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Topographic Controls on N₂O and N₂ Efflux in a Temperate Forest

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Graduate Program in Biology

A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

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TOPOGRAPHIC CONTROLS ON N₂O AND N₂ EFFLUX
IN A TEMPERATE FOREST

(Thesis format: Integrated Article)

by

Eric Mudoto Enanga

Graduate Program in Biology with Environment & Sustainability

A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

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Abstract

The release of greenhouse gases into the atmosphere is becoming an increasingly important problem due to the overwhelming evidence of the relationship between rising greenhouse gas concentrations and increased global temperatures. It is important that we gain a better understanding of the processes controlling the release of N₂O, a powerful greenhouse gas. Topographic influences on soil temperature, moisture, reduction-oxidation (redox) potential, dissolved organic carbon, and nitrate conditions, which in turn influence N₂O release, were investigated in a natural temperate forest ecosystem in Ontario, Canada. Wetland positions were observed to have the conditions most suited for N₂O production (redox potential between +100 and -100 mV). More N₂O was released during the summer from the inner (IW) and outer (OW) wetland positions than from lowland and upland topographic positions. Significant positive relationships between N₂O efflux and precipitation events in both the IW and OW were observed, suggesting that precipitation creates conditions that promote bursts of N₂O release. In addition, substantial biogeochemical activities were observed during the non-growing season under a snow-pack, including denitrification, which can produce N₂O. It is important that we gain a better understanding of the processes controlling the release of N₂O during the non-growing season. Fluxes of N₂ were estimated using the acetylene inhibition technique from wetland positions that had the optimal redox conditions. Adding N₂ and N₂O fluxes to catchment N export not only reduced the discrepancy in N export observed among catchments but also between N inputs and outputs. During summer months, rainfall events can be used to predict N₂O fluxes from the wetland positions within catchments in temperate forests and the IW position had greater potential of reducing N₂O to N₂ compared to the OW and the lowland and upland areas. Failing to account for winter denitrification products may lead to underestimation of annual N losses.

Keywords:

Temperate, Forest, Hydrology, Biogeochemistry, Nitrogen, Topography, Redox

Co-Authorship Statement

This doctoral thesis was funded by a Natural Sciences and Engineering Research Council Discovery Grant to Dr. Irena Creed for a study titled “Influence of hydrology on microbial-mediated biogeochemical hot spots and hot moments in complex landscapes” and a Canadian Foundation for Climate and Atmospheric Sciences grant to Dr. Irena Creed (lead principal investigator), Dr. Sherry Schiff, Dr. Richard Bourbonnière, Dr. Fred Beall, Dr. Dean Jeffries and Dr. Neil Foster for a larger study titled “Roaming GHG Hotspots (CO₂, CH₄ and N₂O: A hydrologically-based method for mapping GHG sources and sinks.”

The thesis contains three manuscripts. In each of these manuscripts, Eric Enanga will be first author, as he helped define the research problem and study design, collected, analyzed and interpreted the data, and wrote the manuscripts. Dr. Irena Creed will be a co-author on each manuscript because of her contributions defining the research problem and study design, interpreting the data, and editing the manuscripts. Dr. Fred Beall will be listed as a co-author on each manuscript for contributing data. Nora Casson will be a co-author on the manuscript resulting from Chapter 2 based on contributions to 2006 and 2007 field data. Tarrah Fairweather will be a co-author on the manuscript resulting from Chapter 3 based on contributions to 2006 and 2007 field data.

Dedication

*I dedicate this thesis to my late father,
who valued education and
who would have been proud to have his name
“Enanga” mentioned alongside a doctorate thesis.*

*I also dedicate this thesis to the rest of my family,
who have been very patient during this journey.*

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List of Abbreviations

α	Bunsen absorption coefficient
BS	Back slope
CAPMoN	Canadian Air and Precipitation Monitoring Network
C	Carbon
C ₂ H ₂	Acetylene
CH ₄	Methane
CO ₂	Carbon dioxide
CR	Crest
D _o	Diffusivity of gas in air
DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
D _s	Effective diffusion coefficient of gas in snow
ρ_{ice}	Density of ice
ρ_{snow}	Density of snow
Eh	Reduction oxidation potential
Fe	Iron
FS	Foot slope
HgCl ₂	Mercuric Chloride
IW	Inner wetland
J	Flux of gas in snow
L	Ostwald solubility coefficient
N	Nitrogen
N ₂	Dinitrogen (molecular nitrogen)
NH ₃	Ammonia

NH_4^+	Ammonium
NH_2OH	Hydroxilamine
N_2O	Nitrous oxide
NO	Nitric Oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NO_x	Nitrogen oxides
O_2	Oxygen
OW	Outer wetland
Φ	Snow porosity
P°	Standard pressure (101.3 kPa)
PTFE	Polytetrafluoroethylene
PVC	PolyVinylChloride
Redox	Reduction oxidation
SH	Shoulder
T°	standard temperature (273.15 K)
TDN	Total Dissolved Nitrogen
TLW	Turkey Lakes Watershed
TS	Toe slope
τ	Complexity of the snow
V°	Volume of gas at standard temperature and pressure
V_{corr}	molar volume corrected for temperature and pressure
WTD	Water Table Depth

Chapter 1

1 Introduction

1.1 Problem Statement

Catchments that receive comparable nitrogen (N) inputs would be expected to export the same amount of N if they experience the same climate, were formed at the same time, have the same parent material, the same vegetation and topography, all factors that govern soil formation (*Jenny, 1941*). Except for topography, catchments in the Turkey Lakes Watershed (TLW) of central Ontario meet the criteria described by *Jenny (1941)*, and the results from over 30 years of research reveals a discrepancy in stream N output among the catchments. Catchments with large, flat and intermittently wet features export less dissolved N in the form of nitrate (NO_3^-), ammonium (NH_4^+) and dissolved organic nitrogen (DON) (*Creed and Beall, 2009*) and the relative proportion of dissolved organic N (DON) is larger than other catchments (*Mengistu et al., 2014*). Previous studies (*Creed et al., 1996; Creed and Band, 1998*) have explored topographic controls on the spatial and temporal variability in stream N export from the TLW catchments. In addition to stream export, however, atmospheric export may provide another pathway through which material can enter or leave the catchment (*Lohse et al., 2009*). Gaseous N efflux has received less attention, possibly contributing to the failure to close N input-output budgets in the catchments (*Creed and Beall, 2009*).

1.2 Scientific Justification

1.2.1 Nitrogen as a Greenhouse Gas

The global N cycle has undergone great alterations during the past century that has resulted in an increase in global reactive N (*Vitousek et al., 1997; Galloway et al., 2004; Rockstrom et al., 2009*). Dinitrogen (N_2) gas constitutes 78% of the atmosphere, but N_2 is an unreactive form of N, because the strong triple bond between the two N atoms requires a substantial amount of energy to break. Due to this substantial energy requirement, atmospheric N_2 is not readily available to primary producers unless fixed by N-fixing organisms or alternatively fixed by industrial processes (e.g., manufactured

fertilizer); hence N is frequently a limiting nutrient to plants despite its abundance in the atmosphere (*Vitousek and Howarth, 1991*).

Historically, the two main global sources of reactive N were from lightning and microbially-mediated biological N fixation that converted N_2 to NH_4^+ (*Delwiche, 1981; Ciais et al., 2013*), which were generally in balance with denitrification returning N_2 to the atmosphere (*Ayres et al., 1994*). Denitrification maintained the equilibrium among the N species while at the same time completing the N cycle. However, human activities associated with the Industrial Revolution have resulted in an increase in reactive N, defined as all other nitrogen species that are not N_2 (*Ciais et al., 2013*) in the atmosphere. For example, reactive N is increasing because of an increasing demand for food and energy to support a growing human population; more fertilizer is needed to meet the increased demand for food (*IPCC, 2013*). This increased fertilizer use combined with poor land management practices (*Syakila and Kroeze, 2011*) results in volatilization of fertilizer applied as N. Furthermore, fossil fuel burning from industrial processes and transportation emit oxides of N into the atmosphere, although recently regulations resulted in a decline in emissions of oxides of N (NO_x), but ammonia is not regulated (*Davidson et al., 2012*). Finally, increased production of crops associated with biological N fixation for example legumes that have root nodules that contain N fixing bacteria has greatly contributed to elevated global reactive N (*Vitousek et al., 1997*) entering ecosystems (*Aber et al., 2003; Lovett and Mitchell, 2004; IPCC, 2013*).

The three major pathways for the production of reactive N are: (1) the Haber-Bosch process that produces ammonia from N_2 for fertilizer and feedstock production; (2) large scale cultivation of legumes and other crops that increase biological N fixation; and (3) combustion of fossil fuels, which converts N_2 and fossil fuel N to N oxides (NO_x) that are emitted to the atmosphere and later deposited to the surface (*Ciais et al., 2013*). Due to atmospheric transport, the eventual deposition of reactive N to the land surface may occur in different ecosystems, sometimes far away from the source of emission (*Sirois et al., 2001; Lovett and Mitchell, 2004*). A minor pathway for the production of reactive N is N-rich sedimentary rocks that release sequestered reactive N, which was initially incorporated into sedimentary rock as organic N or ammonium in silicate minerals

(Morford *et al.*, 2011). Another minor pathway for the production of reactive N is N fixed as nitrate salts over millennia that is re-introduced to the active N pool (Walvoord *et al.*, 2003), although this pathway is more confined to arid areas.

Currently, atmospherically deposited N is estimated at 280 Tg of N yr⁻¹ (Bouwman *et al.*, 2013), which is considered an underestimate because the atmospheric deposition of organic N is not included in the total. A highly uncertain estimate of the organic N fraction of total atmospherically deposited N is 35% (Kanakidou *et al.*, 2012). Only 30 to 60% of the anthropogenically-derived reactive N is returned to the atmosphere via denitrification (N₂ and N₂O) (Galloway *et al.*, 2004; Canfield *et al.*, 2010; Bouwman *et al.*, 2013). The remaining is stored in terrestrial ecosystems (Bouwman *et al.*, 2013).

Recent estimates suggest that globally-averaged atmospheric N₂O concentrations stand at about 325.9 ppb (WMO, 2014) representing an increase of 20% compared to 270 ppb in 1750 from estimates derived from ice cores (Prather *et al.*, 2012). These concentrations are believed to be the highest recorded in the past 800,000 years, with an average radiative forcing (RF) of 0.17 W m⁻² (IPCC, 2013). Once released to the atmosphere, the estimated lifetime for N₂O ranges from about 118 to 131 years, with the main sink occurring through photolysis and oxidation in the stratosphere (Syakila and Kroeze, 2011; Prather *et al.*, 2012) with consequences on the ozone (O₃) layer (Ravishankara *et al.*, 2009). Therefore, it will take more than a century for atmospheric N₂O levels to stabilize in response to controls on N₂O global emissions (Ciais *et al.*, 2013); thus negative impacts on the O₃ layer will continue (Ravishankara *et al.*, 2009; Fleming *et al.*, 2011). In addition, estimates suggest N₂O has a 100-year Global Warming Potential (GWP) of about 265 times that of CO₂ (molecule for molecule). The GWP is the amount of heat trapped by N₂O, which results in warming relative to an equal amount of CO₂ (IPCC, 2013). Therefore, despite its low flux rates and concentration in the atmosphere relative to CO₂, N₂O is having a significant impact on the climate system (Myrhe *et al.*, 2013).

1.2.2 Nitrogen Cycle in Forested Catchments

Recent studies have shown elevated loads of atmospheric N deposition onto temperate forests, with loads approaching those suggested for N saturated systems where N export to the stream is becoming the dominant N output for the system (*Aber et al.*, 1989). *Aber et al.* (1989) defined N saturation as a condition associated with available ammonium and nitrate in excess of total nutritional demand by plants and microbial communities but excluding denitrification. Four hypothetical stages are described that lead to N saturation in forests. Stage 0 of N saturation is when the forest is N limited and there is a tight cycling of N within the system. Stage 1 is when there is rapid growth of trees in response to more N availability. Stage 2 is when the forest is no longer N limited and there is significant loss of N to the atmosphere or aquatic systems. Stage 3 is when the N levels are detrimental to the forest, and the trees show signs of decline or death (*Aber et al.*, 1989).

Several potential fates await atmospherically-deposited N in forest ecosystems (Figure 1.1): (1) it may be assimilated by organisms (microorganisms and higher); (2) it may undergo several transformations that include mineralization, nitrification and denitrification, with N_2O as an obligate intermediate and N_2 as the final product (*Gambrell and Patrick*, 1978; *Fowler et al.*, 2009); or (3) it may be lost to aquatic systems *via* hydrological flows. The potential fate depends in part on the N species that is deposited. Organic N can be assimilated by living things or mineralized to yield NH_4^+ for assimilation, or nitrification while the carbon (C) skeleton is utilized as an energy source when C is limiting, or mobilized *via* hydrological flows within or from the catchment. Deposited NH_4^+ may be taken up by organisms or undergo nitrification under aerobic conditions with the energy generated from oxidation of NH_4^+ to NO_3^- used to fix C by autotrophs (*Wrage et al.*, 2001). Deposited NO_3^- may be taken up by organisms, undergo denitrification under anaerobic conditions, or be exported from the catchment through exiting water (*Wrage et al.*, 2001).

Forested catchments that receive increased reactive N loads necessarily respond with efforts to cope or process it (*Aber et al.*, 1989; *Vitousek*, 2004). At the catchment scale, the forest ecosystem response can result in increased dissolved or gaseous N.

Differences may exist in the dominant dissolved N species among catchments; e.g., catchments with areas that are characterized by highly reducing conditions and high iron (Fe) content in soils may convert NO_3^- through abiotic means to organic forms according to the ferrous wheel hypothesis proposed by *Davidson et al.* (2003). The ferrous wheel hypothesis suggests NO_3^- can be reduced abiotically to NO_2^- coupled with the oxidation of Fe^{2+} to Fe^{3+} with the NO_2^- combining with organic material to form organic N compounds through nitration or nitrosation. *Azhar et al.* (1989) describes nitration as the addition of $-\text{NO}_2$ while nitrosation is the addition of $-\text{NO}$ on aromatic structures. The Fe^{2+} is regenerated from Fe^{3+} by reducing sugars to participate further in the reduction of NO_3^- to NO_2^- , completing the cycle (ferrous wheel).

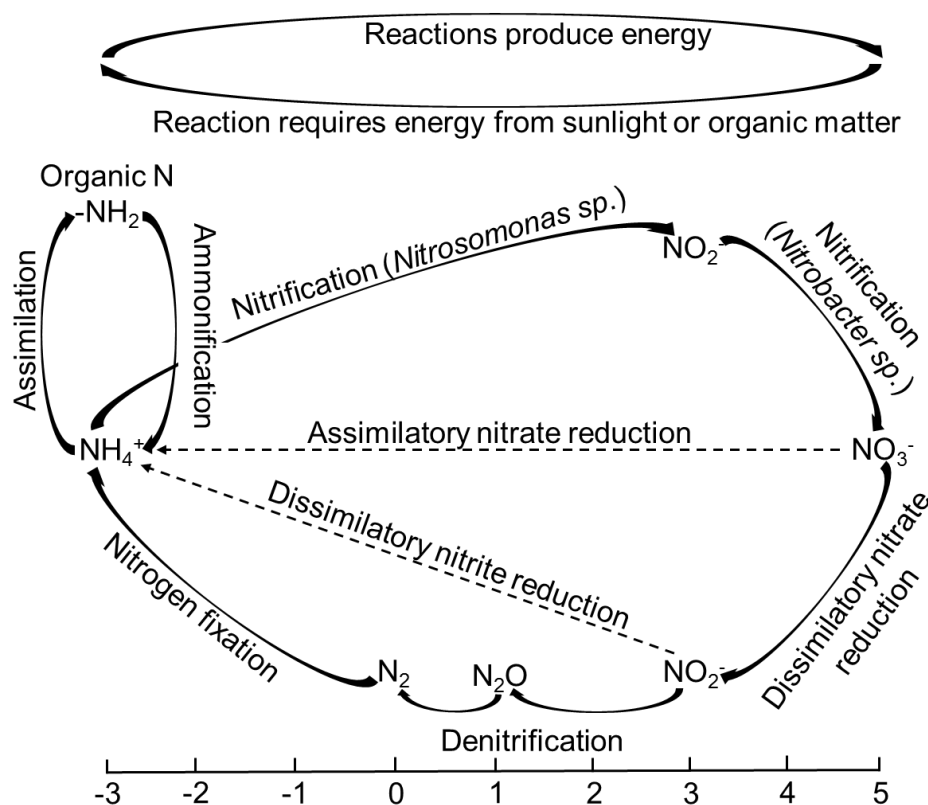


Figure 1.1: The nitrogen cycle modified from *Duff and Triska*, (2000).

The production of N_2O within ecosystems may constitute a disservice (*Burgin et al.*, 2013) due to its association with global warming (*IPCC*, 2013) and ozone depletion (*Ravishankara et al.*, 2009). In N limited systems denitrification removes N converting it

to gaseous forms hence denying plants and animals a limiting nutrient at a local scale. Production of NO_3^- through nitrification and loss to surface water may also constitute an ecosystem disservice as elevated levels of NO_3^- in drinking waters has been associated with “blue baby syndrome” (Avery, 1999; Gupta, 1999; Majumdar, 2003) but also a service for increasing plant available N. Interestingly, denitrification provides an ecosystem service (Burgin *et al.*, 2013) by removing dissolved NO_3^- from water exiting catchments hence shielding receiving aquatic ecosystems from effects of NO_3^- loading (Mosier *et al.*, 1998) and helping close the N cycle when N_2 is the final denitrification product. The same microbial processes that drive denitrification within catchments have found practical application in biological waste water treatment plants to remove inorganic N in waste water before eventual release to surface water (Wrage *et al.*, 2001), providing more ecosystem services.

1.2.3 Gaseous N_2O and N_2 Pathways

Gaseous N production occurs in soil through two main pathways, nitrification and denitrification. During nitrification, N_2O gas is produced as a by-product through decomposition of the intermediates (NH_2OH and NO_2^-) that are part of the nitrification process (Wrage *et al.*, 2001). Denitrification generates the gaseous products, NO , N_2O and N_2 , with N_2O an obligate intermediate product and N_2 the final product (Gambrell and Patrick, 1978; Farquharson and Baldock, 2008).

Nitrification can generally be defined as the oxidation of NH_4^+ to NO_3^- (Hedin *et al.*, 1998) via hydroxylamine (NH_2OH) and nitrite (NO_2^-) intermediates (Wrage *et al.*, 2001). Nitrification is carried out by two groups of autotrophic microorganisms that utilize the energy generated during the process to fix C. The first group are *Nitrosomonas sp.*, which oxidize ammonia up to NO_2^- and are referred to as primary oxidizers. The second group composed of *Nitrobacter sp.* oxidize NO_2^- to NO_3^- and are referred to as secondary oxidizers (Bock *et al.*, 1986; Wrage *et al.*, 2001). Heterotrophic nitrifiers that use C as an energy source and also as a building block are also in existence (Castignetti, 1990) and include fungi and some bacteria (e.g., *Pseudomonas denitrificans*) (Wrage *et al.*, 2001). During nitrification, N_2O is released as a by-product of the oxidation process (Wrage *et al.*, 2001) (Figure 1.2).

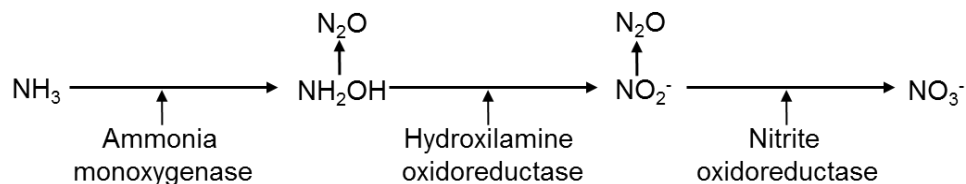


Figure 1.2: Nitrification pathway with enzymes that mediate the process with N_2O production as a by-product (modified from *Wrage et al.*, 2001).

Denitrification is the major process that governs gaseous N production, which consequently aids in closing the N cycle by converting reactive N to N_2 (*Gambrell and Patrick*, 1978), with N_2O as a mandatory intermediate product (*Fowler et al.*, 2009). The process is mainly enzyme catalyzed and is dependent on microbial activity both in terrestrial and aquatic ecosystems (*Wrage et al.*, 2001; *Freing et al.*, 2012); abiotic ammonium nitrate decomposition that occurs in the presence of light is another minor pathway (*Rubasinghege et al.*, 2011) that contributes to N_2O formation.

For microbially-mediated denitrification to occur, the presence of NO_3^- (*Wrage et al.*, 2001; *Farquharson and Baldock et al.*, 2008) and organic C as an energy source are required, because denitrifiers are mainly heterotrophic bacteria (*Firestone and Davidson*, 1989; *Wrage et al.*, 2001). The bacteria include *Pseudomonas*, *Bacillus*, *Thiobacillus*, and *Propionobacterium* (*Firestone*, 1982) which are mainly facultative anaerobes and will preferentially utilize O_2 as an electron acceptor during respiration, but switch to NO_3^- when O_2 levels are low (*Wrage et al.*, 2001; *Farquharson and Baldock*, 2008). The consortium of enzymes that catalyze denitrification include nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Figure 1.3) outlined below (*Hochstein and Tomlinson*, 1988; *Farquharson and Baldock*, 2008).

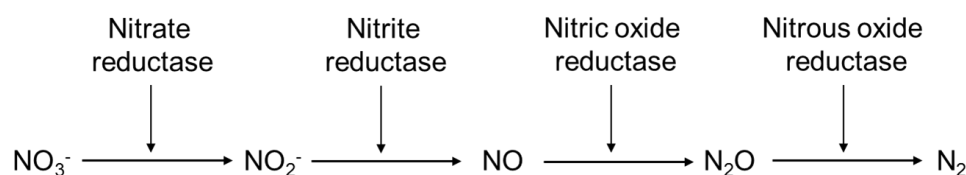


Figure 1.3: Denitrification pathway with enzymes that mediate the process and the intermediate products including N_2O and the final product N_2 (modified from *Wrage et al.*, 2001)

Nitrous oxide production responds to: (1) pH, with low pH favouring N₂O formation with low pH <7 inhibiting nitrous oxide reductase resulting in the accumulation of N₂O (*Knowles*, 1982); (2) NO₃⁻ level, with high NO₃⁻ favouring N₂O formation because reduction of NO₃⁻ to N₂O is the preferred pathway with further reduction of N₂O to N₂ not favoured, resulting in the accumulation of N₂O (*Gambrell and Patrick*, 1978; *Farquharson and Baldock*, 2008); and (3) O₂ level, with relatively high O₂ inhibiting N₂O reductase more strongly than it does the other denitrification enzymes. This results in the production and accumulation of N₂O (*Knowles*, 1982), which may be attributed to the lack of need for further reduction of N₂O to N₂ because of the availability of the other preferred electron acceptors (O₂ and NO₃⁻). Very high O₂ levels inhibit NO₃⁻ reduction resulting in no N₂O production, because O₂ is the preferred electron acceptor due to the associated higher free energy yield (*Hedin et al.*, 1998).

Wrage et al. (2001) proposed three possible denitrification pathways: (1) *Coupled nitrification-denitrification*, where denitrification occurs in two stages performed by two different groups of organisms. One group of organisms oxidize ammonia through the nitrification process with NO₃⁻ as product, while another group of organisms performs the denitrification process of reducing NO₃⁻ to N₂ via NO and N₂O intermediates (nitrite reducers); (2) *Chemodenitrification*, which is a non-biological (abiotic) process that involves chemical decomposition of NH₂OH which is an intermediate in the oxidation of NH₄⁺ to NO₂⁻ or alternatively the decomposition of NO₂⁻ itself with organic amines or Fe²⁺ or Cu²⁺; and (3) *Nitrifier denitrification*, where oxidation of NH₄⁺ to NO₂⁻ during nitrification is followed by the reduction of NO₂⁻ to N₂O and N₂ without going through the NO₃⁻ intermediate. Unlike coupled-nitrification denitrification, where two groups of co-existing microorganisms (nitrifiers and denitrifiers) are involved, in nitrifier denitrification only one group of microorganisms are involved in both nitrification and denitrification (*Wrage et al.*, 2001).

1.2.4 Gaseous N₂O and N₂ Effluxes

Soil under natural vegetation contributes 60% of all naturally derived N₂O and 37% of all total N₂O (natural plus anthropogenic) fluxes (*Ciais et al.*, 2013). Thus soils under natural vegetation, including temperate forests, may contribute a substantial

amount of N₂O compared to all anthropogenic sources combined, and cannot be ignored during N accounting within catchments (*Ciais et al.*, 2013). The flux of N₂O will be influenced by “internal” environmental determinants, such as temperature, moisture, reduction-oxidation potential, nitrogen and carbon supply, pH and O₂ within the forest soils, which may be extremely heterogeneous over space and variable over time at a local scale. The flux of N₂O will also be affected by “external” environmental determinants, such as climate change, because nitrification and denitrification are microbially mediated processes that respond to climatic variables (*Barnard et al.*, 2005; *Singh et al.*, 2010), just like any other living things. These environmental determinants that operate at different spatial and temporal scales make it challenging to come up with regionally or catchment-specific estimates of gaseous N₂O and N₂ fluxes.

Environmental controls that govern N transformations within temperate forests are described below. Soil temperature influences enzyme activity of denitrification processes. As an enzyme-mediated microbial process, denitrification rates increase with increasing temperature up to the optimal temperature before denaturation of the enzyme occurs (*Farquharson and Baldock*, 2008). Sometimes the inverse also occurs depending on the activation energy barrier that needs to be overcome at each stage along the denitrification pathway. For example, if at low temperatures the activation energy barrier for N₂O reduction is higher than for its production, more N₂O may apparently seem to be produced (*Holtan-Hartwig et al.*, 2002), but in reality, N₂O is being allowed to accumulate due to a slowing down in the process that would result in its consumption.

Soil moisture exerts a direct control on N transformations by influencing soil O₂ and the related reduction-oxidation (redox) reactions (*Gambrell and Patrick*, 1978; *Schimel et al.*, 1997; *D’Odorico et al.*, 2003; *Lohse et al.*, 2009), and an indirect control on N transformations by reflecting hydrological flows, which deliver or remove essential precursors, for example dissolved N and dissolved organic carbon (*Lohse et al.*, 2009) from a defined area.

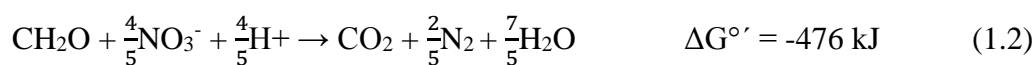
Together, soil temperature and moisture control rates of soil C and N cycling and gas and dissolved solutes loss from catchments through their influence on soil aeration

and redox reactions (*Gambrell and Patrick, 1978; Amundson and Davidson, 1990; Kralova et al., 1992*). Redox reactions occur in all soil metabolic activities and involve the transfer of electrons from reduced substances to oxidized substances with the utilization of energy that is released during oxidation used to satisfy metabolic needs. Organic matter is the preferred electron donor, while electron acceptors are preferred in the following sequence: O₂, NO₃⁻, manganese IV (Mn⁴⁺), iron III (Fe³⁺), sulfate (SO₄²⁻) and carbon dioxide (CO₂), depending on free energy released (*Kralova et al., 1991; Patrick and Jugsujinda, 1992; Stumm and Morgan, 1996; Hedin et al., 1998*). Based on *Hedin et al. (1998)*, most energy from heterotrophic respiration (oxidation of C) is generated when O₂ is the electron acceptor (Equation 1.1) compared to NO₃⁻ (Equation 1.2) then SO₄²⁻ (Equation 1.3) and, finally, methanogenesis (C reduction) yielding the lowest (Equation 1.4a and 1.4b) energy (*Patrick and Jugsujinda, 1992; Hedin et al., 1998*).

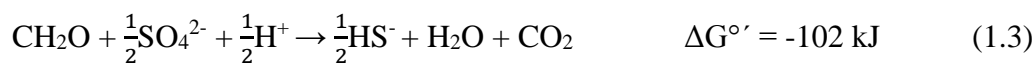
Aerobic respiration:



Nitrate reduction:



Sulfate reduction:



Methanogenesis:



Generally, soil redox potential (Eh) ranges from -300 to +600 mV with aerobic soils ranging from +400 to +600 mV. However, when soils are submerged in water over

long periods of time, the biological demand for O₂ exceeds supply, resulting in lower Eh within the system (*Kralova et al.*, 1992). Soil Eh ranging from -300 to +100 mV has been observed in most reduced soils, whereas Eh ranging from +100 to +400 mV is associated with moderately reduced soils (*Gambrell and Patrick*, 1978).

Factors that decrease O₂ diffusion into the soil (i.e., reduce Eh) are critical in controlling gaseous N fluxes, because of the dependence of denitrification on anaerobic conditions (*Farquharson and Baldock*, 2008; *Lohse et al.*, 2009). Therefore, wet areas are highly redox variable, because of the proximity of oxidized and reduced compounds and the probability of convergence of precursors of reactions that occur in hydrological flows creating these wet areas (*Hedin et al.*, 1998; *Pett-Ridge et al.*, 2006; *Lohse et al.*, 2009). Several studies have assessed the response of denitrification to changing Eh (e.g., *Kralova et al.*, 1992; *Patrick and Jugsujinda*, 1992; *Duca and Koper*, 2012; *Rubol et al.*, 2012). These studies revealed increased denitrification with increased soil moisture that was associated with decreased soil O₂ levels and low Eh (*Lohse et al.*, 2009; *Rubol et al.*, 2012). *Kralova et al.* (1992) reported denitrification being the main biological process controlling N transformations under moderately and strongly reducing conditions (-200 to +200 mV). High N₂ production was observed at -200 mV with no N₂O, whereas N₂O production was first observed at -100 mV and increased steadily with Eh until it peaked at 0 mV and then declined with further increases in Eh until +300 mV. Redox potentials >300 mV did not favour N₂O production (*Kralova et al.*, 1992).

