**Comparison of Simultaneous Soil Profile N₂O Concentration and Surface N₂O
Flux Measurements Overwinter and at Spring Thaw in an Agricultural Soil**

by

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ABSTRACT

Comparison of Simultaneous Soil Profile N₂O Concentration and Surface N₂O Flux Measurements Overwinter and at Spring Thaw in an Agricultural Soil

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A field experiment was carried out in Ontario, Canada to compare independently measured soil N₂O profile concentration and surface N₂O flux measurements overwinter and at spring thaw, to estimate the soil N₂O content accumulation overwinter, and to estimate the magnitude of the contribution of the physical release of trapped N₂O to surface fluxes at spring thaw.

Gas samples at various depths were taken and N₂O concentrations determined, soil profile gaseous N₂O content was calculated by estimating the air-filled pore-space from soil condition measurements, and soil aqueous N₂O content was calculated using liquid water content measurements and applying Henry's Law. Soil N₂O content was found to reach a maximum of ~25 mg N₂O m⁻², and by comparing changes in soil N₂O content to surface fluxes measured using the micrometeorological flux-gradient technique, the physical release of previously produced N₂O was estimated to contribute up to 47% of spring thaw N₂O surface fluxes.

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CHAPTER 1: Introduction

1.1 Introduction

Nitrous oxide (N_2O) is a greenhouse gas with a 100-year GWP (global warming potential) that is 298 times greater than that of CO_2 per mass, and an atmospheric lifetime of 114 years (IPCC 2007). In addition to contributing to the greenhouse effect, N_2O is involved in the destruction of stratospheric ozone, and due to large anthropogenic sources, N_2O has become the dominant anthropogenic ozone-depleting substance (Crutzen 1970; Ravishankara et al. 2009). Atmospheric concentrations of N_2O have increased by 15% since the industrial era and are currently increasing linearly at a rate of 0.25% per year (IPCC 2007). In terms of sources of atmospheric N_2O , it has been estimated that roughly 70% of global annual N_2O emissions are derived from soils and that more than one third of all N_2O emissions are anthropogenic, agriculture constituting the majority of these emissions (Mosier 1994). Globally, N_2O emissions from agricultural soils are estimated to contribute 6.1% to anthropogenic climate forcing (IPCC 2007). In cold climates, after spring thaw, large emissions of N_2O from soils have been found to occur in a multitude of environments including forests, grasslands and agricultural land (Teepe et al. 2000; Holst et al. 2008). These emission events, often termed “bursts” or “flushes”, comprise the highest annual N_2O emission rates and can account for up to 70% of annual N_2O emissions from soils (Wagner-Riddle et al. 1997; Lemke et al. 1998). Spring thaw emissions of N_2O from agricultural soils have been shown to be greater than in other environments such as forests (van Bochove et al. 1996), which is of particular interest considering the contribution agriculture to anthropogenic N_2O emissions.

Spring thaw N_2O emissions have been related to the freezing and thawing of soils, whereby soils subjected to freeze/thaw cycles (FTC) have been shown to exhibit flushes of N_2O

(Goodroad and Keeney 1984). The mechanisms behind these large bursts of N₂O are not well defined and are likely a combination of physical and biological processes (Wang et al. 2008). A fundamental issue concerning these mechanisms, and the dynamics of spring thaw N₂O emissions, is determining whether N₂O is produced once the thawing process begins, or if it is produced during the freezing period and is released at thaw (Wagner-Riddle et al. 2008). As such the mechanisms can be broken down into two groups. First, ‘old’ production, involves the physical release of N₂O trapped in unfrozen soils at depth under a diffusion barrier (ice layer) (Burton and Beauchamp 1994) or within unfrozen water films (Teepe et al. 2001). This physical release would occur as a burst at thaw when the diffusion barrier is destroyed, and the release is of previously produced N₂O. Secondly, “de novo” production comprises the increased production of N₂O due to increased microbial activity at thaw within the surface layer (Nyborg et al. 1997; Lemke et al. 1998; Wagner-Riddle et al. 2010).

1.2 Research Objectives

To date studies have not quantified the relative magnitudes of the two aforementioned processes of “de novo” N₂O production at thaw and physical release of trapped N₂O at thaw. Such quantification would have a fundamental implication for models attempting to describe N₂O emission as spring thaw, as a lack of knowledge has hindered the development of models that can adequately describe spring thaw emissions (Pattey et al. 2007; Wagner-Riddle et al. 2010). Prior research has compared surface N₂O fluxes to soil N₂O concentrations (Maljanen et al. 2007; Wagner-Riddle et al. 2008; Yanai et al. 2011), however much of this research is focused on the effects of snow cover on N₂O emissions, and a comparison of changes in soil N₂O content to surface N₂O emissions has not been made. Furthermore, the majority of prior research relies on the closed-chamber technique to measure N₂O surface emission rates, a technique of

debated effectiveness, and one that lacks the temporal and spatial resolution necessary to follow N₂O emission events (Pattey et al. 2007; Yanai et al. 2011). The objective of this study was to estimate the content of N₂O within the soil profile, and to compare changes in soil N₂O storage to N₂O surface fluxes at spring thaw measured using the micrometeorological flux-gradient technique. Through this comparison the goal was to estimate the relative proportion of N₂O emissions at spring thaw from physical release (old production) and new production.

1.3 Thesis Structure

This thesis is comprised of four chapters. Chapter 1 is a general introduction to the research project and its objectives; chapter 2 is a literature review, highlighting the findings and conclusions of prior research related to this project; chapter 3 describes the research project by outlining the methodology, and both presenting and discussing the findings; and chapter 4 presents the conclusions to the thesis and offers recommendations for future studies.

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CHAPTER 2: Literature Review

2.1 The Involvement of N₂O in the Destruction of Stratospheric Ozone

Like chlorofluorocarbons, N₂O is responsible for the destruction of stratospheric ozone (O₃). As N₂O diffuses upwards from the surface it undergoes photodissociation which produces “odd nitrogen” or NO_x (NO_x = NO + NO₂), and this process is the main source of NO_x in the stratosphere (Crutzen 1970; Dyominov and Zadorozhny 2005). NO, in turn, is directly involved in the process of ozone destruction, where the reactions can be simplified to (Crutzen 1970; Ravishankara et al. 2009):

Ravishankara et al. (2009) found that the ozone-depleting potential of N₂O is roughly one-sixtieth that of CFC-11, the most prevalent chlorofluorocarbon. Though the ozone depleting potential of N₂O is small, the anthropogenic emissions of N₂O are so great (in comparison to the highly regulated CFC emissions) that N₂O has become the dominant anthropogenic ozone-depleting substance (Ravishankara et al. 2009).

2.2 Production Pathways of N₂O in Soils

2.2.1 N₂O Production via Denitrification

As stated by Knowles (1982), “Denitrification refers to the dissimilatory reduction, by essentially aerobic bacteria, of one or both the ionic nitrogen oxides (nitrate, NO₃⁻, and nitrite, NO₂⁻) to the gaseous oxides (nitric oxide, NO, and nitrous oxide, N₂O), which may themselves

be further reduced to dinitrogen (N₂)". In the absence of oxygen, the nitrogen oxides act as terminal electron acceptors and gaseous nitrogen species are major products of these reductive processes (Knowles 1982). Denitrification occurs almost universally in organisms that are primarily aerobic respirers, but which have the ability to shift to denitrification when O₂ is limiting (Schimel and Gullledge 1998). In other words, denitrification is an anaerobic process where bacteria make use of nitrate and nitrite for growth when oxygen is limiting, producing N₂O and N₂ in the process (Galbally 1989). The sequence for denitrification is as follows (Galbally 1989):

Most denitrifying bacteria are heterotrophs, and when O₂ is limiting they make use of NO₃⁻ as their primary electron acceptor to obtain energy from organic compounds (Granli and Bøeckman 1994). Therefore, the presence of dissolved organic carbons (or water-extractable organic carbon, a ready source of electrons) is a necessity for these reductions to occur in soils (Knowles 1982; Galbally 1989):

The proportion of N₂O and N₂ that is produced during denitrification is determined by numerous factors which are often referred to by their impact on the ratio between N₂O and N₂ produced (often discussed as either N₂/N₂O, N₂O/N₂ or N₂O/N₂+N₂O) (Table 2.1) (Firestone and Davidson 1989). Of particular interest here are the effects of temperature, carbon availability and the expression of the N₂O reducing enzyme N₂O-reductase, changes in which are to be discussed as fundamentals behind the N₂O producing mechanisms responsible for freeze-thaw related emissions.

Table 2.1 Factors affecting $\text{N}_2\text{O}/\text{N}_2$ production ratio in soils (table adapted by author from Firestone and Davidson 1989)

Alteration / Factor	Effect on $\text{N}_2\text{O}/\text{N}_2$ Product Ratio
Increasing NO_3^- or NO_2^- concentrations	Increase
Increasing O_2 concentration	Increase
Increasing carbon availability	Decrease
Increasing soil temperature	Decrease
Reducing N_2O -reductase activity	Increase

2.2.2 N₂O Production via Nitrification

Nitrification is the oxidation of ammonium (NH₄⁺) or ammonia (NH₃) to nitrate (NO₃⁻) via nitrite (NO₂⁻) and can be thought of stepwise as (Wrage et al. 2001):

In soils, autotrophic microorganisms are almost entirely responsible for the nitrification that occurs (Bremner 1997). Heterotrophic nitrification, where nitrifiers use organic carbon (C) as both a C and an energy source, can occur in soils and produce N₂O via aerobic denitrification, however it is a minor source of N₂O (Wrage et al. 2001). Autotrophic nitrifiers use CO₂ as a C sources while obtaining their energy through the oxidation of NH₄⁺, which is present in soils due to the mineralization of organic material by other microbes or from fertilization (Granli and Boeckman 1994).

Nitrification is a two-step process, the first of which consists of reducing NH₄⁺ to NO₂⁻ (ammonium oxidation):

and the second step being the further oxidation of NO₂⁻ to NO₃⁻ (nitrite oxidation) (Granli and Bøeckman 1994).

The prior step is performed by the aptly named ammonium oxidizing bacteria who are responsible for one of the two processes by which N_2O is produced through nitrification (Firestone and Davidson 1989). N_2O production via autotrophic nitrification generally occurs through a process termed “nitrifier denitrification” whereby ammonium oxidizing bacteria use NO_2^- as an electron acceptor (reduce) when O_2 is limiting, producing N_2O and N_2 with NO as a possible intermediate (Firestone and Davidson 1989; Wrage et al. 2001). The second pathway for the production of N_2O during autotrophic nitrification occurs by the chemical decomposition of intermediates between NH_4^+ and NO_2^- , such as NH_2OH or even NO_2^- itself (Wrage et al. 2001). This latter process is commonly viewed as a special type of chemodenitrification (to be discussed), though N_2O may also result from the incomplete oxidation of NH_2OH (Wrage et al. 2001). The production of N_2O through nitrification can therefore be thought of as seen in Figure 2.1.

Figure 2.1 Simplified representation of N_2O production pathways via nitrification (and denitrification) in soils (Wrage et al. 2001, used with permission of publisher)

2.2.3 N₂O Production via Chemodenitrification

Chemodenitrification encompasses a number of chemical reactions that represent the chemical decomposition of intermediates from the oxidation of ammonium to nitrite, or nitrite itself, with organic (e.g. amines) or inorganic compounds (eg. Cu²⁺, Fe²⁺) (Firestone and Davidson 1989; Granli and Boeckman 1994; Wrage et al. 2001). The major product of these reactions is NO, such as from the widely documented decomposition of HNO₂ yielding NO and HNO₃ (Firestone and Davidson 1989). N₂O and N₂ can also be produced by these reactions, however chemodenitrification is so closely linked with nitrification it is difficult to determine through which process NO, N₂O and N₂ are produced (Firestone and Davidson 1989). An example of N₂O production is through the decomposition of hydroxylamine (NH₂OH) by MnO₂ (Nelson and Bremner 1970):

As mentioned above, chemodenitrification is strongly related to nitrification as the accumulation of NO₂⁻ in soils (an obligate intermediate of nitrification) often results in its chemical decomposition, especially at low pH (Müller et al. 2003; Mørkved et al. 2007).

2.2.4 N₂O Production Pathways as Contributors to Freeze-Thaw Emissions

With the N₂O producing processes identified, the question remains as to which of these processes are the main contributors to heightened freeze-thaw emissions. In this area a general consensus has been reached, and most of the literature has found denitrification to be the dominant processes inducing freeze-thaw N₂O emissions (Müller et al. 2002; Müller et al. 2003; Ludwig et al. 2004; Öquist et al. 2004; Sehy et al. 2004; Koponen et al. 2006b; Mørkved et al. 2006; Wagner-Riddle et al. 2008). This conclusion has been reached through several methods of

experimentation. Some studies have found that N_2O emissions post freeze-thaw corresponded with high NO_3^- concentrations but not NH_4^+ concentrations, indicating the dominance of denitrification over nitrification (Chen et al. 1995; Müller et al. 2003). Another method used is the inhibition of nitrification in soil cores allowing for the respective contributions of nitrification and denitrification to be determined. Through the use of this method, Priemé and Christensen (2001) found that denitrification was dominant in soil cores from 4 of 5 sites tested, where denitrification was responsible for <40% of the emissions in one site. The strongest evidence as to the production pathways responsible for N_2O production is obtained through the use of ^{15}N tracers. Methodologically similar to comparing N_2O emissions to NO_3^- and NH_4^+ concentrations in the soils, the application of ^{15}N labeled NO_3^- and NH_4^+ allows for the contributions of denitrification and nitrification to be determined based on ^{15}N enrichments found in corresponding N_2O emissions. Using this method Müller et al. (2002) and Wagner-Riddle et al. (2008) identified denitrification as the main contributor to N_2O emissions during soil thawing. In a similar study Ludwig et al. (2004) used ^{15}N tracers to identify that initially 83% of the N_2O flux post freezing-thawing in agricultural soil cores was the result of denitrification, with the proportion being reduced to 72% as the N_2O production rate decreased in time. In addition, again using ^{15}N , Mørkved et al. (2006) found that only 4.4% of freeze-thaw induced N_2O emissions in their lab experiment on agricultural soils originated from nitrification, and that N_2O emissions were inversely related to O_2 concentrations (further indicating the anaerobic process of denitrification as the prevailing process). Though denitrification has been found to be the dominant process inducing the production of N_2O in freeze-thaw emissions, nitrification is still a significant contributor and should not be disregarded as its contribution may

change spatially and temporally as per the findings of Priemé and Christensen (2001) and Ludwig et al. (2004) discussed above.

