Spatial and Temporal Pattern of Nitrous Oxide Flux in an Upland-Bog Watershed

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Dedication

This thesis is dedicated to Bill Zanner, a man of eagerness, joy and inspiration.

Abstract

Problem Statement

This study assessed the influence of landscape type and topographic position on N_2O emissions and the factors that control it.

Objective

The objective was to explain how landscape, topography, vegetation and season influence the controls on N_2O flux across an upland-bog watershed.

Method

Nitrous oxide flux was sampled on each of five upland hillslope positions and the hummock and hollow microtopographic positions in the lagg, lagg with alder (identified as alder) and bog landscape types during 2007 and 2008. Gravimetric water content and depth to water table were measured for each N_2O flux sample at the time of sampling. Additional samples of water and soil were gathered by landscape type and upland hillslope position in June and September 2008 to assess soil and water properties. Additional soil samples by landscape type and upland hillslope position were tested for denitrification potential. The Generalized Estimating Equation was used to statistically analyze N_2O flux, soil moisture content, depth to water table, and air and soil temperature. Univariate Analysis of Variance (ANOVA) and *t*-tests were used to statistically analyze soil for total carbon, total nitrogen, ammonia, nitrate, bulk density

and pH. Univariate ANOVA and *t*-tests were used to analyze water for total carbon, total nitrogen, ammonia, nitrate and pH.

Results

For 2007 the alder landscape type had higher N_2O flux than the bog landscape type; 7.52 *ug* N m^{-2} h^{-1} and 3.13 *ug* N m^{-2} h^{-1} respectively. During 2007, N_2O flux in the alder hollow, 11.65 *ug* N m^{-2} h^{-1}, was greater than the for lagg hummock, alder hummock and bog hummock and hollow. The lagg hollow; 5.27 *ug* N m^{-2} h^{-1} was greater than the bog hummock and hollow. Nitrous oxide flux for 2007 was higher than for 2008.

Depth to water table and DOC were highest for 2007. DOC was greatest and pH was lowest for the bog. The average C:N for the upland was 20 with a positive relationship with denitrification potential. The average C:N for the peatland was 29 with a negative relationship with denitrification potential. There was a positive correlation between upland N_2O flux and soil temperature for 2007 (r^2=0.51) and 2008 (r^2=0.22). There was a negative relationship between 2007 peatland N_2O flux and DOC (r^2=0.32) and a positive relationship between 2007 peatland N_2O flux and dissolved NO_3^- (r^2=0.40).

Denitrification potential for the upland, alder and lagg landscape types was limited by NO_3^-. The alder landscape type appeared to have a more active microbial population than the lagg landscape type because only the alder landscape type had greater denitrification potential for the glucose+nitrate solution than the nitrate solution. Unlike previous studies, the denitrification potential for the bog landscape type was not greater

with NO_3^- solution. There was no difference in denitrification potential for June and September.

Conclusions

The N_2O flux "hotspot" for the bog watershed was the alder hollow microtopographic position, which emitted the highest N_2O flux during 2007. Denitrification potential was greater for the alder and glucose+nitrate solution than the nitrate solution; however soil carbon did not limit denitrification potential. Denitrification potential was limited by NO_3^- for the upland. It was hypothesized that the toeslope would have the highest N_2O flux for the upland. While the toeslope had higher soil total nitrogen and carbon than the other positions, N_2O flux was not significantly greater. The factors controlling N_2O flux in the peatland were not clear, N_2O flux was highest for the alder hollow in 2007 but not 2008. Soil collected from the alder did not have significantly higher denitrification potential than the lagg. Denitrification potential and N_2O flux for the bog were not always lower than the other landscape types pointing to the similar results from other studies indicating perhaps a different community of microbial nitrogen processors not necessarily smaller populations.

Implications

According to the IPCC 2007 assessment on global climate change; there is ample evidence that climate is warming over most of the earth and there is likely to be greater precipitation, higher incidence of storm events and earlier spring snowmelt.

Increased temperatures and organic matter mineralization have the potential to increase N_2O flux because those conditions support nitrification and denitrification. However, increased plant growth would increase competition among vegetation and microorganisms for NO_3^- and NH_4^+ which would decrease substrates of N_2O emissions. This means that N_2O flux increases would likely be limited to the vegetative dormancy, the period of advantage for microbial processing. The conditions that cause earlier spring snow melt may not be the same conditions that could end vegetative dormancy, so this could result in a longer spring period when microbes have the advantage for NO_3^- and NH_4^+ uptake and subsequent N_2O emissions.

Spring snowmelt is the time when water moves through upland soil bringing with it labile carbon, NO_3^- and NH_4^+ to the toeslope and lagg. The water probably has sufficient carbon for active microbial communities and could result in early season N_2O flux peaks.

The hollow microtopographic positions in the lagg and alder landscapes were two places with the highest N_2O flux. Increased temperatures may bring about increased mineralization and increase nitrogen processing rates. Increased precipitation may mean decreased depth to water table. These changes may increase N_2O emissions in the hummocks while saturated conditions in the hollows may jointly limit nitrification which limits denitrification.

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GENERAL INTRODUCTION

The global warming potential for one nitrous oxide (N_2O) molecule has a lifetime of approximately 114 years and has about 296 times greater global warming potential than one molecule of carbon dioxide (CO_2) (IPCC 2007). N_2O is photolyzed to nitric oxide (NO) in the stratosphere which contributes to acid rain and to NO in the troposphere; NO in turn contributes to ozone depletion (Crutzen *et al.* 1970). There is less of an understanding as to what contributes to the atmospheric concentrations of N_2O than that of CO_2 and 65% of the world's N_2O emissions come from soil (IPCC 2007).

Peatlands cover at least 3% of the earth's surface and are present in nearly every region of the world but most of them are in the boreal and temperate regions (Schumann and Joosten 2008). Global warming poses a particular risk to boreal and northern temperate ecosystems (IPCC) which cover about 11% of the earth's surface. Boreal and temperate peatlands store more than 20% and 30% of the world's nitrogen (N) and carbon (C) (Moore 2002, Salm *et al.* 2009) with a majority of the storage residing in bogs (Moore 2002). Currently the contribution of global N_2O emissions from bogs is quite low due in part to the bog's poor nutrient status (Moore 2002), nitrogen (N) availability and processing rates (Bouwman *et al.* 1993, Christensen 1999) but global warming has the potential to significantly increase N_2O emissions (Dinsmore *et al.* 2009). Climate change scenarios most often predict temperature increases which lead to increased organic matter mineralization and availability of ammonium (NH_4^+) and nitrate (NO_3^-) (Shimamura and Takemon 2006); factors that strongly influence N_2O emissions. As temperatures rise, the stocks of soil organic carbon currently considered to be recalcitrant may mineralize which could lead to increased N_2O fluxes (Lu and Chandran 2010).

While maximum N_2O emission from bog systems may never present the risk to global climate change as CO_2, N_2O emissions represent nitrogen loss and understanding the factors that control and influence that loss is complicated and requires continuing study.

Denitrification, nitrification (Aerts and Ludwig 1997, Alm *et al.* 1998, Ambust *et al.* 2006, Ky and Chandran 2010), and chemodenitrification (Bremner *et al.* 1980, De Boer and Kolwalchuk 2001, Kesik *et al.* 2006) are the three processes most likely to emit N_2O from northern peatland landscapes. Nitrification and denitrification pathways leading to N_2O emissions from soil are dependent upon the availability of O_2 (Skiba *et al.* 1993, Ma *et al.* 2007) and supplies of NO_3^- and labile organic carbon (Kang et al 1998). Availability of O_2 is strongly influenced by soil moisture content (Bergsma *et al.* 2002, Bollman and Conrad 1998). Nitrification occurs in drier soil where soil O_2 is not limited. Increases in soil moisture have the potential to limit soil gas exchange. Under these conditions nitrification rates may slow while rates of denitrification increase releasing N_2O gas (Hernandez-Ramirez *et al.* 2009). Chemodenitrification is an abiotic source of N_2O gas (Bremner *et al.* 1980) and rates of chemodenitrification are supported by soil organic carbon (Kappelmeyer *et al.* 2003) just as is denitrification (Parkin 1987). Whereas there is no optimal pH for denitrification (Simek *et al.* 2002), chemodenitrification appears to be limited in soil with pH greater than 4.0 (Yamulki *et al.* 1997).

There is a strong relationship between N_2O flux and topography (Florinsky *et al.* 2005, Groffman and Tiedje 1989) because there is a relationship between landscape position and soil moisture content. Low lying landscape positions tend to collect water from higher elevations. Higher nitrification rates provide more soil NO_3^- for

denitrification. When denitrification is the controlling process, low topographic positions have greater N_2O flux (Yanai *et. al.,* 2003).

Denitrification has long been identified as the main mechanism for nitrate loss from forested systems (Bedard-Haughn *et al.* 2006, Groffman *et al.* 1993, Groffman and Tiedje 1989). Others have found positive correlations between N_2O flux and soil carbon, organic and mineral nitrogen, and soil moisture (Groffman and Tiedje 1989, Yanai *et al.* 2003). The primary control on denitrification in forested systems is soil NO_3^- (Merrill and Zak 1992). There are plant species that have symbiotic relationships with nitrogen-fixing bacteria, which reduce di-nitrogen gas (N_2 gas) to organic nitrogen (Dick *et al.* 2006). In some circumstances N_2O flux is greatest where there are nitrogen fixing plants (Dick *et al.* 2006, Rusch and Rennenberg, 1998).

We need to increase our understanding of how the landscape influences controls on the biogeochemical processes that emit N_2O and how N_2-fixing vegetation affects the flux of N_2O. This study investigated the effects of landscape, vegetation and climate on the factors that control N_2O flux by first measuring N_2O flux in the field across a watershed and then in a controlled environment soil collected from each landscape type and topographic position within the watershed.

CHAPTER 1:

SPATIAL AND TEMPORAL PATTERNS OF N_2O FLUX IN AN UPLAND-BOG WATERSHED

INTRODUCTION

The global warming potential for one nitrous oxide (N_2O) molecule has a lifetime of approximately 114 years (IPCC 2007). N_2O can be photolyzed to nitric oxide (NO) in

the stratosphere, which contributes to acid rain and NO in the troposphere contributes to ozone depletion (Crutzen *et al.* 1970). Doubling the concentration of atmospheric N_2O is projected to lead to a 10% decrease in the ozone layer which would result in a 20% increase in ultraviolet radiation at the earth's surface, increasing risk for skin cancer and other health problems. It has been estimated that 65% of the world's N_2O emissions come from fertilized soil (IPCC 2007).

Northern peatlands store approximately one-third of all soil organic matter globally (Gorham, 1991), even though they cover only 3–5% of the global land area (Artz *et al.* 2008). Almost 60% of the wetlands in the northern temperate and boreal regions are bogs (Moore 2002), which store 20% to 30% of the world's nitrogen (N) and carbon (C) (Salm *et al.* 2009). Climate change scenarios most often predict temperature increases, which lead to increased organic matter mineralization and availability of ammonium (NH_4^+) and nitrate (NO_3^-) (Shimamura & Takemon 2006). Ammonium and nitrate strongly influence N_2O emissions. Currently the contribution of global N_2O emissions from bogs is quite low due in part to the bog's poor nutrient status (Moore 2002), low N availability and slow processing rates (Bouwman *et al.* 1993, Christensen 1999). Global warming has the potential to increase N_2O emissions significantly (Dinsmore *et al.* 2009). As temperatures rise, the stocks of soil organic carbon currently considered to be recalcitrant may mineralize which could lead to increased N_2O fluxes (Lu and Chandran 2010). While maximum N_2O emission from bogs may never present the risk to global climate health as CO_2, the loss of nitrogen from a bog's system could critically undermine species diversity from these unique and fragile ecosystems (UNEP 2002).

Sources and controls for N_2O

Denitrification, nitrification (Aerts and Ludwig 1997, Alm *et al.* 1998, Ambust *et al.* Ky and Chandran 2010), and chemodenitrification (Bremner *et al.* 1980, De Boer and Kolwalchuk 2001, Kesik *et al.* 2006) are the three processes most likely to emit N_2O from an upland-bog watershed. The majority of N_2O from all soil systems is produced through nitrification and denitrification (Ambus *et al.* 2006). Nitrification and denitrification pathways leading to N_2O emissions from soil are dependent upon the availability of O_2 (Skiba *et al.* 1993, Ma *et al.* 2007) and supplies of NO_3^- and organic carbon (Kang *et al.* 1998).

Topography influences N_2O emissions

Generally, lower upland topographic positions have greater N_2O flux (Yanai *et. al.,* 2003) because lower slope positions collect water and are generally have increases soil nitrogen (N) content and organic carbon (Groffman *et. al.,* 1993, Reuter and Bell 2003). Saturated soil with available nitrogen and labile carbon provides soil conditions for the generation of N_2O more than in well drained soil at higher topographic positions (Groffman *et al.* 1993). However, the correlation between N_2O emissions and soil moisture shows mixed effects for peatlands. There was a positive relationship between N_2O and soil moisture for hummocks in a Scottish ombrotrophic bog, but a negative relationship between N_2O and soil moisture for hollows (Dinsmore *et al.* 2009). The difficulty interpreting the different relationships may be due in part to the effect of depth to water table on soil moisture.

In wetland systems where soil N_2O was not inhibited by low levels of NO_3^-; there was a negative relationship between N_2O emissions and depth to water table (Aerts and

Ludwig 1997, Berryman *et al.* 2009) but for bog systems with lower N_2O emissions the relationship with the water table was either negative (Salm *et al.* 2009) or inconclusive (Aerts and Ludwig 1997). The type of peatland (bog vs. fen) can have a dramatic effect on the level of N_2O flux because of variation in hydrology, vegetation, DOC concentrations, pH, and overall nutrient status (Hendzel et. al., 2005), as well as the effect of microtopography on N_2O rates within the peatland (Dinsmore *et al.* 2009). For example, bog hummocks and hollows have different soil temperatures and depth to groundwater and those differences have strong influences on N_2O flux (Dinsmore *et al.* 2009).

Soil moisture and nitrification

Nitrification is by obligate aerobic autotrophs and emits N_2O in addition to the end product, NO_3^- (Ritchie and Nicholas 1972, Wrage *et al.* 2001). Obligate aerobic autotrophs require soil oxygen and therefore low soil moisture to allow an adequate rate of soil gas exchange with the atmosphere. Autotrophic nitrifiers produce NO_3^- as well as N_2O and N_2, but along two different pathways (Wrage *et al.* 2001). The nitrification pathway and the nitrifier denitrification pathway diverge at the step where ammonia (NH_3^+) is converted to nitrite (NO_2^-) (Wrage *et al.* 2001). Nitrifier denitrification is distinct from coupled nitrification-denitrification. Coupled nitrification-denitrification is the production of NO_3^- by aerobic nitrification followed by the anaerobic reduction of the same NO_3^- by denitrification. Heterotrophic nitrification by bacteria and fungi is commonly found under conditions of low pH. Those microorganisms have been found to nitrify as well as denitrify (Wrage *et al.* 2001). Fungi and heterotrophic bacteria in

peatlands have been found to produce NO_3^- with available organic carbon (Gilbert *et al.* 1998).

Denitrification requires nitrification

Denitrification is recognized as the process primarily responsible for N_2O flux from forested soils (Ambus *et. al*., 2005). If soil oxygen is limited, as occurs in saturated soil, then denitrification is the primary N_2O source (Hernandez-Ramirez *et al.* 2009). Denitrification has been found to occur when the soil moisture content is 60% water-filled pore space (WFPS) and above (Hernandez-Ramirez *et al.* 2009). When soil moisture content exceeds 80% WFPS, N_2O is reduced further to N_2. When O_2 is completely absent, N_2O from denitrification eventually ceases because there is no longer any nitrification to produce NO_3^- (Ma *et al.* 2007).

For pristine systems, denitrification is controlled primarily by the availability of NO_3^- (Aerts and Ludwig 1997), which is supplied by nitrification (Wrage *et al.* 2001). However, the presence of N_2O emitting "hotspots" appear to be associated with organic carbon (Parkin 1987) creating oxygen (O_2) limited microsites (van der Heuvel *et al.* 2009, von Arnold *et al.* 2005) in an otherwise low N_2O-producing matrix. The location in a forested bog watershed where low soil oxygen is most likely to be found is at the lowest hydrological position (Groffman and Tiedje 1989), which for an upland-bog watershed is the lagg. The lagg is located as an ecotone between the center of a peatland and the upland toeslope. The lagg has the lowest elevation in the upland-bog watershed and drains both the bog and the upland.

For low nutrient peatland systems with low N_2O emissions, the relationship with the water table was either negative (Salm *et al.* 2009) or inconclusive (Aerts and Ludwig

1997). In a forested upland system, there was no correlation between N_2O production potential of the denitrification enzyme assay (DEA), soil total carbon (STC), soil total N (STN), soil moisture and depth to water table (Von Arnold *et al.* 2005). Others have found a strong correlation between soil moisture and denitrification in a forested wetland (Bedard-Haughn *et. al*. 2006), including associations between soil moisture, soil organic carbon, and denitrification activity (Christensen *et al.* 1990). N_2O flux has high spatial and temporal variability (Van Arnold *et al.* 2005), making it difficult to find statistically significant correlations between factors that control and affect N processes.

Organic carbon and chemodenitrification

Chemodenitrification is the non-enzymatic decomposition of NO_2^- (Kappelmayer *et al.* 2003), and produces greater amounts of N_2O than N_2 in acid soils compared to soil with greater buffering (Bremner *et al.* 1980, Wheatley and Williams 1989). Chemodenitrification is dependent upon low pH (Kappelmeyer *et al.* 2003, Yamulki *et al.* 1997). Carbon strongly promotes chemodenitrification (Kappelmeyer *et al.* 2003, Thorn and Mikita 2000).

Plant species influence soil nitrogen

A number of plant species have symbiotic relationships with atmospheric N_2-fixing bacteria that reduce atmospheric N_2 into ammonium. One plant species common in northern systems is speckled alder (*Alnus incana* spp. *rugosa*.), a tree species commonly found in peatlands. Soil surrounding alder roots has higher N content than soil under non-N_2 fixing trees and even some N_2-fixing crops (Dick *et al.* 2006). Due to the N_2-fixing properties of bacteria in root nodules of alder species growing in pure and mixed stands, soil NO_3^- content was also greater than in non-alder areas, high enough to

exceed plant uptake, resulting in NO_3^- leaching (Compton *et al.* 2003) and increased N_2O flux (Dick *et al.* 2006).

Season influences N_2O flux

In northern landscapes, N_2O flux was higher in spring and fall compared with summer, because plant uptake of inorganic nitrogen was at its lowest during these periods, and therefore vegetation was not as competitive with microbial communities for soil N (Groffman and Tiedje 1989). Spring and fall are typically when soil moisture or water table levels are highest because of lower evapotranspiration, leading to anaerobic conditions and the reduction of NO_3^-. Where evapotranspiration rates remain relatively high later into the fall, N_2O flux in riparian areas was positively correlated with groundwater levels; resulting in the highest N_2O flux in the spring and late winter (Pinay *et al.* 1993).

Hypotheses

It is important to study the flux of N_2O in different topographic positions so that we may increase our understanding of how the landscape influences controls on the biogeochemical processes that produce N_2O. In northern Minnesota, much like other areas in the boreal region, watersheds generally have embedded wetlands. In this study, I assessed a deciduous upland/conifer peatland watershed. For the upland component of the watershed I measured N_2O flux across the topographic gradient of the upland hillslope positions (i.e. summit, shoulder, backslope, footslope, and toeslope positions). The peatland component was a typical northern, domed bog where water flows from the center to the edge of the bog, creating a hydrologically active zone around the bog called the lagg. Upland water also drains to the lagg, creating a relatively nutrient rich zone

surrounding the bog, which supports different vegetation than the bog. Part of that vegetation difference is the presence of alder in some parts of the lagg. Alder influences N_2O flux (Compton *et. al*., 2003, Dick *et. al*., 2006). Both the lagg and the bog have hummock and hollow microtopography that may also influence N_2O flux (Dinsmore *et. al*., 2009).

In this upland/peatland watershed system, I tested the following hypotheses: (1) The lowest upland topographic position, the toeslope, has the highest N_2O flux among upland positions, because of high soil moisture, soil total carbon (STC) and soil total nitrogen (STN); (2) The lagg has higher N_2O flux than the bog and the upland because it is more likely to become saturated and therefore anaerobic and because water flowing from the upland contributes labile DOC and NO_3^-; (3) Lagg with alder has higher N_2O flux than lagg without alder because of N_2-fixing bacteria within alder shrub root nodules, which contribute higher N to the soil than vegetation without N_2-fixing bacteria; (4) Bog and lagg hollows have higher N_2O flux than hummocks because the concave microtopography of the hollow accumulates more water than the convex microtopography of the hummocks, thereby creating the anaerobic conditions that favor denitrification; (5) Amending upland and peatland soil in the field with a glucose+nitrate solution increases N_2O flux over the same soil amended with water only, because peatland and upland soils are NO_3^- limited. Adding one inch rainfall-equivalent water to soil, even though it is well drained, will fill up soil pores until soil matric potential increases to zero decreasing oxygen diffusion and causing oxygen limitation. The addition of glucose+nitrate to the water eliminates the NO_3^- limitation to denitrification. The toeslope, lagg and bog hollows are the topographic positions with the lowest rates of

soil drainage and therefore the potential for the highest denitrification rates. The lagg is most likely to have the highest denitrification rates due to the glucose+nitrate amendment because of drainage of dissolved N and carbon from the upland and bog.

METHODS

Site Description

The study was located within an upland/peatland watershed at the Marcell Experimental Forest (MEF) in north-central Minnesota (Figure 1-1). MEF is a long-term study area located in the US Forest Service's Chippewa National Forest (latitude 47^{o} 32' N, longitude 93 ^{o} 28' W). The Northern Research Station of the USFS has collected environmental data at MEF since 1960. The watershed used in this study was the S2 reference watershed, one of the most highly studied peatland systems in the world (Table 1-1). The lagg for the S2 watershed drains the watershed through a metered weir, 420-m above sea level. The lagg receives water from the upland with the highest elevation at 430 meters above sea level and the bog, where the highest point of the bog is near its center at 422-m above sea level.

The climate of the MEF is subhumid continental, with wide and rapid diurnal and seasonal temperature fluctuations. Average annual air temperature is 3°C (37°F), with extremes of -46°C (-51°F) and 38°C (100°F). Average January temperature is -15°C (5°F), and the average July temperature is 19°C (66°F). Average annual precipitation at the MEF is 78.5 cm, with 75% occurring in the snow-free period (mid-April to early November). An average of 75 rain events occurs each year, but normally only 3 to 4 exceed 2.5 cm (Verry 1975). The S2 watershed contains a 3.2-ha bog dominated by mature black spruce (*Picea mariana*) and a 6.5-ha upland dominated by mature trembling

aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) (Verry 1975). Soils in the watershed are mostly Loxley series (Dysic, frigid Typic Haplosaprist) in the bog and Warba series (mixed, superactive, frigid Haplic Glossufalf) in the upland (Adams *et al.* 2003). The soil pH for the mineral upland was 5.3 ± 0.1 and the organic soil in the bog was 4.2 ± 0.2. The pH of the stream at the watershed outlet was 3.9 ± 0.2.

Experimental design

The sampling design was adapted to the landscape types and topography of the S2 watershed (Figure 1-2). The intention was to thoroughly sample the three landscape types; an upland, lagg, and bog, and the topography associated with each landscape type. Upland topography was defined as the five hillslope positions: the summit, shoulder, backslope, footslope, and toeslope. The peatland part of the watershed has two landscape types,; the lagg and bog. The lagg landscape type is an ecotone between the upland and the bog. The lagg borders the toeslope on the upland side and the bog toward the center of the peatland. The lagg was subdivided into two landscape types based on the presence of alder vegetation, which has N_2-fixing nodules on the roots. The part of the lagg where alder vegetation was absent was labeled the "lagg". The part of the lagg where alder vegetation was present was labeled "alder". The center part of the peatland was labeled the "bog". The lagg, alder and bog were comprised of two microtopographic positions; hummock and hollow, as defined by the geometry of the sphagnum moss growth habit. Where the sphagnum moss grew in a convex, small hill-like configuration, it was labeled a "hummock". Where the sphagnum moss grew so as to form a concave, bowl-like configuration between the hummocks, it was labeled a "hollow".

There were a total of fifteen plots for the upland landscape type, one on each of the hillslope positions with three replicate plots per topographic position and one trace gas sampling chamber per plot. The plots are identified on Figure 1-2 by letters and numbers, the letters identify the upland hillslope topographic position and peatland landscape types; SU (summit), SH (shoulder), B (backslope), F (footslope), and T (toeslope), the lagg positions (L), lagg with alder positions (A), and bog positions (G). The numbers identify the plot replicates: 1to 6.

