denitrifying microorganisms are sensitive to low pH, hence the acidity of this environment may significantly affect the efficiency of N_2O reduction to N_2 (Firestone et al. 1980).

As soils freeze, soil aggregates are disrupted by expanding ice crystallization and during thawing, plant and microbial cells burst due to osmotic gradients (Mazur et al. 1970) and release nutrients (Christensen and Christensen 1991). Relevant to denitrification, this creates a large flush of organic C and N that is rapidly mineralized (Muller 2002). Herrmann and Witter (2002) found FTC increased the amount of C and N mineralized by 2-3 fold. The flush they observed was short-lived and decreased after sequential cycles, suggesting limited pools of easily decomposable material. When NO_3^- and NH_4^+ were added to soil, Muller et al. (2003) observed that they were quickly immobilized, but became available again after a freeze-thaw cycle. These findings support the idea that lysed microbial cells provide nutrients for surviving microbes. A study using ^{13}C labeling showed microbial biomass C contributed ~65% to the C flush, while representing only 5% of the microbial population (Herrmann and Witter 2002).

Causes of N₂O peaks

FTC in agricultural environments create ideal conditions for denitrification by providing nutrient- and substrate-rich, yet O₂ poor, surroundings. Naturally, increases in denitrification activity result in increased emissions of gaseous N. However, it is unclear whether increases in denitrification can account for the magnitude of increases in N₂O emissions typically measured during FTC.

As mentioned previously, N₂O released during denitrification results primarily from the incomplete transformation of N₂O to N₂. The efficiency of this transformation can be expressed as given in Equation 1.1.

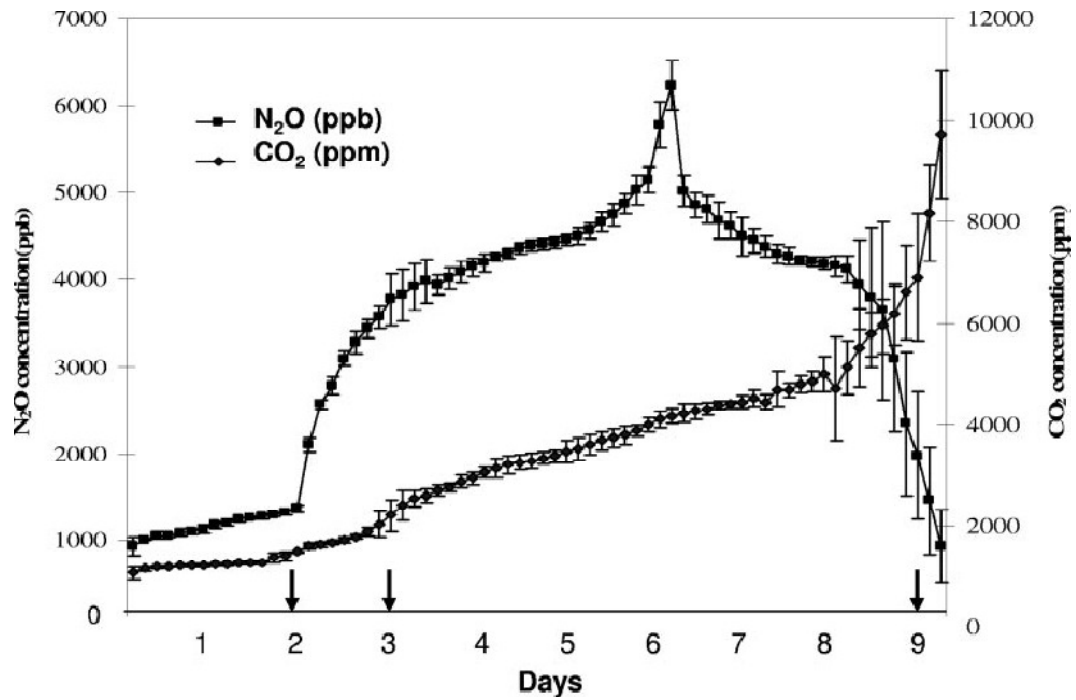
Equation 1.1.

$$r_{N_2O} = \frac{\Delta N_2O}{\Delta N_2O + N_2}$$

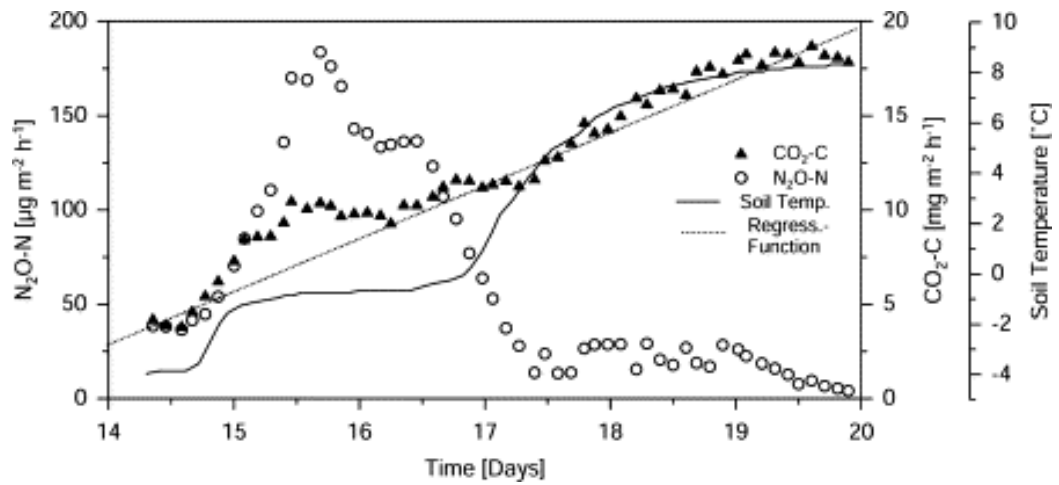
This r_{N_2O} represents the proportion of gaseous N that is emitted as N₂O. This ratio is used as an indicator of denitrifying community function (Cavigelli and Robertson 2000) and N₂O reduction ability (Henault 1998). Using cultured denitrifying bacteria, Firestone et al. (1980) found r_{N_2O} increased with increasing concentrations of NO₃⁻ and NO₂⁻, decreasing pH and increasing O₂ tensions. Betlach and Tiedje (1981) also found increased O₂ tensions to increase r_{N_2O} . Observed effects of FTC on r_{N_2O} from the soil are mixed. While some studies find r_{N_2O} to increase with decreasing temperatures (Bailey and Beauchamp 1973, Keeney et al. 1979, Melin and Nommik 1983) others find no effect of temperature on r_{N_2O} (Holtan-Hartwig et al. 2002, Morkved et al. 2006) and one study shows ratios to increase with time after thawing (Ludwig et al. 2004). The contribution of N₂O reduction inefficiency to high N₂O emissions during soil thawing remains unclear.

While higher N₂O emission rates are found at higher temperatures in laboratory simulations (Castaldi 2000, Dobbie and Smith 2001, Oquist et al. 2004), this is a process different from that measured at the beginning of soil thawing. In these studies, temperatures above 15°C are attributed to increased respiration inducing anaerobic conditions and encouraging denitrification. However, this relationship does not hold at intermediate temperatures and N₂O emissions are commonly shown to decrease as respiration levels increase (Figure 1.9). More relevant to FTC, several studies have found N₂O emissions to be highest between 0 and 2°C (Holtan-Hartwig 2002, Muller et al. 2003, Dorsch 2004, Koponen and Martikainen 2004, Oquist et al. 2004, Drewitt 2007). Despite uncertainty of how temperature will change rN₂O, emissions when soil is between 0-2°C are attributed to a “dysfunction” of N₂O reduction to N₂ in most of these studies, although rN₂O values were not reported.

Holtan–Hartwig et al. (2002) investigated whether nitrous oxide reductase (Nos) had a higher activation energy than nitric oxide reductase (Nor). This would mean that at low temperatures N₂O could be produced, but not reduced. This was not found to be the case. They concluded, “Low temperatures may thus exert a particular challenge to denitrifying communities for some reason, and the effect was found to be the most severe for the N₂O reduction process” (Holtan-Hartwig et al. 2002). Denitrifying communities facing this particular challenge have not been widely studied to determine mechanistically why low temperatures result in high N₂O fluxes. Most of our molecular insight comes from one study by Sharma et al. (2006). Sharma et al. froze and thawed grassland soils in mesocosms in a controlled temperature chamber and measured N₂O and CO₂ emissions in real time. Soil samples were taken over time and characteristics of the microbial community analyzed. Changes in community composition throughout thawing were observed when 16S and 18S rRNA genes and their transcripts were analyzed by denaturing gradient gel



(Sharma et al. 2006)



(Teepe et al. 2001)

Figure 1.9 Two graphs representing commonly seen patterns of decline in N₂O emissions as respiration continues to rise.

electrophoresis (DGGE). DGGE profiles of amplified copy DNA (cDNA) (representing denitrifying gene expression) showed a clear succession towards more complexity in polymerase chain reaction (PCR) amplification products with time after thawing. Most-probable-number (MPN)-PCR was used to quantify these changes in denitrifying gene transcripts. Expression levels of *napA* and *nirS* increased just after thawing began and then decreased as thawing proceeded. No transcript was found for *nosZ*, the gene encoding N₂O reductase. This indicates that *nosZ* was either not expressed, preventing N₂O from being reduced to N₂, or expressed at levels below the detection threshold.

Antecedent factors of N₂O peaks

Spring N₂O peaks are short periods preceded by a winter's worth of C, N and microbial dynamics. Many environmental studies focus on the growing season and exclude winter processes under the assumption that little to nothing is happening during this season. However, soil microbes can remain active down to -20°C (Rivkina et al. 2000, Elberling and Brandt 2003). However, soil temperatures in temperate regions often stay well above this and can even spend significant periods above 0°C depending on how well the ground is insulated and the severity of the winter (Brooks et al. 1998).

During the winter, microbial activity is seldom inhibited by competition with plants and/or lack of soil moisture (Groffman et al. 2001). Studies of N dynamics show a very consistent pattern of increases in inorganic N during the winter followed by a rapid loss of N during spring in alpine (Brooks et al. 1996, Brooks et al. 1998), arctic tundra (Schimel et al. 2004, Schmidt and Lipson 2004) and agricultural (Mahli and Nyborg 1986, Jacinthe et al. 2002, Rich 2007) systems. Spring N losses in these studies occur both through leaching and denitrification. Brooks et al. (1996) and

Schmidt and Lipson (2004) found large increases in microbial biomass in the winter followed by decreased microbial biomass in the spring. None of these trends were found to be true in a northern hardwood forest (Groffman et al. 2001).

There is some evidence that soil temperatures have an important effect on winter N dynamics. Schimel et al. (2004) and Rich (2008) found that lower temperatures (deeper soil freezing) led to lower rates of N mineralization. Rich (2008), Groffman et al. (2001), and Brooks et al. (1998) found that lower temperatures led to higher leaching exports of N in the spring.

Studies examining N₂O emissions also indicate that the freezing period of FTC holds significance to N₂O fluxes during thawing. In laboratory simulations, Teepe et al. (2004) found that longer freeze duration led to higher N₂O emissions and Matzner (2008) found that lower temperatures led to higher N₂O emissions. These results were substantiated in the field as Wagner-Riddle et al. (2007) found that freezing degree hours (time spent below 0°C) was the most significant factor explaining between treatment and between season variability in N₂O emissions ($r^2=0.97$). Similarly, Maljanen et al. (2007) observed significantly higher N₂O fluxes in arable soils that experienced lower temperatures prior to thawing. These studies signify the potential of preceding temperature conditions to explain the magnitude of N₂O fluxes during the thawing phase of FTC.

Winter soil temperatures are greatly affected by soil cover. Soil covers that have been shown to insulate soils against low air temperatures are agricultural residues (Wagner-Riddle et al. 2007) and snow (Groffman et al. 2001, Maljanen et al. 2007, Rich 2008). The effect of snow cover on N dynamics is of concern in the Northeastern United States (Groffman et al. 2001, Rich 2008) due to predictions of less snow cover as the climate changes (Hayhoe et al. 2007). If less snow cover leading to deeper soil freezing results in higher N₂O emissions, it will be a positive

feedback loop reinforcing climate change. This positive feedback loop will need to be accounted for in future climate modeling.