Missing from the above discussion is the process of chemodenitrification and its contribution to N₂O emissions at thaw. The potential for chemodenitrification has been shown to exist in frozen soils (Christianson and Cho 1983), however the resultant N₂O emissions from this process are widely believed to be insignificant (de Bruijn et al. 2009). Goodroad and Keeney (1984) found that chemodenitrification was responsible for less than 10% of N₂O flux at thaw in the grassland soil studied, whereas Röver et al. (1998) found minute emissions from freeze-thawed sterilized soils that may have been the result of chemodenitrification (these emissions were however deemed negligible overall). Furthermore, N₂O production in soils via chemodenitrification is generally of importance only at low pH levels and where nitrite (NO₂⁻) concentrations are high (Mørkved et al. 2007), yet N₂O emissions have been observed to occur without the accumulation of nitrite in soils and at a variety of pH values (Müller et al. 2003). Though in certain soils chemodenitrification may be a minor contributor to freeze-thaw emissions at best, efforts are better placed on understanding what factors lead to the promotion of denitrification as it has been shown repeatedly to be the dominant process.

2.3 Mechanisms Driving Heightened Freeze-Thaw N₂O Emissions

2.3.1 A Combination of Physical and Biological Processes

The phenomenon of heightened N₂O emissions from soils subjected to freeze-thaw cycles, as discussed, is well documented for a variety of environments. The processes behind this phenomenon however, present an area of uncertainty. Here, the processes that are likely contributors to the N₂O bursts at thaw have been divided into five main mechanisms (of which some have further subdivisions): 1) The enhancement of microbial activity due to increased

availability of C and N substrates, 2) The physical release of trapped N_2O by a diffusion barrier, 3) The release of N_2O from soil water due to decreases in solubility with increasing temperature, 4) New production of N_2O triggered in surface layers and 5) The temperature sensitivity of N_2O -reductase. These mechanisms present a variety of processes that fall under both physical and biological classification, and it is apparent that these mechanisms act simultaneously and in combination (Goodroad and Keeney 1984; Matzner and Borken 2008). Furthermore, as per a review by Matzner and Borken (2008), it is likely that the relevance of single mechanisms differ between field and laboratory studies, as well as in different environments. Though the processes at work are expected to be the same in different environments, the parameters governing the emissions and their dynamics will differ (Teepe and Ludwig 2004). The results of a study by van Bochove et al. (1996) agree with this, as much larger over-winter and spring thaw N_2O emissions were observed in agricultural fields as compared to forests in the same winter. In addition, the independent importance of each mechanism also changes in time, space and with a diversity of other dynamic parameters, for example soil water content (Matzner and Borken 2008). This is then confounded by the issue that many of the factors influencing thaw-related emissions cannot or have not been quantified on large enough time and space scales to fully understand the mechanisms behind them (Dörsch et al. 2004; Pattey et al. 2007). Evidently explaining the nature of freeze-thaw related N_2O emissions is a complicated problem, and as stated by Wagner-Riddle et al. (2010): “Whether N_2O is produced mostly in the frozen soil and is released during winter or at thawing, or if it is produced once the thawing process starts, has fundamental implication for models attempting to describe N_2O emission at thaw”. The lack of knowledge discussed has hindered the development of models that can adequately describe spring thaw emissions (Pattey et al. 2007). It is for this reason, combined with the complexity and sporadic nature of N_2O

emissions, that more research on the mechanisms to be discussed is needed for a variety of climates and environments (Pattey et al. 2007; Wang et al. 2008). Furthermore, there is a specific need for studies on the quantification of the relative contributions of previously produced and newly produced N₂O to spring thaw emissions.

2.3.2 Microbial Activity Enhanced by Newly Available C and N Substrates

The first mechanisms to be discussed are based upon the idea that soils subjected to freeze-thaw cycles undergo physical and biological changes that increase the availability of organic C and inorganic N previously unavailable to denitrifiers, thus promoting denitrification and N₂O production. Some of the earliest proponents of these mechanisms include Edwards and Killham (1986), Groffman and Tiedje (1989) and Christensen and Christensen (1991), all of which observed heightened rates of denitrification in soils post freeze-thaw (in both field and laboratory studies). Edwards and Killham (1986), who also observed simultaneous gaseous N losses, hypothesized that their observations were likely the result of increased mobility of C sources due to disruption of soil structure, thus making C more readily available to denitrifiers. Groffman and Tiedje (1989) and Christensen and Christensen (1991) presented what have become two of the main mechanisms, hypothesizing that increased denitrification was likely due to increased C and N substrate availability through: a) the reduction of the stability of soil aggregates and b) the killing and lysis of microbial cells. A third mechanism to be discussed, that has been proposed based predominantly on the findings of Tierney et al (2001), is that the death of fine roots (<1 mm) due to freeze-thaw stresses may also lead to increase in substrate availability as the two former mechanisms (Groffman et al. 2006; de Bruijn et al. 2009).

Studies pertaining to increases in availability of C and N substrate have been performed on a number of different soils, and have yielded mixed results. Through the use of ^{15}N labeled NH_4^+ and NO_3^- in a study set out to identify N transformation processes occurring during various stages of a freezing-thawing episode, it was found that upon freezing there was an increase in NH_4^+ and NO_3^- pools which were accompanied by an increase in ^{15}N excess (Müller et al. 2002). Such an increase can only occur if the released material has a much higher ^{15}N enrichment, and was attributed to the proposed mechanisms of aggregate distribution and lysis of microbial cells, re-releasing NH_4^+ and NO_3^- that was fixed or immobilized shortly after fertilization (Müller et al. 2002). Sehy et al. (2004) failed to recreate the phenomenon of freeze-thaw N_2O emissions by adding readily dissolvable organic carbon (DOC) to simulate the release of nutrients via the mechanisms mentioned. This was either due to an underestimation of the amount of C that becomes available upon thaw, or perhaps that the amount of substrates available is less important than how these substrates are distributed locally (Sehy et al. 2004; Mørkved et al. 2006). Ludwig et al. (2004) subjected soils to combinations of freeze-thaw cycles and the addition of labile C, comparing the results to controls, and found that the changes in nutrient availability due to freezing and thawing were rather small. It is possible that the production of N_2O occurs within microsites where microbial biomass is abundant, thus any substrates liberated via the death of a portion of this biomass (in addition to other sources) will rapidly trigger denitrification in the surviving surrounding community, and these substrates are used up before they can be detected (Sehy et al. 2004; Mørkved et al. 2006). Increased amounts of C substrates available may also promote aerobic respiration and thus CO_2 emissions, which would deplete O_2 supplies in microsites creating anaerobic conditions and promoting denitrification (Mørkved et al. 2006). A proposed mechanism that builds on this idea of microsites is to be discussed later.

Other studies have yielded results contrary to the hypothesis of increased substrates in soil subjected to freeze-thaw cycles fuelling denitrification, and thus N₂O production and emission. Goldberg et al. (2010) disregarded increased nutrient supply as a mechanism behind observed freeze-thaw induced increases in N₂O emissions in forest topsoil's based on measurements of solute concentration taken throughout their study. Elliot and Henry (2009) performed a laboratory experiment (on agricultural soils from London, ON) investigating the effect of freeze-thaw damage on extractable-N in soils (from the proposed lysis of cells, destruction of aggregates and death of fine roots). Their results showed no increase (over control samples) in available N for repeated mild and moderate freezing temperatures (0 to -5°C). Increases were observed from repeated FTC where freezing temperatures were -10°C and where freezing rates were very high. The authors caution that these extreme temperature and freezing rates were unrealistic for the soils collected (as per monitored field conditions) and as such an exaggeration of freezing effects has likely occurred. Exaggerations of this nature may be highly prevalent in freeze-thaw related studies as authors subject soils to freezing temperatures that are unrealistic (Henry 2007).

2.3.2.1 Death of Microbes

The death and lysis of microbial cells due to freeze-thaw stresses, as discussed, is one of the main proposed sub-mechanisms responsible for increased substrate availability post freezing-thawing. This mechanism has been widely adopted by authors to explain stimulated N₂O emissions from freeze-thaw cycles (Christensen and Tiedje 1990; Christensen and Christensen 1991; DeLuca et al. 1992; Burton and Beauchamp 1994; Papen and Butterbach-Bahl 1999; Neilsen et al. 2001; Müller et al. 2002; Müller et al. 2003; Sehy et al. 2004; Koponen et al. 2006a; Goldberg et al. 2008). Skogland et al. (1988) found that up to 70% of microbes were

killed by rapid freeze-thaw cycles in soil samples, and that a few hours after the onset of thaw, CO₂ bursts were measured. The authors assumed that “a fraction of the bacteria are killed, and the surviving cells use the liberated C compounds as nutrients”, explaining the burst in respiration, a phenomenon often termed the “partial sterilization effect”. Though measurements for N₂O were not taken, an increase in C substrate would also increase the potential for denitrification. Christensen and Christensen (1991) observed that extractable C, after an initial FTC in soil samples, increased in non-sterilized samples but not in sterilized samples, which coupled with a measured 40% reduction in denitrifier enzyme biomass, indicates that the increase in extractable C originated from the soil microbial mass. Yanai et al. (2007) also observed partial sterilization (the death of a portion of the microbial community) in 5 out of 6 soil samples subjected to freeze-thaw, and of the 5 samples, 3 experienced stimulated N₂O emissions. Herrmann and Witter (2002) observed a “flush” of C after subjecting soil samples to FTC, and determined that 65% of C was of microbial origin (the source of the other 35% was not determined). Furthermore, it was calculated that the microbial biomass C represented only about 5% of the microbial C in the soil, suggesting that the damage to the microbial population was small (Herrmann and Witter 2002). Other studies have shown that subjecting soils to freeze-thaw treatments created increases in mineral N with concurrent increases of ninhydrin reactive-N, indicative of release from microbial biomass (DeLuca et al. 1992; Schimel and Clein 1996). Brooks et al. (1998) observed a sharp decrease in soil microbial biomass N without a concurrent increase in inorganic N pools. They attribute this phenomenon to the release of N from the lysis of microbial cells (due to the subjection of soils to temperature fluctuations under waning snow packs) and hypothesized that the N was then lost rapidly via hydraulic export and gaseous losses (potentially as N₂O). It has also been proposed that the prolonged nature of heightened N₂O

emissions at spring thaw may be due to a slow leak of nutrients as microbial cells do not lyse immediately (Skogland et al. 1988; Schimel and Clein 1996). Conversely, heightened N₂O emissions have been measured in response to FTC without any notable change in microbial biomass (Neilsen et al. 2001; Groffman et al. 2006; Koponen et al. 2006b; Sharma et al. 2006). Nevertheless, as mentioned previously, these mechanisms and their importance are likely to vary spatially and temporally. Therefore based on the findings discussed, it is apparent that the partial sterilization of the denitrifying community is of primary significance in the explanation of stimulated N₂O emissions by freeze-thaw cycles (Yanai et al. 2007).

2.3.2.2 Aggregate Disintegration

The disintegration of aggregates releasing substrates formerly unavailable to microbial biomass is the second mechanism for increased substrate availability post freezing-thawing to discuss. Freezing and thawing has been shown to decrease aggregate stability (Bullock et al. 1988; Oztas and Fayetorbay 2003) and the breakdown of aggregates is the result of forces exerted by the expansion of ice between soil particles, thus breaking particle-to-particle bonds (Bullock et al. 1988). The effect of freezing on aggregate stability varies significantly with soil type and increases with increasing moisture content in a near-linear fashion (Lehrsch et al. 1991; Oztas and Fayetorbay 2003). The effect is lessened at low moisture contents, as ice crystals are able to complete their growth before applying disruptive forces on the soil matrices (Bullock et al. 1988). The idea of aggregate disintegration in response to freeze/thaw is complicated by the fact that aggregates generally increase in stability with the first few FTC, then decrease in stability with successive cycles thereafter (Lehrsch et al. 1991; Lehrsch 1998). In many parts of the world it is very likely, however, that soils will be subjected to many repeated FTC over-winter and in early spring, especially in surface layers that may freeze and thaw daily (Henry

2007; Henry 2008). Furthermore, the stability of aggregates may increase with freezing-thawing in the presence of high concentrations of dissolved salts (Perfect et al. 1990). The main issue with this mechanism is that although it is often proposed, there exists little direct evidence to support claims (Matzner and Borken 2008). Much support of this mechanism comes from studies that have found increases in N₂O emissions and/or denitrification post freeze-thaw that did not correspond with changes in microbial communities (Neilsen et al. 2001; Sharma et al. 2006). The strongest evidence for this mechanism comes from van Bochove et al. (2000b) and is highly referenced. The authors found that C mineralization and denitrification rates were 95% higher in frozen than unfrozen macroaggregates. This effect was 57% higher in smaller macroaggregates (0.25-2 mm) than in large macroaggregates (2-5 mm), attributed to the higher water content measured in the smaller macroaggregates. The increase in denitrification is likely due to an increase in the availability of mineralizable organic C, the likelihood of which is furthered by a strong correlation between increases in denitrification and CO₂ emissions (van Bochove et al. 2000b). Christensen and Christensen (1991) found that whole soil and aggregates subjected to freeze-thaw treatments released significantly more N₂O than unfrozen samples, however the authors identified that a proportion of this was due to release of C from microbial biomass. Much more research is needed on this topic, of specific interest would be determining the amount of substrates liberated via the disintegration of aggregates subjected to repeated FTC, and the magnitude of N₂O emissions this would induce (Wang et al. 2008).