There were a total of six plots for the lagg landscape type with two trace gas sampling chambers per plot to accommodate the hummock and hollow microtopographic positions within each plot. There were a total of three plots for the alder landscape type with two trace gas sampling chambers per plot to accommodate the hummock and hollow topographic positions. The alder plots were identified on Figure 1-2 as follows: A1, A2 and A3. There were a total of three plots for the bog landscape type with two trace gas sampling chambers per plot to accommodate the hummock and hollow topographic positions. The bog plots were identified on Figure 1-2 as follows; G1, G2 and G3. The letter "G" was used to differentiate the bog plots from the backslope plots.

To facilitate sampling, plots were placed along transects. There were three transects running from the upland summit to the shoulder, backslope, footslope, and toeslope then to the lagg and ending at the bog. These three transects included five plots for the upland and one plot each for the lagg and bog for a total of seven plots with a total of nine trace gas sampling chambers (five chambers for the upland hillslope topographic positions, two chambers for the hummock and two chambers for the hollow for the lagg and bog landscape types). There were six additional plots. There were three plots for the

lagg landscape type with six trace gas sampling chambers to accommodate the hummock and hollow microtopographic positions for each lagg plot. There were three plots for the alder landscape type with six chambers for the hummock and hollow microtopographic positions. In total there were twenty seven plots and thirty nine trace gas sampling chambers.

Static Gas Sampling Chambers

Static gas sampling chambers for measurement of N_2O flux were constructed following recommendations from the GRACEnet chamber-based trace gas flux measurement protocol (Parkin *et al.* 2003). Chamber base collars were constructed from steel and had an inner diameter of 32 cm. Bases were 17 cm deep and inserted 11 cm in the surface soil and remained in the soil through the 2007 and 2008 sampling season. Vegetation in the bog and lagg collars was primarily sphagnum moss, (*Sphagnum spp.),* aulacomnium and polytrichum mosses (*Aulacomnium palustre and Polytrichum juniperinum)*, and forbs such as bluebead (*Clintonia borealis)*, pitcher plant *(Sarracenia purpurea)*, and *cotton grass (Eriophorum spp)* (Bay 1966). Upland collars were placed only over forbs, grasses, and mosses such as twoflower dwarf dandelion (*Krigia biflora* var. biflora), claspleaf twisted stalk (*Streptopus amplexifolius* var. amplexifolius), switchgrass (*Panicum virgatum var. virgatum*), twinflower (*Linnaea borealis spp. americana*), and club moss (*Lycopodium clavatum* L.). The chamber bases were installed more than 24 hours before the first sample was collected.

Chamber samples were collected through a sampling port from a removable, insulated and vented chamber top constructed of stainless steel placed over the chamber base (Figure 1-3). The chamber tops were 33 cm in diameter and 7.5 cm in height.

Chamber tops were covered with reflective insulation to minimize temperature differences between inside and outside of chamber. Closed cell gaskets around the inside perimeter of the chamber top helped prevent leakage during sampling. A stainless steel vent tube helped minimize mass flow of gas from pressure changes inside the chamber during installation, wind passing over the chamber and during sampling. The sampling port had self-closing septa that was replaced every three to four weeks.

Sampling N_2O Gas and Calculating Flux

During 2007, samples were collected on an approximately weekly interval from June 12 to November 2, amounting to 21 samples per sampling chamber. In 2008, 6 samples were collected per gas flux sampling chamber from May 16 to September 11. The sampling season for 2008 was shortened to allow soil amendment with glucose+nitrate solution (see below). All trace gas sampling was conducted between 10:00AM and 2:00PM. The time between 10:00AM and 2:00PM is the time of lowest diurnal temperature variability (Kaye *et al*. 2005). During each sampling event, three gas flux samples were taken beginning immediately after the chamber top was sealed over the base, then at 60 and 120 minutes. The sample volume was 12 mL collected in 9mL vials. The gas samples were brought to the University of Minnesota for analysis on a gas chromatograph (GC) analyzer (5890; Hewlett-Packard, Palo Alto, CA). Samples were analyzed within 14 d of collection.

For every batch of field samples analyzed with the GC, there were vials without field samples containing only ambient air ("ambients") and vials with samples of known N_2O concentration ("standards"). The "standards" were used to calculate the unknown concentrations of N_2O in the other vials. All vials were prepared the same way by being

capped in the same room at the same time under the same conditions. The GC output includes a number labeled the "area count", which correlates with the concentration of N_2O in a known volume of injected gas. A regression between the samples of known N_2O concentration (0.301, 1.57, 3 and 10 *ppm*) is calculated against the associated "area counts" and yielded a slope and intercept that were used to calculate the concentration of the four "ambients". The slope and intercept are used to calculate the N_2O concentration for the area count of each field sample as follows:

Equation1-1.

(field sample "area count") * slope + intercept

Ambient levels of N_2O need to be subtracted from field samples and "standards". The "corrected area count" is the amount of N_2O in the sample only and is calculated using the formula below:

Equation 1-2.

$$\frac{((\text{vol. of sample} + \text{vol. of vial}) * \text{sample area ct} - \text{vol. of vial} * \text{ambient air area ct})}{\text{vol. of sample}}$$

At this point in the calculations there are three corrected area counts representing N_2O concentration for each chamber. The area counts are converted to units of *ppm* by multiplying by the slope and adding the intercept from the regression of the area counts and *ppm* of the standard gas samples:

Equation 1-3.

ppm * slope + intercept

Then a slope is calculated with the three concentrations in *ppm* from above against the deployment time of the chamber measurements resulting in a *ppm*/h value. This *ppm*/h value is converted to *ng* N cm^{-3} h^{-1} by taking into account R, the ideal gas constant,

correcting with the actual air temperature taken at the time the sample was collected and dividing by the volume inside of the assembled chamber base and top. The flux value is converted to a standard unit of flux, ug N m^{-2} h^{-1}, by multiplying by the height of the chamber to convert the volume of cm^3 to cm^2, an area measurement and then multiplied by 10 to convert cm^2 to m^2 in the denominator and *ng* to *ug* in the numerator (1000 *ng* = 1 *ug* and 10,000 cm^2 = 1 m^2). Air and soil temperature were recorded for each sampling session at each chamber. The air temperature was recorded with a metal stemmed thermometer placed on the ground surface close to the chamber. It was assumed that the insulated chamber top and the length of time the chamber top was deployed helped maintain the same air temperature at the soil surface inside the chamber as outside the chamber. Soil temperature was recorded with a metal stemmed thermometer inserted into the soil 5 cm deep next to the chamber. Soil moisture in the upland and depth to the groundwater table in the lagg and bog were recorded at each plot during gas sampling.

Field Denitrification Enzyme Assay (DEA)

On September 24, 2008, a solution of glucose+nitrate, 0.180 g of D-glucose and 0.721 g of KNO_3^- per L of water, was sprinkled in an amount equivalent to 5 cm of rainfall within each chamber base at each plot along the three transects that included five upland hillslope topographic positions; summit, shoulder, backslope, footslope, toeslope and two lagg and bog microtopographic positions, the hummock and the hollow. After all of the chamber bases were amended, the chamber tops were secured and an initial soil gas sample was collected. The second sample and third soil gas samples were collected at 45-minute intervals, whereupon the chamber top was removed and the bases were left open to ambient conditions for 1-h. After 1-h the chamber tops were once again secured

on top of the bases and three soil gas samples were collected at 90-minute intervals. This cycle was repeated four times, the first sample was taken at 1.50 h after the application of the glucose+nitrate solution and the last sample was taken at 9.83 h after the application of the glucose+nitrate solution.

Gas samples were collected in 9 mL vials and brought to the University of Minnesota for analysis on a GC analyzer (model 5890; Hewlett-Packard, Palo Alto, CA) identical in procedure to the routinely collected samples discussed previously.

Soil Sampling and Analysis

Soil was sampled during five sampling sessions; June, July, October 2007, June and September 2008 to determine if there were differences in soil properties among topographic positions and landscape types. There were 15 upland samples collected for each sampling session during 2007 and 30 upland and 18 peatland samples (three samples each for the lagg, alder and bog) collected for June 2008 and 30 upland and 12 peatland samples (lagg and bog) collected for September 2008. There were no substantive differences in the lagg and alder samples collected June 2008 so no alder samples were collected for September. Samples were analyzed for bulk density, pH, inorganic nitrogen, total nitrogen, and total carbon.

The upland cores were separated into two samples, one sample of the A horizon and the second sample of 10-cm beneath the A horizon. There were three cores from each upland landscape position from each of three transects for a total of 30 samples. All mineral soils were sieved in the field moist condition and aggregated by plot for analysis. Peat soil samples from bog and lagg positions were collected in triplicate from each transect. Peat samples were also collected from the triplicate lagg positions that contained

alder. Peat samples were collected using a 50-cm long Macaulay peat auger. Each auger-full of peat soil was divided in half to separate out the 0 to 25-cm sample and the 25 to 50-cm sample. Peat soil was not sieved before aggregating. The three samples from each depth per plot were thoroughly mixed and aggregated into one sample for analysis. Subsamples for STC and STN were air-dried. Subsamples for pH, and inorganic nitrogen were refrigerated in a field moist condition.

Water Sampling and Analysis

Water was sampled during three sampling sessions, October 2007, June and September 2008 to assess whether there were differences in water chemistry among peatland landscape types. There was one well per sampling location; six sampling locations for the lagg and three sample locations each for the alder and bog. Three replicates were sampled during each sampling event. Samples were analyzed for pH, and DNH_4^+, DNO_3^- (Lachat Autoanalyzer, QuikChem 8000, Hach Company, Loveland, CO), and DOC (Shimadzu Total Organic Carbon Analyzer, TOC-V CPH, Shimadzu Instruments Manufacturing Company, New District, Suzhou, Jiangsu People's Republic of China)

Water was drawn and discarded from the well three times before a sample was drawn for testing to reduce the amount of particulates and help to ensure a representative sample for the landscape type.

Statistical analysis

The Generalized Estimating Equation (GEE) is a modified generalized linear model which enables analysis of time series data where the data are not necessarily linearly distributed or where the relationship among the data points is not independent.

SPSS GEE has a Quasi Likelihood Under Independence Model (QIC) value, the purpose of which is to select the GEE model that best fits the data given the assumptions for the data distribution, correlation and other factors. Analyzing the N_2O flux data with scatter plots revealed that the data distributions were not normal. Others have log transformed their datum to fit a general linear model (Ambus *et al.* 2006, Izzauralde *et al.* 2004) but because SPSS has a GEE that can handle non-normally distributed datum; data transformation was not necessary. The data for the N_2O field flux and N_2O field DEA production was best fit with a gamma distribution and a log link function. A gamma distribution fits data with most of the datum near zero. A log link function best fit the N_2O flux data. SPSS has a first order autoregressive (AR1) relationship for time series data, which assumes dependence for observations upon their own past values within the series and that observations closer in time have a different dependence than observations further apart in time. Means were compared with a pair-wise comparison with a Least Squares Difference (LSD) post hoc analysis and a $P \leq 0.10$ level of significance. Soil moisture, depth to water table, and air and soil temperature were analyzed using a GEE model, normal distribution with an independent link function, AR1 working correlation matrix, the post hoc analysis used was LSD pairwise comparison with $P \leq 0.10$ level of significance. Linear regression was used to explain the relationship among N_2O flux and air and soil temperature, STC, DOC, STN, SNH_4^+, SNO_3^-, DTN, DNH^{4+}, DNO_3^- and soil and water pH. Statistical analyses were conducted using SPSS for Windows Release 15.0.0 (SPSS Inc., Chicago, IL, USA).

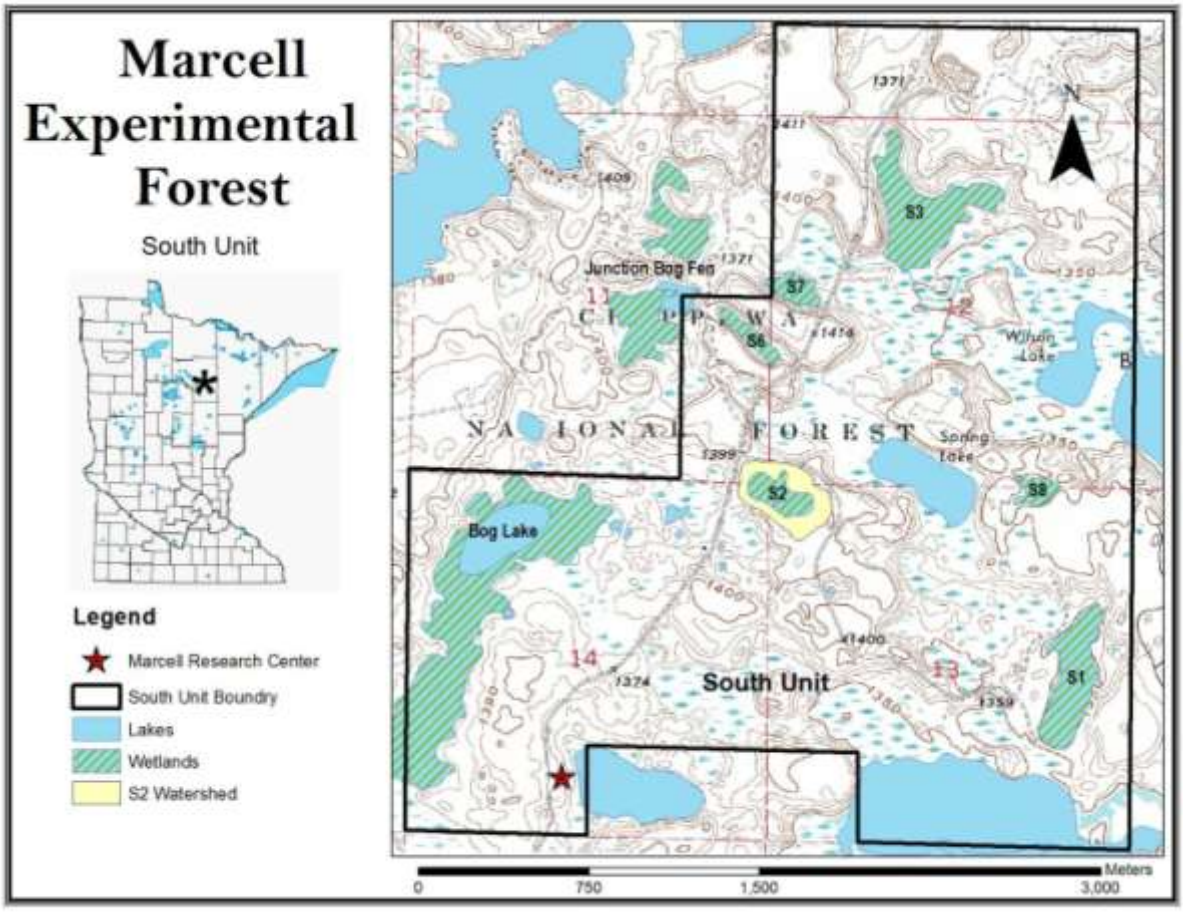


Figure 1-1. The asterisk on the inset map of the state of Minnesota shows the location of Marcell Experimental Forest north of Grand Rapids, Minnesota. The diagram is a depiction of the south unit of the Marcell Experimental Forest . The location of the current study was conducted in the S2 watershed located in the center of the diagram.

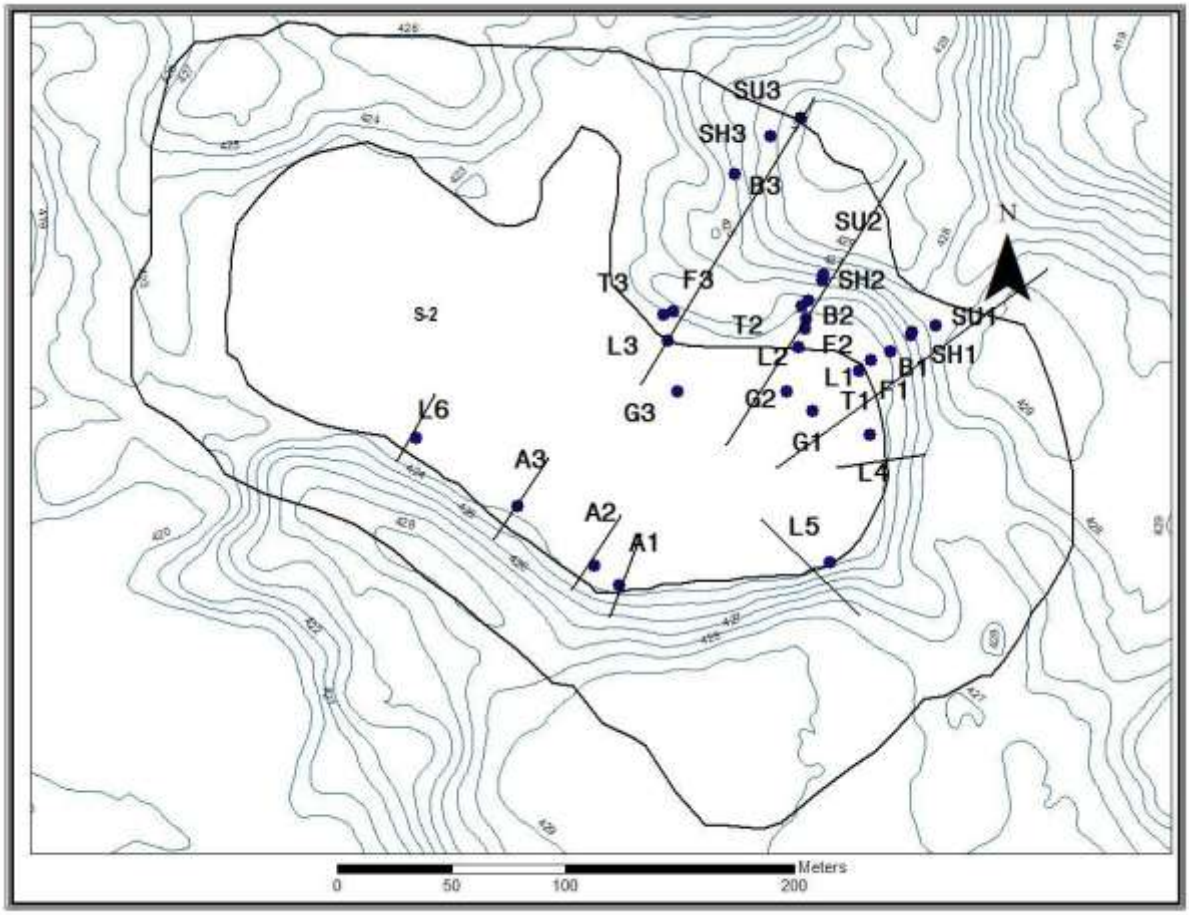


Figure 1-2. The sampling design twenty seven plots and thirty nine trace gas sampling chambers. Three transects plots in the upland, lagg and bog; transect 1: SU1, SH1, B1, F1, T1, L1, G1, transect 2: SU2, SH2, B2, F2, T2, L2, G2 and transect 3: SU3, SH3, B3, F3, T3, L3, G3. There were three lagg plots: L4, L5, L6 and three alder plots; A1, A2 and A3. The plots were located in the S2 watershed of the Marcell Experimental Forest in Grand Rapids, Minnesota.

Figure 1-3. This is the top view of the two part static trace gas sampling chamber. The base of the chamber was a steel collar inserted partway into the ground. The insulated top fit over the base with a gas-tight seal. The top was fitted with a vent tube (seen here as plastic for pre-sampling and later replaced with stainless steel for sampling) and a sampling port with self-closing septa, which was replaced every third week.

RESULTS

N_2O Flux for Upland Hillslope Positions

There were no differences in N_2O flux, air and soil temperature and soil moisture by upland hillslope position for 2007 (Table 1-1, Figure 1-4). In 2008, the toeslope had higher N_2O fluxes than the footslope (Table 1-1). The toeslope, backslope, shoulder and summit had similar N_2O flux. In 2008 air and soil temperature were not different by upland hillslope position. Soil moisture for 2008 was highest for the summit and toeslope. During 2008 the backslope and footslope had higher soil moisture than the shoulder (Table 1-1).

There was significantly higher N_2O flux for 2007 than 2008 for the backslope; N_2O flux for 2008 was higher than for 2007 for the toeslope and there was no difference in N_2O flux by year for the summit, shoulder and backslope (not shown). Air temperature and was higher for 2007 than 2008 but there were no significant differences in soil temperature by year (not shown). There was a positive relationship between 2007 N_2O flux and soil temperature of that year (r^2=0.34). Soil moisture was higher for 2008 than 2007 (not shown).

N_2O Flux Upland, Lagg, Alder and Bog

The alder landscape type had higher N_2O flux than the bog for 2007 (Table 1-2, Figure 1-6). There was similar N_2O flux for the alder, lagg and upland and similar N_2O flux for the upland, lagg and bog. In 2008 the upland had higher N_2O flux than the lagg, alder and bog and N_2O flux for the lagg and alder were not significantly different than for the bog (Table 1-2). Nitrous oxide flux for 2007 (Figure 1-6) was higher than N_2O flux for 2008 (Figure 1-7) for all landscape types (not shown).

In 2007 the upland had higher air and soil temperature than the lagg and alder but it was not significantly different than for the bog (Table 1-2). The bog air and soil temperature were not significantly different than for the other landscape types. In 2008 air and soil temperature did not differ significantly by landscape type (Table 1-2). Air and soil temperature were higher for 2007 than for 2008 (not shown).

N_2O Flux Hummock and Hollow for Lagg, Alder and Bog

The alder hollow had the highest N_2O flux of all peatland microtopography positions for 2007 (Table 1-3, Figure 1-8). There were no significant differences in N_2O flux for the other peatland microtopographic positions for 2007. For 2008, lagg hollow, alder hummock and alder hollow had the highest N_2O flux (Table 1-3) but there were no significant difference in N_2O flux between them and the bog hummock. There was significantly higher N_2O flux for 2007 than N_2O flux for 2008 for each peatland topographic position (not shown).

There were no significant differences in air temperature by peatland microtopographic position for 2007 and 2008 (Table 1-3). Air temperature for 2007 was warmer than air temperature for 2008 (not shown). In 2008 air temperature was warmer than soil temperature (not shown). There were no significant differences in soil temperature for 2008. In 2007, bog hummock and hollows had higher soil temperature than for lagg and alder hollow. Soil temperature for lagg and alder hummock was not significantly warmer than for lagg and alder hollow (Table 1-3). There was a positive relationship between 2007 N_2O flux and soil temperature of which the hollows accounted for more of the relationship (r^2=0.43) than the hummocks (r^2=28). Average soil

temperature for the peatland hollows was lower than for the peatland hummocks for 2007 (not shown).

Field DEA

The biggest difference in N_2O production by upland hillslope position was for the sampling sessions on the day before the application of solution and 1.50 h after the application of solution (Table 1-4). There were very few differences in N_2O production between upland hillslope positions for samples taken after the application of glucose+nitrate solution (Table 1-4 and Figure 1-10).

There were very few differences in response to the application of glucose+nitrate solution by peatland microtopographic position (Table 1-5, Figure 1-11). The bog hummock and hollow had significantly higher production of N_2O at 9.83 hours after the application of solution than before (Table 1-5. Figure 1-11).

Comparisons were made between N_2O production before the application of glucose+nitrate solution and the average of all samples taken after the application of glucose+nitrate solution (not shown). There were no differences in N_2O production before and after the application of glucose+nitrate solution by upland hillslope position and for the entire upland landscape type (not shown). There were no differences in N_2O production before and after the application of glucose+nitrate solution for the lagg and bog landscape types. However, when the lagg hummock and lagg hollow were compared before and after the solution separately from each other, N_2O production was greater after than before the application of solution but not for the bog (Table 1-5).

Soil Properties for Upland Hillslope Positions

Soil pH and bulk density decreased from summit to toeslope and C:N increased from summit to toeslope (Table 1-6, Figure 1-12). There was a significant negative relationship between STC and soil pH (r^2 = 0.52) and STC and bulk density (r^2 = 0.68). There was a positive relationship between 2008 N_2O flux and STN (r^2=0.28) and 2008 N_2O flux and soil NH_4^+ (r^2=0.25). In 2007 and 2008 the toeslope had the highest STC and STN than all other upland hillslope positions. There was very little difference in STC and STN among the footslope, backslope, shoulder and summit positions for 2007 and 2008. STC and STN content for 2007 were lower than for 2008 (not shown). There were no differences in soil NH_4^+ and NO_3^- by upland hillslope position. Soil pH and bulk density were measured for 2007 and 2008 and the difference in soil pH and bulk density by year were not significant; values for 2008 are displayed in Table 1-6.

Soil Properties Upland, Lagg, Alder and Bog

Soil pH was significantly higher for the upland than the lagg, alder and bog (Table 1-7). There were no significant differences in pH for lagg and alder and for alder and bog. Soil bulk density was significantly higher for the upland than the lagg, alder and bog. There were no significant differences in bulk density for the lagg, alder and bog (Table 1-7).

Upland STC was significantly higher for the lagg, alder and bog than the upland (Table 1-7). The lagg and alder had the highest levels of STN, with the upland having the lowest STN content. There were small variations in soil NH_4^+ by landscape type but there were no significant differences in soil NO_3^- by landscape type (Table 1-7).