Purpose of this thesis

My focus in this thesis is on N₂O emission peaks during spring FTC. As mentioned above, there is currently some evidence that less winter soil insulation leading to colder winter soils results in higher N₂O fluxes during this period (Maljanen et al. 2007, Wagner-Riddle et al. 2007). However, this evidence is yet to be further substantiated. I set out to test the following hypothesis:

H1. Less winter snow cover will lead to lower soil temperatures and greater N₂O emissions.

Support for this hypothesis will provide insight into what effects less snow cover from future climate change may have on N₂O emissions. This insight will allow us to recognize a positive feedback loop in N₂O climate forcing, leading to more accurate climate change predictions and design of more effective mitigation strategies.

As described in the agricultural mitigation segment of this chapter, evidence for the effect of cover crops on N₂O emissions is mixed. Winter rye has been found to both increase (Parkin and Kaspar 2006) and decrease (Parkin et al. 2006) N₂O emissions. The effect of winter rye on spring N₂O emissions has not yet been tested. Given that winter rye has been found to be an excellent scavenger of N, a second hypothesis I set out to test is:

H2: Winter rye cover cropping will take up and immobilize NO₃⁻ and thereby lower N₂O fluxes.

Support for this hypothesis would direct us to a specific tool that is readily available to moderate N₂O emissions.

Assuming H1 is supported and further supports previous field studies (Maljanen et al. 2007, Wagner-Riddle et al. 2007), we may ask, why does more winter field cover lead to lower N₂O emissions? Insulation can affect many temperature components such as lowest temperature, freezing rate, freezing depth, duration, frequency and rate of thaw. I will test the influence of one these components – rate of thaw – under my third hypothesis:

H3: A slower thaw rate created by insulation will lead to lower N₂O fluxes.

Support for this hypothesis would give us even more guidance in selecting mitigation management practices. For example, if a slower thaw rate is desired, more agricultural residue could be left on a field or mulch could be applied in winter before thawing. If a slower thaw rate is not desired, early tillage could be recommended to decrease albedo and increase thaw rates.

At a more mechanistic level, the importance of the efficiency in N₂O reduction to N₂ during periods of high N₂O emissions after soil thawing remains unanswered. There is some evidence and much speculation that the magnitude of N₂O emissions after thawing is due to a bigger proportion of N₂O emitted relative to N₂. I test this under my fourth hypothesis:

H4. The ratio of N₂O to N₂O + N₂ emitted will decrease with time after thawing.

Support for this hypothesis would provide evidence that N₂O peaks are caused not only by increases in overall denitrification, but also by decreases in the proportion of N₂O reduced to N₂. This knowledge may help to explain mechanistically why we observe such high N₂O emissions following a freeze-thaw cycle.

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CHAPTER 2

SNOW OR A RYE COVER CROP INCREASE WINTER SOIL TEMPERATURES AND REDUCE NITROUS OXIDE FLUXES IN A NEW YORK CORN FIELD

Abstract

Agriculture is responsible for 58% of the anthropogenic emissions of nitrous oxide (N_2O), a source of stratospheric ozone depletion and a greenhouse gas that contributes to global climate change. In temperate regions, a majority of this N_2O is emitted during spring soil thawing. Climate change models predict that warming trends in the Northeastern U.S. will result in less snow cover, potentially leading to colder soils that may in turn lead to higher N_2O emissions. A winter soil management strategy is needed to mitigate spring N_2O emissions. In this study, I examined the influence of two winter field covers, snow and winter rye, on soil temperature and nitrogen (N) content and subsequent spring N_2O emissions from a NY corn field over two years. A 2 x 2 factorial of rye (+/-) by snow cover (+/-) was established in a randomized complete block design. Nitrous oxide emissions were measured bi-weekly using a static chamber method. The first season (2006-07) was a cold winter (2309 h below 0°C at 8 cm soil depth), historically typical for the region. The snow removal treatment resulted in colder soils and higher N_2O fluxes (73.3 vs. 57.9 $\text{ng N}_2\text{O-N cm}^{-2} \text{ h}^{-1}$). The rye cover had no effect on N_2O emissions. The second season (2007-08) was a much milder winter (1271 h below freezing at 8 cm soil depth), with lower N_2O fluxes overall. Winter rye cover resulted in lower N_2O fluxes (5.9 vs. 33.7 $\text{ng N}_2\text{O-N cm}^{-2} \text{ h}^{-1}$), but snow removal had no effect. These results suggest that if winters remain typically cold in the Northeastern U.S., but snowfall is reduced, we may expect higher N_2O emissions, with winter rye cover unlikely to mitigate this. If, however, less snow cover is due to warmer temperatures as predicted, we may be trending towards lower

spring N₂O emissions where winter rye cover cropping may be a useful mitigation tool.

Introduction

Nitrous oxide (N₂O) is a source of stratospheric ozone depletion and a greenhouse gas with 297 times more climate forcing potential than carbon dioxide (CO₂). However, total N₂O emissions from the landscape are low enough to account for only a fraction of total climate forcing compared to CO₂. Agriculture accounts for only 10-12% of the total anthropogenic greenhouse gas emissions, but is responsible for 58% of the total anthropogenic N₂O emissions (Smith 2007). While agriculture contributes a large proportion of N₂O emissions, changes in management practices also have the potential to change N₂O emission levels (Johnson et al. 2007). Understanding the key sources and mechanisms responsible for generating N₂O in agricultural systems is important for designing mitigation strategies.

In temperate regions, most of the yearly N₂O emissions from agricultural lands occur as soil thaws in the spring. Focusing on reducing N₂O emissions during this time period may result in the most effective management strategies. Freeze-thaw cycles (FTC) during the spring are responsible for 50% (Rover et al. 1998) to 66% (Duxbury et al. 1982) of annual N₂O emissions from agricultural soils. Soil freezing induces soil water redistribution to the surface of the soil as freezing occurs from the top down (Miller 1980). Thawing also occurs from the top down leaving an ice lens that effectively blocks any melt water from draining through the soil matrix (Miller 1980). Freezing and thawing also disrupts soil aggregates, organic matter and soil organisms, which results in a release of carbon (C) and nutrients into the soil solution. Thus, a freeze-thaw cycle creates an oxygen (O₂) poor, yet substrate- and nutrient-rich environment for soil microbial life. Under these conditions, denitrification becomes

the dominant N transformation process (Ludwig et al. 2004, Oquist et al. 2004, Koponen et al. 2006, Morkved et al. 2006).

Denitrification is anaerobic microbial respiration whereby C substrates are oxidized and nitrate (NO_3^-), which serves as the terminal electron acceptor in the absence of O_2 , is sequentially reduced to N_2 . In order for denitrification to occur, O_2 needs to be limited, C substrates and NO_3^- need to be available, and denitrifiers need to be present. The reduction of NO_3^- proceeds as follows: $\text{NO}_3^- \rightarrow$ nitrite (NO_2^-) \rightarrow nitric oxide (NO) \rightarrow nitrous oxide (N_2O) \rightarrow nitrogen (N_2) (Robertson and Groffman 2007). If this sequence is incomplete, N_2O will be released as an end product instead of inert N_2 . The environment created by FTC greatly increases denitrification, and thus increases all of the products generated in this process. However, it is not clear if the relative proportions of different forms of gaseous N emitted remain constant. There is some evidence that the magnitude of N_2O emissions may be caused by a decrease in reduction of N_2O to N_2 (Bailey and Beauchamp 1973, Keeney et al. 1979, Melin and Nommik 1983). It has been noted that the gene responsible for the final step of the sequence, *nosZ*, may be sensitive to low temperatures (Firestone et al. 1980, Sharma et al. 2006). Indeed, several studies have found N_2O emissions to be highest between 0 and 2°C (Holtan-Hartwig et al. 2002, Muller et al. 2003, Dorsch 2004, Koponen and Martikainen 2004, Oquist et al. 2004, Drewitt 2007).

All of these studies found soil temperature to be correlated with N_2O emissions, but less work has been done to examine the importance of preceding temperatures on N_2O fluxes (rates of N_2O emitted per area over time). In a laboratory study, Muller et al. (2003) found the highest N_2O emissions occurred with increases in the length of the freezing period. Teepe et al. (2004) also found increased freezing duration led to increased N_2O peaks in the lab. In the only field study found to address the effects of winter agricultural field cover on N_2O emissions, Wagner-

Riddle et al. (2007) determined hours spent below 0°C (freezing degree hours, FDH) to be the most important factor explaining year to year variability and between treatment differences in winter/spring N₂O fluxes. They showed that no-till management had the most surface residue, trapped the most snow, experienced more FDH, and had lower N₂O fluxes when compared to conventional tillage.

Understanding the implications of a relationship between antecedent temperatures and N₂O emissions is important in regions of the world predicted to experience less snow cover, such as the Northeastern U.S. (Hayhoe et al. 2007). Snow cover is an effective insulator capable of buffering low air temperatures or preventing the ground from freezing at all. Its absence leads to soils experiencing lower soil temperatures, deeper freezing depth and quicker rates of freezing and thawing (Groffman et al. 2001, Rich 2008). The solitary study relating snow cover to spring N₂O emissions (Maljanen et al. 2007) covered one season and provided further evidence that bare soils experience lower winter temperatures and greater frost depths than soils having natural snow cover. These bare soils displayed higher N₂O fluxes during FTC.

Soil covers that we can control include agricultural residue, mulch and cover crops. Of these covers, a living crop has the added benefits of affecting winter soil nutrient and C dynamics. Cover crops may increase N₂O emissions by providing C substrate and inducing an anaerobic environment in the rhizosphere through increased O₂ depletion by root respiration, thus encouraging denitrification (Smith and Tiedje, 1979, Haider et al. 1986, Klemedtsson et al. 1987). However, since FTC environments are already anaerobic, it may be more interesting to note that cover crops may decrease N₂O emissions by limiting available NO₃⁻ (Parkin et al. 2006). Winter rye (*Secale cereale* L.) has been found to be an especially effective N

scavenger (Shipley et al. 1992, Guillard 1995, Strock et al. 2004, Rich 2008) and is readily available for use in the Northeastern U.S.

The results of Maljanen et al. (2007) and Wagner-Riddle et al. (2007) have not been corroborated and, in general, the effects of snow and rye cover on soil temperature and N dynamics remain poorly understood. My purpose in this study was to assess the influence of snow and rye cover on winter soil temperatures and subsequent N₂O fluxes in a New York corn field by testing the following hypotheses:

H1: Less winter snow cover will lead to lower soil temperatures and greater N₂O emissions.

H2: Winter rye cover cropping will take up and immobilize NO₃⁻ and thereby reduce N₂O fluxes.

Materials and Methods

Experimental design

Experimental plots were established in autumn 2005 and maintained through autumn 2008 in an agricultural field near Harford, NY (42.427 N, 76.228 W, elevation 362 m). The mean minimum winter temperature range at the site is -26.1°C to -28.9°C (United States Department of Agriculture Plant Hardiness Zone 5a). The soil is a well-drained Howard gravelly loam (mixed, active, mesic Glossic Haplualf). Corn (Pioneer 54H91; dual purpose; Pioneer Hi-Bred, Johnston, IA) was grown for silage in this field beginning in the spring of 2005, following multiple years of alfalfa cropping. Corn was planted in late April at a density of 81510 seeds ha⁻¹, and fertilized with 84 L ha⁻¹ 30% Nitan (34 kg N ha⁻¹) as a starter fertilizer in 2006-2008 and an additional side-dressing of 233.7 L ha⁻¹ 30% Nitan (92 kg N ha⁻¹) in 2007 and 2008. Corn was harvested in early September. Winter rye was grown from late September through early May, when it was killed chemically. Each year (~1 week after the rye was

killed) the field was chisel-plowed to a depth of 20 cm and disked just before corn planting. Further field management details are given in Rich (2008).