The highly dynamic nature of hydrological and biogeochemical processes that influence N, combined with methodological challenges, has meant scientific understanding of N cycling (especially the gaseous forms; N₂ and N₂O) continues to attract attention (*Groffman et al.*, 2006; *Groffman et al.*, 2009). This can be partly attributed to the relatively varied oxidation states (+5 to -3) in the N cycle (*Duff and Triska*, 2000). Fortunately, catchments provide a natural spatial setting within which N transformations and fate can be studied to gain a better understanding of the processes (*Lohse et al.*, 2009). Because gaseous fluxes respond to location of sampling site relative to hydrological flow paths, research conducted within the catchment setting and at different topographic features can provide a balance and account for material fluxes

within defined spatial and temporal scales (*Lohse et al.*, 2009). More intensive monitoring of gaseous N fluxes in temperate forests is important because it not only helps estimate the global warming effects associated with N₂O fluxes, but also explains N transformation processes. This information can help account for differences in N export among catchments and the imbalance between N inputs and outputs, possibly yielding a gauge of forest stability and health.

Contemporary techniques that are currently in use to measure N₂O efflux involve laser instruments that have high precision, accuracy and sensitivity as well as high temporal resolution (*Butterbach-Bahl et al.*, 2013). Various studies have utilized these micrometeorological techniques (e.g., eddy covariance) in conjunction with tunable diode laser spectrometers that estimate N₂O fluxes over larger areas ranging from 0.5 to 1 ha (*Eugster et al.*, 2007; *Molodovskaya et al.*, 2011). The N₂O flux measurements using these methods provide continuous observations of N₂O flux data that is less variable. However, eddy covariance and tunable diode laser spectrometer techniques are not appropriate in hilly terrain like the TLW. For this reason, a more traditional chamber technique was used. Chamber methods have been and continue to be widely used in measuring gas fluxes (*Davidson et al.*, 2002; *Filippa et al.*, 2009; *Vilain et al.*, 2010; *Parkin et al.*, 2010; *Hall et al.*, 2014), because they are simple to use, less costly and provides the flexibility of studying treatment effects in addition to specific process studies (*Butterbach-Bahl et al.*, 2013) in less homogenous terrain.

1.3 Goal and Hypotheses

The goal of this thesis is to develop a predictive understanding of gaseous N fluxes from temperate forested catchments. The central premise of this thesis is that improved estimates of soil N₂O efflux from forested landscapes require consideration of topographic controls on nitrogen cycling and routing processes. Topography influences N transformations by controlling soil temperature, moisture and redox conditions and regulates NO₃⁻ and DOC availability while helping create anaerobic conditions required for denitrification to occur.

Two catchments in the TLW were used as a model system, similar to that suggested by *Vitousek* (2004), to understand N inputs, transformations and outputs among catchments, considering they receive similar N input and topography is the main variable, in response to different environmental factors. The two catchments were in close proximity (~1 km apart) with one of the catchments (c38) containing a large proportion of its area as wetland while the other (c35) contained a small percentage of wetland. In order to better understand the N transformations within the catchments and their fates, we simultaneously explored both the hydrological and biogeochemical interactions as suggested by *Lohse et al.* (2009) and monitored dissolved and gaseous N export.

Three hypothesis were addressed:

(1) During the growing season (snow free season), topography redistributes water and water-soluble precursors (NO_3^- , DOC) of formation of gaseous N species, creating hotspots of soil N_2O efflux in topographic features that accumulate water in response to summer storms. To test this hypothesis, N_2O fluxes during the growing season (June to September) were measured from 2006 to 2010 at three different transects with steep, moderate and gentle slopes. The relationship between N_2O efflux and rainfall, at different topographic positions, was assessed to determine if precipitation influences N_2O fluxes within catchments in the TLW.

(2) During the dormant season (snow season), water and water-soluble precursors (NO_3^- , DOC) of N gases are evenly distributed across topographic features until they are rapidly flushed from the catchment during spring melt. To test this hypothesis, gas samples were collected at different topographic positions within Catchment 38 (c38) under the snow pack to estimate N_2O fluxes. In addition, dissolved N species including dissolved N_2O was estimated in stream water exiting the catchment.

(3) The major differences in nitrogen export among the catchments are due to variations in the flux of denitrification products ($\text{N}_2 + \text{N}_2\text{O}$). To test this hypothesis, dissolved N (NO_3^- , NH_4^+ and DON) export patterns were assessed from two catchments: one with a large proportion of wetland (~25 %) and the other a small wetland proportion

(~ 1 %) within the TLW. The N export patterns were compared and $N_2 + N_2O$ was measured from the c38 wetland positions then applied the relationship between topographic position with N_2O and N_2 flux in c38 to the other catchment (c35) relative to the size of wetland area. Data for the 2006 water year (June 01, 2006 to May 31, 2007) were used for these estimates since it contained a continuous record for all N species.

The thesis findings are expected to help assess the ability of temperate forests to process the reactive loads of N that are deposited on them during snow free and under a snow pack, understand the relative importance of dissolved *vs.* gaseous fluxes from forested catchments N budgets, and close the N input *vs.* output N budget of forested catchments.

1.4 Thesis Organization

This thesis has been prepared in the integrated article format and consists of three manuscripts related to the three main questions. The introduction (Chapter 1) provides an overview of the research problem, including the hypotheses and questions that form the basis of the thesis. The first manuscript (Chapter 2) investigates growing season N fluxes, and shows how summer storms and associated changes in soil temperature, moisture and reduction-oxidation potential lead to gaseous N_2O efflux episodes from forest soils, with the largest episodes occurring in wetland features. The second manuscript (Chapter 3) investigates dormant season N fluxes, and shows that gaseous N_2O efflux occurs from forest soils underneath the snowpack, and that the ripening of the snowpack in readiness for spring melt leads to mobilization of the precursors and products of denitrification contributing to dissolved N_2O export from the streams during snowmelt. The third manuscript (Chapter 4) incorporates the new estimates of N_2O and N_2 efflux in previous estimates of N export from forested catchments and evaluates if by so doing the apparent difference in N export patterns among forested catchments is reduced. The chapter also evaluates if addition of denitrification products N_2+N_2O to N outputs reduces the discrepancy between N inputs and outputs. The last chapter (Chapter 5) summarizes the major conclusions of the thesis and identifies future research directions.

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Chapter 2

2 Summer Storms Trigger Soil N₂O Efflux Episodes in Forested Catchments

On forested landscapes, topography influences both biogeochemical and hydrological processes that in turn affect the storage of N within the forest ecosystem, as well as the export of N both to surface waters and to the atmosphere. This chapter tests the hypothesis that during the snow free season (i.e., growing season), topography redistributes water and the water-soluble precursors (i.e., nitrate (NO₃⁻) and dissolved organic carbon (DOC) for the formation of gaseous N species, forming hotspots of soil N₂O efflux in topographic features that accumulate water in response to summer storms. Storm water that exceeds the forest canopy interception capacity falls to the forest floor, converges downslope in topographic flat areas and depressions, resulting in an increase in soil moisture content and an associated decrease in reduction-oxidation (redox) potential that favours the formation of reduced forms of N including N₂O. The size of storms is used to predict the magnitude of N₂O effluxes during these storm events.

2.1 Introduction

Atmospheric deposition is the main source of elevated nitrogen (N) levels in terrestrial and aquatic ecosystems (IPCC, 2013). The atmospheric deposition of reactive N is generally attributed to the wide use of nitrogenous compounds in agricultural and industrial processes (Vitousek *et al.*, 1997; Gruber and Galloway, 2008; Schlesinger, 2009; Canfield *et al.*, 2010; IPCC, 2013), which can disperse far from the source through the atmosphere with consequences for both terrestrial and aquatic ecosystems (Vitousek *et al.*, 1997; Sirois *et al.*, 2001; Kulkarni *et al.*, 2008). Despite decreases in anthropogenic N emissions (Environment Canada, 2012), elevated N levels continue to affect temperate forests (Sirois *et al.*, 2001). Atmospherically deposited N in forests can be stored in the soil, transformed by organisms, exported in dissolved forms to surface waters, or exported in gaseous forms through denitrification to nitrous oxide (N₂O) and dinitrogen (N₂) gas to the atmosphere (Figure 2.1; Gambrell and Patrick, 1978; Wrage *et al.*, 2001; Yanai *et al.*, 2013).

Northern forests are a major world biome (*Natural Resources Canada*, 2014) and understanding the fate of atmospherically deposited N is important because exports to aquatic and atmospheric systems can have negative effects on society. In particular, N₂O, which is the third largest well-mixed greenhouse gas contributor to global warming (*Elkins and Dutton*, 2011) and the most significant ozone-depleting agent, (*Ravishankara et al.*, 2009; *UNEP*, 2013) deserves attention. N₂O emissions have increased by 20% from pre-industrial levels (*IPCC*, 2013), and they are projected to double by 2050 (*UNEP*, 2013). These increases in N₂O efflux are dominated by emissions from soils treated with synthetic and organic N fertilizer (*Davidson*, 2009; *Syakila and Kroeze*, 2011), but forest soils, especially those in wetland dominated areas, may also contribute substantial amounts of N₂O (*Ambus and Robertson*, 2006).

The denitrification of reactive N to N₂O is a natural microbial process that predominantly occurs in soils (*Gambrell and Patrick*, 1978; *Firestone and Tiedje*, 1979). Denitrification is favoured in flooded soils, where there is an ample supply of labile organic matter to serve as an energy source and support considerable microbial activity at optimum temperature, but the oxygen re-supply rate is inadequate to satisfy the demand for terminal electron acceptors in response to microbial respiration. This lowers the redox potential into ranges that favour the reduction of NO₃⁻ to N₂O or N₂ (*Gambrell and Patrick*, 1978; *Lohse et al.*, 2009; *Burgin et al.*, 2011) (Figure 2.1).

On forested landscapes, soil water availability regulates soil N cycling rates and pathways (*Lohse et al.*, 2009; *Burgin et al.*, 2011). Precipitation regimes influence soil water availability (*Li et al.*, 1992; *Zhu et al.*, 2014). Once a rain event exceeds the interception capacity of a forest canopy, excess water increases soil moisture, thereby lowering redox potential and increasing the likelihood that denitrification will occur. Recent estimates suggest that the frequency of intense and heavy rainfall events has increased in North America and Europe (*Hamburg et al.*, 2013; *IPCC*, 2013). This may influence N₂O efflux during the growing season at continental scales. Considering the episodic nature of soil N₂O fluxes (*Farquharson and Baldock*, 2008) and spatial and temporal variation (hot spots and hot moments, *McClain et al.*, 2003), investigating the effects of rainfall events may provide valuable information about N₂O efflux estimates by

capturing the hot moment. Several studies have reported increased N_2O production with increased rainfall using modeling techniques (*Li et al.*, 1992), laboratory experiments (*Rubol et al.*, 2012; *Hall, et al.*, 2013) and field experiments (*Vilain et al.*, 2010).

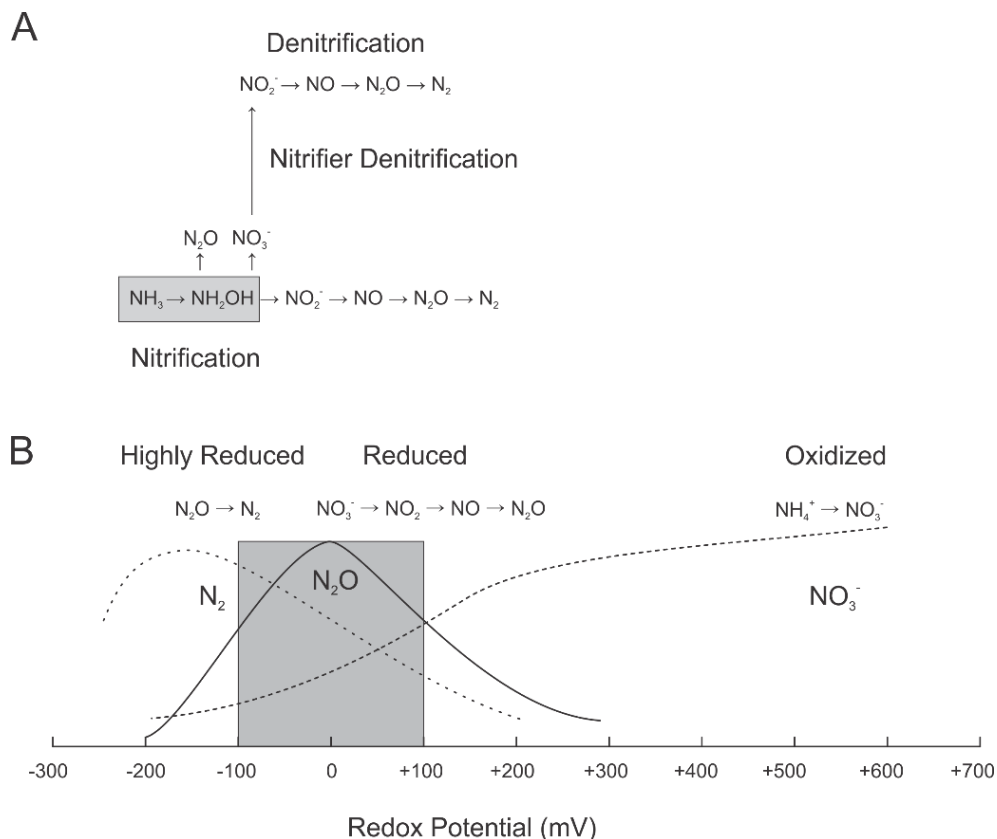


Figure 2.1: Conceptual figure of (A) biochemical pathways for denitrification (modified from *Wrage et al.*, 2001) and (B) relationship between reduction-oxidation (redox) potential conditions and the end products of denitrification (*Kralova et al.*, 1992).

In forested landscapes, precipitation that reaches the forest floor is redistributed due to topography (*Zhu et al.*, 2014). Topography plays an important role in regulating not only soil nutrient pools but also temperature and moisture of soils (*Stewart et al.*, 2014), thereby influencing microbial biomass and N cycling processes (*Ambus*, 1998; *Hazlett and Foster* 2002; *Gu et al.*, 2011; *Stewart et al.*, 2014). Precipitation-induced saturation of soils results in low O_2 concentrations, causing low redox potentials that favour N_2O production, and prolonged saturation can lead to further reduction of N_2O to N_2 (*Gambrell and Patrick*, 1978; *Firestone and Tiedje*, 1979).

There is a need to understand the controls on denitrification and develop better estimates of gaseous N export from forested catchments (*Pickett and Cadenasso, 1995; Ambus and Robertson, 2006; Yanai et al., 2013*). This need has been made more important because the scientific understanding of N cycling has not kept pace with other biogeochemical processes due to spatial and temporal heterogeneity (*McClain et al., 2003*) and methodological challenges (*Groffman et al., 2006*) encountered when measuring and estimating N₂O fluxes. Understanding the links between topography and redox potential as well as their impacts on N₂O production may lead to improved estimates of N₂O efflux from forest landscapes. Accounting for topographically influenced gaseous N export may help explain observed differences in dissolved N export from forested catchments that have similar forest type and age and receive the same amount of N deposition yet show substantial differences in N export (*Creed and Beall, 2009*).

The purpose of this study was to explore topographic controls on temporal and spatial patterns of redox potential and their relation to N₂O production along a hillslope during the growing season in a temperate forest. It was hypothesized that during the non-growing season (snow free season), topography redistributes water and water-soluble precursors nitrate (NO₃⁻) and dissolved organic carbon (DOC) of formation of gaseous N species creating hotspots of soil N₂O efflux in topographic features that accumulate water in response to summer storms. To test the hypothesis, the following questions were asked: Q1: Does soil N₂O efflux vary with topographic feature (uplands, lowlands, wetlands)? Q2: Do variations in the soil physical (temperature, moisture) or chemical (redox potential, nitrate, DOC) conditions within topographic features relate to soil N₂O efflux? Q3: Do episodic variations in the soil physical or chemical conditions due to summer storms correlate with changes in the rates of soil N₂O efflux?

The hypothesis and associated questions were tested in the forested landscape of the Great Lakes-St. Lawrence forest region at the northern edge of the temperate forest biome of North America.

2.2 Study Area

The Turkey Lakes Watershed (TLW) ($47^{\circ}03'00''\text{N}$ and $84^{\circ}25'00''\text{W}$) is located near the eastern shore of Lake Superior in the Algoma Highlands of Central Ontario, 60 km north of Sault Ste. Marie (Figure 2.2). The watershed occupies 10.5 km^2 of the northern edge of the Great Lakes-St. Lawrence forest region and mainly comprises an uneven-aged, mature to over-mature, old-growth hardwood stand. The need to study the effects of acid rain on terrestrial and aquatic ecosystems led to the establishment of this study site in 1980 (Jeffries *et al.* 1988; Jeffries, 2002).

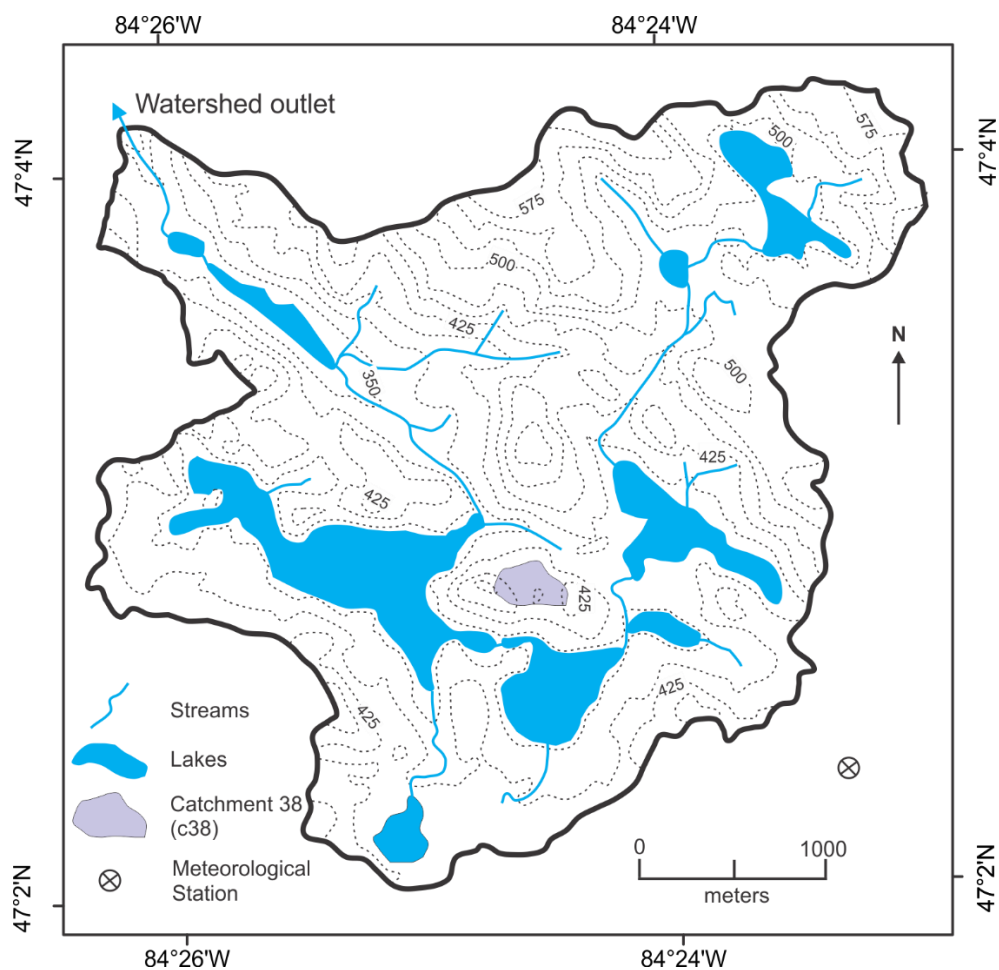


Figure 2.2: Map of the Turkey Lakes Watershed near Sault Ste. Marie, Ontario, Canada.

The TLW is situated in close proximity to the eastern shoreline of Lake Superior, ensuring that a continental climate dominates the watershed area, with a mean total annual precipitation of 1189 mm and mean annual temperature of 4.6°C , based on the

30-year (1981-2010) data record from the meteorological recording station that is located just outside the watershed. Total annual precipitation and stream discharge have generally decreased over this 30-year time span (Mengistu *et al.*, 2014), while there has been a gradual increasing trend in the mean annual temperature (Figure 2.3).

The topographic relief at the TLW is about 400 m, ranging from 644 m at the apex of Batchawana Mountain to 244 m at the outlet of the Batchawana River that drains into Lake Superior (Creed *et al.*, 2002). Precambrian metamorphosed basalt forms the base upon which the watershed rests, with greenstone and granite comprising most of the bedrock (Jeffries *et al.*, 1988). A thin discontinuous till of varying depth overlays the bedrock, ranging from < 1m in high elevation areas, gradually increasing to 1-2 m in lower elevation areas and culminating in a substantial depth of up to 65 m in depressions.

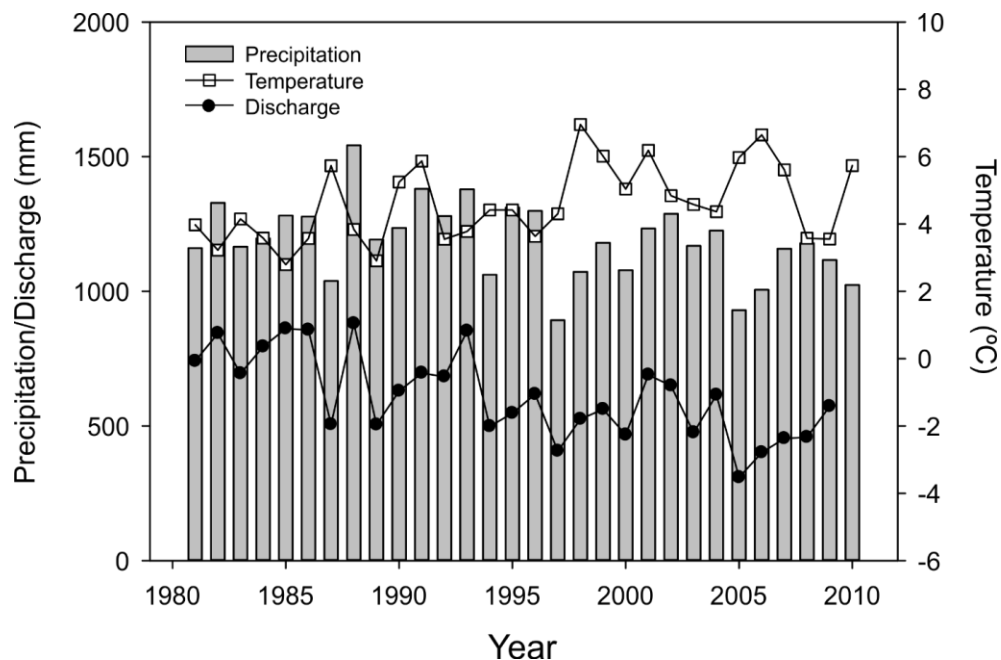


Figure 2.3: Cumulative annual precipitation and discharge and average annual temperature in the Turkey Lakes watershed from 1981 to 2010.

Bedrock-controlled depressions and areas adjacent to streams and lakes are dominated with dispersed pockets of Ferric Humisols with Orthic Ferro-Humic and Humo-Ferric podzols as the dominant soils (Canada Soil Survey Committee, 1978). The forest stand is dominated by 90% sugar maple (*Acer saccharum* Marsh.), dotted with patches of white pine (*Pinus strobus* L.), yellow birch (*Betula alleghaniensis* Britton),

ironwood (*Ostrya virginiana* (Mill.) K. Koch), white spruce (*Picea glauca* Moench Voss.), and red oak (*Quercus rubra* L.) in upland areas. Sugar maple dominates in the wetland areas, which are interspersed with some eastern white cedar (*Thuja occidentalis* L.), balsam fir (*Abies balsamea* (L.) Mill.), and black ash (*Fraxinus nigra* Marsh.), (Wickware and Cowell, 1985).

This study complements one conducted by Webster (2007), who explored topographic effects on soil carbon dioxide efflux from the same forested landscape (Webster et al. 2008a; Webster et al., 2008b).

2.3 Methods

2.3.1 Experimental Design

Within the TLW, catchment 38 (c38) occupies an area of 0.063 km² that includes a major (25% of catchment area) wetland (Figure 2.4). Distinct topographic features can be identified along hillslopes that drain into the wetland, which fall into the general categories of frequently dry uplands, intermittently dry and wet lowlands, and frequently wet wetlands.

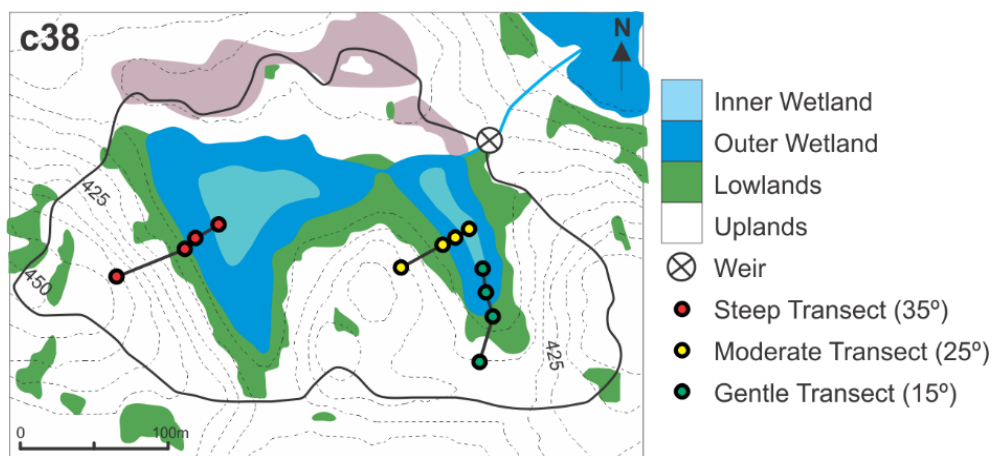


Figure 2.4: Map of catchment 38 (c38) in the Turkey Lakes Watershed.

Three transects were established with different slopes: the steep transect has a slope of approximately 35°, the moderate transect has a slope of approximately 25°, and the gentle transect has a slope of approximately 15°. All three transects were

instrumented to monitor environmental conditions and collect gas samples at four topographic positions: Inner Wetland (IW), Outer Wetland (OW), Lowlands (comprising Toeslope (TS) and Foothills (FS)), and Uplands (comprising Backslope (BS), Shoulder (SH) and Crest (CR)) (*Webster et al.*, 2011). Samples were collected from early June to late September from 2006 to 2010. Initially, one collar was placed at each of the IW, OW, Lowlands (in the TS position) and Uplands (in the BS position) on each of the three transects. In 2008, two additional replicate collars were placed at each of the wetland positions (IW and OW). Each sampling site (topographic position) was instrumented for synoptic sampling of soil N₂O efflux, synoptic monitoring of soil solution for dissolved N species and dissolved organic carbon (DOC), and continuous monitoring of soil temperature, moisture and redox potential.

2.3.2 Data Collection

Meteorological data including daily total precipitation (using a standard rain gauge) and daily average air temperature were obtained from the Canadian Air and Precipitation Monitoring Network (CAPMoN) station operated by Environment Canada and located just outside the TLW (latitude, 47°02'06" N; longitude, 84°22'52" W).

Ground based static chambers (*Hutchinson and Livingston*, 2001) were used to monitor soil N₂O efflux at each sampling site. Square collars measuring 45.7 × 45.7 cm and enclosing 2,088 cm² were inserted 10-20 cm into the soil. Samples were collected between 10 am and 2 pm EST being representative of the daily mean average (*Vilain et al.*, 2010) in each of the years from 2006 to 2010. Vented PVC chambers (14.6 L) fitted with a quick connect base fitting and tubing were inverted over the collars for an hour to allow N₂O to build up (*Holland et al.*, 1999). The vent was necessary to inhibit the air disturbance during chamber placement onto the collar and to address concerns over negative and positive pressure changes during sampling that would affect accumulation of the target gas (N₂O) during sampling (*Hutchinson and Livingston*, 2001).

Gas samples were collected at time 0, 20, 40 and 60 minutes from each of the collars using a 30 mL syringe fitted with a needle. Prior to sample collection, the sampling tube was flushed five times with 30 mL of air from the chamber to clear tubing

of previous sample and ensure mixing of the air inside the chamber. Gas samples equivalent to 30 mL of chamber air were then drawn into a sampling syringe – 5 mL of the sample was flushed through the sampling needle to rinse it of any residual atmospheric air. The remaining portion, 25 mL of the sample was injected into sealed 12.1 mL pre-evacuated Exetainers[®] that contained a small amount of magnesium perchlorate as a desiccant, and was then transported to the laboratory for analysis.

Gas sample analysis for measuring N₂O was performed using gas chromatography on a SRI 8610C (SRI Inc., Las Vegas, NV) equipped with an electron capture detector. The make-up gas comprised 10% methane, with the remaining 90% of the balance made up of argon with helium as the carrier gas. Samples were loaded into a Combi-PAL auto sampler (CTC Analytics, Switzerland), and calibration standards (5 ppm N₂O) were analyzed after every 12 samples (*DeSimone et al.*, 2010). The samples were analyzed with 2.5 mL of sample injected into the gas chromatograph, and N₂O concentrations were determined by comparison of peak area of the sample and the average peak area of the calibration standard run before and after the sample using 5 ppm N₂O standard:

$$C_g = \frac{A}{R} \quad (2.1)$$

where C_g is the concentration of the sample (ppm), A is the sample N₂O peak area from the gas chromatogram and R represents the peak area of standard per unit concentration (ppm) of N₂O:

$$R = \frac{\mu}{a} \quad (2.2)$$

where μ is the mean peak area derived from the standards run before and after the sample while “ a ” is the actual concentration of the standard (5 ppm).

