

**N mineralization and nitrification in Alberta bogs  
as a function of time since wildfire and increasing  
anthropogenic N deposition**

A thesis presented to the faculty of the Department of Biology at  
Villanova University

In partial fulfillment of the requirements for the degree of Master  
of Science in Biology

By

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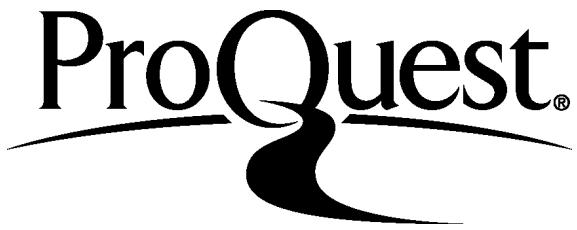
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## **ACKNOWLEDGEMENTS**

I would foremost like to thank my advisor, Dr. R. Kelman Wieder, for giving me the opportunity to be a Master's student, and for working closely with me through the completion of my degree. I would also like to thank Dr. Melanie Vile, for advising me and being a member of my committee. I thank Dr. Adam Langley for being a member of my committee and supporting my project, especially in regard to attempting gross N mineralization experiments. Additionally, I would like to thank Dr. Samantha Chapman and Dr. Michael Russell for helping me to place my research in the context of their classes and assisting me in an unofficial capacity. I would like to thank Villanova University and the National Science Foundation for their support of my research.

The work presented in this thesis would be impossible without the members of the Wieder/Vile lab. I owe many thanks to Kimberli Scott, Kelly McMillen, Cara Albright, Mikah Schlesinger, and Hope Fillingim for their help in the field and in the lab. I would also like to thank Jeremy Hartsock, and other former members of the lab, as their work laid a foundation for me to pursue this research. I also owe a debt of gratitude for all current and former undergraduate assistants, but particularly Agrima Poudel and John O'Connor. I would also like to thank Athabasca University for the use of their facilities, and Elaine Goth-Birkigt for all of her assistance.

Finally, I would like to thank my family and friends for their support throughout my academic career. I could not have accomplished all that I have without my parents, Gregg and Ellen Stuart.

## BIOGRAPHICAL SKETCH

I was born in Grand Rapids, Michigan. After completing high school, I attended Bryn Mawr College in Bryn Mawr, Pennsylvania. I majored in history of art, but maintained a passion for biology and the sciences and took courses in chemistry, biology, and statistics. In the summer of 2012, I was hired as a field research assistant for the Wieder/Vile lab and spent my summer in the bogs of Canada. My work that summer solidified my interest in biology, and encouraged me to pursue science more seriously. The following summer, I again worked as a field assistant and was invited to join the lab as a Master's student. I was very grateful for this opportunity, as I was passionate about the research being performed and the environment in which we worked.

My research was funded through the National Science Foundation and a Summer Graduate Fellowship through Villanova University. I spent two summers in Alberta measuring rates of net nitrification and ammonification, and during the second summer performed a pilot study on the possibility of performing gross N mineralization experiments. I am planning to present my work at the Society of Wetland Scientists conference in May 2016.

After completing my Master's degree, I have accepted a PhD position in Biology and Ecosystem Science at Northern Arizona University under the direction of Dr. Michelle Mack. I intend to continue studying N cycling in high latitude environments, this time in Alaska. Though I will greatly miss the peatlands of Alberta, I am looking forward to continuing to explore some of the core questions of my research in a new ecosystem.

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## ABSTRACT

*The pristine peatlands of Alberta, Canada are currently experiencing an increase in atmospheric nitrogen (N) deposition coupled with a rise in the intensity and frequency of wildfires. Given the global importance of peatlands as a large net carbon (C) sink relative to their land area, understanding nutrient cycling dynamics and the effect of these disturbances is paramount. Obtaining accurate rates of N mineralization and nitrification can augment our current understanding of N-limitation, plant-microbe competition, and nutrient availability in bogs. Net N mineralization was quantified (buried bag technique) in the summers of 2013, 2014, and 2015 along a chronosequence of five bog sites that represent 2 to 113 years since fire (YSF) in 2013. Each site contains six replicate plots of four different N treatments: control, 0, 10, and 20 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Significant differences in rates of net ammonification and nitrification were observed in site and round of measurement, but not in N treatment. Net DIN production was higher at older sites (net ammonification added to net nitrification, averaged across year, sampling round, and treatment in µg N m<sup>-2</sup> d<sup>-1</sup>, was 933, 736, 137, 723, and 140 at 113, 73, 32, 12, and 2 YSF, respectively), which is counter to the hypothesis that available N, and thus net N mineralization, would be higher in recently burned bogs due to the slower recovery rate of mycorrhizal vegetation and lower C:N ratios due to labile C release from roots post-fire. Measurement of gross N ammonification, nitrification, and microbial N consumption rates could indicate whether the low net N mineralization rates reported for these bog sites are associated with slow or rapid turnover of the dissolved inorganic N pool.*

## *Overview*

Peatlands, while occupying only 3-4% of the earth's land surface, hold 25-30% of the terrestrial soil C stores (Gorham 1991; Clymo *et al.* 1998; Yu 2012; Limpens *et al.* 2006). Anthropogenic disturbances facing peatlands, such as increasing temperatures, nitrogen (N) deposition, and more frequent wildfires, could be particularly detrimental on a global scale due to the C storage that peatlands provide (Limpens and Brenendse 2003; Bragazza *et al.* 2006; Wieder *et al.* 2009; Turetsky *et al.* 2011). About 87% of the world's peatlands are found in the boreal zone (Vitt 2006). Canada alone contains about  $1.24 \times 10^6 \text{ km}^2$  of peatlands, which is roughly 10% of the global total (Joosten and Clark 2002). Extreme latitudes in both the northern and southern hemispheres are critical to the atmospheric C cycle and are likely to see pronounced effects from global climate changes more quickly than other latitudes (IPCC 2013).

Assessing current peatland nutrient cycling regimes, and predicting future changes, can help determine the effects that anthropogenic disturbances will have on global climate trends. N limitation is classically considered to be one of the defining traits of terrestrial ecosystems (Vitousek and Howarth 1991). However, whether boreal peatlands are N limited remains uncertain. Recent research suggests that N<sub>2</sub> fixation by prokaryotes associated with peat-forming *Sphagnum* moss has been a historically underestimated N source to peatlands (Vile *et al.* 2014). N mineralization and ammonification in boreal peat has been assumed to be low, which contributes to the paradigm of N limitation. Quantifying steps in the N cycle, such as ammonification and nitrification, can augment our current understanding of N cycling processes in

*Sphagnum*-dominated bogs.

Historically, N deposition in the western boreal region has been low compared to eastern North America and Europe (Wieder *et al.* 2010). Inorganic N inputs to ecosystems are increasing regionally in Alberta due to the expansion of the Alberta Oil Sands (Wieder *et al.* 2010; Vile and Wieder 2010). Anthropogenic N inputs in Europe and eastern North America have been higher than western North America due to early, large scale industrialization and appears to have affected nutrient cycling in those peatlands (Limpens and Berendse 2003; Gunnarsson *et al.* 2004; Bonnett *et al.* 2010). Concurrently, the number and severity of wildfires are increasing (Flannigan 2009). The post-fire recovery of plant and microbial communities also affects nutrient cycling, and may threaten the C-sink abilities of peatlands through several mechanisms (Wieder *et al.* 2009).

The goal of the research presented in this thesis is to obtain net rates of N mineralization and nitrification in a series of experimental sites that capture bogs at different stages of recovery from wildfire and are amended with artificial N deposition treatments. Additionally, the possibility of obtaining gross ammonification and nitrification rates was explored through a small pilot study of  $^{15}\text{N}$  isotope-pool dilution, which is still relatively untested in peat soils. The first part of this thesis will provide background information on peatlands as well as recent biogeochemical research being performed in these ecosystems. The second part will present the research executed in bog sites over three summer field seasons in Alberta, Canada. The third part presents the pilot study performed to assess the possibility of obtaining gross N mineralization rates.

## Part I: Peatland ecology and context of current research

### *Defining Peatlands*

Peatlands classically are defined as areas where the rate of net primary productivity (NPP) exceeds the rate of decomposition in the peat column (Clymo 1971).

The long-term C storage capability of peatlands is due to the accumulation of organic matter that occurs over long spans of time (Clymo 1971; Wieder *et al.* 2006). The slow decomposition of the peat profile, often attributed to cold, anoxic conditions, is crucial in the formation of peat (Gorham 1991; Gignac and Vitt 1994; Halsey *et al.* 2000). The biologically active surface of the peat, or acrotelm, is where plant growth occurs (Ingram 1978; Clymo 1984). About 5-10% of the organic material produced in the decomposition-prone surface will move into the anaerobic catotelm layer beneath, where decomposition will slow considerably (Clymo 1984; Gorham 1991; Warner *et al.* 1993; Vardy 2000).

Peatlands can be divided into morphometric types based upon characteristics such as hydrology and flora (Vitt 2006). Bogs are ombrogenous, meaning that nutrient and water inputs come from atmospheric deposition as opposed to groundwater and/or surface water runoff (Damman 1986; Vitt 2006). Fens are fed by geogenous waters and thus are minerotrophic, as water sources such as groundwater or runoff from surrounding upland waters transmit a higher amount of dissolved anions and cations. Fens can be further subdivided by the plant species that thrive there, and, consequently, the characteristics promoted by the presence of those plants. True mosses characterize rich fens, while poor fens, as well as bogs, are dominated by peat-building *Sphagnum* mosses (Vitt 2000). Bogs have drier surface conditions and a thicker aerobic acrotelm layer than

fens (Vitt 2006). Accordingly, bogs are more susceptible than fens to changes in elemental composition of wet and dry atmospheric deposition (Malmer and Wallén 2004).

### *The Composition of Bogs*

*Sphagnum* mosses are the pivotal species around which bogs are organized (Rydin *et al.* 2006). *Sphagnum* is key in both the development and maintenance of bogs, as its morphology directly supports the creation of the environment in which it thrives (van Breemen 1995; Vile *et al.* 2011). Each *Sphagnum* shoot is comprised of living, photosynthetic cells in the capitulum at the peat surface as well as dead hyaline cells for water storage (Vitt 2006). The **poikilohydric** mosses protect themselves from desiccation through a combination of efficient water storage and capillary action (Hayward and Clymo 1982; Titus and Wagner 1984). The density of the capitula at the peat surface (2-7 shoots cm<sup>-2</sup>) limits competition for vertically accessible resources such as atmospheric deposition and sunlight and increases water storage capacity (Vitt 2006). The disassociation of hydrogen ions from the cell wall, along with interactions with rainwater chemistry, makes the environment acidic, with a pH of 3.5-4.5 in the water of true bogs (Clymo 1984; McKnight *et al.* 1985). Thus, the wet, anoxic, and acidic environment promoted by *Sphagnum* slows the decay of plant material, especially in the catotelm layer. *Sphagnum* in bogs acts as a **gatekeeper** for nutrients entering the environment from **atmospheric deposition** (Lamers *et al.* 2000). In order to effectively compete with vascular plants, most of which are able to grow taller and possibly outcompete the bryophytes by gaining greater access to sunlight and shading the area below, *Sphagnum*

Poikilohydry is the lack of ability to maintain and/or regulate water content to achieve homeostasis of cells and tissue connected with quick equilibration of cell/tissue water content to that of the environment.

utilizes incoming nitrogen before it enters the rhizosphere and becomes accessible to vascular plants (Bonnett *et al.* 2010). The peat mosses are able to survive in nutrient-poor environments because of the efficiency with which they capture nutrients that enter the system (Aldous 2002; Bridgman 2002). Nutrient limitation is propagated through the ecosystem by these mechanisms (Bragazza and Limpens 2004; Wiedermann *et al.* 2009; Fritz 2014).

A distinctive consequence of differences in the growth of different *Sphagnum* species is the microtopography of bogs, or the formation of high hummocks and lower hollows in close proximity to one another. Different species of *Sphagnum* thrive in the various niches created through this variation (Andrus 1986; Gignac *et al.* 1998). Species that typically occupy the hummock positions, such as *S. fuscum* and *S. capillifolium*, generally have better capillary transport and a decreased tendency toward decay due to a higher amount of structural carbohydrates relative to hollow species (Rydin 1993; Turetsky *et al.* 2008). Hollow species are unable to grow much higher than the water table and experience more frequent desiccation, but also have higher rates of photosynthesis (Titus *et al.* 1983; Rydin 1993; Gerdol 1995). The ability of *Sphagnum* to fill different micro-niche bog environments ensures nearly complete coverage of the peat surface by moss (Gignac 1992; Vitt 2006).

In North American true bogs, *Picea mariana* is the overwhelmingly predominant species of tree found (Clymo *et al.* 1984; Vitt 2006). *Picea*, along with shrub species such as *Ledum groenlandicum*, *Chamaedaphne calyculata*, *Vaccinium oxycoccus*, *Vaccinium vitis-idaea* and *Andromeda glaucophylla*, rely on mycorrhizal root structures to obtain the nutrients necessary for their growth (Thormann 2006a). Ericoid mycorrhizae, associated

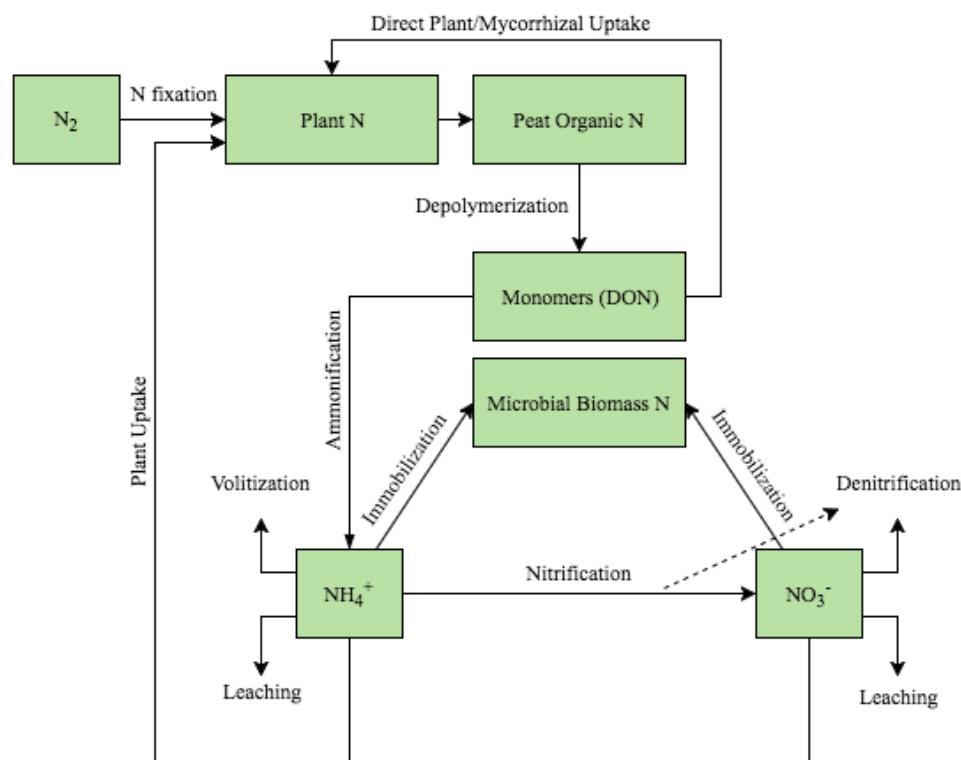
with ericaceous shrubs present in low litter quality environments, contribute to the breakdown of organic material (Read 1991). The ectomycorrhizae associated with *Picea* are primarily responsible for acquisition of nutrient resources, with limited saprotrophic abilities (Read 1991; Thormann 2006b). Alternatively, plants may rely on deep root systems. *Rubus chamaemorus*, *Smilacina trifolia*, and *Eriophorum vaginatum*, which may be non-mycorrhizal, send long roots into the peat matrix to acquire nutrients (Vasander and Kettunen 2006). Bogs also may contain small carnivorous plants, such as *Drosera* spp. and *Sarracenia* spp., which rely partially on the abundant supply of insects that populate the bogs (Bragazza and Limpens 2004). Vascular plants constitute a potential source of competition for the peat mosses, but also provide structure around which the microtopography of the bog can develop relative to the water table (Rydin *et al.* 2006).