The field experiment was set up as a 2 x 2 factorial, randomized complete block design (RCBD) with two cropping systems treatments (maize/winter fallow and maize/winter rye) and two snow treatments (natural snow depth and snow removed). Each treatment was replicated four times, resulting in 16 (10 x 10 m) plots. Snow was removed within 2 d after each precipitation event with a shovel or leaf blower. An attempt was made to leave a thin layer (1 to 2 cm) of snow in the snow removal plots to minimize damage from foot traffic and to retain albedo for keeping the soils cool.

Temperature measurements

Soil temperatures were collected and averaged every hour by a Campbell CR3000 data logger (Campbell Scientific, Logan, UT) with thermocouples placed at 8 cm depth. Air temperature data were available from a National Oceanic and Atmospheric Agency (NOAA) weather monitoring station (US Climate Reference Network station identification: NY Ithaca 13E) located about 1 km from the experimental plots.

N₂O flux measurements

In 2007 and 2008, gas samples used to measure N₂O fluxes (ng N₂O-N cm⁻³ h⁻¹) from the soil were collected from 3 of the 4 experimental blocks (12 plots) using the static chamber method as described by Venterea et al. (2005). Briefly, static chambers measuring 53 x 32 x 8.6 cm were placed over the soil and sealed for 1 h. A 25 cc sample of the headspace gas was drawn from the chamber with a syringe every 30 min after sealing. In 2007, samples were taken every 8-10 d between February and May, and on days 1, 2, 3, 5, 7, and 10 of the spring thaw. Gas samples were stored in

25 ml pre-evacuated vials with butyl rubber stoppers and transported to the ARS North Central Soil Conservation Laboratory in Morris, MN, where 10 cc of manufactured ambient air were injected into each vial to pressurize them. The samples were then analyzed by gas chromatography using a ^{63}Ni electron capture detector (ECD) (Varian GC Model CP-3800, Varian, Inc., Palo Alto, CA). In 2008, the chambers were duplicated within each plot and samples were taken roughly every two weeks from February through April. The samples were then analyzed at Cornell University by gas chromatography using a ^{63}Ni electron capture detector (ECD) (Varian GC Model 3700, Varian, Inc.) as described by Terry et al. (1981).

Ancillary soil measurements

Some environmental variables were measured as part of a separate, but related, study on winter C and N dynamics conducted in the same plots. Greater detail and, in most cases, protocols can be found in Rich (2008). Eight soil cores were taken in mid-April at 0-15 cm depth and composited for each plot. Standard soil tests were conducted to quantify macro- and micro-nutrients using a Morgan extraction. Additional soil N and C measurements relevant to denitrification were also taken as follows. Potentially mineralizable nitrogen (PMN) was determined using the method described by Drinkwater et al. (1996). Microbial biomass N (MB-N) was determined in 2007 using a simultaneous chloroform fumigation-extraction procedure (CFE) as described by Witt et al. (2000). Percent organic matter (%OM) was estimated as loss-on-ignition (Cambardella et al. 2000). Active carbon (AC), a measure of management sensitive soil C pools, was analyzed using the rapid colorimetric protocol developed by Weil et al. (2003).

Data analysis

Number of hours spent below 0°C at 8 cm depth (freezing degree hours, FDH), the lowest temperature recorded at 8 cm depth (minimum temperatures, MT), and the average minimum temperature (AMT) were determined for each of the four replicate blocks of the experiment and the effect of treatments on these was tested through use of analyses of variance (ANOVA). A ‘freeze’ in this study was defined as a period in which the maximum soil temperature reached $\leq -0.1^{\circ}\text{C}$ (to account somewhat for freezing point depression) for at least 2 consecutive days and a ‘thaw’ was defined as a period in which the maximum soil temperature reached $\geq -0.1^{\circ}\text{C}$ for at least 2 consecutive days. Frequency of FTC was determined using these criteria. Extent of co-linearity between FDH, MT, and AMT was determined through linear regression.

Nitrous oxide flux data that were greater than two standard deviations from the mean were excluded from data analysis. Remaining nitrous oxide fluxes were averaged for each year across all dates in each plot and treatment effects were tested by use of ANOVA. The correlation between FDH, MT, and AMT and average N₂O flux was determined through linear regression.

Treatment effects on PMN, MB-N, and NO₃⁻ were also tested by use of ANOVA. ANOVA was run for an RCBD and p-values of ≤ 0.1 were considered significant. A series of t-tests were used to test comparisons of further interest. All analyses were performed in JMP 7 (SAS Institute, Cary, NC)

Results

Temperature variables

Snow removal resulted in colder soils in 2006-2007 (Figure 2.1), with lower MT ($p < 0.0001$), more FDH ($p = 0.02$), and more FTC ($p = 0.003$) experienced in plots where snow was removed. Snow removal in 2007-2008 resulted in more FDH

($p=0.02$) and a higher number of FTC ($p=0.08$), but not significantly lower MT (Table 2.1). Soil in the spring warmed more quickly in snow removed plots (Figure 2.1). Rye cover cropping did not affect soil temperatures with the exception of fewer FTC ($p=0.08$) during 2007-2008. In the winter of 2006-2007 air temperature was below 0°C for a longer time, while 2007-2008 had several periods of warming during the winter (Figure 2.1). Accordingly, 2007-2008 had fewer FDH and FTC than 2006-2007, although MT were similar.

N₂O fluxes

N_2O fluxes in 2007 ranged from -449 to $1194 \text{ ng N cm}^{-2} \text{ h}^{-1}$. No significant differences between individual treatments were found using Tukey's test (Fig. 2.2A). Plots where snow was removed had significantly higher N_2O fluxes than those with natural snow cover when averaged across cover crop treatments (t-test, $p = 0.07$). No differences in N_2O fluxes were observed between rye and fallow plots when averaged across snow cover treatments.

N_2O fluxes in 2008 averaged an order of magnitude lower than 2007 and ranged from -24 to $97 \text{ ng N cm}^{-2} \text{ h}^{-1}$ (Figure 2.2B). There was less variability in the 2008 data and significant differences between the treatments were observed. A comparison of individual treatment means (Tukey's test) showed that for plots where snow was removed, fallow plots produced significantly higher N_2O fluxes than plots with a rye cover crop, which explains the significant cover crop x snow cover interaction ($p=0.02$, Table 2.1). Fallow plots had significantly higher fluxes than rye cover plots when averaged across snow cover treatments ($p=0.0045$). Snow cover treatments showed no significant differences when averaged across cover crop treatments. The variables AMT, FDH, and MT were correlated with each other ($r^2 = 0.81$ to 0.99), indicating colinearity. No significant relationships were found between

any of the temperature variables and N₂O fluxes. It is interesting to note that the data from 2008 seemed to display a relationship between FDH and N₂O fluxes (Figure 2.2B), with the exception of the rye, snow-removed treatment. When these data were removed from the 2008 analysis, the results showed a strong FDH to N₂O flux relationship ($r^2 = 0.98$, $p = 0.08$) (Table 2.2). This treatment was removed from analysis because it may be confounding the FDH to N₂O relationship based on evidence discussed in a later section (discussion).

Ancillary soil data

Rye plots had significantly more PMN in 2007 than the fallow plots; whereas, in 2008, the snow-removed plots had more PMN than those with snow retained. The snow-removed plots had higher NO₃⁻ concentrations than those with snow retained in both 2007 and 2008 (Table 2.1). Active carbon levels and %OM did not differ between treatments for either year (Table 2.1).

Discussion

Less snow cover resulted in colder soil temperatures. This observation was consistent with previous studies (Groffman et al. 2001, Maljanen et al. 2007) and illustrates how climate change may affect Northeastern U.S. soils in the future. In addition to lower soil temperatures, longer soil freezing duration and more FTC, soils with snow removed warmed faster than snow-covered soils during warming cycles (Figure 2.1). Although anticipated snow cover effects were observed in both years, N₂O flux responses were significant only in relation to snow cover in 2007 and only in relation to rye cover cropping in 2008.

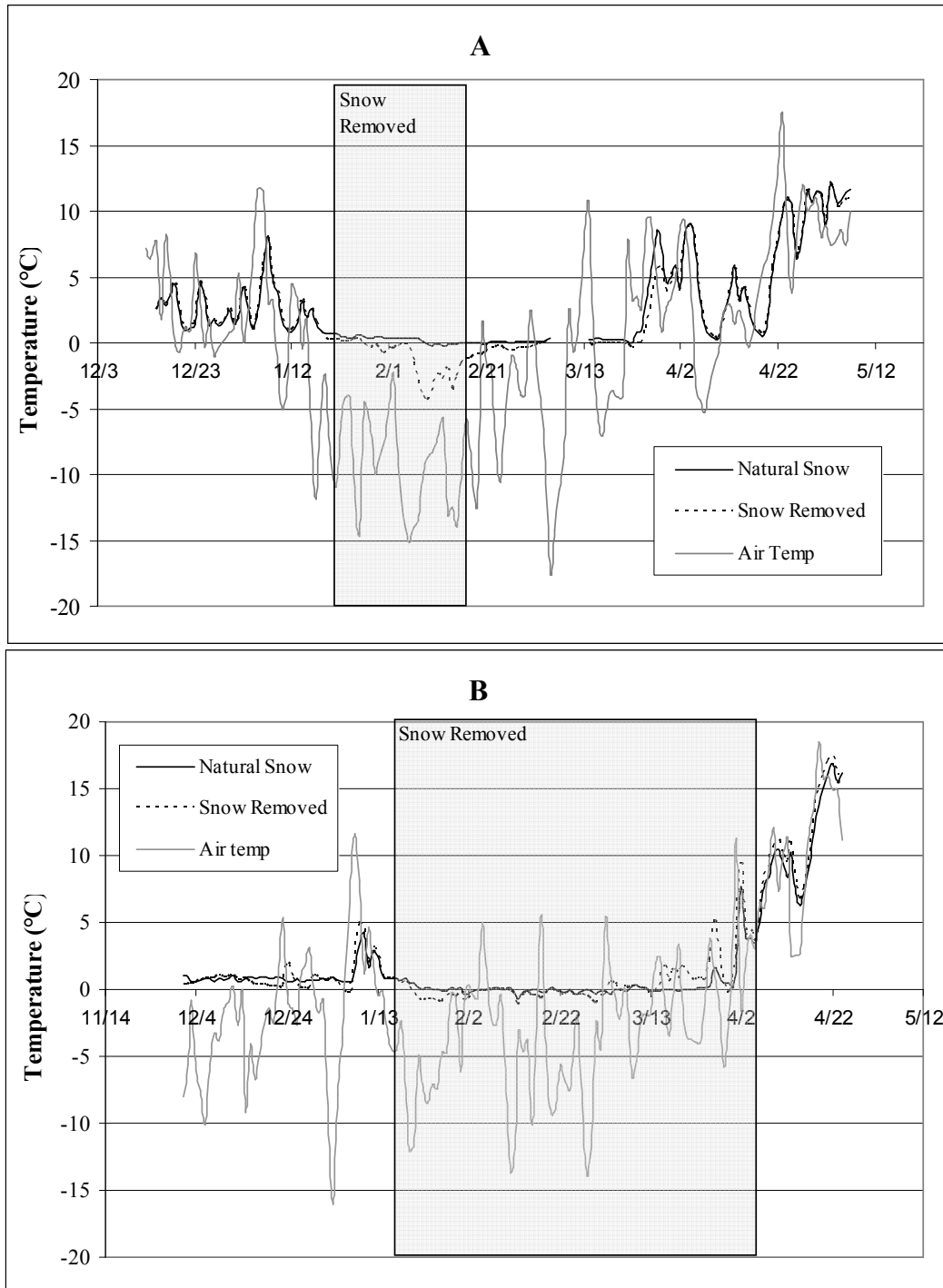


Figure 2.1 Air and soil temperature (at 8 cm depth) recorded in (A) 2006-2007 and (B) 2007-2008.