N₂O fluxes were determined by calculating the linear regression of the slope of N₂O concentration within the chambers with time (*Mathias et al.*, 1980; *Conen and Smith*, 2000; *Hutchinson and Livingston*, 2001; *Hall et al.*, 2014). The N₂O fluxes were scaled up to the total headspace volume derived from the addition of chamber volume

and the collar volume above the soil surface and cross-sectional area of the collar, and corrected for ambient pressure and temperature:

$$F_{corr} = \left(\frac{F \times V}{V_{corr} \times A} \right) \times 28 \frac{mg}{mmol} \quad (2.3)$$

where F_{corr} is the final corrected N₂O flux (mg-N m⁻² hour⁻¹), F is the N₂O flux measured in the field (mL N₂O L⁻¹ hour⁻¹), V is the total volume of the chamber plus the collar (liters), A is the cross-sectional area of the collar (m²), 28 mg mmol⁻¹ is the molar mass of nitrogen in N₂O and V_{corr} is the molar volume of N₂O corrected for temperature and pressure (mL mmol⁻¹) and measured during the time the samples were collected.

$$V_{corr} = \frac{22.414 \times P^{\circ} \times T}{T^{\circ} \times P} \quad (2.4)$$

where T (K) and P (hPa) are the actual air temperature and pressure at the time the samples were collected, T° and P° are 273.16 K and 101.32 kPa (1013.2 hPa) and 22.414 is the molar volume, in liters, of N₂O at standard temperature (T°) and pressure (P°).

To determine the effects of soil environment on N₂O gas transformations, soil temperature (2005-2010), soil moisture (2005-2010), and soil redox potential (2005) were measured at each sampling site and water table depth was measured at the centre of the wetland (2005-2010). Each sampling site was fitted with probes 5 cm into the mineral soil for continuous monitoring of soil temperature, moisture and redox potential. A thermocouple wire enclosed in a 0.634 cm inner diameter copper tube filled with epoxy was used to measure soil temperature. Water Content Reflectometers (WCR) were used to measure soil moisture (CS 616, Campbell Scientific Canada Corp., Alberta, Canada). Later, WCR output was converted to volumetric water content using a manufacturer provided calibration equation for upland soils and by *Yoshikawa et al.* (2004) for wetland soils. Redox potential was determined by measuring the voltage between a platinum electrode and a potassium chloride reference electrode. All the instruments were wired to data loggers (Campbell Scientific CR10X) using multiplexors, which were powered by batteries charged by solar panels. Wetland water table depth was continuously measured

using a water level logger (WT-HR Water Height Data Logger, TruTrack Inc., Christchurch, NZ). All environmental data were collected every five minutes and averaged every 30 minutes by the data logger.

Soil pore water samples for nutrient determination were collected at each of the sampling positions using suction lysimeters (Model 1900, Soil Moisture Corp., Santa Barbara CA). Triplicate shallow lysimeters were installed at each topographic position and up to a depth of 10 cm into the mineral soil or peat. Prior to installation, lysimeters were acid-washed using 10% hydrochloric acid and rinsed using deionized water (10 times), and then flushed with soil pore water *in situ* prior to sample collection.

A vacuum of 50 kPa was applied to each lysimeter 24 hours prior to sample collection, after which the soil pore water samples were collected in acid-washed polycarbonate bottles using a hand vacuum pump. All samples were transported back to the laboratory and refrigerated; the samples were then filtered through 0.45 μm polysulfone membrane filters (Supor 450, Pall Gelman Science, Michigan, USA), then frozen for transport and storage until the day they were analyzed. Analysis of water samples for NO_3^- and NH_4^+ was done at UWO using flow-injection colorimetry (Lachat QuikChem 8000, Milwaukee, WI), DOC was determined by oxidative combustion coupled with infrared detection while total nitrogen was done by chemiluminescence analyses (TOC-TN auto analyzer, Shimadzu Japan).

2.3.3 Data Analyses

Only positive N_2O measurements were included in the analysis. This is consistent with the non-inclusion of soil uptake N_2O (negative N_2O fluxes) in the budget by *Mosier et al.* (1998). The publication of *Mosier et al.* (1998) served as a reference, outlining that soil uptake of N_2O would not be included in soil N_2O budgets until better information became available. In our study, percentage of negative N_2O fluxes was low (16%) relative to the total number which was 21 % less than the values reported by *Chapuis-Lardy et al.* (2007) which stood at 37 % while *Verchot et al.* (1999) reported recording up to 65 % of negative fluxes. Therefore, excluding the negative fluxes did not greatly affect our N_2O flux estimates (*E. Enanga*, Unpubl. Data).

All statistics were performed using SigmaPlot 12 software (Systat, San Jose, CA). The data were analyzed individually by transect, as well as averaged across the three transects (i.e., one value per topographic position per day). Statistical significance was assessed at the $p < 0.05$. Box whisker plots were used to assess the variations in soil N₂O efflux and the environmental controls on soil N₂O efflux including temperature, moisture, redox potential, NO₃⁻ and DOC concentrations among different topographic positions.

To investigate the effects of rain events on N₂O efflux, only days where the precipitation events were greater than or equal to 3 mm that exceeds the canopy interception capacity, (*Carlyle-Moses & Price, 1999; Price & Carlyle-Moses, 2003*) were assessed. And also the water table had to be less than 10 mm above the ground surface, for the N₂O fluxes to be included in the regression, based on *Jorgensen and Elberling (2012)* who used the 10 mm threshold in their study. The relationship between precipitation events and N₂O efflux was assessed using nonlinear regression with a power function for all four topographic positions. An Analysis of Covariance (ANCOVA) was performed to determine if the precipitation vs. N₂O relationship varied among topographic positions.

2.4 Results

There has been a declining trend in precipitation and discharge in the TLW since 1981 (Figure 2.3). However, among the years being investigated in this study (2005-2010), precipitation and discharge were lowest in 2005 and tended to increase from 2006 to 2010. From June to September in 2005, there were considerable differences in soil temperature among topographic positions when averaged across transects with the IW and OW topographic positions warmer than the lowlands and uplands (Figure 2.5). When separated by transect, there were no consistent patterns among the topographic positions. Soil temperature was highest in the upland position on the steep slope transect, the OW and lowland in the moderate slope transect, and in the IW and OW in the gentle slope transect.

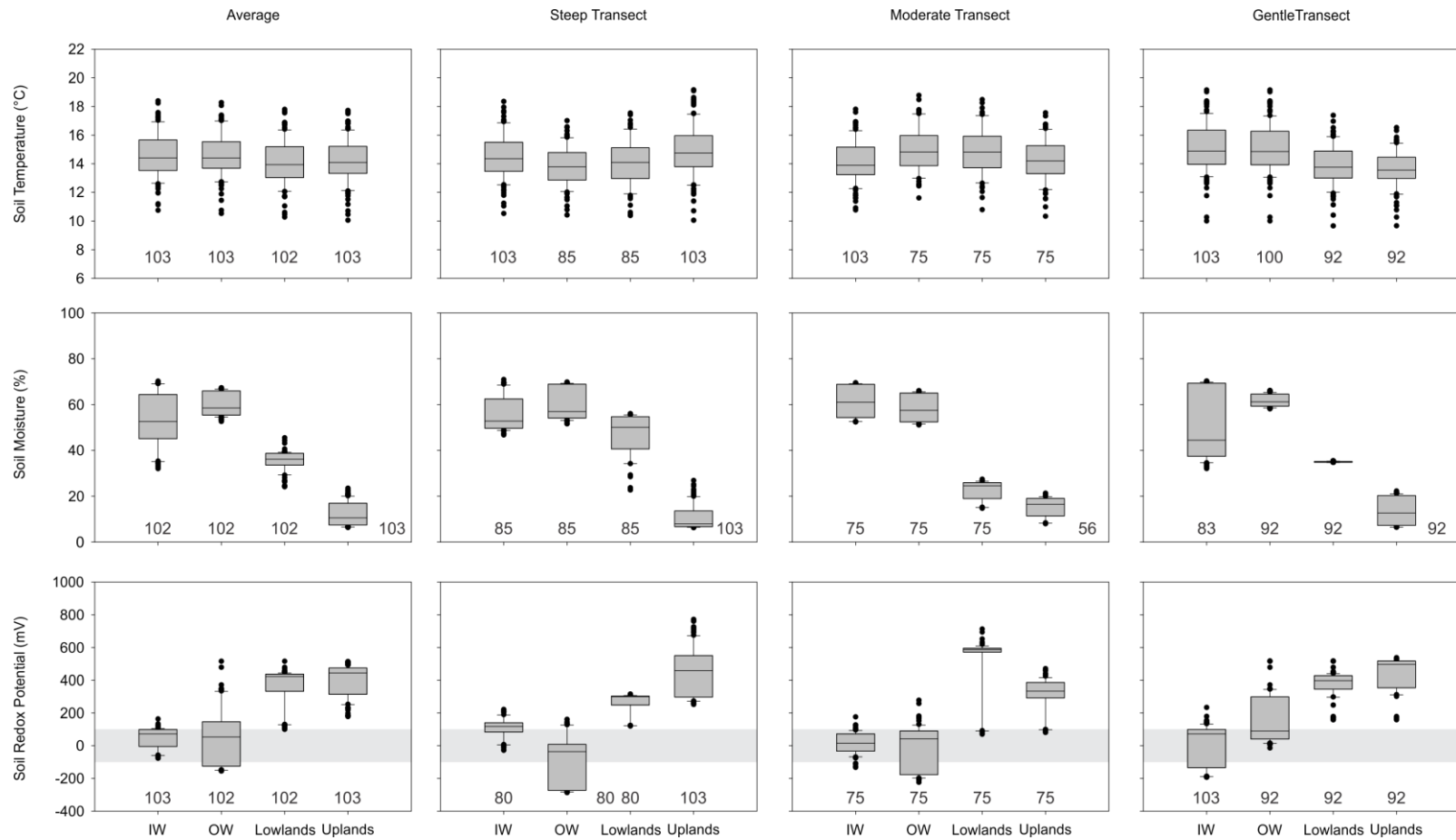


Figure 2.5: Soil temperature, moisture, and redox potential from 2005 in the inner wetland (IW), outer wetland (OW), lowlands, and uplands for topographic positions averaged across three transects and for individual transects. The sample sizes are indicated below each box.

There were variations in soil moisture among topographic positions. The OW had the highest moisture averaged across the three transects. There was also a discernable difference in redox potential at each topographic position. Redox potential was higher in the lowland and upland positions than in the wetlands (IW and OW) both when the three transects were averaged and when each transect was considered separately (Figure 2.5). On average, the IW and OW positions had redox potentials within the +100 to -100 mV range where denitrification occurs most often (Figure 2.1, 2.5), but among the transects, the redox potential in the IW and OW positions of the moderate slope transect were most consistently within this range (Figure 2.5).

Based on our observations, redox potential is the better indicator of potential N₂O production than temperature. Unfortunately we only had redox potential data for 2005, when the full suite of environmental controls was monitored. Therefore, we looked at the relationship between soil moisture (which we had for all years) versus redox potential in 2005 to see if a significant relationship existed. We observed that soil moisture of about 60% was associated with redox potential of -100 to +100 mV where peak N₂O production often occurs. We used soil moisture as a proxy for redox conditions that lead to N₂O production and observed that these conditions occurred predominantly in the IW and OW positions (Figure 2.6).

There were noticeable differences in soil N₂O efflux among the topographic positions in data from 2006 to 2010. The OW position recorded higher soil N₂O efflux than all other positions (Figure 2.7). The soil moisture patterns from 2006 to 2010 closely approximate those of the soil N₂O efflux patterns, while DOC and NO₃⁻ patterns did not show a direct association with N₂O (Figure 2.8). The soil moisture and soil N₂O efflux patterns were similar (Figures 2.7, 2.8), there was less variation in soil moisture (coefficient of dispersion, which is the inter-quartile range divided by median) of 0.24, 0.04, 0.14, and 0.18, respectively than in soil N₂O efflux (coefficients of dispersion of 1.08, 1.45, 1.33, and 1.09 in the IW, OW, lowlands, and uplands, respectively). To investigate what other factor could be influencing soil N₂O efflux, we investigated the relationship between precipitation (which influences moisture) and soil N₂O efflux.

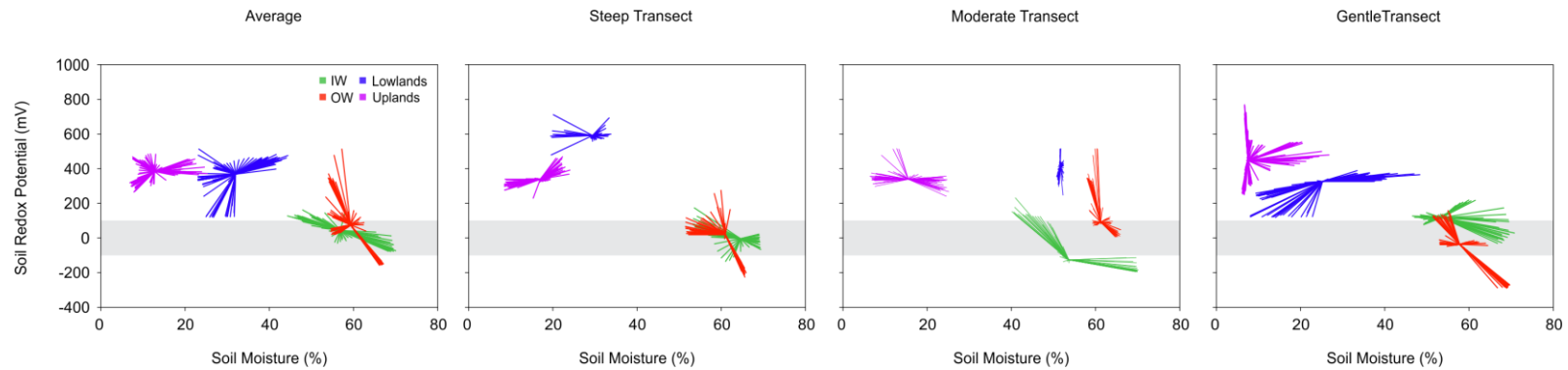


Figure 2.6: Relationship between reduction-oxidation potential and soil moisture in 2005 averaged across transects and in individual transects with topographic position medians as centroids on which the individual data points are centered.

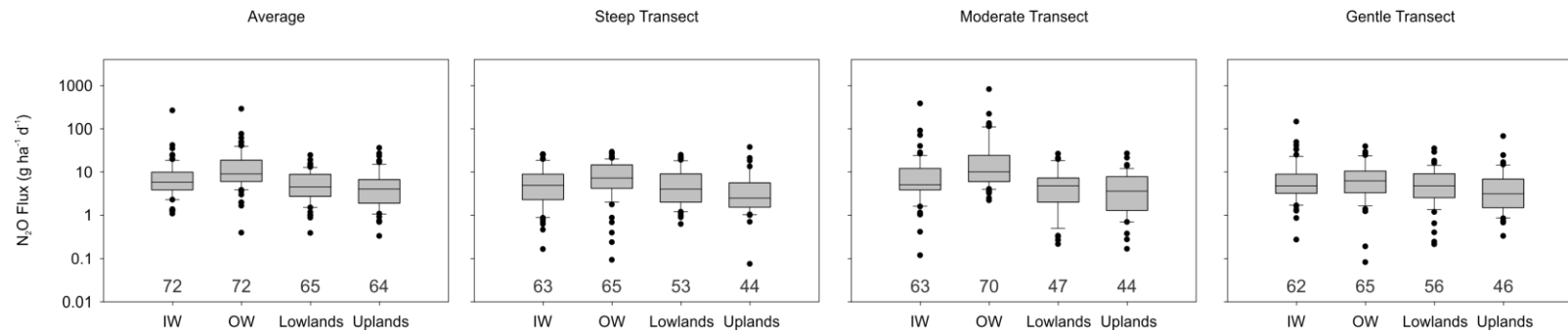


Figure 2.7: Soil N₂O efflux from 2006-2010 in the inner wetland (IW), outer wetland (OW), lowlands, and uplands topographic positions averaged across three transects and for individual transects, and sample sizes are indicated below the boxes.

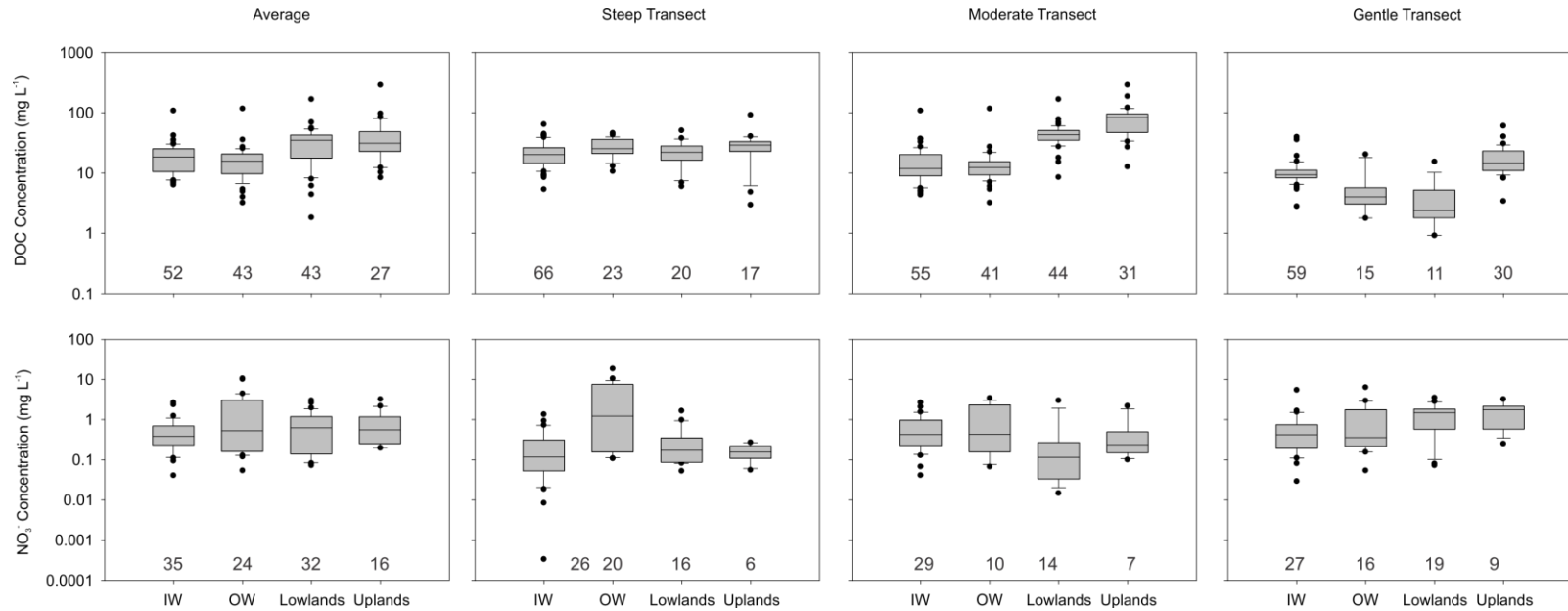


Figure 2.8: Dissolved organic carbon (DOC) and nitrate (NO₃⁻) from 2006-2010 in the inner wetland (IW), outer wetland (OW), lowlands and uplands topographic positions averaged across three transects and for individual transects on or within one day of N₂O sampling, with sample sizes indicated below the box whisker plots.

Precipitation was based on rainfall measured on the day before and day of measurement in an attempt to capture precipitation that may have fallen over the night prior to the measurements that took place between 10 am and 2 pm. There were significant relationships between precipitation and soil N₂O efflux in the IW and OW topographic positions when considering days when a precipitation event was greater than or equal to 3 mm and when the water table depth was less than 10 mm (Figure 2.9). The magnitude of precipitation explained 62% of the variation in soil N₂O efflux from the IW position and 72% of the variation in the OW position with at most 42 triplicate measurements averaged to derive the regression. There were no significant relationships between the magnitude of precipitation and the soil N₂O efflux from the lowlands and uplands positions.

An ANCOVA of the relationships between the magnitude of precipitation and soil N₂O efflux showed that there were significant differences between the IW and OW topographic positions ($df=1$, $F=22.16$, $p<0.001$) and no significant interaction effect. While we used same day plus previous day precipitation to investigate the relationship between N₂O efflux and precipitation, there were some days where the majority of the precipitation fell the day before sampling. Removing these days from the investigation improved the variation explained in the IW and OW (to 77% and 83%, respectively), but left us with only six data points.

2.5 Discussion

A changing climate is likely to influence the physical conditions in temperate forested ecosystems that promote the release of N₂O, a powerful greenhouse gas (*Elkins and Dutton, 2011; Syakila and Kroeze, 2011; IPCC, 2013*). We investigated spatial and temporal patterns in the physical conditions that promote N₂O release during the summer growing season. We found that topography is a key determinant of soil N₂O efflux because of its role in redistributing precipitation that falls below the forest canopy into topographic flats and depressions and thereby creating redox conditions needed for soil N₂O production. We observed that rain falling at the different topographic positions results in different N₂O fluxes. With projected increases in the magnitude and frequency of precipitation events due to climate change within the region (*Hamburg et al., 2013*),

there will likely be corresponding increases in soil N₂O efflux as long as the frequency of rainfall is not high enough to flush NO₃⁻ out of the system instead allowing it to undergo denitrification.

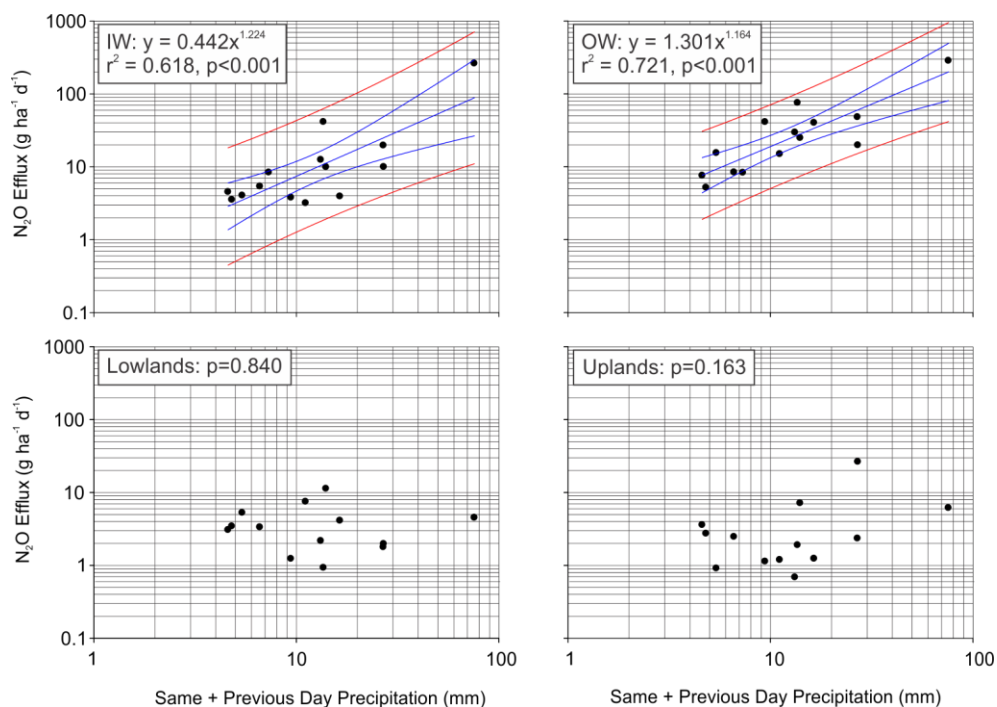


Figure 2.9: Relationship between precipitation events and soil N₂O efflux on days where same day plus previous day precipitation exceeded 3 mm and the water table depth was less than 10 mm. The red lines represent the 95% confidence interval for the points while the blue outer-lines the regression line, with the center line representing the non-linear regression line of best fit.

The wetland areas recorded redox potentials in the lower ranges (<300 mV) that would support denitrification with N₂O formation (Kralova *et al.*, 1992). The OW position experienced more variable redox potentials that oscillated between oxic (>400 mV) and anoxic (<400 mV). Therefore, there were dry spells (redox >400 mV) during which nitrification would occur (Wrage *et al.*, 2001; Hazlett and Foster, 2002; Foster *et al.*, 2005; Snider *et al.*, 2009) to replenish the NO₃⁻ that was depleted during denitrification that corresponded with wet periods (redox <400 mV). Our results suggest rain events would raise the soil moisture content in the surface soils with corresponding decrease in the redox potential, while at the same time bringing in more NO₃⁻ and DOC with vertical flux of water and the lateral flux due to movement of water from the

bordering upland areas (*Cirimo and McDonnell, 1997; Lohse et al., 2009*) creating conditions that favour N₂O production. The relationship between size of rainfall event and soil N₂O flux was stronger at the OW, probably due to the *in situ* production of precursors (NO₃⁻ and DOC) of denitrification when redox was high and from the *ex situ* production of precursors from upland areas that are transported to the OW during periods of hydrologic connectivity when lateral movements of water and nutrients occurs (*Cirimo and McDonnell, 1997; Hill et al. 2000; Lohse et al., 2009; Creed and Beall, 2009; Mengistu et al., 2014*).

The IW position recorded a narrower range of redox potentials compared to the OW position. This may have resulted in greater depletion of NO₃⁻ with minimal to no replenishment of NO₃⁻ because of lack of great variability in redox that may have replenished exhausted precursors through nitrification. Subsequently further reduction of N₂O to N₂ in the absence of adequate NO₃⁻ (*Wrage et al., 2001*) was favoured; this may explain the lower N₂O fluxes observed from the IW position relative to the OW position. In addition the location of the IW relative to the OW position (IW is surrounded by OW) along the hillslope continuum ensured that the IW's main source of inputs through lateral flow is from the OW position. The OW position may be rich in N₂O due to its rapid processing of NO₃⁻, hence the IW may be receiving inputs depleted in NO₃⁻ and rich in N₂O from the OW position and therefore relegated to the role of, mainly, completing the denitrification process by further reducing N₂O to N₂ (*Gambrell and Patrick, 1978; Wrage et al., 2001*).

The OW position recorded much more variable redox potentials (i.e., greater range of redox) compared to the IW position which may have resulted in OW generating its own precursors (NO₃⁻) during the dry spell (high redox periods) through nitrification in addition to receiving more from neighbouring lowland and upland areas during wet conditions through lateral flow of water. This coupled with the high moisture and low redox potential in the OW resulted in reduction (conversion) of the “double dose” abundance of NO₃⁻ to predominantly N₂O. While the IW is surrounded by the OW hence shielded from fresh inputs of NO₃⁻ and DOC from uplands and left to scavenge for remnants (N₂O from the OW) and its own N₂O (*in situ* generated N₂O) which may have

been further reduced to N_2 for lack of a better alternative (NO_3^-) electron acceptor. Alternatively the position of OW relative to other topographic positions means it is in direct contact with material including precursors from uplands because it lies within the hydrologic flow pathway (Lohse *et al.*, 2009).

The upland and lowland topographic features generally recorded higher redox potential values (>300 mV) compared to the wetland areas, hence denitrification was not the favoured process (Kralova *et al.*, 1992; Foster *et al.*, 2005) at this position because the soils are better aerated (Morse *et al.*, 2014). Oxygen is the preferred electron acceptor at the lowland and upland areas and not NO_3^- (Kralova *et al.*, 1992) hence N_2O efflux was low and did not respond in any systematic or significant way to rainfall events. Rain water falling on this lowland and upland areas, with inclined slopes, would flow to the wetland areas and at the same time transport NO_3^- to the wetland areas (Lohse *et al.*, 2009) with OW position as the major recipient due to its location and as evidenced by the generally much more variable NO_3^- concentration in soil pore water at the OW compared to the other topographic positions (Figure 2.8).

The steeper slope transect seemed to have most of the NO_3^- produced at the upland transported downslope to the OW (Cirimo and McDonnell, 1997) compared to the gentler slope transect which generally recorded lower NO_3^- concentrations at the OW (Figure 2.8). And the moderate slope transect did not show major variations in NO_3^- concentrations among the different positions while the gentle transect did not favor transport to the wetland areas.

The significant positive soil N_2O efflux response to rainfall events > 3 mm suggests that rainfall influences key physical and chemical parameters that promote N_2O efflux. This include increasing soil moisture content, reducing O_2 concentration due to rain water occupying some of the soil pore spaces resulting in low redox potential (Li *et al.*, 1992; Liptzin and Silver, 2009; Rubol *et al.*, 2012), and also causing water table depth to rise. In addition, in the OW position, which has the highest soil N_2O efflux, there is very little variation in soil moisture, but quite a bit of variation in soil N_2O efflux (as indicated by the coefficients of dispersion), which suggests that rainfall may be

controlling soil N₂O flux by transporting the upland and lowland precursors (NO₃⁻ and DOC) to the wetland which then triggers soil N₂O efflux.

Rainfall events that exceed the canopy interception capacity (*Carlyle-Moses and Price, 1999; Price and Carlyle-Moses, 2003*) reach the forest floor triggering N transformation processes that lead to significant soil N₂O efflux from wetland positions. These rainfall events only trigger soil N₂O efflux episodes if the water table depth is < 10 mm (1 cm) because at higher water table depths, nutrients would bypass the bio-active layer and get flushed from the catchment into the stream (*Creed et al., 1996*). Water table depths > 10 mm above the ground surface led to saturated soils with slow diffusion of N₂O (*Teh et al., 2011; Arah et al., 1997*), which may have contributed to the low N₂O measurements in the wetland due to reduction to N₂ (*Wrage et al., 2001*).

The range of redox potentials of the OW position on the moderate slope transect, was narrower and fell within -100 to +100 mV (Figure 2.5, 2.6), while the steep and gentle slopes had values falling relatively lower and higher respectively. Therefore, based on soil N₂O efflux from the OW topographic positions, the steep slope may be too steep and the gentle slope may be too gentle to support N₂O as a final denitrification product with the moderate slope transect “just right” for N₂O production and consequent efflux from the soil.

The inextricable link between rainfall, the wetland soil redox potential (and associated moisture), and the elevated soil N₂O efflux combined with the increased reactive N in the atmosphere suggests that soil N₂O efflux will continue to be a substantial component of the N cycle.