Water Properties Lagg, Alder and Bog

For 2007 and 2008, there were no significant differences in depth to water table by peatland landscape type (Table 1-8) but depth to water table was greater for 2007 than

2008 (not shown). In 2007, the bog had lower water pH than the lagg and alder, which were similar but in 2008 the bog and alder had similar water pH, but the lagg was less acidic (Table 1-8). In 2007 and 2008, DOC was higher for the bog than the lagg and alder, which had similar levels. DOC levels were significantly higher for 2007 than for 2008 (not shown). There were no significant differences in DTN by peatland landscape type for 2007 and 2008 (Table 1-8). The lagg and bog had higher levels of DTN in 2007 than 2008 but there was no change for the alder (not shown). There was no difference in dissolved NO_3^- (DNO_3^-) by peatland landscape type in 2007 but in 2008 the bog had significantly lower levels than the lagg and alder. There were higher levels of DNO_3^- for the lagg and alder in 2007 than in 2008. In 2007 the bog had higher levels of dissolved NH_4^+ (DNH_4^+) but in 2008 there were no differences in DNH_4^+ by peatland landscape type (Table 1-8) .

In 2007, there was a negative relationship between N_2O flux and DOC, DOC and DNO_3^-, DOC and DTN, and DOC and water pH (Table 1-9). In 2007, there was a positive relationship between N_2O flux and DNO_3^- (Table 1-9). In 2008, there was a positive relationship between N_2O flux and DNH_4^+ (Table 1-9).

Table 1-1. N_2O flux, air and soil temperature and soil water content by upland hillslope position for the S2 watershed at the Marcell Experimental Forest for 2007 and 2008. N_2O flux for each topographic hillslope position was the average of three replicates for twenty one samples for 2007 and six samples for 2008 with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance and are ranked alphabetically in descending order within each sampling year

Hillslope position	N_2O flux		Air temp.		Soil temp.	
	2007	2008	2007	2008	2007	2008
	$ug\ N\ m^{-2}\ h^{-1}$			ºC		
Summit	3.93 (0.29) a	3.05 (0.81) bc	18.8 (0.9) a	13.6 (1.6) a	13.2 (0.3) a	13.2 (0.3) a
Shoulder	3.80 (0.79) a	2.85 (0.05) b	18.1 (0.9) a	13.4 (1.6) a	12.8 (0.5) a	12.8 (0.5) a
Backslope	3.76 (1.10) a	2.69 (0.69) bc	18.3 (0.9) a	14.1 (1.6) a	13.4 (0.4) a	13.4 (0.4) a
Footslope	4.38 (0.80) a	2.08 (0.36) c	18.5 (0.9) a	15.2 (1.6) a	13.1 (0.5) a	13.1 (0.5) a
Toeslope	3.70 (1.04) a	3.99 (0.74) ab	18.3 (0.9) a	13.5 (1.6) a	12.0 (0.3) a	12.0 (0.3) a

Hillslope position	Soil moisture	
	2007	2008
	$H_2O_g/soil_g$	
Summit	0.18 (0.01) b	0.33 (0.07) a
Shoulder	0.16 (0.01) b	0.20 (0.01) c
Backslope	0.17 (0.004) b	0.22 (0.01) b
Footslope	0.18 (0.001) b	0.24 (0.01) b
Toeslope	0.29 (0.02) a	0.50 (0.12) a

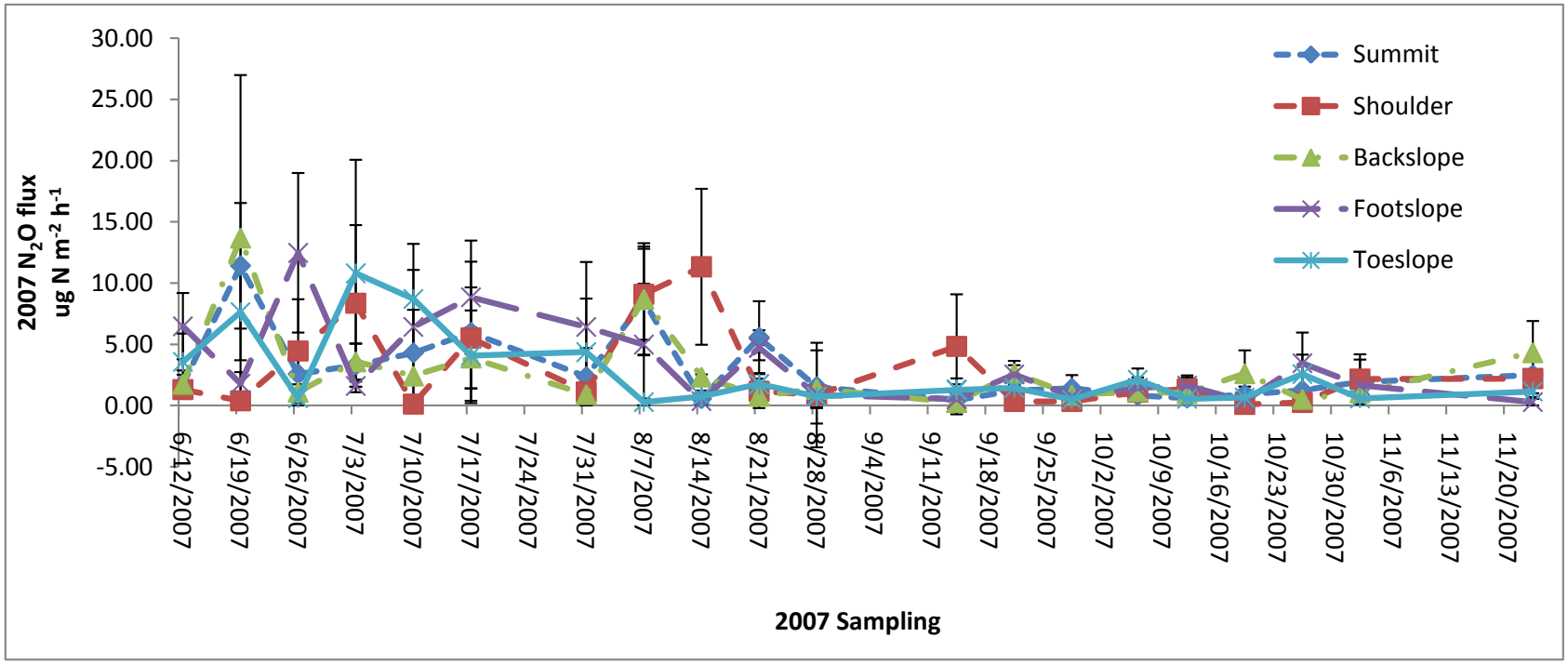


Figure 1-4. N_2O flux for the upland hillslope topographic position for 2007 for the S2 watershed of the Marcell Experimental Forest , Grand Rapids, MN. Average N_2O flux for each topographic position was an average of three replicates for twenty one samples from June 12, 2007 to November 2, 2007. The bars respresent the standard error of the mean.

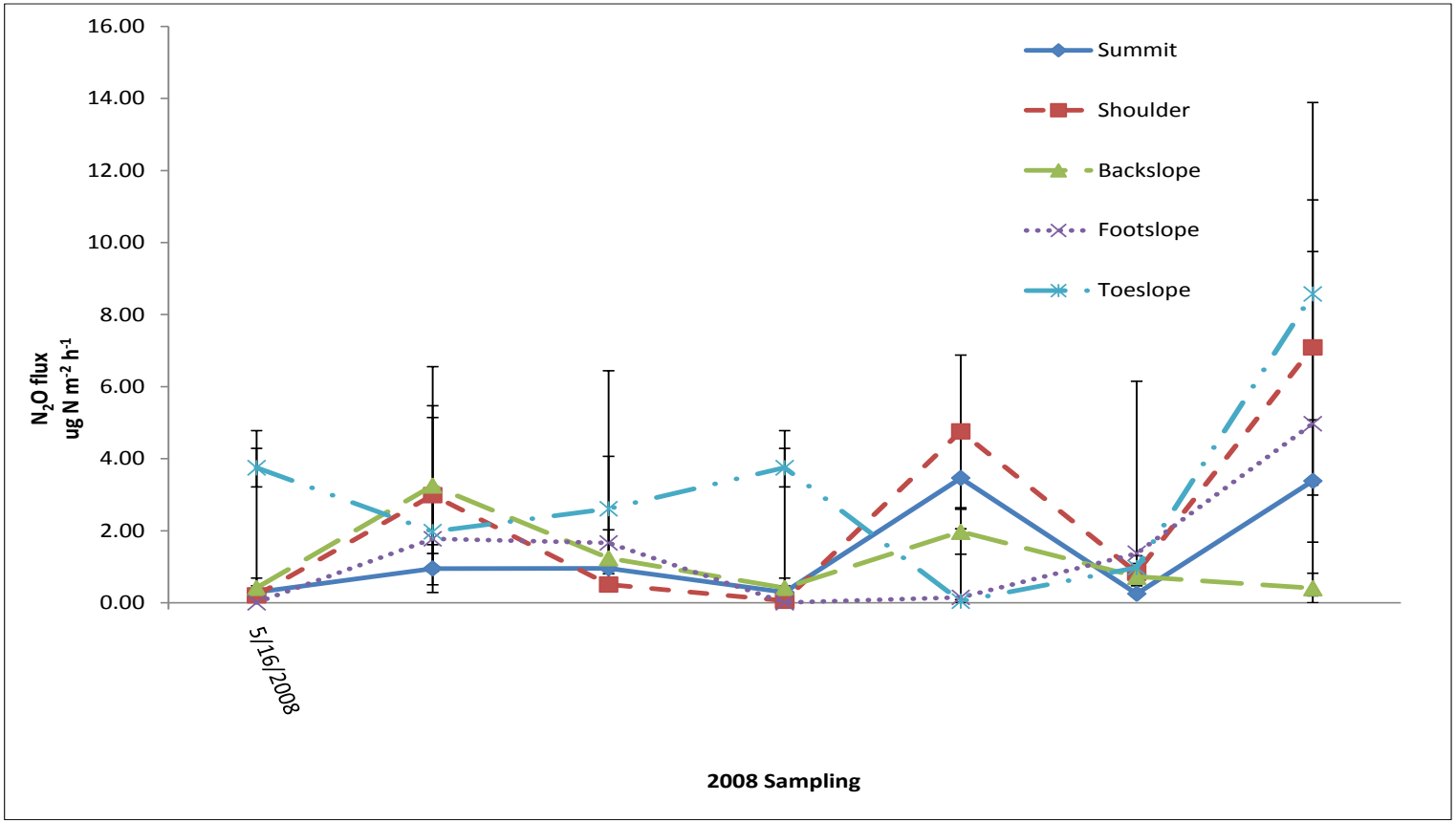


Figure 1-5. N_2O flux for the upland hillslope topographic position for 2008 for the S2 watershed of the Marcell Experimental Forest , Grand Rapids, MN. Average N_2O flux for each topographic position was an average of three replicates for six samples from May 16, 2008 to September 16, 2008. The bars respresent the standard error of the mean.

Table 1-2. N_2O flux for the upland, lagg and bog landscape types of the S2 watershed at the Marcell Experimental Forest sampled 2007 and 2008. N_2O flux was the average of fifteen samples for the upland, six samples for the lagg and three samples for the bog with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance and are ranked alphabetically in descending order within each sampling year.

Landscape type	N_2O flux		Air temperature		Soil temperature	
	$ug\ N\ m^{-2}\ h^{-1}$		^oC			
	2007	2008	2007	2008	2007	2008
Upland	3.91 (0.39) bc	2.93 (0.31) a	18.4 (0.4) a	13.9 (0.8) a	14.7 (0.3) a	12.9 (0.4) a
Lagg	4.46 (0.58) bc	1.82 (0.30) b	17.2 (0.5) b	13.1 (0.9) a	13.9 (0.3) b	12.7 (0.5) a
Alder	7.52 (2.56) ab	1.92 (0.18) b	16.9 (0.7) b	12.3 (1.3) a	13.6 (0.2) b	12.3 (0.7) a
Bog	3.13 (0.58) c	1.85 (0.28) b	18.1 (0.7) ab	13.1 (1.2) a	14.4 (0.4) ab	12.0 (0.7) a

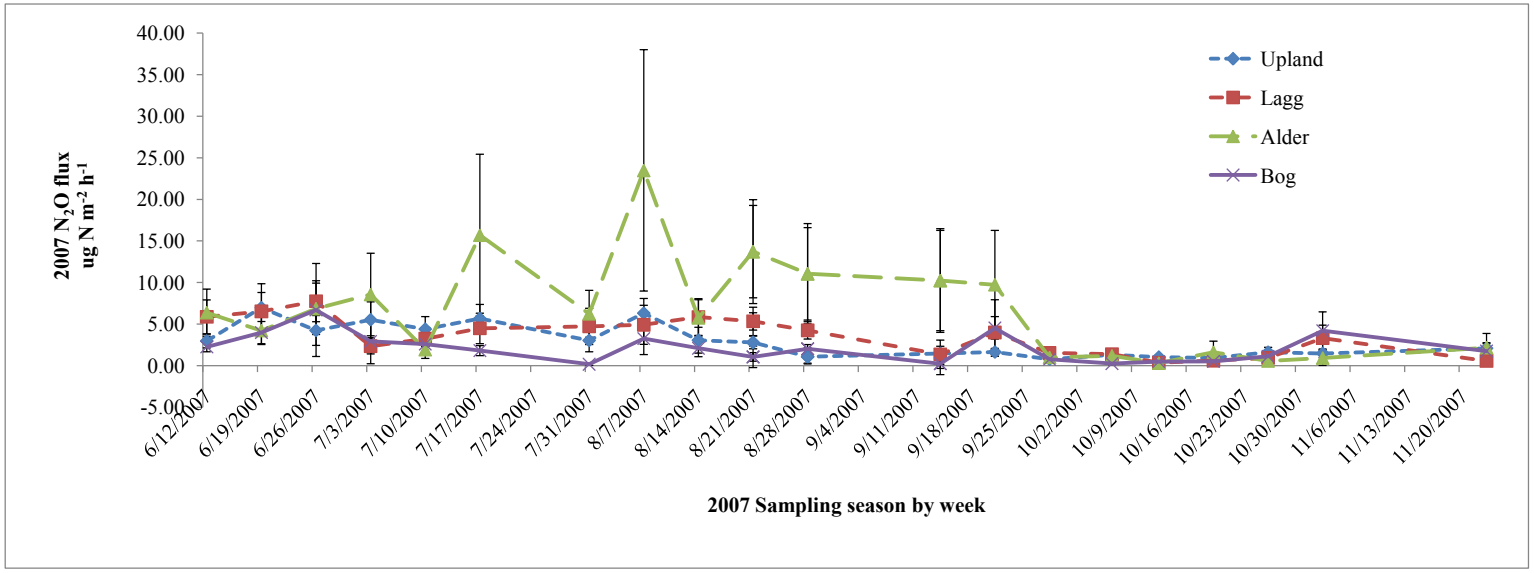


Figure 1-6. N_2O flux for the upland, lagg, alder and bog landscape types for 2007 for the S2 watershed of the Marcell Experimental Forest , Grand Rapids, MN. Average N_2O flux for the upland landscape type was the average of fifteen replicates for twenty-one samples; N_2O average for the lagg landscape type was the average of six replicates for twenty-one samples and N_2O average for the alder and bog were the average of three replicates for twenty-one samples from June 12, 2007 to November 2, 2007. The error bars respresent the standard error of the mean.

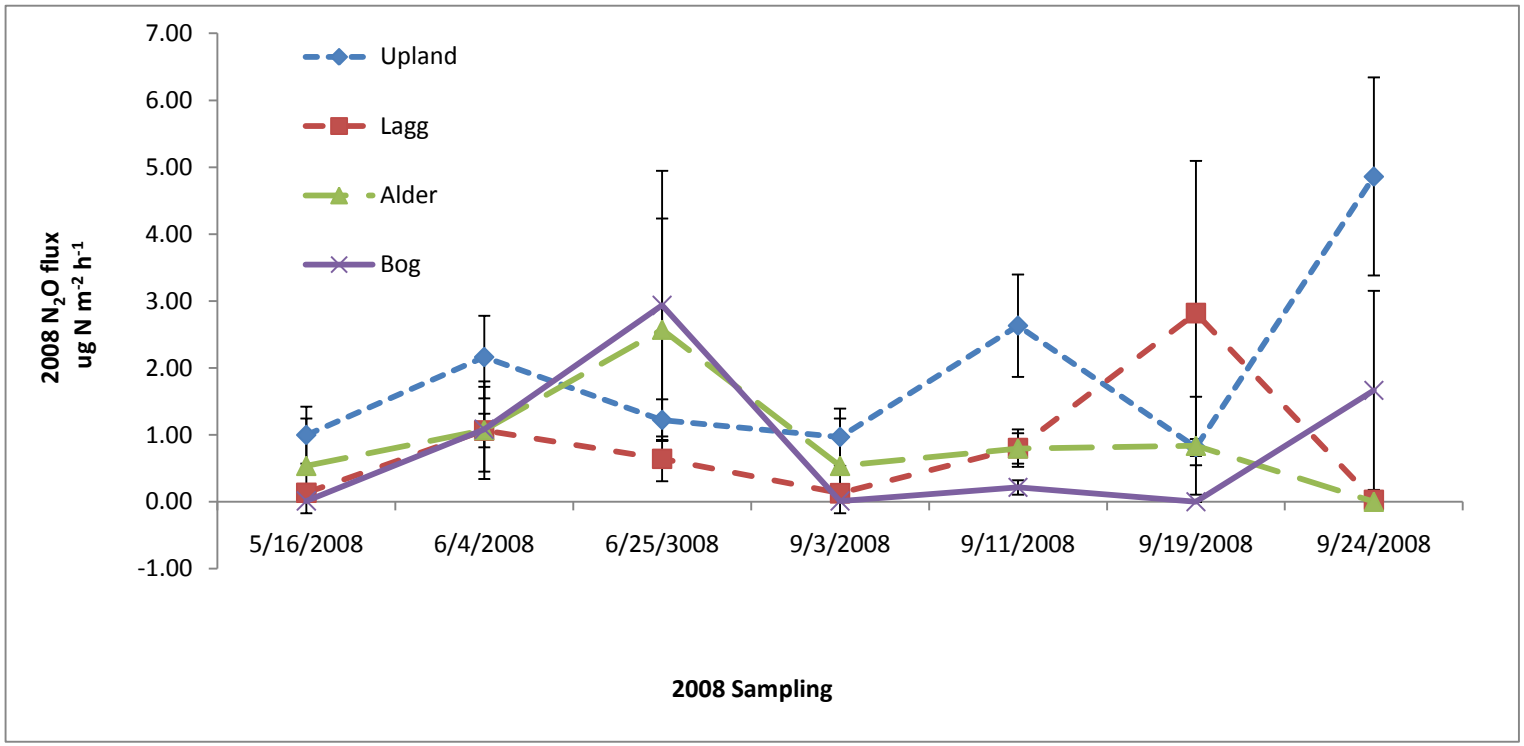


Figure 1-7. N_2O flux for the upland, lagg, alder and bog landscape types for 2008 for the S2 watershed of the Marcell Experimental Forest , Grand Rapids, MN. Average N_2O flux for the upland landscape type was the average of fifteen replicates for six samples; N_2O average for the lagg landscape type was the average of six replicates for six samples and N_2O average for the alder and bog were the average of three replicates for six samples from May 16, 2008 to September 24, 2008. The bars respresent the standard error of the mean.

Table 1-3. N_2O flux for peatland microtopography of the S2 watershed at the Marcell Experimental Forest sampled 2007. N_2O flux was the average of six samples for the lagg and three samples for the lagg with alder (alder) and bog with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance and are ranked alphabetically in descending order for each soil property.

Peatland topography	N_2O flux		Air temperature		Soil temperature	
	$ug\ N_2O\ m^{-2}\ h^{-1}$				°C	
	2007	2008	2007	2008	2007	2008
Lagg hummock	3.65 (0.69) bc	1.48 (0.79) b	17.50 (1.55) a	13.66 (1.23) a	14.53 (0.44) ab	13.03 (0.69) a
Lagg hollow	5.27 (0.82) ab	2.16 (0.56) a	16.98 (1.49) a	12.59 (1.23) a	13.34 (0.44) b	12.45 (0.69) a
Alder hummock	3.41 (0.22) c	1.81 (0.13) a	17.02 (1.51) a	12.70 (1.74) a	13.72 (0.63) ab	12.73 (0.97) a
Alder hollow	11.65 (3.87) a	2.04 (0.33) a	16.85(1.61) a	11.89 (1.74) a	13.41 (0.63) b	11.89 (0.97) a
Bog hummock	2.96 (0.58) c	2.22 (0.44) a	18.47 (1.79) a	13.61 (1.74) a	14.95 (0.63) a	11.98 (0.97) a
Bog hollow	3.31 (0.99) bc	1.47 (0.18) ab	17.82 (1.63) a	12.68 (1.74) a	13.86 (0.62) a	11.98 (0.97) a

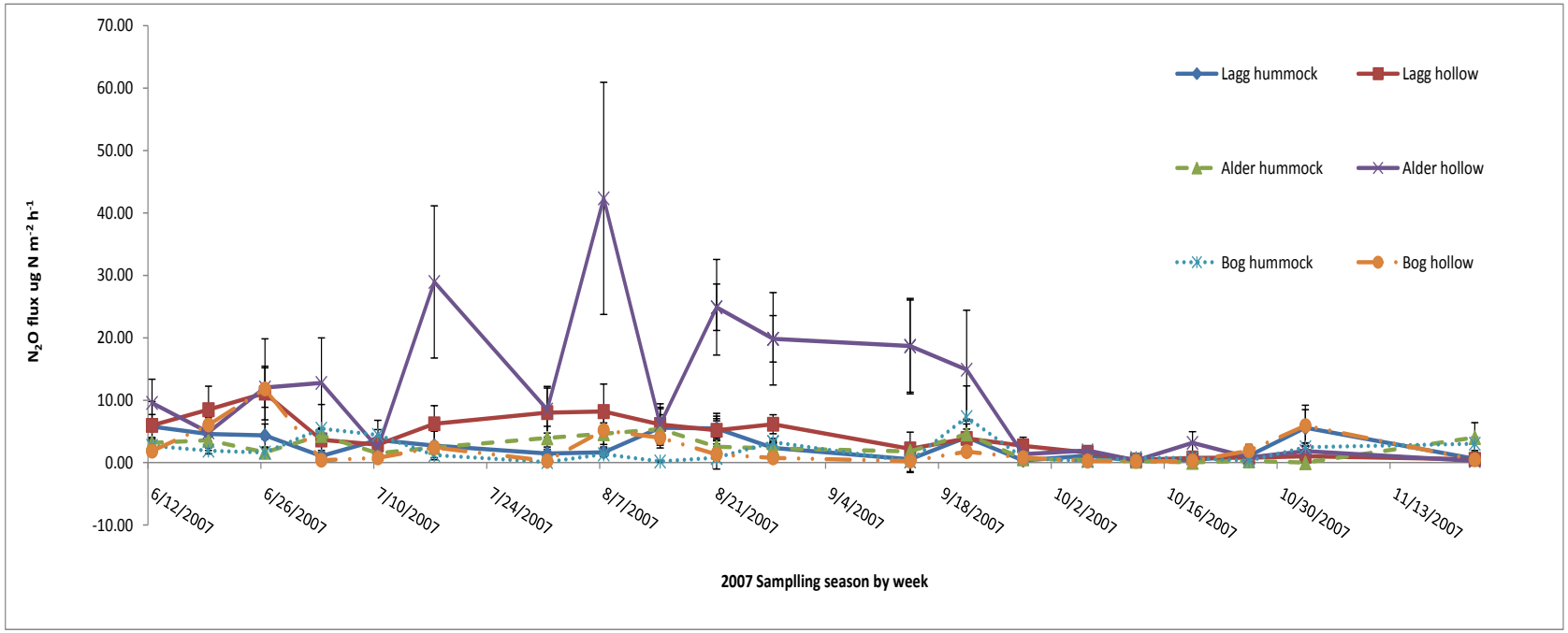


Figure 1-8. N_2O flux for the lagg hummock , lagg hollow, alder hummock, alder hollow, bog hummock and bog hollow for 2007 for the S2 watershed of the Marcell Experimental Forest , Grand Rapids, MN. The marks on the line represent the average N_2O flux for that landscape type and microtopographic position on the date sampled with the bar representing the standard error for the average.