2.3.2.3 Death of Fine Roots

In a Northern hardwood forest Tierney et al. (2001) observed an overwinter fine root (<1 mm in diameter) mortality rate of ~ 14%, and ~ 28% in plots where snow was removed. Roots killed overwinter decompose rapidly (the median rate measured to be 35 days) and can present a

substantial source of C and N to the soil system (Tierney et al. 2001). It has been suggested in papers thereafter that this phenomenon may then present a new supply of substrates to denitrifiers, promoting denitrification and N₂O emissions (Groffman et al. 2006; Maljanen et al. 2007; de Bruijn et al. 2009). This proposition, however, has been met with some challenge. Matzner and Borken (2008), in a review of the findings of Tierney et al. (2001), state that it is unlikely that soil forest C and N pools after thawing will significantly increase due to rapid decomposition of fine roots. Groffman et al. (2006), in a study on the same soils as Tierney et al. (2001), proposed that increased N₂O emissions from soils subjected to snow cover removal were due to the death of fine roots decreasing plant uptake, thus increasing levels of inorganic N in the soil and promoting denitrification and nitrification. The major issue with this suggestion, however, is that no significant increases in denitrification or nitrification were measured (Groffman et al. 2006; Matzner and Borken 2008). Most pertinent to this study, the mortality of fine roots in response to freeze-thaw stresses would only be important in permanently vegetated areas such as forests (as studied by Tierney et al. 2001), and thus would not be significant in agricultural fields (Matzner and Borken 2008).

2.3.3 Physical Release of Trapped N₂O by a Diffusion Barrier

Overwinter production and emission of N₂O, much like spring thaw emission “bursts”, has been observed in a variety of environments (Goodroad and Keeney 1984; Wagner-Riddle et al. 1997; Teepe et al. 2000; van Bochove et al. 2000a; Dörsch et al. 2004; Öquist et al. 2004; Goldberg et al. 2010). Emissions have also been observed at the time of freezing, often as bursts in the same manner as at spring thaw (Röver et al. 1998; Papen and Butterbach-Bahl 1999; Teepe et al. 2000; Teepe et al. 2001). The idea behind this class of mechanisms is that a portion

of N₂O produced overwinter or at freezing may become “trapped” and is then subsequently released at spring thaw.

Bremner et al. (1980), upon observing large N₂O emissions once frozen topsoils thawed in the spring, hypothesized that this occurrence was possibly due to release of N₂O produced and trapped in unfrozen subsoils overwinter. Through the use of argon tracer gas diffused into the soil, it has been shown that ice layers formed at the surface and within the soil profiles can constitute gas-impermeable diffusion barriers (van Bochove et al. 2001). Furthermore, high concentrations of gaseous N₂O accumulating overwinter within the soil atmosphere followed by sharp decreases in concentrations in the spring have been observed in a number of studies (Goodroad and Keeney 1984; Burton and Beauchamp 1994; Heincke and Kaupenjohann 1999; van Bochove et al. 2001; Dörsch et al. 2004; Drewitt and Warland 2007; Maljanen et al. 2007; Wagner-Riddle et al. 2008; Goldberg et al. 2010). This indicates that the overwinter N₂O production rate exceeds the combined rate of diffusion and relocation (Burton and Beauchamp 1994). Unfortunately, efforts to correlate changes in N₂O concentrations in soil air with emissions at the surface have yielded inconsistent findings (Heincke and Kaupenjohann 1999). Drewitt and Warland (2007) and Wagner-Riddle et al. (2008) both observed the buildup of N₂O within the soil atmosphere overwinter, and both observed rapid decreases in soil gaseous N₂O concentrations at the onset of thaw. In the latter case the decrease in soil N₂O concentrations did not correspond with observed peaks in surface N₂O fluxes. It is likely that much of the N₂O trapped within the soil profile was subject to complete denitrification and was further reduced to N₂, as evidenced by an increase in ¹⁵N₂ concentrations at depth (Wagner-Riddle et al. 2008). Conversely, it has also been observed that soil gas concentrations increased rapidly post freezing of the surface layer of soils, followed by an initial burst of N₂O observed

which coincided with the melting of the surface layer and a decrease in soil gaseous N₂O concentrations, hence a physical release, then a subsequent burst thereafter likely the result of increased microbial activity (Teepe and Ludwig 2004; Maljanen et al. 2007; Wagner-Riddle et al. 2010). It has been proposed that the soil profile can be effectively divided into two distinct sections by a frozen layer within, the first being a surface layer, characterized by intense temperature fluctuations and water accumulation during thaw, and the second a freely drained subsurface region (Burton and Beauchamp 1994). Production occurs at depth where, due to temperature buffering, the soil remains unfrozen, and N₂O accumulation occurs underneath the frozen layer within the subsurface region due to the reduced diffusion and hence “sealing effect” (Cates and Keeney 1987; Burton and Beauchamp 1994; Kaiser et al. 1998). It has been suggested this trapped N₂O is then released at thaw coinciding with the destruction of the frozen layer (Burton and Beauchamp 1994). Teepe et al. (2004) found in a lab experiment that decreasing the thawing time of a freeze-thaw cycle slowed the release of N₂O, suggesting that the destruction of a diffusion barrier is an important process. It is then possible that in many circumstances this mechanism contributes to the prolonged heightened N₂O emissions observed at spring thaw, but is not responsible for the intense bursts.

A slight variation of this mechanism has been brought forth, the difference being where the N₂O is being produced. Presented originally as a mechanism leading to overwinter emissions, the production of N₂O may occur in unfrozen microsites (nutrient rich water-films that persist even when the soil is frozen) as opposed to in unfrozen subsoils, and is then trapped (Papen and Butterbach-Bahl 1999; Teepe et al. 2001). Due to interactions with the surface of soil particles, water films are tightly bound to particles and will exist as unfrozen water well below 0°C (Edwards and Cresser 1992). It has been found that 8-20% of soil water remained unfrozen

despite constant soil temperatures of -5°C , and that significant amounts of liquid soil water can exist down to -20°C (Edwards and Cresser 1992; Rivinka et al. 2000). Teepe et al. (2001) and Papen and Butterbach-Bahl (1999) proposed that these water films, which are surrounded by ice, present the ideal location for denitrification to occur. The surrounding ice limits diffusion creating anaerobic conditions, and nutrient availability is high due to occlusion of ions from ice (Edwards and Cresser 1992). Combining this with a supply of nutrients from the proposed mechanism of nutrient release from the stresses of freezing, there is a strong possibility that these water films would be highly enriched with substrates (Koponen and Martikainen 2004). The surrounding ice will also limit diffusion out allowing for the buildup of N_2O , which may then be released as the diffusion barriers are destroyed at spring thaw. Moreover, microbes can remain physiologically active provided unfrozen water remains in the soil profile (Coxson and Parkinson 1987; Rivinka et al. 2000; Schimel and Mikan 2005). Panikov et al. (2006) found that microbial respiration occurred in arctic soils down to -39°C , suggesting a specialized ecological group of microorganisms adapted to extreme conditions and life at sub-zero conditions exists. Since not all microbial species can be cultivated in the laboratory (Panikov et al. 2006), there may also exist strains of denitrifying bacteria that are adapted to extreme conditions and could produce N_2O in supercooled water films. Also, increases in organic C substrates decrease the threshold temperature at which denitrification occurs in sub-zero soils, a likelihood in nutrient rich water films (Dorland and Beauchamp 1991). The production of N_2O is likely to occur in both of the discussed locations (waterfilms and in the unfrozen sub-layer) simultaneously (Teepe et al. 2001), and there has yet to be any study that differentiates N_2O production between them.

2.3.4 Release of N₂O from Soil Water due to Change in Solubility with Temperature

The solubility of N₂O is inversely related to temperature in a non-linear relationship (Weiss and Price 1980). At 0°C the solubility of N₂O is roughly twice the solubility at 19°C, whereas a further increase from 19 to 38°C results in a mere 18% decrease in solubility (Heincke and Kaupenjohann 1999). The storage of N₂O in soil water is at a maximum when soil water is at the freezing point, and an increase in temperature will decrease the solubility of N₂O in water, resulting in degassing (Weeks and McMahon 2007). Goodroad and Keeney (1984) found that soil cores treated with chloroform (to inhibit microbial activity), when warmed from 1 to 10°C, released N₂O that according to their calculations was almost entirely due to decreased N₂O solubility. Burton and Beauchamp (1994) identified that surface N₂O flux events in the early spring periods may be due, in part, to these changes in N₂O solubility associated with soil warming. Van Bochove et al. (200b) noticed an increased ability of agricultural soils to denitrify NO₃⁻ in the surface layers underneath snow-packs and yet concurrent high concentrations of N₂O in the deep soil. They presented the idea of a solubility gradient whereby N₂O is degassed from soil water that is moving downward from the surface and warming along the temperature gradient. Measurements of the amount of N₂O that is present in soil solution overwinter and at spring thaw, as well as the proportion degassing from solution as soils warm presents another area in which more study is required.

2.3.5 Production Triggered in Thawed Surface Layer

This mechanism is similar to the first category of mechanisms discussed in that it is based on the idea of promoting denitrification and the “de novo” production of N₂O as opposed to the release of stored N₂O. As discussed earlier, Burton and Beauchamp (1994) proposed a division of the soil into two distinct regions by an ice layer, of interest here is the surface layer,

characterized by intense temperature fluctuations and water accumulation during thaw. The initial critical condition for the initiation of a major freeze-thaw related flux event appears to be the presence of a shallow, water saturated layer of soil perched atop a layer of frozen soil below (which impedes drainage) (Nyborg et al. 1997). This creates anaerobic conditions (due to high water content reducing oxygen diffusion) and promotes denitrification (Koponen and Martikainen 2004). Wagner-Riddle et al. (2008) performed an experiment using chambers, micrometeorological techniques, soil gas probes, in situ soil columns, and the injection of ^{15}N labeled NO_3^- at two depths. Their findings allowed them to link heightened surface N_2O fluxes at spring thaw to production in the surface layer due to observed higher ^{15}N enrichments of N_2O released in SL treatments ($^{15}\text{NO}_3^-$ added at depth of 0-5 cm) as compared to the DL treatments ($^{15}\text{NO}_3^-$ added at 12 to 17 cm depth). Though ^{15}N enriched N_2O concentrations were measured at depth, they did not correspond to the highest surface fluxes, and therefore the authors conclude that peak N_2O emissions at spring thaw was predominantly newly-produced N_2O within the surface layer, not the release of N_2O trapped beneath frozen layers. Furthermore, studies have shown spring thaw N_2O emissions occur when the surface layer thaws but underlying layers of soil remain frozen, indicating the source to be biological activity in the surface layer (Lemke et al. 1998; Koponen et al. 2006a; Maljanen et al. 2007; Dusenbury et al. 2008). Wagner-Riddle et al. (2010) found that heightened N_2O emissions observed at spring thaw in March were associated with surface processes in the soil as they corresponded to the phase changes from ice to liquid as measured by heat pulse probes at 5 cm depth. Other studies have noted daily fluctuations of N_2O emissions in the spring that corresponded to the daily freezing and thawing of the surface layer of soils with diurnal temperature fluctuations, which again indicates production via denitrification in the surface layer (Dörsch et al. 2004; Holst et al. 2008). It is

quite likely that production triggered in the surface layer is associated with increases in substrates as proposed in the first mechanisms discussed (Holst et al. 2008). Since unfrozen water content increases drastically with soil temperature near -2°C , it is therefore also likely that microbial thaw (increasing microbial production) begins around this temperature, complicating the determination of when spring thaw has begun (Romanovsky and Osterkamp 2000; Schimel and Mikan 2005). It has also been proposed that the new production of N_2O may occur at different times than a physical release as per studies that observed two distinct pulses of N_2O emission in an agricultural soil at spring-thaw (Teepe and Ludwig 2004; Maljanen et al. 2007). In the case of Maljanen et al. (2007) the first, smaller, pulse occurred concurrently with a decrease in soil N_2O concentrations and was attributed to the physical release of trapped N_2O due to the destruction of a diffusion barrier. The second, larger, pulse was likely the result of new production in the surface layer (Maljanen et al. 2007). Teepe and Ludwig (2004) also attributed an initial burst to a physical release due to the rapid nature of the event, both increasing and decreasing abruptly. A second peak followed three days later and decreased gradually, thus indicating increased microbial activity as its source (production was not directly linked to surface processes however). These conclusions were however based primarily on circumstantial evidence, such as the timing of events, as neither study quantified either the change in soil N_2O content or microbial production. As discussed earlier, to improve our modeling capabilities of N_2O such that models can predict spring thaw N_2O emissions, a better understanding of the relative contribution of old vs. new production of N_2O is needed (Wagner-Riddle et al. 2010).

2.3.6 Temperature Sensitivity of N_2O -reductase

The N_2O reducing enzyme N_2O -reductase may be more susceptible to low temperatures than enzymes involved in production of N_2O (NO_3^- , NO_2^- , and NO reductase), which would

reduce the N_2/N_2O product ratio and possibly increase N_2O emissions at low temperatures (Holtan-Hartwig et al. 2002). It has been found that the N_2/N_2O ratio increased markedly with increased soil temperature post thaw, indicative of the expression of N_2O -reductase increasing with temperature. Müller et al. (2003) noted a lag time between increasing soil temperatures, water content and N_2 emissions (increasing water content promoting complete denitrification to N_2) suggesting that it may take some time for the *de novo* synthesis of N_2O denitrifying enzymes to occur. However, the *de novo* synthesis of the reductase enzyme, though it may occur, is not a prerequisite for heightened N_2O emissions (Teepe et al. 2001). Other authors have also attributed their observations to the inhibition of N_2O -reductase with temperature (Goldberg et al. 2010), however there is a lack of direct evidence. Yanai et al. (2007) found that in only one of six tested soil samples repeated FTC inhibited the reduction of N_2O , which was likely associated with the temperature sensitivity of N_2O -reductase. Additionally, Morkved et al. (2006) found no alteration in the $N_2O/(N_2 + N_2O)$ ratio as a result of FTC, and observed no selective damage to N_2O -reductase.