2.6 Conclusion

Denitrification is a key process of the forest N cycle with N₂O contributing to N transformations in the TLW forest soils. While denitrification occurs at all topographic positions in the forest, it is more pronounced in wetland areas where moisture and redox conditions are conducive for denitrification to occur. The wetland areas have greater potential for accumulation of denitrification precursors mainly NO₃⁻ and labile C (DOC)

due to their relative position compared to the inclined upland areas in addition to the alternating redox potential between oxic and anoxic zones. Because of the strong relationship observed between precipitation events and soil N₂O efflux at the wetland positions (IW and OW), incorporation of precipitation events and wetland extents into soil N₂O efflux models will lead to better estimates of N₂O fluxes from forested catchments.

2.7 References

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Chapter 3

3 Winter Soils Produce N₂O that is Exported in Gaseous and Dissolved Forms from Forested Catchments

In this chapter, the difference in soil nitrous oxide (N₂O) effluxes among hillslope topographic positions underneath the snowpack are explored. The hypothesis tested is that during the dormant season (snow season), water and water-soluble precursors nitrate (NO₃⁻) and dissolved organic carbon (DOC) of N gases are evenly distributed across topographic features until they are rapidly flushed from the catchment during spring melt. The significance of this chapter is that it determines if microbial activity that is ongoing under a snow pack produces considerable amounts of soil N₂O efflux, and if so, how this N₂O is partitioned between gaseous N₂O efflux to the atmosphere and dissolved N₂O export to streams.

3.1 Introduction

There are elevated levels of reactive nitrogen (N) compounds in natural systems due to atmospheric N deposition (IPCC, 2013). Consequently, forest systems in eastern North America tend towards N-saturation instead of the N-limitation typically associated with terrestrial systems (Aber *et al.*, 1989; Stoddard, 1994; Venterea *et al.*, 2004; Galloway *et al.*, 2008, Goodale *et al.*, 2011). These elevated levels promote N biogeochemical processes, including denitrification, which produces nitrous oxide (N₂O), a powerful greenhouse gas (Aber *et al.*, 1989; Stoddard, 1994; Smith, 1997; Vitousek *et al.*, 1997; IPCC, 2013). Production of N₂O is dependent on the reduction-oxidation (redox) potential of the soil environment, which is favored in the range of -100 mV to +100 mV (Gambrell and Patrick, 1978; Kralova *et al.*, 1992), a range typically found in wetlands (Patrick and Jugsujinda, 1992). Therefore, catchments with substantial wetland areas have greater potential for high rates of denitrification, and consequently N₂O flux to the atmosphere and export to surface waters (Groffman *et al.*, 2003; Ullah and Zinati, 2006; Paré and Bedard-Haughn, 2012).

Biogeochemical processes during the non-growing season can substantially contribute to N transformations (Brooks and Williams, 1999; Jones, 1999; Filippa *et al.*,

2009; Williams and Helmig, 2009; Groffman *et al.*, 2011), contrary to the perception of the non-growing season as a period of diminished microbial activity (Campbell *et al.*, 2005). Soil N₂O efflux has been reported to occur at temperatures as low as -2°C (Dorland and Beauchamp, 1991) and NO₃⁻ consumption at -4°C (Malhi *et al.*, 1990). Because N₂O production is mediated by several enzyme driven microbial processes, it can be used as an indicator of biological activity that result in N transformations in snow-covered soils (Brooks and Williams, 1999).

During the non-growing season, reduced plant demand for N may allow for N to be more readily available for microbial uptake, favoring accelerated microbial activity (Reay *et al.*, 2008). This enhanced biogeochemical microbial activity would generate electrons, lowering the redox potential of the surrounding area when microbial respiration consumes O₂ faster than it can be replenished (Gambrell and Patrick, 1978; DeLaune and Reddy, 2005; Farquharson and Baldock, 2008). Consequently, a shift from O₂ as the final electron acceptor to NO₃⁻ results in extensive production of N intermediates and products, including N₂O during the transformations (Jones, 1999; Sommerfeld *et al.*, 1993; Farquharson and Baldock, 2008; Wrage *et al.*, 2001).

Soils underneath a snowpack are more likely to be saturated because soil temperatures are slightly higher than snow temperatures (Sommerfeld *et al.*, 1996), which may promote snowmelt at the soil-snow interface and also promote diffusion of water vapor from the colder snowpack to the warmer surface soil layer (Feng *et al.*, 2001; Sommerfeld *et al.*, 1996). Saturated soils where most soil pore spaces are filled with water instead of air and where the rate of O₂ diffusion into the soil pore spaces is lower than utilization can also create pockets of low redox potential (Gambrell and Patrick, 1978; Groffman *et al.*, 2009; Burgin *et al.*, 2011). Nitrous oxide formed in these pockets of low redox potential may be released as gas to the atmosphere or flushed into streams as dissolved N₂O (Heincke and Kaupenjohann, 1999) when there is hydrologic connectivity between uplands and streams. Which is similar to the NO₃⁻ flushing hypothesis (Creed *et al.*, 1996) that is associated with buildup of NO₃⁻ in soil pore water prior to peak discharge, before being flushed out during snow melt that corresponds to peak discharge.

In forest systems with the same climate, geology, and biota, topography may cause variation in biogeochemical processes within a watershed (*Jenny, 1941; Amundson and Jenny, 1997*). Topography may influence the physical and chemical controls on biological N transformations within forested catchments (*Hazlett and Foster, 2002; Vilain et al., 2010; Gu et al., 2011; Stewart et al., 2014*), including soil moisture and redox potential, the two major factors that govern production and consumption of N₂O within a catchment (*Holtan-Hartwig, 2002*). Variation in these soil environmental conditions may favour different N transformation processes at different topographic positions (*Vilain et al., 2010; Paré and Bedard-Haughn, 2012; Stewart et al., 2014*). Improving our understanding of the variation in N₂O production at different topographic positions and under the snow-pack will contribute to improved N budgets in temperate forested ecosystems.

The purpose of this study was to quantify non-growing season N₂O flux at different topographic positions with inherent differences in soil moisture and redox conditions and also stream export. The study builds upon previous work that investigated variation in other forms of dissolved N, including NO₃⁻, ammonium (NH₄⁺) and dissolved organic nitrogen (DON), among catchments (*Creed and Band, 1998; Beall et al., 2001; Creed and Beall, 2009*). It was hypothesized that during the dormant season (snow season), water and water-soluble precursors (NO₃⁻, DOC) of N gases are evenly distributed across topographic features until they are rapidly flushed from the catchment during spring melt. The following three questions were addressed – Q1: Does snow N₂O efflux vary with topographic feature? Q2: Do underlying soil physical (temperature, moisture) or chemical (redox potential, nitrate, DOC) conditions in topographic features relate to overlying snow N₂O efflux? Q3: Does spring melt cause changes in soil physical or chemical conditions that increase gaseous N₂O efflux or dissolved N₂O efflux in streams exiting the catchments? The hypothesis and associated questions were tested in the forested landscape of the Great Lakes-St. Lawrence forest region at the northern edge of the temperate forest biome of North America.

3.2 Study Area

The Turkey Lakes Watershed (TLW) is located approximately 60 km north of Sault Ste. Marie in the Algoma Highlands of central Ontario and occupies an area of 10.5 km². Streams originating from the watershed drain into the Batchawana River and ultimately into Lake Superior. The continental climate experienced within the watershed is strongly influenced by its proximity to Lake Superior (*Jeffries et al.* 1988). A snowpack generally persists from November through April. Peak discharge occurs in April-May during spring snowmelt with another peak occurring again in October-November during autumn storms.

The topography of the watershed is controlled by bedrock that is composed primarily of greenstone with small outcrops of granite (*Jeffries et al.*, 1988). The topographic relief of the watershed is 400 m, from its highest point at Batchawana Mountain (644 m a.s.l.) down to the outlet (244 m a.s.l.) (*Creed et al.* 2002) at the mouth of the Batchawana River. Glacial till is deposited on the bedrock, from which mostly Orthic Ferro-Humic and Humo-Ferric podzolic soils have developed with intermittent pockets of Ferric Humisols found in bedrock-controlled depressions and adjacent to streams and lakes (*Canada Soil Survey Committee*, 1978). The watershed is covered by an uneven-aged mature hardwood forest with 90% of the canopy dominated by sugar maple (*Acer saccharum* Marsh) (*Creed et al.*, 2002).

The TLW monitoring program was initiated in 1980 to study the effects of acid rain deposition on both terrestrial and aquatic ecosystems (*Jeffries*, 2002). Within the TLW, 13 experimental headwater catchments have been extensively monitored for stream chemistry and discharge since 1981, however we chose one catchment (c38) for intensive study. The catchment (c38) was chosen because it encompasses well-defined and discrete topographic features. This study builds on *Enanga et al.* (Chapter 2), where the topographic effects on growing season soil N₂O efflux were explored with the aim of drawing comparisons between growing season and non growing season N processes and transformations.

3.3 Methods

Precipitation and air temperature were monitored continuously from the Canadian Air and Precipitation Monitoring Network (CAPMoN) station operated by Environment Canada just outside the TLW (latitude, 47°02'06" N; longitude, 84°22'52" W).

3.3.1 Experimental Design

Catchment 38 (c38) has a total area of 6.33 ha, with the uplands draining into a 1.58 ha wetland (Figure 3.1). A hillslope transect was established on the moderate slope with four topographic positions: the center of the wetland (inner wetland, IW), the outer ring of the wetland (outer wetland, OW), the lowland (toeslope, TS and footslope, FS), and the uplands (backslope, BS, shoulder, SH and crest, CR) (Figure 3.1).

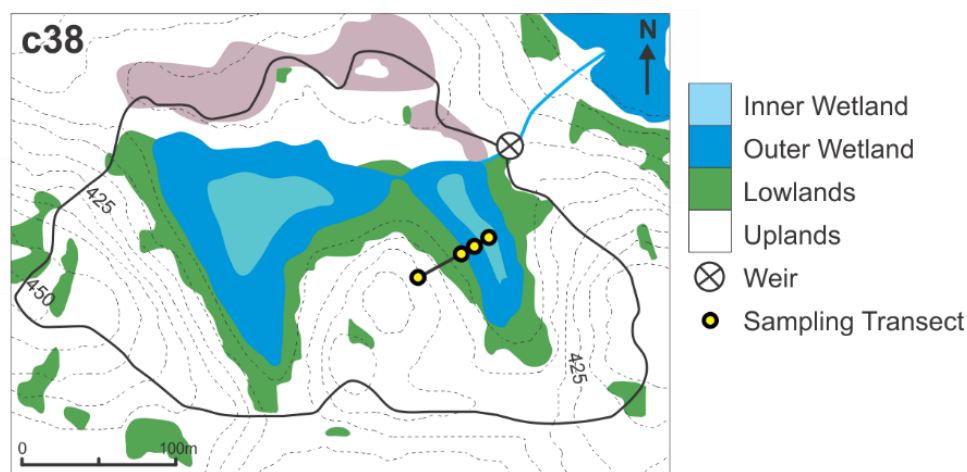


Figure 3.1: Catchment 38 (c38) in the Turkey Lakes Watershed.

Each position was instrumented for regular sampling of gaseous N_2O efflux (from the soil surface and through the snowpack). Sampling equipment for gas sampling under a snow-pack was installed prior to snowpack formation, and removed after the snowpack melted. Samples were collected from October 2006 to May 2007, daily during snowmelt periods and bi-weekly otherwise. Samples were collected between 1000h and 1400h which is a time considered to be representative of the daily mean (Vilain *et al.*, 2010). Soil temperature, moisture and redox potential were monitored continuously at a depth of 5 cm into the mineral soil in lowlands and uplands or into the peat in wetlands. Soil pore

water was collected from lysimeters installed between 5 and 10 cm depth for analysis of dissolved N species (NO_3^-) and dissolved organic carbon (DOC). Gas samples for soil N_2O efflux measurement were collected the same day the soil pore water was sampled. Boardwalks were used to access all instruments to prevent disturbance and potential compaction of soil during sampling.

3.3.2 Soil Environment

Soil temperature, 5 cm beneath the forest floor was measured using a thermocouple wire embedded into a 0.63 cm internal diameter copper tube with epoxy, while soil moisture was measured using water content reflectometers (CS 616, Campbell Scientific Canada Corp., Alberta, Canada). Redox potential was determined by measuring the voltage between a potassium chloride reference electrode and a platinum electrode. The thermocouples, water content reflectometers, and electrodes were all wired to Campbell Scientific CR10X data loggers with AM16/32 relay multiplexors, which derived their power from batteries that were charged by 30 W solar panels. All the environmental data were recorded every 5 min and averaged every 30 min by the data loggers. Water content reflectometer output was converted to volumetric water content based on calibration equations from the manufacturer and from the literature for the mineral soil and peat (*Yoshikawa et al.* 2004) in the c38 wetland. The wetland water table was continuously measured at the center of the wetland using a water level logger (WT-HR Water Height Data Logger, TruTrack Inc., Christchurch, NZ).

3.3.3 Soil Dissolved Nitrogen Concentrations

Shallow lysimeters were installed in triplicates at each topographic position at a depth of 5 to 10 cm in the soil beneath the forest floor. Prior to installation and sampling, all lysimeters were acid-washed once using 10% hydrochloric acid and rinsed with distilled water 30 times and deionized water 10 times. In addition, the first liter of soil pore water flushed through the lysimeters after installation was discarded, assuming that this first volume was modified by soil binding sites, and that subsequent samples were thereafter not modified.

Soil pore water was collected using suction lysimeters (Model 1900, Soil Moisture Corp., California, USA) after every three days during snowmelt periods and every two weeks during the other periods. On each sampling occasion, the lysimeters were primed by applying a 50 kPa vacuum, and samples were collected 24 hours later using acid-washed polycarbonate bottles. The samples were transported to the laboratory where each sample was filtered through 0.45 μm polysulfone membrane filter (Supor 450, Pall Gelman Science, Michigan, USA). Samples were then frozen for transport and storage until the time of analysis.

Nitrate concentrations were analyzed on a Technicon Autoanalyzer II (Technicon Corp., NY, USA) using the cadmium reduction method while ammonium concentrations were analyzed using sodium nitroprusside methods (*Keeney and Nelson, 1982*). Total dissolved N (TDN) analysis was done on the filtered samples using an Antek 9000VN Total Nitrogen analyzer (Antek Instruments, Texas, USA) equipped with an auto sampler. DON concentration (mg of N L^{-1}) was determined as a difference between TDN and dissolved inorganic N, which was estimated as the sum of dissolved NO_3^- and NH_4^+ species. DOC was determined following acidification ($\text{pH} < 2$, 1N HCl), then samples subjected to high temperature catalytic oxidation and detected using infrared techniques (TOC 5000A, Shimadzu Corp, Tokyo, Japan). The water chemical analyses were conducted at the Water Chemistry Laboratory at the Great Lakes Forestry Centre in Sault Ste. Marie, Ontario.

3.3.4 Soil Gaseous Nitrogen Efflux

When there was a snowpack, gas samples were collected from various depths within the snowpack at each topographic position. Three PVC tubes measuring 1.25 m with six sampling ports attached to each tube at heights of 5, 10, 25, 50, 75 and 100 cm, measured from the bottom of the PVC tube, were placed at each topographic position. Each of the sampling ports had 4 m of microbore PTFE (Polytetrafluoroethylene) tubing attached. The PTFE tubing was inserted through the PVC tube and was attached to a nearby tree approximately 1.5 m above the ground to enable gas sampling to occur when the snowpack was over a meter. Foam pipe insulation was used to enclose exposed tubing to prevent damage from animals and potential freezing of moisture in the tube.

Swagelok® fittings, sealed with septa, were attached to the end of each tubing and secured on a tree. Gas samples collection was done by first inserting a needle through both the septum and the Swagelok® fitting and into the tube. The line was purged of old air prior to sample collection by pulling 5 mL of air from the tubing after which 25 mL of snowpack air were withdrawn from the tubing and injected into a 12.1 mL evacuated extainer. Each extainer contained 0.1 g of magnesium perchlorate as a desiccant to absorb any moisture residues within the gas samples.

Gaseous N₂O fluxes from the soil that diffused through the snowpack to the atmosphere were calculated based on Fick's first law of diffusion:

$$J = -D_s \left(\frac{dc}{dz} \right) \quad (3.1)$$

where, J is the flux of N₂O ($\mu\text{mol cm}^{-2} \text{s}^{-1}$), $\frac{dc}{dz}$ is the average change in concentration of N₂O with the vertical depth of snow and D_s ($\text{cm}^2 \text{s}^{-1}$) is the effective diffusion coefficient of gas in snow:

$$D_s = -\phi\tau D_0 \quad (3.2)$$

where, τ is the tortuosity or complexity of the snow (Equation 3.2), D_0 is the diffusivity of gas in air ($0.139 \text{ cm}^2 \text{s}^{-1}$ for N₂O) (Sommerfeld *et al.*, 1993) and ϕ is the porosity of the snow:

$$\phi = 1 - (\partial_{\text{snow}}/\partial_{\text{ice}}) \quad (3.3)$$

where, ∂_{snow} (g cm^{-3}) is the density of snow determined using a snow cutter (volume 78.5 cm^3) at 5, 10, 25, 50, 75 and 100 cm within the snowpack and ∂_{ice} is the density of ice (0.9168 g cm^{-3}).

$$\frac{D_s}{\tau} = -\phi D_0 \quad (3.4)$$

where, τ is the tortuosity (Filippa *et al.*, 2009) of the snow and ϕ is the porosity of the snow (Equation 3.3).

When there was no snowpack, gas samples were collected using the static chamber method (*Livingston and Hutchinson, 1995*). Round PVC collars (30 cm I.D.), each enclosing a soil area of 707.1 cm², were inserted level 10 to 20 cm into the soil. The volume of air in each collar over the soil surface was determined on each sampling date by measuring the distance from the top of the collar to the soil (or water in flooded collars). Vented PVC flux chambers (14 L) (*Hutchinson and Livingston, 2001*) were placed on top of the collars. Air samples were collected from each chamber plus collar at 0, 15, 30, 45 and 60 min. Prior to each sample being collected, the sampling tube was purged five times with 30 mL of chamber and collar air, which also ensured mixing of the contents of the chamber and collar. Then, 30 mL of chamber plus collar air was drawn into the syringe, 5 mL of sample air was flushed through the needle to rinse it of any residue of atmospheric air with the remaining 25 mL of gas sample injected into a 12.1 mL evacuated Exetainer® containing 0.1 g desiccant.

Nitrous oxide fluxes were calculated by linear regression of the slope of increasing N₂O concentration in the chambers plus collar with time (*Mathias et al., 1980; Conen and Smith, 2000*). The N₂O concentration was scaled according to the total headspace volume derived from the sum of the volume of the collar above the soil surface plus the chamber volume, cross-sectional area of the collar, and corrected for ambient pressure and temperature:

$$F_{corr} = \left(\frac{F \times V}{V_{corr} \times A} \right) \times 28 \mu\text{g}/\mu\text{mol} \quad (3.5)$$

where, F_{corr} is the final corrected N₂O flux ($\mu\text{g of N m}^{-2} \text{ min}^{-1}$), F is the flux measured in the field ($\mu\text{L L}^{-1} \text{ min}^{-1}$), V is the volume of the chamber and collar combined (liters), A is the cross-sectional area of the collar (m^2) and V_{corr} is the molar volume of N₂O corrected for temperature and pressure ($\mu\text{L } \mu\text{mol}^{-1}$) at the time of sample collection:

$$V_{corr} = \frac{22.414 \times P^\circ \times T}{T^\circ \times P} \quad (3.6)$$

where, T (K) and P (hPa) are the air temperature and pressure at the time of measurement, P° (standard pressure) and T° (standard temperature) are 1013 hPa and

273.15 K, respectively, and 22.414 is the volume, in liters, occupied by one mole of gas at standard pressure (P°) and temperature (T°).

Nitrous oxide concentrations were measured using an SRI 8610C gas chromatograph (SRI Instruments, California, USA) equipped with an electron capture detector. P5 (5% methane, with argon making up the remaining 95% balance) or P10 (10% methane, with argon making up the remaining 90% balance) were used as the make-up gas, and He was used as the carrier gas. The gas chromatograph was equipped with a 1 m pre-column packed with 80/100 mesh HayeSep D and a 2 m main column packed with 80/100 mesh HayeSep D. Analysis of all gas samples was done within two months from the day the samples were collected.

Samples were withdrawn from the Exetainers® by flushing a 10 mL syringe and needle with He and allowing the sample to flow freely out of the over pressurized vial (i.e. 25 mL of gas sample contained in a 12.1 mL capacity vial). Five mL of gas sample were manually injected into the gas chromatograph where N₂O concentrations were determined by comparing the sample peak area with the average peak area of the standards analyzed before and after the sample using a one-point calibration of 5 ppm N₂O standard:

$$C_g = \frac{A}{R} \quad (3.7)$$

where, C_g is the concentration of the sample (ppm), A is the sample peak area from the gas chromatograph and R is the average peak area of the check standards run before and after the sample and corresponding to a concentration of 1 ppm of the N₂O standard:

$$R = \frac{\mu}{a} \quad (3.8)$$

where, μ is the average peak area from the gas chromatograph of the standards run before and after the sample and a is the actual concentration of the standard (5 ppm) that was used.

3.3.5 Stream Dissolved Nitrogen Export

Discharge exiting the catchment was measured continuously at the installed 90° V-notch weir, stilling well, with a Stevens A-71 water level recorder used to continuously record stream stage. Stream stage was then converted to mean daily discharge using rating curves.

Stream discharge was monitored daily, and samples for dissolved N species (N_2O and NO_3^-) and dissolved organic carbon (DOC) were collected daily during snowmelt periods and every two weeks during non-snow melt periods. Filling 125 mL acid-washed polycarbonate bottles with stream water discharging from the catchment collected water samples. The water samples were filtered and analyzed in the same way as the soil solution samples. Fluxes of all N species (mg of N L^{-1}) were calculated by multiplying the concentration of N species (mg of N L^{-1}) by discharge (L s^{-1}) and dividing the resultant flux by the area of the catchment (ha) then adding up the daily fluxes (per unit of catchment area) to arrive at the whole year fluxes.

Water samples for dissolved N_2O measurement were collected at the weir by completely filling a 120 mL serum bottle under water and inserting a rubber stopper while still holding the bottle under water, therefore, ensuring no air bubbles were trapped in the bottle. The temperature of the stream and also atmospheric air pressure were recorded during sample collection. In the laboratory, samples were preserved with 0.15 mL of 0.5% mercuric chloride (HgCl_2), which is an inhibitor of microbial activity (*Holl et al.*, 2005). The samples were left to equilibrate and attain room temperature at which point the bottles, with sample in them, were inverted and 20 mL of helium was injected into the sample bottle while simultaneously withdrawing or displacing 20 ml of sample water. The bottle and its contents were manually shaken for 2 minutes to allow the water and He headspace to equilibrate. Then, gas samples were withdrawn from the headspace and analyzed for N_2O concentration.

Concentrations of dissolved N_2O in the water samples were calculated using the following equation derived from *Tiedje* (1982):

$$M = C_g[V_g + (V_l \times L)] \quad (3.9)$$

where, M represents the total amount of N_2O in both the water and headspace (μmol), C_g is the concentration of N_2O in the headspace (ppm), V_g is the volume of the headspace (mL), V_l is the volume of water (mL) and L is the Ostwald solubility coefficient described by *Battino et al.* (1996):

$$L = \left(\frac{\alpha T}{T^\circ}\right) \left(\frac{P^\circ}{P}\right) \quad (3.10)$$

where, L represents the Ostwald coefficient, α is the Bunsen absorption coefficient (*Tiedje*, 1982), T is the temperature at the time of measurement (K), T° is 273.15 K, P is the pressure at the time of measurement (hPa) and P° is 1013 hPa.

Excess dissolved N_2O concentrations were then calculated by subtracting the saturation value for N_2O based on the air pressure and stream temperature at the time of sample collection from the measured N_2O concentration. Thereafter, the N_2O saturation was calculated using the following equation:

$$[N_2O]_S = [N_2O]_A \times L \quad (3.11)$$

where, $[N_2O]_S$ represents the saturated N_2O concentration (ppm), $[N_2O]_A$ is the ambient N_2O concentration (0.314 ppm) and L is the Ostwald coefficient given in Equation 3.10.

3.3.6 Data Analyses

Variations in soil temperature, moisture, redox potential, NO_3^- and DOC among topographic positions were assessed using a combination of box whisker plots and time series plots. Spearman rank correlations were performed between soil physical and chemical parameters and dissolved N_2O flux in the stream. Spearman rank correlations were also performed between soil physical and chemical parameters and gaseous soil N_2O efflux. All plots and statistical analyses were performed using SigmaPlot 12 (Systat Software, San Jose, CA), and significance was assessed at $p < 0.05$ level.

3.4 Results

Temperature steadily decreased from a high of approximately 15°C in October to a low of -20°C in February and again rose to > 20°C in May (Figure 3.2). Peaks in discharge were coincident with large rain and mixed rain-snow precipitation events, with low discharge during February and most of March when precipitation was only as snow (Figure 3.2).

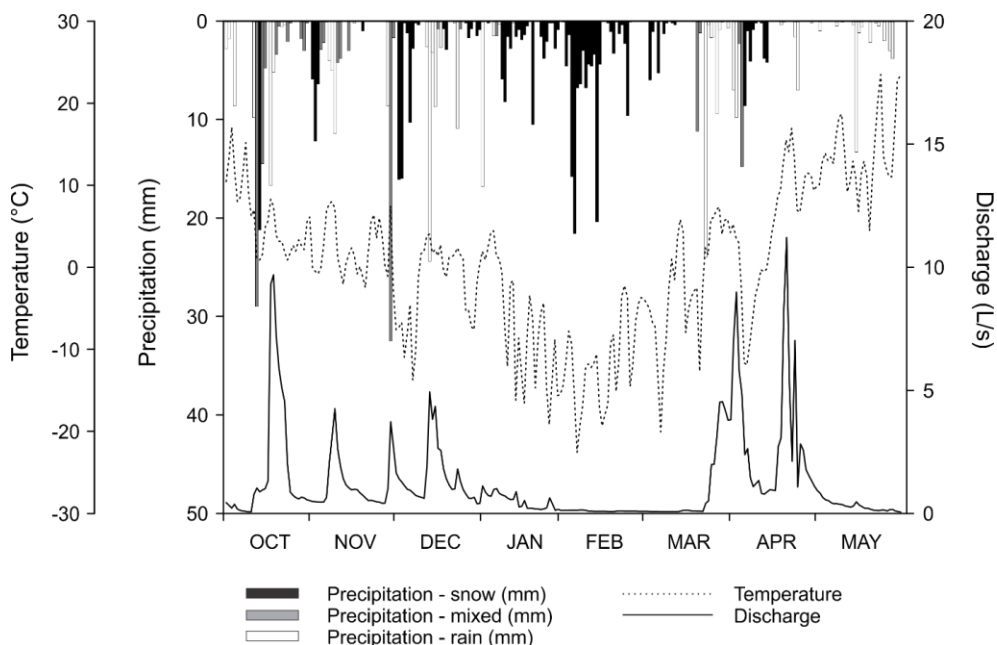


Figure 3.2: Daily average temperature, cumulative precipitation and cumulative discharge in c38 located within Turkey Lakes Watershed, from October 2006 to May 2007.

Snow depth generally increased over the non-growing season until approximately mid-February when it began to recede; the lowland and upland positions generally had deeper snow than the wetland positions (Figure 3.3).

Snow density generally increased throughout the sampling period, with one notable decrease in January that corresponded with increase in snow depth (Figure 3.3). Water table depth fluctuated from October to December, steadily decreased in January, and stabilized at approximately -10 cm in February and early March before increasing again in mid-March (Figure 3.3). Soil N₂O fluxes were variable at the different topographic positions, (Figure 3.4), however, the OW position recorded both the highest

(32.506 g-N ha⁻¹ d⁻¹) and lowest (0.073 g-N ha⁻¹ d⁻¹) N₂O effluxes. The IW N₂O fluxes ranged from 10.162 g-N ha⁻¹ d⁻¹ to 0.123 g-N ha⁻¹ d⁻¹, lowland N₂O fluxes ranged from 9.283 g-N ha⁻¹ d⁻¹ to 0.221 g-N ha⁻¹ d⁻¹, whereas the upland N₂O fluxes ranged from 14.974 g-N ha⁻¹ d⁻¹ to 0.137 g-N ha⁻¹ d⁻¹.