### *N Cycling in Bogs*

N is an essential element for plant life. It is the most common element present in the air we breathe and yet also the most common limiting nutrient for plant growth. The N that exists in the atmosphere is N<sub>2</sub>, a form of N rendered inert by all organisms except bacteria due to the strong triple bond between the two N atoms (Schlesinger 1997). The processes that plants and microbes undergo to obtain, convert, and use N are collectively known as the N cycle (**Fig. 1.1**).

The bogs of northwestern boreal Canada traditionally have been considered N-limited (Wassen *et al.* 1995; Bragazza *et al.* 2004; Wieder 2006). As bogs are unconnected to groundwater sources, N acquisition occurs via microbial N<sub>2</sub> fixation or

atmospheric deposition of both inorganic and organic forms of N (Chapin 2003; Vitt 2006; Limpens *et al.* 2006; Morford *et al.* 2011; Vile *et al.* 2014). Due to the low mean temperature and acidity of peat, atmospheric deposition has been assumed to be more important than N<sub>2</sub>-fixation (Limpens *et al.* 2006), as boreal peatlands formerly were considered inhospitable to N<sub>2</sub>-fixing bacteria (Houlton 2008). These bacteria convert atmospheric N<sub>2</sub> into ammonium, an inorganic form used by plants.



**Figure 1.1** The nitrogen cycle, adapted from Schimel and Bennett (2004) and Norton (2000). Dashed line indicates “leaky pipe” to denitrification.

Until the recent expansion of oil sands development north of Fort McMurray, Alberta, Canada, N deposition rates in Alberta were less than 2 kg ha<sup>-1</sup> yr<sup>-1</sup> (Vitt *et al.*

2000; Hazewinkel *et al.* 2008; Wieder *et al.* 2012; Wieder *et al.* 2015, in press).

However, the assumption of N limitation in *Sphagnum* itself is belied by high concentrations of N in both the peat and the moss.  $^{210}\text{Pb}$ -dated cores indicate that the observed *Sphagnum* NPP cannot be sustained exclusively by the low-level atmospheric deposition rates observed in Alberta (Vitt *et al.* 2003; Wieder *et al.* 2010; Vile *et al.* 2014). Recent data suggest that  $\text{N}_2$  fixation plays a much greater role in *Sphagnum* N acquisition than previously expected, while the vascular constituents of bogs are very much N limited. Members of the Vile lab have found rates of  $\text{N}_2$ -fixation in Alberta bogs of 4.8-62.3 kg N  $\text{ha}^{-1} \text{ yr}^{-1}$  and may be connected to methanotrophic bacteria (Vile *et al.* 2014).

Nitrogen mineralization is the microbial conversion of organic forms of N to inorganic forms of N, which refers to the ammonification of organic N from proteins or amino acids to  $\text{NH}_4^+$ . The nitrification  $\text{NH}_4^+$  to  $\text{NO}_3^-$ , the subsequent step in the N cycle, is sometimes included in mineralization, though this thesis will distinguish between ammonification and nitrification (McGill and Cole 1981). As illustrated in Figure 1, the depolymerization of organic N into monomers is essential for mineralization to proceed. The quality and quantity of DON produced within the system may be a primary control on the rate of N mineralization (Schimel and Bennett 2004). The availability of labile DON as a controlling factor in rates of mineralization may be particularly important as the waterlogged, acidic nature of bogs, combined with low-quality litter and *Sphagnum*'s antimicrobial properties, leads to slow decomposition rates of organic matter (Wieder and Vitt 2006).

With a high C:N ratio of 45:1 reported for *Sphagnum*-dominated bogs, net ammonification and nitrification rates are low, and tend to favor net immobilization (Chapin *et al.* 2003; Wieder *et al.* 2010; Hartsock 2013). The C needs associated with assimilation, growth, and energy requirements for microbes means that N cycling often is closely related to the availability of labile C resources (Murphy 2003). Nitrification, the oxidation reaction that transforms  $\text{NH}_4^+$  into  $\text{NO}_2^-$  and then into  $\text{NO}_3^-$ , is limited by available  $\text{NH}_4^+$  (Schlesinger 1997; Limpens *et al.* 2006). Plants take up both  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , although some plants show preferences for one form of inorganic N over the other (Sheppard 2014). In the typical bog condition of cold, wet, acidic peat, nitrification rates are considered to be low and difficult to measure accurately (Regina 1997).

Production of  $\text{N}_2$  or  $\text{N}_2\text{O}$  also can occur via denitrification, or the microbial reduction of  $\text{NO}_3^-$ , complicating the issue of which process produces the  $\text{N}_2\text{O}$ . The “leaky pipe” theory of gaseous N production describes a pipe whose thickness is determined by the rates of both nitrification and denitrification, with the size of the holes in the pipe that leak nitrogen being determined by abiotic factors such as soil water content (Firestone *et al.* 1980; Davidson *et al.* 2000).

Nitrogen that is mineralized has several possible fates: uptake by plants, nitrification, and immobilization by microbes. In N-limited systems, measured rates of net ammonification and nitrification may be low due to microbial immobilization, or uptake of inorganic nitrogen by the microbial community (Schimel and Bennett 2004). The classic paradigm of nitrogen uptake has held that plants only used inorganic forms of nitrogen such as ammonium or nitrate (Schimel and Bennett 2004). While plants may primarily use inorganic N, dissolved organic nitrogen (DON) uptake is considered to be a

potential, but difficult to quantify, source of plant N (Jones *et al.* 2004; Bragazza and Limpens 2004; Jones and Willett 2005; Kielland *et al.* 2006; Kranabetter *et al.* 2007). In bog environments, mycorrhizal roots take up DON (Thormann 2006a). Mycorrhizal DON uptake helps vascular plants to compete for resources in *Sphagnum*-dominated peatlands.

Net ammonification and nitrification rates are a measure of the production of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N minus microbial immobilization, whereas gross rates of ammonification and nitrification are determined by the total amount of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N produced without excluding inorganic N uptake, by a unit of weight or volume over a set amount of time (Hart *et al.* 1994). Net N mineralization rates were, for many years, thought to be an accurate measure of plant available N, as DIN was considered to be the only important N input for plants from the soil (Schimel and Bennett 2004). It was also assumed that microbes outcompeted plants for available  $\text{NH}_4^+$ . Both of these assumptions have been challenged, due to evidence for plant DON uptake, long-term N competition taking into account microbial die-off events, and the potentially confounding nature of gross production and gross consumption in net rates (Schimel and Bennett 2004).

### *Anthropogenic Disturbances in Alberta Bogs*

Our current understanding of N cycling in Alberta bogs will be affected by recent land use changes, climate change, and increased human activity. Canada is currently the top exporter of oil to the United States (United States Energy Information Administration 2012). The Athabasca Oil Sands Region (AOSR, Fig.1.2) is host to one of the world's largest petroleum deposits, with an estimate of 173 billion recoverable barrels (Alberta Environment 2008). While the oil sands have been known to exist since the nineteenth

century, mining did not begin until the 1960's, and has expanded greatly in recent decades (Krim 2003). Because the petroleum of the oil sands is in the form of bitumen, a semi-solid, viscous oil substance, the process of extraction and refinement is energy inefficient and expensive relative to mining of traditional oil reservoirs (Weinhold 2011). While relatively little land space will be used for surface mining due to the successful use of *in-situ* extraction techniques, the sharp increase in human activity and industrial emissions has the potential to affect peatlands in several ways, including the possible diminishment of bog CO<sub>2</sub>-C sink strength (Cleary *et al.* 2005; Lee and Cheng 2009; Wieder *et al.* 2009).



**Figure 1.2** Athabasca Oil Sands Region, centered on the mining center at Fort McMurray. Map from Alberta Environment and Sustainable Resource Development (<http://esrd.alberta.ca/>).

Inorganic N deposition (mainly  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) has increased from rates below 2  $\text{kg ha}^{-1} \text{yr}^{-1}$  to levels between 2-5  $\text{kg ha}^{-1} \text{yr}^{-1}$ , a trend that will continue to increase as the size and scope of oil sands development grows (Wieder *et al.* 2010; Vile and Wieder 2010; Wieder *et al.* 2015, *in press*). Bogs in Europe and eastern North America have been exposed to higher levels of N deposition than bogs in northwestern Canada in the modern industrial age, some for more than a century (Wiedermann *et al.* 2009). N deposition rates in populated, industrialized areas such as these can range from 5-45  $\text{kg ha}^{-1} \text{yr}^{-1}$  (Aerts *et al.* 1999). Studies in Europe have concluded that increased N deposition affects bog ecosystems negatively, both in plant response and microbial activity. The observation of increased levels of DON in peat pore water as N deposition increases indicates that *Sphagnum* may be leaching organic N in response to N saturation (Bragazza and Limpens 2004). The response of *Sphagnum*-dominated bogs to N deposition has been characterized as triphasic (Lamers 2000; Berendse *et al.* 2001; Heijmans *et al.* 2002). The naturally N-limited *Sphagnum* layer first shows increased growth in response to the fertilizing effects of nitrogen. After an initial spurt of productivity, another limiting factor, such as phosphorus, may prevent additional growth. In the second phase, *Sphagnum* will continue to act as a nutrient filter by storing excess N as evidenced by increased *Sphagnum* N concentration, but as the third phase is entered, *Sphagnum* will reach an N-saturation point and proceed to leach N into the rhizosphere, where it becomes available to vascular plants. Given access to unprecedented levels of bioavailable N, vascular plants could outcompete the bryophytes and cause the collapse of the C sink (Bubier *et al.* 2003; Juutinen 2010; Larmola 2013). Whether collapse is a function of the amount of N or the length of the exposure time is not clearly known

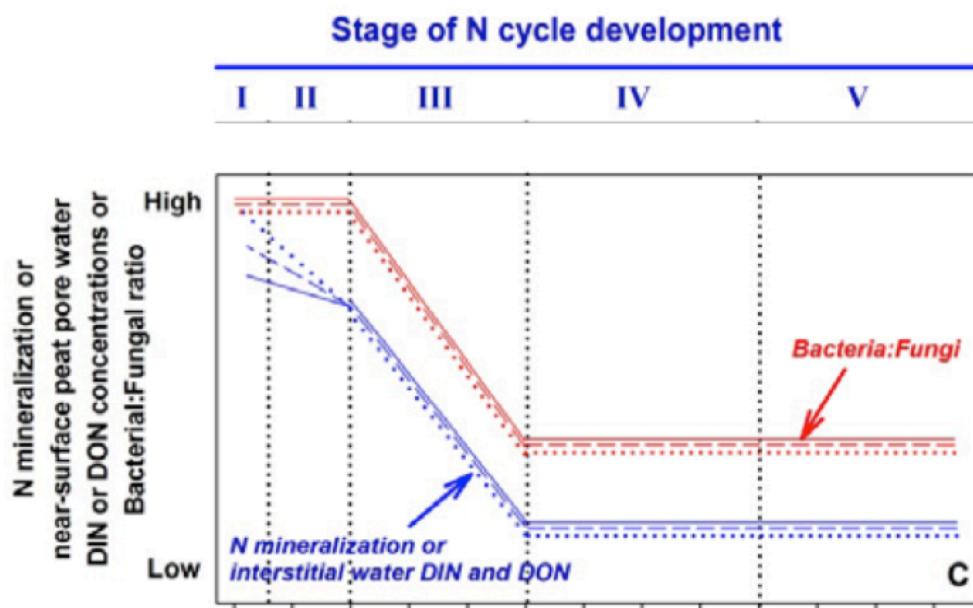
(Wiedermann *et al.* 2009). Both DIN and DON concentrations in peatland porewaters have, in Europe, been shown to increase as N deposition increases (Bragazza and Limpens 2004).

Concurrent with increasing atmospheric N deposition is an increase in the frequency and intensity of wildfires in the boreal region (Flannigan 2009). The heightened intensity of global warming in high latitudes can contribute to a lowering of the water table level in peatlands (Gorham 1991). Increased air temperatures and human activity in the boreal region also increase fire incidence. It is predicted that in the next 85 years, incidences of boreal fire will increase by 50%, and in a matter of less than 20 years could greatly outpace the capabilities of current fire control teams (Flannigan 2009). Temperature elevation and drought increase the severity of fires that occur, leading to a loss of  $16.8 \text{ kg C m}^{-2}$  from peatlands, or 450 years worth of peat accumulation, during individual fire events (Turetsky *et al.* 2011). In addition to the initial pulse of carbon added to the atmosphere, burned bogs will continue to act as a net C source instead of a sink for about 20 after fire, as bog vegetation recovers (Wieder *et al.* 2009). The current estimate of fire return for bogs in Alberta is  $123 \pm 26$  years (Turetsky *et al.* 2011). If the fire return interval were to decrease to less than 61 years, Alberta bogs, as a whole, would be a net source of C to the atmosphere rather than a net sink (Wieder *et al.* 2009).

### *Current Research: Addressing Unknowns of N Cycling in a Changing Province*

N cycling in peat bogs as a function of time since fire has been largely unexplored, particularly in conjunction with anthropogenic N deposition. Wieder and Vile (2012) hypothesized that N cycling in Alberta bogs will change in stages

corresponding to the recovery of plant species post-wildfire. Immediately following a fire, *Sphagnum* species and ericaceous shrubs are often damaged, and black spruce trees are dead. Mineralization levels were hypothesized to be high, due to a release in useable C and elevated surface peat temperatures following the destruction of vegetation. The increased presence of labile C following fire was expected to augment N mineralization rates. Five years after fire, the *Sphagnum* mosses will return to 100% cover, and initial increases in mineralization were hypothesized to taper off after labile C released by fire has been utilized. Initial recovery of black spruce trees, and subsequent increases in competition for organic nitrogen via mycorrhizal roots, will further decrease N mineralization rates 20-50 post-fire. After 50 years, full black spruce recovery will have taken place and net N mineralization levels should remain constant, and low, through the rest of the bog's life cycle (Fig.1.3). As nitrification rates are related to available  $\text{NH}_4^+$ , the same trend was predicted to apply to post-fire nitrification.



**Figure 1.3** Predicted rates of net mineralization following fire by stages based on years since fire, from Wieder and Vile (2012).

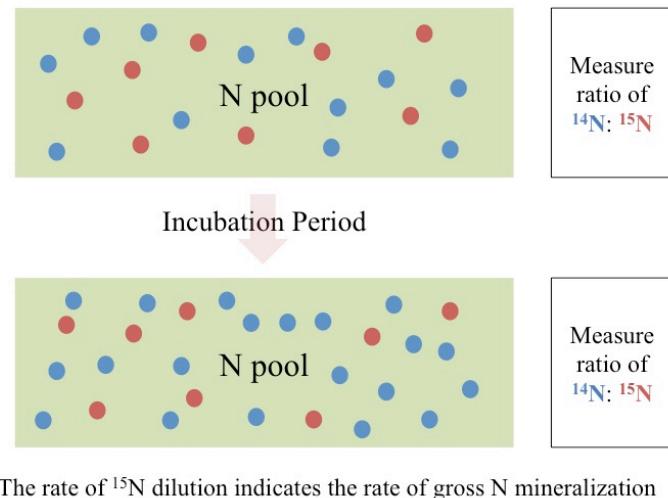
Increased atmospheric N deposition is hypothesized to lead to more net N immobilization, which could be attributed either to decreased ammonification and nitrification due to changing C:N ratios, or to increased microbial uptake fueled by the increase in available N. There may be an interactive effect between inorganic N deposition and years since fire, based on rates of mycorrhizal plant recovery interacting with increased available inorganic nitrogen. These predictions are in fact based on predicting different, but connected, processes; DON breakdown, gross ammonification and nitrification, and microbial N immobilization.