2.4 Freeze-Thaw Induced N_2O Emissions as Affected by Soil Conditions

2.4.1 Freezing Temperature (and Insulating Effects)

The effect of freezing temperature on N_2O emissions appears to be rather strong, whereby soils frozen at -15°C were shown to have released 6.7 to 12 times more N_2O upon thaw than soils frozen at -1.5°C (Koponen and Martikainen 2004). Neilsen et al. (2001) performed a study on hardwood forest soils where soils subjected to a harsh -13°C freeze yielded increased N_2O emissions, however a mild freeze of -3°C did not. In an analysis of measurements over several field seasons in agricultural soils, Wagner-Riddle et al. (2007) found a linear relationship between accumulated degree-hours $<0^\circ\text{C}$ at 5 cm soil depth and accumulated N_2O emissions

overwinter, such that winters with a higher degree of freezing yielded greater N₂O emissions. Based on such findings, it has been cautioned that laboratory freeze-thaw experiments that are subjecting soils to temperatures that are far more extreme than they would be under real conditions may be leading to the exaggeration of results (Henry 2007). Therefore, factors such as insulating effects of snow cover and litter layer should be taken into account as well as climatic characteristics and timing of sample collection when performing laboratory experiments, such that soils are subjected to temperatures that are realistic based on their environments (Henry 2007). Furthermore, Hu et al. (2006) found that in a comparison with traditional omni-directional (from all sides) freezing methods, their uni-directional (more realistic to field conditions) method yielded much lower N₂O emissions events that lasted for much shorter durations. The authors attribute the higher emissions in the omni-directional method to more severe and rapid freezing. In terms of insulating effects, it is known that the removal of snow leads to colder soil temperatures as it removes the insulating effects a snow-pack presents (Hardy et al. 2001). Therefore, the removal of snow from plots has shown to significantly increase N₂O emissions both overwinter and at spring thaw from soils as compared to plots where snow-packs were left intact (Groffman et al. 2006; Maljanen et al. 2007; Wagner-Riddle et al. 2007). This is of noteworthy importance as climate change is likely to reduce the accumulation of snow packs in many regions, thus reducing the insulating effect and subjecting soils to more severe and deeper freezing (Henry 2007; Henry 2008). Moreover, the removal of a litter-layer that accompanies conventional agricultural practices has been shown to increase N₂O emissions due in part to the removal of the insulating effect of the litter-layer itself as well as its ability to trap snow (Hardy et al. 2001). The reason for the increases in N₂O emissions with more severe freezing temperatures is hypothesized to be associated with the mechanism of increased substrate

availability, as more severe temperatures will put greater stresses on microbial communities and allow for a greater buildup of ice to instigate aggregate disintegration (Koponen and Martikainen 2004; Maljanen et al. 2007; Goldberg et al. 2008).

2.4.2 Freezing Duration

The effect of freezing duration has also been found to have a considerable effect on N₂O emissions upon thaw. Teepe et al. (2004) noted that the freezing of three different agricultural soils for one day yielded negligible N₂O emissions whereas a notable increase in emission occurred if samples were frozen for 2.4 days. Freezing for 11.5 days provided a further increase of emissions of 2 to 5 times. Similarly, Sigurindy et al. (2009) observed 9 freeze-thaw cycles in an agricultural site in Central New York, 7 of which were accompanied by N₂O emission bursts. The two FTC that did not cause spikes in N₂O emissions were argued to have had too brief of freezing to cause heightened emissions, whereas the two largest emission events were associated with the initial event (after winter-long freeze) and the second after a long intermittent freezing event (Sigurindy et al. 2009). Another study found that forest soils subjected to a short freezing period (3 days) yielded cumulative N₂O emissions that were up to 22% less than long freezing period (11 days) (Teepe and Ludwig 2004). Additionally, Papen and Butterbach-Bahl (1999) studied overwinter and spring thaw N₂O emissions for two consecutive winters (1995 and 1996) finding that in the spring of 1996 much larger “flushes” of N₂O emissions occurred as compared to 1995, attributed to a much longer frost period during the winter of 1996. A possible explanation for this phenomenon is that the release of nutrients increases with increasing time of freezing (Butterbach-Bahl et al. 2001; Teepe et al. 2004). There is, however, lack of information concerning the effect of freezing duration on microbial populations, aggregate destruction and fine root mortality as most studies are performed in laboratories with brief freezing periods.

Another possible explanation is that an increased division of facultative denitrifying bacteria switch to anaerobic processes over time within the anaerobic conditions of the frozen soil, allowing for denitrification to occur in frozen soils and a buildup of N₂O in the soil atmosphere which would be released upon thaw (Teepe et al. 2004). Furthermore, since denitrification occurs in organisms that are primarily aerobic respirers but which have the ability to shift to denitrification when O₂ is limiting (Schimel and Gulledge 1998), the slow increase of O₂ concentrations in frozen soil might create a lag time before anaerobic conditions are present and the switch to denitrification occurs.

2.4.3 Successive Freeze-thaw Cycles

Successive freeze-thaw cycles and their effects on N₂O emissions is an area that has yielded a variety of results. Coxson and Parkinson (1987) observed 60+ FTC in the topsoil of a forest floor in Alberta within a period from December to March. Similarly, soil in many areas globally are subjected to several freeze-thaw events, and current climate change predictions, which as discussed may reduce snow cover due to predicted warmer and drier winters in many regions, will then also subject soils in many areas to an increased number of freeze-thaw cycles (Henry 2007; Henry 2008). It is for this reason that understanding the effects of successive FTC on soil physical properties, nutrients, and greenhouse gas emission dynamics is of utmost importance (Henry 2007). Röver et al. (1998) found in field and associated laboratory experiments that N₂O emissions increased moderately after an initial soil freeze-thaw event, and the largest spike in emissions was observed after the second FTC. Conversely, it has been found in other studies that the initial freeze-thaw event produced the greatest emissions, which then decreased steadily with each successive cycle thereafter (Sigurindy et al. 2009). Chen et al. (1995) found in a lab experiment on agricultural soils that three successive freeze-thaw cycles

resulted in increased “pulses” of N₂O emissions. A large decrease in the magnitude of N₂O fluxes occurred from the first FTC to the second (0.88 kg day⁻¹ h⁻¹ and 0.48 kg day⁻¹ h⁻¹ respectively), the difference between the second and third however was much less pronounced (the third being 0.42 kg day⁻¹ h⁻¹) (Chen et al. 1995). In agreement with these observations it was found in two Finnish agricultural soils that peak N₂O emissions occurred after either the first or second freeze-thaw cycles, and decreased to a fairly constant level with further cycles (Koponen et al. 2006a). Nyborg et al. (1997) found no increase in N₂O emissions after the second FTC in the spring in their field study and hypothesized the existence of a “recovery” period between large events. Results of an experiment by Sigurindy et al. (2009) agree with this hypothesis as of 9 observed FTC in an agricultural soil, the two of greatest magnitude corresponded to the first FTC and the second to a FTC with the longest intermittent freezing duration between events. In contrast to the findings above, it has been found that successive FTC did not result in decreased N₂O emissions (Teepe et al. 2004). The reason behind decreases in N₂O emissions with successive FTC, a common observation, has been hypothesized to be associated with a decreasing supply of nutrients (Koponen et al. 2006b). In agreement with this, Hermann and Witter (2002) found that “flushes” of C that occurred in soils post freezing-thawing were the highest in the first FTC and decreased significantly after the fourth cycle. It therefore appears that the pool of substrates susceptible to freeze-thaw is limited (Herrmann and Witter 2002). Furthermore, the idea of a necessary recovery period between large emission events may then be associated with the recovery of the microbial community, allowing for subsequent killing and reclaiming of nutrients as discussed (Koponen et al. 2006b).

2.4.4 Soil Water Content

High water content of thawing soil will increase the anaerobic volume, enhancing denitrification, and thus the water content of soils, having a drastic effect on N₂O emissions (Mørkved et al. 2006). In a laboratory experiment, intact soil samples moistened to 65% water content and subjected to FTC were observed to yield cumulative emissions 14 times greater than the samples at field moisture content (Koponen and Martikainen 2004). In a field study, Müller et al. (2003) measured N₂O emissions from unfertilized soils at both a “wet” and “dry” site, the N₂O fluxes upon thaw being much larger from the saturated “wet” site. Teepe et al. (2004) found that N₂O emissions from FTC in agricultural soils decreased with decreasing moisture content, the greatest emissions occurring at a water-filled pore space (WFPS) of 64%. The relationship between moisture content and N₂O emissions appears to hold true up to a threshold water content (Teepe et al. 2004; Sigurindy et al. 2009). Beyond a WFPS of 70%-76% N₂O emissions have been observed to decrease drastically (Teepe et al. 2004; Sigurindy et al. 2009) and optimum water content for N₂O emissions has been suggested to be between 40%-70% WFPS (Sigurindy et al. 2009). The drop off in N₂O emissions above a threshold water content is related to an increase in the N₂/N₂O ratio, hence increasing anaerobiosis to a point which promotes the complete denitrification of N₂O to N₂ (Davidson 1991; Teepe et al. 2004). Though the effect of water content on freeze-thaw N₂O emissions is pronounced, it has been found that the effects of freezing temperature and duration seem to over-ride that of water content (Hu et al. 2006).

2.4.5 Soil Texture

The effect of soil texture on freeze-thaw related N₂O emissions is an area that has had little direct study. Freeze-thaw effects on soil physical properties are a function of many factors

such as: rate of freezing, freezing temperature, soil water content, soil bulk density, number of FTC, etc. (Herrmann and Witter 2002). Therefore, though many freeze-thaw N₂O studies have been performed on a variety of soils, the highly different conditions these soils were subjected to in prior experiments makes it difficult to compare results and attribute differences in findings specifically to changes in soil structure as related to soil texture. Nevertheless, a study by Teepe et al. (2004) set out to directly study the effect of soil texture on freeze-thaw N₂O emissions directly. By testing three different soil textures, loamy, sandy and silty, they found minor differences in N₂O emissions between the soil types after subjecting them to FTC. Their results indicated that soil texture had a small influence on N₂O emissions, notably after small freezing durations (< 3 days) where the sandy soil had the highest emissions likely due to a lower water content than the soils with more clay content, therefore taking less time for the soil to freeze thoroughly (Teepe et al. 2004).

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CHAPTER 3: Comparison of Simultaneous Soil Profile N₂O Concentration and Surface N₂O Flux Measurements Overwinter and At Spring Thaw In An Agricultural Soil

3.1 Introduction

Atmospheric concentrations of nitrous oxide, an atmospheric gas both responsible for contributing to the greenhouse effect and the destruction of atmospheric ozone, have increased by 15% since the industrial era and are currently increasing linearly at a rate of 0.25% per year (IPCC 2007; Ravishankara et al. 2009). As such, global research efforts have been made to identify the sources of N₂O production, and it has been found that up to 70% of global annual N₂O emission are derived from soils, in turn making agriculture the largest anthropogenic source of N₂O emissions (Mosier 1994). The production of N₂O in soils occurs via the microbial processes of nitrification and denitrification (Firestone and Davidson 1989), and it has been found that subjecting soils to freeze-thaw cycles increases N₂O losses such that up to 70% of the annual N₂O flux from agricultural soils in cold climates can be emitted at spring-thaw (Wagner-Riddle et al. 1997). The mechanisms driving these freeze-thaw related N₂O emissions are poorly understood, and of fundamental importance is determining whether the majority of N₂O emissions are produced prior to spring thaw and then released, or if they are produced at the onset of spring thaw (Wagner-Riddle et al. 2010).

To date studies have not quantified the relative magnitudes of previously and newly produced N₂O at thaw. Such quantification would have a fundamental implication for models attempting to describe N₂O emission as spring thaw, as a lack of knowledge has hindered the development of models that can adequately describe spring thaw emissions (Pattey et al. 2007; Wagner-Riddle et al. 2010). Prior research has compared surface N₂O fluxes to soil N₂O concentrations (Maljanen et al. 2007; Wagner-Riddle et al. 2008; Yanai et

al. 2011), however much of this research is focused on the effects of snow cover on N₂O emissions, and a comparison of changes in soil N₂O content to independently measured surface N₂O emissions has not been made. Furthermore, the majority of prior research relies on the closed-chamber technique to measure N₂O surface emission rates, a technique that lacks the temporal and spatial resolution necessary to follow N₂O emission events (Pattey et al. 2007; Yanai et al. 2011). The objective of this study was to estimate the content of N₂O within the soil profile, and to compare changes in soil N₂O content to N₂O surface fluxes at spring thaw measured using the micrometeorological flux-gradient technique. Through this comparison the goal was to estimate the relative proportion of N₂O emissions at spring thaw from physical release (old production) and new production.

3.2 Materials and Methods

3.2.1 Field Site

Measurements for this study were carried out at the Elora Research Station (latitude 43°39'N longitude 80°25'W, elevation 376m) in Centre Wellington, ON from November 2010 through May 2011, where the study area consisted of one 1.5 ha plot (within a 32 ha area) under a corn-soybean-winter wheat rotation. Within this plot the harvest of soybeans and planting of winter wheat occurred in October 2010, preceded by corn in 2008 and 2009. The study plot was managed using conventional practices, involving spring or fall plowing and N-fertilization at planting according to provincial guidelines (no fertilizer was applied at soybean planting in spring 2010), and was one of four plots used in a larger study focused on N₂O emissions from differing agricultural treatments. The region in which the study site is found has an average annual air temperature of 6.7°C, an average annual precipitation of 908 mm (765 mm rain, 159 mm snow), and over the period of November to April averages 148 d with minimum air

temperature <0°C and 68 d with snow cover >5 cm (Environment Canada 2012). The soil at this site is classified as an imperfectly drained Guelph silt loam (29% sand 52% silt, 19% clay) with an average pH of 7.6 (water) and a carbon content of roughly 30 g C kg⁻¹ within the top 10 cm. Further descriptions of the research site and crop management practices can be found in Wagner-Riddle et al. (2007) and Jayasundara et al. (2007).