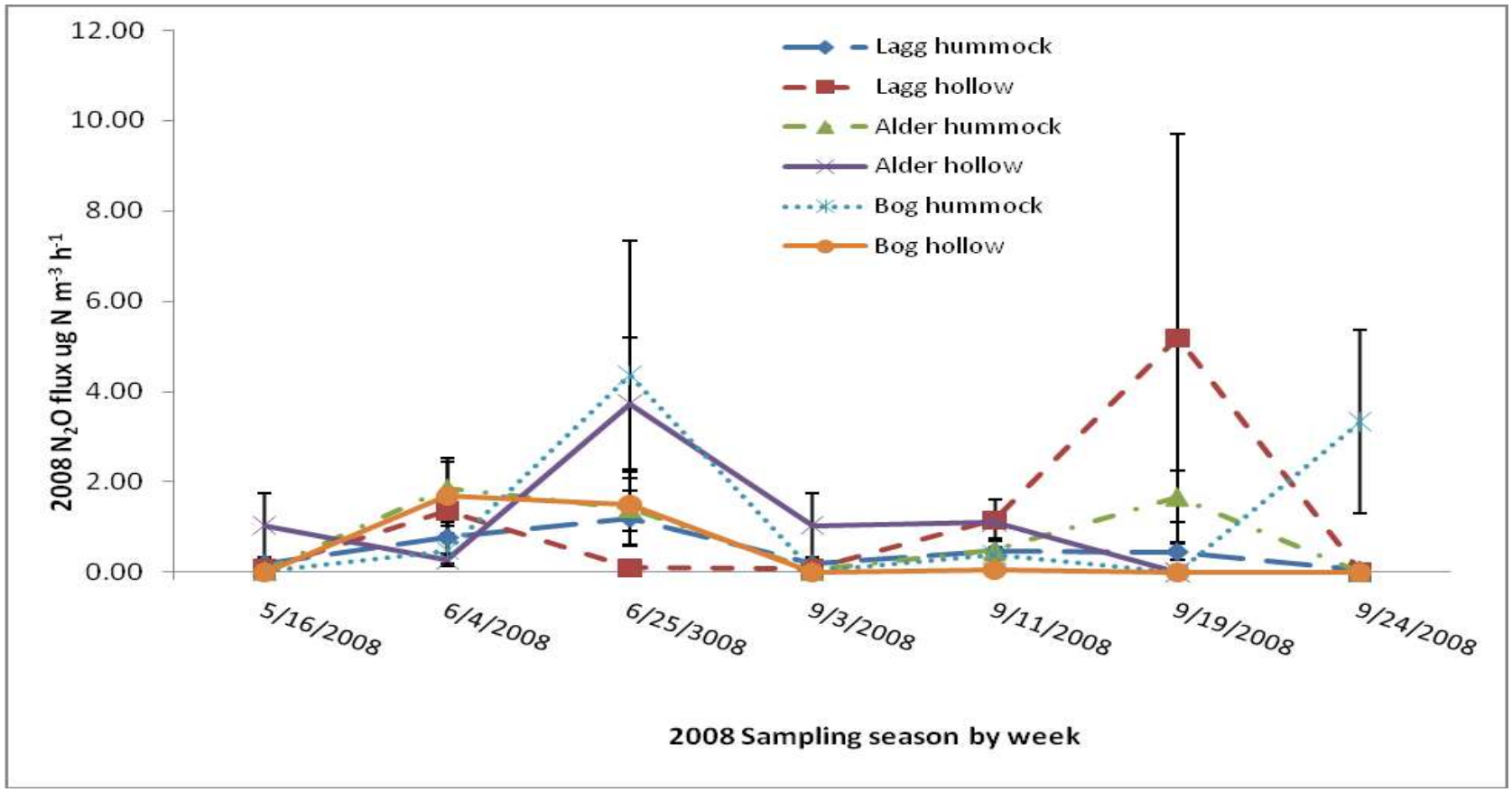


Figure 1-9. N_2O flux for the lagg hummock, lagg hollow, alder hummock, alder hollow, bog hummock and bog hollow for 2008 for the S2 watershed of the Marcell Experimental Forest , Grand Rapids, MN. The marks on the line represent the average N_2O flux for that landscape type and microtopographic position on the date sampled with the bar representing the standard error for the average.

Table 1-4. N_2O flux before and after the application of glucose+nitrate solution for the upland hillslope positions of the S2 watershed in the Marcell Experimental Forest, Grand Rapids, MN. The N_2O flux used for comparison of the effect of the solution was sampled after a 2.5 cm rainfall the day before the glucose+nitrate solution was applied ("Day before"). There were four samples after the application of glucose+nitrate solution, identified as the number of hours after the solution was applied; 1.50 h, 3.50 h, 6.50 h and 9.83 and an average N_2O flux for all samples after the application of the glucose+nitrate solution ("After application"). Average N_2O flux was the average of three samplings with the standard error in parentheses. Average N_2O flux for "After application" was the average of nine samplings after the application of the glucose+nitrate solution, an asterisk (*) represents a significant difference to "Day before" at the $P \leq 0.10$ level of significance. Letters represent statistical differences at the $P \leq 0.10$ level of significance and are ranked alphabetically in descending order by time for each upland hillslope position for the first half of the table and by upland hillslope position for each sampling time in the second half of the table.

Sampling schedule	Summit	Shoulder	Backslope	Footslope	Toeslope
			ug N m^{-2} h^{-1}		
Day before	7.58 (3.40) b	5.60 (1.68) b	8.92 (7.28) b	0.23 (0.19) c	17.72 (6.69) b
Hours after application of solution					
1.50 h	31.68 (12.85) a	17.99 (9.78) ab	10.39 (8.48) ab	31.76 (5.83) a	10.86 (0.33) ab
3.50 h	4.63 (2.57) b	8.41 (5.58) b	7.10 (4.53) b	9.59 (3.85) b	8.60 (4.10) ab
6.50 h	15.88 (8.26) ab	29.65 (7.87) a	20.84 (9.90) a	24.79 (13.66) ab	48.71 (37.37) ab
9.83 h	3.45 (2.14) b	4.06 (3.00) b	2.69 (1.65) b	12.62 (6.28) b	3.76 (0.70) b
After application	17.39 (6.74)	18.68 (5.75)	12.78 (5.30)	22.05 (6.33) *	22.72 (14.80)

	Sampling schedule					
Hillslope position	Day before	1.50 h	3.50 h	6.50 h	9.83 h	After application
			ug N m^{-2} h^{-1}			
Summit	7.58 (3.40) b	31.68 (12.85) a	4.63 (2.57) a	15.88 (8.26) a	3.45 (2.14) a	17.39 (6.74)
Shoulder	5.60 (1.68) b	17.99 (9.78) ab	8.41 (5.58) a	29.65 (7.87) a	4.06 (3.00) a	18.68 (5.75)
Backslope	8.92 (7.28) abc	10.39 (8.48) b	7.10 (4.53) a	20.84 (9.90) a	2.69 (1.65) a	12.78 (5.30)
Footslope	0.23 (0.19) c	31.76 (5.83) a	9.59 (3.85) a	24.79 (13.66) a	12.62 (6.28) a	22.05 (6.33) *
Toeslope	17.72 (6.69) a	10.86 (0.33) b	8.60 (4.10) a	48.71 (37.37) a	3.76 (0.70) a	22.72 (14.80)

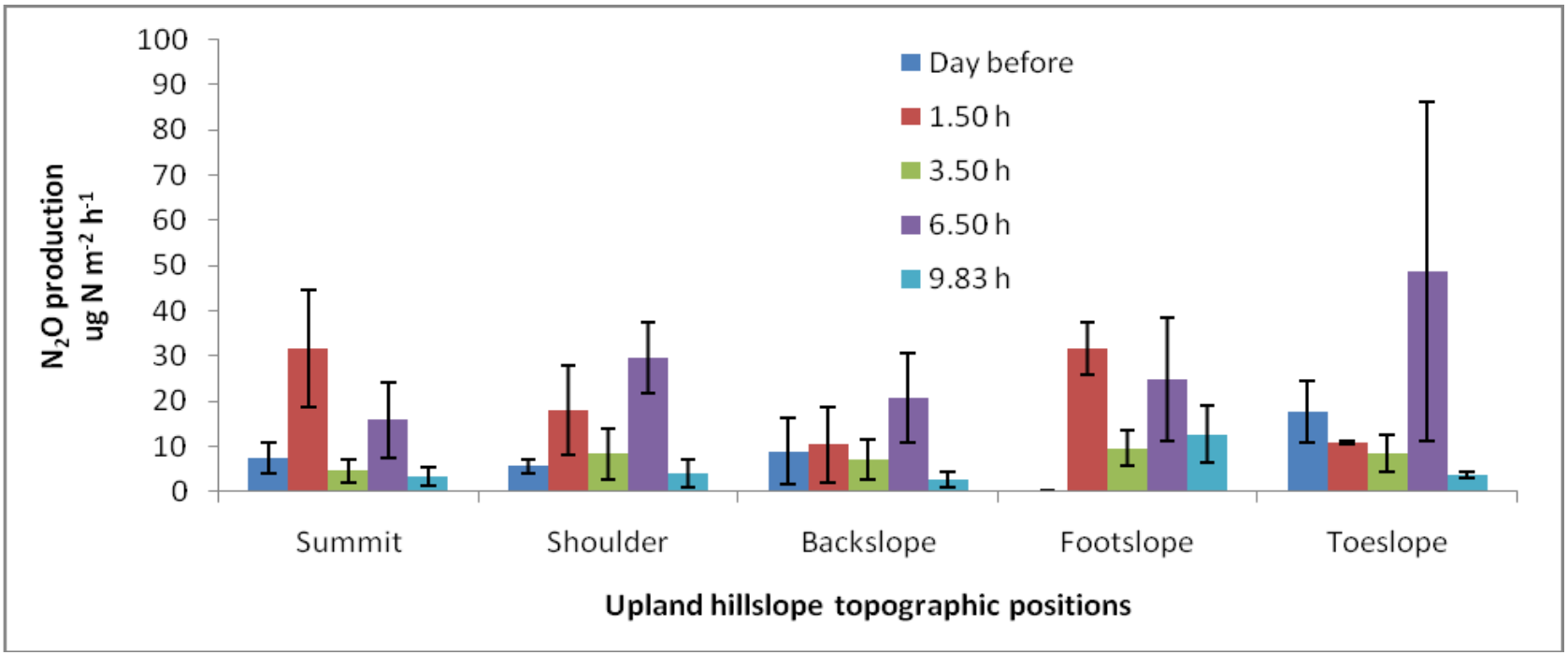


Figure 1-10. Nitrous oxide production before and after the application of a glucose+nitrate solution in the trace gas sampling chamber bases installed at the five upland hillslope topographic positions in the S2 watershed in the Marcell Experimental Forest, Grand Rapids, MN on September 25, 2008. Nitrous oxide production was the average of three replicates per hillslope position and the error bars represent the standard error. The legend identifies the timing of the N_2O production sampling; the day before the application of a glucose+nitrate solution and the number of hours after the application of a glucose+nitrate solution.

Table 1-5. N_2O flux before and after the application of glucose+nitrate solution for the peatland microtopographic position of the S2 watershed in the Marcell Experimental Forest, Grand Rapids, MN. The N_2O flux used for comparison of the effect of the solution was sampled after a 2.5 cm rainfall the day before the glucose+nitrate solution was applied ("Day before"). There were four samples after the application of glucose+nitrate solution, identified as the number of hours after the solution was applied; 1.50 h, 3.50 h, 6.50 h and 9.83. Average N_2O flux was the average of three samplings with the standard error in parentheses for "Day before" and the individual times after application of solution. Average N_2O flux for "After application" was the average of nine samplings after the application of the glucose+nitrate solution, an asterisk (*) represents a significant difference to "Day before" at the $P \leq 0.10$ level of significance. Letters represent statistical differences at the $P \leq 0.10$ level of significance and are ranked alphabetically in descending order by time for each peatland microtopographic position for the first half of the table and by peatland topographic position for each sampling time in the second half of the table.

Sampling schedule	Lagg hummock	Lagg hollow	Bog hummock	Bog hollow
	Average (SE) $ug\ N_2O\ m^{-2}\ h^{-1}$			
Day before	0.19 (0.15) c	11.63 (7.18) abc	3.10 (1.56) b	0.01 (0.0E6) c
Hours after application of solution				
1.50 h	7.64 (4.07) ab	7.01 (2.11) b	3.05 (1.29) b	7.06 (3.44) b
3.50 h	10.97 (1.98) a	11.59 (4.48) ab	2.29 (1.75) b	0.76 (0.36) b
6.50 h	14.21 (8.18) a	9.43 (3.20) b	4.96 (1.73) b	13.66 (8.03) a
9.83 h	3.67 (0.53) b	2.75 (1.15) c	17.57 (4.45) a	26.71 (10.02) a
After application	10.94 (3.44)*	9.34 (2.19)	3.43 (1.06)	7.16 (3.61)

	Sampling schedule					
Hillslope position	Day before	1.50 h	3.50 h	6.50 h	9.83 h	After application
Lagg hummock	0.19 (0.15) b	7.64 (4.07) a	10.97 (1.98) a	14.21 (8.18) a	3.67 (0.53) b	10.94 (3.44)*
Lagg hollow	11.63 (7.18) a	7.01 (2.11) a	11.59 (4.48) a	9.43 (3.20) a	2.75 (1.15) b	9.34 (2.19)
Bog hummock	3.10 (1.56) a	3.05 (1.29) a	2.29 (1.75) b	4.96 (1.73) a	17.57 (4.45) a	3.43 (1.06)
Bog hollow	0.01 (0.00) b	7.06 (3.44) a	0.76 (0.36) b	13.66 (8.03) a	26.71 (10.02) a	7.16 (3.61)

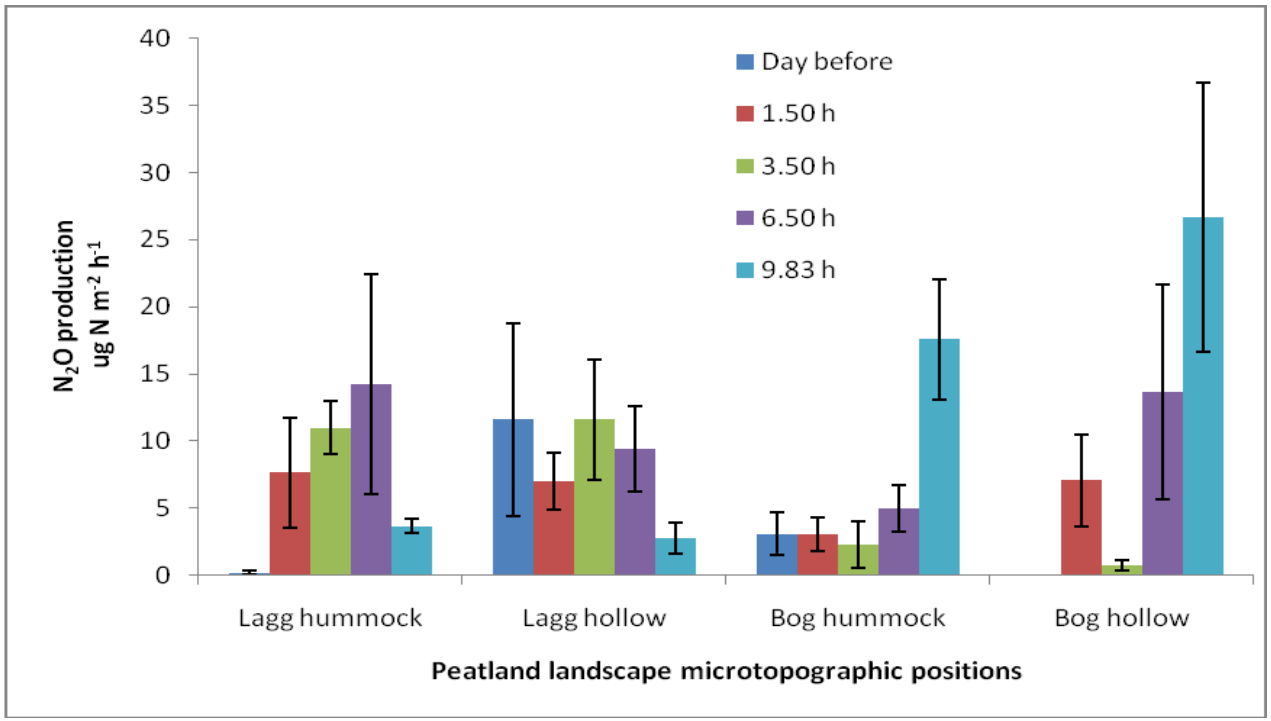
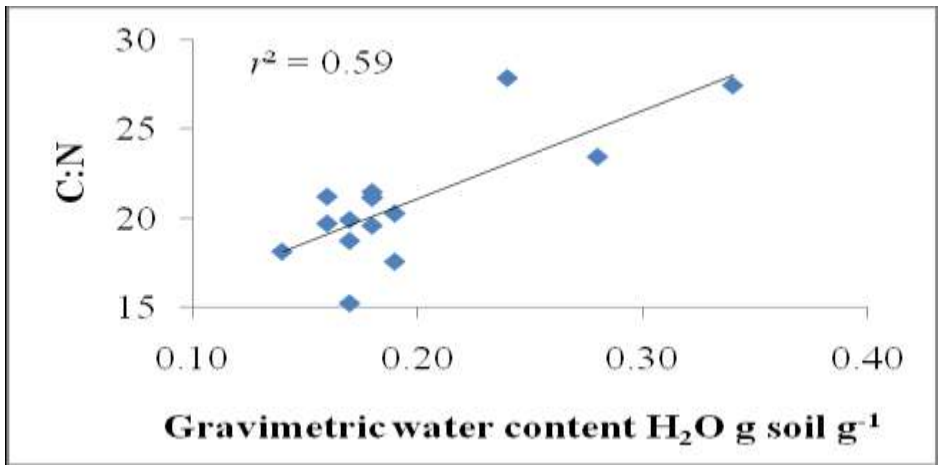


Figure 1-11. Nitrous oxide production before and after the application of a glucose+nitrate solution in the trace gas sampling chamber bases installed at the four peatland microtopographic positions in the S2 watershed in the Marcell Experimental Forest, Grand Rapids, MN on September 25, 2008. Nitrous oxide production was the average of three replicates per microtopographic position and the error bars represent the standard error. The legend identifies the timing of the N_2O production sampling; day before the application of a glucose+nitrate solution and the number of hours after the application of a glucose+nitrate solution.

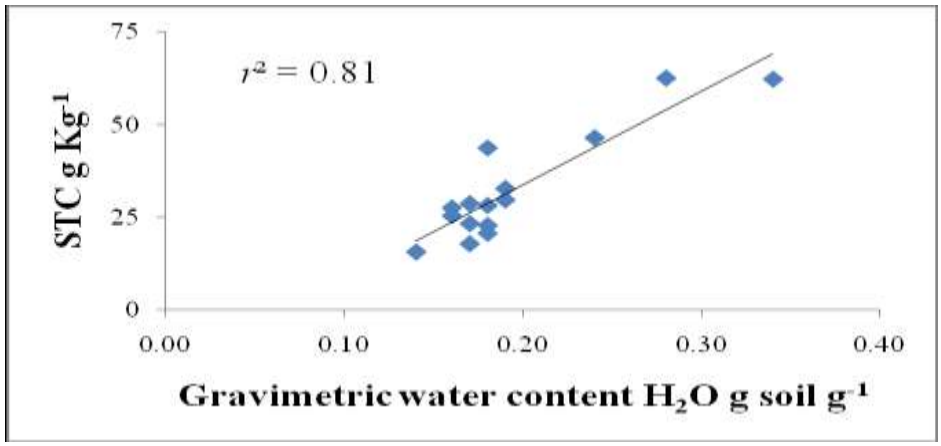
Table 1-6. Soil properties for the upland by hillslope topographic position of the S2 watershed at the Marcell Experimental Forest. Soil pH and bulk density were the average of three samples and 2007 soil total carbon (STC) and soil total nitrogen (STN) were the average of nine samples with the standard error in parentheses. 2008 STC, STN, soil NH_4^+ (SNH_4^+) and soil NO_3^- (SNO_3^-) were the average of six samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance and are ranked alphabetically in descending order. The letters b.d. in place of a numerical value indicate values below the detection limit (0.02 mg kg^{-1})

Position	2008 Soil pH	2008 Bulk density
	pH	Mg m^{-3}
Summit	5.60 (0.13) a	0.77 (0.04) a
Shoulder	5.57 (0.17) a	0.72 (0.05) a
Backslope	5.41 (0.16) a	0.66 (0.07) b
Footslope	5.14 (0.16) b	0.61 (0.03) b
Toeslope	4.81 (0.26) b	0.43 (0.07) c

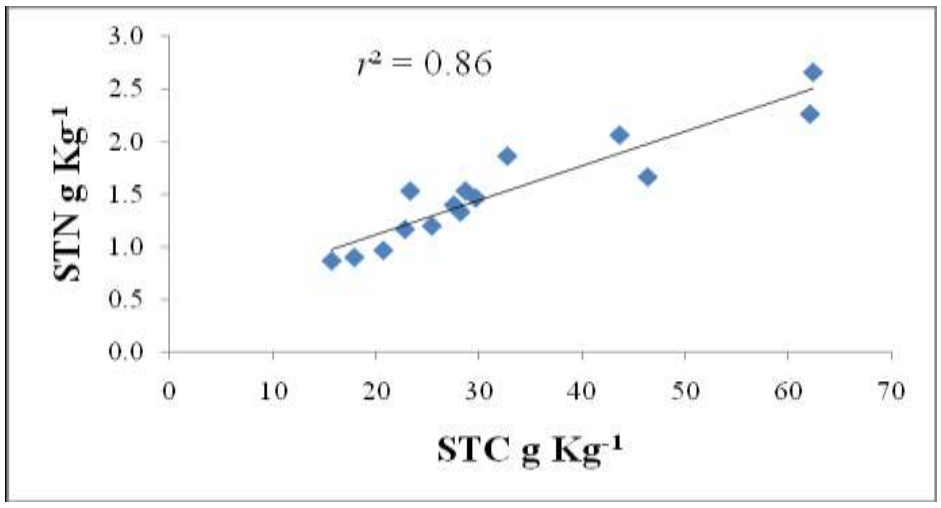
Position	2007 STC	2008 STC	2007 STN	2008 STN	2008 SNH_4^+	2008 SNO_3^-
	g kg^{-1}				mg kg^{-1}	
Summit	34.67 (4.55) b	44.96 (6.23) b	1.78 (0.17) b	2.21 (0.22) b	4.93 (2.82) a	0.32 (0.32) a
Shoulder	22.91 (2.43) c	42.81 (4.72) b	1.29 (0.14) c	2.16 (1.15) b	7.30 (5.16) a	0.01 (0.01) a
Backslope	27.44 (1.53) bc	47.91 (7.28) b	1.36 (0.08) c	2.41 (0.28) b	5.83 (3.69) a	b.d.
Footslope	20.49 (1.61) c	63.61 (9.08) b	1.04 (0.08) c	2.65 (0.30) b	1.53 (1.02) a	b.d.
Toeslope	57.01 (4.51) a	107.59 (14.64) a	2.16 (0.24) a	4.37 (0.64) a	5.61 (3.56) a	b.d.



(a)



(b)



(c)

Figure 1-12. There were strong relationships between soil properties in the upland (a) positive relationship between soil moisture content and C:N, (b) a positive relationship between soil moisture content and soil total carbon and (c) positive relationship between STC and STN. Averages are for 45 samples collected from the A horizon of the upland in the S2 watershed of the Marcell Experimental Forest, Grand Rapids, MN.

Table 1-7. Soil properties for the A horizon of the upland landscape type and 0-25 cm depth for the lagg, alder and bog of the S2 watershed at the Marcell Experimental Forest. Soil pH and bulk density for the upland were the average of fifteen samples and 2007 soil total carbon (STC) and soil total nitrogen (STN) were the average of forty five samples with the standard error in parentheses. 2008 STC, STN soil NH_4^+ (SNH_4^+) and soil NO_3^- (SNO_3^-) for the upland was the average of thirty samples with the standard error in parentheses. Soil pH, bulk density, and 2008 STC, STN, SNH_4^+ and SNO_3^- for the lagg, alder and bog were the average of six samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance and are ranked alphabetically in descending order. The letters b.d. in place of a numerical value indicate values below the detection limit (0.02 mg kg^{-1})

Position	2008 Soil pH	2008 Bulk density	2008 STC	2008 STN
		Mg m^{-3}	g kg^{-1}	
Upland	5.31 (0.9) a	0.64 (0.04) a	61.38 (5.87) b	2.76 (0.21) c
Lagg	4.51 (0.16) b	0.12 (0.02) b	439.15 (4.94) a	16.50 (0.39) a
Alder	4.42 (0.02) bc	0.02 (0.01) b	445.39 (9.39) a	17.90 (0.75) a
Bog	3.87 (0.25) c	0.10 (0.00) b	454.62 (3.65) a	13.88 (0.61) b

Position	2008 SNH_4^+	2008 SNO_3^-
	mg kg^{-1}	
Upland	5.08 (1.56) ab	0.01 (0.01)
Lagg	1.00 (0.31) b	b.d.
Alder	10.96 (4.59) a	b.d.
Bog	6.99 (2.89) ab	b.d.