Measurement of net N mineralization, particularly in environments thought to be at least partially N-limited, may obscure the importance of DON in plant-microbial resource competition (Schimel and Bennett 2004). Employing gross N mineralization measurement techniques could shed light on hypotheses of the true rate of nutrient turnover. *Sphagnum* NPP is fueled by higher-than-expected rates of N<sub>2</sub> fixation (Vile *et al.* 2014), but the fate of that N once it enters the rhizosphere cannot be fully addressed solely with net N mineralization rates. The assumption that nutrient turnover is inherently slow instead of a symptom of high competition for N resources could be challenged by gross N mineralization measurements. I hypothesized that the studied bogs would demonstrate high rates of inorganic N production and consumption, particularly in older bogs with established mycorrhizal networks contributing to the breakdown of organic N provided in part by high N content in *Sphagnum*.

A firm consensus of expected rates of net N mineralization under changing N deposition conditions has not been reached, particularly for peatlands with historically

low N deposition rates outside of Europe and eastern North America (Updegraff 1995; Bayley and Thormann 2005; Fellman and D'Amore 2007). Gross N mineralization rates have not been explored in Alberta bog ecosystems due to the costs and challenges associated with performing gross mineralization experiments (Kaye and Hart 1997; Schimel and Bennett 2004).

Gross N mineralization rates are both more informative and more difficult to measure than net N mineralization. In order to obtain an accurate rate of gross N mineralization, the process must be observed without alteration, as it would occur in the absence of experimental measures. To avoid augmenting the available substrate pools, as organic  $^{15}\text{N}$  tracers may, gross N mineralization can be measured using an isotope dilution technique (Davidson 1991). Isotope dilution approaches to quantifying N mineralization, unlike isotope tracer experiments, theoretically do not add substrates for nitrification, which would inflate the measured rate of N mineralization and alter the natural process through the implementation of the experiment, particularly if the process is naturally substrate-limited. Measuring gross ammonification and nitrification involves adding isotope-labeled inorganic N compound ( $^{15}\text{NH}_4^+$  or  $^{15}\text{NO}_3^-$ ) to a closed system, and quantifying the rate at which the atom % of the isotopically labeled product is diluted through the production of the non-isotopically labeled product (**Fig. 1.4**).



**Figure 1.4** Illustration of the principle of isotope dilution, where an isotopically labeled product is added to a closed system and its rate of dilution is measured.

There are several important underlying assumptions regarding isotope dilution mineralization measurements, as follows:

1. Gross mineralization rates are constant through the incubation period
2. Microbes do not discriminate between different stable N isotopes, viz. isotope fractionation does not occur at significant levels
3.  $\text{NH}_4^+$  or  $\text{NO}_3^-$  taken up by microbes is not remineralized during experiment

Kirkham and Bartholomew (1955) and Bjarnason (1988) generally confirmed that the first assumption is true, as long as soil samples were not exposed to extra moisture or substrate as a result of adding the isotope solution. Samples that are already moist, such as peat, will not be prone to this sort of error. Fractionation is defined as the general processes that control the relative abundance of stable isotopes through preferential processing of the lighter of two isotopes, as heavier isotopes form stronger chemical bonds than light isotopes. The second assumption is not strictly true, but N isotope

fractionation occurs at extremely low levels, particularly during short incubation times (Delwiche and Steyn 1970). The third assumption is the most likely to be violated. While several methods indicate that a sample time of 1-3 days is ideal, colder, boreal peat bogs are thought to have a much slower rate of nutrient turnover compared to mineral soils so longer incubation times may be needed (Chapin *et al.* 2011).

Gross N mineralization experiments have been performed in peat soils, and one study was previously performed in Alberta peatlands. Generally, however, gross ammonification and nitrification rates in peatlands have not been explored, or have been quantified with mixed samples of peat and mineral soils. Part of the reason may be that it is difficult to add enough  $^{15}\text{N}$  to be measurable while not augmenting  $\text{NH}_4^+ \text{-N}$  and/or  $\text{NO}_3^- \text{-N}$  pools, thus inflating the rates of the processes being observed (Davidson 1991).

Wray and Bailey (2008) added 6 mL of 98atom%  $^{15}\text{N}$  at a concentration of 35  $\mu\text{g/L}$  to 5  $\times$  10 cm core from Alberta peatlands. Kwak (2015), working with mixed peat and mineral Alberta soils, added 2 ml of 10atom%  $^{15}\text{N}$  to 30 g of mixed soil collected from a field site from the 1-10cm depth layer. The findings of peat soil or mixed peat and mineral soil gross N mineralization experiments are summarized in Table 1.1. Hart *et al.* (1994) suggests adding 2 mg isotopic N  $\text{kg}^{-1}$  dry soil. Given the extremely low dry bulk density and high volumetric water content of *Sphagnum* peat, addition amounts for this study were intentionally kept very low.

<b>Location</b>	<b>Sample Type</b>	<b>Reported Rates (mg N kg<sup>-1</sup> d<sup>-1</sup>)*</b>		<b>Reference</b>
		<b>NH<sub>4</sub><sup>+</sup>-N</b>	<b>NO<sub>3</sub><sup>-</sup>-N</b>	
Ontario, Canada	Peatland conifer stand soil	18-19	Negative rate	Westbrook and Devito (2004)
Alberta, Canada	Peat/mineral mix, mine reclamation	2.3	0.8	McMillan (2007)
Alberta, Canada	Floating/Non-floating peatland	202 /27 *	250/31 *	Wray and Bayley (2008)
Alberta, Canada	Peat/mineral mix, mine reclamation	2-12	0-5	Kwak (2015)

\*Rates for Wray and Bayley (2008) reported by bulk density, mg N m<sup>-2</sup> d<sup>-1</sup>.

**Table 1.1** Review of past <sup>15</sup>N pool dilution studies performed on boreal peat samples with reported rates of gross ammonification and nitrification.

The form of NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> added may also play a role in the rates obtained. Kwak (2015) chose to add <sup>15</sup>NH<sub>4</sub>NO<sub>3</sub> or NH<sub>4</sub><sup>15</sup>NO<sub>3</sub> depending upon whether a sample was being used to measure ammonification or nitrification, based upon the principle that this would allow all fluxes to be more comparable to one another. Traditionally, (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and K<sup>15</sup>NO<sub>3</sub> have been used so that the non-isotopically labeled NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> pools are not increased (Davidson 1991; Murphy 2003). However, in some soils obtaining potential rates may be more informative than actual rates, and addition of isotopically-labeled N may change microbial partitioning in terms of immobilization (Murphy 2003).

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## **Part II: Net ammonification and nitrification experiments**

### *Introduction*

Peatlands are of greater importance to global C budgets than their relative land area would suggest, due to their ability to accumulate and store C for long periods of time (Wieder *et al.* 2006; Yu 2012). The boreal zone hosts 85% of the world's peatlands (Wieder *et al.* 2006), and is currently experiencing anthropogenic disturbances such as warming temperatures, increased wildfire frequency, and elevated N deposition (Limpens and Brenendse 2003; Bragazza *et al.* 2006; Turetsky *et al.* 2011). Understanding current N cycling regimes and predicting future shifts that will accompany human disturbances are paramount to making accurate predictions about future C release or storage capacity (Wieder *et al.* 2009).

Net ammonification is the conversion of organic N monomers into inorganic  $\text{NH}_4^+$ , and net N nitrification is the conversion of  $\text{NH}_4^+$  to  $\text{NO}_3^-$ , an oxidation reaction by microbes for their energy needs (McGill and Cole 1981). Net N mineralization, by strict definition and in this thesis, is the conversion of organic N to inorganic N via ammonification, but the term is often broadly used to refer to both processes (Schimel and Bennett 2004). Both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are bioavailable to plants for their N requirements, and some plants may demonstrate a preference for one over the other (Ashton *et al.* 2008, Sheppard *et al.* 2014).

Boreal peatlands are dominated by *Sphagnum* moss in the understory (Rydin *et al.*

2006), which promotes the cold, wet, anoxic conditions that allow for long-term C storage (van Breemen 1995; Vile *et al.* 2011). Vascular plants, such as the primary bog tree *P. mariana*, and ericaceous shrubs are secondary competitors for N resources and rely on strategies such as mycorrhizal associations, deep roots, and DON uptake to maintain their existence (Thormann 2006a; Vitt 2006; Wiedermann *et al.* 2009; Bonnett *et al.* 2010). Increasing high latitude temperatures, increased inorganic N inputs via atmospheric deposition, and increased wildfire frequency may disrupt nutrient cycling and cause species shifts (Wieder *et al.* 2012). Past research on post-fire C regime changes indicates that C cycling changes after wildfire events (Wieder *et al.* 2009); Given the relationship between C and N in the rhizosphere, such as the tendency for net N mineralization to increase when C:N ratio exceeds that required for microbial growth, the resultant impact of nutrient availability and C:N ratios may affect N cycling (Limpens *et al.* 2006).

Increased human activity and the expansion of the oil sands in Alberta are contributing to increased atmospheric N deposition in an area that historically has had low N deposition rates (Vitt *et al.* 2000; Hazewinkel *et al.* 2008; Wieder *et al.* 2012; Wieder *et al.* 2015, in press). Given access to unprecedented levels of bioavailable N, vascular plants could outcompete the bryophytes and cause the collapse of the C sink (Bubier *et al.* 2003; Juutinen 2010; Larmola 2013). The response of bogs to anthropogenic N deposition in boreal western Canada is still undetermined, due to the ecological differences between these bogs and those previously studied under high N deposition levels in Europe and eastern North America (Wiedermann *et al.* 2009). Due to the role of peat C:N ratios, changing dissolved organic nitrogen (DON) quality, and the

effect of increased inorganic N concentrations, net ammonification and nitrification rates could be a part of feedback loops wherein changing process rates speed up or slow down other ecosystem changes. DON quality and quantity is a limiting factor in N mineralization (Schimel and Bennett 2004). Net DON production reflects input to the soil via decomposition, which is considered slow in bogs, minus outflow via microbial conversions or direct plant uptake (Vitt 2006; Jones *et al.* 2004; Kielland *et al.* 2006; Kranabetter *et al.* 2007)

The type of N resources available in bogs may help make predictions for future species shifts and thus C storage ability. Vile *et al.* (2014) demonstrated that the rate of N accumulation in *Sphagnum* moss in Alberta bogs cannot be accounted for solely through atmospheric deposition, and that the N accumulation observed in peat bogs is associated with N<sub>2</sub>-fixation. Determining the fate of N once it enters the rhizosphere, and the movement of organic material through other constituents of the bog ecosystem, brings a renewed purpose to the study of N mineralization rates.

### *Objectives/Hypotheses*

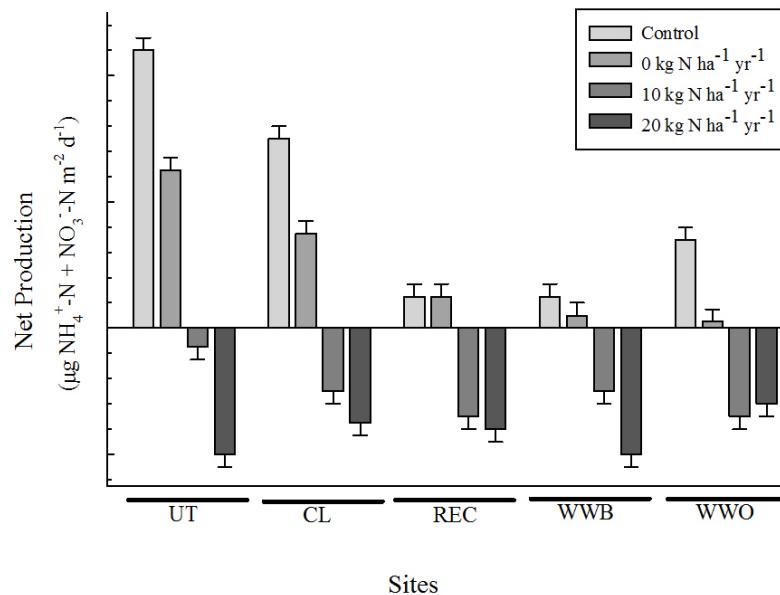
The overall objective of this project is to examine the combined and interactive effects of post-fire recovery and augmented N deposition on one aspect of N cycling, mineralization, by obtaining accurate rates of net ammonification, net nitrification, and net DON production in bogs. Described below are the key questions being asked in this thesis, along with my hypotheses and rationales.

**How will net ammonification and nitrification rates change as a function of time since fire? How will net N mineralization rates change as a function of experimental N deposition? Will there be an interactive effect of time since fire and N deposition?**

Wieder and Vile (2012) predicted at the outset of the current research in Alberta bogs that time since wildfire would have a significant effect on net ammonification and nitrification rates due to shifting C:N ratios and the recovery rate of different plant species(**Fig. 1.3**). Wildfire can release labile nutrients from the roots of burned trees, while shrubs and mosses may be desiccated or singed but typically recover more quickly (Benscoter and Vitt 2008). The slow recovery rate of plants with mycorrhizal roots is hypothesized to lead to a high bacterial:fungal ratio immediately after fire. I hypothesized that net N mineralization rates would be highest in recently burned (Stage I; **Fig. 1.3**) bogs due to the absence of mycorrhizal competition for DON, and that rates would decrease until the full recovery of black spruce occurs (Stage IV), at which point rates would remain stable and low, and that nitrification would display a similar trend.

The addition of  $\text{NH}_4\text{NO}_3$  to experimental plots is expected to significantly affect net N ammonification rates, but not nitrification rates, as  $\text{NO}_3^-$  is a microbial byproduct of  $\text{NH}_4^+$  oxidation rather than a nutrient source (McGill and Cole 1981). The C:N ratio in boreal bogs is high, so increasing N deposition would likely increase microbial immobilization. It is important to consider the potential interaction between age of bog since wildfire and N deposition; due to the recovery of *Sphagnum* and, more importantly,  $\text{N}_2$ -fixing microbial associates in *Sphagnum*, the increased DIN effects will mostly be seen in young bogs. I hypothesized that there would be a significant interaction between

years since fire (YSF) and N treatment, with N treatment effects being more pronounced in younger bogs (**Fig. 2.1**).



**Figure 2.1** Hypothesized site and N treatment interaction effects on net ammonification and nitrification. Legend indicates experimental levels of N deposition. Bars are arranged by site, in order from youngest to oldest.