3.2.2 Surface N₂O Flux Measurements

Vertical surface N₂O fluxes (F_{N₂O}) were measured using the micrometeorological flux-gradient methodology Wagner-Riddle et al. (1996):

$$F_{N_2O} = \frac{u_* k \Delta C}{\left[\ln\left(\frac{z_2 - d}{z_1 - d}\right) - \psi_{h_2} + \psi_{h_1} \right]} \quad [1]$$

where u^* is the friction velocity, k is the von Karman constant (= 0.4), ΔC is the difference in nitrous oxide concentrations between heights z_2 and z_1 (obtained using a tunable diode laser trace gas analyzer; TGA 100, Campbell Scientific, Logan, UT), d is the displacement height, and ψ_{h_1} and ψ_{h_2} are integrated Monin-Obukhov similarity functions for heat for sampling heights z_1 and z_2 respectively. These functions were calculated using the stability parameter $(z-d)/L$, where L is the Obukhov length, according to empirical expressions derived from Dyer and Hicks (1970) and Paulson (1970), as reported in Wagner-Riddle et al. (1996). Hourly friction velocity and sensible heat flux (needed to calculate the Obukhov length and to estimate the stability functions), were calculated using a sonic anemometer (CSAT3, Campbell Scientific, Logan, UT, USA). A tower with four cup anemometers (F460, Climatronics Corp., Newton, PA, USA) was used to record hourly wind speed profiles. Data from both sonic and cup anemometers were used in the logarithmic wind profile equation to solve for d and roughness length (z_0). Mean d and z_0 were

then calculated for specific periods, taking into account changes in surface conditions over the study period. These means were directly used to calculate flux, and to estimate u^* with measured wind speeds in the logarithmic wind profile equation. The latter calculation was necessary when the sonic anemometer did not yield data, due to rainy or foggy conditions). For those periods, stability corrections in the wind profile equation were calculated using an estimate of sensible heat flux based on measured net radiation (CNR1, Kipp and Zonen, Campbell Scientific, Edmonton, AB). N_2O fluxes were calculated as half-hourly and daily averages.

3.2.3 Soil Profile Measurements

Profile measurements were taken in four locations within the study area, consisting of probes installed above and below the soil surface. Probes were installed November 10th, 2010 and removed May 1st, 2011.

3.2.3.1 Temperature and Water Content

Soil temperature and soil water content were measured as half hourly averages in profiles at four locations within the 1.5 ha plot. At each location soil thermocouples (TC; copper-constantan) and soil water content reflectometers (WCR; CS616, Campbell Scientific (Edmonton, AB) were installed horizontally at depths of 5, 10 and 15 cm. The manufacturer's calibration curve was used to determine the volumetric water content from the WCR. Heat Pulse Probes (HPP) were installed at four locations at a depth of 2 cm for a near surface measurement of soil temperature. Additionally, data from WCR and TC installed as part of another study were made available, where the WCR were installed vertically from 10-40 cm, 40-70 cm, and 70-100 cm, and the TC were installed at depths of 25 cm, 55 cm and 80 cm. Surface temperature was determined using an infra-red thermometer installed 1.5 m above the soil surface at each location.

3.2.3.2 Soil Gas Concentration Measurements

Soil gases were sampled via diffusive equilibrium samplers which consisted of silicone tubing (roughly 20 cm long and 1 cm in diameter, permeable to gas but not liquid) sealed at one end, and attached at the other to polyurethane tubing (3 mm outer diameter, 1.8 mm inner diameter) long enough to be run to the surface and attached to a stopcock. Six soil gas probes were installed at each location, for a total of 24 probes. Two probes were installed at 5 cm in each location, as well as one probe at each depth of 10, 15, 30 and 60 cm. The probes were installed by digging an 80 cm deep pit at each location, drilling holes in the wall of the pit of slightly larger diameter and length than the probes, and then inserting them. The drill holes were plugged with bentonite clay, the polyurethane tubing with stopcocks was run to the surface and tied to a yardstick, and the pits were refilled. TC and WCR were installed within the walls of the same pits at each location. For more information concerning probe construction and installation refer to Kammann et al. (2001).

Soil gas samples were collected weekly over winter, starting November 24th, 2010 and with increasing frequency approaching the main thaw event, up to daily sampling during spring thaw in March 2011. Sampling was performed using 10 mL syringes with stopcocks by drawing all of the air within each probe (6 mL samples on average). After a sample was drawn from a probe, the stopcock on the syringe was closed, the probe was refilled with ambient air and then its stopcock was closed. The new (ambient) air within the probe would diffusively re-equilibrate with the soil atmosphere through the silicone membrane between samplings. After all probes were sampled the 6 mL samples in the syringes were taken to a trailer adjacent to the sample plot and promptly transferred to 3 mL vacutainers (Labco Exetainer, High Wycombe, UK). Once in vacutainers the samples were shipped to the Department of Soil Science at the University of

Manitoba where they were analyzed for N₂O concentrations using a gas chromatograph (GC)(Varian GP3800; Varian Canada, Mississauga, ON). For more information on gas analysis refer to Tenuta et al. (2010).

3.2.4 Soil Coring and Sampling

Soil cores were collected monthly over-winter and bi-weekly approaching thaw using a hydraulic soil core extractor. Nine samples were taken from the study plot at each sampling, and were sub divided into 0-15 cm and 15-30 cm samples. For all samples, bulk density and gravimetric water content were measured after bringing cores to room temperature, and were averaged. Hence, gravimetric water content included liquid water present in cores while frozen, as well as ice which had been converted to liquid water.

3.2.5 Supporting Data

Snow depth on the ground was measured at several points at each location with a ruler each time soil gas was sampled. Half-hourly net radiation was measured within the study plot using a Kipp and Zonen CNR-1 net radiometer (Campbell Scientific, Edmonton, AB). Air temperature was obtained from the Elora Environment Canada automated reference climate station located 100 m from the study plot.

3.2.6 Soil N₂O Content Calculations

3.2.6.1 Profile Breakdown

To calculate the N₂O content within the soil profile on a per location basis, first the soil profile had to be broken down into layers for which the N₂O concentrations measured by the soil gas probes would be representative. Next, soil water content (from WCR) and soil temperature (from TC) estimates were needed for each layer. Finally, from soil cores, bulk density and

gravimetric water content were also needed. The division of the layers, and the measurements associated to these layers are presented in Table 3.1. At 70 cm the soil profile became very rich in clay, and as such there was likely little N₂O stored found below that depth due to decreased pore space. Furthermore, N₂O storage below 70cm is unlikely to contribute to surface emissions. Calculations were therefore done to a depth of 70 cm. Since coring samples were taken to a depth of 30 cm, and samples were divided into 0-15 cm and 15-30 cm; values from the 15-30 cm were assumed to be representative of lower layers. A comparison of bulk density values from this study and values obtained from soil cores taken in 2009 at the study site (unpublished data) show this assumption to be reasonable. Average bulk density at 15-30 cm over this study period was found to be 1.29 g cm⁻³, while the 2009 cores yielded a value of 1.31 g cm⁻³, and the average bulk density from 30-80 cm was found to be 1.21 g cm⁻³.

Table 3.1 Breakdown of measurements used for %AFPS calculations by layer and instrument depth. %GWC_{core} and BD_{core} are gravimetric water content and bulk density respectively, obtained from soil core sampling.

Layer	N ₂ O Concentration	Soil Temperature	Soil Water Content	%GWC _{core} and BD _{core}
	Soil Gas Probe	TC	WCR	Soil Cores
0-7.5 cm	Avg. of two at 5 cm	5 cm	5 cm	0 – 15 cm
7.5 - 12.5 cm	10 cm	10 cm	10 cm	0 – 15 cm
12.5 - 22.5 cm	15 cm	15 cm	15 cm	15 – 30 cm
22.5 - 45 cm	30 cm	25 cm	10-40 cm	15 – 30 cm
45 - 70 cm	60 cm	55 cm	40-70cm	15 – 30 cm

3.2.6.2 Air Filled Porosity

An estimate of air filled porosity (V_{air}) was needed to calculate soil N_2O content in each layer. This required an estimate of the constituents of the soil profile (solids, ice, liquid water and air) on a volumetric basis. To determine the proportion of the soil occupied by solids (soil particles) the following was used:

$$V_{\text{sol}} = 100 \times \frac{\rho_{\text{bulk}}}{\rho_{\text{part}}} \quad [2]$$

where V_{sol} indicates the portion of the soil occupied by soil particles on a percent by volume basis, ρ_{bulk} is bulk density in g cm^{-3} , and ρ_{part} is particle density, a value of 2.65 g cm^{-3} (quartz) was used;

Next, to calculate the fraction of the soil profile occupied by ice, first percent gravimetric moisture content from core samples ($G_{\text{H}_2\text{O}}$) had to be converted to a volumetric measure ($V_{\text{H}_2\text{O}}$) using:

$$V_{\text{H}_2\text{O}} = G_{\text{H}_2\text{O}} \left(\frac{\rho_{\text{bulk}}}{\rho_{\text{water}}} \right) \quad [3]$$

where ρ_{water} is the density of water, a value of 0.9998 g cm^{-3} (for $T=0^\circ\text{C}$) was used, and $V_{\text{H}_2\text{O}}$ is the percent volumetric water content comprised of all water in the core samples (liquid water and ice converted to liquid water) .

Then, percent ice by volume (V_{ice}) was calculated by finding the difference between $V_{\text{H}_2\text{O}}$ and the volumetric liquid water content from the WCR (V_{liq}), both of which were on a liquid water basis, and converting this difference back to ice:

$$V_{\text{ice}} = (V_{\text{H}_2\text{O}} - V_{\text{liq}}) \frac{\rho_{\text{water}}}{\rho_{\text{ice}}} \quad [4]$$

where ρ_{ice} is the density of ice, and a value of 0.9167 g cm^{-3} (for $T=0^\circ\text{C}$) was used.

Finally, the percent air filled pore space (V_{air}) was calculated by finding the remaining volume, not occupied by solids (V_{sol}), liquid water (V_{liq}) or ice (V_{ice}):

$$V_{air} = 100 - V_{sol} - V_{liq} - V_{ice} \quad [5]$$

The above calculations were carried out for each gas sampling time. Since V_{liq} was an automated measurement (from water content reflectometers) taken as half-hourly averages, the value for the half-hour in which gas sampling occurred was used. Conversely, G_{H_2O} and ρ_{bulk} were measurements based on physically sampled soil cores. As such, G_{H_2O} and ρ_{bulk} were assumed to have changed linearly between soil core samplings, and values of G_{H_2O} and ρ_{bulk} from these linear trends were used for each gas sampling time.

3.2.6.3 N₂O Content

N₂O content was calculated on a per m² basis, where total N₂O content was the sum of gaseous N₂O content and aqueous N₂O content. To calculate gaseous N₂O content (mg N₂O m⁻²) the following was used:

$$\text{Gaseous N}_2\text{O Content} = [\text{N}_2\text{O}] \times \text{LayerThickness} \times \text{UnitArea} \times \left(\frac{\%V_{air}}{100} \right) \quad [6]$$

where $[\text{N}_2\text{O}]$ is N₂O concentration in mg N₂O m⁻³, layer thickness is in m and unit area is 1 m².

Henry's Law modified for closed systems was used to calculate aqueous N₂O content, as Henry's Law does not directly apply to closed vessels where volume of gas dissolved in liquid is independent of partial pressure (Moraghan and Buresh 1977; Heincke and Kaupenjohann 1999):

$$M_{aq} = M_{gas} \beta \frac{V_{liq}}{V_{air}} \quad [7]$$

where M_{aq} is mass of N_2O dissolved ($g\ m^{-2}$), M_{gas} is mass of N_2O in the soil atmosphere ($g\ m^{-2}$), and β is solubility of N_2O ($m^2\ N_2O\ m^{-2}\ H_2O$).

The solubility of N_2O is dependent on temperature, and this relationship can be described by the following equations (Weiss and Price 1980):

$$\ln(K_o) = A1 + A2 \left(\frac{100}{T} \right) + A3 \ln \left(\frac{T}{100} \right) \quad [8]$$

$$\beta = K_o V^+ \quad [9]$$

where K_o is a solubility coefficient in $mol\ L^{-1}$ (using atmospheric pressure, 101325 Pa), β is solubility as per equation [7], V^+ is the molar volume (the volume of one mole of pure real gas at STP, $24.4\ L\ mol^{-1}$ for N_2O), T is temperature (K), and $A1$, $A2$ and $A3$ are constants equal to -62.7062, 97.3066 and 24.1406, respectively (Weiss and Price 1980).

3.3 Results

3.3.1 Environmental Conditions Winter 2010/2011

Conditions at the beginning of sampling in November 2010 were cool with soil temperatures between 1 - 8°C and air temperatures averaging 5°C. In December 2010, conditions became much colder and persisted through January 2011, with decreasing trends in air and soil temperature, and soil liquid water content (Figure 3.1). January 1st was an unseasonably warm day reaching 10°C, causing significant melting of the snow pack, and a spike in 5 cm soil water content (Figure 3.1a). In February cold conditions continued and the snow pack built to a peak of 32.5 cm by February 7th. Mid-month, a warm period occurred resulting in snow melt, however conditions following this period remained cold, with air temperatures persistently below 0°C through the end of February, and a snow pack of < 10 cm depth.

In March 2011 conditions began to warm, with frequent air temperature highs of 0°C to 5°C. By mid-month, air temperatures peaked above 10°C, surface temperature reached 8.3°C, and soil liquid water content increased sharply (Figure 3.1a, b). This was followed by air temperatures constantly below -2°C, and surface temperatures below -4°C. By the end of March the air temperature returned to above 0°C and through April daily highs were all above 0°C. On April 1st the snow pack had disappeared, surface temperature rose to 6.2°C and soil temperatures began to exhibit diurnal fluctuations with peaks >0°C.

Figure 3.1 Snow depth, 5 cm soil water content, 5 cm soil temperature, surface temperature and daily average N_2O surface flux over the study period, from November 2010 to May 2011. (a) snow depth, dashed line; soil water content, solid line (b) soil temperature, solid line; surface temperature, dashed line

3.3.2 N₂O Surface Flux and Soil N₂O Concentrations

From January 2011 through until the end of February 2011 daily average N₂O surface fluxes were mostly between 0 and 7 ng N₂O m⁻² s⁻¹ (Figure 3.1c). Values in the early part of March 2011 followed the same pattern, however after mid March, two peaks or “heightened emission events” occurred, the first event with a peak rate of 17.7 ng N₂O m⁻² s⁻¹ on day 76, and the second event reaching a peak daily average rate of 25.1 ng N₂O m⁻² s⁻¹ day 89 (Figure 3.1c).