Table 1-8. Water properties for the lagg, alder and bog peatland landscape types for the S2 watershed at the Marcell Experimental Forest 2007 and 2008. Depth to water table was an average of twenty one samples for 2007 and six samples for 2008, there were six replicates for the lagg and three replicates for the alder and bog with the standard error in parentheses. Peatland water pH (water pH), dissolved organic carbon (DOC), dissolved total nitrogen (DTN), dissolved NH_4^+ (DNH_4^+) and dissolved NO_3^- (DNO_3^-) for 2007 were the average of six samples for the lagg and three samples for the alder and bog and for 2008 an average of twelve samples for the lagg and six samples for the alder and bog with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance and are ranked alphabetically in descending order for each water property.

Landscape type	Depth to water table		pH water	
	cm			
	2007	2008	2007	2008
Lagg	16.76 (1.56) a	7.99 (0.42) a	4.9 (0.2) a	5.1 (0.1) a
Alder	17.84 (1.87) a	8.07 (0.18) a	4.8 (0.1) a	4.5 (0.3) b
Bog	16.07 (0.30) a	8.43 (0.79) a	3.9 (0.1) b	4.3 (0.2) b

Landscape type	DOC		DTN	
	ppm			
	2007	2008	2007	2008
Lagg	91.4 (7.4) b	49.99 (0.93) b	1.11 (0.10) a	0.7 (0.2) a
Alder	79.7 (1.3) b	47.62 (0.64) b	1.20 (0.10) a	1.2 (0.2) a
Bog	107.2 (1.3) a	62.84 (2.69) a	0.99 (0.15) a	0.6 (0.2) a

Landscape type	DNH_4^+		DNO_3^-	
	ppm			
	2007	2008	2007	2008
Lagg	0.25 (0.07) b	0.36 (0.06) a	0.06 (0.02) a	0.43 (0.17) a
Alder	0.25 (0.09) b	0.26 (0.10) a	0.07 (0.01) a	0.62 (0.19) a
Bog	0.55 (0.16) a	0.80 (0.32) a	0.04 (0.003) a	0.04 (0.02) b

Table 1-9. The relationships between peatland N_2O flux, DOC, dissolved total N (DTN), dissolved NO_3^- (DNO_3^-) and water pH, for years 2007 and 2008. There were twelve samples for each variable within each regression.

Variables				2007
Dependent	Independent	*P*	r^2	Regression
N_2O flux	DOC	0.04	0.36	y = 14.93 – 0.11 x
N_2O flux	DNO_3^-	0.03	0.40	y = 1.43 + 62.69 x
DNO_3^-	DOC	0.02	0.42	y = 0.17 – 0.001 x
DTN	DOC	0.02	0.41	y = 1.92 – 0.009 x
Water pH	DOC	0.001	0.71	y = 7.11 – 0.03 x

Variables				2008
Dependent	Independent	*P*	r^2	Regression
N_2O	DNH_4^+	0.01	0.67	y = 0.97 + 3.25 x

DISCUSSION

Upland hillslope positions

Contrary to my first hypothesis, the upland toeslope position did not have greater N_2O flux than other hill slope positions in 2007, but during 2008 the toeslope had higher N_2O flux than the footslope. The conditions that support higher N_2O flux (soil moisture, STC, STN) were greatest at the toeslope for 2007 and 2008 but soil NH_4^+ and NO_3^- were not significantly higher at the toeslope and not significantly correlated to N_2O flux. The lack of a significant relationship between 2007 N_2O flux and soil NH_4^+ and NO_3^- could be because N_2O flux and soil NH_4^+ and NO_3^- were not significantly different by hillslope position; in addition, soil NO_3^- values were very low. A lab assay found that denitrification potential did not differ by upland hillslope position (Chapter Two).

There were poor correlations ($P < 0.10$) between upland soil properties and N_2O flux. Insignificant relationships between STN and N_2O are not uncommon (Aerts and Ludwig 1997, Dinsmore *et al.* 2009). The significant relationships with N_2O flux were limited to peatland N_2O flux and peatland water chemistry, which will be discussed later.

Upland N_2O flux by year

There was higher N_2O flux for 2007 than 2008 for all landscape types. Air temperature was lower for 2008 than 2007, potentially lowering microbial activity overall, however there is sufficient evidence for N_2O flux under snow cover and from frozen soil (Maljanen *et al.* 2009, Yashiro *et al.* 2006, Yates *et al.* 2006), so it is unlikely that the relatively small difference in air temperature between 2007 and 2008 was the only cause for lower N_2O flux 2008.

There was higher soil moisture recorded for 2008 than 2007 and it was expected that the higher soil moisture would have supported higher nitrification rates, which in turn would have supported higher denitrification rates. In addition, higher soil moisture could have increased the probability of a combination of available, SNH_4^+, SNO_3^- and STC (Baker and Vervier 2004) further supporting nitrification and denitrification. Perhaps higher soil moisture increased substrate availability on the microsite scale, because there was a weak but positive correlation between upland N_2O flux and soil NH_4^+ (r^2=0.25) for 2008 (Bedard-Hauhgn *et al.* 2006, Hernandez-Ramirez *et al.* 2009). However, according to precipitation records 2008 was not wetter than 2007. Sampling for 2007 included spring, summer and fall while sampling for 2008 was limited to spring and fall, periods of higher rainfall.

When soil moisture levels are higher but less than 60% water-filled pore space (highest soil moisture for 2007 and 2008 was for the toeslope, 0.29 and 0.50 %WFPS, respectively (Pihlatie etal., 2004, Yates *et al.* 2006) denitrification products have a lower N_2O:N_2 ratio especially when soil carbon is not limited (Chapter Two, Groffman and Tiedje 1989). Therefore, it is possible that N_2O flux was lower for 2008 than 2007 was because sampling was limited to periods of higher soil moisture with lower N_2O:N_2 ratios.

Peatland N_2O flux by year

Perhaps the higher N_2O flux for the peatland during 2007 was because there was a greater depth of the profile above the water table, supporting nitrification, or perhaps nitrification rates were greater, providing more NO_3^- substrate for denitrification. Nitrification rates increased in peatlands when there was a natural drawdown of the water

table (Alm *et al.* 1999). This is because there is a better gas exchange between the soil and the atmosphere, increasing soil aeration, which supports aerobic decomposition of the peat. An increase in decomposition increases release of CO_2, supplying the energy for autotrophic nitrifiers (Poughon *et al.* 2000). Repeated wet-dry cycles increase N mineralization and N availability for microbial processing (Venterink *et al.* 2002) with the potential for subsequent N_2O flux. Denitrification is an important source of N_2O from drained peatlands (Aerts and Ludwig 1997), due in part to the increase of available STC for denitrification in the anaerobic microsites (Bergsma *et al.* 2002). Freeman *et al.* (1993) replicated an experiment with intact peat cores and simulated drought and flooding and in all cases; drought increased N_2O flux whereas flooding lowered N_2O flux. It was hypothesized that drought increased peat N mineralization rates and production of available inorganic N which supported nitrification in aerobic sites and denitrification in anaerobic sites. It appears that conditions where there is simultaneous nitrification and denitrification will produce greater N_2O flux than denitrification alone, perhaps because denitrification is limited by NO_3^- (Groffman *et al.* 2009, Öquist *et al.* 2007, Wrage *et al.*, 2001).

Inadvertent timing of sampling with rainfall may have given the impression that 2008 was wetter than 2007. It is possible that sampling during 2007 events coincided with denitrification "hot moments" (i.e., increased) denitrification in response to pulses of organic matter mineralization and nitrification. But it is possible that sampling during 2008 occurred closer in time to rainfall events but too soon for nitrification pulses. There were no significant relationships between N_2O flux, upland soil moisture and precipitation (not shown).

Comparing N_2O flux between systems

Average N_2O flux for the S2 upland was 3.91 *ug* N m^{-2} h^{-1} for 2007 and 2.93 *ug* N m^{-2} h^{-1} for 2008, which is higher than for more northerly systems such as a larch taiga (0.03 µg N_2O m^{-2} h^{-1}, Takakai *et al.* 2008). The larch tiaga in the study by Takakai *et al.* (2008) had an annual precipitation of only 230 mm and an average annual temperature of -10°C; cooler and drier than MEF. Nitrous oxide flux for a temperate Norway spruce forest was 11.98 ng N_2O m^{-2} h^{-1} (Bagherzadeh *et al.* 2008), one order of magnitude greater than this study. The Bagherzadeh *et al.* (2008) study site had higher annual precipitation (1050 mm), warmer average temperatures (6.5°C) and higher soil carbon (soil organic carbon) and soil total nitrogen content (280 g kg^{-1} and 13 g kg^{-1} respectively) than the study by Takakai *et al.* (2008). The conditions for the Bagherzadeh *et al.* (2008) studys potentially support higher nitrification rates and greater soil NO_3^- stocks to support higher denitrification rates.

The coefficient of variation for this study's upland N_2O flux in Minnesota's Marcell Experimental Forest was 0.05 to 2.61, which is in a similar range to a study by Lamers *et al.* (2007b) a Norway spruce stand growing under similar conditions to the Bagherzadeh *et al.* (2008) study with a coefficient of variability ranging from 0.55 to 1.42 and N_2O fluxes between 0.2 and 3.9 *ug* N m^{-2} h^{-1}. In a peatland in Scotland similar in vegetation and hummock and hollow topography as the S2 study site, Dinsmore *et al.* (2009) measured an average N_2O flux of 0.24 *ug* m^{-2} h^{-1} with a maximum coefficient of variation of 3.1. The Dinsmore *et al.* (2009) peatland received much less precipitation than MEF, 1016 mm of precipitation per year as compared to the precipitation in MEF of 785 mm. Maximum and minimum temperatures reported for July and January for the

Scotland study site were 19°C and 0.7°C respectively, as opposed to maximum and minimum July and January temperatures for MEF of 38°C and -46°C respectively. It seems that N_2O flux variability is positively associated with the magnitude of the N_2O flux. It is not necessarily the case that higher average N_2O fluxes in different systems will have higher variability than other systems, perhaps it is the magnitude of the N content which has more influence (Ambus and Robertson 2006). Perhaps this means that the source of N_2O variability is associated with moments and places in the system when the controlling factors coincide and not the systems themselves. In other words, for instance, to some degree soil moisture can support higher nitrification rates, but lower temperatures may slow them down. To the degree increased soil moisture makes up for cooler temperatures, N_2O flux may have less variability because of stable sources of NO_3^- substrate. However, if low soil moisture is also associated with cooler temperatures during certain times of the year, then both factors work toward decreasing NO_3^- substrate and increasing the range of N_2O flux.

Upland, lagg and bog

The results for 2007 did not provide evidence to support the second hypothesis that N_2O flux for the lagg would be higher than for the upland; however 2007 N_2O flux for the alder was greater than for the bog. Nitrous oxide flux for 2008 was greater for the upland than for the lagg and bog. While the upland had lower N_2O flux for 2008 than 2007 it did not decrease as much as the lagg and alder and was in part why the upland had greater N_2O flux than the peatland for 2008. Organic soil has a higher N_2O consumption rate than mineral soil (Fraiser *et al.* 2010) and may explain why there was a greater decrease in peatland than upland N_2O flux in 2008.

Nitrous oxide flux during 2008 for the lagg, alder and bog were not significantly different from each other, probably because there was less of a difference in N_2O flux between the hummock and hollow for each peatland landscape type during 2008. During 2008 there was much less difference in N_2O flux between hummocks and hollows and this removed the differences in N_2O flux by peatland landscape type that was seen in 2007. Lower N_2O flux for 2008 had less of an effect on upland hillslope positions than peatland microtopographic positions, resulting in greater 2008 N_2O flux for the upland than the peatland landscape types.

What Fraiser *et al.* (2010) refers to as N_2O consumption may also be interpreted as denitrification in organic soil is more likely to go to completion with a higher N_2:N_2O ratio than mineral soil. It is possible that when denitrification occurs in organic soils it is associated with the breakdown of the peat and the availability of more labile carbon than that would be associated with drier conditions, such as what occurred during 2007, not the lower depth to water table during 2008. It appears that nitrification was limited during 2008, which limited denitrification. However, it is possible that 2008 sampling dates coincided with times of low flux as it appears that 2007 N_2O flux had a relative peak in July and August, the missing sampling months during 2008.

Soil total carbon and STN were lower for the upland than the peatland landscape types. This difference appears to be associated with mineral versus organic soil because there were fewer differences in STC and STN among peatland landscape types than between upland and peatland landscape types. Where there is higher STC and STN there is the potential for higher N processing rates (Damman 1978, Godwin and Conway 1939). Lower soil pH for the lagg and alder as compared to the upland may not have

been low enough to suppress nitrification (Nicol *et al.* 2008), although pH affects dqifferent populations differently (D'Angelo and Reddy 1998, Mitchell *et al.* 2003). Lower levels of STN in the upland may be associated with lower microbial populations than the lagg and alder (Basiliko and Moore 2006, Bohlen *et al.* 2001) and perhaps the lower potential for nitrification and denitrification rates and N_2O emissions as seen in 2007. However, denitrification in the upland and peatland were not limited by levels of STC, the energy source for denitrifying microorganisms (Chapter Two, Korom 1992). It is more likely that nitrification, the producer of NO_3^-, the denitrification substrate, is the primary controller of denitrification and N_2O flux (Wrage *et al.* 2001). Therefore, the conditions which influence nitrification rates influence denitrification rates.

It could be that increased soil water had a smaller impact on the nitrification potential in the upland than in the lagg and alder hollows. The upland is a mineral soil with good drainage and was not saturated during 2008, but the water table was closer to the surface during 2008 than in 2007. This meant that less of the soil profile was conducive to nitrification in the hollows during 2008. In contrast, it could be that the lower N_2O flux reflected a lower N_2O:N_2 ratio rather than lower nitrification and denitrification rates, per se.

Another aspect of decreasing the depth to water table during 2008 is the possibly of a dilution effect on the soil chemistry that may influence N_2O flux. Raising the water table may dilute substrates and reduced contact between microorganisms (Fisher *et al.* 1998). There were higher amounts of dissolved NO_3^- in the lagg and alder during 2008 than 2007. This may mean lowered substrate availability to the microorganisms on the soil surface.

During 2007 there was higher DOC for all peatland landscape types and lower DNO_3^- for the lagg and alder than during 2008. For the year 2007 there was a negative relationship between N_2O flux and DOC and a positive relationship between N_2O flux and DNO_3^- while during 2008 these relationships were insignificant, perhaps owing to the lower levels of DOC except there were higher levels of DNO_3^- in the lagg and alder. The data does not present an explanation as to why there appeared to be a "dilution factor" for DOC but not DNO_3^-. There was a negative relationship between DOC and pH; the bog had higher DOC and lower pH than the lagg and alder with higher denitrification potential which has been interpreted as greater microbial activity. Microbial activity has been found to be negatively influenced by low pH and microbes adapted to low pH have been found to have slower processing rates. This may explain in part why the bog landscape type with the lower pH and higher DOC had low N_2O flux and denitrification potential.

Alder and lagg

The alder hollow microtopographic position had one of the highest N_2O flux values for 2007 while at the same time the alder hummock had one of the lowest N_2O fluxes. These results do not unequivocally support the third hypothesis, that the alder landscape type will have higher N_2O flux than the lagg landscape type because the alder increases available soil N (Compton *et al.* 2003). The Compton *et al.* (2003) study also found that leaching through the root zone increased available soil nutrients. But soil and water samples from the alder and lagg did not differ significantly. Water moves through the lagg and alder landscape types as it flows around the lagg mixing with water from

non alder areas of the lagg on its way toward the watershed outlet thereby removing any potential differences in water chemistry between the lagg and alder landscape types.

Chemodenitrification is also supported by STC but lagg soil pH was less acid than the chemodenitrification threshold of 4.0 (Kappelmeyer *et al.* 2003). Therefore it is less likely that chemodenitrification was a source of N_2O flux for the lagg, but it may have contributed to the N_2O flux for the bog, which had soil and water pH closer to 4.

Hummock and hollow

The fourth hypothesis stating that peatland hollows will be the sites for higher N_2O flux than hummocks had mixed results. The alder hollows had the highest N_2O flux for the peatland in 2007 but this difference was missing in 2008. There was more difference in N_2O flux between the hummock and hollow during 2007 than 2008 perhaps because of the lower water table. It seems that it is the peatland hollows that made the difference in N_2O flux between peatland landscape types, not the peatland hummocks. The upland had similar N_2O flux to the peatland hummocks, a microtopographic position with greater drainage potential than the peatland.

It seems then that the dissolved substrates are more influential on N_2O flux than the non-dissolved substrates. There were no significant relationships between soil chemistry and N_2O flux for the upland and the peatland; the only significant relationships with N_2O flux were between the peatland and water chemistry. Others have found significant relationships between soil chemistry and N_2O flux (Baker and Vervier 2004, Bougon *et al.* 2009).

Aerobic conditions support nitrification and lower N_2O flux but N_2O flux increases when the soil has been saturated and then brought back to an aerobic state (Vor

et al. 2003). Perhaps the lower water table for 2007 was not so much evidence of lower precipitation for 2007 because precipitation was not significantly different for 2007 as 2008 (MEF and NOAA data not shown) but of greater water table fluctuations. It is possible that fluctuations in the water table during 2007 promoted higher N_2O flux (Vor *et al.* 2003) which may have more of an influence on hollows than hummocks because of their proximity to the water table. Davidsson *et al.* (2002) found drained peat soil produced higher N_2O emissions than saturated peat soil. This is consistent with Wrage *et al.* (2001), who made the distinction between coupled nitrification-denitrification, and others who have studied the other relationships between nitrification and denitrification (Bollman and Conrad 1998, Fraiser *et al.* 2010)

Field DEA

The addition of a glucose+nitrate solution to the upland did not consistently produce greater N_2O emissions. There were only differences in N_2O emissions before and after application of solution for the footslope hillslope position and the lagg hummock microtopographic position. Typically, when DEA experiments are conducted in the laboratory, acetylene (C_2H_2) is injected into the sample, the purpose is to inhibit nitrification and complete reduction of N_2O to N_2 (Chapter Two). DEA experiments that compare N_2O flux with and without C_2H_2 indicate that there are higher levels of N_2O from denitrification when C_2H_2 is applied and higher levels of N_2 from denitrification when no C_2H_2 is applied (Hunt *et al.* 2007); incomplete denitrification produces higher N_2O:N_2 ratios (Ullah and Zinati 2006).

Possibly the reason why the footslope and lagg hummock were the only two positions to have greater N_2O emissions after the application of solution as compared to

before were low N_2O emission before the application of solution and low variability of emissions after application of solution. The bog hollow had similar low emissions before the application of solution but emissions after the solution were highly variable eliminating significant differences between before and after. The pattern of response was inconsistent and not clear, making it difficult to interpret the results; perhaps the addition of C_2H_2 would have helped by retarding denitrification and increasing the N_2O:N_2 ratio. It may be argued that microbial activity limited N_2O emissions, but because denitrification potential did not increase with additions of glucose to the NO_3^- solution, except for the alder, it is not entirely clear that low rates of microbial activity limited N_2O emissions.

CONCLUSION

There was higher N_2O flux for 2007, the year with deeper water table depth and lower soil moisture content. Since N_2O flux is most likely produced through coupled nitrification - denitrification wherein nitrification produces NO_3^-, the substrate for denitrification, the year with changing soil moisture most likely produced higher rates of NO_3^- than a consistently wetter year. In addition, fluctuating water table levels spurs organic carbon mineralization and nitrification in the peatland allowing for the highest N_2O flux in the watershed, particularly for the alder hollows. However, removal of plant roots from soil samples collected from the lagg and alder landscape types probably removed the differences in soil chemistry.

Although there was higher STC and STN at the upland toeslope, N_2O flux for the toeslope was not greater than the other hillslope positions. This was probably because STC did not limit N_2O flux in any of the hillslope positions. It is likely that nitrification

rates are also similar across hillslope positions because denitrification potential was not different by upland hillslope position.

Adding glucose+nitrate to field sampling chambers does not necessarily increase N_2O production most likely because denitrification was allowed to go to completion without the application of C_2H_2.

POTENTIAL DENITRIFICATION RATES BY LANDSCAPE TYPE FOR AN UPLAND-BOG WATERSHED

INTRODUCTION

Denitrification is the main mechanism for nitrate loss from forested systems (Bedard-Haughn *et al.* 2006, Groffman *et al.* 1993, Groffman and Tiedje 1989) and nitrate is the primary control on denitrification in forested systems (Merrill and Zak 1992). Limits on nitrate supply include organic matter mineralization rates, which are very low in bogs (Verhoeven *et al.* 1990, Vitousek *et al.* 1982).

Korom (1992) listed four basic requirements for denitrification: (1) the presence of inorganic nitrogen; (2) bacteria capable of metabolizing nitrate (NO_3^-) as an electron acceptor; (3) organic carbon as the electron donor; and (4) restricted oxygen availability. Landscape factors, such as hydrology, topography and soil properties, confound a straightforward accounting of Korom's list of requirements (Groffman *et al.* 1993, Hafner and Groffman 2005, Hill *et al.* 2000, Hill *et al.* 2004). It becomes apparent that more information is necessary to understand N_2O production because denitrification responds to changes in these landscape factors.

Soil physical properties influence soil chemical properties

Soil texture controls hydraulic conductivity, which determines hydrological flow (Hafner and Groffman 2005, Hill *et al.* 2004). Hydrological flow affects the distribution of NO_3^- within the soil profile (Hill *et al.* 2004, Groffman *et al.* 1993). In upland soil, Groffman *et al. (*1993) consistently found lower N_2O emissions at well-drained summits as opposed to greater N_2O emissions at poorly drained toeslopes. Studies have shown that the largest loss of available nitrogen was at the top of the hillslope via microbial immobilization whereas the largest loss of available nitrogen at the bottom of the hillslope was due to denitrification (Groffman *et al.* 1993). Because NO_3^- moves with water; the highest denitrification rates occur where NO_3^- intersects with organic carbon (Baker and Vervier 2004). The co-occurrence of NO_3^- and organic carbon may be due to *in situ* mineralization or intersecting hydrological flow paths. The location in the landscape that is most likely to have the highest N_2O emissions due to higher *in situ* mineralization of organic matter and intersecting flow paths is the lowest part of the landscape (Groffman *et al.* 1993, Hill *et al.* 2004, Izaurralde *et al.* 2004, Jørgensen *et al.*2004) which is the lagg in an upland-bog watershed. Upland-bog watersheds are comprised of three distinct landscape types; upland, lagg and bog. The lagg is the lowest landscape elevation and lies at the circumference around the bog next to the upland toeslope. The lagg receives water with dissolved nutrients and carbon from the continuously throughout the year from the bog, but only in runoff during spring snowmelt from the upland (Kolka *et al.* 2001, Verry and Timmons 1982).

Davidsson and Ståhl (2000) found higher denitrification rates in the presence of organic soil particles than with dissolved organic carbon. They concluded that microbial populations occupied the surfaces of the organic matter particles, thus being in better

contact with NO_3^- supplies. It may also be that these organic matter particulates are the anaerobic microsites described by Parkin's classic paper (1987). Parkin discovered that microbial respiration consumes oxygen in the small pore spaces of the soil aggregate faster than oxygen can diffuse from the matrix into the aggregate. This creates anaerobic microsites within an otherwise oxic matrix and likely the site for denitrification and N_2O emissions.

There are three conditions that retard air exchange between the atmosphere and soil; soil wetness, depth in the profile and the accumulation of soil CO_2. Soil water impedes the movement of soil air from the atmosphere to the spaces between soil particles. Deeper soil has lower partial pressure of air than soil near the surface because in part, the tortuous path air has to travel from the atmosphere to soil depth. Denitrifiers require soil organic carbon (SOC) for reduction of NO_3^- (Davidsson and Ståhl 2004); SOC and NO_3^- decrease with soil depth so denitrifier populations would be expected to decline with depth (Alvarez and Alvarez 2001).

Landscape factors influence denitrification

Upland-bog watersheds are excellent landscapes for studying how gradients influence the conditions that support denitrification. The catena of soil from the mineral upland to the organic lagg and bog may be sampled for SOC and NO_3^-, two major factors that control denitrification. Bogs are primarily sphagnum moss, a plant and the soil's parent material, which creates acid organic soils that tend to inhibit microbial activity (Williams and Crawford 1983). Upland mineral soils are less acid and more rapid rates of microbial activity favoring organic matter mineralization and nitrification. The lagg, the lowest elevation in the watershed, has a unique vegetative community within the

watershed. The lagg community is the only community in the watershed that may be populated by alder, a plant with nitrogen fixing nodules on its roots that may enhance soil N. Alder species compete and propagate extremely well in flooded areas like the lagg (Dirr 1998).

Previous studies have assessed the importance of SOC and soil NO_3^- on denitrifier activity. N_2O evolution was highest from soils developed under wet-dry cycles (Garcia-Montiel *et al.* 2003, Hunter and Faulkner 2001). It was explained that during the dry cycles microbial nitrifiers used the sources of SOC for energy and that during the wet cycles, the heterotrophic denitrifiers were then able to take advantage of the sources of NO_3^- for denitrification. Garcia-Montiel *et al.* (2003) and Hunter and Faulkner (2001) suggested that forest soils with lower pH may be populated by heterotrophic nitrifiers such as fungi, and that the fungal decomposition activity on non-water soluble sources of SOC (i.e. cellulose) may explain the low correlation between N_2O emissions and SOC. Results from Chapter One found no significant correlation between STC and N_2O flux. Denitrifiers are facultative aerobic heterotrophs that do not rely on denitrification for growth (Rich *et al.* 2003) and this may help explain the high spatial and temporal variability of denitrification (Hunter and Faulkner 2001).