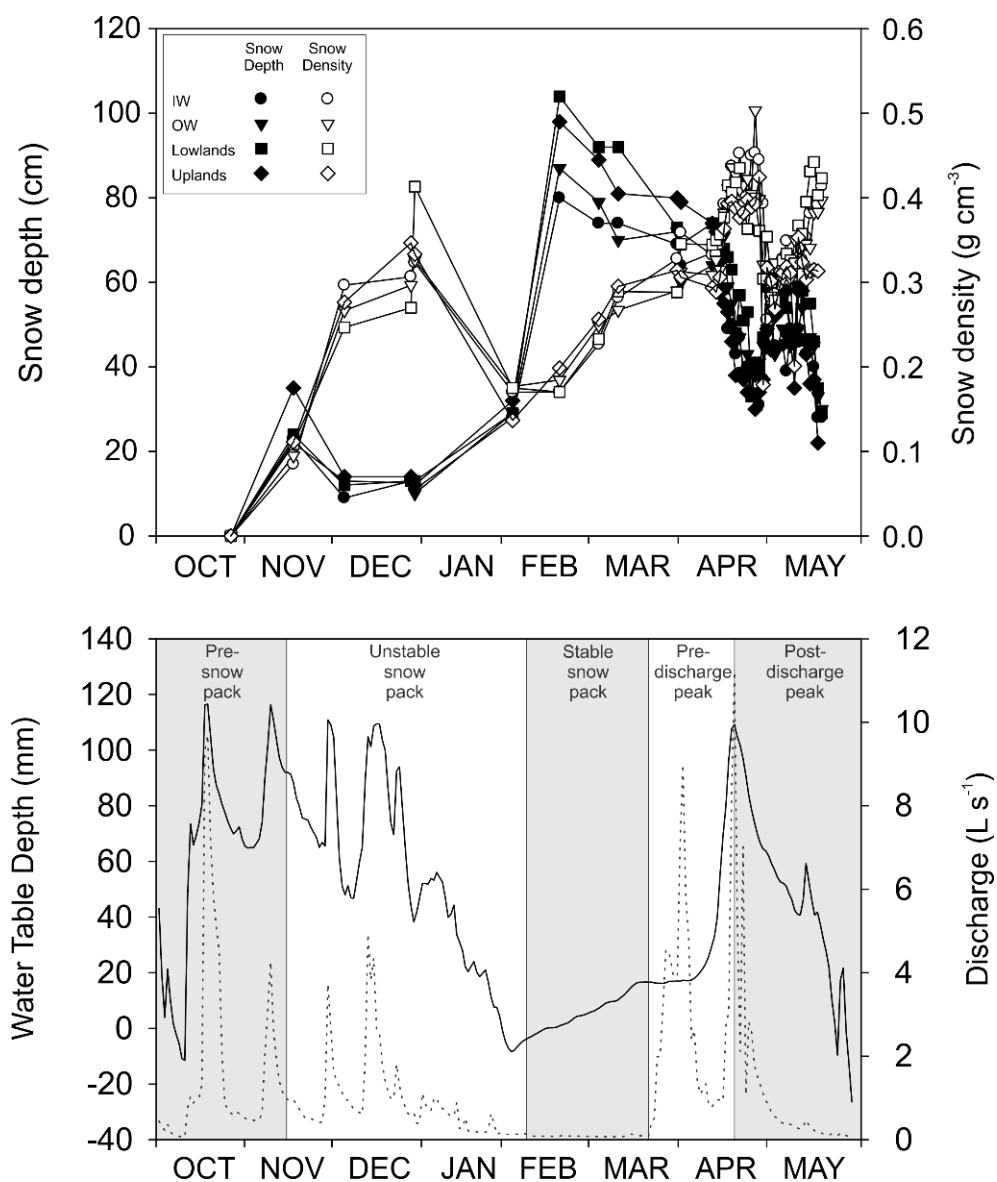


Figure 3.3: (Top) Daily snow depth and snow density, and (Bottom) water table depth and discharge from October 2006 to May 2007 in c38 of the Turkey Lakes Watershed.

However, more pronounced variations were observed when dissolved N₂O fluxes in the stream exiting the catchment were compared to the soil N₂O fluxes. Comparing stream dissolved N₂O flux with soil N₂O fluxes at different topographic positions

revealed that stream dissolved N₂O efflux was noticeably lower (10 times lower) than soil N₂O flux at all four topographic positions, although soil N₂O fluxes among the topographic positions were within comparable ranges (Figure 3.4).

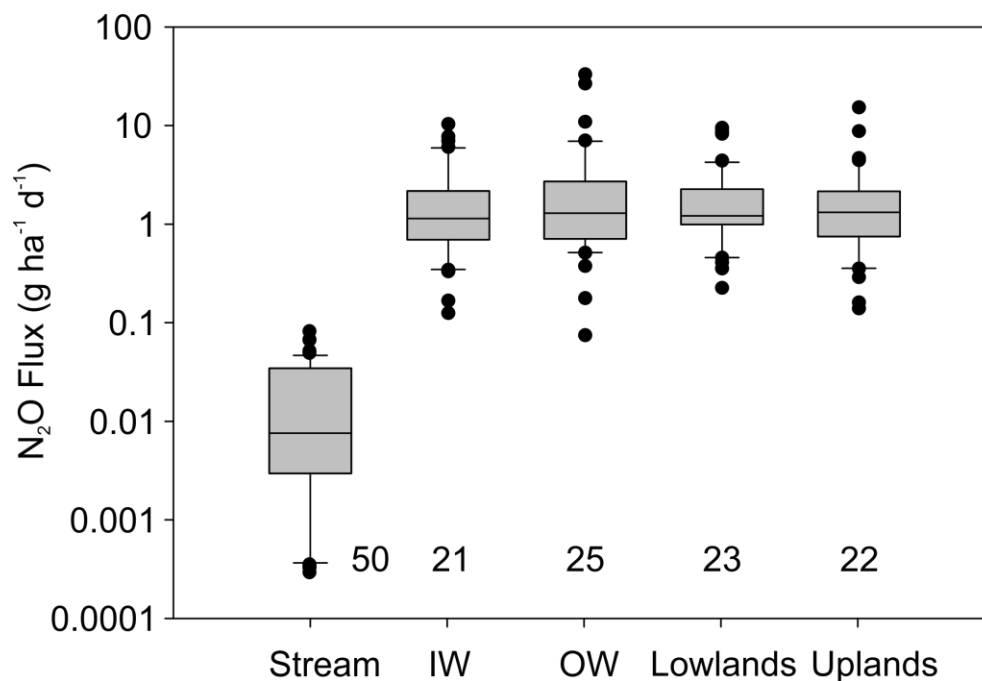


Figure 3.4: Box plots of stream and soil N₂O fluxes in c38 of the Turkey Lakes Watershed from October 2006 to May 2007, with sample size at the bottom of the plots.

Soil temperature at all topographic positions was approximately 10°C in October and rapidly declined during the month, and eventually stabilized and ranged between 0°C and 2°C from December to mid-April (Figure 3.5). The minimum temperature of -0.28°C was observed at the uplands and it is the only topographic position that recorded temperatures below zero and also recorded the lowest maximum temperature of 11.97°C, the IW recorded a minimum of 0.18°C and maximum 12.74°C, the OW minimum of 0.36°C and maximum of 13.29°C, the lowland minimum was 0.24°C and maximum of 13.84°C.

There was variation in soil temperature among topographic positions. The OW position recorded some of the highest temperatures compared to the lowlands and uplands but not much different from the IW. The uplands recorded some of the lowest

temperature readings with the other topographic positions showing closer similarities in the temperature patterns.

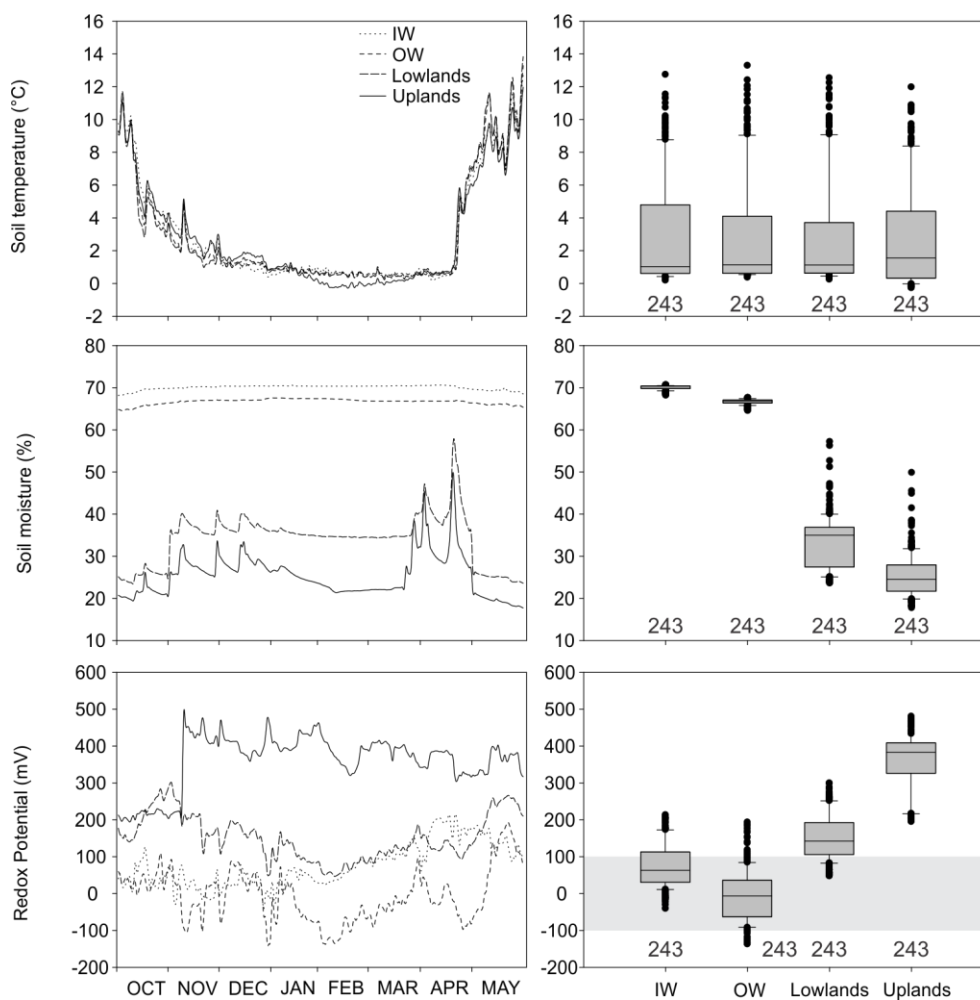


Figure 3.5: Time series and box plots of daily soil temperature, moisture and redox potential in c38 of the Turkey Lakes Watershed from October 2006 to May 2007 with sample sizes indicated below the box whisker plots.

There were visible differences in soil moisture content among all four topographic positions. Soil moisture in the IW and OW positions remained between 71 and 68 %, respectively, throughout the non-growing season, whereas at the lowland and upland positions it fluctuated more, with lowlands recording a minimum of 24 and maximum of 57% and uplands a minimum of 18 and maximum of 50% (Figure 3.5). Redox potential also fluctuated over the course of the non-growing season, with both the IW and OW within the -100 to +100 mV that favors denitrification. However, the OW position was most consistently in the -100 to +100 mV range (Chapter 2) that favours N_2O as the final

denitrification product (Figure 3.5). The redox potentials ranged from: 0 to +100 mV from October to February, after which they typically ranged from +100 to +200 mV in the IW positions; +193 mV to -137 mV in the OW positions; +299 mV to +47 mV in the lowlands; and +480 mV to +194 mV in the uplands. There were considerable differences in redox potential among all four topographic positions.

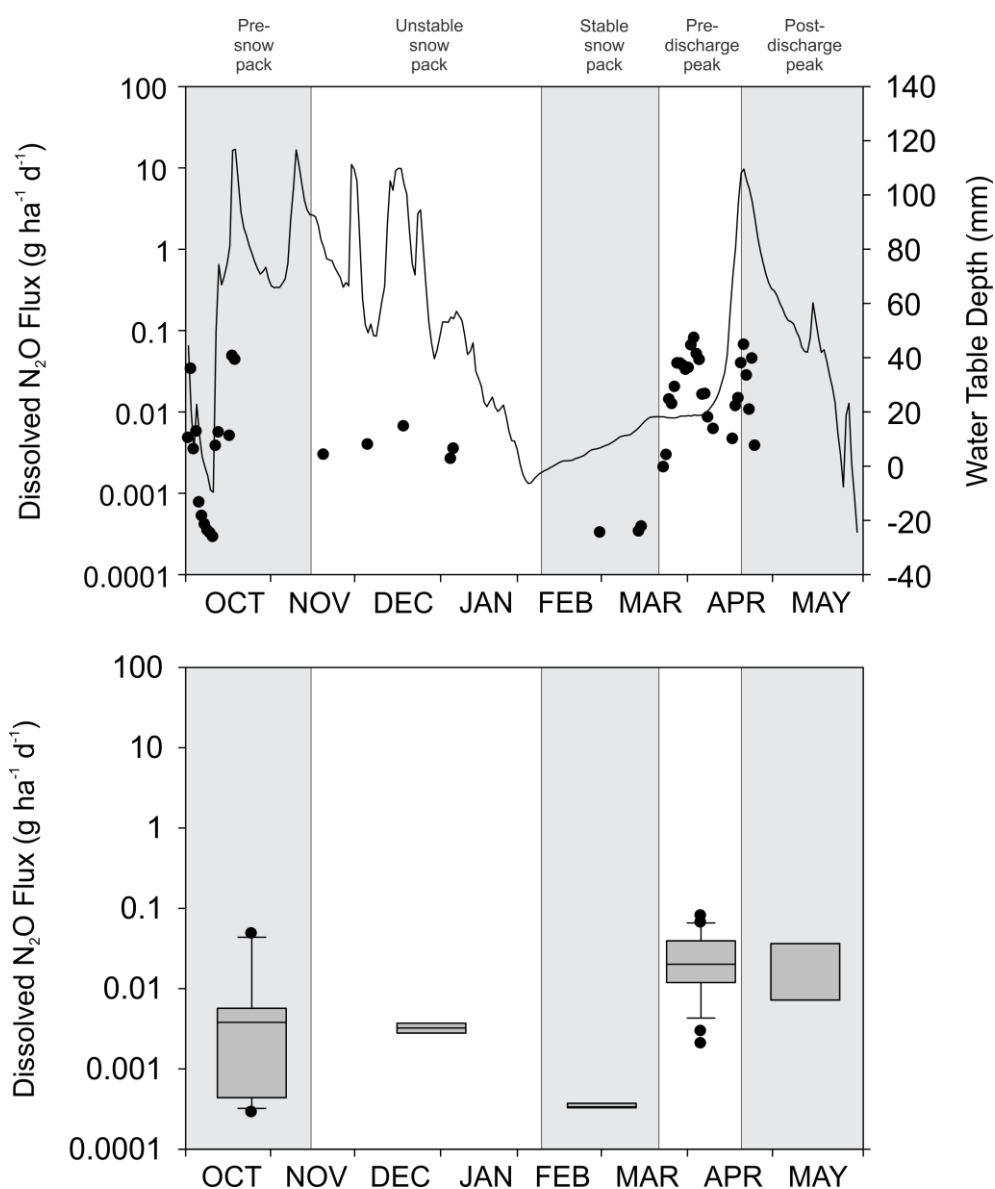


Figure 3.6: Time series of stream N_2O fluxes and water table depth in c38 of the Turkey Lakes Watershed from October 2006 to May 2007, with the time series divided based on the timing of snow cover, discharge and water table depth.

The peak in dissolved N_2O flux coincided with the rising water table depth and the period prior to peak in stream discharge and preceding spring snowmelt (Figure 3.6). Otherwise no specific pattern was observed in dissolved N_2O flux prior to the peak just before peak in discharge. The highest individual soil N_2O efflux values were observed from the OW position relative to the other topographic positions. But in general, there was a uniform distribution of soil N_2O efflux during the non-growing season (Figure 3.7), although the highest variation (large spread in values) in soil N_2O effluxes were observed during pre-peak discharge period, which also coincided with pre-peak water table depth.

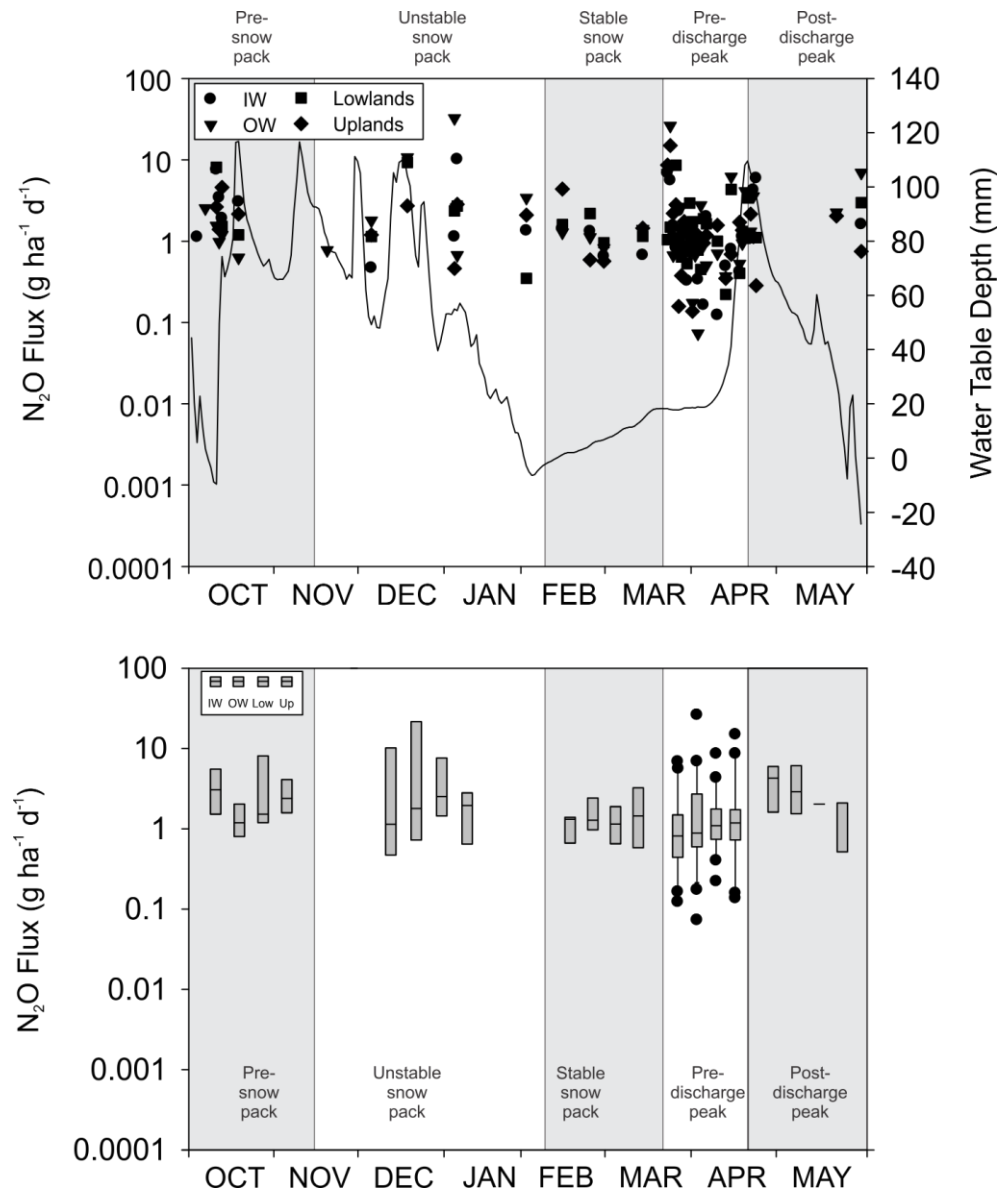


Figure 3.7: Time series of soil N_2O fluxes and water table depth in c38 of the Turkey Lakes Watershed from October 2006 to May 2007, with the time series divided based on the timing of snow cover, discharge and water table depth.

Soil pore water DOC and NO_3^- concentrations were highest at the upland areas and lowest at the OW position (Figure 3.8). Time series of NO_3^- concentrations revealed higher concentrations coinciding with pre-melt with NO_3^- concentrations higher at the upland position (Figure 3.9) while DOC did not follow any specific pattern.

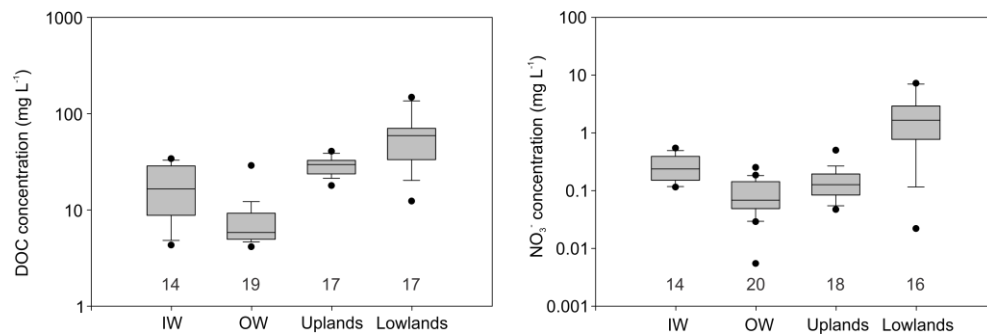


Figure 3.8: Box plots of DOC and NO_3^- concentrations in c38 of the Turkey Lakes Watershed from October 2006 to May 2007, and sample sizes are indicated below the boxes.

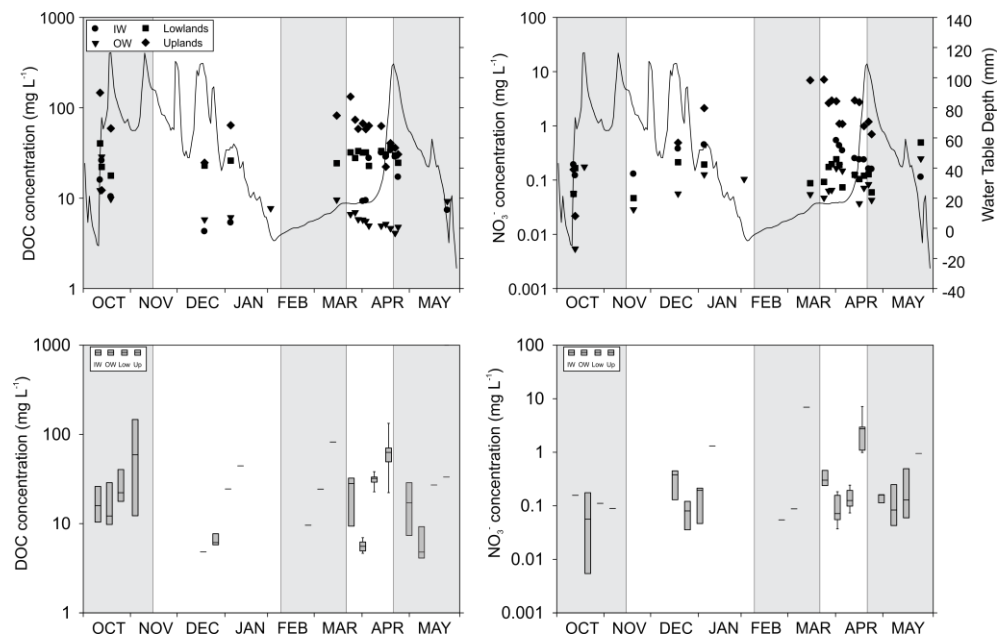


Figure 3.9: Time series of NO_3^- and DOC concentrations and water table depth in c38 of the Turkey Lakes Watershed from October 2006 to May 2007, with the time series divided based on the timing of snow cover, discharge and water table depth (Figure 3.3).

Stream dissolved N₂O flux was negatively correlated to soil temperature at the OW position ($r = -0.290$, $p = 0.031$), positively correlated to soil moisture at the IW position ($r = 0.335$, $p = 0.012$), OW position ($r = 0.346$, $p = 0.009$), lowland position ($r = 0.675$, $p < 0.001$) and upland position ($r = 0.741$, $p < 0.001$), and positively correlated with soil redox potential at the IW position ($r = 0.408$, $p = 0.002$). Stream dissolved N₂O flux was also positively correlated with soil pore water NO₃⁻ concentration at the OW position ($r = 0.480$, $p = 0.043$). Stream NO₃⁻ flux and dissolved N₂O flux were also positively correlated ($r = 0.285$, $p = 0.033$) (Table 3.1). There is only one WTD measurement at the center of the wetland within the catchment and one set of values of dissolved N₂O exiting the catchment hence the constant value of dissolved N₂O flux correlation with WTD. NH₄⁺ concentrations were not correlated to stream N₂O fluxes (data not shown)

Table 3.1: Spearman rank correlation coefficients between stream dissolved N₂O flux (g ha⁻¹) and different control variables within the stream and catchment based (soil variables) controls at each topographic position.

Control Variable	Stream		IW		OW		Lowlands		Uplands	
	r	p	r	p	r	p	r	p	r	P
Stream temperature (C)	-0.041	0.801	-	-	-	-	-	-	-	-
Stream NO ₃ ⁻	0.285	0.033	-	-	-	-	-	-	-	-
Stream DOC	-0.203	0.133	-	-	-	-	-	-	-	-
Water table depth (mm)	-	-	0.397	0.003	0.397	0.003	0.397	0.003	0.397	0.003
Soil moisture (%)	-	-	0.335	0.012	0.346	0.009	0.675	<0.001	0.741	<0.001
Soil temperature (°C)	-	-	-0.086	0.527	-0.290	0.031	-0.139	0.305	-0.171	0.206
Soil redox (mV)	-	-	0.408	0.002	0.176	0.193	0.211	0.117	-0.056	0.683
Soil pore water NO ₃ ⁻	-	-	0.159	0.591	0.480	0.043	0.282	0.266	-0.288	0.272
Soil pore water DOC	-	-	-0.077	0.792	-0.360	0.151	-0.186	0.466	-0.363	0.148

Soil N₂O efflux showed variable responses to soil physical and chemical properties at each topographic position (Table 3.2). Soil N₂O efflux at the lowland position significantly increased with an increase in soil temperature ($r=0.336$, $p=0.045$). Soil N₂O efflux tended to decrease with increase in soil moisture both at the IW position ($r = -0.606$, $p < 0.001$) and uplands ($r = -0.345$, $p=0.034$). The pattern that was observed with no significant differences in soil N₂O flux among the different topographic positions

was repeated with no strong correlation in physical and chemical factors on soils N₂O flux. Similarly soil NH₄⁺ concentrations at the different topographic positions did not show any significant correlations with soils N₂O fluxes (data not shown).

Table 3.2: Spearman rank correlations between soil N₂O efflux and each of the soil physical and chemical parameters at each topographic positions. The pattern that was observed with no significant differences in soil N₂O flux among the different topographic positions was repeated with no strong correlation in physical and chemical factors on soils N₂O flux.

Control Variable	IW		OW		Lowlands		Uplands	
	r	p	r	p	r	p	r	P
Water table depth (mm)	0.003	0.984	-0.113	0.485	0.032	0.849	0.018	0.913
Moisture (%)	-0.606	<0.001	0.047	0.774	-0.186	0.279	-0.345	0.034
Soil Temperature (C)	0.258	0.128	0.261	0.103	0.336	0.045	0.041	0.807
Redox potential (mV)	-0.265	0.117	-0.014	0.937	0.102	0.552	-0.028	0.866
Soil pore water NO ₃ ⁻	-0.583	0.087	-0.479	0.058	0.287	0.352	-0.231	0.416
Soil pore water DOC	-0.115	0.733	0.304	0.263	-0.379	0.192	-0.289	0.287

3.5 Discussion

The N transformation processes that occur under a snowpack can make a substantial contribution to a catchment's N budget (*Brooks and Williams, 1999; Jones, 1999; Filipa et al., 2009; Williams and Helmig, 2009; Groffman et al., 2011*). We explored how topographic heterogeneity underneath the snowpack influence N transformations during the snowpack period in a temperate forested catchment. Despite variation in soil moisture and redox potential at different topographic positions, there were no major differences in soil N₂O effluxes among the topographic positions, although soil N₂O effluxes were much higher than stream N₂O fluxes. We observed that more soil N₂O efflux was released from soils compared to the stream, suggesting that soil N₂O efflux under the snowpack is an important component of N transformations and should be incorporated into annual N budgets. Agricultural systems report much more substantial dissolved N₂O fluxes (*Dowdell et al., 1979; Baulch et al., 2012*) and soil N₂O fluxes (*Dorsch et al., 2004*) compared to natural forested systems (*Davidson and Swank, 1990; Yanai et al., 2013*).

Physical characteristics (e.g., temperature, moisture, redox potential) within the soil matrix are potentially strong determinants of N transformation processes (Saggar *et al.*, 2013). Beneath the snow pack, the soil temperature was typically higher than 0°C (Figure 3.2) throughout the winter and the shoulder months of October and May. On a few occasions it dipped slightly below zero (-0.28°C) at the uplands, but this is still higher than -2°C at which N₂O production was observed (Dorland and Beauchamp, 1991) or -4°C at which NO₃⁻ consumption was observed by Malhi *et al.* (1990). There were relatively minor variations in soil temperature among the topographic positions that may have contributed to the lack of discernable variations in soil N₂O effluxes among the topographic positions. Major differences in temperatures would have altered both nitrification and denitrification rates because both are enzyme driven reactions (Wrage *et al.*, 2001). Because denitrification is the main N₂O producing pathway in TLW (Spoelstra *et al.*, 2001) and is enzymatically mediated (Keeney *et al.*, 1979; Firestone, 1982; Holtan-Hartwig *et al.*, 2000), considerable differences in soil temperatures at the different topographic positions would most likely have led to noticeable differences in soil N₂O fluxes.

Higher soil moisture generally coincided with lower soil redox potentials consistent with other reports (e.g., Lohse *et al.*, 2009; Rubol *et al.*, 2012). When comparisons were made among topographic positions, the OW position had lower soil moisture compared to the IW and yet lower redox potentials than the IW position. This may suggest that greater microbial activity at the OW position is generating a higher electron density resulting in lower redox (Farquharson and Baldock, 2008) in the OW despite the slightly lower moisture compared to the IW. Because the OW position most often had favourable redox potential and high moisture, high rates of N₂O production were expected to occur at this position relative to the other positions (Snider *et al.*, 2009). Which may suggest, that despite similarities in conditions (moisture and redox) at the IW and OW, the IW may have lacked the appropriate microbial community (Holtan-Hartwig *et al.*, 2000) that would vigorously support N₂O accumulation.

Chemical characteristics (e.g., NO₃⁻ and DOC) within the soil matrix are also potentially strong determinants of N transformation processes in addition to the physical

parameters. There were differences in the NO_3^- and DOC concentrations in soil pore water at different topographic positions, though there were no clear patterns over the non-growing season, even with substantial changes in water table depth. The highest observed NO_3^- and DOC in soil pore water concentrations were noticed at the upland position and lowest at OW position. This suggests that nitrification is the major process occurring at the upland position as observed by *Spoelstra et al.* (2001), with accumulation of NO_3^- in the uplands. High rates of nitrification in the uplands have been shown in previous studies within the region (*Foster et al.*, 1986; *Foster et al.*, 2005; *Spoelstra et al.*, 2001; *Spoelstra et al.*, 2007) and consistent with findings that report that sugar maple support high rates of nitrification compared to other hardwood species (*Lovett and Mitchell*, 2004). Consequently, the recent shift to NH_4^+ as the dominant inorganic N species in atmospheric N deposition reported by *Davidson et al.* (2012), is likely to further promote nitrification due to the abundance of the precursor (deposited NH_4^+).