Soil N₂O concentrations were low in the early part of the sampling period, with background values below 1 ppm at all depths until early January 2011. Through February a slight increase in N₂O concentrations within the soil profile occurred, and by day 40 average concentrations at each depth were between roughly 2 to 6 ppm. On day 50, N₂O concentrations throughout the soil profile began increasing, and this trend continued well through mid March (Figure 3.2). On day 69 the average 5 cm N₂O concentration reached a maximum of 289 ppm (Figure 3.2a). Peak average concentrations occurred slightly later at deeper depths, the 10 cm maximum of 131 ppm occurred on day 74, as did the 15 cm maximum of 33 ppm and the 60 cm maximum of 19 ppm (Figure 3.2b, c, e). The 30 cm average concentration data showed a slightly different trend, with a peak of 22 ppm occurring even later on day 77 (Figure 3.2d). After reaching peak values, the 5 cm N₂O concentrations began to decrease rapidly, reaching 2.5 ppm by day 80 with a slow decrease afterwards to below 1 ppm (Figure 3.2a). N₂O concentrations at 10 cm followed a similar pattern, though decreases were slower, decreasing rapidly after day 74 to 10.8 ppm by day 80, then decreasing slowly to 0.7 ppm by day 100 (Figure 3.2b). The 15 cm N₂O concentration also decreased rapidly after day 74, then decreased slowly to background levels by day 105 (Figure 3.2c). Soil N₂O concentrations at 30 and 60 cm took the longest to return to background value. At 30 cm, decreases in N₂O concentration began rapidly after day

77, then slowly decreased to background levels by day 108 (Figure 3.2d), while at 60 cm decreases occurred slowly (with large fluctuations) after day 74, reaching background levels by day 108 (Figure 3.2e).

Figure 3.2 Soil N_2O concentrations from diffusive samplers at multiple depths over the study period, from November 2010 to May 2011. (a) 5 cm depth (b) 10 cm depth (c) 15 cm depth (d) 30 cm depth (e) 60 cm depth. Error bars represent the standard error of the mean. Please note variable scaling.

3.3.3 Soil N₂O Content

In the following sections soil N₂O content will be discussed, where the terms *total* soil N₂O content is the sum of both gaseous and aqueous content, and *whole profile* N₂O content refers to content summed from 0 to 70 cm. Unless otherwise specified it is to be assumed that mention of N₂O content without either of the aforementioned qualifiers refers to whole profile total soil N₂O content.

In early January whole profile total soil N₂O content was low, at a value of 0.58 mg N₂O m⁻² on day 4, where roughly 60% of this N₂O was found between 22.5 and 70 cm depth. Through January and into mid February N₂O content increased slowly, reaching 2.36 mg N₂O m⁻² on day 50. Shortly after day 50, N₂O content began to increase more rapidly, reaching a maximum of 25.09 mg N₂O m⁻² by day 71 (Figure 3.3). At this time whole profile gaseous and aqueous N₂O content were 10.92 mg N₂O m⁻² and 14.17 mg N₂O m⁻² respectively. Furthermore, at this time 56% of total whole profile N₂O content was in the 0-7.5 cm layer, 15% in the 7.5-12.5 cm layers, 7% in the 12.5-22.5 cm layer, 11.0% in the 22.5-45 cm layer and 12% in the 45-70 cm layer. Mid-March the greatest decreases in N₂O content occurred for total, gaseous and aqueous N₂O content, where total N₂O content for the profile was reduced to 8.5 mg N₂O m⁻² (Figure 3.3) by day 80, and 3% of this content existed in the 0-7.5 cm layer, and 49% existed in the 45-70 cm layers. This rapid decrease and change in N₂O distribution reflects the decreases in N₂O concentrations measured in the surface layers. After day 79, whole profile decreases in N₂O content occurred more slowly, reflecting the slow decreases seen in the deeper layers, with total N₂O content for the whole profile reaching background values between days 105 and 108.

Figure 3.3 Whole profile gaseous, aqueous and total soil N_2O content vs. time, from January to April, 2012

3.3.4 Dynamics of N₂O Surface Flux, Soil Conditions and Soil N₂O Content

From soil N₂O content data it was clear that accumulation occurred after the warm period in February, while depletion began rapidly after a period of warming mid-March coinciding with increasing N₂O surface fluxes. As such, to determine what triggered the buildup of N₂O soil, conditions in mid-February were examined, and to determine the relationship between surface N₂O fluxes and change in soil N₂O content, conditions from mid-March through to early April were considered. In the latter, two peaks in N₂O surface emissions (termed “events” here) from days 71-80 and days 80-92, respectively, were examined in detail.

During the warm period in mid-February air temperatures fluctuated between 4°C and 7°C, returning below 0°C in the early morning of day 50 (Figure 3.4). The snow pack ablated completely by day 50 and a sharp increase in soil liquid water content at 5 cm occurred, with soil temperature at ~0.2°C and surface temperatures consistently between 0 and 2°C (Figure 3.4 a, b). Conditions following day 50 remained cold, air temperatures were persistently below 0°C through the end of February, soil liquid water content decreased to values similar to prior to the warm period, and the snow pack depth increased to 5 cm. From the onset of the warm conditions, total N₂O content in the soil began to increase slowly, and shortly after the return of cold conditions (day 50) N₂O content began to increase more rapidly (Figure 3.4). Throughout this period, while soil N₂O content began to increase, the N₂O surface flux remained small, with the majority of daily averages values less than 5 ng N₂O m⁻² between days 40 and 60 (Figure 3.4 c).

In mid-March total soil N₂O content was at a maximum on day 71 to 74, while the snow cover was still >6 cm (Figure 3.5 c). Warming of air temperature on day 74 through to day 81 triggered a decrease in snow depth, followed by surface warming and increasing soil liquid water content (Figure 3.5 a, b). At the same time soil N₂O content decreased (stabilizing by day 79),

and N₂O surface emissions increased, peaking on day 76, then decreasing to a minimum on day 80 (Figure 3.5). This warm period represents the first heightened N₂O emission event analyzed, defined as lasting from day 71 (a time of low flux, and maximum soil N₂O content) to day 80, when the daily average N₂O surface flux stopped decreasing. Over this first event the change in whole profile total soil N₂O content was -16.59 mg N₂O m⁻², while accumulated N₂O surface emissions were 8.57 mg N₂O m⁻² (Table 3.2).

By day 82 and through to day 88, air and surface temperature highs were <0°C, and snow cover began to build while soil liquid water content decreased (Figure 3.5 a, b). Concurrently, N₂O surface fluxes began increasing, remaining >16 ng N₂O m⁻² s⁻¹ from day 85-89, reaching the highest value during the study period on day 89 (Figure 3.5 c). Warm conditions returned on day 89 with air and surface temperatures >0°C, which continued to increase through mid-April, resulting in increasing soil water content as further melting occurred, while the N₂O surface flux rate decreased rapidly, stabilizing by day 92 (Figure 3.5). This period is the second emission event analyzed, starting from the low N₂O emissions on day 80 and extending to day 92 where daily flux values returned to background levels. Over this second event the change in whole profile total soil N₂O content was -1.51 mg N₂O m⁻², while accumulated N₂O surface emissions were 12.74 mg N₂O m⁻² (Table 3.2).

Table 3.2 Change in total soil N₂O content by layer and accumulated surface N₂O emissions over Event 1 and Event 2 ($\pm$ standard error of the mean)

Days	Accumulated Surface Emissions (mg N ₂ O m ⁻²)	Change in Total Soil N ₂ O Content (mg N ₂ O m ⁻²)											
		Whole Profile		0-7.5cm		7.5-12.5cm		12.5-22.5cm		22.5-45cm		45-70cm	
Event 1													
71-73	0.16	-2.15	± 2.16	-3.26	± 1.73	0.02	± 0.13	0.28	± 0.20	0.42	± 0.23	0.38	± 0.20
73-74	0.32	-0.64	± 0.68	-1.17	± 0.55	-0.28	± 0.27	0.25	± 0.13	0.16	± 0.26	0.39	± 0.25
74-75	0.48	-4.14	± 0.65	-2.20	± 2.06	-0.83	± 0.81	-0.41	± 0.37	-0.32	± 0.27	-0.38	± 0.28
75-76	1.48	-1.83	± 0.86	-1.81	± 1.49	-0.04	± 0.17	0.12	± 0.07	0.12	± 0.06	-0.22	± 0.31
76-77	1.65	0.95	± 1.06	-0.43	± 1.46	-0.24	± 0.26	0.06	± 0.07	1.28	± 1.05	0.27	± 0.50
77-78	1.91	-5.54	± 2.30	-2.47	± 0.61	-1.05	± 0.33	-0.99	± 0.35	-0.98	± 1.75	-0.05	± 0.41
78-79	1.70	-3.65	± 0.75	-1.81	± 0.90	-0.57	± 0.78	0.20	± 0.08	-1.87	± 0.82	0.39	± 0.32
79-80	0.88	0.41	± 1.21	-0.50	± 0.22	-0.36	± 0.29	0.08	± 0.14	0.70	± 0.51	0.50	± 0.46
Sum: 71-80	8.57	-16.59	± 2.33	-13.66	± 2.09	-3.34	± 1.73	-0.40	± 0.34	-0.47	± 0.22	1.28	± 1.08

Table 3.2 cont. Change in total soil N₂O content by layer and accumulated surface N₂O emissions over Event 1 and Event 2 ($\pm$ standard error of the mean)

Days	Accumulated Surface Emissions (mg N ₂ O m ⁻²)	Change in Total Soil N ₂ O Content (mg N ₂ O m ⁻²)											
		Whole Profile		0-7.5cm		7.5-12.5cm		12.5-22.5cm		22.5-45cm		45-70cm	
Event 2													
80-81	0.26	0.23	± 0.60	0.37	± 0.22	0.11	± 0.17	0.11	± 0.42	-0.69	± 0.38	0.32	± 0.27
81-82	0.49	-0.13	± 0.74	0.05	± 0.24	0.19	± 0.38	0.28	± 0.29	-0.12	± 0.06	-0.53	± 0.44
82-84	1.96	-2.28	± 1.03	-0.49	± 0.33	-0.63	± 0.34	-0.89	± 0.92	0.22	± 0.13	-0.48	± 0.19
84-86	3.13	-1.66	± 1.17	-0.04	± 0.01	-0.07	± 0.02	-0.32	± 0.09	0.43	± 0.27	-1.65	± 1.37
86-88	2.38	1.30	± 1.48	-0.01	± 0.01	-0.03	± 0.02	0.06	± 0.19	0.02	± 0.39	1.26	± 1.19
88-89	1.29	-0.19	± 0.28	0.06	± 0.01	0.02	± 0.02	0.04	± 0.05	-0.07	± 0.24	-0.24	± 0.19
89-90	1.40	1.08	± 0.55	0.26	± 0.06	0.16	± 0.07	0.20	± 0.06	0.26	± 0.27	0.20	± 0.21
90-91	1.17	0.42	± 0.35	0.25	± 0.09	0.18	± 0.09	0.15	± 0.15	0.29	± 0.20	-0.44	± 0.45
91-92	0.67	-0.29	± 0.35	-0.22	± 0.11	-0.05	± 0.05	-0.20	± 0.17	0.32	± 0.34	-0.15	± 0.07
Sum: 80-92	12.74	-1.51	± 1.55	0.23	± 1.08	-0.12	± 0.21	-0.57	± 0.31	0.67	± 1.09	-1.72	± 0.96

Figure 3.4 Snow depth, 5 cm soil water content, 5 cm soil temperature, surface temperature, total soil N₂O content and daily average N₂O surface flux from day 45 to day 65. (a) snow depth, solid line with closed circles; soil water content, solid line without markers (b) soil temperature, solid line; surface temperature, dashed line (c) total soil N₂O content, solid line with closed circles; daily average N₂O surface flux, dashed line with closed triangles. Error bars represent the standard error of the mean.

Figure 3.5 Snow depth, 5 cm soil water content, 5 cm soil temperature, surface temperature, total soil N₂O content and daily average N₂O surface flux from day 70 to day 95. (a) snow depth, solid line with closed circles; soil water content, solid line without markers (b) soil temperature, solid line; surface temperature, dashed line (c) total soil N₂O content, solid line with closed circles; daily average N₂O surface flux, dashed line with closed triangles. Error bars represent the standard error of the mean.

3.4 Discussion

3.4.1 Soil N₂O Content Accumulation

To understand and evaluate the physical release of built-up N₂O in soils, first an understanding of what conditions cause the build-up of N₂O is needed. High concentrations of gaseous N₂O accumulating overwinter within the soil atmosphere followed by sharp decreases in concentrations in the spring have been observed in a number of studies (Goodroad and Keeney 1984; Burton and Beauchamp 1994; van Bochove et al. 2001; Drewitt and Warland 2007; Maljanen et al. 2007; Wagner-Riddle et al. 2008; Goldberg et al. 2010). It has been suggested that such accumulations overwinter are due to ice layers formed in the soil profile creating diffusion barriers that hinder the exchange of gas. These built-up gases are released in the spring when the diffusion barrier is destroyed (Bremner et al. 1980; Cates and Keeney 1987; Burton and Beauchamp 1994). Through the use of argon tracer gas diffused into the soil, van Bochove et al. (2001) showed that ice layers formed at the surface and within the soil profile can constitute gas-impermeable diffusion barriers. Results from this study strongly agree with the ideas presented in prior research, while providing field measurements of the conditions that led to the accumulation of N₂O in the soil profile. In this study, from the beginning of gas sampling (day 319) through January and into early February N₂O concentrations increased slowly to a maximum of ~2 to 6 ppm, despite the buildup of snow cover and freezing temperatures within the soil profile persisting since December. However, after the brief warm period mid February from days 48 to 50 and the subsequent decrease in temperatures, N₂O concentrations in the soil profile began to increase rapidly (Figure 3.4). During the warm period the snow cover melted completely, 5 cm soil temperature was near 0°C, and a sharp increase in soil water content at 5 cm occurred. Since unfrozen water content increases drastically with soil temperature near -2°C (Romanovsky and

Osterkamp 2000), these measurements show thawing within the surface layers. During this period water from snowmelt would have distributed across the soil surface, and begun infiltrating the thawing soil. Subsequently, the rapid decrease in air temperature coupled with decreases in surface temperature and 5 cm liquid water content from day 49 to the morning of day 50, indicate freezing began taking place (Figure 3.4 a). The melt-water at the surface and within surface layers would have frozen, forming an ice layer. It appears that this ice layer (or cap) constituted an effective diffusion barrier allowing for the observed increases in N_2O concentrations observed from day 50 onward.