Parson's *et al.* (1991) examined the differences in a Denitrification Enzyme Assay (DEA) by rates of N_2O production between well drained and poorly drained soil and Hunt *et al.* (2007) investigated DEA for three soil depths. Parsons *et al.* (1991) found good correlation between denitrification and microbial respiration rates as measured by CO_2 evolution. Parkin *et al.* (1991) also noted that while the two study soils were well supplied with SOC; denitrification was limited by soil carbon because DEA rates

increased with additional carbon. Hunt *et al.* (2007) found SOC, soil total N and C:N were good predictors of DEA rates. They found regressions between DEA rates and soil total carbon and soil total N were significant, regressions with SOC were better predictors of DEA variability. Hunt *et al.* (2007) found higher DEA rates for soils with C:N of 25 or less and this was even when there were wide variations in DEA rates across the landscape. Higher rates of incomplete denitrification were attributed to soil from the lower profile where the C:N was greater than 25. Hunt also found higher DEA rates when soil was incubated with glucose+nitrate solution indicating DEA was limited by both carbon and nitrogen, although denitrification rates were not significantly correlated with soil NO_3^-.

Why a DEA for a forested upland-bog watershed

Denitrification studies often focus on agricultural soils or soils impacted by additions of nitrogen. Agricultural practices add nitrogen fertilizers, sometimes in excess of crop uptake and knowing the fate of nitrogen is important (Zanner and Bloom 1995). Yet, our knowledge of how nitrogen is processed in systems without agricultural inputs may provide important information for understanding how topography influences critical nitrogen processes.

Microbial activities determine whether a system is a source or sink for N_2O. On a molecular basis, N_2O has more influence on global climate change than CO_2 (Freeman *et al.* 1993). This makes understanding denitrification and the conditions that influence denitrification over the course of the year important (Anderson and Peterson 2009). Understanding how denitrifying microorganisms process ambient levels of nitrogen across a landscape over time is vital background information. This is especially true

when the landscape has soil that has developed from different parent materials, moisture regimes and vegetative cover, those factors that influence two controlling factors for denitrification; soil NO_3^- and SOC (Dinsmore *et al.* 2009, Hunt *et al.* 2003, Klemedtsson *et al.* 2005).

Denitrification is notoriously variable (Garcia-Montiel *et al.* 2003, Hunt *et al.* 2007, Hunter and Faulkner 2001, Rich *et al.* 2003). Laboratory experiments are specifically designed to control for the condition variability, although laboratory DEA studies using forest soil have high variability (Yanai *et al.* 2003). One method for handling soil heterogeneity is to employ a stratified random sampling such as in this investigation.

Hypotheses

The purpose of the laboratory DEA test is to isolate the important controls on denitrification through testing of the following hypotheses: (1) Denitrification potential for the upland is limited by soil NO_3^- (Christensen *et al.* 1990, Henrich and Haselwandter 1997). Therefore, denitrification rates will be greater when soil from the A horizon and 10 cm below the A horizon is incubated with nitrate solution. The toeslope will have the highest denitrification potential of the upland hillslope positions because the toeslope is least limited in soil total nitrogen; (2) Denitrification potential for the lagg will be greater than for the upland and the bog. While denitrification is not limited by SOC for the lagg and bog, the lagg has higher biotic diversity, microbial activity and therefore larger denitrifier populations and higher soil NO_3^- than the bog. Bog soil has an acid pH, inhibiting microbial denitrification, while lagg and upland soil pH is less acid. The lagg is the lowest elevation of the entire watershed and experiences wet-dry cycles throughout

the year, triggering organic matter mineralization events that produce denitrification substrates; SOC and soil NO_3^- (Venterink *et al.* 2002); (3) Denitrification potential for the lagg with alder (from here on labeled alder) is higher than all other landscape types. *Alnus spp*. has N-fixing bacteria in its root nodules which supports bacterial production of mineral N in the rhizosphere. The alder landscape position is expected to have the highest total nitrogen and NO_3^- of the entire watershed, thereby supporting the highest denitrification rates; (4) Denitrification potential for the peatland 0-25 cm depth is greater than for the 25-50 cm depth just as denitrification potential across the upland is highest for the A horizon as compared to 10 cm below the A horizon. Plant litter falls on the surface and is readily available to aerobic decomposers, supplying STC and soil NO_3^- to denitrifier populations. In addition, plant roots occupy the top layer of soil which makes a positive contribution to soil nutrient status and sources of labile organic carbon for microbial denitrification; (5) Denitrification activity in fall is higher than during the growing season. Soil collected in the summer during active plant uptake of NO_3^- will restrict NO_3^- availability for denitrifier activity. Soil collected in the fall after leaf fall will have greater soil NO_3^- than during the summer resulting in more soil NO_3^- available to denitrifiers.

This study is being conducted in an upland-bog watershed with an aspen-birch forest upland surrounding a slightly convex bog having a black spruce and larch forest. The zone between the outer edge of the bog and the lower edge of the upland is called the lagg. During snowmelt and extremely high rainfall events, water from the upland enters the lagg and given the slightly convex shape of the bog, water moves more or less continuously from the center of the bog toward the lagg. The lagg is comprised of two

zones with shrub differentiated by the presence of alder. This study is designed to assess potential and actual N_2O production over the course of a year, by season, landscape type and topographic position.

METHODS

Site Description

The study was located within an upland-peatland watershed at the Marcell Experimental Forest (MEF) in north-central Minnesota (Figure 2-1). MEF is a long-term study area located in the US Forest Service's Chippewa National Forest. The Northern Research Station of the USFS has collected environmental data at MEF since 1961. The watershed used in this study was the S2 watershed, one of the most studied peatland systems in the world (Figure 2-1).

The climate of the MEF is subhumid continental, with wide and rapid diurnal and seasonal temperature fluctuations. Average annual air temperature is 3°C (37°F), with extremes of -46°C (-51°F) and 38°C (100°F). Average January temperature is -15°C (5°F), and the average July temperature is 19°C (66°F). Average annual precipitation at the MEF is 78.5 cm, with 75% occurring in the snow-free period (mid-April to early November). In 1975 there was an average of 75 rainstorms each year of which normally only 3 - 4 exceeds 2.5 cm (Verry 1975); since then, according to data from NOAA (www.ncdc.noaa.gov/oa/climate/normal/usnormals.html) there was an increase in the number of days with heavier than average precipitation and higher average precipitation than reported in 1975. The S2 watershed contains a 3.2-ha bog dominated by mature black spruce (*Picea mariana*) (pH at the watershed outlet is 3.9 ± 0.2) and a 6.5-ha upland dominated by mature trembling aspen (*Populus tremuloides*) and paper birch

(*Betula papyrifera*) (Verry 1975). Soil in the watershed is mostly the Loxley series (Dysic, frigid Typic Haplosaprist) in the bog and the Warba series (mixed, superactive, frigid Haplic Glossufalf) in the upland (Soil Survey Staff 2009).

Sampling design

The sample design was based on the topography of the S2 watershed. Three transects ran from the upland summit down slope through the lagg to the bog. There was one sample plot per upland slope position: summit, shoulder, backslope, footslope and toeslope, one plot in the lagg and one plot in the bog. There were three more lagg plots with in an area of the lagg that had N_2- fixing vegetation (Speckled alder (*Alnus incana spp.rugosa*). In total 24 sample sites were located (Figure 2-2).

Vegetation in the bog and lagg was primarily mosses and moss-like vegetation such as sphagnum moss (*Sphagnum spp.),* aulacomnium and polytrichum mosses (*Aulacomnium palustre* and *Polytrichum juniperinum)*, and forbs, such as bluebead (*Clintonia borealis)*, pitcher plant *(Sarracenia purpurea)* and cotton grass *(Eriophorum spp)* (Bay 1966). Upland ground cover was forbs, grasses and mosses such as twoflower dwarf dandelion (*Krigia biflora* var. *biflora*), claspleaf twisted stalk (*Streptopus amplexifolius* var. *amplexifolius*), switchgrass (*Panicum virgatum* var. *virgatum*), twinflower (*Linnaea borealis spp. americana*), and club moss (*Lycopodium clavatum* L.) in a mature trembling aspen-paper birch forest.

Soil Sampling and Analysis

Soil was sampled twice, June 2008 and September 2008, for the purpose of comparing DEA results for soil collected during the growing season and soil collected

after the growing season. For laboratory incubations, a soil core was collected from each upland plot that included the A-horizon and at least 10-cm beneath the A-horizon. The core was separated into two samples, one sample of the A-horizon and the second sample 10-cm beneath the A-horizon. There were three cores from each upland landscape position from each of three transects for a total of 30 samples, 15 samples from the A-horizon and 15 samples from 10 cm below the A-horizon. All mineral soils were sieved in the field moist condition before aggregating. Peat soil samples were collected from two lagg positions; lagg with alder and lagg without alder, and one bog position. Soil samples were collected using a 50-cm long Macaulay peat auger. Each auger-full of peat soil was divided in half to separate out the 0 to 25-cm sample and the 25 to 50-cm sample. Peat soil was not sieved in the field moist condition before aggregating, but any non-peat inclusions such as twigs, plant tops or pieces of undecomposed wood were removed by hand. The three samples from each depth per plot were then thoroughly mixed and aggregated into one. Each peatland position had triplicate soil samples resulting from the three transects or 18 samples in total (3 positions X 2 depths X 3 transects). Samples were analyzed for bulk density, pH, exchangeable nitrogen species, total nitrogen, and total carbon. Because of low DEA results for deeper soils in June, September samples were collected from the A-horizon in upland soils and the top 25-cm of soil in peatlands, and because soil chemistry and DEA results were similar between lagg and alder samples, no alder samples were collected in September.

Denitrification Enzyme Assay (DEA)

Denitrification enzyme assay (DEA) with C_2H_2 but without chlorophenicol was used to determine denitrification potential on a watershed scale by comparing the flux of

N_2O gas for upland, lagg, alder and bog under ideal laboratory conditions (Groffman *et al.* 1992). The factors that control microbial activity and, therefore, denitrification, include the amount of nitrate available, the level of oxygen in the soil atmosphere, and the amount and lability of the organic carbon. The DEA method used for this study was a modification of Robertson and Tiedje (1987) for the purpose of accommodating mineral and organic soil. To assess DEA in upland and peatland soils, four lab treatments were investigated. Those four treatments included the no addition control, a carbon addition (labile, organic carbon in the form of glucose), a nitrate addition, and a glucose+nitrate addition. The control treatment was a soil mixed with double distilled water. The carbon treatment was a soil mixed in a solution of 0.180-g of D-glucose for every 1-L of water. The NO_3^- treatment was a soil mixed in a solution of 0.721-g of KNO_3^- for every 1-L of water. The glucose+nitrate treatment was a soil mixed with a solution of 0.180-g of D-glucose and 0.721-g of KNO_3^- for every 1-L of water. The ratio of solution to soil was 10 mL of solution added to 10 g of mineral soil or 5 g of organic soil. Each triplicate hillslope position soil was run in duplicate for each lab treatment, with laboratory blanks consisting of solution without any soil added.

The soil solution was placed in 20-mL serum bottles and sealed with an aluminum cap and rubber septum and incubated at 25^oC. Prior to incubation, the bottles were purged of air and replaced with dinitrogen (N_2) gas to create anaerobic conditions. The purging apparatus consisted of a manifold of 10 hypodermic needles connected to a pump and a N_2 tank. This allowed air to be pumped out of the bottles to a negative pressure of 26.7 atm and then injected with N_2 gas to a positive pressure of 15 atm. This cycle was repeated three times before the final serum bottle pressurization of N_2 was released to 0.5

atm. In the final two minutes of pressurization, acetylene (C_2H_2) was run through a gas cleansing apparatus of two Erlenmeyer flasks filled about a-third of the way with water and then vented through a hypodermic needle inserted into the lid of a canning jar. When the needle was removed, C_2H_2 was allowed to remain in the jar until a sufficient amount was collected to inject the 10 serum bottles with 12-mL of C_2H_2 each. Acetylene stops the denitrification process from going to completion so that the amount of N_2O gas produced indicates the total denitrification activity. The serum bottles were then placed on a shaker table for 30 minutes before a 9-mL gas sample from the headspace in the serum bottle was withdrawn for the time zero sample. The bottles remained on the shaker table for the next hour and were sampled at half-hour intervals for the second and final samples.

Gas samples were analyzed within 7 days of collection with a gas chromatograph (GC) analyzer (5890; Hewlett-Packard, Palo Alto, CA). The area count under the curve produced by the GC is correlated with the total concentration of N_2O gas in the vial. The area count is corrected for the N_2O present in the ambient air in the vial. The corrected area count is used in regression equations to predict N_2O concentrations. Concentrations in *ppm* were converted to ng N g^{-1} hr^{-1} based on headspace volume over the incubation time and mass of soil. Headspace volume was calculated by subtracting the volume of soil (including soil moisture content) and the volume of the solution from the volume of the serum bottle.

Statistical analysis

All statistical analyses were conducted using SPSS for Windows, Release 15.0.0 (SPSS Inc., Chicago, IL, USA). Determination of the differences between mean N_2O

production, SOC, total soil N, soil NH_4^+, soil NO_3^- and soil pH were done using an ANOVA assuming a normal distribution and equal variances among treatments with Tukey multiple range test and determination of homogeneous groups at the $P \leq 0.10$ level of significance. Laboratory DEA rates were correlated with field N_2O production and field DEA rates. Laboratory DEA denitrification rates were analyzed for relationships with soil depth, topographic position, landscape type, SOC, total soil N, soil NO_3^- and soil pH. Linear regression analysis between lab DEA rates, field N_2O production, soil chemistry and field DEA N_2O production were considered significant at the $P \leq 0.10$ level of significance.

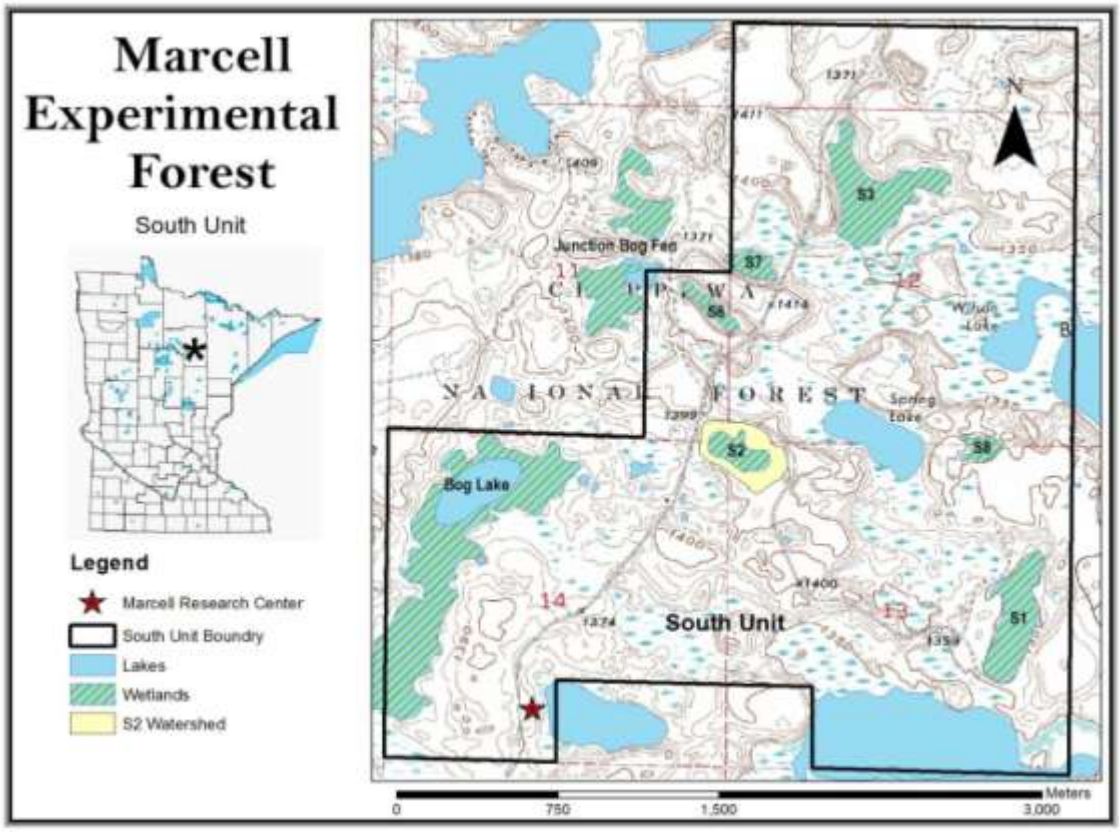


Figure 2-1. The asterisk on the inset map of the state of Minnesota shows the location of Marcell Experimental Forest north of Grand Rapids, Minnesota. The diagram is a depiction of the south unit of the Marcell Experimental Forest . The location of the current study was conducted in the S2 watershed located in the center of the diagram.

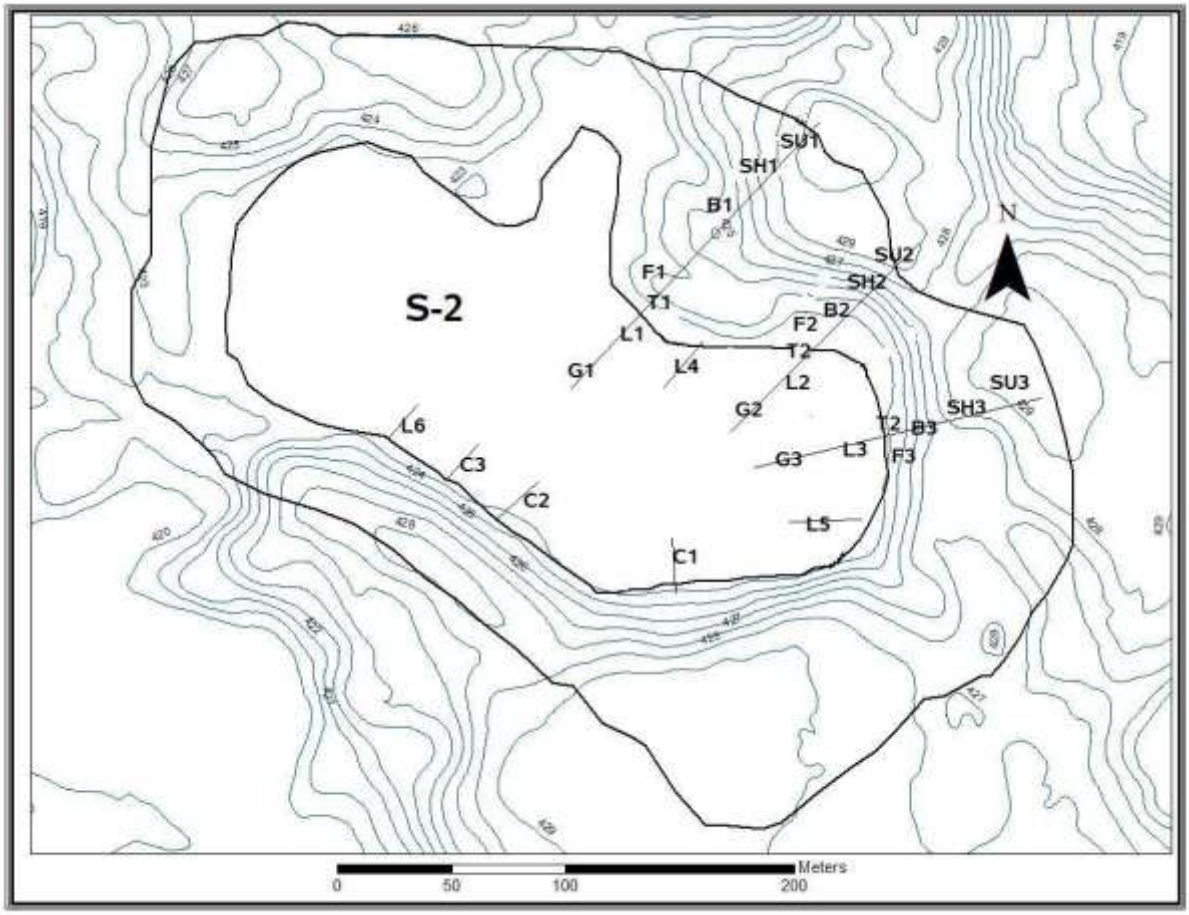


Figure 2-2. The sampling design twenty seven plots and thirty nine trace gas sampling chambers. Three transects plots in the upland, lagg and bog; transect 1: SU1, SH1, B1, F1, T1, L1, G1, transect 2: SU2, SH2, B2, F2, T2, L2, G2 and transect 3: SU3, SH3, B3, F3, T3, L3, G3. There were three lagg plots: L4, L5, L6 and three alder plots; C1, C2 and C3. The plots were located in the S2 watershed of the Marcell Experimental Forest in Grand Rapids, Minnesota.

RESULTS

June 2008 N_2O production in upland A horizon hillslope positions

There were no differences in N_2O production by upland hillslope position for all solutions (Table 2-1). The pattern of N_2O production for each upland hillslope position between the solutions show that when there was a difference, the solutions which supported highest N_2O production were the nitrate and glucose+nitrate solutions (Table 2-2). This was the pattern for the summit, backslope and toeslope. There were no differences by solution for the shoulder. The footslope had higher N_2O production for the nitrate solution but N_2O emission for the glucose+nitrate solution was not significantly different than the glucose and control solutions (Table 2-2).

September 2008 N_2O production in upland A horizon hillslope positions

There were no differences in N_2O production among upland hillslope positions for the control and glucose solutions (Table 2-3).. Incubating soil in the nitrate solution resulted in higher N_2O production for the toeslope

The nitrate solution had the highest N_2O production and the control solution had the lowest N_2O production for each upland hillslope position except for the backslope where there were no differences in N_2O production by solution (Table 2-4).

June 2008 vs. September 2008 N_2O production in upland A horizon hillslope positions

N_2O production by upland A horizon hillslope positions for June and September were not significantly different (not shown).

June 2008 N_2O production 10 cm below the upland A horizon hillslope positions

There was no consistent pattern among hillslope positions for N_2O production by solution. The control solution for the toeslope was greater than for the backslope (Table

2-5). For the glucose, nitrate and glucose+nitrate solutions, the toeslope was greater than the footslope and the shoulder (Table 2-5). The control solution produced the highest N_2O production for the shoulder, backslope and toeslope. There were mixed results for the summit and footslope (Table 2-6).

June 2008 N_2O production A horizon vs. 10 cm below the upland A horizon hillslope positions

There were higher levels of N_2O production for the A horizon summit, shoulder, backslope and footslope than for soil collected from 10 cm below the A horizon when incubated with nitrate solution; otherwise there were no significant differences in N_2O production by soil depth (not shown).

June 2008 N_2O production upland A horizon, 0-25 cm lagg, 0-25 cm alder and 0-25 cm bog

The N_2O production for the alder was highest for the nitrate solution (Table 2-7). The bog had the highest N_2O production for the glucose solution although there was no difference in N_2O production by solution for the bog. The glucose+nitrate solution produced the highest N_2O production for the alder and the lowest N_2O production for the upland and bog (Table 2-7).

The upland had the highest N_2O production for the nitrate and glucose+nitrate solutions (Table 2-8). The lagg had the highest production for the nitrate and glucose+nitrate solutions. The alder had the highest N_2O production for the glucose+nitrate solution. There were no significant differences in N_2O production by solution for the bog (Table 2-8).

September 2008 N_2O production upland A horizon, 0-25 cm lagg and 0-25 cm bog

There were no differences in N_2O production by landscape type for the control solution (Table 2-9). The lagg had the highest N_2O production and the bog had the lowest N_2O production for the glucose and nitrate solutions. The upland and lagg had the highest N_2O production and the bog had the lowest N_2O production for the nitrate solution. (Table 2-9).

The highest N_2O production for the upland was for the nitrate solution with no significant differences for the control and glucose solutions (Table 2-10). The highest N_2O production for the lagg was for the glucose solution and no significant differences for the control and nitrate solutions. There were no differences in N_2O production by solution for the bog landscape type (Table 2-10).

June 2008 vs. September 2008 N_2O production upland A horizon, 0-25 cm lagg, 0-25 cm alder and 0-25 cm bog

There were no significant differences in N_2O production for the upland and bog by date for the control, glucose and nitrate solutions. There was significantly higher N_2O production in the lagg for the September control and glucose solutions than during June.