The NO_3^- and DOC accumulated at the uplands can potentially be transported down slope, first, to the OW then IW with movement of water (*Cirno and McDonell*, 1997; *Hill et al.*, 2000; *Lohse et al.*, 2009). Faster flow likely occurs on steeper slopes and slower movement in the gentler slopes in the lower areas (lowlands, OW and IW) influencing redox potential (*Grimm et al.*, 2003) with greater potential for NO_3^- transformation to gaseous forms and more N_2O efflux at the OW as witnessed in the summer (Chapter 2). In addition, utilization of the accumulated NO_3^- at the OW may result in episodes of high rates of soil N_2O efflux since high moisture and low redox is topography dependent (*Beven and Kirkby*, 1979). Alternatively NO_3^- and DOC in the OW position could also be flushed out (*Creed et al.*, 1996) to the stream when there is hydrologic connection (coincident with spring melt period) because of the close proximity of the OW to the stream relative to, the centrally located, IW position. This observation is supported by previous studies showing streams exiting catchments in the TLW to be rich in NO_3^- of biological origin (nitrification) based on ^{15}N studies even during snow melt (*Spoelstra et al.*, 2001; *Spoelstra et al.*, 2007) further suggesting active nitrification during winter.

At both wetland positions, high soil moisture ensures that anaerobic conditions persist over long periods of time, so NO_3^- is the electron acceptor during microbial respiration and DOC is electron donor (*Gambrell and Patrick, 1978; Kralova et al., 1992*). Thus, the low NO_3^- concentration observed at the IW and OW positions may be attributed to the rate of consumption of NO_3^- exceeding its replenishment, because reducing conditions persistent at these topographic positions, under a snow pack, do not support replenishment of NO_3^- by nitrification due to low O_2 (*Wrage et al., 2001*).

Despite differences in soil conditions (redox, moisture, soil pore water NO_3^- and DOC concentration), there were no discernable differences in soil N_2O fluxes among the different topographic positions, which suggest that temperature was the overriding environmental factor as there were no observable differences in temperature among the different topographic positions. Coupled with environmental conditions that were relatively stable during the non-growing season, with an almost continuous snow-pack that varied little among the different topographic positions (Figure 3.3), it may not be surprising for similar N_2O fluxes among different topographic positions to be evident.

One alternative explanation for the observed no differences in N_2O fluxes among topographic positions may be attributed to constant snowmelt at the snow-soil surface interface (*Sommerfeld et al., 1996*) maintaining a constant supply of moisture, and therefore favourable denitrification conditions among the different topographic conditions. Another alternative explanation is that the rate of N_2O accumulation in the uplands and lowlands may appear similar to the rate of N_2O production at the wetland positions. However, in reality, the rate of consumption (conversion of N_2O to N_2) at the lower redox potentials (high moisture content) observed at the wetland positions means that the wetland positions actually produced more N_2O , which was at the same time being consumed (reduced to N_2) due to the prevailing low redox potential compared to the lowlands and uplands. In other words, we may have estimated all the N_2O production at the uplands and lowlands, whereas we estimated the net N_2O production (since some of the N_2O produced had been converted to N_2) in the wetlands.

The patterns of NO_3^- and DOC along the hillslope suggest that these substrates may limit denitrification during the non-growing season. Wetlands were relatively nutrient-poor with optimal soil moisture and redox potential for denitrification, whereas lowlands and uplands were nutrient-rich with sub-optimal soil moisture and redox potential. This may imply that the different topographic positions lacked at least one of the optimal conditions (moisture/redox or NO_3^- /DOC pairs) resulting in no noticeable differences in measured soil N_2O effluxes among the topographic positions. Alternatively, the lack of the right quantity and quality of precursors (NO_3^- and DOC) needed (*Wrage et al.*, 2001) with quality of DOC most likely a major determinant may be the overriding factor given the similar concentrations of NO_3^- and DOC in the two positions at the two wetland positions (IW and OW) relative to the lowlands and uplands.

Significant correlations between soil moisture and stream dissolved N_2O flux at each of the topographic positions may suggest that soil moisture was the dominant driver controlling dissolved N_2O flux observed in the stream. This is consistent with observations that moisture is an important variable in N_2O production and soil N_2O can be lost from the catchment in discharge waters (*Heincke and Kaupenjohann*, 1999; *Chapuis-Lardy et al.*, 2007). The steady increase in the strength of the spearman rank correlations between soil moisture and dissolved N_2O flux from the IW to the uplands positions ($r=0.335, 0.346, 0.675$ and 0.741) may be an indication that moisture has a greater effect on dissolved N_2O export to the stream. The magnitude of the moisture effect on dissolved N_2O is more pronounced in the lowlands and uplands because it provides the medium through which dissolved N_2O can move from the uplands to the lowlands and out of the catchment *via* the OW (*Kliwer and Gilliam*, 1995; *Heincke and Kaupenjohann*, 1999; *Chapuis-Lardy et al.*, 2007).

Dissolved N_2O flux in the stream was approximately 10 times lower than soil N_2O flux, which is consistent with findings by *Davidson and Swank* (1990) that dissolved N_2O may be an insignificant pathway of N_2O loss from forested catchments. However other studies in streams exiting agricultural catchments have shown higher dissolved N_2O fluxes relative to stream surface area (*Dowdell et al.*, 1979; *Baulch et al.*, 2011). Dissolved N_2O flux appeared to peak just prior to peak in discharge and snowmelt an

indication that most of the N_2O was being flushed out from the catchment (soil) as water exits the catchment similar to the NO_3^- flushing observed in TLW by *Creed et al.* (1996). This observation is further supported by the positive response of dissolved stream N_2O flux to rising water table, which is consistent with observations that high water table impedes escape of gaseous N_2O to the atmosphere with higher likelihood of export/loss to surface waters (*Heincke and Kaupenjohann, 1999*).

The observed high dissolved N_2O flux coincident with spring melt may also suggest that the high water flow rates associated with snowmelt result in less residence time for N_2O dissolved in soil pore water in the system hence more N_2O exiting the catchment in dissolved water during spring melt (*Dowdell et al., 1979; Schnabel and Stout, 1994; Clough et al., 2005*). In addition, the increasing snow density with decreasing snow depth just prior to spring melt may have further impeded diffusion of N_2O gas from underneath the snow resulting in more export of dissolved N_2O to the stream. The concentration of dissolved N_2O in stream water exiting the catchment was on average $0.396 \mu\text{g} \cdot \text{L}^{-1}$ which was similar to measurements observed by *Davidson and Swank* (1990) that ranged from 0.31 to $0.48 \mu\text{g} \cdot \text{N} \cdot \text{L}^{-1}$. However, these results should be interpreted with caution because of the likelihood of underestimation of dissolved N_2O loss due to the likelihood of rapid off-gassing of dissolved N_2O when water is exposed to the atmosphere (*Davidson and Firestone, 1988; Reay et al., 2003*).

Generally, the fate of N_2O produced in soils include: (1) diffusion from the soils to the atmosphere; (2) dissolution in water that may be exported *via* discharge waters; or (3) reduction to N_2 (*Heincke and Kaupenjohann, 1999; Clough et al., 2005*). All the three fates seem to have been active during the non growing period, with diffusion from the soil in gaseous phase in the amount of $310 \text{ g ha}^{-1} \text{ yr}^{-1}$ from the catchment surface, dissolved N_2O in an amount up to $2 \text{ g ha}^{-1} \text{ yr}^{-1}$ in catchment discharge waters. It is likely that the snow pack may have served to slow down diffusion of gaseous N_2O leading to probable further reduction of N_2O to N_2 in the wetland positions where favourable redox potentials occurred and loss in dissolved form through stream water exiting the catchment during snow melt and periods just prior to peak discharge. Low temperatures observed

during the shoulder months of October and May ensured similar N₂O fluxes throughout the sampling period irrespective of the presence or absence of snow.

3.6 Conclusion

In sugar maple forests, N cycling processes leading to formation of the potent greenhouse gas N₂O continue under a snowpack. Soil N₂O efflux was substantial under the snowpack and was uniformly distributed along topographic positions. In contrast, dissolved N₂O flux to the stream was 10 times less than soil N₂O efflux, with the dissolved N₂O flux exhibiting a strong temporal pattern and with most dissolved N₂O effluxes occurring during spring melt. These low dissolved N₂O fluxes occurred despite inhibition of N₂O diffusion from the soil surface by a combination of snow cover and high water content and also despite the relatively higher solubility of N₂O in water. Soil N₂O efflux remained the single most important N₂O loss pathway from the catchment. It is, therefore, important to include winter N₂O fluxes in annual budgets because they are likely to lead to better estimates of catchment N loss and help enhance understanding of the fate of atmospherically deposited N in forested landscapes within temperate regions.

3.7 References

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Chapter 4

4 Nitrous Oxide and Dinitrogen: The Missing Flux in Nitrogen Budgets of Forested Catchments

In this chapter, denitrification products are estimated along a hillslope's topographic positions to enable accounting of both dissolved and gaseous nitrogen (N) export from forested catchments. This chapter tests the hypothesis that the inclusion of denitrification products (N_2+N_2O) in the N export equations accounts for the discrepancy in N export patterns observed among catchments, which in turn helps account for most of the atmospherically-deposited N. The significance of this chapter is that it presents a more inclusive estimate of N exports that may account for the discrepancy in N export among catchments and also between inputs and outputs while also providing information useful for assessing forest stability and health.

4.1 Introduction

Persistent elevated atmospheric nitrogen (N) deposition may alter the structure and function of receiving ecosystems, which may change N export patterns as systems respond to N saturation (Aber *et al.*, 1989; Stoddard *et al.*, 1994; Duncan *et al.*, 2013). Nitrogen-saturated systems have more N than can be recycled by plants and microbes (Aber *et al.*, 1989), resulting in leaching or export to surface waters in dissolved forms and to the atmosphere in gaseous forms (Stoddard *et al.*, 1994; Mulholland *et al.*, 2008), rather than the efficient cycling of N observed in N-limited systems (Aber *et al.*, 1989). Furthermore, extreme cases of N saturation may lead to forest decline and potential plant death in response to diminished plant uptake with enhanced N output from the system (Aber *et al.*, 1989; Reay *et al.*, 2008; Duncan *et al.*, 2013).

A predictive understanding of N transformations at different spatial and temporal scales remains a major scientific challenge (Galloway *et al.*, 2004; Groffman *et al.*, 2009; Davidson *et al.*, 2012; Duncan *et al.*, 2013). Partitioning N within ecosystems into its different species based on inputs and outputs is an essential first step towards quantifying the different forms of N in the system, (Foster *et al.*, 2005). Our ability to track the fate of N in catchments is incomplete (Galloway *et al.*, 2004; Duncan *et al.*, 2013) – despite

increased reactive N in ecosystems due to human activity (*Vitousek et al.*, 1997; *Kulkarni et al.*, 2008; *Gruber and Galloway*, 2008; *Davidson et al.*, 2012), there remains a portion of atmospherically-deposited N that cannot be accounted for that is often referred to as the “missing N” in catchment N budgets (*Galloway et al.*, 2004, *Yanai et al.*, 2013).

Dissolved N export from catchments to surface waters has received considerable attention (*Dillon et al.*, 1991; *Fenn et al.*, 1998; *Creed and Band*, 1998; *Beall et al.*, 2001; *Christopher et al.*, 2008); however, gaseous N export has not received comparable attention even though soils are known to be the dominant source of atmospheric N₂O (*Butterbach-Bahl, et al.*, 2013), a potent greenhouse gas and the major depleting agent for the stratospheric ozone layer (*Ravishankara et al.*, 2009; *IPCC*, 2013). A better understanding of gaseous N emissions from ecosystems is needed, because N₂O is a major concern for governments involved in international agreements on climate change (*IPCC*, 2013) and for the protection of ozone layer in the stratosphere (*Ravishankara et al.*, 2009).

Topography can play an important role in N transformations (*Zak et al.*, 1991; *Ambus*, 1998; *Hazlett and Foster*, 2002) and in both dissolved (*Creed and Beall*, 2009) and gaseous (*Duncan et al.*, 2013) N export. Soil temperature, moisture and redox potential exert a strong influence on soil N₂O effluxes (*Saggar et al.*, 2013). During the growing season, rainfall strongly influences the soil environment resulting in elevated N₂O fluxes (Chapter 2). During the non-growing season, snowfall accumulates forming a snowpack under which a significant pool of N₂O can accumulate (Chapter 3). The N₂O may be further reduced to N₂ during heterotrophic respiration when NO₃⁻ is in limited supply (*Bryan*, 1981). Denitrification processes play a critical role in converting reactive N back to the unreactive form (N₂) and helps complete the N cycle (*Aber et al.*, 1989; *Vitousek*, 2004; *IPCC.*, 2013).

Measuring N₂ directly in the field is a challenge given the high background levels in the atmosphere (*Groffman et al.*, 2006). However, several methods exist which can be used to estimate N₂. The acetylene (C₂H₂) inhibition technique (*Yoshinari et al.*, 1977) is based on the ability of C₂H₂ to inhibit the enzyme, nitrous oxide reductase, which is

responsible for the reduction of N_2O to N_2 , allowing N_2O to accumulate. Therefore, N_2 production via denitrification is estimated as the difference between C_2H_2 treated and untreated samples and the method has been used extensively (e.g. *Ryden et al.*, 1979; *Zaman and Nguyen*, 2010; *Blanco-Jarvio et al.*, 2011; *Sun et al.*, 2012). A potential drawback of this method is that C_2H_2 also inhibits nitrification and may underestimate N_2 if NO_3^- is limiting and if the major replenishment source of NO_3^- is nitrification.

Other methods used to estimate N_2 include stable isotope tracing with labelled $^{15}\text{NO}_3^-$, which is introduced into the soil and then the production of both $^{15}\text{N}_2\text{O}$ and $^{15}\text{N}_2$ are measured (*Well et al.*, 2005; *Yang et al.*, 2014). Alternatively labelled $^{15}\text{N}_2\text{O}$ can be introduced in the soil and its consumption tracked over time (*Clough et al.*, 2005). Both of these stable isotope methods may result in overestimation of denitrification due to high availability of introduced substrate either as $^{15}\text{NO}_3^-$ or $^{15}\text{N}_2\text{O}$ altering reaction rates (*Chapuis-Lardy et al.*, 2007). Yet other methods are being developed using molecular techniques, where the presence of the gene code for nitrous oxide reductase is used as an indicator for N_2O reduction (*Zumft et al.*, 1990), however the presence of a gene may not necessarily give an indication whether the enzyme is active (*Chapuis-Lardy et al.*, 2007). The C_2H_2 technique is commonly used because of its relative ease of application (*Saggar et al.*, 2013).

Several studies have assessed N budgets in forests with varied results, some reporting greater N export than deposited (*Chestnut et al.*, 1999) and others reporting less N export than is deposited (*Dillon et al.*, 1991; *Yanai et al.*, 2013) consistent with a healthy and stable ecosystem (*Odum*, 1969; *Vitousek and Reiners*, 1975). Soil N_2O efflux from forest soils can comprise a substantial portion of N budgets (*Ciais et al.*, 2013) and, when combined with N_2 efflux, can account for as much as 40% of atmospherically deposited N (*Bouwmann et al.*, 2013). Quantifying soil N_2O and N_2 losses throughout the year should help capture both the spatial and temporal variation in N export patterns and improve estimates of N budgets in ecosystems.

The purpose of this study was to quantify the magnitude of N export from forested catchments and its partitioning between dissolved and gaseous phases. By

improving estimates of N export, we expect to explain the discrepancy observed in dissolved N export patterns among catchments within a specific region, and to discover and explain the fate of atmospherically deposited N. It was hypothesized that the major differences in dissolved N export among the catchments are due to variations in the flux of denitrification products ($N_2 + N_2O$). The following questions were assessed: Q1: Do denitrification products ($N_2 + N_2O$) account for a substantial portion of catchment N outputs? Q2: Do denitrification products ($N_2 + N_2O$) explain the observed discrepancy between dissolved and particulate N inputs and N outputs (i.e., the missing N in N budgets)? This work builds on previous studies (*Creed and Beall, 2009; Mengistu et al., 2014*) that reported differences in dissolved N export among catchments in the forested landscape of the Great Lakes-St. Lawrence forest region at the northern edge of the temperate forest biome of North America.

4.2 Study Area

The Turkey Lakes Watershed (TLW) is located near the eastern shore of Lake Superior in the Algoma Highlands of Central Ontario ($47^{\circ}03'00''N$ and $84^{\circ}25'00''W$), 60 km north of Sault Ste. Marie (Figure 4.1). It occupies 10.5 km^2 of the northern edge of the Great Lakes-St. Lawrence forest region and was established in 1980 to study the effects of acid rain on both terrestrial and aquatic ecosystems (*Jeffries, 2002*).

Topographic relief of the area is approximately 400 m, ranging from 644 m at the apex of Batchawana Mountain and descending to 244 m at the outlet into Lake Superior (*Creed et al., 2002*). A thin discontinuous till of varying depth overlays the bedrock with depth ranging from < 1 m at high elevations, 1-2 m at lower elevations and up to a substantial 65 m in depressions. Dispersed pockets of Ferric Humisols are found in bedrock-controlled depressions and adjacent to streams and lakes, and Orthic Ferro-Humic and Humo-Ferric podzolic soils are dominant (*Canada Soil Survey Committee, 1978*). Precambrian metamorphosed basalt forms the base upon which the watershed rests with greenstone and granite comprising the bedrock (*Jeffries, 1988*), as the underlay that dominates the central Ontario region (*Natural Resources Canada, 2014*).

The TLW contains an uneven-aged, mature-to over-mature, and old-growth hardwood trees. It is dominated by up to 90% with sugar maple (*Acer saccharum* Marsh.), which is dotted with yellow birch (*Betula alleghaniensis* Britton), white pine (*Pinus strobus* L.), white spruce (*Picea glauca* Moench Voss.), ironwood (*Ostrya virginiana* (Mill.) K. Koch), and red oak (*Quercus rubra* L.) in the upland areas. Sugar maple dominates in the wetland areas mixed, with some eastern white cedar (*Thuja occidentalis* L.), black ash (*Fraxinus nigra* Marsh.), and balsam fir (*Abies balsamea* (L.) Mill.) (Wickware and Cowell, 1985).

4.3 Methods

4.3.1 Experimental Design

Dissolved N outputs were monitored in two experimental catchments in the TLW with gaseous N output intensively monitored from catchment 38 (c38). Catchment 38 (c38) occupies an area of 6.33 ha and contains a large wetland (25% by area), and catchment 35 (c35) occupies an area of 3.12 ha with almost no wetland (1% by area) (Figure 4.1, Table 4.1).

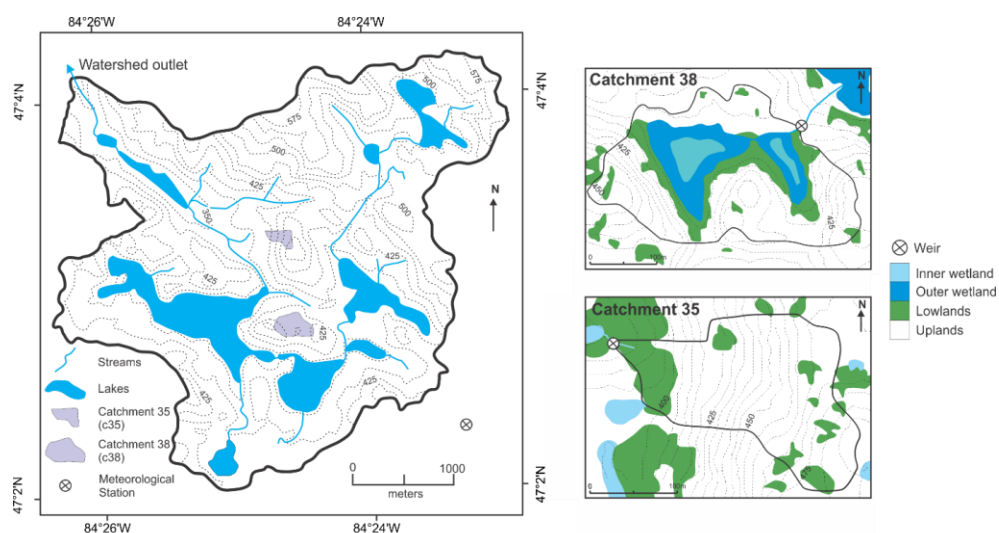


Figure 4.1: Map of the Turkey Lakes Watershed in Central Ontario, Canada showing the two catchments assessed in the present study (c38 and c35).

Dissolved N output was monitored by collecting water samples from weirs at the outlets of both catchments. Stream NO_3^- and NH_4^- were measured from June 2005 to May

2010. Gaseous N₂O output was studied intensively in c38 because it contains a large wetland and encompasses all topographic features of interest. Three hillslope transects with different slopes (gentle (15°), moderate (25°), and steep (35°)) were established with sampling sites at the inner wetland (IW), outer wetland (OW), lowlands (toeslope and footslope) and uplands (backslope, shoulder and crest) positions (Figure 2.1). Gas samples for soil N₂O efflux measurements were collected during the growing season from June to September from 2006 to 2010 at all three transects, and during the non-growing season from October 2006 to May 2007 at the moderately sloped transect. A combination of methods was used during snow covered and snow free seasons to estimate fluxes similar to those used in previous studies (e.g., *van Bochove et al.*, 2000; *Filippa et al.*, 2009). The concentration gradient method described by *Sommerfeld et al.* (1993) was used during snow covered periods and chamber methods (*Holland et al.*, 1999) before the snowpack formed and when the snow had melted.

Table 4.1: Land area of catchments c38 and c35 by topographic position.

Catchment	IW ha (%)	OW ha (%)	Lowlands ha (%)	Uplands ha (%)	Total (ha)
c38	0.66 (10.4%)	0.92 (14.5%)	0.94 (14.8%)	3.81 (60.2%)	6.33
c35	0.01 (0.3%)	0.02 (0.6%)	0.57 (18.3%)	2.52 (80.8%)	3.12

4.3.2 Atmospheric Nitrogen Inputs

Atmospheric N deposition measurements have been conducted at the nearby Canadian Air and Precipitation Monitoring Network (CAPMoN) station operated by Environment Canada since 1981 (Figure 4.1). Daily measurements of precipitation were made using a standard rain gauge and Nipher-shielded snow gauge, while samples for wet deposition measurements were collected using a wet-only precipitation chemistry sampler, and samples for daily air chemistry measurements were collected using filter packs. Dry deposition values were calculated using daily deposition velocities (*Jefferies pers. comm.*). The CAPMoN air concentration measurements included nitrate (NO₃⁻) and ammonium (NH₄⁺). Nitrate, nitric acid (HNO₃) and NH₄⁺ measurements were determined from precipitation and dry air (*Sirois and Vet*, 1988). Total organic nitrogen (TON) was

estimated as 15% of total nitrogen (TN), which was based on average regional deposition of TON (*Dillon et al.*, 1991, *Jefferies and Semkin pers. comm.*). Daily air temperature and pressure was obtained from the same meteorological station.

4.3.3 Dissolved Nitrogen Outputs

Stream outflow from the study catchments has been monitored by the Canadian Forest Service since 1980. Each stream has a V-notch weir, stilling well, and Stevens A-71 water level recorder installed to continuously record stream stage. Stream stage was converted to mean daily discharge by rating curves and later converted to unit area discharge by dividing by the catchment area. The values were summed to achieve yearly estimates, with linear regression used to estimate missing values (*F. Beall, pers. comm.*).

Water samples for determination of NO_3^- , NH_4^+ , and DON were collected from streams exiting the two catchments (c35 and c38) daily during spring melt and weekly or every two weeks for the remainder of the year. Water samples were analyzed at the Water Chemistry Laboratory at the Great Lakes Forestry Centre using standardized procedures and quality-control methods (see *Creed et al.*, 1996)

Similarly, water samples for N_2O determination were collected at the 90° V-notch weir of the streams flowing out of c38 and c35 daily during snowmelt periods and every two weeks during the remainder of the year. Serum bottles were completely filled with water (120 mL), with a rubber stopper inserted while still holding the bottle under water to ensure no air bubbles were trapped in the sampling bottle. Stream temperature and air pressure were recorded at the time of water sample collection. In the lab, samples were preserved with 0.15 mL of 0.5% mercuric chloride (HgCl_2) to inhibit microbial activity (*Holl et al.*, 2005). The samples were allowed to sit on the lab bench until they attained room temperature, at which point 20 mL of water were withdrawn and simultaneously replaced with 20 mL of helium (He) gas. Bottles were manually shaken for 2 min to attain equilibrium between the N_2O dissolved in water and in the He headspace volume. Then, gas samples were withdrawn from the headspace using a syringe attached to a needle, which was pierced through the rubber septa and analyzed for N_2O concentration.

Concentrations of N₂O dissolved in the water samples were calculated using the following equation from *Tiedje* (1982):

$$M = C_g[V_g + (V_\ell \times L)] \quad (4.1)$$

where, M is the total amount of N₂O distributed in both the water and He headspace (μmol), C_g represents the concentration of N₂O in the He headspace (ppm), V_g is the volume of the headspace (mL), V_ℓ is the volume of water (mL) and L is the Ostwald solubility coefficient derived from *Battino et al.*, 1996:

$$L = \left(\frac{\alpha T}{T^\circ}\right) \left(\frac{P^\circ}{P}\right) \quad (4.2)$$

where, L represents the Ostwald coefficient, α stands for the Bunsen absorption coefficient (*Tiedje*, 1982), T is the temperature at the time of measurement (K), T° is 273.15 K, while P is the pressure at the time of measurement (hPa) and P° is 1013.2 hPa.

The excess dissolved N₂O concentration was then calculated by subtracting the saturation value for N₂O corresponding to the air pressure and stream temperature at the time of sample collection from the measured N₂O concentration. N₂O saturation was calculated using the following equation:

$$[N_2O]_S = [N_2O]_A \times L \quad (4.3)$$

where, $[N_2O]_S$ stands for the saturated N₂O concentration (ppm), $[N_2O]_A$ denotes the ambient N₂O concentration (0.314 ppm) and L represents the Ostwald coefficient given in Equation 4.2.

Annual dissolved N outputs for the water years (June 1st to May 31st) from 2005 to 2010 were calculated by summing the measurement-based fluxes for NO₃⁻, NH₄⁺, and DON for each year and the estimated flux for dissolved N₂O was derived from the 2006-2007 non-growing season (October 1st, 2006 to May 31st, 2007) and assumed to be constant in each year and applied to all years (i.e. 2005, 2007, 2008, 2009 and 2010). Dissolved N₂O fluxes in c38 and c35 were measured daily during spring melt and at least once every two weeks between June 2006 and May 2007. Daily dissolved N₂O effluxes

were estimated using interpolation, and the data for 2006 water year (June 2006 to May 2007) were applied to all years. All estimated N₂O fluxes were summed to estimate total N₂O efflux for each year (2005 to 2010) from June to September. The October 2006 to May 2007 non-growing season dissolved N₂O flux was used to estimate the 2005, 2007, 2008, 2009 and 2010 annual N export.

4.3.4 Gaseous Nitrogen Outputs

Soil N₂O Efflux During the Snow-Free Period

Gas samples were collected using the static chamber method (*Holland et al.*, 1999) with square metal collars measuring 45.7 × 45.7 cm installed level and 10 to 20 cm in the soil. The volume of air in the collar was determined on each sampling date prior to collecting gas samples by measuring the height from the top of the collar to the soil surface (or water surface for flooded collars) and multiplying by the cross-sectional area. Vented PVC chambers were placed on top of the collar for one hour to allow a buildup of N₂O (*Holland et al.*, 1999). Air samples were collected at time 0, 20, 40 and 60 minutes following chamber placement. Prior to each sample collection, the sampling tube was flushed five times with 30 mL of air from the chamber to ensure mixing of the air inside the chamber. Thirty milliliters of chamber air were then drawn into a sampling syringe, then 5 mL of air were flushed through the sampling needle and the remaining 25 mL were injected into sealed 12.1 mL Exetainers, which contained a small amount of magnesium perchlorate as a desiccant.

Nitrous oxide gas samples were analyzed using a SRI 8610C gas chromatograph (SRI 8610C, Las Vegas, NV) equipped with an electron capture device with 10% methane and 90% argon as the make-up gas and helium as the carrier gas. Samples were loaded into the auto sampler and 2.5 mL of sample was manually injected into the gas chromatograph. The concentrations of N₂O were determined by comparing the average peak area of standards (5 ppm) run before and after the sample to the peak area of the sample using a one-point calibration with a 5 ppm N₂O standard:

$$C_g = \frac{A}{R} \quad (4.4)$$

where C_g denotes the concentration of the sample (ppm), A is the peak area from the gas chromatogram and R is the response of the average peak area of the standard corresponding to 1 ppm of N_2O :

$$R = \frac{\mu}{a} \quad (4.5)$$

where μ is the average peak area of the standards run before and after the sample and a is the actual concentration of the standard (5 ppm).

Soil N_2O fluxes were determined by calculating the linear regression of the slope of N_2O concentration in the chambers with time (*Mathias et al.*, 1980; *Conen and Smith*, 2000; *Hutchinson and Livingston*, 2001; *Hall et al.*, 2014), scaled up to total chamber plus collar volume and related to cross-sectional area of the collar and corrected for ambient pressure and temperature:

$$F_{corr} = \left(\frac{F \times V}{V_{corr} \times A} \right) \times 28 \frac{mg}{mmol} \quad (4.6)$$

where F_{corr} is the final corrected N_2O flux (mg of N m^{-2} $hour^{-1}$), F is the flux measured in the field (ml L^{-1} $hour^{-1}$), V is the total volume of the chamber and the collar (in liters), A is the cross-sectional area of the collar (m^2), 28 mg $mmol^{-1}$ is the molar mass of nitrogen in N_2O and V_{corr} is the volume occupied by one mole of N_2O and corrected for temperature and pressure (mL $mmol^{-1}$)

$$V_{corr} = \frac{22.414 \times P^o \times T}{T^o \times P} \quad (4.7)$$

where T (K) and P (hPa) are the air temperature and pressure at the time of measurement, T^o and P^o are standard temperature and pressure (273.16 K and 1013.2 hPa respectively) and 22.414 is the volume occupied by one mole of a gas (N_2O in this study) at standard temperature (T^o) and pressure (P^o).