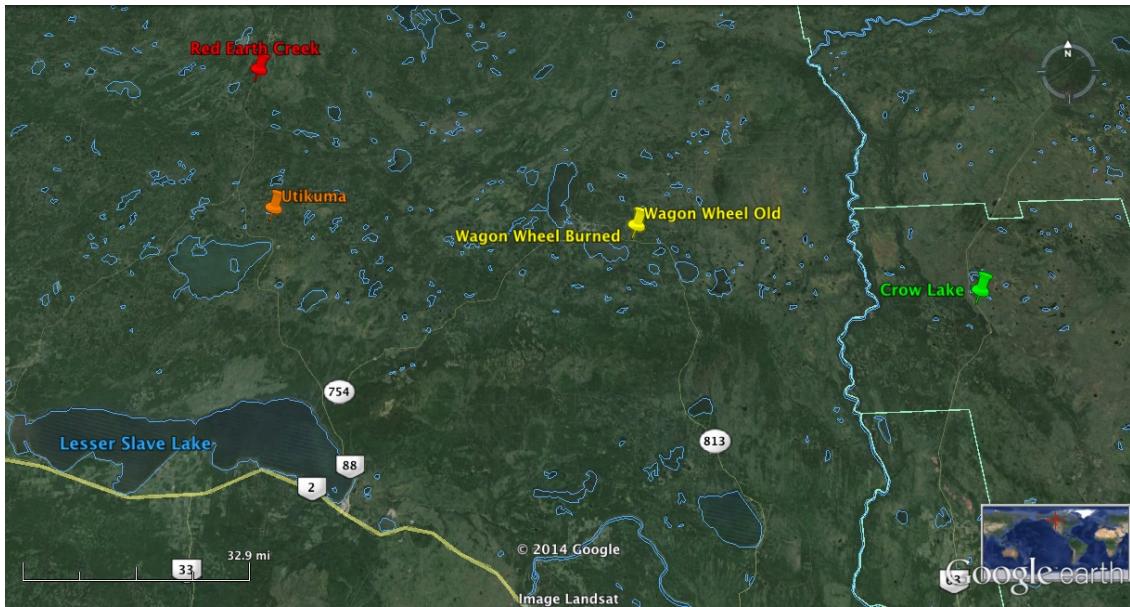
N mineralization rates may depend upon the amount of DON biologically available. While this study will measure total N and use that concentration to calculate DON, not all DON is considered biologically available due to the level of recalcitrance (Schimel and Bennet 2004). DON concentrations are higher than DIN concentrations in bog porewater (Bragazza and Limpens 2004); DON is not commonly measured in N mineralization studies despite its importance in peat soils, as it is not important in mineral soils (Zsolnay 2003; Schimel and Bennett 2004).

## Methods

To better understand N cycling in bogs post-fire and the attendant effects of increased N deposition simultaneously, Dr. Wieder and Dr. Vile have received NSF funding to carry out an extensive study of the functioning of five Alberta bogs in different stages of post-fire recovery. The use of a chronosequence, or substituting location for time, is appropriate for Alberta bogs, as they are floristically similar (Wieder *et al.* 2009). Five bog sites in Alberta, Canada were selected in 2013 as representatives of bogs in different stages of recovery following wildfires (Table 2.1, **Fig. 2.2**). The sites represent a span of 4 to 111 years of age since time of last wildfire as of 2015, and have been divided into five distinct stages based on fire recovery (Table 2.1).

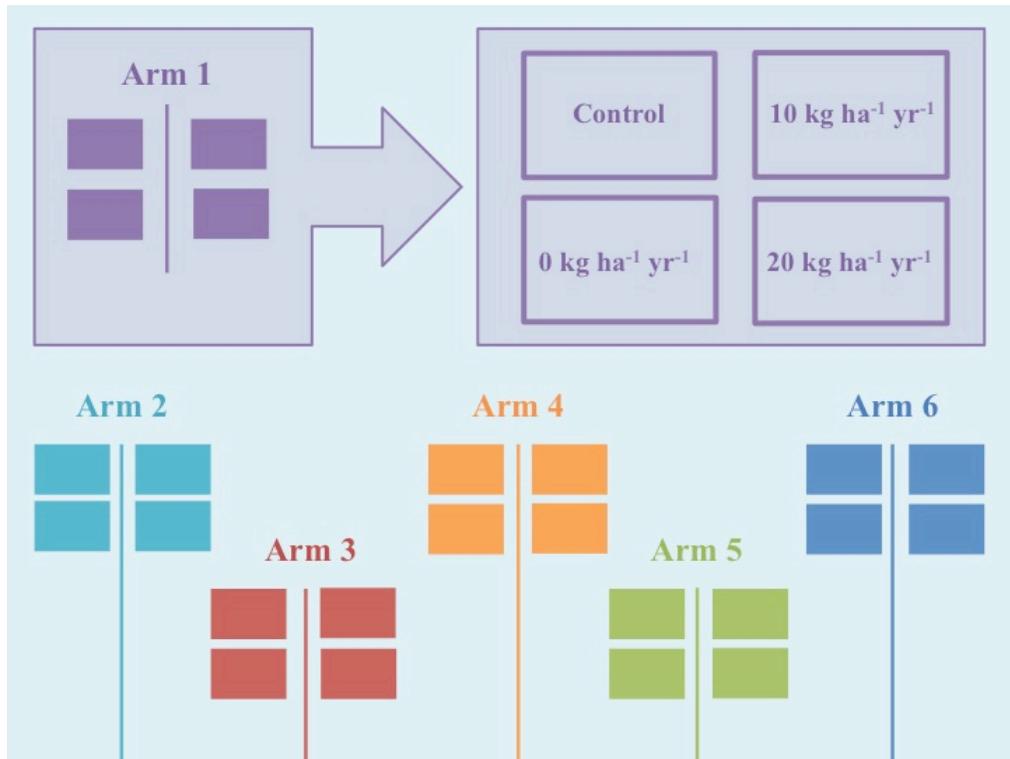
Site Name	Latitude	Longitude	YSF (in 2013)	Stage
Utikuma (UT)	55°59' N	115°17' W	2	I
Crow Lake (CL)	55°43' N	112°12' W	12	II
Red Earth Creek (REC)	56°22' N	115°17' W	32	III
Wagon Wheel Burned (WWB)	55°43' N	112°12' W	73	IV
Wagon Wheel Old (WWO)	55°43' N	112°12' W	113	V

**Table 2.1.** Table of locations and age characteristics of bogs used in this research.



**Figure 2.2** Aerial map of locations of NSF study sites utilized in this thesis. Map generated with Google Earth.

At each of these sites, six arms of four plots were established, for 24  $2.4 \times 4.8$  m plots total at each site (**Fig. 2.3**). On each arm, the four plots receive one of four N treatments. Each arm has one plot of each of the following treatments:  $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ,  $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ,  $0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (just  $\text{H}_2\text{O}$ ), and a complete control receiving neither water nor N. Since 2013 for all sites except UT, which began receiving N treatments in 2012, the plots have been and will continue to be fertilized a total of 8 times per year during the summer for the duration of the study. N treatments are applied as  $\text{NH}_4\text{NO}_3$  dissolved in 4 L of  $\text{H}_2\text{O}$  and applied to the plots with backpack sprayers, and represents only a slight augmentation in  $\text{H}_2\text{O}$  input over the background level of precipitation.



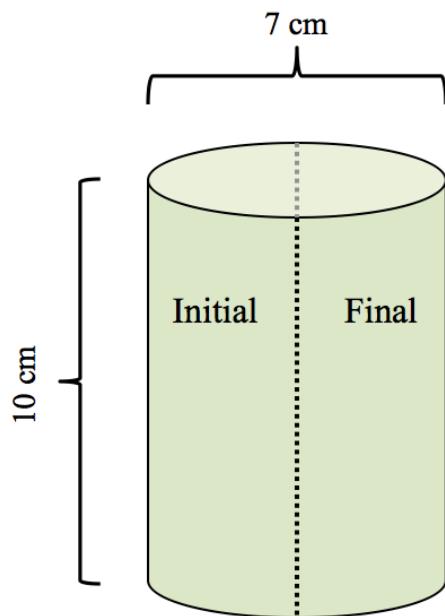
**Figure 2.3** Schematic sample site design for UT, CL, REC, WWB, and WWO. Arms (6) are indicated by different colors. Each arm has one control treatment and three nitrogen treatments ( $0$ ,  $10$ , and  $20\text{ kg ha}^{-1}\text{ yr}^{-1}$ ). Plots are delineated by wooden boards and encompass  $18\text{ m}^2$ . This figure does not accurately reflect distances between plots or represent plots to scale.

In the summers of 2013, 2014, and 2015, net ammonification and nitrification measurements were performed in each of the plots described above. Two sampling rounds were performed during each of these summer field seasons, and a third round was performed in 2015 (Table 2.2).

<b>Year</b>	<b>Round</b>	<b>Start dates</b>	<b>End dates</b>
<b>2013</b>	1	6/24-6/29	7/28-8/1
	2	7/24-7/30	9/11-9/15
<b>2014</b>	1	5/24-5/26	6/26-6/28
	2	6/25-6/27	7/25-7/27
<b>2015</b>	1	5/25-5/27	6/25-6/27
	2	6/24-6/26	7/28-7/29
	3	8/9-8/11	10/12-10/16

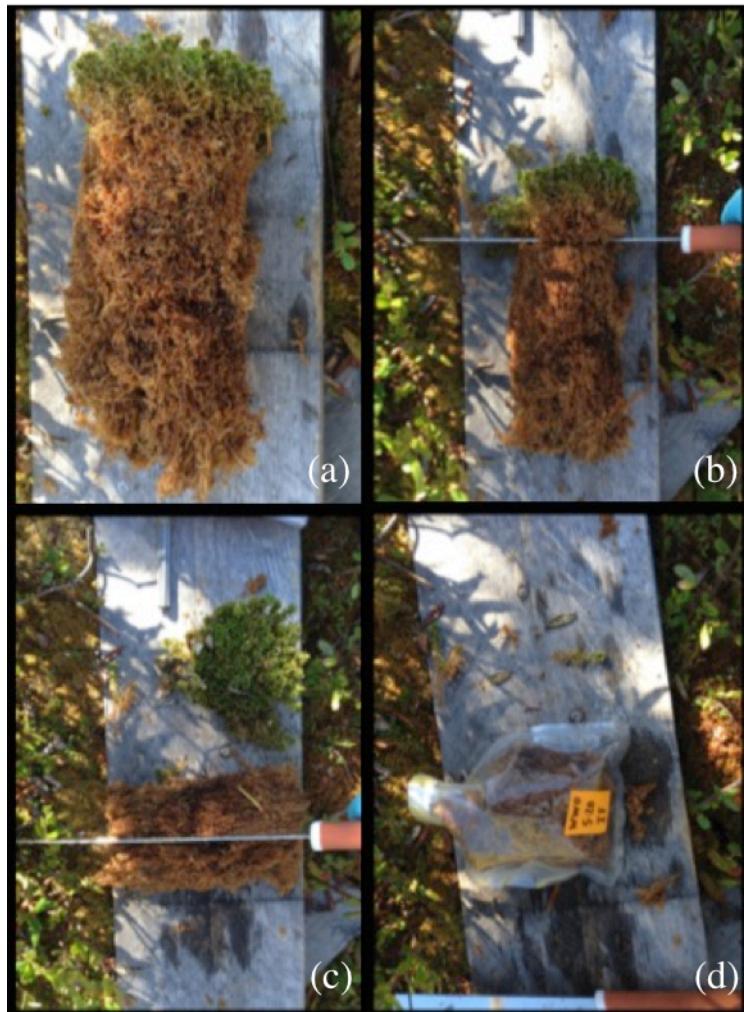
**Table 2.2** Dates of N mineralization measurement rounds in the three years of data included in this thesis.

Net mineralization was quantified using the buried bag method (Robertson 1999; Bayley and Thormann 2005). A single surface peat core of 10 cm in length 7.5 cm diameter, not including the active, living layer of *Sphagnum*, was taken from each experimental plot and divided in half (**Fig. 2.4, 2.5**). One half was immediately placed into a Whirl-Pak® polyethylene bag and taken back to the lab at Athabasca University for extraction. The other half was placed in a Whirl-Pak® polyethylene bag and reburied beneath the peat for approximately one month. For both initial and final samples, one 1 M KCl extraction of the peat sample was performed within 3 days of collection from the field. Samples were refrigerated in between collection from the field and time of extraction.



**Figure 2.4** Core divisions for net mineralization protocol.

Field moist samples were extracted with 1 M KCl for one hour and then vacuum filtered through a Whatman 42 filter paper using Büchner funnels. Extract solution volume was measured, transferred to bottles, and frozen for transport back to Villanova University. The extracted peat sample was dried at 60° C and weighed. Extract solutions were analyzed for the concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . A Seal III autoanalyzer was used to determine  $\text{NH}_4^+$  concentrations using alkaline phenol and  $\text{NO}_3^-$  concentrations using the hydrazine reduction method. Extracts were analyzed for total dissolved N using a Shimadzu catalytic combustion TC/TN analyzer. Inorganic N concentrations were subtracted from total N concentration to determine DON concentration.



**Figure 2.5** (a) A peat core is taken from a plot, and (b) the active layer is removed. (c) The core is then divided in half, and each half (d) is placed in a Whirl-Pak™ bag. The initial sample is immediately transported back to the lab, while the final sample is replaced and covered by the active layer previously removed from the core.

Rates of net ammonification and nitrification were determined by subtracting initial  $\text{NH}_4^+$ -N or  $\text{NO}_3^-$ -N from the corresponding final concentration. Rates are reported in  $\mu\text{g m}^{-2} \text{ d}^{-1}$ , based on the peat bulk density. Statistical analyses were performed in SAS using PROC MIXED MODEL. Data were statistically analyzed as a repeated measures

rmANOVA, as the same plots were repeatedly measured over time and each plot represents an individual experimental unit, with main effects of site, N treatment, and sampling round as a repeated effect. *A posteriori* comparisons were made using Tukey's HSD approach.

## Results

### Initial Extractable NH<sub>4</sub><sup>+</sup>-N and Net Ammonification

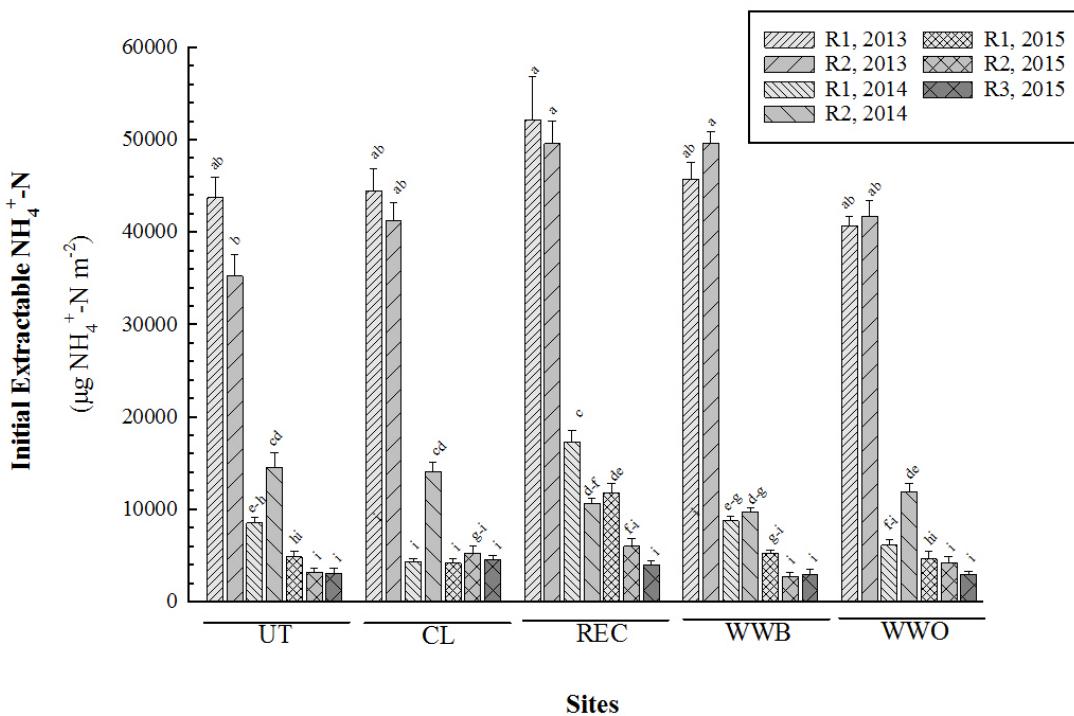
Initial extractable NH<sub>4</sub><sup>+</sup>-N, averaged across all sites, treatments, and sampling rounds, was  $17,721 \pm 647 \text{ } \mu\text{g NH}_4^+ \text{-N m}^{-2}$ . Initial extractable NH<sub>4</sub><sup>+</sup>-N exhibited significant round  $\times$  site interactions ( $p < 0.0001$ , **Fig. 2.6**), and round  $\times$  N treatment interactions ( $p = 0.0464$ , **Fig. 2.7**). Despite the round  $\times$  site interaction, in general initial extractable NH<sub>4</sub><sup>+</sup>-N was higher in 2013 than in 2014 or 2015; For any given sampling round, site differences were few and did not exhibit a pattern of clear change as a function of time since fire (**Fig. 2.6**). Similarly, the round  $\times$  N treatment interaction displayed a pattern of higher initial extractable NH<sub>4</sub><sup>+</sup>-N concentrations in 2013 than in 2014 or 2015 and for any given sampling round, there was no effect of N treatment (**Fig. 2.7**). There was no significant round  $\times$  site  $\times$  N treatment interaction ( $p = 0.6321$ ).