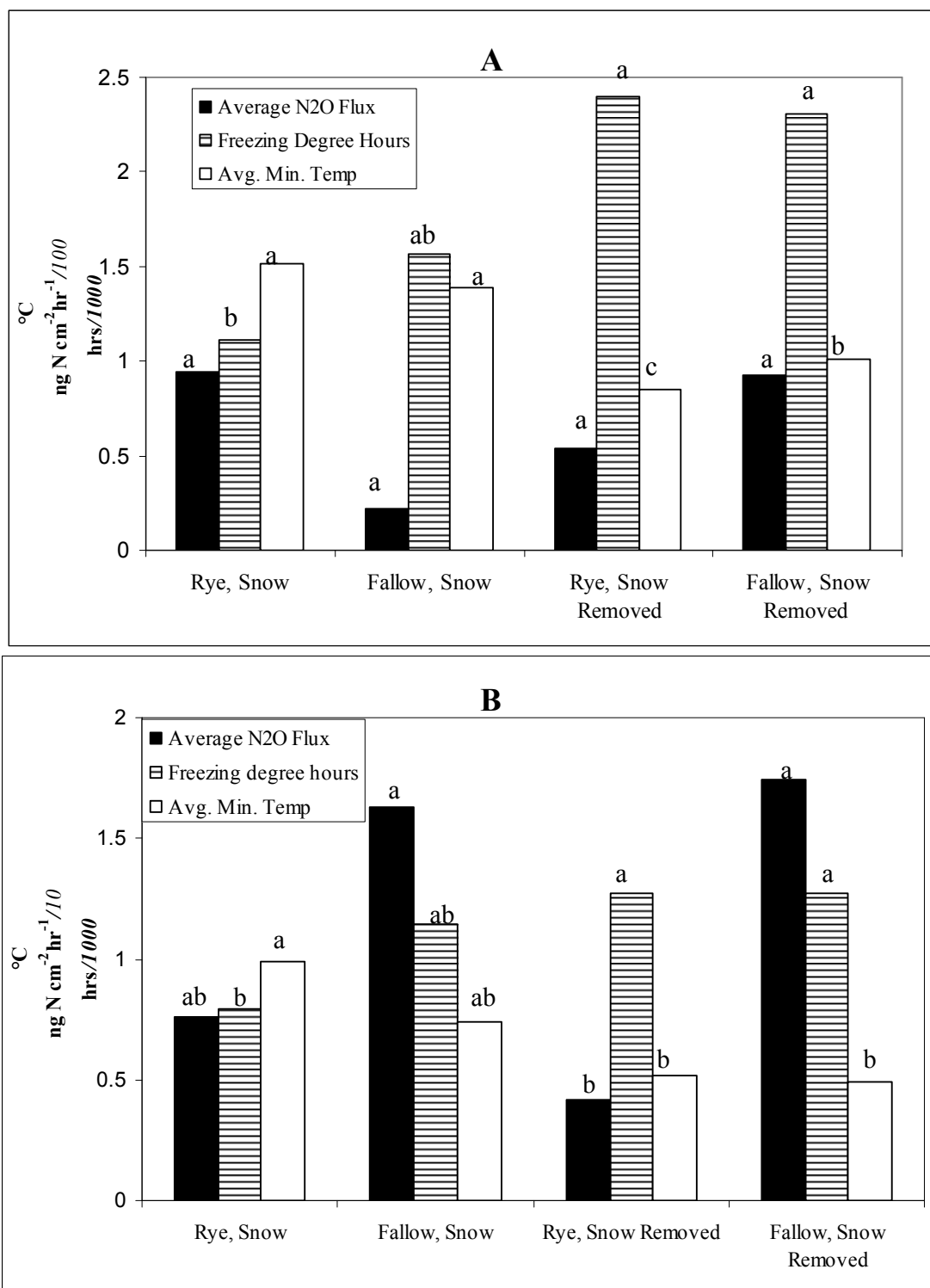


Figure 2.2 Average N₂O fluxes ($\text{ng N}_2\text{O cm}^{-2} \text{h}^{-1} \cdot (\text{A})100^{-1} \cdot (\text{B})10^{-1}$), freezing degree hours ($\text{h} \cdot 1000^{-1}$), and average minimum soil temperatures ($^{\circ}\text{C}$) for the four field treatments in (A) 2006-2007 and (B) 2007-2008. Different letters denote significant differences within categories.

Table 2.1 Summary of temperature factors, soil N and C variables, and N₂O fluxes in 2006-07 and 2007-08. Measurements include lowest minimum temperature (MT), freezing degree hours (FDH), freeze-thaw cycles (FTC), nitrate (NO₃⁻), potentially mineralizable nitrogen (PMN), percent organic matter (%OM), active carbon (AC) and N₂O flux. Temperature data were collected Nov. to May each year, N and C measurements were taken in mid-April each year, and N₂O fluxes were measured Feb. to May each year. Individual contrasts were cover crop: winter rye vs. fallow; snow cover: snow removal vs. ambient snow; and cover crop x snow cover interactions. Values in parentheses are standard deviations. † 2006-2007 data from Rich (2008). * significant at p≤0.10, ** significant at p≤0.05.

Year	Treatment	MT (°C)	FDH (h)	FTC (No.)	†NO ₃ (mg kg ⁻¹)	†PMN (µg N g soil ⁻¹ wk ⁻¹)	†%OM	†AC (mg C kg ⁻¹)	N ₂ O (ng N ₂ O-N cm ⁻² h ⁻¹)
2006- 2007	Ambient	-0.1	1112	1.00	12.5	9.2	3.2	590	94.0 (282.6)
	Snow	-0.7	1562	0.75	11.2	6.3	3.2	683	21.7 (81.7)
	Snow	-6.7	2400	3.75	15.7	8.7	3.2	665	53.8 (283.7)
	Removed Fallow	-5.1	2309	2.25	14.4	6.6	3.2	785	92.8 (249.4)
	Effects					p-value			
	Cover crop	0.32	0.45	0.16	**0.01	*0.07	0.76	0.14	0.18
	Snow cover	**<0.0001	**0.02	**0.003	0.97	0.92	0.79	0.21	0.97
	Interaction	0.26	0.12	0.3	0.28	0.72	0.96	0.84	0.26
2007- 2008	Ambient	-1.1	792	0	36.3	5.2	4.0	538	7.6 (11.7)
	Snow	-2.1	1147	0.75	36.0	4.9	3.8	537	16.3 (28.4)
	Snow	-7.2	1275	0.75	39.3	6.3	3.7	531	4.2 (9.6)
	Removed Fallow	-5.2	1271	1.75	40.	6.5	4.2	532	17.4 (27.5)
	Effects					p-value			
	Cover crop	0.97	0.13	*0.08	0.47	*0.10	0.56	0.99	**0.05
	Snow cover	0.14	**0.02	*0.08	0.87	0.77	0.96	0.76	0.40
	Interaction	0.44	0.12	0.79	0.91	0.95	0.17	0.97	**0.02

Table 2.2. Correlation coefficients resulting from linear regression between the temperature variables: average minimum temperature (AMT), freezing degree hours (FDH) and lowest minimum temperature (MT), and average N₂O flux.

§ with the rye, snow removed treatment excluded.

* significant at $p \leq 0.10$; ** significant at $p \leq 0.05$.

		AMT	FDH	MT
2006- 2007	FDH	**0.96	--	--
	MT	**0.99	**0.92	--
	Avg N ₂ O flux	< 0.01	< 0.01	< 0.01
2007- 2008	FDH	**0.94	--	--
	MT	**0.87	**0.81	--
	Avg N ₂ O flux	0.03	0.07	0.03
	§ Avg N ₂ O flux	0.83	*0.98	0.84

Snow cover, temperature and N₂O fluxes

As hypothesized, snow removal and the concomitant colder soil temperatures led to higher N₂O fluxes in 2007, confirming results reported by Wagner-Riddle et al. (2007) and Maljanen et al. (2007). Evidence of a relationship between low soil temperatures and high N₂O emissions was further supported by differences in N₂O fluxes between the two years. Soils in 2007 experienced a colder winter with more FDH and a higher number of FTC than 2008 and showed N₂O fluxes that were nearly an order of magnitude higher than fluxes measured in 2008.

Climate change implications for N₂O fluxes in the Northeastern U.S. are complex. In winters where temperatures are similar to each other, our results suggest that winters with less snow cover will have higher N₂O emissions. However, less snow cover is likely to be associated with warmer winters (Hayhoe et al. 2007). Thus, warmer winter air temperatures could counter-act the effects of less snow cover. The ultimate effect of climate change on soil temperatures and N₂O dynamics will depend on the relative magnitude of direct climate change effects associated with warmer air

temperatures vs. indirect effects on soil temperatures determined by the effects on snow depth, snow cover duration, and FTC.

While there is strong evidence that soil temperature does affect N₂O emissions, the importance of individual temperature variables such as MT, FDH, or frequency of FTC could not be elucidated from these data. No significant correlations between the temperature variables measured and N₂O emissions were found, but these relationships may be confounded by the effects of the rye cover crop on NO₃⁻ uptake at different soil temperatures. This latter possibility is suggested by evidence of a correlation between temperature and N₂O emissions when the rye plus snow-removed plots were excluded from the analysis in the 2008 data set. Most likely, all of these temperature parameters interact to influence N₂O emissions.

Lower minimum temperatures have generally been found to increase C and N turnover according to a recent review by Matzner et al. (2008), although a certain temperature threshold could not be defined. In arable soils, -5°C has been found to decrease microbial biomass (Herrmann and Witter 2002, Dorsch et al. 2004), but this temperature has no effect on microbial biomass in alpine and arctic environments (Lipson et al. 2000, Grogan et al. 2004). Respiration has been found to proceed at temperatures as low as -18°C in arctic (Eberling and Brandt 2003) and -20°C in permafrost (Rivkina et al. 2000) environments. However, little is known about how the lowest minimum temperature affects the activity and survival of soil microbial life, let alone denitrifying communities, in temperate regions.

Freezing duration was found to increase N₂O emissions in a laboratory simulation in which N₂O fluxes were 2-5 times higher in soil cores frozen 11.5 d as compared to cores frozen for 2.4 d (Teepe et al. 2004). Similarly, Muller et al. (2003) found increased N₂O fluxes with the more hours that soils spent below 0°C. MT and FDH may be operationally similar mechanisms underlying increased N₂O fluxes. All

studies linking lowest minimum temperature or freeze duration to N₂O fluxes attribute higher fluxes to increased damage to soil aggregates and plant and microbial cells. Aggregate disruption and cell lysis result in a higher flush of nutrients and C substrates for anaerobic respiration via denitrification. Teepe et al. (2004) also speculated that longer freezing duration may lead to a gradual depletion of O₂ dissolved in soil water microfilms as microbes respire. This would cause more facultative anaerobes to shift to anaerobic respiration over time and more microbes to be in the process of reverting to aerobic respiration during thawing. A similar O₂ dynamic may occur as lower temperatures freeze more water, leaving less O₂ and forcing more facultative microbes to respire anaerobically.

N₂O fluxes have been found to decrease with sequential FTC (Matzner et al. 2008), but it has not been determined how only a few intense FTC might compare to many FTC in terms of cumulative emissions. Typically, studies on FTC frequency serve the purpose of showing N₂O emissions following FTC depend upon availability of labile sources of C and N, as these may be depleted quickly during each sequential FTC (Herrmann and Witter 2002).

Although the primary mechanisms and temperature variable(s) controlling spring N₂O emissions remain unknown, the results of this study give some direction to land managers on how to mitigate spring N₂O emissions from their land. Snow cover led to lower N₂O emissions during a historically typical winter with sustained air temperatures below freezing, but did not affect N₂O emissions during a winter without sustained air temperatures below freezing. This indicates that management practices that promote an insulating cover (such as leaving more agricultural residue or mulching) may lead to lower spring N₂O emissions during a cold winter, while not threatening to lead to higher N₂O emissions if the winter is not as cold as historical winters.