June 2008 N_2O production 10 cm below upland A horizon, 25-50 cm lagg, 25-50 cm alder and 25-50 cm bog

Incubation with the control solution produced significantly higher rates of N_2O for the 25-50 cm bog than the 25-50 cm lagg and alder (Table 2-11) The lowest N_2O production was for 10 cm below the A horizon upland (Table 2-11). The glucose solution produced the highest rates of N_2O production for the 25-50 cm lagg, alder and bog while the lowest N_2O production was for 10 cm below the A horizon upland. Incubation with nitrate and glucose+nitrate solutions produced more N_2O for the 25-50 cm alder than the 25-50 cm lagg, 25-50 cm bog and 10 cm below the A horizon upland. There were no

significant differences in N_2O production for the 25-50 cm bog and 10 cm below the A horizon upland for the nitrate and glucose+nitrate solutions (Table 2-11).

N_2O production was highest when incubated with control solution and lower when incubated with the other solutions for all landscape types (Table 2-12).

June 2008 N_2O production upland A horizon, 0-25 cm lagg and 0-25 cm bog vs. 10 cm below upland A horizon, 25-50 cm lagg and 25-50 cm bog

Nitrous oxide production for the upland was greater for the A horizon than 10 cm below for the nitrate and glucose+nitrate solutions except for the control solution which produced the highest levels of N_2O for 10 cm below the A horizon (not shown). There was no difference in N_2O production for the glucose solution by upland soil depth (not shown).

Nitrous oxide production was greater for 25-50 cm than 0-25 cm alder for the control solution; there were no differences in N_2O production for the other DEA solutions (not shown). There was no difference in N_2O production for the lagg and bog by depth (not shown).

Soil properties June and September A horizon upland

Soil pH and bulk density increased with hillslope elevation whereas STC and STN was greatest for the toeslope (Table 2-13). The toe and footslope had the lowest pH and the toeslope had the lowest bulk density of the hillslope positions. The toeslope had the highest STC and STN for both June and September. There were no significant differences in soil NH_4^+ (SNH_4^+) and soil NO_3^- (SNO_3^-) by upland hillslope position (Table 2-13).

Upland STC and SNH_4^+ were higher for June than September but there were no significant differences for upland STN and SNO_3^- by date (not shown).

Soil properties 10 cm below A horizon upland hillslope positions

There were no significant differences in soil pH, bulk density and soil NO_3^- (SNO_3^-) by upland hillslope position for the upland 10 cm below the A horizon (Table 2-14). There was significantly more soil total carbon (STC) for the toeslope than the other hillslope positions. The shoulder had the lowest soil total N (STN). There was no pattern for soil NH_4^+ (SNH_4^+) by upland hillslope position (Table 2-14).

Soil properties June and September upland A horizon, 0-25 cm lagg, 0-25 cm alder and 0-25 cm bog

The upland had higher soil pH and bulk density than the lagg, alder and bog (Table 2-15). The upland had lower soil total carbon (STC), soil total nitrogen (STN) than the lagg, alder and bog for June and September (Table 2-15). The lagg and alder had higher STN than the bog for June but there was no difference in STN between the lagg and bog for September. There was no difference in SNH_4^+ by landscape type for June. The bog had higher SNH_4^+ than the upland and lagg for September. There was no difference in SNO_3^- by landscape type for June and September (Table 2-15).

Soil properties June 10 cm below the A horizon upland, 25-50 cm lagg, 25-50 cm alder and 25-50 cm bog

There were no significant differences in soil pH by landscape type but upland bulk density was significantly greater than the peatland landscape types (Table 2-16). Total soil carbon for the bog was significantly greater than for the other landscape types, with the upland having the lowest soil total carbon content. The lagg, alder and bog landscape types had similar levels of soil total nitrogen and the upland had the lowest soil total nitrogen content. There were similarities in soil NH_4^+ content among landscape

types except for the bog which had the lowest soil NH_4^+ content. Soil NO_3^- was below the detection limit for all landscape types.

Soil properties June 2008 A horizon upland, 0-25 cm lagg, 0-25 cm alder and 0-25 cm bog vs. 10 cm below A horizon upland, 25-50 cm lagg, 25-50 cm alder and 25-50 cm bog

The upland had higher levels of STC and STN in the A horizon than 10 cm below the A horizon. The alder landscape type had significantly higher pH for 25-50 cm than 0-25 cm (data not shown). Soil bulk density was statistically greater for the 25-50 cm for lagg and bog but not for alder (data not shown).

There was significantly more STC for 25-50 cm than 0-25 cm for the lagg and bog (data not shown). There was significantly more STN for 25-50 cm than 0-25 cm for lagg, alder and bog. Soil NO_3^- was not significantly greater than the detection limit and there were no statistical differences in soil NO_3^- by peatland landscape types by depth (data not shown).

Regression Analysis

The average C:N for the upland was below 25 and the average C:N for the peatland was greater than 25. There was a positive relationship between N_2O production for the nitrate solution and C:N for the upland and a weak, negative relationship between N_2O production for the nitrate solution and C:N for the peatland (Figures 2-3 and 2-4).

Table 2-1. N_2O production for June 2008 by upland hillslope position for the A horizon for the S2 watershed in the Marcell Experimental Forest , Grand Rapids, MN. N_2O production was the average of three measurements over time for each of three samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among positions and are ranked alphabetically in descending order.

Hillslope position	Control		Glucose		Nitrate		Glucose+Nitrate	
					$ng\ N\ g^{-1}h^{-1}$			
Summit	71.9 (59.0)	a	11.1 (5.9)	a	200.2 (15.7)	a	291.9 (110.9)	a
Shoulder	76.1 (57.8)	a	55.1 (54.2)	a	127.6 (39.9)	a	223.0 (94.1)	a
Backslope	9.0 (8.2)	a	2.2 (0.4)	a	189.7 (4.4)	a	212.2 (24.5)	a
Footslope	5.4 (4.8)	a	4.3 (2.0)	a	275.2 (98.8)	a	116.5 (39.6)	a
Toeslope	0.9 (0.8)	a	9.3 (4.7)	a	380.2 (95.0)	a	755.7 (463.2)	a

Table 2-2. N_2O production for June 2008 by DEA solution for the A horizon of each upland hillslope position for the S2 watershed in the Marcell Experimental Forest , Grand Rapids, MN. N_2O production was the average of three measurements over time for each of three samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among solutions and are ranked alphabetically in descending order.

DEA solution	Summit		Shoulder		Backslope		Footslope		Toeslope	
					ng N $g^{-1}h^{-1}$					
Control	71.9 (59.0)	bc	76.1 (57.8)	a	9.0 (8.2)	b	5.4 (4.8)	b	0.9 (0.8)	b
Glucose	11.1 (5.9)	c	55.1 (54.2)	a	2.2 (0.4)	b	4.3 (2.0)	b	9.3 (4.7)	b
Nitrate	200.2 (15.7)	ab	127.6 (39.9)	a	189.7 (4.4)	a	275.2 (98.8)	a	380.2 (95.0)	ab
Glucose+Nitrate	291.9 (110.9)	a	223.0 (94.1)	a	212.2 (24.5)	a	116.5 (39.6)	b	755.7 (463.2)	a

Table 2-3. N_2O production for September 2008 by upland hillslope position for the A horizon of the S2 watershed in the Marcell Experimental Forest, Grand Rapids, MN. N_2O production was the average of three measurements over time for each of three samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among positions and are ranked alphabetically in descending order.

Hillslope position	Control		Glucose		Nitrate	
			$ng\ N\ g^{-1}h^{-1}$			
Summit	2.3 (1.9)	a	50.2 (36.9)	a	194.7 (22.4)	ab
Shoulder	11.4 (4.6)	a	32.9 (6.8)	a	123.9 (33.2)	b
Backslope	26.7 (23.5)	a	126.4 (109.2)	a	210.2 (42.7)	ab
Footslope	11.5 (5.8)	a	16.4 (13.3)	a	252.2 (81.5)	ab
Toeslope	6.0 (3.4)	a	15.8 (8.7)	a	368.3 (141.4)	a

Table 2-4. N_2O production for September 2008 by DEA solution for the upland A horizon hillslope positions of the S2 watershed in the Marcell Experimental Forest, Grand Rapids, MN. N_2O production was the average of three measurements over time for each of three samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among solutions and are ranked alphabetically in descending order.

DEA Solution	Summit	Shoulder	Backslope	Footslope	Toeslope
		$ng\ N\ g^{-1}h^{-1}$			
Control	2.3 (1.9) b	11.4 (4.6) b	26.7 (23.5) a	11.5 (5.8) b	6.0 (3.3) b
Glucose	50.2 (36.9) ab	32.9 (6.8) ab	123.4 (109.2) a	16.4 (13.3) ab	15.8 (8.7) ab
Nitrate	194.7 (22.4) a	123.9 (32.2) a	210.2 (42.7) a	252.2 (81.5) a	368.3 (141.4) a

Table 2-5. N_2O production for June 2008 by upland hillslope position for 10 cm below the A horizon for the S2 watershed in the Marcell Experimental Forest, Grand Rapids, MN. N_2O production was the average of three measurements over time for each of three samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among positions and are ranked alphabetically in descending order.

Hillslope position	Control	Glucose	Nitrate	Glucose+Nitrate
		$ng\ N\ g^{-1}h^{-1}$		
Summit	185.0 (127.6) a	11.9 (7.5) ab	5.5 (2.7) ab	21.1 (9.4) ab
Shoulder	98.6 (31.6) a	8.1 (6.0) ab	1.3 (0.4) b	5.2 (3.5) b
Backslope	51.0 (23.6) a	3.8 (2.4) b	7.5 (5.3) ab	16.1 (8.6) ab
Footslope	58.5 (32.6) a	18.4 (8.9) ab	1.6 (0.2) b	5.4 (3.6) b
Toeslope	77.6 (22.4) a	23.5 (9.4) a	10.7 (2.7) a	34.3 (16.3) a

Table 2-6. N_2O production for June 2008 by DEA solution for 10 cm below the A horizon in the S2 watershed of the Marcell Experimental Forest, Grand Rapids, MN. N_2O production was the average of three measurements over time for each of three samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among solutions and are ranked alphabetically in descending order.

DEA solution	Summit	Shoulder	Backslope	Footslope	Toeslope
		$ng\ N\ g^{-1}h^{-1}$			
Control	185.0 (127.6) a	98.6 (31.6) a	51.0 (23.6) a	58.5 (32.6) a	77.6 (22.4) a
Glucose	11.89 (7.6) b	8.1 (6.0) b	3.8 (2.4) b	18.4 (8.9) a	23.5 (9.4) b
Nitrate	5.48 (2.7) b	1.3 (0.4) b	7.5 (5.3) b	1.6 (0.2) ab	10.7 (2.6) b
Glucose+Nitrate	21.1 (9.4) ab	5.2 (3.5) b	16.1 (8.6) b	5.4 (3.6) b	34.3 (16.3) b

Table 2-7. N_2O production for the upland A horizon, 0-25 cm lagg, 0-25 cm alder and 0-25 cm bog landscape types of the S2 watershed at the Marcell Experimental Forest June 2008. N_2O production was the average of three samples over time for fifteen samples for the upland and three samples each for the lagg, alder and bog with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among landscape types and are ranked alphabetically in descending order.

Landscape type	Control	Glucose	Nitrate	Glucose+Nitrate
			$ng\ N\ g^{-1}h^{-1}$	
Upland	16.4 (10.6) b	16.4 (10.6) b	234.6 (33.6) b	319.8 (102.0) c
Lagg	12.1 (9.2) b	90.1 (33.8) b	703.4 (324.0) a	1375.5 (853.8) b
Alder	147.5 (81.0) a	37.7 (20.0) b	551.6 (343.2) a	2570.9 (1139.6) ab
Bog	93.1 (72.1) b	221.2 (110.2) a	58.4 (25.5) b	99.5 (51.1) c

Table 2-8. N_2O production for the upland A horizon, 0-25 cm lagg, 0-25 cm alder and 0-25 cm bog landscape types of the S2 watershed at the Marcell Experimental Forest June 2008. N_2O production for the control, glucose, nitrate and glucose+nitrate treatments according to the lab DEA protocol were the average of three samples over time for fifteen samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among solutions and are ranked alphabetically in descending order.

DEA Solution	Upland	Lagg	Alder	Bog
			$ng\ N\ g^{-1}h^{-1}$	
Control	16.4 (10.6) b	12.1 (9.2) b	147.5 (81.0) b	93.1 (72.1) a
Glucose	16.4 (10.6) b	90.1 (33.8) b	37.7 (20.0) b	221.2 (110.2) a
Nitrate	234.6 (33.6) a	703.4 (324.0) ab	551.6 (343.2) b	58.4 (25.5) a
Glucose+Nitrate	319.8 (102.0) a	1375.5 (853.8) a	2570.9 (1139.6) a	99.5 (51.1) a

Table 2-9. N_2O production for September 2008 by landscape type for the upland A horizon, 0-25 cm lagg and 0-25 cm bog landscape types of the S2 watershed at the Marcell Experimental Forest . N_2O production for the control treatment according to the lab DEA protocol was the average of three samples over time for fifteen samples for the upland and three samples each for the lagg and bog with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among landscape types and are ranked alphabetically in descending order.

Landscape type	Control	Glucose	Nitrate
		$ng\ N\ g^{-1}h^{-1}$	
Upland	11.56 (4.8) a	48.32 (22.5) b	229.87 (36.4) a
Lagg	175.67 (44.8) a	893.64 (273.2) a	388.76 (144.4) a
Bog	94.69 (12.3) a	203.45 (91.3) b	69.77 (28.2) b

Table 2-10. N_2O production for September 2008 by DEA solution for the upland A horizon landscape type of the S2 watershed at the Marcell Experimental Forest . N_2O production for the control, glucose, nitrate and glucose+nitrate treatments according to the lab DEA protocol were the average of three samples over time for fifteen samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among solutions and are ranked alphabetically in descending order.

DEA Solution	Upland	Lagg	Bog
		$ng\ N\ g^{-1}h^{-1}$	
Control	11.56 (4.8) b	175.67 (44.8) b	94.69 (12.3) a
Glucose	48.32 (22.5) b	893.64 (273.2) a	203.45 (91.3) a
Nitrate	229.87 (36.4) a	388.76 (144.4) b	69.77 (28.2) a

Table 2-11. N_2O production for June 2008 by landscape type for 10 cm below the A horizon upland and 25-50 cm lagg and 25-50 cm bog landscape types of the S2 watershed at the Marcell Experimental Forest . N_2O production was the average of three samples over time for fifteen samples for the upland and three samples each for the lagg and bog with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among landscape types and are ranked alphabetically in descending order.

	Control	Glucose	Nitrate	Glucose+Nitrate
Landscape type		$ng\ N\ g^{-1}h^{-1}$		
Upland	94.1 (26.9) c	13.1 (3.4) b	5.3 (1.5) c	16.4 (4.6) c
Lagg	1174.9 (492.6) b	300.3 (88.4) a	120.7 (34.4) b	198.9 (16.4) a
Alder	1162.7 (309.2) b	228.9 (146.0) a	174.1 (56.2) a	164.5 (15.8) b
Bog	2608.1 (1363.2) a	224.8 (195.8) a	13.7 (5.4) c	20.9 (0.1) c

Table 2-12. N_2O production for June 2008 by DEA solution for 10 cm below the upland A horizon, 25-50 cm lagg and 25-50 cm bog landscape types of the S2 watershed at the Marcell Experimental Forest. N_2O production for the control, glucose, nitrate and glucose+nitrate treatments according to the lab DEA protocol were the average of three samples over time for fifteen samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among solutions and are ranked alphabetically in descending order.

	Upland	Lagg	Alder	Bog
DEA Solution		*ng N $g^{-1}h^{-1}$*		
Control	94.1 (26.9) a	1174.9 (492.6) a	1162.7 (309.2) a	2608.1 (1363.2) a
Glucose	13.1 (3.4) b	300.3 (88.4) b	228.9 (146.0) b	224.8 (195.8) b
Nitrate	5.3 (1.5) b	120.7 (34.4) b	174.1 (56.2) b	224.8 (195.8) b
Glucose+Nitrate	16.4 (4.6) b	198.9 (16.4) b	164.5 (15.8) b	20.9 (0.1) b

Table 2-13. Soil pH and bulk density for the A horizon upland hillslope positions of the S2 watershed at the Marcell Experimental Forest sampled June and September 2008. Soil pH was the average of six samples and bulk density was the average of three samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among positions and are ranked alphabetically in descending order. The letters b.d. in place of a numerical value indicate values below the detection limit (0.02 mg kg^{-1})

	2008					
Hillslope position	June pH	June Bulk density	June STC	September STC	June STN	September STN
		Mg m^{-3}	g kg^{-1}			
Summit	5.60 (0.13) a	0.77 (0.04) a	54.04 (8.80) b	35.88 (5.83) b	2.47 (0.37) b	1.96 (0.20) b
Shoulder	5.57 (0.17) a	0.72 (0.06) a	43.34 (6.47) b	42.29 (8.33) b	2.09 (0.16) b	2.24 (0.27) b
Backslope	5.41 (0.16) a	0.65 (0.07) b	54.74 (12.47) b	41.08 (7.93) b	2.68 (0.38) b	2.14 (0.41) b
Footslope	5.14 (0.16) b	0.61 (0.03) b	71.36 (14.30) b	55.86 (12.14) b	2.87 (0.43) b	2.43 (0.46) b
Toeslope	4.81 (0.26) b	0.43 (0.07) c	121.66 (23.49) a	93.52 (17.95) a	4.93 (1.08) a	3.81 (0.75) a

	2008			
Hillslope position	June SNH_4^+	September SNH_4^+	June SNO_3^-	September SNO_3^-
	mg kg^{-1}			
Summit	8.13 (5.32) a	1.72 (1.09) a	b.d.	0.63 (0.63)
Shoulder	13.58 (9.63) a	1.02 (1.02) a	0.01 (0.01)	b.d.
Backslope	11.65 (5.85) a	b.d.	b.d.	b.d.
Footslope	3.05 (1.71) a	b.d.	b.d.	b.d.
Toeslope	11.22 (5.66) a	b.d.	b.d.	b.d.

Table 2-14. Soil pH and bulk density for 10 cm below the A horizon upland hillslope positions of the S2 watershed at the Marcell Experimental Forest sampled June 2008. Soil pH was the average of six samples and bulk density, soil total carbon (STC), soil total nitrogen (STN), soil NH_4^+ (SNH_4^+) and soil NO_3^- (SNO_3^-) were the average of three samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among positions and are ranked alphabetically in descending order. The letters b.d. in place of a numerical value indicate values below the detection limit (0.02 mg Kg^{-1}).

Hillslope position	pH	Bulk density	2008 June STC	STN	SNH_4^+	SNO_3^-
		Mg m^{-3}	g kg^{-1}		mg Kg^{-1}	
Summit	5.37 (0.32) a	1.23 (0.09) a	9.73 (1.79) b	0.64 (0.14) a	3.07 (1.54) b	b.d.
Shoulder	5.46 (0.34) a	1.22 (0.20) a	5.50 (0.51) b	0.36 (0.01) b	6.43 (0.29) a	0.01 (0.01)
Backslope	5.19 (0.30) a	1.45 (0.09) a	9.64 (2.67) b	0.74 (0.19) a	8.93 (2.18) a	b.d.
Footslope	5.16 (0.39) a	1.44 (0.17) a	8.85 (1.32) b	0.51 (0.05) a	1.53 (0.77) b	b.d.
Toeslope	4.95 (0.49) a	1.46 (0.10) a	12.65 (2.47) a	0.65 (0.14) a	5.50 (2.18) ab	b.d.

Table 2-15. Soil pH, bulk density, soil total carbon (STC), soil total nitrogen (STN), soil NH_4^+ (SNH_4^+) and soil NO_3^- (SNO_3^-) for A horizon upland, 0-25 cm lagg, 0-25 cm alder and 0-25 cm bog of the S2 watershed at the Marcell Experimental Forest sampled June 2008. Soil pH was the average of six samples and bulk density, soil total carbon (STC), soil total nitrogen (STN), soil NH_4^+ (SNH_4^+) and soil NO_3^- (SNO_3^-) were the average of three samples with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among landscape types and are ranked alphabetically in descending order. Alder soil samples were not collected in September and is represented with a dash, "-". The letters b.d. in place of a numerical value indicate values below the detection limit (0.02 mg Kg^{-1}).

	2008					
	June		June	September	June	September
Landscape type	pH	Bulk density	STC	STC	STN	STN
		$Mg\ m^{-3}$		$g\ kg^{-1}$		
Upland	5.31 (0.09) a	0.64 (0.04) a	69.03 (9.20) b	53.72 (7.04) b	3.01 (0.34) c	2.52 (0.25) b
Lagg	4.51 (0.16) b	0.12 (0.02) b	439.39 (9.71) a	439.91 (5.25) a	17.21 (0.29) a	15.79 (0.39) a
Alder	4.42 (0.02) bc	0.02 (0.01) b	445.39 (9.39) a	-	17.90 (0.75) a	-
Bog	3.87 (0.25) c	0.10 (0.00) b	448.76 (0.26) a	460.48 (5.70) a	13.09 (0.80) b	14.67 (0.78) a

	2008			
	June	September	June	September
Landscape type	SNH_4^+	SNH_4^+	SNO_3^-	SNO_3^-
		$mg\ kg^{-1}$		
Upland	9.53 (2.53) a	0.55 (0.32) b	0.06 (0.04)	0.06 (0.04) a
Lagg	1.47 (0.29) a	0.53 (0.41) b	b.d.	b.d.
Alder	10.96 (4.59) a	-	b.d.	0.01 (0.01) a
Bog	10.85 (4.70) a	3.13 (2.22) a	b.d.	b.d.

Table 2-16. Soil pH, bulk density, soil total carbon (STC), soil total nitrogen (STN), soil NH_4^+ (SNH_4^+) and soil NO_3^- (SNO_3^-) for 10 cm below the A horizon upland, 0-25 cm lagg, 25-50 cm alder and 25-50 cm bog of the S2 watershed at the Marcell Experimental Forest sampled June 2008. Soil pH was the average of fifteen samples for the upland and three samples for the lagg, alder and bog with the standard error in parentheses. Letters represent statistical differences at the $P \leq 0.10$ level of significance among landscape types and are ranked alphabetically in descending order. The letters b.d. in place of a numerical value indicate values below the detection limit (0.02 mg Kg^{-1}).

Landscape type	pH	Bulk density	STC	STN	SNH_4^+	SNO_3^-
		2008 June				
		$Mg\ m^{-3}$	$g\ Kg^{-1}$		$mg\ Kg^{-1}$	
Upland	5.23 (0.15) a	1.36 (0.06) a	9.28 (0.95) d	0.58 (0.06) b	5.09 (0.91) ab	b.d.
Lagg	5.08 (0.62) a	0.21 (0.01) b	463.70 (3.29) b	16.40 (0.23) a	9.18 (8.27) a	b.d.
Alder	4.71 (0.03) a	0.02 (0.01) b	434.00 (15.24) c	16.35 (0.58) a	5.13 (4.91) ab	b.d.
Bog	4.89 (0.49) a	0.18 (0.01) b	492.21 (7.53) a	15.96 (0.23) a	0.27 (0.27) b	b.d.

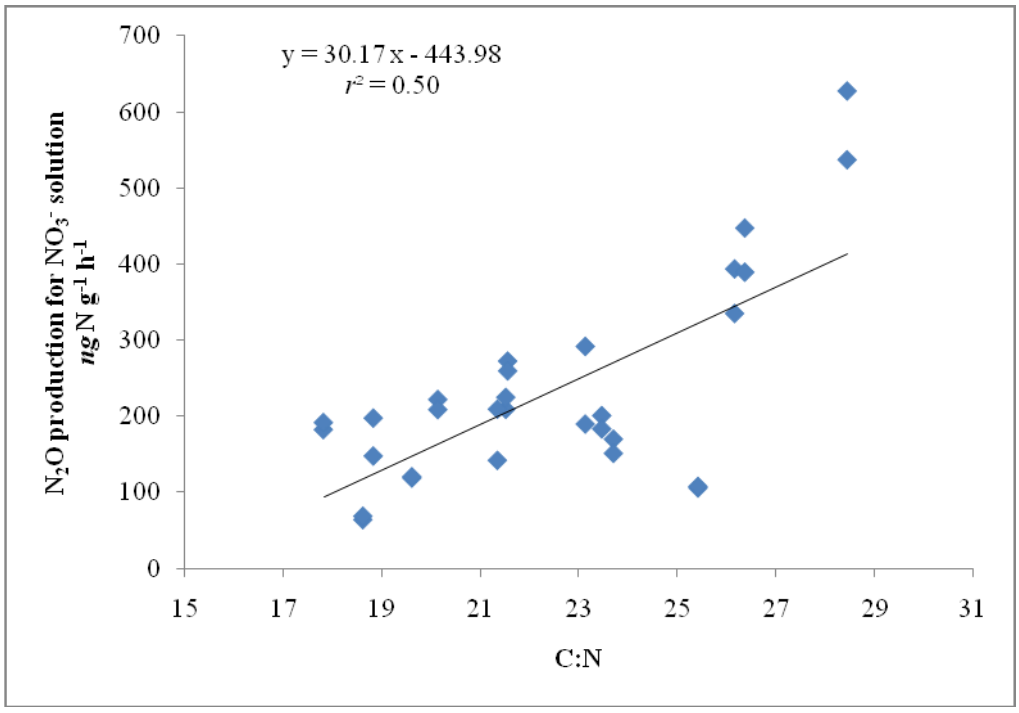


Figure 2-3. N_2O production for the nitrate incubation versus C:N for upland A horizon for the June and September soil samples of the S2 watershed at the Marcell Experimental Forest , Grand Rapids, MN.