An unanswered question about the overwinter buildup of N_2O within soils is where within the soil profile the majority of N_2O released at thaw is produced. It has been suggested that production occurs at depth where, due to temperature buffering, the soil remains unfrozen, and N_2O accumulation occurs underneath the frozen layer within the subsurface region due to the reduced diffusion and hence sealing effect (Cates and Keeney 1987; Burton and Beauchamp 1994; Kaiser et al. 1998). Presented as a mechanism leading to overwinter emissions, the production of N_2O may also occur in unfrozen microsites within frozen soil (nutrient rich water-films that persist even when the soil is frozen) as opposed to in unfrozen subsoils, and may then become trapped in air filled pore spaces within the ice by diffusion barriers (Papen and Butterbach-Bahl 1999; Teepe et al. 2001). Due to interactions with the surface of soil particles, water films are tightly bound to particles and will exist as unfrozen water well below 0°C (Edwards and Cresser 1992). It has been found that 8-20% of soil water remained unfrozen despite constant soil temperatures of -5°C , and that significant amounts of soil water can exist down to -20°C (Edwards and Cresser 1992; Rivinka et al. 2000). Teepe et al. (2001) and Papen and Butterbach-Bahl (1999) proposed that these water films, which are surrounded by ice,

present the ideal location for denitrification to occur since surrounding ice limits diffusion and creates anaerobic conditions, and nutrient availability is high due to occlusion of ions from ice and the death and lysis of microbes (Christensen and Christensen 1991; Edwards and Cresser 1992). Teepe et al. (2001) hypothesized that surrounding ice would also limit diffusion out of the unfrozen sites allowing for the buildup of N₂O, which would then be released as the diffusion barriers are destroyed at thaw. Results from this study do show that production is occurring at depth, as increases in N₂O concentrations occurred down to 60 cm, well below the freezing depth. Interestingly, however, N₂O concentrations measured in this study decreased with depth. N₂O concentrations at 5 and 10 cm reached maximum average values of 289 and 131 ppm respectively, while average maximum values at 15, 30 and 60 cm were 33, 22 and 19 ppm, respectively (Figure 3.2). This opposes the findings of several other studies where N₂O concentrations were found to be greater at depth (Burton and Beauchamp 1994; van Bochove et al. 2001; Goldberg et al. 2010), while supporting the idea of trapped N₂O production in unfrozen water films. During the N₂O accumulation period, roughly day 50 to 71, soil temperatures at 5, 10 and 15 cm dropped to lows of -4.3, -2.6 and -1.4°C, respectively. In a prior experiment by Wagner-Riddle et al. (2010), at the same location as this study, it was found that for this soil (managed using conventional till practices) pore water was frozen when soil temperature < -2°C, below which point the liquid water remaining is predominantly present as thin films adhered to soil particles as discussed. Measured soil temperatures < -2°C at 5 and 10cm then demonstrate that there was potential for production in unfrozen microsites, especially at shallow depths, and high N₂O concentrations at 5 and 10 cm suggests that N₂O produced in these water films accumulated in the frozen matrix. These results confirm the hypothesis that N₂O can be produced

and trapped within the frozen layer, and that production in both the frozen layer and at depth occur simultaneously, as was suggested by Teepe et al. (2001).

The rate of N₂O surface emission from day 4 to day 50 was 0.63 mg m⁻² day⁻¹, while a slight increase in total soil N₂O content occurred at a rate of 0.04 mg m⁻² day⁻¹ (Table 3.3). After day 50 the accumulation of N₂O began as discussed above, and peak N₂O content in the profile occurred on day 71 (Figure 3.4). Between these days the average surface N₂O emissions rate was 0.35 mg m⁻² day⁻¹, roughly half of the rate from day 4 to day 50, while the rate of change in N₂O content in the soil increased to 1.09 mg m⁻² day⁻¹. If N₂O is accumulating in the soil, then N₂O net production (net production = production – consumption) must be greater than losses of N₂O through relocation. If it is assumed that from day 4 to day 71 no significant amounts of N₂O downward transport occurred, it would mean that the sum of the rate of N₂O emissions at the surface and rate of soil N₂O content buildup would equal the rate of net N₂O production. For day 4 to 50 the net production rate was therefore found to be 0.67 mg m⁻² day⁻¹ while from day 50 to 71 the production rate was 1.44 mg m⁻² day⁻¹ (Table 3.3). This shows that the accumulation of N₂O content between days 50 and 71 was not just the result of a fraction of produced N₂O unable to escape to the atmosphere becoming trapped under a diffusion barrier, but that N₂O production was also heightened. Of the 22.73 mg N₂O m⁻² increase in N₂O content from day 50 to 71, 16.49 mg N₂O m⁻² (73%) was in the uppermost 12.5 cm of the soil profile. Reconsidering the evidence of production in unfrozen water films within the upper frozen layers discussed above, this result suggests that unfrozen water films not only provided a location for N₂O production, but promoted N₂O production. Increases in N₂O production could be associated with the promotion of denitrification due to anaerobic conditions caused by the presence of a diffusion barrier limiting O₂ supply (Teepe et al. 2001; Yanai et al. 2011), increased availability of C substrate

due to death and lysis of microbial cells (Herrmann and Witter 2002), increased nutrient concentrations due to exclusion from growing ice (Edwards and Cresser 1992) and the suppression of the N_2O reducing enzyme N_2O -reductase at low temperatures (Holtan-Hartwig et al. 2002).

Table 3.3 Surface N₂O emissions, soil N₂O content change and net N₂O production rate estimates from day 4 to day 50 and day 50 to day 71.

	Elapsed Time	Accumulated Surface N ₂ O Emissions	Surface N ₂ O Emission Rate	Change in Soil Total N ₂ O Content	Content Change Rate	Net Production Rate
	Days	mg N ₂ O m ⁻²	mg N ₂ O m ⁻² day ⁻¹	mg N ₂ O m ⁻²	mg N ₂ O m ⁻² day ⁻¹	mg N ₂ O m ⁻² day ⁻¹
Day 4-50	46.0	29.01	0.63	1.79	0.04	0.67
Day 50-71	20.9	7.33	0.35	22.73	1.09	1.44

3.4.2 Soil N₂O Content Depletion

3.4.2.1 Event 1

The initial and largest decreases in soil profile N₂O content were coincident in time to the first flux event from day 71 to 80, which as discussed prior occurred during a period of warming and soil melting, characterized by snow melt, increasing water content and soil warming (Figure 3.5). Specifically, at the start of the thaw period (days 71 - 75) N₂O content in the soil profile began to decrease, while the N₂O surface fluxes increased. During this time, melting of the ice barrier at the surface would have been occurring slowly, and trapped gases would have begun to “leak” out. On day 76, the highest daily average flux in Event 1 occurred, which corresponds with the intensification of the thaw due to increased temperature and loss of snow pack, and the highest rate of decreasing soil N₂O content (days 75 - 79). Measurements suggest the ice barrier would have melted quickly and all the way through (soil temperature at 25 cm and 55 cm reached 0.1 and 0.7°C respectively) allowing trapped gases to escape more freely (diffusion was likely still limited to some extent by high soil water content). This indicates that surface emissions in the first event were predominantly the result of the physical release of N₂O.

Prior studies have suggested initial N₂O emissions at thaw correspond with the thawing of the profile and decreasing N₂O concentrations, indicating a physical release (Goodroad and Keeney 1984; Burton and Beauchamp 1994; van Bochove et al. 2001; Drewitt and Warland 2007; Maljanen et al. 2007). However, within this prior research few studies directly compared N₂O concentrations to independently measured surface fluxes, and a comparison of the magnitude of soil N₂O content to independently measured surface fluxes has not been made. Accumulated N₂O surface emissions during Event 1 were 8.57 mg N₂O m⁻², while the change in whole profile N₂O content was -16.59 mg N₂O m⁻² (Table 3.2). This net loss in profile N₂O

content was due to a loss of $17.00 \text{ mg N}_2\text{O m}^{-2}$ in the 0 to 12.5 cm layer, and a small gain of $1.28 \text{ mg N}_2\text{O m}^{-2}$ in the 45 to 75 cm layer. These results imply that the surface flux corresponded to ~52% of the loss of N_2O content in the soil profile, if net production is assumed to be negligible. This further supports the notion that N_2O emissions during the first event were the result of the physical release, and that the surface N_2O emissions at the surface were predominantly the release of N_2O trapped within the upper layers of the soil profile. Interestingly, it also shows that roughly half of the soil N_2O content was lost via other mechanisms. The increase in N_2O content within the lowest layer during the first event may indicate that N_2O was carried downwards through the profile with melt-water, however an increase of $1.28 \text{ mg N}_2\text{O m}^{-2}$ does not account for the remaining soil N_2O content change. Decreases in N_2O content in the deepest layer only began shortly after Event 1, it is therefore plausible that moist conditions continued to hinder the release of N_2O at depth, although the ice barrier had melted. The more likely potential sink is that with increasing temperatures, moist anaerobic conditions and an abundance of N_2O , complete denitrification (to N_2) was occurring, providing the main N_2O consumption mechanism as per the findings of Wagner-Riddle et al. (2008) using ^{15}N labelled tracers at the same study location.

3.4.2.2 Event 2

Starting from a low flux on day 80, daily average flux increased steadily to a peak on day 89, and then decreased rapidly. The mass balance for Event 2 (day 80 - 92) indicated an accumulated N_2O emission 8.4 times greater than the loss in N_2O content in the profile (12.74 vs. $1.51 \text{ mg N}_2\text{O m}^{-2}$). This result shows Event 2 consisted of fluxes that were predominantly due to “de novo” production. These results validate the hypotheses presented in studies by Maljanen et al. (2007) and Teepe and Ludwig (2004), who suggested that of their observed events the first

were due to physical release and those thereafter were due to new production. It is worth noting that during the second event in this study, the whole profile loss of $1.51 \text{ mg N}_2\text{O m}^{-2}$ consisted of a loss of $1.72 \text{ mg N}_2\text{O m}^{-2}$ in the 45 to 70cm, while layers within 0-45 cm fluctuated between small sinks and sources (Table 3.2). This shows that the contribution of the physical release of N_2O to surface emission may have continued but on a much reduced scale, as N_2O trapped at depth could have slowly diffused upwards.

As the analysis of Event 2 indicated that “de novo” production was dominant, it is important to investigate the conditions under which this net production was occurring. Air temperature during Event 2 were mostly below -2°C , a thin layer of snow ($\sim 13 \text{ cm}$) covered the soil, and soil temperature data during this period showed diurnal patterns with daily maximum soil temperatures of roughly 0°C , -0.1°C and -0.1°C at 2, 5 and 10 cm, respectively. This, combined with observed decreasing soil water content at 5 and 10 cm but not 15 cm, suggests that while freezing may have been occurring it did not penetrate deep into the soil during this secondary freezing period. During Event 1 incoming solar radiation reached daily maximum of $\sim 750 \text{ W m}^{-2}$, while from day 83 through to 90 daily maximum values increased to $\sim 900 \text{ W m}^{-2}$. Therefore, due to high rates of solar radiation diffusing through the thin snow pack, the top few millimeters of soil could have been thawing diurnally, and within this surface layer N_2O production may have been occurring, consistent with the findings of Wagner-Riddle et al. (2010) on the same soils.

3.4.3 Relative Proportions of Physical N_2O Release and “De Novo” Production

Using the estimates of N_2O content and mass balances discussed, an estimate of the relative contribution of the physical release of N_2O and new N_2O production to the surface emissions can be made. Over the two emission events, days 71-92, $21.31 \text{ mg N}_2\text{O m}^{-2}$ was

emitted (Table 3.2). Based on the assumption that the first event was purely the result of the physical release of previously produced N_2O , the physical release would have accounted for all $8.57 \text{ mg N}_2\text{O m}^{-2}$ emitted at the surface (Table 3.2). During the second event $12.74 \text{ mg N}_2\text{O m}^{-2}$ was emitted at the surface, while a loss of $1.51 \text{ mg N}_2\text{O m}^{-2}$ was observed in the soil (Table 3.2). These estimates then show that overall $10.08 \text{ mg N}_2\text{O m}^{-2}$ ($8.57 + 1.51 \text{ mg N}_2\text{O m}^{-2}$) was due to the physical release, comprising 47% of the spring thaw emissions, while $11.23 \text{ mg N}_2\text{O m}^{-2}$ ($12.74 - 1.51 \text{ mg N}_2\text{O m}^{-2}$) was due to “de-novo” production, comprising 53% of emissions. The assumption that surface emissions during Event 1 were purely the physical release is reasonable as prior studies have suggested a lag time between the physical release and “de novo” production (Teepe and Ludwig 2004; Maljanen et al. 2007). Furthermore, if the reduction of N_2O to N_2 was occurring as discussed, it is likely that consumption was greater than production and thus net production was <0 . However, it is possible that overall the contribution of new microbial production was greater than 53%, as one cannot be certain that net microbial production was not >0 during the first event as it could not be separated from the change in N_2O content in the soil profile. Also, it is possible that the loss of N_2O content over the second event, largely at depth, was not fully released to the surface. If one were to assume that over Event 2, as in Event 1, only 52% of the N_2O content loss was to the surface, then the microbial production during Event 2 would be $11.96 (12.74 - 0.78 \text{ mg N}_2\text{O m}^{-2})$, where $0.78 = 0.52 \times 1.51 \text{ mg N}_2\text{O m}^{-2}$). This would change the overall estimates to 44% of the spring thaw N_2O emissions being due to the physical release, and 56% being due to “de novo” production. These estimates then indicate the maximum potential contribution of the physical release is just under half of the emissions at thaw, while showing the relative importance of the physical release decreases with time, as it accounted for

up to 12% of the N₂O emission in the second event (if all the N₂O content loss in Event 2 was to the surface).