Rye cover cropping, NO₃⁻ and N₂O fluxes

Use of a rye cover crop led to lower N₂O fluxes in 2008. This partly supports my second hypothesis; that a rye cover crop would immobilize NO₃⁻, and thereby reduce N₂O fluxes. There is conflicting evidence for NO₃⁻ immobilization, however. When NO₃⁻ was measured during the latter half of the N₂O sampling period (mid-April), rye plots had more NO₃⁻ (p=0.01) and PMN (p=0.07) than the fallow plots in 2007 and similar levels of NO₃⁻ and more PMN (p=0.1) than the fallow plots in 2008. There was more available inorganic N in the rye plots even with an average rye N uptake of 40 kg N ha⁻¹ in 2007 and 62 kg N ha⁻¹ in 2008 (Rich 2007). More available NO₃⁻ in the rye plots is most likely due to decomposition of fine roots killed during the winter freeze and subsequent N mineralization when soil temperatures were once again consistently above freezing. The rye covered plots leached less NO₃⁻ than the fallow plots, especially when snow was retained. In contrast to NO₃⁻ and PMN data, less NO₃⁻ leaching suggests that the rye plots had less NO₃⁻ in the soil solution than fallow plots most likely due to N uptake by the rye (Rich 2008).

Higher NO₃⁻ leaching in rye plots without snow cover compared to plots with snow cover was attributed by Rich (2008) to more fine root mortality which likely decreased N uptake ability and increased availability of high-N plant tissue for decomposition as was found in a forested ecosystem by Tierney et al. (2001). If NO₃⁻-N were more available due to root damage, we might also expect higher N₂O fluxes in these plots. Instead, rye plots without snow showed lower N₂O fluxes than rye plots with snow in both years (although not significantly different). It is possible that leaching was the dominant NO₃⁻ loss process in rye, snow removed plots, limiting the availability of NO₃⁻ as an electron acceptor for anaerobic respiration.

Discrepancies between N recovered in soil, water, gas, and biomass are most likely due to timing of sampling. Nitrous oxide fluxes and leachate samples were collected throughout late winter and early spring, while NO_3^- and PMN were determined for only one day in mid-April and biomass was collected in early May, although the N in the biomass was accumulated throughout the autumn and spring. In the Northeastern U.S., FTC, precipitation events, snowmelt, the beginning of vegetative growth and increases in microbial activity during the spring create very dynamic N conditions (Rich 2007). Soil sampling at a single time point for N availability indices likely did not represent how well rye cover cropping was able to immobilize NO_3^- and thereby decrease N_2O emissions.

Stronger evidence of N immobilization by rye may be seen in the amount of N contained in harvested rye compared to the fallow plots in which no biomass N existed. A colder winter in 2006-2007 may have inhibited N uptake by rye (as shown by fewer kg N ha^{-1} in 2007 than in 2008), decreasing the potential mitigating effect of rye on denitrification and contributing to higher N_2O fluxes than those measured in 2008. Milder temperatures in 2007-2008 may have enabled rye to immobilize more N, while downplaying the differences between snow treatments. This would indicate that, in 2008, rye did, in fact, affect N_2O emissions by immobilizing NO_3^- .

Conclusions

Results reported here provide evidence that during cold winters, as historically experienced in the Northeastern U.S., the absence of snow will likely lead to colder soils and higher N_2O fluxes. However, if the predicted winter warming trends for the Northeastern U.S. occur, soil temperatures and subsequent N_2O emissions may not be affected by snow cover. Much will depend on year-to-year variation in the relative magnitude of the deviation in air temperatures as compared to the deviation in snow

cover and soil freezing dynamics from historical averages. Further examination of the individual and combined effects of the lowest temperature attained and freezing duration and frequency is needed to elucidate the most likely mechanisms underlying the relationship between soil temperature and N₂O fluxes.

In a scenario of warmer winters as exemplified by the 2008 season, results of this experiment offer strong evidence that a winter rye cover crop can help to reduce N₂O emissions. Winter cover cropping also increased N uptake and reduced NO₃⁻ leaching (Rich 2008); and could improve soil structure, stop erosion (Langdale 1991), increase soil organic matter, sequester C, conserve soil moisture (Lal et al. 1991), and increase biodiversity (Altieri 1999). In the interests of reducing N losses to the atmosphere and waterways, and improving soil health, winter cover cropping is a valuable tool and should be incorporated into future agricultural land management plans.

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CHAPTER 3
THAWING DYNAMICS ALTER NITROUS OXIDE FLUXES AND NITROUS
OXIDE REDUCTION EFFICIENCY

Abstract

Nitrous oxide (N_2O) catalyzes reactions leading to stratospheric ozone depletion and is an important greenhouse gas whose emission from soil is especially high during spring freeze-thaw cycles (FTC) in temperate regions. A better understanding of the factors that drive higher N_2O emissions during these periods may lead to knowledge useful for designing effective mitigation strategies. A laboratory-simulated FTC in soil microcosms was used to measure the ratio of N_2O to total gaseous N emitted ($r\text{N}_2\text{O}$) during periods of high N_2O emissions. I found that $r\text{N}_2\text{O}$ decreased ($0.64 \rightarrow 0.0$) over time after thawing. This result suggests that a lack of reduction of N_2O to N_2 during denitrification may be contributing to the high N_2O emissions measured during soil thawing. The simulated FTC experiment was also used to examine the effect of soil thawing rate on N_2O emissions. Recent field work (Chapter 2) indicated that temperature buffering created by an insulating soil cover during the winter may lead to lower N_2O emissions in the spring. Such buffering may result in higher minimum soil temperatures, shorter freeze durations, fewer FTC and slower rates of freezing and thawing. Rate of soil thawing was examined here. Slower thawing led to higher N_2O emissions (1200 vs. $750 \text{ ng N}_2\text{O-N cm}^{-2} \text{ h}^{-1}$). This result suggests that slower thawing is not the sole mechanism responsible for lower N_2O emissions in agricultural fields with more soil cover. Further work should focus on interactions between temperature-dependent variables that lead to higher N_2O emissions during spring thawing in order to refine potential mitigation strategies and improve climate modeling efforts.

Introduction

Nitrous oxide (N_2O) is both a greenhouse gas and a catalyst of stratospheric ozone depletion. It ranks 4th behind carbon dioxide (CO_2), methane (CH_4), and dichlorodifluoromethane (CFC-12) in global climate change forcing (Forster et al. 2007). Unlike these other gases, N_2O comes mainly from the soil (70%) (Conrad 1996). Of the N_2O emissions associated with human activity, 60% come from agricultural lands (Smith 2007).

Nitrous oxide is a by-product of several microbially-mediated nitrogen (N) transformations, including N mineralization, nitrification, anaerobic ammonium oxidation (anammox) and denitrification (Roberston and Groffman 2007). During anaerobic microbial respiration via the denitrification pathway, nitrate (NO_3^-) is sequentially reduced to N_2 gas as microbes use the intermediary N compounds as terminal electron acceptors (TEA) (Figure 3.1). In order for denitrification to occur, oxygen (O_2) must be limited, NO_3^- must be available as a TEA, carbon (C) must be available as a substrate and the microorganisms capable of denitrification must be present (Smith and Tiedje 1979). While N_2 is generally the final product of denitrification, the reduction series can be incomplete, resulting in the release of N_2O . Thus, denitrification events yield a combination of N_2 and N_2O as end products.

Denitrification is the dominant N transformation during soil freeze-thaw cycles (FTC) (Ludwig et al. 2004, Oquist et al. 2004, Koponen et al. 2006, Morkved et al. 2006); times when unique physical, chemical and biological conditions prevail. These conditions contribute to the phenomenon of high N_2O emissions in the spring that can contribute to up to 66% of annual emissions in agricultural soils (Duxbury et al. 1982).

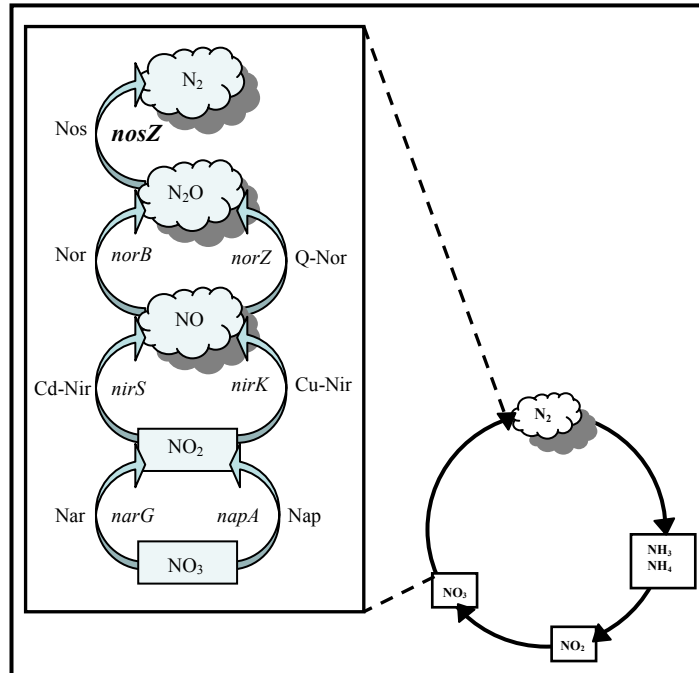


Figure 3.1 Denitrification pathway as part of the N cycle. Enzyme abbreviations appear to the outside of each step in the pathway. Names for the corresponding genes appear on the inside of each step in the pathway.

When soil freezes from the top downwards, water redistribution to the top of the soil profile is induced. This creates an ice lens. Upon thawing from the top downwards, the ice lens blocks snow melt-water from draining. Such an FTC creates saturated to super-saturated soil conditions (Miller 1980). Soil aggregates, organic matter and microbial cells are also disrupted/ruptured during an FTC. This releases a flush of carbon C substrates and nutrients into the soil solution (Christensen and Christensen 1991, Herrmann and Witter 2002). As the soil solution freezes, solutes are excluded from the ice, but retained within microfilms, causing nutrient concentrations to increase and pH to decrease. During thawing, these solutes are slowly diluted and pH returns to previous levels (Stahli and Stadler 1997). All of these conditions combined create the ideal environment for denitrification.

It is uncertain if increases in denitrification can account for the magnitude of increases in N₂O emissions typically measured during FTC. It is possible that in addition to an increase in the overall rate of denitrification, there may be an increase in the proportion of gaseous N that is emitted as N₂O instead of N₂ (Holtan-Hartwig et al. 2002). This relationship can be expressed as a ratio:

Equation 3.1
$$rN_2O = \frac{\Delta N_2O}{\Delta N_2O + N_2}$$

Observed effects of FTC on rN₂O from the soil are inconsistent. While some studies find that rN₂O increases with decreasing temperature (Bailey and Beauchamp 1973, Keeney et al. 1979, Melin and Nommik 1983, Muller et al. 2003); other studies find no effect of temperature on rN₂O (Holtan-Hartwig et al. 2002, Morkved et al. 2006). Ludwig et al. (2004) showed rN₂O increased with time after thawing, while Muller et al. 2003) showed rN₂O decreased over time after thawing. Thus, the contribution of N₂O reduction inefficiency to high N₂O emissions during thawing remains unclear. One purpose behind this study was to further investigate changes in rN₂O during and after soil thawing. I hypothesized that rN₂O would decrease with time after thawing, providing evidence that peak periods of N₂O emissions are caused not only by increases in denitrification, but also by decreases in the proportion of N₂O reduced to N₂. To test this hypothesis, I simulated a freeze-thaw cycle in soil microcosms contained in a temperature controlled chamber.

In addition to examining rN₂O under controlled conditions, this simulated FTC allowed me to measure the effect of soil thawing rates on N₂O emissions. Evidence that the rate at which the soil thaws may be important to N₂O emissions comes from a few recent field studies. Wagner-Riddle et al. (2007) found that agricultural soils with more surface residue cover during the winter spent fewer hours below 0°C and emitted

less total N₂O. Similarly, Maljanen et al. (2007) and Dietzel (Chapter 2) found that more snow cover led to warmer soils and lower N₂O emissions in agricultural fields. These studies indicate that spring N₂O emissions may be controlled by managing soil cover via adding residues or growing cover crops or by changes in snow cover resulting from climate change.

The relationships between soil cover, soil temperature, and N₂O fluxes are not well defined. The insulating effects of soil cover may result in higher minimum soil temperatures, shorter freezing duration, fewer FTC, and/or slower rates of freezing and thawing. Determining which of these temperature components affects N₂O emissions more strongly will give us mechanistic insight into how soil FTC affect N₂O emissions and will help guide management decisions and climate change predictions. One temperature component, the rate of soil thawing, was used to test the hypothesis that a slower thaw rate caused by soil insulation will lead to lower N₂O emissions.