Net rates of ammonification across all rounds, sites, and N treatments averaged  $389 \pm 36 \text{ } \mu\text{g NH}_4^+ \text{-N m}^{-2} \text{ d}^{-1}$ . In the repeated measures ANOVA, there was a significant round  $\times$  site interaction ( $p < 0.0001$ , **Fig. 2.8**), but no significant N treatment ( $p = 0.5146$ ), round  $\times$  N treatment interaction ( $p = 0.4678$ ), site  $\times$  N treatment interaction ( $p = 0.1809$ ) or round  $\times$  site  $\times$  N treatment interaction ( $p = 0.8894$ ). The round  $\times$  site interaction revealed

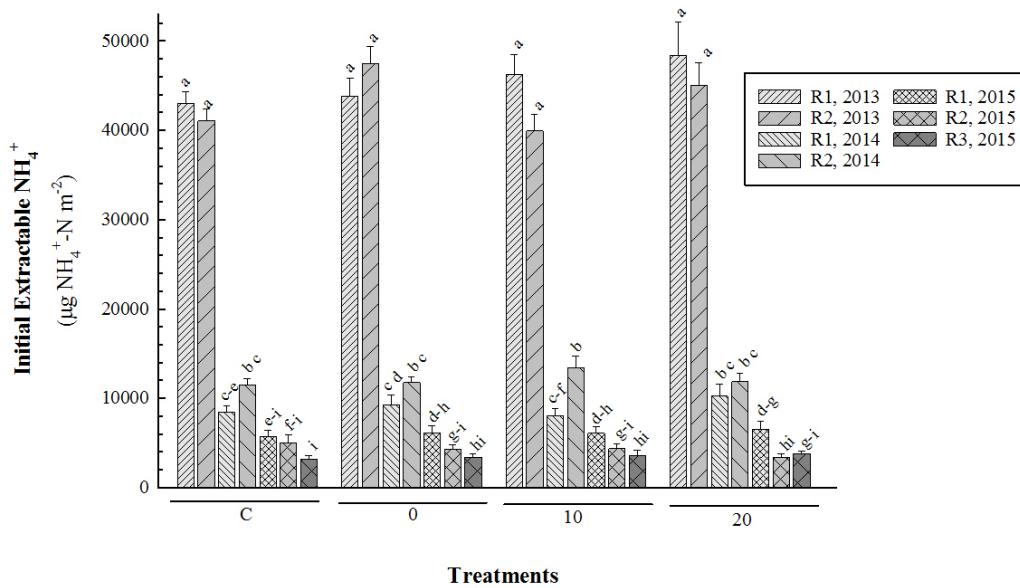
no site differences in 2013 or 2015, but net ammonification rates were lower at the youngest site (UT) than several of the older sites (WWB and WWO) in 2014 (**Fig. 2.8**). Net ammonification was negatively correlated with initial extractable  $\text{NH}_4^+$ -N and initial extractable DON concentration. This correlation can be most plainly observed through REC round 1 and 2 in 2013 and WWB round 2 2013. All three are significantly higher than all other round  $\times$  site means for initial extractable  $\text{NH}_4^+$ -N concentration, and those same groups have the three lowest mean net  $\text{NH}_4^+$ -N immobilization means.

	Initial Extractable $\text{NH}_4^+$ -N	Initial Extractable $\text{NO}_3^-$ -N	Initial Extractable DON	Net Nitrification
<b>Pearson Correlation Coefficient</b>	-0.23143	-0.27215	-0.17423	0.61366
<b>p value</b>	< 0.0001	< 0.0001	< 0.0001	< 0.0001

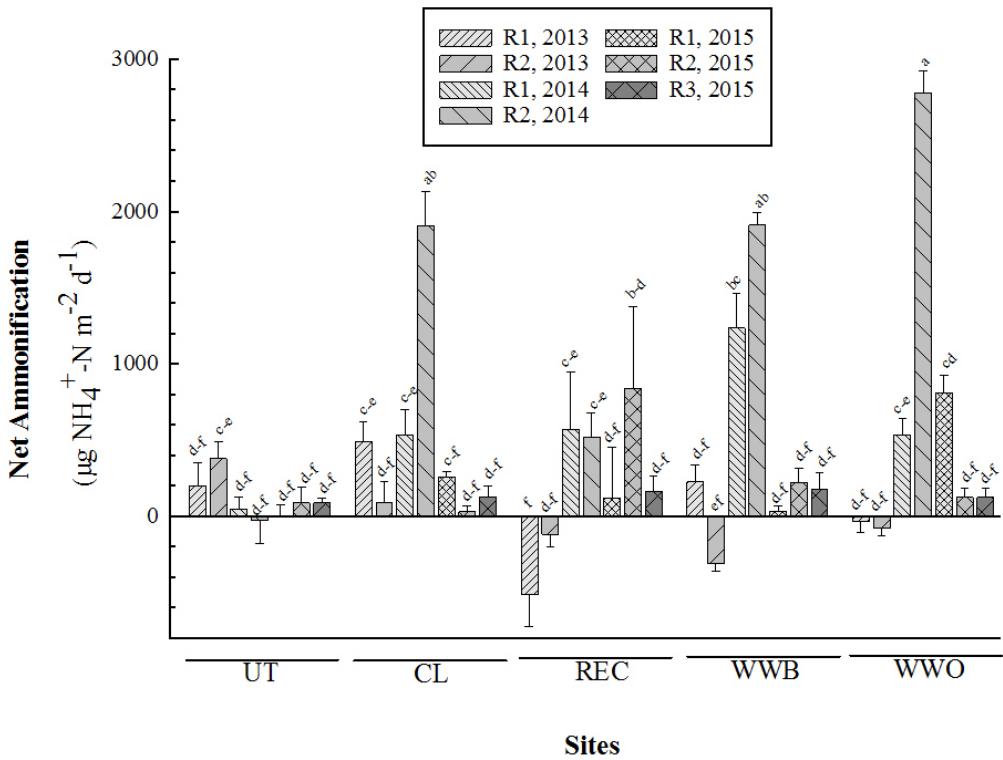
**Table 2.3** Pearson correlation coefficient and *p* values for Net ammonification rates. Row one indicates the comparison variable.



**Figure 2.6** Average initial extractable  $\text{NH}_4^+$  by round and site. Significance indicated by letters above each individual bar were determined using Tukey's HSD.



**Figure 2.7** Average initial extractable  $\text{NH}_4^+$  by round and N treatment. Significance indicated by letters above each individual bar were determined using Tukey's HSD.



**Figure 2.8** Average net ammonification rate by round and site. Significance indicated by letters above each individual bar were determined using Tukey's HSD.

### Initial Extractable $\text{NO}_3^-$ -N and Net Nitrification

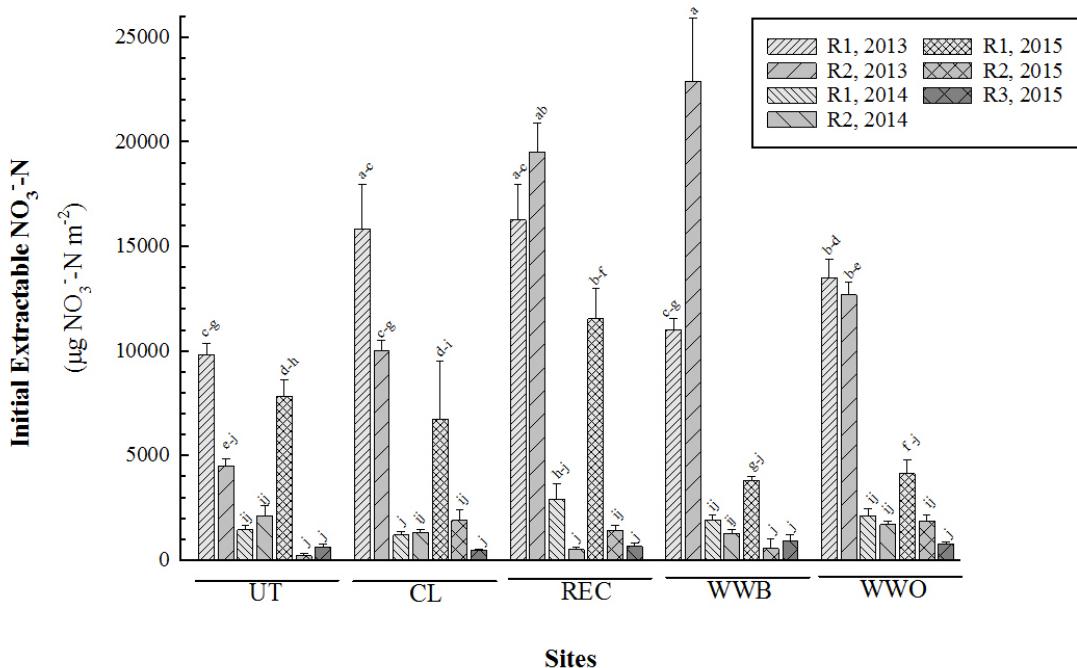
Initial extractable  $\text{NO}_3^-$ -N, across all sites, treatments, and rounds was  $5,615 \pm 267 \mu\text{g } \text{NO}_3^-$ -N  $\text{m}^{-2}$ . A significant round  $\times$  site interaction was exhibited ( $p < 0.0001$ , Fig. 2.9). No significant N treatment effect ( $p = 0.4383$ ), site  $\times$  N treatment ( $p = 0.0820$ ), round  $\times$  N treatment ( $p = 0.1920$ ), or site  $\times$  round  $\times$  N treatment ( $p = 0.8397$ ) interactions were obtained. As with initial extractable  $\text{NH}_4^+$ -N, at most sites, concentrations in 2013 were significantly higher than in 2014 and 2015. When comparing between two rounds in

2013, the younger sites (UT and CL) had higher concentrations for round one, earlier in the growing season, than at the second measurement time. Conversely, older sites (REC, WWB, and WWO) were either not significantly different, or higher in the second round. In the first round of 2015, initial extractable  $\text{NO}_3^-$ -N concentrations were higher in younger sites than in WWB and WWO (**Fig. 2.9**).

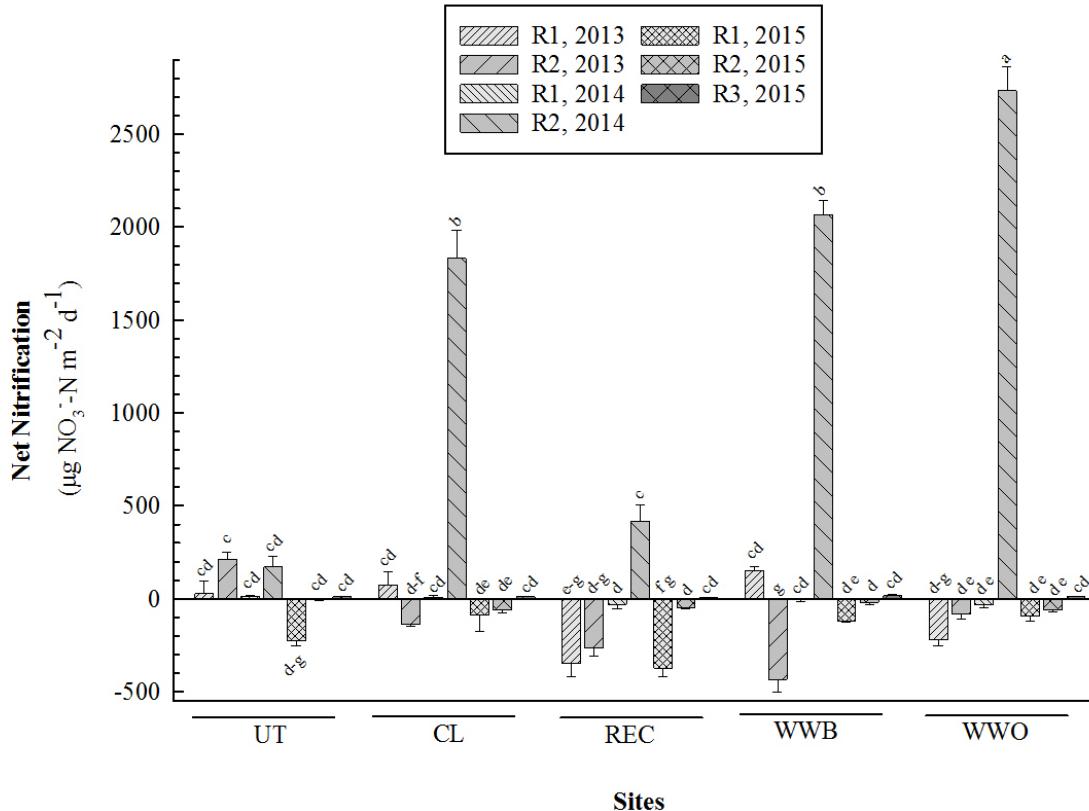
Average net nitrification across all rounds, sites, and treatments was  $147 \pm 24 \mu\text{g NO}_3^-$ -N  $\text{m}^{-2} \text{d}^{-1}$ . A significant round  $\times$  site interaction was observed ( $p < 0.0001$ , **Fig. 2.10**). No treatment effect was exhibited ( $p = 0.5453$ ), and no site  $\times$  N treatment ( $p = 0.9613$ ), round  $\times$  N treatment ( $p = 0.4140$ ), or site  $\times$  N treatment  $\times$  round ( $p = 0.9257$ ) interactions were observed. The three highest round  $\times$  site means occurred in the same three groups as the highest net ammonification round  $\times$  site means (**Fig. 2.8**, **Fig. 2.10**). All 2015 round  $\times$  site means were either negative (indicating net  $\text{NO}_3^-$ -N immobilization) or very close to zero, and exhibited few significant differences. However, a visual trend of the lowest rates occurring in round one and the highest rates in round three is present. Net nitrification was negatively correlated with initial extractable  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N, and positively correlated with net ammonification.

	Initial Extractable $\text{NH}_4^+$ -N	Initial Extractable $\text{NO}_3^-$ -N	Initial Extractable DON	Net Ammonifi- cation
<b>Pearson Correlation Coefficient</b>	-0.18364	-0.37807	-0.26158	0.61366
<b><i>p</i> value</b>	< 0.0001	< 0.0001	< 0.0001	< 0.0001

**Table 2.4** Pearson correlation coefficient and *p* values for net nitrification rates. Row one indicates the comparison variable.



**Figure 2.9** Average initial extractable  $\text{NO}_3^-$  by round and site. Significance indicated by letters above each individual bar were determined using Tukey's HSD.



**Figure 2.10** Average net nitrification by round and site. Significance indicated by letters above each individual bar were determined using Tukey's HSD.