Soil N₂O Efflux During the Snow Period

When a snow pack was present gas samples were collected every two weeks during non-melt periods and daily during snowmelt. Gas samples were taken at various depths within the snowpack at each of the topographic positions (IW, OW, lowlands and uplands) in c38. Three 1.25 m tall PVC tubes with six sampling ports attached to each tube at heights of 5, 10, 25, 50, 75 and 100 cm (measured from the bottom of the PVC tube) were placed at each position. Each sampling port had 4 m of microbore Polytetrafluoroethylene tubing attached. The 4 m of tubing was inserted through the PVC tube and was attached to a nearby tree approximately 1.5 m above the ground to enable gas flux sampling when the snowpack was over a meter. The tubing was enclosed in foam pipe insulation to prevent exposure and potential damage from animals. The end of the tubing attached to the tree had Swagelok© fittings attached and sealed with a septum. Gas samples were collected by first inserting a needle through both the septum and the Swagelok© fitting and into the tube. Five milliliters of air were pulled from the tubing into a syringe to purge the line of old air, then 25 mL of snowpack air were sucked from the tubing and injected into a 12.1 mL evacuated exetainer. Each exetainer contained 0.1 g of magnesium perchlorate to absorb any residues of moisture from the samples.

Fluxes of N₂O gas from the soil, through the snowpack to the atmosphere were calculated following Fick's first law of diffusion:

$$J = -D_s \left(\frac{dc}{dz} \right) \quad (4.8)$$

where, J is the N₂O flux ($\mu\text{mol cm}^{-2} \text{s}^{-1}$), $\frac{dc}{dz}$ is the average vertical change in the concentration of N₂O with sampling depth in snow and D_s ($\text{cm}^2 \text{s}^{-1}$) is the effective diffusion coefficient of gas in snow:

$$\frac{D_s}{\tau} = -\phi D_0 \quad (4.9)$$

where, τ is the tortuosity or complexity of the snow (Equation 4.11), D_0 is the diffusivity of gas in air ($0.139 \text{ cm}^2 \text{ s}^{-1}$ for N_2O) (Sommerfeld *et al.*, 1993) and ϕ is the porosity of the snow:

$$\phi = 1 - (\partial_{\text{snow}}/\partial_{\text{ice}}) \quad (4.10)$$

where, ∂_{snow} (g/cm^3) is the density of snow determined using a snow cutter (volume 78.5 cm^3) at 5, 10, 25, 50, 75 and 100 cm within the snowpack and ∂_{ice} is the density of ice (0.9168 g cm^{-3}).

$$\tau = \phi^{1/3} \quad (4.11)$$

where, τ represents the tortuosity of the snow (Filippa *et al.*, 2009) and ϕ represents the porosity of the snow (Equation 4.10).

Soil N₂ Efflux

The acetylene inhibition technique was used to estimate N_2 production in the wetland of c38. Twenty-five square metal collars ($45.7 \times 45.7 \text{ cm}$) were installed: 5 controls in the IW and OW, 10 replicate treatments in the IW position, and 10 replicate treatments in the OW position. They were left to settle for a year before gas samples were collected. The 20 treatment collars were subjected to 30% volume of acetylene relative to the headspace volume. The 30% headspace volume acetylene (C_2H_2) concentration was determined using a field-based optimization experiment conducted at 0%, 10%, 20%, 30%, 40%, and 50% C_2H_2 . The 30% C_2H_2 volume relative to headspace volume was the most suitable concentration because it had the most rapid increase in N_2O that reached a maximum after one hour.

The total headspace volume of each collar plus chamber combination was calculated by, first measuring the average collar height above the soil and multiplying it by the cross-sectional area. The chamber volume was added to the calculated headspace collar volume to arrive at the total headspace volume. The percentage of C_2H_2 to be pumped in to the headspace volume of each collar plus chamber combination was then calculated:

$$V_{total} = V_{chamber} + V_{collar} \quad (4.12)$$

Therefore;

$$V_{C_2H_2} = V_{total} \times \frac{30}{100} \quad (4.13)$$

where V_{total} is total headspace volume, $V_{chamber}$ is the chamber volume, V_{collar} is the collar volume above the soil surface and $V_{C_2H_2}$ is the calculated volume of C_2H_2 to be added that would constitute 30% by volume of the total headspace volume (V_{total}).

The soil moisture content within each collar was increased by sprinkling with simulated rain that was equivalent to 10 mm using MilliQ (nanopore) water prior to C_2H_2 treatment. The collars had grooves on the side that restricted the chamber from sliding off and to hold water to establish a seal between the collar and chamber. The grooves were filled with water to establish a seal between the chamber and collar (Zhu *et al.*, 2005), and then acetylene gas was released into the headspace via the gas sampling port. The flow and hence volume of C_2H_2 was measured ($m^3 \text{ sec}^{-1}$) using a flow meter to deliver a constant rate of gas until 30% of the headspace volume was achieved.

Acetylene gas was allowed to percolate into the soil for one hour, after which the chambers were lifted off the collars and aired out by swinging them in the air five times to eliminate any residual C_2H_2 within the chamber and to give the collar time to air out. The chamber was replaced on the collar, and gas samples were collected immediately after placing the chamber onto the collar (time zero) and at 15-minute intervals for one hour. Control collars had no C_2H_2 added and samples were collected at 15-minute intervals for one hour similar to the treated collars. Nitrous oxide samples were analyzed on a SRI 8610C (SRI 8610C, Las Vegas, NV) GC equipped with an electron capture device with 10% methane, and 90% argon as the make-up gas and helium as the carrier gas.

Dinitrogen (N_2) was estimated for each collar as the difference in N_2O produced from the C_2H_2 treated and untreated (control) collars (Ryden *et al.*, 1979; Firestone *et al.*, 1980; Zaman and Nguyen, 2010; Sun *et al.*, 2012):

$$N_2 = N_2O_{acetylene} - N_2O_{untreated (control)} \quad (4.14)$$

where, $N_2O_{acetylene}$ is the N_2O measured from C_2H_2 treated collars (representing N_2+N_2O) measured as N_2O , and $N_2O_{untreated (control)}$ is the N_2O measured from the untreated (control) collars and represents the net N_2O that remains after some of it is converted to N_2 . The $N_2:N_2O$ ratio was calculated for each collar based on N_2 derived from Equation 4.14 above.

Total Gaseous Nitrogen Outputs

Annual gaseous N outputs for the water years (June to May) from 2005 to 2010 were calculated by summing the fluxes during growing season (June to September) and the non-growing season (October, 2006 to May, 2007) in the IW, OW, lowland and upland topographic positions. Fluxes during the growing season were determined for rain days (i.e., when rainfall exceeded 3 mm based on rainfall collected on the day of measurement plus the previous day (in order to capture rainfall that may have fallen over night) and when the water table depth was less than 10 mm) and non-rain days or when rainfall was below 3 mm. For rain days, non-linear regression models were developed establishing relationships between the magnitude of rainfall and IW or OW soil N_2O efflux in c38 from 2006 to 2010 (Chapter 2). These regression models were applied to estimate soil N_2O effluxes for all storm days in the c38 and c35 catchments during the growing season from 2005 to 2010. The soil N_2O effluxes for the IW and OW on non-storm days and the lowlands and uplands on both storm and non-storm days were estimated as the median observed N_2O fluxes for the given year (2006-2010) and the median for 2006 to 2010 was used for 2005 (when soil N_2O efflux was not measured). The resulting soil N_2O effluxes were scaled for the proportion of area of the IW, OW, lowland, and upland positions in c38 and c35 (Table 4.1).

For the non-growing season (October to May), the soil N_2O efflux data were based on the median of N_2O flux measurements from October 2006 to May 2007 in the IW, OW, lowland and upland positions. The resulting soil N_2O effluxes were scaled for the catchment area in c38 and c35 (Table 4.1).

Total N₂ efflux was estimated in the IW and OW on summer rain days by multiplying the N₂O effluxes by the field-derived N₂:N₂O ratios. It was also estimated during all days of the winter because the surface of the soil under a snow pack is constantly saturated (*Sommerfeld et al.*, 1996), creating the conditions for N₂ formation (*Gambrell and Patrick*, 1978; *Holtan-Hartwig et al.*, 2002). We did not estimate N₂ during non-rain days or in the uplands and lowlands because the conditions would not have favoured the formation of N₂ based on observed soil O₂ concentrations that were high approaching atmospheric concentrations (*Morse et al.*, 2014) and redox potentials that were low (Chapter 2 and 3).

4.3.5 Data Analyses

Differences in N₂+N₂O efflux at different topographic positions were assessed using box whisker plots. Bar charts were used to compare N output patterns between catchments while a line was used to denote N inputs with standard deviations used to assess the variability within and also between N inputs and N outputs. All plots and data analyses were performed using SigmaPlot 12 (Systat Software, San Jose, CA).

4.4 Results

The number of rain days (precipitation events from June to September where the sum of two-day precipitation was greater than 3 mm) in each year varied over the past 30 years, with lows of 36 rain storm events in 1997 and highs of 72 rain storm events in 2010 (Figure 4.2). No specific pattern was observed between the number of storm events and the amount of precipitation received during a specific year but the period between 2007 and 2010 recorded an increasing trend in the number of storm events. The year 2010 recorded the highest number of storms to date with the only other year recording substantial storms being 1986. The lowest number of storms was observed in 2007 during the study period, the only other year that recorded a lower number of storms prior to 2007 was 1997, with (36) still the lowest number of storms recorded to date.

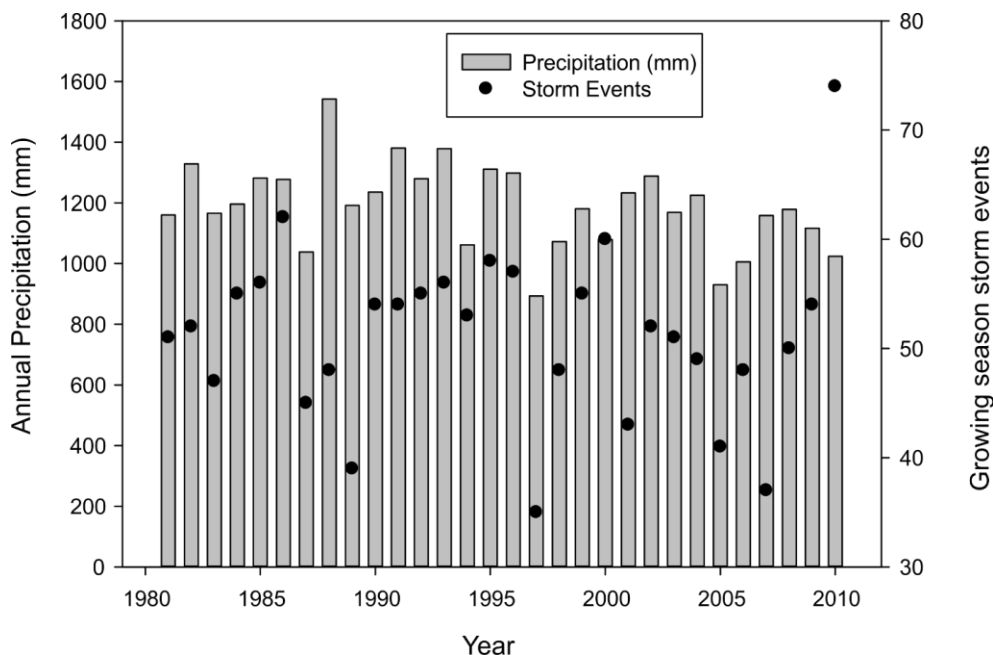


Figure 4.2: Annual precipitation patterns in the Turkey Lakes Watershed with the number of storm events during the growing season (June to September).

Based on the acetylene-inhibition experiment, the $N_2:N_2O$ ratio in the IW (19.6:1) was higher than the $N_2:N_2O$ ratio in the OW (10.1:1) (Table 4.2). The minimum ratio observed at the IW position was higher than the minimum value observed at the OW position. Similarly, the maximum ratio at the IW was higher than that observed at the OW position, with greater variability in the ratios observed from the IW compared to the OW based on the standard deviations.

Table 4.2: Ratios of N_2 to N_2O in the inner wetland (IW) and outer wetland (OW) topographic positions determined using a field based acetylene inhibition experiment.

Wetland Position	Average	Standard Dev	Minimum	Maximum
Inner Wetland (n=10)	19.585	9.659	6.831	33.616
Outer Wetland (n=10)	10.056	6.265	3.986	21.785

Using the average ratios derived for the IW and OW positions to calculate N_2+N_2O efflux from the different topographic positions, the N_2+N_2O efflux from the wetlands in c38 was more compared to fluxes from the lowlands and uplands (Figure 4.3). Comparison between the two sampling periods showed, the N_2+N_2O efflux was more in the IW and OW positions of c38 compared to the lowlands and uplands

positions, both in June to September (2006-2010) and October to May (2006/2007) (Figure 4.3). Considering the two wetland positions (IW and OW), during June to September the OW position exported more N_2+N_2O compared to the IW but during October to May there was a reversal with the IW exporting more N_2+N_2O compared to the OW position.

There was no noticeable difference in N_2+N_2O efflux between the lowlands and uplands either during June to September or October to May sampling periods. There was a considerable difference in the N_2+N_2O fluxes when compared among sampling periods with more fluxes observed from lowlands and uplands during the period of June to September compared to October to May.

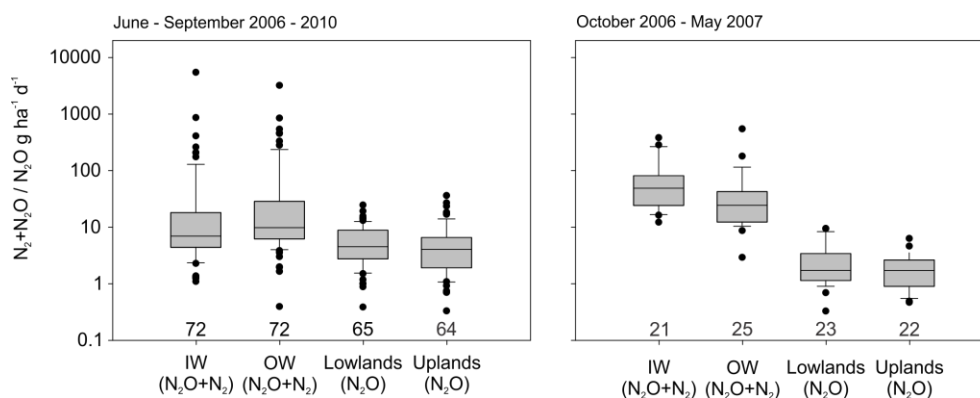


Figure 4.3: Soil N_2O and N_2O+N_2 effluxes across a topographic profile during the growing season (June to September) from 2006 to 2010 and the non-growing season in October 2006 to May 2007. The sample sizes are indicated below the boxes.

Growing season (June to September) soil N_2O efflux estimates from the two catchments were comparable ($0.77 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in c38 and $0.60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in c35), while N_2 flux estimates were much greater in c38 ($1.47 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) than in c35 ($0.06 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) (Table 4.3). Similarly, non-growing season (October to May) soil N_2O efflux estimates from the two catchments were comparable ($0.31 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in c38 and $0.24 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in c35), while N_2 estimates were much larger in c38 ($1.03 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) than in c35 ($0.06 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) (Table 4.3). Stream dissolved N_2O fluxes were extremely small compared to the soil gaseous N_2O fluxes.

Table 4.3: Average annual N budget, with inputs for the entire Turkey Lakes Watershed and exports for catchments c38 and c35, of water (precipitation for input, discharge for exports), nitrate (NO_3^-), ammonium (NH_4^+), organic N (Total Organic Nitrogen for input, dissolved organic nitrogen for exports), N_2O (soil and dissolved) and N_2 . Total Organic Nitrogen input was estimated as 15% of total nitrogen input (*Dillon et al.* 1991, *Jefferies and Semkin* pers. comm.).

Nitrogen species (kg ha^{-1})	Input			Exports					
	Turkey Lakes Watershed			c38			c35		
	Growing Season	Non-Growing season	Total	Growing Season	Non-Growing Season	Total	Growing Season	Non-Growing Season	Total
Water (mm)	358.67	692.75	1051.42	53.2	405.22	458.42	37.35	396.8	434.15
NO_3^-	1.22	2.98	4.20	0.02	0.96	0.98	0.22	4.54	4.76
NH_4^+	1.45	2.76	4.21	0.003	0.060	0.063	0.001	0.065	0.066
Organic N	0.47	1.01	1.48	0.33	1.57	1.90	0.04	0.46	0.50
N_2O (Soil)	-	-	-	0.77	0.31	1.08	0.60	0.24	0.84
N_2O (Dissolved)	-	-	-	0.0002	0.0016	0.0018	0.000004	0.0017	0.0017
N_2	-	-	-	1.47	1.03	2.50	0.04	0.06	0.10
Total N	3.14	6.75	9.89	2.59	3.93	6.52	0.90	5.37	6.27

There was considerably more export of dissolved organic and inorganic N species in the non-growing season compared to the growing season. Export of NO_3^- was noticeably larger in c35, the catchment with a very small wetland; export of NH_4^+ was similar in both catchments; and export of DON was distinctively larger in c38, the catchment with a large wetland (Table 4.3).

While there were differences in the average annual export of different N species in the two catchments, the total amount of N exported from the two catchments was similar (Figure 4.4) when denitrification products N_2 and N_2O were added to the total N export and when only positive fluxes were considered. However, even when negative fluxes were included in the total N fluxes, the differences between the catchments and also between inputs and outputs were narrowed (Figure 4.5).

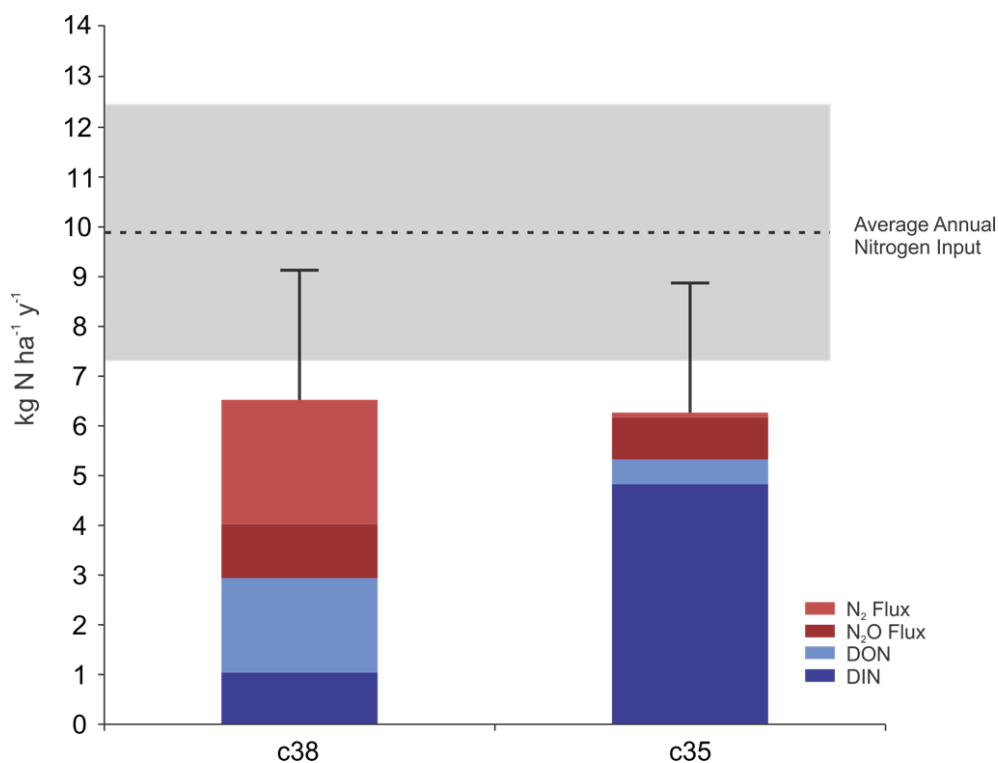


Figure 4.4: Average annual N budget for the c38 and c35 catchments in the Turkey Lakes Watershed. The bars represent the standard deviations of the N outputs with the grey shaded area representing the standard deviation of N inputs.

The average annual N input from 2005 to 2010 was $9.89 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, and the total N output was $6.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ from c38 (65.9% of N input) and $6.3 \text{ kg ha}^{-1} \text{ yr}^{-1}$

from c35 (63.3% of N input). The composition of the total N output differed, with $\text{N}_2 + \text{N}_2\text{O}$ comprising 54.9 % of N output from c38 but only 15.0% from c35. The NO_3^- , NH_4^+ , and DON comprised the remaining 45.1% of N output from c38 and 85.0% of N output from c35. The standard deviations between the N outputs and inputs reveal no overlap with the average N inputs and outputs.

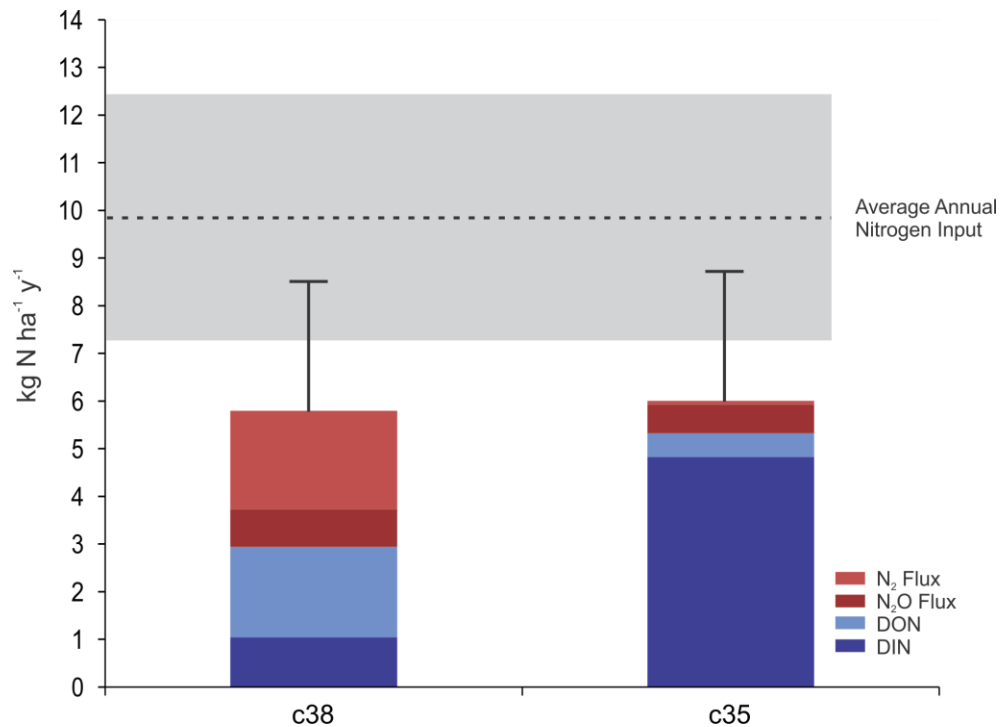


Figure 4.5: The annual N export data when negative values are included in the budget. The bars represent the standard deviations of the N outputs with the grey shaded area representing the standard deviation of N inputs.

During the period from 2005 to 2010, there was considerable variation in annual N inputs and outputs (Figure 4.6). The two catchments varied in the annual N budgets with c38 showing greater differences between inputs and outputs in 2005 and 2007 while c35 showed greater differences in 2005 and 2006. With the exception of 2007 when c35 recorded a small difference in N inputs and outputs, both catchments recorded consistent and considerably low differences between inputs and outputs in 2009. The same year 2009 recorded the lowest atmospheric N inputs when all the year 2005, 2006, 2007, 2008, 2009 and 2010 are considered.

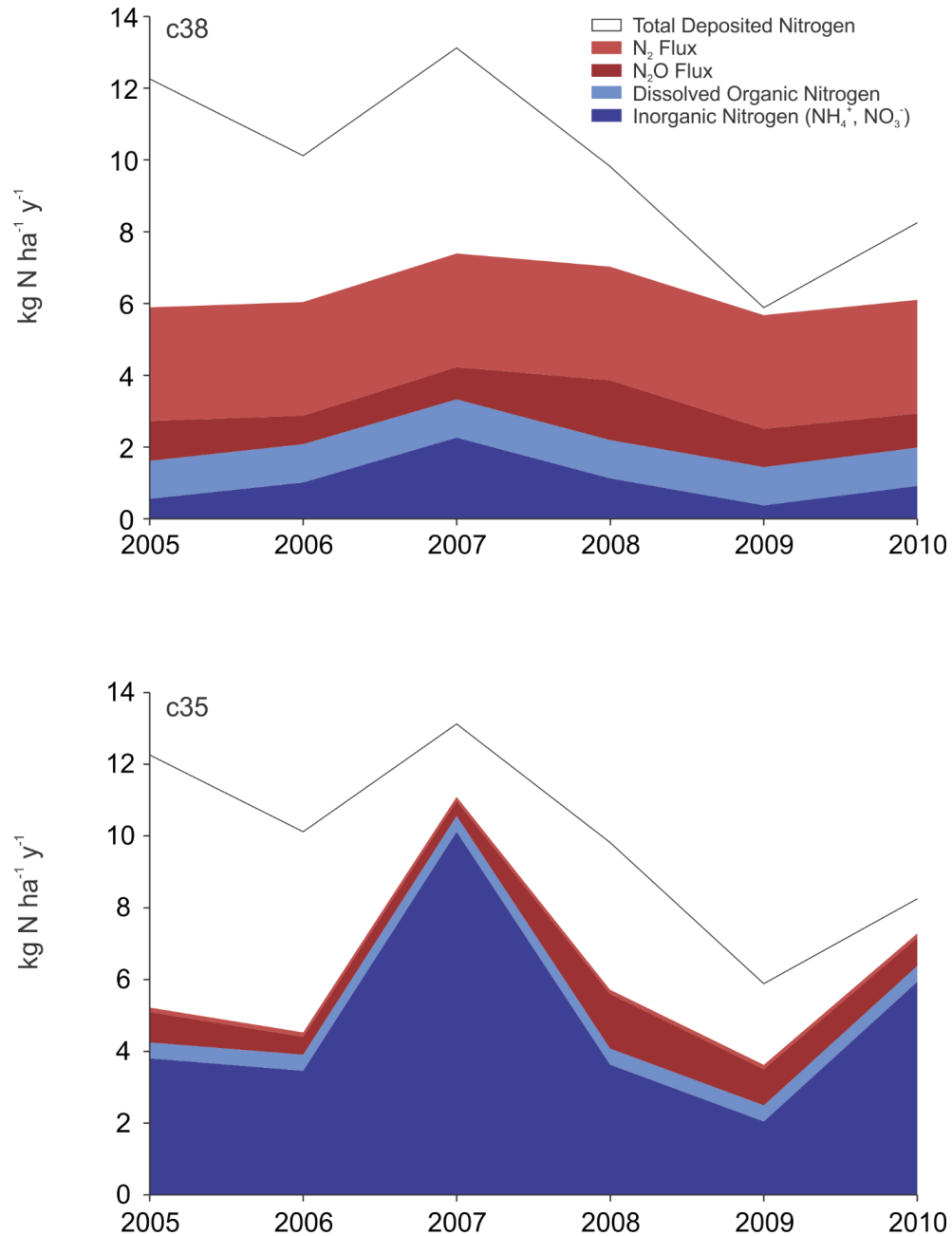


Figure 4.6: Annual N budgets for the c38 and c35 catchments in the Turkey Lakes Watershed.

Time series of monthly N inputs and outputs exposed peaks in dissolved inorganic and organic N outputs in the spring, and to a lesser extent, the autumn (Figure 4.7). More dissolved N output occurred during periods when catchment discharge is high (spring snowmelt and autumn storms). In contrast, monthly N output of N₂O and N₂ was largest in the summer, particularly in the catchment with a major wetland (c38) (Figure 4.7). The

catchment with the smaller wetland (c35) exported more inorganic N dominated with NO_3^- while c38 (larger wetland) exported more organic N and denitrification products, $\text{N}_2 + \text{N}_2\text{O}$.