Materials and Methods

Experimental logistics

One hundred and two intact soil cores containing a Howard gravelly silt loam (mixed, active, mesic Glossic Haplualf) were excavated from a NY cornfield in mid-May, 2008. The cores were contained in 5 cm diam x 30 cm long PVC pipes and taken to a depth of 20 cm to leave a 10 cm headspace (196 ml) to facilitate gas flux measurements. The field had been planted with corn for the previous two years, which followed several years of alfalfa. In the prior growing season (2007), the field received 34 kg N ha⁻¹ starter fertilizer and 92 kg N ha⁻¹ side-dressing from 30% Nitran, an inorganic fertilizer. These were the same experimental plots used by Rich (2008) and Dietzel (Chapter 2).

The intact soil cores were packed into a plywood-sided, metal-bottomed box measuring 90 x 90 x 30 cm (l x w x h). Two cores near the middle of the box were fitted with thermocouples at 0, 2, 5, 10, 15 and 20 cm depths to record temperature changes throughout the profile of the soil cores. Thermocouples were connected to a CR3000 datalogger (Campbell Scientific, Logan, UT). Fifteen centimeters were left between the edge of the box and the cores. This space and the interstitial spaces between the cores were packed with soil to a 20 cm height at a bulk density similar to that of the soil in the cores (1.31 g cm^{-3}). Twenty liters of deionized water were added to the bottom of the box, saturating the bottom 6 cm of soil inside the cores.

The box was housed inside a refrigerated shipping container (Mitsubishi 1994, Model CPE15-3BAIIF, Kanagawa, Japan). The shipping container had been retrofitted with heaters. The temperature was monitored and controlled by climate control software (Labview 8.5, National Instruments, Dallas, TX). To simulate a possible New York FTC, the software was programmed to reduce the air temperature from 16°C to -10°C over 30 h. The temperature was to be held at -10°C for 24 h and then increased to 13°C gradually over 24 h, thus keeping the air temperature below 0°C for a total of 50 h.

During the temperature increase, at 1°C air temperature, deionized water of the same temperature ($\sim 30 \text{ ml}$) was added to every core until the soil in the core was visibly supersaturated, thus simulating snowmelt. Cores were monitored visually until no water remained on the surface of the soil of any core, after which 2 ml soil samples were taken every 24 h from cores included specifically to monitor soil water content. Soil subsamples were dried and weighed to determine water-filled pore space (WFPS) (Robertson and Groffman 2007).

Cores were left in place and randomly allocated to five separate groups. Group 1 cores were used for ancillary measurements – these housed the thermocouples and

provided soil samples for WFPS measurements. Group 2 was used to measure N_2O fluxes (rate of N_2O emitted per unit area over time, $\text{ng N}_2\text{O-N cm}^{-2} \text{ h}^{-1}$) during and after soil thawing and to establish how long after thawing N_2O fluxes peaked. Groups 3-5 were used to estimate $r\text{N}_2\text{O}$ at different times over the experiment by use of a standard core method (Groffman et al. 2006). To measure $r\text{N}_2\text{O}$, half of the cores used at each time point were injected with acetylene (C_2H_2), which prevents the reduction of N_2O to N_2 and thus provides an estimate of total gaseous N losses. The other half of the cores used at each time point were injected with N_2 and these cores provided an estimate of the N_2O flux itself. Group 3 cores were used for $r\text{N}_2\text{O}$ estimates between 30-48 h, Group 4 cores were used for this test between 104-122 h and Group 5 cores were used for these estimates from 176-194 h after the air temperature reached 0°C .

N_2O flux measurements

Group 1 contained 20 randomly selected cores. Flux measurements were taken from these cores beginning when the air temperature reached 0°C during reheating (hour 0). Measurements were made using a static core method (Groffman et al. 2006). Each core was covered with parafilm and then capped with a PVC cap fitted with an autoclaved butyl rubber stopper. Seven milliliters of headspace gas were drawn out with a syringe at 0, 30, and 60 min after capping and stored in 3 ml Vacutainers (no additive, Becton, Dickinson and Co. Franklin Lakes, NJ). Flux measurements were repeated every 12 h for 196 h. The N_2O concentration in the sampled headspace gas was determined within 7 d of sampling by use of a ^{86}Ni equipped gas chromatograph (Varian GC Model 3700, Varian, Inc., Palo Alto, CA) as described by Terry et al. (1989). Calibration curves were created using custom standards mixed in 3 ml Vacutainers. Fluxes of N_2O from the cores were calculated from linear regression of the change in headspace N_2O concentration over the three sampling times (0, 30, 60

min). This rate of change in headspace concentration was divided by the soil surface area to determine $\text{ng N}_2\text{O cm}^{-2} \text{hr}^{-1}$. The Hutchinson-Mosier equation was used when the concentration gradient met the required conditions (Hutchinson and Mosier 1981).

rN₂O

Groups 3, 4 and 5 contained 24 cores each and were used to determine $r\text{N}_2\text{O}$ by use of the C_2H_2 block method (Groffman et al. 2006). Acetylene prevents N_2O from being reduced to N_2 , thus N_2O produced is the sum of the $\text{N}_2\text{O} + \text{N}_2$ produced. Cores were sealed as described above with an added layer of parafilm on the outside of the cap. The headspace of half of the cores in each group received a 10% volume of C_2H_2 and the other half of the cores in each group received a 10% volume of N_2 . Both gases were pumped with a syringe to mix them within the cores upon injection. A 7 ml headspace gas sample was drawn 6, 12, and 18 h after C_2H_2 or N_2 was injected. Gas samples were stored and N_2O concentration determined as described above. The $r\text{N}_2\text{O}$ was determined by dividing the mean non-blocked fluxes (N_2O emissions) by the mean C_2H_2 blocked fluxes ($\text{N}_2\text{O} + \text{N}_2$ emissions) (Equation 3.1). The first C_2H_2 block was run from hours 30-48, the second from hours 104-122, the third from hours 176-194.

Rate of soil thaw

To test the effect of insulation and a slower thaw rate on N_2O fluxes, hydrophobic cotton insulation (~5 cm) was randomly added to the surface of half of the cores (10 of 20) in Group 2 after the soil was frozen, but before it began to thaw. Cores with and without cotton insulation were distributed evenly with respect to distance from the center of the box. One of the cores that was fitted with thermocouples at various depths also received insulation, but was not otherwise

sampled. The N₂O fluxes during peak times were compared between treatments over time using a restricted maximum likelihood model (REML) in JMP 7.0 (SAS Institute, Cary, NC).

Results

The freeze-thaw cycle simulation and insulation treatments were established as planned. The air temperatures in the shipping container reached -10°C and soil temperatures at all depths reached -6°C. Freezing occurred from the top downwards and from the bottom upwards as shown in (Figure 3.2A). Thawing also occurred from both directions, with the 5 cm depth being the last soil depth to thaw after 69 and 75.5 h for non-insulated and insulated cores, respectively (Figure 3.2B). Insulated cores took 6.5 h longer to thaw at 5 cm depth than the non-insulated cores. Water was visible on the surface of the cores at 76 h, but absent at 84 h, indicating the ice lenses were breaking and water began draining during these eight hours. This coincided with the thaw at 5 cm. After the ice lens melted and standing water drained, the top 5 cm of the cores maintained ~65% WFPS.

rN₂O

The proportion of gaseous N that was emitted as N₂O decreased over time after thawing (Figure 3.3A). Between 30 and 48 h, fluxes averaged 1.8 and 2.9 ng N₂O-N cm⁻² h⁻¹ in non-blocked and C₂H₂ blocked cores, respectively, for an rN₂O of 0.64. Between 104 and 122 h, fluxes averaged 66.3 and 173.7 ng N₂O-N cm⁻² h⁻¹ in non-blocked and C₂H₂ blocked cores, respectively, for an rN₂O of 0.38. Between 104 and 122 h, fluxes averaged -432.6 and -141.1 ng N₂O-N cm⁻² h⁻¹ in non-blocked and C₂H₂ blocked cores, respectively. Since the amount of N₂O produced relative to the amount of N₂O + N₂ produced was the value of interest, and neither could be detected in the

third time period, the rN_2O was considered equal to 0.0. Nitrous oxide fluxes measured for the acetylene block method differed greatly between time points (Figure 3.3B, Table 3.1).

Rate of soil thaw

Insulated cores had higher N_2O fluxes during peak times than non-insulated cores ($p=0.03$) (Figure 3.4). Nitrous oxide emissions began at $85 (\pm 7)$ and $87 (\pm 3)$ h and peaked at $156 (\pm 7)$ and $148 (\pm 7)$ h for the non-insulated and insulated cores, respectively. An N_2O peak was measured in each core, but with much variability between cores. Peak fluxes ranged from 225 to $3115 \text{ ng } N_2O\text{-N cm}^{-2} \text{ h}^{-1}$. Insulated cores also peaked sooner than non-insulated cores (Figure 3.4).

Discussion

rN_2O

The proportion of gaseous N emitted as N_2O relative to all gaseous N emissions decreased with time after thawing as shown by decreasing rN_2O , as hypothesized. This indicates that decreased reduction of N_2O to N_2 may contribute to the magnitude of N_2O peaks measured following a freeze-thaw cycle. Decreased rN_2O with time after thawing is consistent with trends found by Muller et al. (2003) in which rN_2O decreased over time after thawing. However, in their study soil temperature continued to increase over time, whereas the soil temperature in my experiment increased to 12°C over 136 h and remained constant thereafter while N_2O fluxes continued to rise. My results point to a combination of changes in the activity of nitrous oxide reductase (Nos) that occurred both in response to temperature and over time because the temperature was unchanging as N_2O fluxes continued to rise.

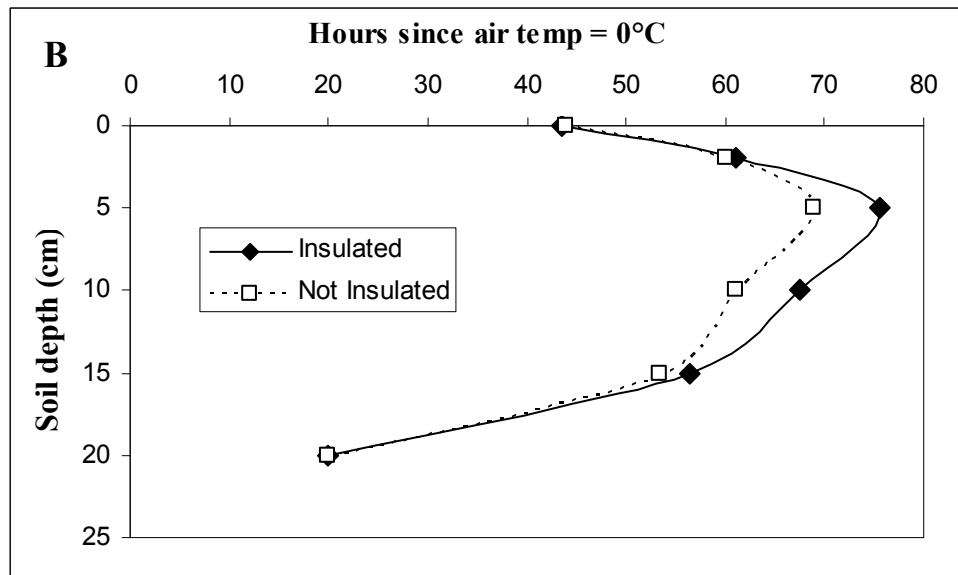
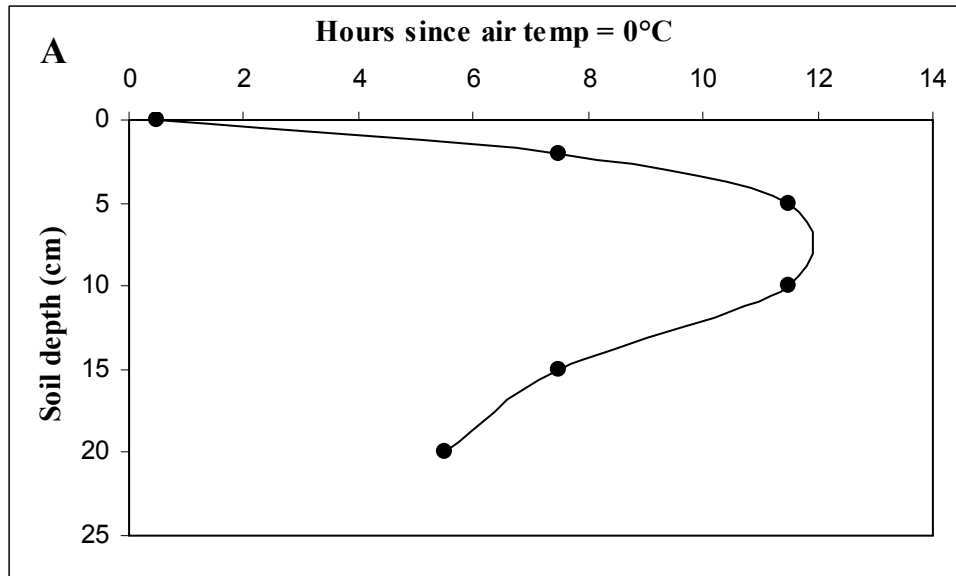


Figure 3.2 Time taken for soil to (A) freeze at different soil depths and (B) thaw at different soil depths after the air temperature was reduced to 0°C or raised to 0°C during freezing and thawing, respectively. Data come from two cores before (A) and after (B) the insulation treatment was implemented.