### Initial Extractable DON and Net DON Production

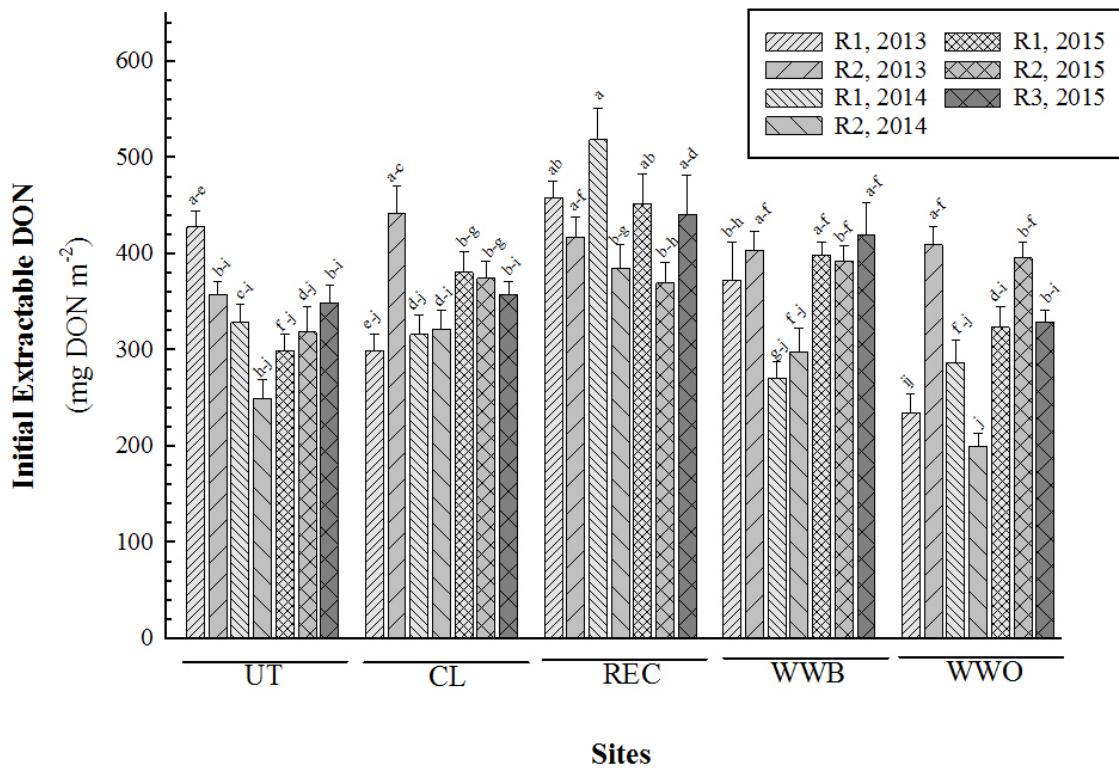
Average initial extractable DON, across all sites, rounds, and treatments, was  $359,897 \pm 4401 \mu\text{g N m}^{-2}$ . A significant round  $\times$  site interaction was observed ( $p<0.0001$ , Fig. 2.11). No treatment effect was exhibited ( $p=0.4440$ ), and no site  $\times$  N treatment ( $p=0.7090$ ), round  $\times$  N treatment ( $p=0.1987$ ), or site  $\times$  N treatment  $\times$  round ( $p=0.8311$ ) interactions were observed. REC, a Stage III bog, generally had the highest initial extractable DON concentration as compared to other sites within the same round.

Otherwise, despite the round  $\times$  site interaction, clear site differences do not emerge.

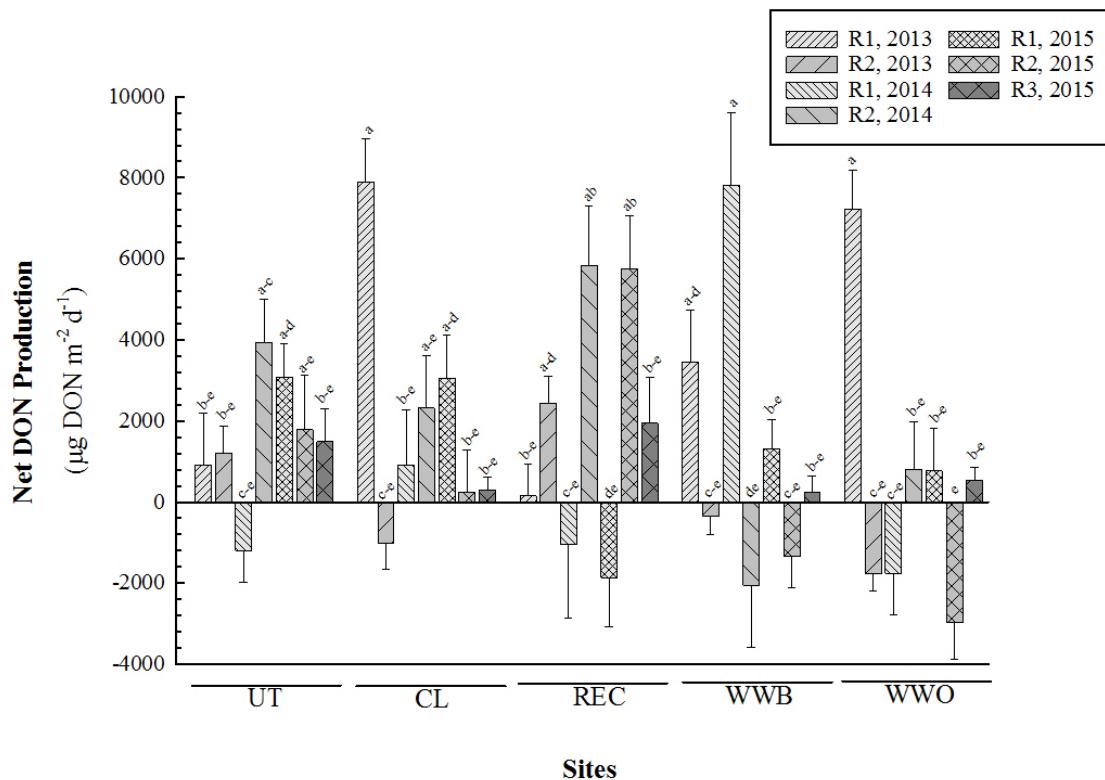
Net DON production, averaged across sites and treatments, was  $1413 \pm 200 \mu\text{g N m}^{-2} \text{ d}^{-1}$ . The round  $\times$  site interaction was significant ( $p < 0.0001$ , **Fig. 2.12**). No treatment effect was exhibited ( $p = 0.9786$ ), and no site  $\times$  N treatment ( $p = 0.6938$ ), round  $\times$  N treatment ( $p = 0.2197$ ), or site  $\times$  N treatment  $\times$  round ( $p = 0.6635$ ) interactions were observed. Despite the significant round  $\times$  site interaction, a clear YSF fire effect was not apparent. Older sites did tend to exhibit more negative rates than younger sites. Net DON production was negatively correlated with initial extractable DON concentration, and positively correlated with net ammonification.

	Initial Extractable $\text{NH}_4^+ \text{-N}$	Initial Extractable $\text{NO}_3^- \text{-N}$	Initial Extractable DON	Net Ammonifi- cation	Net Nitrification
<b>Pearson Correlation Coefficient</b>	-0.00718	-0.02591	-0.57376	0.21414	0.03822
<b><i>p</i> value</b>	0.8361	0.4555	< 0.0001	< 0.0001	0.2709

**Table 2.5** Pearson correlation coefficient and *p* values for net DON production rates. Row one indicates the comparison variable.



**Figure 2.11** Average initial DON concentration by round and site. Significance indicated by letters above each individual bar were determined using Tukey's HSD.

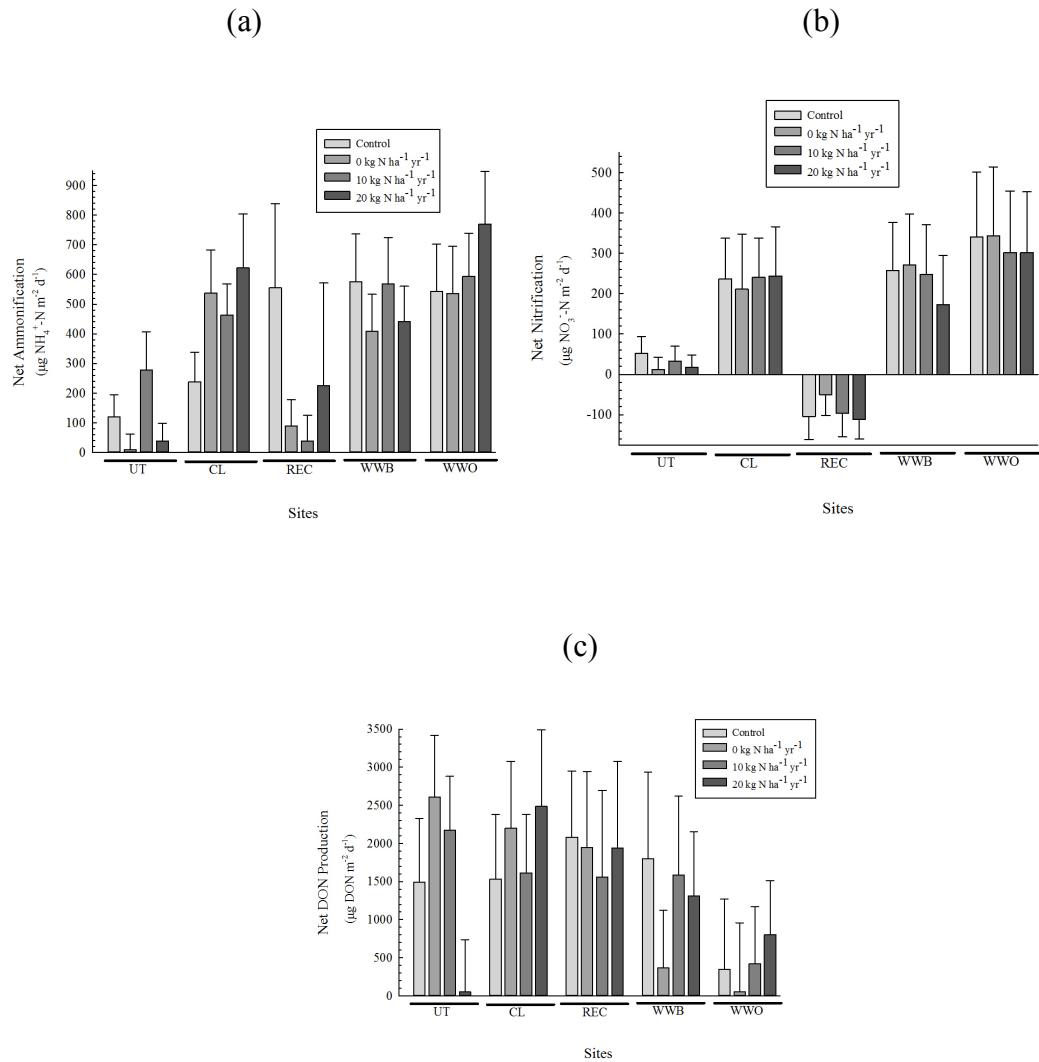


**Figure 2.12** Average net DON concentration by round and site. Significance indicated by letters above each individual bar were determined using Tukey's HSD.

### Treatment Effects

As stated above, few treatment effects were observed in the repeated measures ANOVA for any variable. When averaged across round and N treatment, net ammonification (in  $\mu\text{g NH}_4^+ \text{-N m}^{-2} \text{d}^{-1}$ ,  $\pm$  SE), in order from the youngest to the oldest site, was  $110 \pm 42$ ,  $489 \pm 67$ ,  $228 \pm 117$ ,  $498 \pm 70$ , and  $610 \pm 80$ . A similar trend is seen in net nitrification when averaged across round and N treatment (in  $\mu\text{g NO}_3^- \text{-N m}^{-2} \text{d}^{-1}$ ,  $\pm$  SE), in order from the youngest to the oldest site, which was  $29 \pm 17$ ,  $233 \pm 57$ ,  $-90 \pm 27$ ,  $237 \pm 61$ , and  $322 \pm 78$ . The absence of an N treatment is clear through statistical analyses. Averaged across all sites and rounds, net ammonification (in  $\mu\text{g NH}_4^+ \text{-N m}^{-2} \text{d}^{-1}$ )

$^1$ ,  $\pm$  SE) for treatments C, 0, 10, and 20 kg NH<sub>4</sub>NO<sub>3</sub> ha<sup>-1</sup> yr<sup>-1</sup>, was 426  $\pm$  76, 319  $\pm$  56, 391  $\pm$  58, and 419  $\pm$  91. Averaged across all sites and rounds, net nitrification (in  $\mu\text{g NO}_3^-$ -N m<sup>-2</sup> d<sup>-1</sup>,  $\pm$  SE) for treatments C, 0, 10, and 20 kg NH<sub>4</sub>NO<sub>3</sub> ha<sup>-1</sup> yr<sup>-1</sup>, was 156  $\pm$  48, 159  $\pm$  53, 148  $\pm$  47, and 125  $\pm$  48. A site  $\times$  treatment interaction is absent (**Fig. 2.13**).



**Figure 2.13** Net ammonification (a), nitrification (b), and DON production (c) averaged across all rounds by site and treatment. Error bars are SE.

SITE	TRT	RND	Net NH <sub>4</sub> -N ( $\mu\text{g N m}^{-2} \text{d}^{-1}$ )	Net NO <sub>3</sub> -N ( $\mu\text{g N m}^{-2} \text{d}^{-1}$ )	Init. DON ( $\text{mg N m}^{-2}$ )
UT	C	1	-6 ± 238	-5 ± 129	455.7 ± 74.2
UT	0	1	450 ± 185	139 ± 139	283.3 ± 56.8
UT	10	1	326 ± 474	-32 ± 154	256.1 ± 102.9
UT	20	1	36 ± 274	13 ± 138	282.7 ± 49.5
CL	C	1	612 ± 172	213 ± 90	360.5 ± 56.8
CL	0	1	474 ± 201	39 ± 41	395.1 ± 33.2
CL	10	1	464 ± 169	188 ± 76	385.2 ± 41.2
CL	20	1	403 ± 457	-150 ± 267	423.1 ± 80.8
REC	C	1	-129 ± 171	-237 ± 103	174.9 ± 70.1
REC	0	1	-342 ± 337	-293 ± 129	170.1 ± 76.3
REC	10	1	-564 ± 332	-420 ± 135	448.2 ± 17.4
REC	20	1	-1016 ± 699	-439 ± 217	118.7 ± 81.1
WWB	C	1	373 ± 387	150 ± 26	453.8 ± 24.5
WWB	0	1	79 ± 186	172 ± 65	459.0 ± 42.1
WWB	10	1	128 ± 93	145 ± 46	416.4 ± 105.2
WWB	20	1	317 ± 139	137 ± 49	402.6 ± 34.4
WWO	C	1	41 ± 110	-173 ± 49	216.3 ± 39.6
WWO	0	1	29 ± 179	-199 ± 75	275.2 ± 40.4
WWO	10	1	-145 ± 116	-242 ± 54	242.7 ± 31.7
WWO	20	1	-68 ± 185	-263 ± 97	201.6 ± 50.8
UT	C	2	497 ± 258	164 ± 62	458.2 ± 46.7
UT	0	2	-86 ± 148	110 ± 49	492.7 ± 52.0
UT	10	2	695 ± 290	345 ± 108	344.4 ± 75.0
UT	20	2	404 ± 48	239 ± 46	434.5 ± 28.4
CL	C	2	-198 ± 96	-155 ± 20	434.5 ± 45.5
CL	0	2	219 ± 415	-172 ± 28	430.6 ± 54.6
CL	10	2	135 ± 161	-95 ± 13	266.6 ± 27.8
CL	20	2	191 ± 348	-135 ± 20	390.8 ± 37.6
REC	C	2	167 ± 100	-165 ± 82	455.2 ± 15.4
REC	0	2	12 ± 191	-197 ± 94	365.5 ± 45.6
REC	10	2	-228 ± 123	-321 ± 71	333.8 ± 68.2
REC	20	2	-445 ± 167	-385 ± 71	405.1 ± 23.2
WWB	C	2	-145 ± 89	-314 ± 15	332.1 ± 14.5
WWB	0	2	-301 ± 83	-365 ± 18	430.2 ± 34.2
WWB	10	2	-349 ± 135	-406 ± 71	359.3 ± 33.4
WWB	20	2	-452 ± 83	-652 ± 258	400.4 ± 18.7
WWO	C	2	16 ± 86	-34 ± 67	403.6 ± 15.7
WWO	0	2	-169 ± 115	-115 ± 50	460.2 ± 70.0
WWO	10	2	-7 ± 59	-81 ± 31	402.0 ± 20.8
WWO	20	2	-152 ± 137	-92 ± 64	413.3 ± 43.7