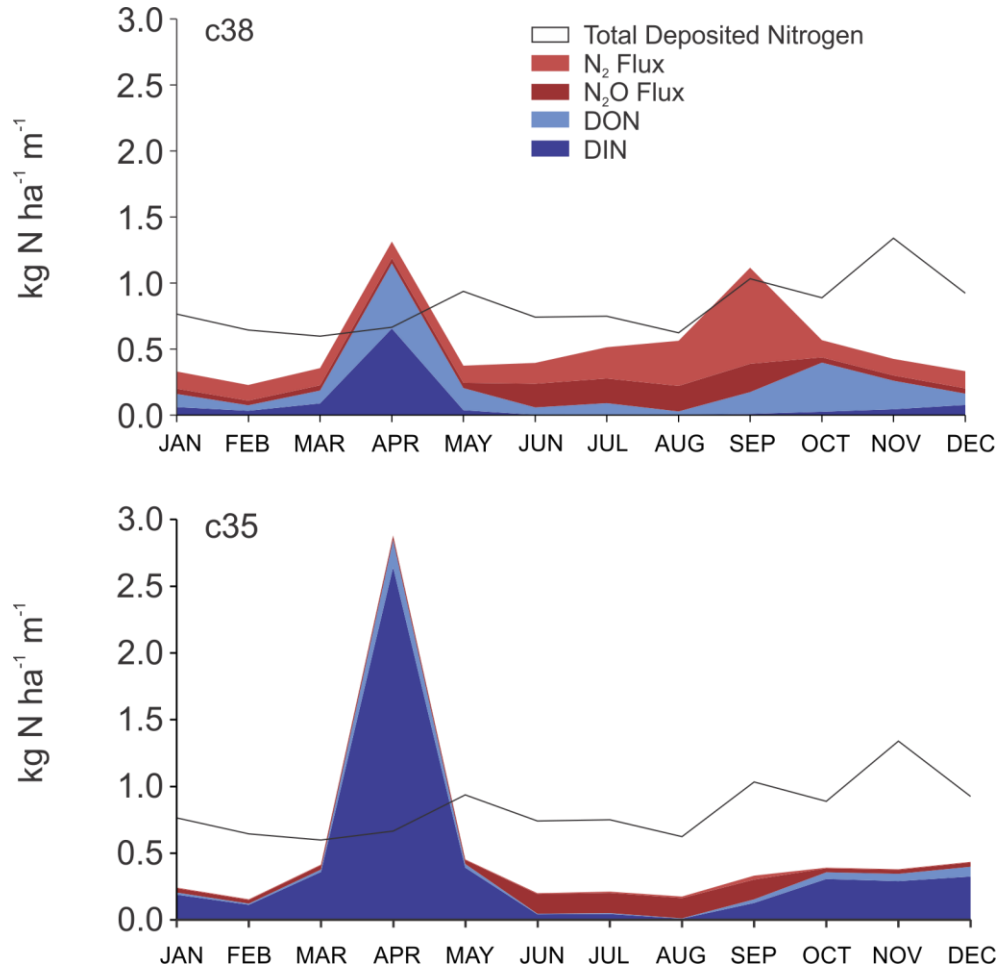


Figure 4.7: Monthly N budgets for the c38 and c35 catchments in the Turkey Lakes Watershed.

4.5 Discussion

Estimates of N inputs vs. N outputs are important because they provide insights on the functioning of an ecosystem. This is particularly important when considering atmospheric N deposition that has been elevated since the industrial revolution (*Aber et al.*, 1989; *Galloway et al.*, 2008; *Davidson et al.*, 2012) and the potential fate of this elevated atmospheric N deposition on forested landscapes (*Aber et al.*, 1989) and on N loading to surface waters (*Kliewer and Gilliam*, 1995; *Vidon et al.*, 2009; *Burgin et al.*,

2013). We investigated dissolved and gaseous N output patterns between two catchments: one with a large wetland (c38) and one with a very small wetland (c35). When both dissolved and gaseous N outputs were considered, the average annual N output from both catchments was similar; however, the proportion of different N species that comprised these N budgets was different, and this difference was explained by the different proportions of wetlands in the two catchments.

More NO_3^- was exported from c35 than c38, which suggests that whereas NO_3^- is being flushed from c35, NO_3^- is being transformed to other forms of N in c38 (*Creed et al.*, 1996; *Creed and Beall.*, 2009). The NO_3^- may be converted to DON (e.g., ferrous wheel hypothesis of *Davidson et al.*, 2003) or to N_2O or N_2 through denitrification (*Gambrell and Patrick*, 1978; *Wrage et al.*, 2001). Indeed, we observed higher N outputs of DON, N_2O , and N_2 from c38 compared to c35. Both catchments exported similar amounts of NH_4^+ , although in very small amounts, which is consistent with observations made by *Vitousek and Reiners* (1975) and *Foster et al.* (2005).

Measurements of N_2O and N_2 are notoriously difficult (*Groffman et al.*, 2009). We measured N_2O directly from different topographic positions within c38 but estimated N_2 from wetland positions using the acetylene inhibition technique described by *Tiedje et al.* (1989). Our estimates of $\text{N}_2:\text{N}_2\text{O}$ ratios were quite variable (Table 4.2), although well within the wide ranges (1:100 to 500:1) reported by *Addy et al.* (1999) (Table 4.4). We used the same $\text{N}_2:\text{N}_2\text{O}$ ratio generated during the growing season to estimate N_2 fluxes during the non-growing season.

In our study the main difference between the snow free season and the snow season at the different topographic positions is temperature, with appreciably lower temperatures observed during the snow period. Some studies observe a decrease in N_2O efflux with a decline in soil temperature – due to lower microbiological activity and higher solubility of N_2O and thus lower gaseous N_2O efflux (*Heincke & Kaupenjohann*, 1999). In this scenario, there is likely to be lower N_2O efflux during the snow season, because the produced N_2O will spend more time in the dissolved form (*Heincke & Kaupenjohann*, 1999) increasing the chances of it being reduced further to N_2 . Therefore

using the same $N_2:N_2O$ ratio generated during the summer to estimate N_2 during the winter may overestimate N_2 , if we consider higher temperatures to favour complete denitrification to N_2 as suggested by *Saggar et al.* (2013).

Table 4.4: Different methods and techniques that have been used to estimate $N_2:N_2O$ ratios and the varied results that have been obtained over the years.

$N_2:N_2O$ ratio	Methods	Substrate	Controlling variables	Reference
0.01 to 500	^{15}N -labelled N_2 and N_2O collected from mesocosms	Riparian and wetland soils	pH, NO_3^- concentration	<i>Addy et al.</i> , 1999
0.2 to 100	Direct N_2 measurement from intact cores	Mineral soil from spruce beech forests	Soil water, soil temperature	<i>Butterbach-Bahl et al.</i> , 2002
21 to 220	Direct N_2 measurement from intact cores	Forest floor and AH from a beech forest	Soil water, soil temperature, pH	<i>Dannenmann et al.</i> , 2008
0.5 to 17	^{15}N -labelled N_2 and direct N_2O measured from intact cores	Agricultural soil	C and NO_3^- availability, water filled pore space, soil texture	<i>Del Grosso et al.</i> , 2000
0 to 9	Acetylene block applied to soil suspensions	Agricultural soil	Redox potential	<i>Kralova et al.</i> , 1992
>130	Direct N_2 measurement from intact cores	Static chambers for N_2O ; intact soil cores for N_2	Species, water table height	<i>Mander et al.</i> , 2003
0.81 to 200	^{13}N -labelled gases measured from intact cores	Litter from pine and beech forests	Land use	<i>Speir et al.</i> , 1999
3.5 to 9	Acetylene block applied to intact cores	Soil from riparian zone of bottomland hardwood forest	Soil water	<i>Ullah et al.</i> , 2005

Table 4.4: Continued from previous page

N ₂ :N ₂ O ratio	Methods	Substrate	Controlling variables	Reference
0.1 to 550	Acetylene block applied to repacked cores	A horizon from agricultural soil	Soil water, C availability	<i>Weier et al.</i> , 1993
0.30 to 1.3	Acetylene block applied to moist sieved soil	Pasture soil upper 10 cm	NO ₃ ⁻ and C	<i>Deendoven and Anderson</i> , 1995
0.3	Acetylene block	Agricultural soil upper 15 cm	NO ₃ ⁻	<i>Blanco-Jarvio et al.</i> , 2011
0.2 to 2	Acetylene block applied to intact cores	Pasture soil	NO ₃ ⁻ and pH	<i>Zaman and Nguyen</i> , 2010
5.4 to 48	¹⁵ N applied to hand mixed soil	Tropical forest 10 cm soils	NO ₃ ⁻ , moisture	<i>Yang et al.</i> , 2014
0.68 to 7.1	Acetylene block applied to mixed and sieved soil	Forest and grassland soils 20 cm	pH	<i>Sun et al.</i> , 2012
0.24 to 71	Acetylene block applied to intact cores	Agriculture soil	Moisture, NO ₃ ⁻	<i>Menéndez et al.</i> , 2008
5 to 55	Direct N ₂ measurement from intact cores	Agriculture soil	NO ₃ ⁻ , moisture	<i>Scheer et al.</i> , 2009
3 to 10	Direct N ₂ measurement from intact cores	Agriculture soils	NO ₃ ⁻	<i>Wang et al.</i> , 2013

However, if we consider the higher solubility (residence time) of N₂O in dissolved form during the snow period, then the lower conversion rate of N₂O to N₂ at lower temperatures will be counterbalanced by the longer residence time of N₂O due to higher solubility at lower temperatures. This counteracting effect may result in the constancy in the N₂:N₂O ratio. Other studies report a decline in N₂O efflux with increasing temperature - due to differences in microbiological responses that consume

and produce N_2O to temperature and formation of dew at night favouring further reduction of N_2O to N_2 (*Donoso et al.*, 1993). This finding also supports our use of the same ratio as the best estimate since there is no specific pattern of response to temperature and therefore a field derived estimate approximates the actual ratio better in the absence of a ratio derived in the winter.

Our annual gaseous N_2O efflux estimates from the catchments ranged from 0.86 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ to 1.08 $\text{kg N ha}^{-1} \text{ yr}^{-1}$, which is within reasonable range of the values reported for another temperate deciduous forest by *Yanai et al.* (2013) that ranged from 0.27 and 1.4 $\text{kg N ha}^{-1} \text{ yr}^{-1}$, but lower than those reported from highly N-saturated European forests of 3 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ (*Brumme et al.*, 1999; *Groffman et al.*, 2006; *Yanai et al.*, 2013).

Our results suggest that wetlands influence denitrification processes, with a higher $\text{N}_2:\text{N}_2\text{O}$ ratio in the IW position. This may suggest that nutrients arriving at the IW position are depleted in NO_3^- after passing through the adjacent OW (Chapter 2), and therefore more N_2O is being used as an electron acceptor and reduced to N_2 in the absence of the preferred O_2 or NO_3^- in the IW (*Gambrell and Patrick.*, 1978). In a related study, *Morse et al.* (2014) measured lower O_2 at IW compared to OW position, providing empirical support for potential for N_2O to be reduced to N_2 at greater rates in the IW compared to the OW.

In c35, monthly N export patterns show peaks in dissolved inorganic N coinciding with spring snowmelt and to a lesser extent with autumn storms, consistent with previous findings by *Creed et al.* (1996) and *Creed and Band* (1998a, b). This phenomena may be explained by the longer hydrologic flushing time observed in c35 compared to c38, which means more N was hydrologically flushed into the stream from c35 than c38 because of the larger available flushable area (*Creed and Beall*, 2009). However, there was considerable accumulation of atmospherically deposited N during the rest of the year. In c38, monthly N export patterns also show peaks in dissolved inorganic N (as well as dissolved organic N) during the spring snowmelt and fall storms, although to a lesser degree than c35. While most of the dissolved N export coincided with spring melt,

interception of the drainage waters by the wetland likely results in retention and conversion of N to other forms (e.g., DON) (*Creed and Beall, 2009; Mengistu et al., 2014*). The coincidence in the timing of peak DON export from c38 with peak NO_3^- export from c35 suggests that NO_3^- is converted to DON before export (Figure 4.7). However, the shorter flushing time in c38 (*Creed and Beall, 2009*) may also mean that N that has accumulated during the winter is not exported as efficiently as in c35, allowing gaseous forms of N ($\text{N}_2+\text{N}_2\text{O}$) during the warmer conditions (*Farquharson and Baldock, 2008; Holtan-Hartwig et al., 2002*) of the summer to occur. In contrast to c35, there was considerable export of N in the form of N_2O and N_2 from c38 mitigating the accumulation of atmospherically deposited N during the summer, but not so during the winter.

The species composition of atmospheric N deposition is shifting appreciably from NO_3^- to NH_4^+ . *Davidson et al. (2012)* reported a shift from NO_3^- to NH_4^+ as the major N species, which may help explain the observed discrepancy in N export between inputs and outputs because of the lower mobility of NH_4^+ compared to NO_3^- within soils and therefore the greater retention of NH_4^+ and by extension deposited N within the soil (*Foster et al., 2005*). In addition, wetlands are more likely to retain more deposited NH_4^+ due to the anaerobic conditions that do not favor nitrification (*Wrage et al., 2001*). While NH_4^+ may also be volatilized and contribute to gaseous N output in the form of NH_3 gas, this potential N transformation pathway was not measured or estimated. However, given the relatively low pH values observed in soil pore water collected from all topographic positions (pH 3.09 to 7.69), volatilization may not be a major pathway of N loss since only $\text{pH}>8$ would favour volatilization.

Our research findings are consistent with other studies that have looked at N budgets within forested catchments. Specifically, gaseous N_2O efflux accounted for 30% of the total gaseous N flux in c38 and 89% in c35, consistent with observations made at Hubbard Brook by *Kulkarni et al. (2013)*. $\text{N}_2\text{O}+\text{N}_2$ contributed 55% of total N export in c38 whereas c35 contributed 15% of total N export, with the discrepancy between the catchments decreasing when $\text{N}_2+\text{N}_2\text{O}$ was included in the total N export. Furthermore, gaseous $\text{N}_2\text{O}+\text{N}_2$ export represented 36% in c38 and 10% in c38 of the total atmospherically-deposited N, which is consistent with *Bouwmann et al. (2013)*, who

estimated N_2+N_2O export as 40% of deposited N. Dissolved N_2O efflux was < 0.5% of the total N_2O efflux in both catchments, consistent with observations made by *Davidson and Swank* (1990), suggesting that dissolved N_2O efflux to streams may not be a significant pathway of N export in forested catchments.

However, average annual N input was larger than average annual N output from both catchments in all observed years 2005 to 2010; on average, the catchments exported about $6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, leaving at least $3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ unaccounted for. The potential fates of this “missing N” include: (1) dry deposited N that falls on leaf surfaces that may not reach the forest floor but may be processed on the leaf surface (*Uscola et al.*, 2014). This may help explain further the higher N export observed during the winter months including spring melt because there are no leaves to intercept the deposited N and process therefore there is a higher likelihood of all the deposited N reaching the forest floor with resultant export from the catchment; (2) DON, NH_4^+ or NO_3^- immobilized by soil microorganisms (*Robinson*, 1986; *Wild*, 1988; *Foster et al.*, 2005; *Davidson et al.*, 2012); (3) NO_3^- loss to ground waters (*Davidson et al.*, 2012) which may be more in wetlands than uplands, because of the greater probability of leaching of dissolved NO_3^- into ground water in wetlands compared to the relatively drier soils of the uplands; (4) dissolved N_2O loss when plants take up and transpire water (*Mosier et al.*, 1990).

Wetland dominated catchments will have higher rates of transpiration and therefore if dissolved N_2O can be taken up by plant roots and transpired it is more likely to be more substantial in the summer than in the winter due to less transpiration loss in the winter (less leaves). We did not estimate N_2O that could be lost through transpiration in the summer. The same cannot be said of N_2 , since N_2O is much more soluble than N_2 with *Kliewer and Gilliam* (1995) reporting the solubility of N_2O at 1.30 milliliters of N_2O gas at STP absorbed by 1 ml of water while 0.023 milliliters of N_2 would be absorbed by 1 ml of water. Therefore if the solubility of the gases can be used to estimate relative loss of N_2O compared to N_2 through plant transpiration, then there will be 57 times more N_2O lost for every 1 ml of water transpired compared to N_2 ; and (5) Given the ability of dissolved N_2O to rapidly off-gas when exposed to the atmosphere (*Heincke and Kaupenjohann*, 1999), we may not have captured all the dissolved N_2O by the time of

analysis (Fairweather, 2007) resulting in under estimation of N export and hence the discrepancy in N inputs compared to outputs. The fact that N input exceeded N output suggests that the catchments may still be within the range of healthy and stable ecosystems (Odum, 1969; Vitousek and Reiners, 1975; Aber *et al.*, 1989).

Annually, a similar pattern of less N output than input was observed in both c35 and c38 in all the years 2005 to 2010. However in 2009, the difference between N inputs and outputs was considerably smaller in both c35 and c38, which coincided with lowest atmospheric N inputs. This may suggest that the lower N inputs coupled with a relatively high number of both storm events and precipitation volume (Figure 4.2) ensured that a large proportion of the atmospherically deposited N was either denitrified in response to a greater number of storm events or exported to surface water in response to high precipitation volume. The high proportion of N₂ output relative to N inputs in 2009 may suggest that starving the system of N inputs from deposition may favour N₂ as the final denitrification product. Because under saturated conditions, NO₃⁻ will be depleted with higher likelihood of further reduction of N₂O to N₂ (Gambrell and Patrick, 1978; Wrage *et al.*, 2001).

4.6 Conclusion

Estimates of N budgets in temperate forested catchments are improved by accounting for denitrification products N₂O and N₂ fluxes. While there remains a considerable amount of atmospherically-deposited N that is not accounted for, by accounting for N₂O and N₂ we have narrowed the observed differences in N outputs between catchments with and without wetlands. The presence of wetlands contributes denitrification products (N₂O and N₂) to the composition of N outputs from catchments. These denitrification products account for substantial amounts of both atmospherically-deposited N (10 to 36% of N input) and to N in discharge waters (15 and 55% of N output). With the apparent discrepancy in N outputs among catchments now reasonably explained, future work should focus on the remaining substantial discrepancy between N inputs and N outputs from catchments in temperate forested landscapes.

4.7 References

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Chapter 5

5 Conclusions

5.1 Research Findings

Gaseous N is a significant form of export of nitrogen (N) from catchments at the northern limit of the temperate forest. This dissertation explored the influence of topographic features on N₂O fluxes during snow and snow free periods in response to physical and chemical controls. The results show that denitrification occurs at all topographic positions in the forest, but is more pronounced in wetland areas.

The influence of topographic position is based on precipitation type with rain having a greater effect on N₂O flux during the snow-free periods triggering a response in N₂O fluxes. Snow pack seemed to act more as a physical barrier in diffusion of N₂O and mobilizing and exporting it during spring melt in dissolved form. Therefore, these two processes complimented each other to ensure that N₂O was produced all year round.

During the snow free periods, rainfall “triggers” the formation of optimal moisture and redox conditions in these wetland areas; rainfall greater than the canopy interception capacity is a good predictor of N₂O efflux from the wetland positions as evidenced by the strong nonlinear regression between rainfall ≥ 3 mm and N₂O flux. Furthermore, appreciably greater N₂O fluxes were observed from the edge of the wetland (OW) compared to other topographic positions, further validating the influence of topographic position on N₂O fluxes. Therefore, better estimates of N₂O fluxes can be obtained when the wetland is further resolved into the edge (OW) versus the center (IW) of the wetland instead of considering both as one homogenous unit. Failing to estimate N₂O fluxes based on proportion of IW and OW relative to the total wetland area may result in inaccurate estimates depending on the relative size of the IW and OW compared to total wetland area.

Snowfall does not stop the denitrification process and ignoring winter N transformations can lead to underestimation of annual N export from these catchments. We detected considerable N cycling processes under the snowpack, with gaseous N₂O

efflux occurring through and from the snowpack and dissolved N_2O (in addition to NO_3^- , NH_4^+ and dissolved organic N) exported from the catchment in discharge waters. The wetland positions consistently registered redox potential measurements that were within the ranges that favor N_2O and even N_2 production during the snow season and similar to those observed during the snow free season. These redox values corresponded to high moisture content that remained consistently high under a snowpack in the wetland positions ($> 67\%$), while in the upland areas the fluctuations were larger; nevertheless all positions had comparable N_2O fluxes. Considering the low redox potentials observed in the wetland positions (IW and OW), there is high likelihood that denitrification was proceeding to completion (reduction of N_2O to N_2) similar to what was observed during the snow free season.

Within wetlands, there are differences in the denitrification potentials. Acetylene inhibition technique was used to estimate the ratio of $\text{N}_2:\text{N}_2\text{O}$. The IW had greater potential for reducing N_2O to N_2 compared to the OW position, with the $\text{N}_2:\text{N}_2\text{O}$ ratio higher in the IW compared to the OW position. Application of the $\text{N}_2:\text{N}_2\text{O}$ ratio developed during the summer to estimate both summer and winter N_2 resulted in the wetland areas exporting appreciably more denitrification products than the lowland and upland areas.

Consideration of, both the gaseous and dissolved N fluxes from the catchments reduced the discrepancy in N export among catchments that has been previously reported (*Creed and Beall, 2009*). Wetland production of N_2O is an ecosystem disservice (*Burgin et al., 2013*) due to its global warming and stratospheric ozone depletion effects and may also deprive plants much-needed N in N limited systems. However, the benefits of denitrification removing reactive N from water exiting forested wetlands may outweigh the costs associated with global warming and stratospheric ozone depletion. Wetlands transform reactive N to less reactive forms, reducing NO_3^- in surface waters reducing the potential for “blue baby syndrome” (*Avery, 1999*) and reducing potential for eutrophication of receiving water bodies and algal bloom proliferation therefore providing an ecosystem service.

More effort needs to be targeted towards balancing of wetland functions to maximize ecosystem services and minimize ecosystem disservices, which heavily depends on reduction of atmospheric N deposition. Under low N deposition, denitrification is likely to go to completion returning inert N_2 to the atmosphere, because lower concentrations of NO_3^- in the system favors N_2 as the final denitrification product providing an ecosystem service in return. However, under high atmospheric N deposition, N_2O would be favoured as the final denitrification product due to high availability of reactive N resulting in no need to further reduce N_2O to N_2 due to the lower energy yield associated with the process (*Hedin et al.*, 1998). The key is to deprive the system of anthropogenic forms of N (NH_4^+ , DON, or NO_3^-) that favor completion of denitrification with N_2 as the final product (ecosystem service), otherwise abundance of reactive N will favour N_2O as a final denitrification product (an ecosystem disservice due to global warming and ozone depletion).

Depriving the system of anthropogenic N will ensure transformation to N_2 is proportionately favored, because irrespective of the form of N that is deposited, it can be transformed from DON to NH_4^+ through ammonification and then to NO_3^- through nitrification, further increasing the NO_3^- concentration in the system with sugar maple more prone to this conversion (*Lovett and Mitchell*, 2004; *Foster et al.*, 2005). Getting the right combination of NO_3^- and other N species will ensure denitrification goes to N_2 in a similar way that controlling biological wastewater treatment plants have done (*Wrage et al.*, 2001) with the right ratios of O_2 , NO_3^- and microorganisms.

High nitrification rates are associated with sugar maple forest (*Foster et al.*, 2005; *Lovett and Mitchell*, 2004). Abundant supply of NO_3^- combined with the right redox conditions and energy source (DOC), denitrification can be a major N transformation pathway. Based on this realization, generalization of the results obtained in this study to other temperate forests that are not dominated with sugar maple should be done with caution. This is because there is a likelihood of overestimating denitrification products from non-sugar maple dominated forests since sugar maple forest are better equipped to generate one of the major denitrification precursors NO_3^- to support the process.

5.2 Anticipated Significance

This study contributes to understanding N transformations and the fate of transformed N within temperate forest soils during snow-covered and snow-free periods. This work contributes to (1) understanding topographic regulation of the environmental controls on N transformations including gaseous N₂O; and (2) using catchment topographic properties to explain discrepancies in N export patterns among catchments. This work included developing a relationship between rainfall and soil N₂O efflux for the months between June and September over five years (2006-2010). A field based N₂:N₂O ratio from the wetland positions within the TLW was developed and used to estimate N₂ produced from N₂O measurements during both the snow-covered and snow-free seasons. With the developed relationship between precipitation and N₂O efflux and the field based N₂:N₂O ratio within the wetland position, the relationships may be used to estimate N₂O fluxes during rainfall events and therefore help account for some of the deposited N in forested catchments. The relationships developed can be used to estimate denitrification in sugar maple dominated temperate forests given the unique high nitrification rates and estimate N budgets among catchments. Our results may also be used to assess forest stability and health (*Odum, 1979*) with the TLW seeming to be in a relatively healthy and stable state due to on average lower N loss (export) compared to N inputs.

5.3 Recommendations for Future Research

A significant gap between N input and N outputs remained. Further research is needed to determine the fate of atmospherically deposited N that is not accounted for by dissolved and gaseous forms of N output. Potential mechanisms that may account for the unaccounted for atmospherically deposited N include the following:

(1) Atmospheric N inputs that are deposited and processed on the sugar maple leaves similar to observations made on other deciduous and coniferous leaves (*Uscola, et al., 2014*).

(2) Atmospherically deposited N that is in the form of NH₄⁺ (or transformed to NH₄⁺) would have a high likelihood of attaching to the negatively charged clay and

organic particles due to its positive charge, contributing to “missing N” (*Foster et al.*, 2005). This process may be particularly important given the observed recent shift to NH_4^+ as a substantial portion of the deposited N (*Davidson et al.*, 2012) caused by the fact that NO_3^- emissions are now regulated, while NH_4^+ is not regulated and fertilizer application is leading to some of the applied NH_4^+ as fertilizer being lost to the atmosphere through volatilization and later falling as atmospheric deposition (*Davidson et al.*, 2012).

(3) Atmospherically deposited N that is in the form of NO_3^- (or transformed to NO_3^-) could be transformed to DON via the ferrous wheel hypothesis which involves Fe^{2+} catalyzing reduction of nitrate to nitrite while it is oxidized to Fe^{3+} , and then reduced forms of carbon regenerate Fe^{2+} by reducing Fe^{3+} . The nitrate that is reduced to nitrite reacts with dissolved organic matter through nitration and nitrosation of aromatic ring structures to produce dissolved organic nitrogen (*Davidson et al.*, 2003), a hypothesis that should be tested given the availability of iron in the TLW soils (*Creed unpublished data*) and the observed higher DON from the catchment with the larger proportion of wetland (c38) compared to c35 (*Creed and Beall*, 2009).

(4) Dissolved N_2O is taken up by plant roots and eventually emitted to the atmosphere through transpiration (*Mosier et al.*, 1990), especially during the summer months when transpiration rates are high and N_2O production is also relatively high.

(5) N is leached into groundwater. Although while the catchments in TLW are “bedrock lined” (*Jeffries et al.*, 1988), there may be fractures in the bedrock based on *Schiff et al.* (2002) who described similar observations in their study which may result in loss of N to groundwater via deeper flow paths.

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Appendix 1: Calculation of Annual Nitrogen Fluxes

We calculated summer soil N₂O efflux rates based on the regression equation derived from storm events. We then calculated the non-storm efflux rates from the median of the remaining soil N₂O efflux values. The N₂ was estimated from the N₂:N₂O ratio from the acetylene experiments and added to soil N₂O efflux during storm events for the wetland positions only. The ratio was also used to estimate N₂ for the soil N₂O efflux during the winter months for the wetland positions only. Median values derived from all data days at each topographic positions were used to estimate soil N₂O efflux from the different topographic positions during the winter months.

Table A.1: Summary of the method used to estimate gaseous export at the different topographic position during the different seasons (June to September and October to May) and storm and non-storm days.

	Growing Season (June to September)				Non-growing season (October to May)	
	Storm Days		Non-Storm Days			
	N ₂ O	N ₂	N ₂ O	N ₂	N ₂ O	N ₂
IW	Regression with precipitation	Ratio	c38 IW Median	-	c38 IW Median	Ratio
OW	Regression with precipitation	Ratio	c38 OW Median	-	c38 OW Median	Ratio
Lowlands	c38 Lowlands Median	-	c38 Lowlands Median	-	c38 Lowlands Median	-
Uplands	c38 BS Uplands Median	-	c38 Uplands Median	-	c38 Uplands Median	-
Stream	c38/c35 Actual measurements/ s/ interpolations	-	c38/c35 Actual measurements/ s/ interpolations	-	c38/c35 Actual measurements/ interpolations	-

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PUBLICATIONS

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Enanga, E. M., N. Casson, R. J. Sorichetti, I. F. Creed, W. A. Shivoga, and C.G. Trick. Community perceptions and attitudes towards ecosystem health in Lake Naivasha, Kenya. *In prep.*

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PRESENTATIONS

1. Creed, I. F., and **E. M. Enanga** (2014), Climate change and the fate of nitrogen on northern temperate forests. American Geophysical Union Fall Meeting, December 15-19, San Francisco, CA. **Invited Talk.**
2. Creed, I. F., **E. M. Enanga**, P. Badiou, and M. Gloutney (2014), How can we slow the rate of wetlands on human dominated landscapes? A new market opportunity for farmers? Lattornell Symposium, November 18-20, 2014, Nottawasaga Inn, ON. **Invited Talk.**
3. **Enanga, E. M.**, I. F. Creed, P. Badiou, and M. Gloutney (2014), Investigating carbon storage and accumulation rates of wetlands within the agricultural landscape of Southern Ontario. Great Lakes Wetlands Day, February 04, 2014, Radisson Hotel Toronto East, ON. **Invited Talk.**
4. **Enanga, E. M.**, and I. F. Creed (2013), Climate warming and atmospheric pollution effects on denitrification rates from forested wetlands. American Geophysical Union Fall Meeting, December 9-13, San Francisco, CA. **Poster.**
5. Creed I. F., **E. M. Enanga**, P. Badiou, and D. Lobb (2013), Slowing the rate of loss of mineral wetlands on human dominated landscapes: diversification of farmers markets to include carbon. American Geophysical Union Fall Meeting, December 9-13, San Francisco, CA. **Invited Talk.**
6. Morse, J. L., Beall F., Creed I. F., **Enanga E. M.**, Fernandez I., Groffman P. (2013), Soil denitrification fluxes and oxygen dynamics in three contrasting northeastern North American forests. Ecological Society of America, August 4-9, Minneapolis, MN.

7. **Enanga, E. M.**, and I. F. Creed (2013), Significance of nitrous oxide and dinitrogen efflux from saturated forest soils in regulating water quality. 12th North American Forest Soils Conference, June 12 – 20, Whitefish, MT. **Poster.**
8. Creed, I. F., **E. M. Enanga**, S. G. Mengistu, F. D. Beall, and P. Hazlett (2011), Role of redox surfaces in explaining catchment nitrogen export across multiple spatial and temporal scales. American Geophysical Union Fall Meeting, December 5-9, San Francisco, CA. **Invited Talk.**
9. **Enanga, E. M.**, N. J. Casson, and I. F. Creed (2011), Rain induced nitrous oxide and dinitrogen emissions account for differences in dissolved nitrogen export from forested catchments. Gordon Research Conference on Catchment Sciences: Catchments as Sentinels of Global Change, July 10-15, Bates College, Lewiston, Maine, USA. **Poster.**
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