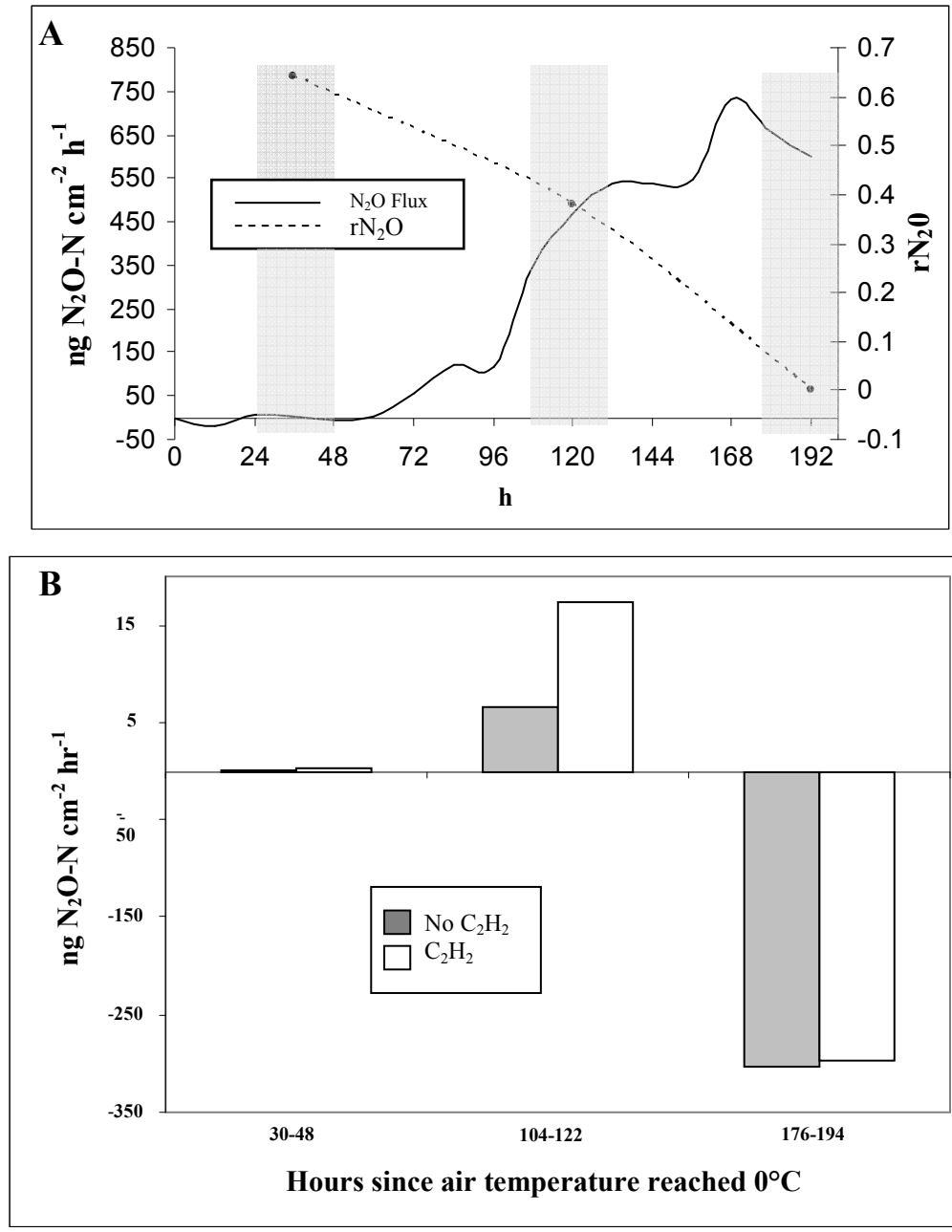


Figure 3.3 Changes in rN₂O over time after air temperature reached 0°C. Correspondence of rN₂O with N₂O flux activity is shown in (A). Bars in (A) represent time periods when rN₂O measurements were taken. rN₂O is derived from soil core Group 3 and N₂O flux activity from soil core Group 2 (non-insulated cores). N₂O flux measurements averaged over non-blocked and C₂H₂ blocked soil cores for each period are shown in (B).

Table 3.1. Headspace concentrations of N₂O from cores without (N₂O) and with (N₂O + N₂) C₂H₂ injected 6, 12, and 18 h after injection. Ambient headspace N concentrations were assumed to be 11 ng N cm⁻³. N₂O fluxes for these time periods are also shown, but were derived by finding fluxes for individual soil cores and then averaging.

Sampling period	Hour	N ₂ O (ng N cm ⁻³)	Std. Dev	N ₂ O + N ₂ (ng N cm ⁻³)	Std. Dev	rN ₂ O
30-48	6	23	2.0	21	3.2	
	12	21	12.2	20	2.6	
	18	28	10.8	25	3.0	
N ₂ O flux (ng N ₂ O-N cm ⁻² h ⁻¹)		2	2.8	3	4.2	0.64
104-122	6	102	86.9	162	174.9	
	12	118	103.2	193	132.4	
	18	180	141.4	320	197.3	
N ₂ O flux (ng N ₂ O-N cm ⁻² h ⁻¹)		66	136.2	173	126.0	0.38
176-194	6	294	119.8	354	194.4	
	12	192	77.9	203	101.4	
	18	181	74.8	193	96.5	
N ₂ O flux (ng N ₂ O-N cm ⁻² h ⁻¹)		-304	490.7	-297	408.0	0

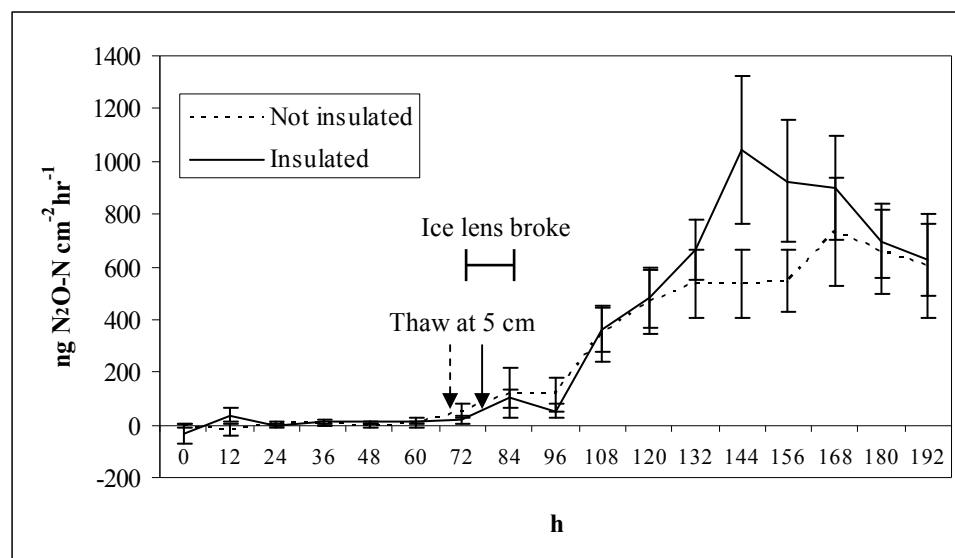


Figure 3.4 Mean N₂O fluxes after the air temperature reaches 0°C (0 h) for non-insulated and insulated soil core treatments.

Other studies addressing reduction of N_2O to N_2 with respect to time after an event use the onset of anaerobiosis as the seminal event. The enzymes catalyzing reduction of NO_3^- and NO_2^- to N_2O (nitrate reductase and nitrite reductase) have been found to be synthesized within 5 h of anaerobiosis, but the enzyme catalyzing the reduction of N_2O to N_2 (nitrous oxide reductase, Nos) is not synthesized until 16-33 h after O_2 is depleted (Smith and Tiedje 1979, Firestone and Tiedje 1979, Dendooven and Anderson 1994). In addition, nitrate and nitrite reductases have been found to be very persistent in soils, while nitrous oxide reductase is less persistent (Smith and Parson 1985, Dendooven and Anderson 1994). Similar enzyme dynamics, if they are occurring during soil thawing, could help to explain the initially high $r\text{N}_2\text{O}$ measured once soil thawing began. Enzymes responsible for the production of N_2O (nitric oxide reductases) may be preserved during freezing and quickly activated upon thawing. Meanwhile the enzyme responsible for N_2O reduction (Nos) may not be preserved and may take much longer to be synthesized. The resulting increases in production and decreases in the reduction of N_2O would be reflected in a high value for $r\text{N}_2\text{O}$ and high N_2O peaks, as were measured in this study.

Evidence further supporting the contribution of large $r\text{N}_2\text{O}$ to high N_2O fluxes can be seen in the relationship of general flux activity to changes in $r\text{N}_2\text{O}$ (Figure 3.3A). The $r\text{N}_2\text{O}$ measured during 30-48 h occurred while most of the soil was frozen and its contribution to total N_2O emissions was small. However, the second $r\text{N}_2\text{O}$ (0.38) was measured between 104-122 h while N_2O fluxes were trending upwards and the third $r\text{N}_2\text{O}$ (0.0) was measured between 176-194 h while N_2O fluxes were trending downwards. This downward trend in N_2O flux activity coincided with no detectable production of N_2O from the cores used to determine $r\text{N}_2\text{O}$. These results

suggest that the observed decreases in N₂O fluxes could have resulted from increases in reduction of N₂O to N₂.

A decrease in rN₂O does not necessarily mean more N₂O is being reduced because it could also indicate that less N₂O is being produced. However, in this study, reduction of N₂O to N₂ was reflected in the negative N₂O fluxes measured during the third rN₂O measurement period. In these cores, headspace N₂O concentrations increased during the initial 6 h incubation (Table 3.1), indicating production of this gas within the core. The cores remained sealed and over the next 12 h, the N₂O concentration in the headspace decreased; indicating that the existing N₂O was being “consumed”, most likely by its reduction to N₂.