**Table 2.6** Table of average ammonification, nitrification, and initial DON by site, treatment, and round for 2013.

SITE	TRT	RND	Net NH <sub>4</sub> -N ( $\mu\text{g N m}^{-2} \text{d}^{-1}$ )	Net NO <sub>3</sub> -N ( $\mu\text{g N m}^{-2} \text{d}^{-1}$ )	Init. DON ( $\text{mg N m}^{-2}$ )
UT	C	1	-27 ± 71	-7 ± 23	393.4 ± 38.6
UT	0	1	46 ± 131	7 ± 14	314.7 ± 17.8
UT	10	1	70 ± 180	43 ± 13	277.6 ± 34.4
UT	20	1	106 ± 239	12 ± 11	320.2 ± 40.4
CL	C	1	295 ± 82	-13 ± 17	264.4 ± 22.2
CL	0	1	325 ± 55	-4 ± 15	343.1 ± 59.1
CL	10	1	555 ± 195	22 ± 27	318.3 ± 27.4
CL	20	1	956 ± 645	28 ± 18	336.9 ± 42.9
REC	C	1	1839 ± 1470	-79 ± 80	504.7 ± 62.1
REC	0	1	123 ± 145	-31 ± 22	584.4 ± 56.9
REC	10	1	268 ± 131	23 ± 19	543.6 ± 99.0
REC	20	1	53 ± 128	-36 ± 33	545.9 ± 103.6
WWB	C	1	1691 ± 629	6 ± 17	439.4 ± 35.2
WWB	0	1	539 ± 122	-4 ± 44	299.3 ± 38.0
WWB	10	1	1553 ± 549	-6 ± 21	225.7 ± 24.4
WWB	20	1	1153 ± 256	1 ± 24	300.4 ± 39.7
WWO	C	1	224 ± 38	-44 ± 22	362.6 ± 65.3
WWO	0	1	376 ± 92	-61 ± 43	273.0 ± 37.0
WWO	10	1	565 ± 210	-12 ± 21	283.3 ± 43.5
WWO	20	1	967 ± 341	-19 ± 23	227.0 ± 16.1
UT	C	2	360 ± 355	422 ± 183	254.9 ± 38.7
UT	0	2	-220 ± 140	60 ± 66	216.2 ± 37.9
UT	10	2	44 ± 469	69 ± 65	223.8 ± 51.5
UT	20	2	-293 ± 80	128 ± 22	303.3 ± 21.2
CL	C	2	1472 ± 178	1722 ± 195	344.7 ± 45.4
CL	0	2	2197 ± 556	2024 ± 410	321.7 ± 26.5
CL	10	2	1608 ± 387	1605 ± 319	349.2 ± 37.9
CL	20	2	2351 ± 580	1978 ± 290	270.3 ± 40.6
REC	C	2	543 ± 360	406 ± 205	397.9 ± 32.9
REC	0	2	477 ± 280	473 ± 214	369.4 ± 49.0
REC	10	2	553 ± 279	480 ± 205	368.1 ± 67.4
REC	20	2	505 ± 426	305 ± 98	404.6 ± 55.7
WWB	C	2	1942 ± 245	2064 ± 206	335.9 ± 54.2
WWB	0	2	1992 ± 143	2193 ± 117	288.7 ± 55.4
WWB	10	2	1918 ± 182	2115 ± 148	298.5 ± 50.4
WWB	20	2	1797 ± 93	1898 ± 160	268.2 ± 42.4
WWO	C	2	2906 ± 233	2776 ± 309	211.5 ± 25.1
WWO	0	2	2687 ± 370	2913 ± 358	174.3 ± 40.2
WWO	10	2	2460 ± 284	2627 ± 228	208.2 ± 20.7
WWO	20	2	3063 ± 219	2624 ± 149	205.8 ± 15.8

**Table 2.7** Table of average ammonification, nitrification, and initial DON by site, treatment, and round for 2014.

SITE	TRT	RND	Net NH <sub>4</sub> -N ( $\mu\text{g N m}^{-2} \text{d}^{-1}$ )	Net NO <sub>3</sub> -N ( $\mu\text{g N m}^{-2} \text{d}^{-1}$ )	Init. DON ( $\text{mg N m}^{-2}$ )
UT	C	1	-16 ± 51	-206 ± 36	256.6 ± 19.8
UT	0	1	-108 ± 51	-237 ± 74	268.1 ± 10.4
UT	10	1	246 ± 255	-195 ± 33	309.6 ± 52.4
UT	20	1	-105 ± 53	-273 ± 72	359.5 ± 30.7
CL	C	1	262 ± 91	-34 ± 34	376.2 ± 24.3
CL	0	1	306 ± 49	-362 ± 353	339.1 ± 33.2
CL	10	1	230 ± 68	5 ± 30	353.3 ± 38.8
CL	20	1	228 ± 72	36 ± 40	452.5 ± 58.8
REC	C	1	1036 ± 1312	-609 ± 106	506.6 ± 74.9
REC	0	1	-70 ± 190	-258 ± 61	386.7 ± 9.3
REC	10	1	-192 ± 65	-429 ± 78	450.9 ± 35.2
REC	20	1	-285 ± 104	-195 ± 32	462.1 ± 96.1
WWB	C	1	45 ± 83	-120 ± 18	400.0 ± 31.9
WWB	0	1	67 ± 90	-124 ± 10	423.5 ± 18.0
WWB	10	1	25 ± 75	-124 ± 18	378.2 ± 25.5
WWB	20	1	-8 ± 37	-102 ± 7	391.7 ± 33.9
WWO	C	1	596 ± 174	-97 ± 37	332.9 ± 38.2
WWO	0	1	787 ± 256	-80 ± 50	356.3 ± 37.5
WWO	10	1	887 ± 287	-118 ± 68	316.9 ± 31.6
WWO	20	1	964 ± 253	-81 ± 52	286.9 ± 61.9
UT	C	2	-14 ± 45	-4 ± 3	345.2 ± 42.4
UT	0	2	-28 ± 32	-6 ± 6	354.9 ± 68.9
UT	10	2	456 ± 454	-7 ± 8	234.8 ± 58.3
UT	20	2	0 ± 24	-8 ± 6	324.7 ± 33.7
CL	C	2	-60 ± 93	-78 ± 49	374.2 ± 33.8
CL	0	2	85 ± 95	-56 ± 34	376.8 ± 32.1
CL	10	2	24 ± 92	-57 ± 28	350.9 ± 38.6
CL	20	2	64 ± 25	-59 ± 22	396.0 ± 39.7
REC	C	2	378 ± 149	-58 ± 13	407.7 ± 59.9
REC	0	2	419 ± 256	-48 ± 12	344.4 ± 39.4
REC	10	2	201 ± 40	-50 ± 16	356.0 ± 47.4
REC	20	2	2358 ± 2160	-33 ± 24	368.1 ± 33.6
WWB	C	2	72 ± 39	-1 ± 1	346.9 ± 35.5
WWB	0	2	427 ± 347	-1 ± 1	405.5 ± 20.5
WWB	10	2	256 ± 74	-3 ± 3	368.8 ± 26.0
WWB	20	2	135 ± 60	-66 ± 60	446.1 ± 33.1
WWO	C	2	21 ± 72	-53 ± 17	359.9 ± 27.5
WWO	0	2	40 ± 39	-64 ± 22	434.0 ± 36.5
WWO	10	2	267 ± 178	-71 ± 23	412.6 ± 43.8

WWO	20	2	$167 \pm 117$	$-60 \pm 22$	$373.9 \pm 13.6$
UT	C	3	$52 \pm 22$	$6 \pm 7$	$338.7 \pm 26.9$
UT	0	3	$70 \pm 68$	$23 \pm 8$	$261.9 \pm 16.9$
UT	10	3	$106 \pm 81$	$8 \pm 6$	$398.4 \pm 47.0$
UT	20	3	$119 \pm 85$	$9 \pm 2$	$380.5 \pm 21.8$
CL	C	3	$-37 \pm 12$	$8 \pm 5$	$355.5 \pm 25.0$
CL	0	3	$153 \pm 156$	$11 \pm 6$	$388.0 \pm 26.3$
CL	10	3	$228 \pm 196$	$10 \pm 3$	$330.6 \pm 31.0$
CL	20	3	$164 \pm 131$	$14 \pm 5$	$356.2 \pm 24.1$
REC	C	3	$52 \pm 19$	$15 \pm 9$	$417.9 \pm 54.5$
REC	0	3	$9 \pm 9$	$3 \pm 3$	$394.5 \pm 66.7$
REC	10	3	$179 \pm 195$	$4 \pm 4$	$419.5 \pm 19.8$
REC	20	3	$410 \pm 363$	$4 \pm 9$	$531.9 \pm 141.7$
WWB	C	3	$50 \pm 28$	$19 \pm 8$	$379.1 \pm 23.4$
WWB	0	3	$60 \pm 28$	$32 \pm 20$	$392.0 \pm 18.6$
WWB	10	3	$445 \pm 449$	$21 \pm 5$	$484.8 \pm 114.4$
WWB	20	3	$149 \pm 69$	$-8 \pm 20$	$423.1 \pm 64.9$
WWO	C	3	$-2 \pm 10$	$10 \pm 5$	$323.4 \pm 42.3$
WWO	0	3	$1 \pm 13$	$10 \pm 4$	$329.0 \pm 18.4$
WWO	10	3	$133 \pm 70$	$17 \pm 2$	$314.6 \pm 11.4$
WWO	20	3	$443 \pm 278$	$7 \pm 4$	$347.2 \pm 20.8$

**Table 2.8** Table of average ammonification, nitrification, and initial DON by site, treatment, and round for 2015.

### *Discussion*

Most net N mineralization measurements associated with bogs have been made either in Europe or eastern North America (Aerts 1999). Western boreal Canadian bogs, however, are both drier and have lower rates of N deposition (Bayley and Thormann 2005; Fellman and D'Amore 2007). Bayley and Thormann (2005) measured net N mineralization in an Alberta bog at a depth of 0-5 cm and found rates of  $2.4 \text{ mg N kg dry soil}^{-1} \text{ d}^{-1}$ . Fellman and D'Amore (2007), working in a bog in southeastern Alaska, measured rates of  $0.41 \text{ mg N kg dry soil}^{-1} \text{ d}^{-1}$  for the same process with depth to 10 cm. Grigal and Homann (1994), working in a shallow peatland in Minnesota, recorded net N

mineralization rates of 0.80-7.70 kg ha<sup>-1</sup> yr<sup>-1</sup>. However, yearly rates may not be dependable, as they are extrapolated based on rates per day and not accounting for seasonal differences, and the 10 cm cores used in the study contained both peat and mineral soil (Grigal and Homann 1994). Chapin (2003) made net N mineralization measurements using the same methods in this thesis. NO<sub>3</sub><sup>-</sup>-N was undetectable in most samples, and ammonification was 0.5-1.0 g NH<sub>4</sub><sup>+</sup>-N m<sup>-3</sup> d<sup>-1</sup> (Chapin *et al.* 2003). Past measurements made in the Wieder lab by Hartsock (2013) aligned more closely with the rates observed in Alaska, averaging 0.45 mg N kg dry soil<sup>-1</sup> d<sup>-1</sup>. I predicted that net ammonification rates would be generally higher than net nitrification rates.

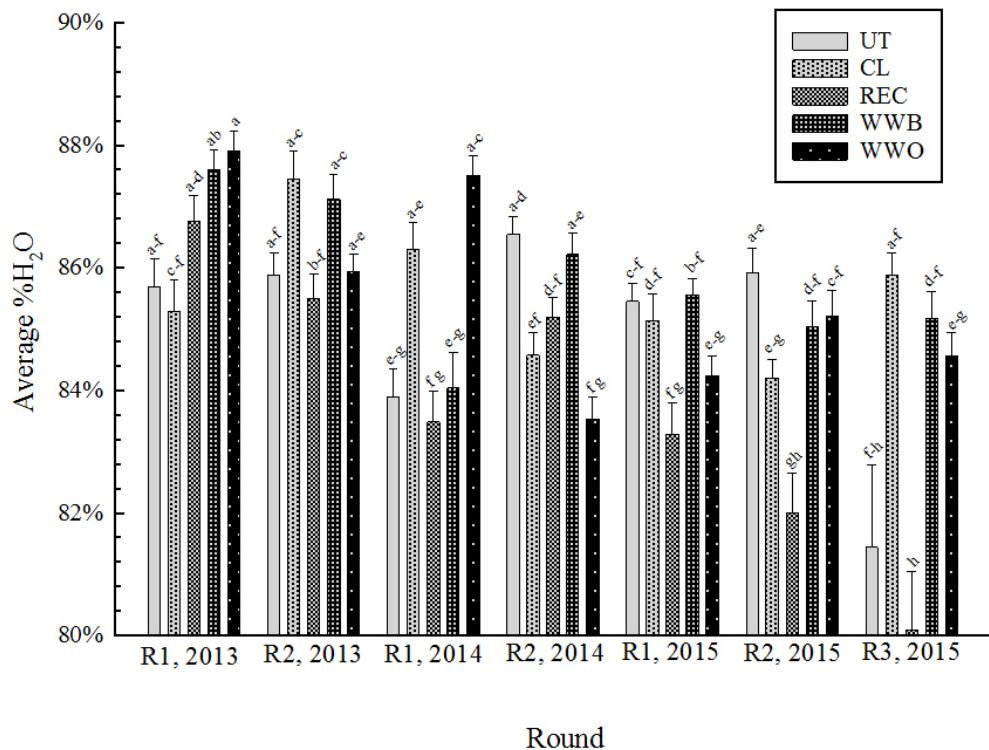
The net nitrification and ammonification rates contained in this thesis were determined using bulk density and reported on a m<sup>2</sup> basis as opposed to dry peat weight basis. This allows rates to be determined by m<sup>-2</sup> d<sup>-1</sup> basis instead of per kg of dry soil, a metric that can be more clearly understood when applied to peat *in situ*. When converted to mg N kg<sup>-1</sup> d<sup>-1</sup>, the rates recorded in this thesis appear to be slightly lower, but close to, measurements made by Hartsock (2013), and the bog study in southeastern Alaska bogs (Fellman and D'Amore 2007). After conversion, average net ammonification and nitrification across all sites, treatments, and rounds added together was 0.269 mg N kg<sup>-1</sup> d<sup>-1</sup>. Additionally, as expected, ammonification was generally higher than nitrification. Nitrification measurements were more likely to yield net immobilization rates, though net immobilization was observed in NH<sub>4</sub><sup>+</sup>-N transformations as well.