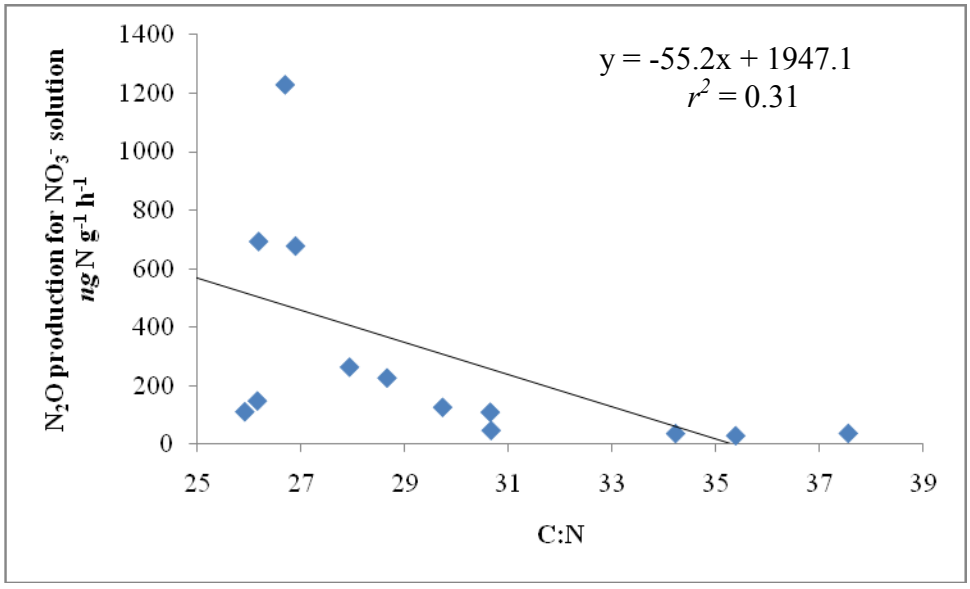


Figure 2-4. N_2O production for the nitrate solutions versus C:N for 0-25 cm lagg, alder and bog landscape types for the June and September soil samples of the S2 watershed at the Marcell Experimental Forest , Grand Rapids, MN.

DISCUSSION

For the upland A horizon, there is evidence to support the first hypothesis that, soil NO_3^- is the primary factor limiting denitrification potential. The upland A horizon DEA potential was consistently greater when soil was incubated with nitrate solution. There was no significant increase in N_2O production when it was incubated with glucose+nitrate solution as compared to the nitrate solution which may mean that soil carbon levels did not limit N_2O production. Soil total carbon was greater for the toeslope than the other hillslope positions but there was not a significant increase in DEA potential for the toeslope compared to the other hillslope positions because soil total carbon did not limit denitrification potential in all of the hillslope positions. There was no difference in denitrification potential or N_2O flux (Chapter One) between upland hillslope positions as suggested by Groffman *et al.* (1993) due to hillslope drainage class, most likely because of the small differences in elevation between the summit and the toeslope. The toeslope was consistently wetter than the other hillslope positions with the highest levels of STC and STN (Chapter One).

Evidence for the second hypothesis; denitrification potential for the lagg will be greater than for the upland and the bog had mixed results. The lagg and alder landscape types responded to the nitrate and glucose+nitrate solution with greater N_2O production than the other landscape types. The lagg and alder are the lowest elevations in the landscape and accept dissolved nutrients from the upland and bog (Kolka *et al.* 2001) however there are differences in the quality and quantity of the organic carbon that flows to the lagg by season. The Kolka *et al.* (2001) study did not investigate NO_3^- flow *per sé*,

but they found a distinct difference in the chemical qualities of bog flow versus upland flow that reached the lagg. Perhaps these differences explain why the lagg responded differently in June than in September. The influx of labile carbon and NO_3^- may contribute to conditions that support higher microbial communities in the lagg and alder landscape types than the upland and bog landscape types.

The bog had higher N_2O production than the lagg and upland for the control and glucose solutions. There were no soil properties that explained the increased production, soil NO_3^- was slightly above detection limits for the upland and below the detection limit for the peatland landscape types. Soil total nitrogen was lower for the bog than the lagg although the upland had the lowest soil total nitrogen. Typically bog soil is considered to have lower populations of bacteria than upland and lagg soil, and it was unexpected that heterotrophic fungi would respond with higher denitrification levels. Perhaps it would help explain this result if the response of bog microbes to denitrifying conditions was investigated.

The lagg had higher N_2O production than the upland and bog for the nitrate and glucose+nitrate solution. It appears that the addition of nitrate increased microbial activity and the native levels of carbon are sufficient to support denitrification activities. It is likely that greater N_2O production from lagg soil over upland and bog soil is explained by larger denitrifier populations due to the large carbon supply and influx of dissolved substrates from the upland and bog as well as the relatively high vegetative diversity present in the lagg.

Comparing denitrification potential between the upland, lagg, alder and bog showed that the alder, when incubated with nitrate solution; produced higher amounts of N_2O. Perhaps the microbial community in the soil surrounding the root nodules expands to soil further from the root itself and remained behind when the roots were removed. The alder microbial community increased N_2O production for the glucose+nitrate solution but not the nitrate solution. This may indicate that the denitrifying microbial community was able to respond to the addition of nitrate only when more labile carbon was supplied. This supports the ideas presented earlier that bacterial denitrifiers are less abundant in the peatland but will respond to more labile carbon than that supplied by peat soil. The same response was seen in the upland soil and to a lesser extent in the lagg soil. This may be another piece of evidence showing a linkage between the upland and lagg as the bog showed no increase in denitrification potential by the addition of glucose and nitrate.

There was no difference in denitrification potential for the bog by solution for June and September. A poor microbial response to glucose (Fisk *et al.* 2003) and nutrients (Bubier *et al.* 2003) have been found in other studies. The bog usually produced the least N_2O of the peatland landscape types even though the bog had similar soil chemistry to the other peatland landscape types. A common perception is that the bog has poor microbial responses because microbial processes slow under conditions of a low pH, i.e., below 4.00. However, bog soil pH was not significantly lower than the alder soil pH. More recent research has found evidence that the bog may have a different community of denitrifiers than upland systems and even the lagg (Fisk *et al.* 2003,

Mitchell *et al.* 2003, Williams and Crawford 1983). This means the bog will have different microbial communities with differences in processing (Fisk et al, 2003). Therefore, tests developed using soil from upland, mineral systems may need to be modified to effectively test bog soil and interpret the results accurately.

Evidence did not support the third hypothesis that stated the alder landscape type would have higher denitrification potential than the lagg landscape type. The alder landscape type did not have higher N_2O flux than the lagg landscape type in Chapter One and this was because of the low N_2O flux for the alder hummock. Apparently the influence of the root nodules on N_2O emissions is limited and removing them from the soil sample removes the difference between the alder and lagg soil samples.

The fourth hypothesis stated that denitrification potential for the top layer of soil would have a higher denitrification potential than the lower layer of soil. The control solution produced higher N_2O for 10 cm below the A horizon than the A horizon although the A horizon glucose and nitrate solutions produced higher N_2O gas than for10 cm below the A horizon. There was more SOC in the A horizon than 10 cm below the A horizon, it is possible that the quality of the carbon below the A horizon was more labile (Shrestha *et al.* 2007) to support greater denitrification rates (Murray *et al.* 2004, Pang and Cho 1984) over the limited time of the incubation. Dissolved organic carbon may percolate through soil layers (Uselman *et al.* 2007) resulting in more labile organic carbon in the lower soil layers than closer to the surface (Shrestha *et al.* 2007). However, then one would have expected a greater N_2O response with the addition of glucose to the solution for 10 cm below the A horizon which was not the case. Denitrification potential

for the glucose solution for the soil 10 cm below the A horizon was lower than for the control solution as was also the case for the nitrate and glucose+nitrate solutions. High concentrations of NO_3^- have been shown to suppress denitrification when there is insufficient SOC (Hunt *et al.* 2006, Terry and Tate III 1980) but the glucose+nitrate solution did not produce as much N_2O gas as the control solution for the 10 cm below the A horizon, indicating that the microbial community 10 cm below the A horizon was not able to process the added carbon and NO_3^-. There was no difference in N_2O production by upland depth for the glucose solution perhaps because the soil above and below the A horizon had sufficient carbon to process the small amounts of native NO_3^-. Parsons *et al.* (1991) found that significantly higher denitrification potential between treatments when there were no significant differences in levels of soil NO_3^-. This may mean that microbial communities are able to process exceedingly small amounts of soil NO_3^- below the detection limit. Perhaps the most likely explanation for higher N_2O production for 10 cm below the A horizon over the A horizon was that the microbial community in 10 cm below the A horizon peaked earlier than for the A horizon.

Alder soil collected from 25-50 cm had higher N_2O production than the 0-25 cm depth when incubated with control solution otherwise 0-25 cm peatland soil produced higher N_2O emissions than 25-50 cm peatland soil. Peat soil with higher carbon and soil moisture has been found to produce higher levels of N_2O whereas peat soil with lower carbon and moisture levels produces higher amounts of N_2 (Wray and Bayley 2007). The DEA experiment controls soil moisture so that all samples have the same soil moisture content plus C_2H_2 was added to the soil and solution mixture to inhibit further reduction

of N_2O to N_2. The lower part of the alder profile had higher levels of SOC than the upper part of the profile. The addition of glucose for the 0-25 cm layer of all peatland soils to the control and nitrate solutions did not produce an increase in denitrification potential so it is reasonable to conclude that there was sufficient SOC in the 0-25 cm layer. Soil NO_3^- was not significantly different between alder 0-25 cm and 25-50 cm depths however, it is altogether possible that denitrifying populations were able to respond to levels of soil NO_3^- below the detection limits (Priha *et al.* 1999) and differences in soil properties not measured by this study.

The fifth hypothesis; denitrification potential in fall is greater than during the growing season also had mixed results. There was higher N_2O production after the growing season (September soil sampling) than during the growing season (June soil sampling) for the lagg incubated in the control and glucose solutions but not for the upland and bog. There were no significant differences in SOC and soil NO_3^- between the two sampling dates. According to the hypothesis based on Groffman and Tiedje (1989); soil NO_3^- after the growing season will be higher than levels of soil NO_3^- during the growing season because during the growing season plants outcompete microorganisms for soil NO_3^-. Differences in soil NO_3^- may be below the detection limit but still evidence higher rates for N_2O production for one treatment than another (Parsons *et al.* 1991). Applying the reasoning of Groffman and Tiedje (1989) it is likely that in fall there are larger microbial populations than June and these larger populations were able to respond to increased levels of labile carbon and NO_3^-.

Hunt *et al.* (2007) suggested that a C:N ratio of 25 was the threshold over which no significant N_2O production occurred and cited Klemedtsson *et al*. (2005) in which they said C:N ratios over 25 suppress N_2O production They said that the C:N ratio was a "robust threshold controller of nitrous oxide production". Hunt's study areas included several mineral soil, riparian buffers with high nitrogen loading. Therefore his statement about a "robust threshold" cannot necessarily be applied to peatland systems because it gives the impression that when soil has a C:N above 25 it will produce lower N_2O emissions than soil with a lower C:N under the same conditions. This study found higher N_2O flux in the field and higher denitrification potential for organic soil with a C:N greater than 25 and greater than upland mineral soil with a C:N below 25.

However, Klemedtsson *et al.* (2005) studied drained peatlands where drained peat soil had higher organic matter mineralization and N_2O production rates than peat soil with higher water tables (Alm *et al.*1999, Ciu, et al,. 2005). Mineralized peat soil and newly formed peat soil differ in their chemical qualities (Artz *et al.* 2008) which probably influences denitrifier activity and denitrification rates. The results from this investigation showed a significant but weak, positive relationship for the upland (Figure 2-3) between N_2O production and a C:N below 25 and a significant but weak, negative relationship between N_2O production and a C:N for the peatland (Figure 2-4).

The evidence for soil NO_3^- as the factor limiting denitrification potential from the DEA experiment was mixed for peatland landscape types at the 0-25 cm depth. The lagg had higher N_2O production for the glucose+nitrate solution than the control and glucose solution but not the nitrate solution which had similar production as the control and

glucose solutions. In addition, the bog produced similar levels of N_2O across all DEA solutions. The alder incubated in glucose+nitrate solution had the highest N_2O production yet N_2O production for the the nitrate solution was similar to the control and glucose solutions. Levels of STC were similar for all peatland landscape types and were in amounts that clearly did not limit peatland N_2O production in addition the control and glucose solutions had similar levels of N_2O production. This was a similar result to Davidsson *et al.* (2002) in which additions of glucose did not stimulate denitrification potential.

Peatlands have long been known to be nutrient limited ecosystems (Godwin and Conway 1939). The lack of response to the nitrate solution may be because the denitrifying microbial community in the lagg and bog have enzymes adapted to breaking down more complicated molecules like cellulose, such as in peat soil, rather than glucose and that's why adding glucose, a labile carbon, did not trigger higher activity (Garcia-Montiel *et al.* 2003, Hunter and Faulkner 2001, Rich *et al.* 2003). Earlier reports have shown a lack of increased N_2O production for the bog after additions of NO_3^- due to small denitrifier populations (Hashidoko *et al.* 2008). However, a study by Davidsson *et al.* (2002) found a large increase in denitrification potential with the addition of NO_3^- via floodwater. In Chapter One, soil properties had poor relationships to N_2O production while peatland water chemistry had strong relationships with N_2O production; however the addition of NO_3^- was in solution.

High DEA activity suggests a large denitrifying microbial population (Fisher *et al.* 1998) as shown in these results when the lagg and alder had higher rates of N_2O

production than the bog for the nitrate and glucose+nitrate solutions. Larger populations of microbial fungi have been found in association with birch roots than spruce roots (Priha *et al.* 1999); birch trees are found in the lagg but not in the bog while spruce is found in both the lagg and bog. This suggests that soil NO_3^- limited DEA potential for the lagg and alder but denitrifying enzyme levels limited N_2O production for the bog.

Bacterial communities differ according to hydrological conditions (Bougon *et al.* 2009, Iribar et al. 2008). Depth to water table was not significantly different for the lagg and bog when measured along with the N_2O production sampling for 2007 and 2008 (Chapter One), but there are other hydrological relationships that were not measured in this study. Water from the bog to the lagg moves through saturated peat but water in the lagg often moves as surface flow which is likely to have a higher rate of flow with a higher nutrient content than the saturated flow; peaking in the spring before leaf out and fall after leaf fall (Verry and Timmons 1982). It is possible that these seasonal hydrological flow peaks as surface flow in the lagg that trigger DOC flow peaks concurrent with seasonal nutrient flow peaks and these together cause of spikes of microbial population growth (Fisher *et al.* 1998). This could be why the lagg had higher N_2O production for the control and glucose solutions after the growing season rather than during the growing season. It could also be why lagg had higher N_2O production than the bog after the growing season and not during the growing season.

Rates of denitrification for the wetland forested edges have lower rates of denitrification, between 1.0 and 2.0 ng N g^{-1} h^{-1} (Hernandez and Mitsch 2007) than this study in a raised, ombrotrophic bog in the Marcell Experimental Forest. An earlier

denitrification study in S2 bog in the Marcell Experimental Forest by Urban *et al.* (1988) found maximum denitrification potentials near 570 N g^{-1} h^{-1}, while Thoreau's bog (Hemond 1983) reported average denitrification rates of 3 ng N g^{-1} h^{-1}. Clearly, denitrification rates for peat soil vary widely even between studies of the same site.

CONCLUSION

For the upland, soil NO_3^- appeared to be the primary factor limiting denitrification potential for the A horizon and ambient SOC levels did not limit N_2O production for the A horizon. Interestingly, N_2O production for 10 cm below the A horizon control solution was often greater than for the glucose and nitrate solutions. Earlier studies have found evidence that surplus levels of NO_3^- suppress N_2O production when SOC levels are inadequate to fuel denitrification but the glucose+nitrate solution did not always produce higher N_2O emissions than the nitrate solution. Nitrous oxide production for all A horizon upland hillslope positions except the toeslope, incubated in nitrate solution, were greater than for 10 cm below the A horizon, otherwise there were no significant differences in N_2O production by depth. These mixed results may be due to the inherently high variation in laboratory DEA experiments or perhaps it suggests further investigation into the differences in soil chemistry between the A horizon and 10 cm below the A horizon and its influence on denitrifier communities.

The higher N_2O production for peatland 25-50 cm over N_2O production for 0-25 cm may be due to higher levels of SOC for the 25-50 cm depth, however levels of STC were not considered limiting for denitrification. Similarly there was higher N_2O production after the growing season than during the growing season for the lagg.

Perhaps, there were higher levels of NO_3^- after the growing season, albeit below detection limits, but sufficient to produce higher N_2O. It is also possible that the lack of competition between the denitrifier microbes and vegetation after leaf fall allowed an increase in microbial populations. Denitrifier communities for lagg soil incubated in control solution collected September appeared to be more responsive than denitrifier communities for June bog soil but not for the glucose, nitrate and glucose+nitrate solutions. There were no differences by date for soil carbon, nitrogen, NH_4^+ and NO_3^- therefore it is not surprising that adding glucose and NO_3^- did not have an effect, and given the short incubation time microbial populations might not have had enough time to grow in response to the additions. If increased denitrification potential was due to microbial activity it was because the lagg had a larger denitrifier population and perhaps because there was less competition between microbes and vegetation for soil nutrients. The lagg has a larger population of deciduous, woody vegetation than the bog and so a greater difference in vegetation respiration between after and during the growing season.

The advantage of alder soil over lagg soil appears to be limited to the alder roots with their N-fixing root nodules as denitrification potential and soil properties were not consistently different for the two lagg landscape types.

There was a positive but weak relationship between denitrification potential and the C:N ratio for the upland where C:N was below 25 and a negative but weak relationship between denitrification potential and the C:N for the lagg where C:N was greater than 25. Although there was a negative relationship between N_2O and C:N for the

peatland, N_2O production was higher for the lagg than for the upland. Peat soil appears to have a higher denitrification potential than mineral soil.

GENERAL SUMMARY

Nitrous oxide flux in the field was limited by NO_3^- which was produced via nitrification and reduced to N_2O by denitrification. The highest N_2O fluxes in an upland-bog watershed were in the alder hollow microtopographic position. The hollows are the microtopographic positions with the highest N_2O flux perhaps because of wetter conditions supporting denitrification and perhaps because the microorganisms on the soil surfaces have a closer association with nitrification and denitrification substrates. Water table levels adjust with precipitation and evaporation and uptake. As the water table recedes the probability of organic matter mineralization and nitrification increases with the influx of atmospheric air. Nitrification occurs over a wide range of soil moisture conditions but requires soil air, and with precipitation the water table rises back toward the surface of the hollows gradually saturating the soil and decreasing soil-atmosphere gas exchange triggering the denitrification of the nitrate stocks.

Similar conditions and processes are at work in the upland mineral soil too. Rainfall events wet the soil but because the S2 upland is well drained saturation is unlikely to occur. But as water percolates down through the soil, nitrification and mineralization activities are activated in soil microsites which may use up the soil oxygen faster than the CO_2 and other gases can be displaced by incoming atmospheric oxygen. As soil oxygen stress increases nitrification slows down and denitrification picks up, however, denitrification is limited by the nitrification activity that had gone on before.

The S2 upland soil is coarse textured and rarely becomes anaerobic thereby limiting denitrification to microniches rather than the entire matrix as what may be possible in the peatland at and somewhat below the water table. This relationship between nitrification and denitrification is called "coupled nitrification-denitrification" and is what is considered the primary source of N_2O flux in pristine landscapes such as this upland-bog watershed. This is why nitrate is the primary controlling factor for N_2O flux in this upland-bog watershed.

Soil carbon, another of the primary factors controlling N_2O flux is adequate for all N processes and the formation of N_2O gas. There are locations in the upland-bog watershed that have more soil total carbon than other areas, i.e., the toeslope for the uplands and the peatlands for the entire watershed, it occurs in an excess to reduce the native supplies of NO_3^-. The addition of glucose+nitrate solution to trace gas sampling chambers throughout the upland, lagg and bog did not consistently increase N_2O outputs. However, it is probably because the available carbon resident in the soil plus the added glucose that supported complete denitrification and the emissions of N_2 rather than N_2O gas, making it difficult to assess denitrification potential differences in the field without the addition of an inhibitor such as C_2H_2.

Denitrification potential assessed in a laboratory setting demonstrates that the lagg and alder landscape types have the highest denitrification enzyme activity of the entire watershed. Denitrification potential for bog soil was not influenced by additions of glucose and nitrate solutions. There is some controversy as to whether or not the bog has small microbial populations or there are larger microbial populations but their processing

rates are inhibited by low pH; clearly microbial populations in bogs are poorly understood. What is known from this study is that the lagg and alder have a higher denitrification potential once nitrate is supplied, suggesting that they have the highest functioning microbial populations. This study also underscores the results from previous studies that have found significant differences in bog microbial populations and a need to investigate them differently than other systems.

Perhaps high variability in N_2O production is what made it look like the soil collected from 10 cm below the A horizon had a higher denitrification potential for the control solution than soil from the A horizon of the upland hillslope. There may be an answer in the quality of carbon at depth and its relationship to denitrifying microorganisms. Again, there was higher production for the 25-50 cm alder soil over the 0-25 cm alder soil incubated in the control solution but there were no difference in denitrification potential by depth for the lagg and bog. Clearly, more work needs to be done to understand differences in soil chemistry and its effect on microbes.

Another interesting finding is the positive but weak relationship between denitrification potential and the C:N ratio for the upland the negative but weak relationship between denitrification potential and the C:N for the lagg. The C:N ratio for the upland was below 25 and the C:N for the lagg was above than 25 in addition N_2O flux was higher for the lagg than for the upland. There has been work done to identify microbial population differences between upland mineral soil and peatland organic soil; perhaps the differences lie in the functional differences between autotrophic bacteria and heterotrophic fungi.

FURTHER WORK

I would propose further study into the controls on N_2O emissions for peatland soil since denitrification has been identified as the primary cause of N_2O emissions for forest soils (Bedard-Haughn *et al.* 2006, Groffman *et al.* 1993, Groffman and Tiedje 1989). This study's DEA results showed no significant increase in N_2O emissions when incubated in nitrate solution even though peatlands are known to be nutrient limited (Godwin and Conway 1939, McQueen and Wilson 2000). The most logical explanation seems to be that the peatland's small microbial community (Hashidoko *et al.* 2008) required longer incubation to reach maximum rates of emissions (Bernal *et al.* 2007, Jun-Qiang *et al.* 2008). A long incubation time, i.e., eight hours (Bernal *et al.* 2007) with headspace sampling at one hour intervals is more likely to capture maximum N_2O emissions plus multiple sampling at one hour intervals would provide a robust flux calculation and be more likely to find statistical differences. The second piece of the experiment would be a description of the microbial populations and a better understanding of the functional differences in the populations. The following methods would be used on the soil samples before incubation and at hourly intervals throughout the incubation. The method to identify complete microbial populations is called PCRA (polymerase chain reaction-amplified) which identifies population genetics (Muyzer *et al.* 1993). A method using antibiotics and enrichment techniques differentiates active populations from dormant ones (Luna *et al.* 2002).

This study's DEA results also showed that alder soil from the 25-50 cm depth produced higher levels of N_2O than the 0-25 cm layer for the control solution. There was

no support for this finding in the literature. There is an interaction between soil carbon and soil texture on fungal and bacterial biomass and activity (Giardina *et al.* 2001) for mineral soils, perhaps there is also an influence on carbon quality on microbial communities, populations and processing rates. Venterea *et al.* (2003) found strong relationships between elevation, forest tree cover, nitrogen mineralization rates, respiration and C:N ratios; that when a forest cover species had a negative correlation with nitrification and soil NO_3^- content then there was a positive correlation with C:N and a negative correlation with N-mineralization. Perhaps continuing this work on alder with its N-fixing nodules in a peat soil could help explain the result of this study. Perhaps there are answers in the very nature of peat soils. Artz *et al.* (2008) found that lignin-to-carbohydrate ratio increased with depth and peat age. Typically higher plant material lignin content is negatively correlated with decomposition rates but calcium content was also important (Hobbie *et al.* 2006). Perhaps techniques such as the DEA that were developed for comparing microbial processing in mineral soil are not as useful for organic soil. Again, understanding the functional microbial communities in mineral and peat, particularly fungal and bacterial activity in is important before one can compare results between mineral and peat soil (Laughlin and Stevens 2002).

Either of the aforementioned experiments would fill in the gaps of our knowledge on the differences in mineral and peat soil.

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