Peak half-hourly averaged fluxes for Event 1 and Event 2 reached 57.15 and 60.6 ng N₂O m⁻² s⁻¹ respectively, each occurring the same day as the peak daily average N₂O emission of 17.7 and 25.1 ng N₂O m⁻² s⁻¹ for Event 1 and Event 2, respectively. Prior field studies on Canadian agricultural soils have observed much larger flux events at spring thaw, van Bochove et al. (2000a) observed peak flux of 216 ng N₂O m⁻² s⁻¹ in a Quebec soil while Burton and Beauchamp (1994) observed a peak rate of 346 ng N₂O m⁻² s⁻¹ in a Southern Ontario soil (each calculated daily from soil N₂O concentration gradients and estimated diffusion coefficients). Earlier studies performed at the same location as this study measured daily average surface N₂O fluxes above 100 ng N₂O m⁻² s⁻¹ at spring thaw (Wagner-Riddle et al. 1997; Wagner-Riddle et al. 2010). In terms of soil gas concentrations, Drewitt and Warland (2007) observed fluctuations in soil N₂O concentrations between 65 and 85 ppm at 25 cm overwinter at the same site, and their measurements were larger than but on the same order of magnitude as measurements made at 15 and 30 cm in this study. Although a substantial accumulation of N₂O within the soil profile was observed during this study, increases in N₂O surface fluxes at thaw were arguably small compared to prior findings. It must be remembered that as the relative importance of the mechanisms that cause heightened freeze/thaw related N₂O emissions are variable (Matzner and Borken 2008), the relative proportions of physically release N₂O and newly produced N₂O are likely highly variable. As peak spring thaw N₂O surface flux rates at thaw have been attributed to microbial production (Wagner-Riddle et al. 2008), findings here likely represent a spring thaw where “de novo” production at thaw was relatively small. This is likely due to the winter being fairly mild, as the accumulated freezing degree-hours at 5 cm depth from November to April was

1963 (Wagner-Riddle et al. 2007). This is lower than many prior years in the conventional till plots at the same location as this study plot (the majority being from 2000-4000), as were cumulative N₂O emissions over Nov to Apr at ~0.42 kg N ha⁻¹ (prior year emissions generally roughly 0.75 - 1.5 kg N ha⁻¹) (Wagner-Riddle et al. 2007). Therefore, though the estimates made show that 44 to 47% of the surface emissions observed were the result of the physical release, it is possible that microbial production was much greater in prior studies. Furthermore, the formation of a gas impermeable ice layer will not necessarily happen in every environment, or every winter, again indicating that the physical release is potentially less important in many other situations. These results could then represent the upper limit of the relative contribution of the physical release, as the observed winter involved the formation of an ice layer and substantial N₂O accumulation in the soil profile, but arguably low rates of “de novo” N₂O production at thaw.

3.5 References

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CHAPTER 4: Conclusions and Recommendations for Future Studies

The objectives of this research were foremost to estimate the content of N₂O within the soil profile, to compare changes in soil N₂O content to N₂O surface fluxes at spring thaw measured using the micrometeorological flux-gradient technique, and to estimate the relative proportion of N₂O emissions at spring thaw from physical release (old production) and new production based on this comparison. Using soil profile conditions and N₂O concentration measurements, an estimate of soil N₂O content accumulation was made, and was estimated to have reached a maximum of ~25 mg N₂O m⁻² overwinter under an ice layer that limited diffusion between the soil and atmosphere. Furthermore, it was found that N₂O accumulation was the greatest within the surface layers of the soil profile, suggesting that N₂O was produced in unfrozen water films within the frozen matrix and was trapped by ice layers presenting a diffusion barrier. It was also found that following the sealing of the surface with ice, the rate of N₂O production increased, suggesting that production of N₂O in unfrozen water films heightened N₂O production. At depth, N₂O concentrations also increased well above background (though less than in the surface layers), displaying that production occurred both in unfrozen water film and unfrozen subsoils.

Upon spring thaw, the destruction of the ice layer within the surface layer corresponded with a decrease in soil N₂O content and an initial increase in surface fluxes, suggesting physical release of N₂O to the atmosphere at thaw, while a second peak in N₂O fluxes was attributed to “de novo” production. By performing a simple mass balance it was shown that the physical release of trapped N₂O can constitute a large portion of spring thaw surface N₂O emissions, which was estimated to be up to 47% of spring thaw emissions in this study. It was suggested, however, that this estimate is likely representative of conditions that allowed for a large

proportion of spring thaw N₂O emissions to be due to physical release, and that it may be less important in other situations. Moreover, the contribution of the physical release was found to decrease in time, as emissions shifted to being the result of “de novo” microbial production, the mass balance confirming that the second N₂O emission event was largely due to new production. Findings also illustrated that roughly half of N₂O accumulated within the soil profile was not released to the surface at thaw, but was lost via other pathways, potentially via complete denitrification.

As discussed in the literature review, the study of the mechanisms leading to freeze-thaw related N₂O emissions is a complex problem, and one that spans several scientific disciplines including soil science, soil physics, and microbiology. Though many mechanisms have been proposed, efforts need to be placed on quantifying these mechanisms on large time and space scales to better understand their contributions, for example quantifying the release of nutrients from freeze-thaw cycles due to aggregate disintegration and death of microbes, as well as the magnitude of N₂O emissions resultant. I believe this study is a step in the right direction concerning this issue, and I suggest the specific need for more studies that independently measure surface fluxes and soil N₂O content, in more environments, over multiple seasons, and potentially using a larger amount of samplers and sensors to improve the estimates. With a better understanding of the fundamental issue of the relative contributions of ‘old’ production and “de novo” production to spring thaw N₂O emissions, further analysis could focus on identifying how environmental conditions such as freezing temperature, freezing duration, and soil water content affect the contribution of these two pathways, as well as the more specific mechanisms that drive the increase in N₂O production. Such studies would likely be large and lengthy, however it presents a great opportunity for both collaborative and multidisciplinary work.

APPENDIX A: Supporting Data

Table A.1 Bulk density and gravimetric soil moisture measurements from soil sampling and laboratory analysis

Date	Day of Year	Bulk Density (g cm^{-3})		Gravimetric Soil Moisture (% by Weight)	
		0-15 cm	15-30 cm	0-15 cm	15-30 cm
2010-12-16	350	1.02	1.56	34.61	21.33
2011-01-10	10	0.91	1.47	38.52	27.16
2011-03-02	61	0.75	1.11	57.70	41.68
2011-03-17	76	0.95	1.07	50.30	45.73
2011-04-03	93	0.95	1.07	27.59	27.59
2011-04-13	103	0.95	1.07	22.47	21.39
2011-04-25	115	0.95	1.07	23.68	22.68

Figure A.1 N_2O solubility coefficient K_o vs Temperature

Figure A.2 Incoming short wave and net radiation vs time from day 50 to day 100

Figure A.3 Soil liquid volumetric water content vs time for 5, 10 and 15 cm depths. 5 cm, solid line ; 10 cm, dotted line ; 15 cm, dashed line

Figure A.4 Soil temperature vs time for 5, 10 and 15 cm depths. 5 cm, solid line ; 10 cm, dotted line ; 15 cm, dashed line

Figure A.5 Soil liquid volumetric water content vs time for 10-40 cm and 40-70 cm depths. 10-40 cm, solid line ; 40-70 cm, dashed line

Figure A.6 Soil temperature vs time for 25 and 55 cm depths. 25 cm, solid line ; 55 cm, dashed line

Figure A.7 Soil constituents by volume vs time for 0-7.5 cm layer, as per calculations in section 3.2.6.2. Solids, thin solid line ; liquid water, dashed line ; ice, dotted line ; air, thick solid line

Figure A.8 Soil constituents by volume vs time for 7.5-12.5 cm layer, as per calculations in section 3.2.6.2. Solids, thin solid line ; liquid water, dashed line ; ice, dotted line ; air, thick solid line

Figure A.9 Soil constituents by volume vs time for 12.5-22.5 cm layer, as per calculations in section 3.2.6.2. Solids, thin solid line ; liquid water, dashed line ; ice, dotted line ; air, thick solid line

Figure A.10 Soil constituents by volume vs time for 22.5-45 cm layer, as per calculations in section 3.2.6.2. Solids, thin solid line ; liquid water, dashed line ; ice, dotted line ; air, thick solid line

Figure A.11 Soil constituents by volume vs time for 45-70 cm layer, as per calculations in section 3.2.6.2. Solids, thin solid line ; liquid water, dashed line ; ice, dotted line ; air, thick solid line

APPENDIX B: Example Soil N₂O Content Calculation

This appendix contains an example calculation of whole profile total soil N₂O content done stepwise. This example is carried out for one sampling time and for one replicate, whereas numbers reported in this thesis are the average of all four replicates for each sampling time. Equations outlined in this thesis are referenced where applicable.

Table B.1 Example calculation, Step 1 – Calculate air-filled-pore-space (V_{air})

						Eq. 2	Eq. 3	Eq. 4	Eq. 5
Time (day)	Probe #	Depth (cm)	ρ_b (g cm ⁻³)	$G_{\text{H}_2\text{O}}$ (%)	V_{liq} (%)	V_{sol} (%)	$V_{\text{H}_2\text{O}}$ (%)	V_{ice} (%)	V_{air} (%)
70.33	1	5	0.87	53.25	11.71	32.88	46.48	37.86	17.55
70.33	2	5	0.87	53.25	11.71	32.88	46.48	37.86	17.55
70.33	3	10	0.87	53.25	7.37	32.88	46.48	42.59	17.16
70.33	4	15	0.98	48.68	21.08	36.94	47.74	29.03	12.94
70.33	5	30	1.09	44.11	20.60	41.01	48.01	29.86	8.54
70.33	6	60	1.09	44.11	23.15	41.01	48.01	27.08	8.76

Table B.2 Example calculation, Step 2 – Convert N₂O concentrations from ppm to g m⁻³ using the perfect gas law equation

Time (day)	Probe #	Depth (cm)	N ₂ O Conc. (ppm)	Soil T (C)	Soil T (K)	Molar Mass (g mol ⁻¹)	Pressure (Pa)	N ₂ O Partial Press. (Pa)	N ₂ O Conc. (g m ⁻³)
70.33	1	5	214.56	-0.25	272.90	44.0129	101300	21.73	0.422
70.33	2	5	311.66	-0.25	272.90	44.0129	101300	31.57	0.612
70.33	3	10	220.75	-0.21	272.94	44.0129	101300	22.37	0.434
70.33	4	15	6.49	-0.13	273.02	44.0129	101300	0.66	0.013
70.33	5	30	2.94	-0.30	272.85	44.0129	101300	0.30	0.006
70.33	6	60	2.48	0.70	273.85	44.0129	101300	0.25	0.005

Table B.3 Example calculation, Step 3 – Calculate gaseous N₂O content by layer

								Eq. 6
Time (Day)	Probe #	Depth (cm)	Layer (cm)	Layer Thickness (m)	N ₂ O Conc. (g m ⁻³)	V _{air} (%)	Unit Surface Area (m ²)	Gaseous N ₂ O Content (g m ⁻²)
70.33	1	5	0-7.5	0.08	0.422	17.55	1.00	5.55x10 ⁻³
70.33	2	5	0-7.5	0.08	0.613	17.55	1.00	8.06 x10 ⁻³
70.33	3	10	7.5-12.5	0.05	0.434	17.16	1.00	3.72 x10 ⁻³
70.33	4	15	12.5-22.5	0.10	0.013	12.94	1.00	1.64 x10 ⁻⁴
70.33	5	30	22.5-45	0.23	0.006	8.54	1.00	1.11 x10 ⁻⁴
70.33	6	60	45-70	0.25	0.005	8.76	1.00	1.07 x10 ⁻⁴

Table B.4 Example calculation, Step 4 – Calculate aqueous N₂O content by layer

Time (Day)	Probe #	Layer (cm)	Soil T (K)	$V_{\text{liq}} / V_{\text{air}}$	Eq. 9	Eq. 8	Eq. 7
					$\beta \text{ (L}_{\text{N}_2\text{O}} \text{ L}_{\text{Soln}}^{-1})$	$K_o \text{ (mol L}^{-1} \text{ atm}^{-1})$	Aqueous N ₂ O Content (g m ⁻²)
70.33	1	0-7.5	272.90	0.67	1.343	0.060	4.97 x10 ⁻³
70.33	2	0-7.5	272.90	0.67	1.343	0.060	7.22 x10 ⁻³
70.33	3	7.5-12.5	272.94	0.43	1.341	0.060	2.14 x10 ⁻³
70.33	4	12.5-22.5	273.02	1.63	1.337	0.060	3.58 x10 ⁻⁴
70.33	5	22.5-45	272.85	2.41	1.346	0.060	3.62 x10 ⁻⁴
70.33	6	45-70	273.85	2.64	1.295	0.058	3.67 x10 ⁻⁴

Table B.5 Example calculation, Step 5 – Calculate whole profile total N₂O content

Time (Day)	Probe #	Layer (cm)	Gaseous N ₂ O Content (g m ⁻²)	Aqueous N ₂ O Content (g m ⁻²)	Total N ₂ O Content (by layer) (g m ⁻²)
70.33	1	0-7.5	5.55x10 ⁻³	4.97 x10 ⁻³	1.05 x10 ⁻²
70.33	2	0-7.5	8.06 x10 ⁻³	7.22 x10 ⁻³	1.53 x10 ⁻²
70.33	3	7.5-12.5	3.72 x10 ⁻³	2.14 x10 ⁻³	5.86 x10 ⁻³
70.33	4	12.5-22.5	1.64 x10 ⁻⁴	3.58 x10 ⁻⁴	5.22 x10 ⁻⁴
70.33	5	22.5-45	1.11 x10 ⁻⁴	3.62 x10 ⁻⁴	4.73 x10 ⁻⁴
70.33	6	45-70	1.07 x10 ⁻⁴	3.67 x10 ⁻⁴	4.75 x10 ⁻⁴

Calculating whole profile (0-70cm) N₂O content is then done by summing across the layers, using the average of the two probes at 5cm for the 0-7.5 cm layer. Doing so yields a whole profile gaseous N₂O content of 0.011 g m⁻², and whole profile aqueous N₂O content of 0.009 g m⁻², and a whole profile total N₂O content of 0.020 g m⁻² or 20.23 mg m⁻².