Previous studies in which denitrification enzyme activities were examined have relied on measurements of gaseous N emissions as indicators of enzyme activity (Smith and Tiedje 1979, Firestone and Tiedje 1979, Dendooven and Anderson 1994, Holtan-Hartwig et al. 2002). Molecular techniques now allow more direct measurements of activity. In essence, mRNA coding for the various reductases of interest can be extracted from soil and reverse-transcribed into cDNA. The cDNA can then be used in PCR experiments and the resulting amplicons used for other analyses, such as cloning and sequencing. The number of copies of the mRNA that directs the synthesis of the necessary denitrification enzymes can be estimated using qPCR, the results of which indicate how much of a given enzyme is being produced in the soil sampled. Sharma et al. (2006) was the first study where this technique was used for this purpose. They found increased expression of nitrate- and nitrite- reductase genes (*narG* or *napA* and *nirS* or *nirK*, respectively) two days after soil thawing, with decreased expression after the third and ninth days. No expression of *nosZ*, the gene coding for nitrous oxide reductase, was detected in the 9 days following the thaw, suggesting it was either not produced or was produced in very small amounts, below

the limit of detection of their assay. Little or no production of Nos in the soils in their study would explain the high rN₂O typically measured during thawing.

Decreases in N₂O flux and increases in N₂O reduction to N₂ could also be due to a lack of NO₃⁻ availability (Weier et al. 1993). As NO₃⁻-N in the soil solution is used as a TEA, its availability decreases. When NO₃⁻ availability is limited, N₂O becomes the more abundant electron acceptor. However, only some microbes can reduce both NO₃⁻ and N₂O (Throback 2006). If NO₃⁻ depletion was the cause of lower N₂O fluxes, this could indicate a microbial community shift from denitrifiers only able to reduce NO₃⁻ to N₂O to denitrifiers able to reduce N₂O to N₂. This or other mechanisms responsible for the high rN₂O values measured immediately following freezing have yet to be determined. My results provide evidence that a high rN₂O may explain the high spring N₂O emissions observed. This suggests mitigation strategies should not only aim to reduce denitrification, but to reduce rN₂O as well.

Rate of soil thaw

Insulated cores had higher N₂O fluxes than non-insulated cores, although the opposite result was hypothesized. The insulated cores were expected to have lower N₂O fluxes based on the field results in which lower N₂O fluxes were measured in plots with soil cover (Chapter 2, Wagner-Riddle et al. 2007, Maljnen et al. 2007). Higher fluxes in insulated cores may have been the result of more cellular damage to microbes. This would be compatible with basic cryobiological theory that slower temperature change during thawing results in greater cellular damage due to ice recrystallization (Mazur 1970). More damage may have resulted in a greater loss of microbial biomass and a larger nutrient flush. Intracellular damage has also been speculated to occur (Holtan-Hartwig 2002, Morkved 2006), inhibiting the ability of microbes to reduce N₂O. Because only some microbes possess the gene required for

reducing N₂O (Throback 2006), another possibility is that there was greater mortality or intracellular damage experienced by this sub-population within the soil microbial community.

These laboratory results contrast with results from field trials where covered (insulated) soil had lower N₂O fluxes than bare soil (Wagner-Riddle 2007, Maljanen 2007, Chapter 2). Field soils experience many conditions not replicated in this simulation, such as differences in freezing rates, freezing duration and frequency and the lowest temperature attained. Contrasting what factors were controlled in the laboratory microcosm study and those that were not may provide insight into which field processes most strongly affect N₂O emissions. In the simulated FTC, rate of thaw and timing of when the ice lens broke were the only derived temperature components that differed between the two treatments. Here, rate of thaw yielded results that were opposite to those observed in the field. This indicates that, when duration and depth of freezing are held constant, insulation retards thawing and increase N₂O emissions. Rate of thawing under field conditions will depend on the insulation (soil cover), but will also depend on depth and duration of freezing, which are also mediated by soil cover. Other temperature dependent variables, such as freezing rate, depth of freezing, freezing duration, frequency of FTC, and the lowest temperature attained may have a stronger influence on N₂O flux rates under field conditions. Establishing which temperature variables most strongly influence spring N₂O emissions will enable us to design management strategies that manipulate these variables to mitigate N₂O emissions. Evidence from this study suggests that soils should be managed to thaw at a faster rate or reduce the intensity of freezing events.

Conclusions

The amount of N₂O emitted relative to total gaseous N emitted (rN₂O) in the laboratory simulation decreased with time after thawing (0.64→0.0). Higher reduction of N₂O to N₂ coincided with decreases in N₂O flux measurements. This suggests that N₂O peaks that occur following a freeze-thaw cycle may in part be due to a lack of reduction of N₂O to N₂. This mechanism may explain why N₂O is emitted in such large amounts during spring soil thawing. With this in mind, mitigation strategies should be designed that will not only help to decrease overall denitrification in the spring, but will also create conditions that will lead to more efficient reduction of N₂O to N₂.

Insulation delayed thawing and led to higher N₂O fluxes. This was possibly due to damage suffered by microbial communities with thawing occurring at a slower rate, leading to a larger flush of substrate and nutrients for denitrification. This may relate to field situations where the ground freezes first and then receives a cover of insulation, such as snow or mulch. Thus, increasing soil insulation after soils have already frozen should not be recommended as a management practice because it is unlikely to mitigate N₂O emissions and may actually exacerbate them. In other scenarios studied, where soils experienced differing freezing rates, duration, frequency and lowest temperatures, insulation decreased N₂O emissions (Wagner-Riddle et al. 2007, Maljenen et al. 2007, Chapter 2), most likely because insulation reduced the depth to which the soil froze, and thus reduced the time needed for the soil to thaw. Further research investigating the combination of temperature dependent variables that are the primary drivers of spring N₂O emissions is needed so that we can design and test more effective management strategies to reduce spring N₂O emissions from agricultural landscapes.

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CHAPTER 4

CONCLUSIONS AND FUTURE RESEARCH

Key Findings

Nitrous oxide (N_2O) plays a major negative role in global climate change (Denman et al. 2007). In this thesis, I set out to examine the period of greatest N_2O emissions in temperate agricultural fields, which is during spring freeze-thaw cycles (FTC). Much of what determines the magnitude of N_2O emissions during FTC is not well understood, preventing us from effectively mitigating N_2O emissions during this period. Also, a poor understanding of the sources of this harmful gas inhibits accurate predictions of future climate change. In this thesis, I addressed four questions designed to (a) improve our understanding of the controls on N_2O emissions during spring soil thawing, (b) test the efficacy of a rye cover crop as a promising management strategy to reduce N_2O emissions, and (c) clarify how emissions may change as a result of climate change predictions.

First, I asked if less snow cover, as predicted for the Northeastern U.S., leads to higher N_2O emissions in the spring. I measured and contrasted N_2O fluxes between experimental field plots with and without snow cover and with and without a rye cover crop. I measured N_2O fluxes during FTC during a typical cold winter and a milder winter, similar to what is predicted due to climate change. I found evidence that during cold winters, as historically experienced in the Northeastern U.S., the absence of snow will lead to colder soils and higher N_2O fluxes. However, if predicted winter warming trends occur, soil temperatures and subsequent N_2O emissions may not be affected by snow cover. As climate change leads to warmer winters and warmer soils, results of this study suggest we may expect lower N_2O emissions compared to past years. Much will depend on year-to-year variation in the relative magnitude of the

deviation in air temperatures from the historical averages vs. the deviations of snow cover and soil freezing dynamics from historical averages.

During this experiment, I also examined one management option by testing the hypothesis that winter rye cover cropping will take up and immobilize NO_3^- and thereby lower N_2O fluxes. N_2O fluxes were measured in field plots with and without rye during the typically cold and the milder winter. In the colder winter (2006-2007), rye cover cropping had no detectable effect on spring N_2O emissions. However, during the milder winter (2007-2008), rye cover cropping resulted in lower N_2O fluxes, providing evidence that in a scenario of warmer winters, a winter rye cover crop could help to reduce N_2O emissions. If climate changes lead towards winters with milder temperatures, winter rye may be considered as a valuable management tool for reducing high spring N_2O emissions.

Following the evidence that less snow cover leads to higher N_2O emissions, I next asked if this was due to a faster rate of thaw during the spring. To answer this question, I used intact soil cores to simulate a freeze-thaw cycle and, after freezing but before thawing, added insulation to the surfaces of half of the cores. Measurement of soil temperatures and resulting N_2O fluxes showed that the cores with insulation thawed at a slower rate and had greater N_2O peaks. This finding was contrary to what was expected based on the field results. Soils with insulation in the field had lower N_2O emissions and soils with insulation in the freeze-thaw cycle simulation had higher N_2O emissions. Insulation in the field can lead to effects on a multitude of freezing components besides the rate of thaw. These include a slower freezing rate, decreased lowest minimum temperature, decreased depth of freezing and reduced duration of freezing. Insulation will also affect the frequency of FTC the soil experiences by protecting soil from freezing in the first place. Because slower rate of thaw, as experienced in insulated soils, was not found to lead to lower N_2O emissions, it may

be that the intensity (depth and duration) of soil freezing or one of other temperature components is more important in lowering spring N₂O emissions from insulated field soils.

The simulated FTC allowed me to ask a basic question concerning the occurrence of high N₂O emissions following FTC's. I asked if the magnitude of N₂O emissions following FTC's was due to a higher proportion of N₂O being emitted relative to N₂ gas. In other words, do FTC affect the last step in the denitrification pathway, N₂O reduction to N₂, and thus increase the proportion of N₂O emitted relative to N₂? During and after thawing, I measured the ratio of N₂O to N₂O + N₂ (rN₂O) and found the proportion of gaseous N emitted as N₂O decreased with time after thawing. This decrease corresponded to decreases in the N₂O flux measured, indicating that high emissions may in part be due to an inefficient reduction of N₂O to N₂ when soils are still very cold following soil thawing. This suggests that management strategies targeting mitigation of spring N₂O emissions should not focus only on reducing denitrification, but also consider ways reduce rN₂O.

Future Work

My work has provided some evidence that less insulation affects how antecedent temperature factors modify spring N₂O emissions as colder soils lead to higher emissions. However, there is evidence from field studies in which insulated soils had lower emissions (Wagner-Riddle et al. 2007, Maljanen et al. 2007, Chapter 2) that doesn't support a slower thawing rate being the primary temperature factor leading to high spring emissions. Having already tested the possibility that slower soil thawing may lead to lower N₂O emissions, more work should be done to identify which of the other temperature components is the primary control over the magnitude of N₂O emissions during spring thaws. Different winter field covers such as

agricultural residues, standing vegetation and mulch should also be tested to determine the most effective winter insulation management strategies.

Results of this study suggest that winter rye cover cropping during a mild winter diminishes N₂O emissions during the spring thaw. This is the first study to test the effects of winter rye cover cropping on N₂O emissions and these results need to be further corroborated. Research should also be directed toward determining the potential of winter rye and other cover crops to mitigate N₂O emissions in different hardiness zones.

The finding that a large rN₂O may contribute to high N₂O emissions during thawing gives us insight into why N₂O emissions are so high during the spring, but we still do not know, mechanistically, why rN₂O_s are so large. There are many possible explanations behind an increase in the proportion of N₂O emitted relative to total gaseous N during denitrification that have not yet been studied and that we have recently developed the technology to investigate. Reduction of N₂O to N₂ may be decreased by the absence of microbes possessing the *nosZ* gene. Lack of induction of this gene, or induction of this gene, but dysfunction of the enzyme it produces are other possibilities. Molecular methods may enable us to examine the presence of this gene in the soil microbial populations through DNA detection, the activity of this gene through mRNA detection and the presence and activity of Nos through various enzyme assays (Wallenstein et al. 2006). These methods should be used to further establish the mechanisms responsible for high N₂O emissions from soil during spring thawing.

Broader Implications

Nitrous oxide contributes to global climate change which has had a series of negative effects on this planet, including sea level rise, increased drought and more

frequent heat waves (Rosenzweig et al. 2007). As global climate change progresses, these negative effects are predicted to be amplified (Schneider et al. 2007).

Agricultural land is a major source of N₂O (Smith et al. 2007), but also a major source of food to sustain this planet. Management strategies need to be found that both mitigate N₂O emissions and allow food production to continue. Understanding the effects of winter soil cover on N₂O emissions during the highest period of emissions in temperate regions and why these emissions are so high will allow targeted design of N₂O mitigation strategies. My work suggests that field management strategies that increase soil insulation during the winter should be encouraged. In this thesis, I also provide evidence that climate change itself may result in lower N₂O emissions in temperate regions by causing higher minimum winter temperatures leading to lower N₂O emissions.

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