To account for as many possible sources of variation as possible, average percent H<sub>2</sub>O was determined for all samples. Percent H<sub>2</sub>O was calculated by subtracting the dry core weight from the initial moist weight over the initial moist weight. There was a

significant round  $\times$  site effect ( $p < 0.0001$ ) that showed a pattern of inter-annual variation (Fig. 2.13). The general trend appears to be consistently higher percent H<sub>2</sub>O measurements from 2013 that get progressively lower in later years. REC is significantly lower than other sites in 2015, but despite the round  $\times$  site interaction, no clear YSF effect emerges (Fig. 2.13). The reasons for these variations may lay partially in the weather of these separate years. Through both the rainfall records (<http://fortmcmurray.weatherstats.ca/charts/precipitation-5years.html>) and the percent H<sub>2</sub>O percentages (determined by wet sample weight-dry sample weight/wet sample weight  $\times$  100, with initial and final % averaged together per round), it is clear that 2013 was the wettest year and 2015 was the driest. Average percent H<sub>2</sub>O was negatively correlated with net ammonification, nitrification, and DON production.

	Net Ammonification	Net Nitrification	Net DON Production
<b>Pearson Correlation Coefficient</b>	-0.18364	-0.37807	-0.26158
<b><i>p</i> value</b>	< 0.0001	< 0.0001	< 0.0001

**Table 2.9** Pearson correlation coefficient and *p* values for average percent H<sub>2</sub>O. Row one indicates the comparison variable.



**Figure 2.14** Percent H<sub>2</sub>O (wet-dry sample weight/wet sample  $\times 100$ ) weight by site and round. Significant differences represented by letters above individual columns were determined using Tukey's HSD *a posteriori* comparisons.

Few definitive site patterns were observed in the data. When a site effect was observed, most notably in net ammonification in 2014, the effect was the opposite of what was hypothesized, that net ammonification would decrease along a chronosequence of time since fire. The oldest sites, WWB and WWO, had the highest recorded rates of net ammonification. While not statistically significant, the visual trend of net ammonification in 2013 is more consistent with the original hypothesis.

One of the rationales for my original hypothesis on the effect of time since wildfire was possible root nutrient exudation resulting from wildfire. However, those nutrient resources may not last for the entire duration of Stage I. Once newly available post-fire resources were exhausted, net N mineralization rates may have slowed due to a lack of bioavailable DON. Alternatively, C resources made available by fire may have been exhausted, shifting the C:N ratio and resulting in an initial, but brief, window of conditions conducive to net ammonification. The differences in net N mineralization may also be related to post-fire moss species dominance. *Sphagnum* moss is considered a gatekeeper species of nutrients entering the rhizosphere; in the absence of *Sphagnum* dominance, inorganic N inputs to the bog may increase microbial immobilization. Complete *Sphagnum* recovery is preceded by the dominance of true mosses, such as *Polytrichum strictum*, and is not dominant until 20 YSF, while older successional bogs (YSF>80) are associated with feather mosses (Benscoter and Vitt 2008). Additionally, post-fire microbial community recovery in upland boreal forests, in terms of composition and diversity, is predicted to take 11 years to return to a pre-fire state (Xiang *et al.* 2014). Net ammonification was positively correlated with net nitrification, which supports the hypothesis that increased or decreased ammonification would be reflected in nitrification rates.

Counter to my initial hypothesis, very few N treatment effects, or YSF × N treatment interactions, were observed across three summers of data. Generally, experimental N addition did not affect net N mineralization rates and did not have a significant interaction with YSF. This may be attributable to the N treatments not significantly altering the quantity or quality of DON consistently, and the absence of a

change in pore water N concentrations despite treatments. The fate of experimentally applied NH<sub>4</sub>NO<sub>3</sub> in the plots is not yet known; however, if additions are not affecting any of the above mentioned environmental conditions, there may not be a consistent effect of N treatment yet observed. Additionally, NH<sub>4</sub>NO<sub>3</sub> additions may down regulate other steps of the N cycle, such as N<sub>2</sub> fixation. If, as predicted in triphasic models (Lamers 2000; Berendse *et al.* 2001; Heijmans *et al.* 2002), N deposition can result in species shifts, that may affect ammonification rates through the reduced cover of the nutrient-controlling *Sphagnum* and make DON resources more accessible. The significant negative correlation observed between net ammonification and initial extractable NH<sub>4</sub><sup>+</sup>-N (McGill and Cole 1981) seems to suggest that the principle behind the N treatment hypothesis is correct. However, the significant negative correlation between net nitrification and initial extractable NO<sub>3</sub><sup>-</sup>-N is unexpected, as NO<sub>3</sub><sup>-</sup>-N concentration would not be expected to have any connection to nitrification rates. It is possible that initial concentration is a reflection of, instead of a precondition to, nitrification rates. As would be expected, ammonification is positively correlated with net DON production.

The total net inorganic N input to the rhizosphere through mineralization was 536 ± 60 µg N m<sup>-2</sup> d<sup>-1</sup>, and the total net N input was 1949 ± 260 µg N m<sup>-2</sup> d<sup>-1</sup>. To adequately account for observed rates of NPP, 40-50 kg N ha<sup>-1</sup> yr<sup>-1</sup> is needed, but deposition rates alone, long considered the major N source of ombrotrophic bogs, cannot account for this budget (Vile *et al.* 2014). Total N inputs through ammonification, mineralization, and DON, as measured by this thesis, are a scant 0.019 kg N ha<sup>-1</sup> d<sup>-1</sup> on average, and 3.5 kg N ha<sup>-1</sup> yr<sup>-1</sup> based on a 180 day growth season. Vile *et al.* (2014) showed that N<sub>2</sub>-fixation was a previously underestimated N source to ombrotrophic bogs, and could fully account

for the N inputs to *Sphagnum*. Changing rates of N mineralization, therefore, may have greater effects on vascular plants competing in bogs.

### *Conclusions*

The unexpected patterns (or lack thereof) observed in net N mineralization and net nitrification rates in relation to YSF and N treatment raises new questions of interest for the fate of nutrients in the rhizosphere. Looking at gross N fluxes, as well as the quality of nutrient resources available to plants and microbes would further illuminate essential unknowns about N cycling in bogs. Given the global importance of peatland ecosystems to the terrestrial C budget and their fragile position amid mounting anthropogenic disturbances, it is important to be able to predict changes in nutrient cycling regimes and the potential species shifts. The potential for positive feedback cycles between those two factors, nutrient cycling and altering plant communities, has the ability to impact C storage abilities across boreal latitudes or to increase the effect of current anthropogenic disruptions.

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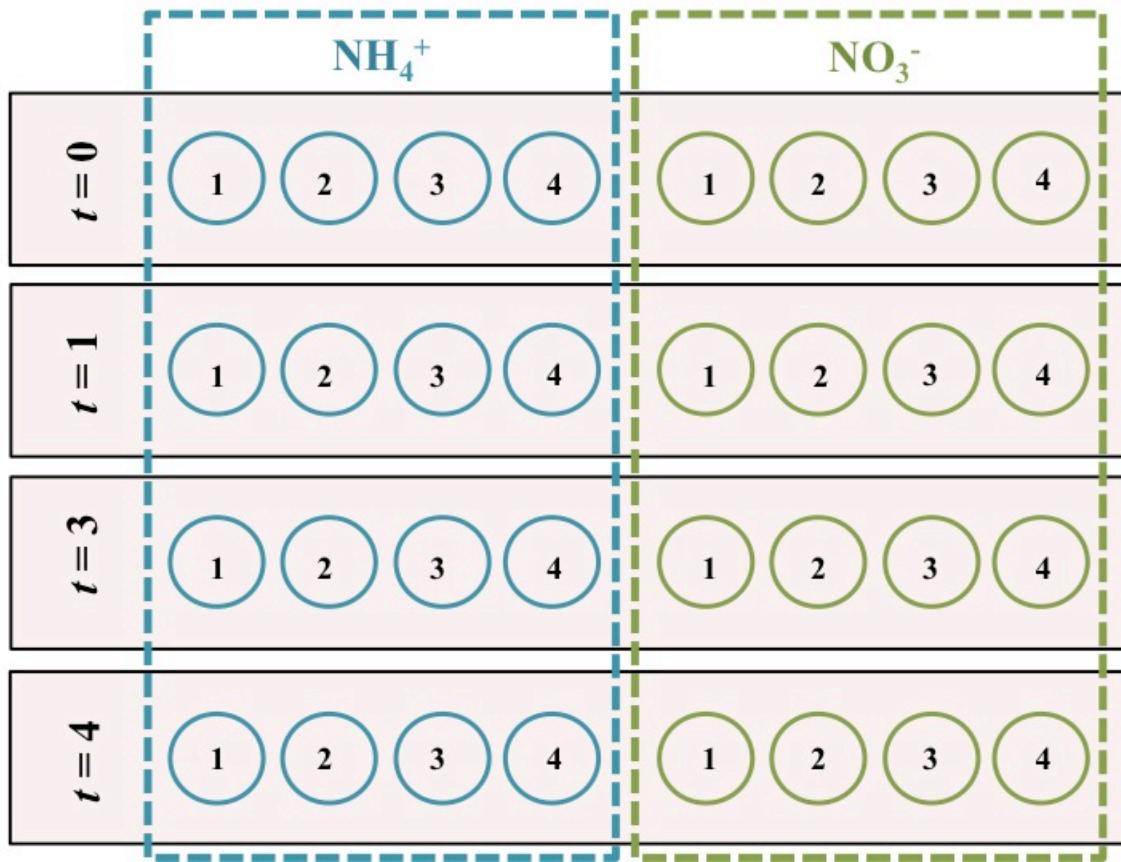
## **Part III: Gross N mineralization and gross nitrification experiments**

### *Introduction*

Net N mineralization measurements have long been assumed to be a good proxy for plant-available N with two assumptions: one, that microbes outcompete plants for inorganic N resources, and two, that plants do not use DON (Jones and Kielland 2002; Schimel and Bennett 2004). While net N mineralization rates are still useful indices of N availability, both assumptions have been called into question in recent research. While it is still assumed that microbes will initially outcompete plants, this does not account for microbial die-off events, giving the longer-lived plants a lifespan advantage (Kaye and Hart 1997). DON uptake by plants in nutrient poor soils or through mycorrhizal associations has been observed in northern latitude  $^{15}\text{N}$  tracer studies (Näsholm *et al.* 2009). Obtaining gross mineralization and nitrification rates, especially in places where rates of N turnover may be underestimated using net measurement, would give a more accurate picture of available N. In Part II, I reported that net ammonification, nitrification, and DON production, across all sites and rounds, is about  $3.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  for a 180 day growing season. While *Sphagnum* N needs can be accounted for through N<sub>2</sub>-fixation, vascular plant uptake indicates that annual N demand of growth is much greater than net mineralization rates. I performed a pilot study on peat collected from a middle-aged bog (WWB, 73 YSF) to examine whether the  $^{15}\text{N}$  pool dilution technique could be used to quantify gross N mineralization rates in peat bogs.

## Methods

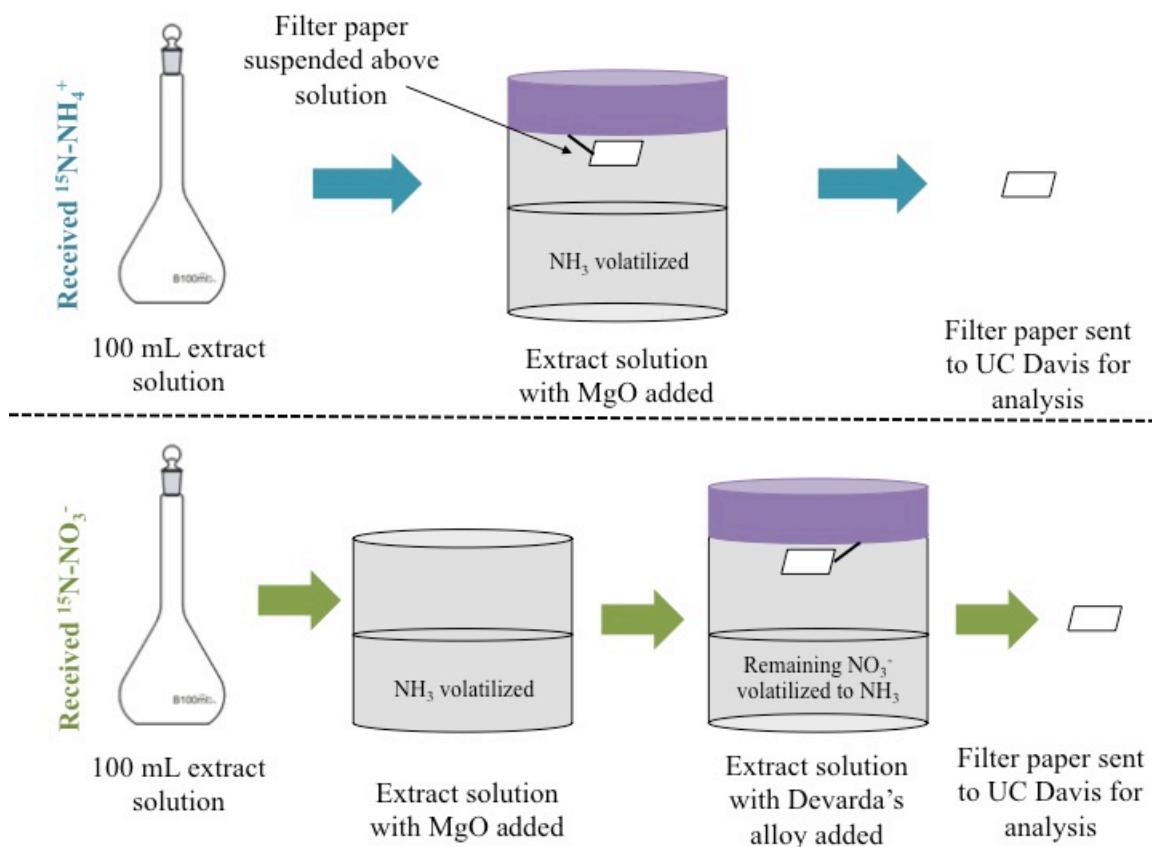
Peat to a depth of roughly 15 cm was collected from the field site and then transferred back to the laboratory at Athabasca University. Several time points were chosen as incubation periods, according to suggestions from Davidson (1991) and Hart (1994). While a 24-hour incubation was used by the pioneers of the isotope-dilution gross N mineralization method (Davidson 1991; Hart *et al.* 1994), a 48-hour incubation has been used in boreal studies containing peat (Westbrook and Devito 2004; McMillan 2007). At  $t=0$ , 16 cores (with the top green layer 2-4 cm removed, as described in net N mineralization methods) received 3 mL of solution containing 40 µg of 98atom%  $(^{15}\text{NH}_4)_2\text{SO}_4$  and 16 other cores received 3 ml of solution containing 40 µg 98atom%  $\text{K}^{15}\text{NO}_3$ . Solution was injected evenly through core using a syringe and a spinal needle. Four cores of each N treatment ( $^{15}\text{NH}_4^+$  or  $^{15}\text{NO}_3^-$ ) were immediately removed from the peat, given a scoop of KCl, and placed on ice before being transported to the laboratory for extraction. This process was repeated four times for each time point ( $t_1=24$  hours,  $t_3=72$  hours,  $t_4=96$  hours). All samples  $t > 0$  were compared to the corresponding  $t_0$  as the initial sample (**Fig. 3.1**).



**Figure 3.1** Experimental design of gross N mineralization pilot study. Each circle represents one peat core. Four replicates were taken for both treatments at each time point, and each sample after  $t=0$  was compared to the corresponding numbered core as the initial sample. The blue dashed box indicates samples that received  $^{15}\text{N}-\text{NH}_4^+$  and the green dashed box indicates samples that received  $^{15}\text{N}-\text{NO}_3^-$ . Black boxes indicate the time point at which samples were removed from incubation and extracted with KCl.

Extractions were performed as described above for net N mineralization technique, and 100 mL of each sample was used for the following steps. For samples to which  $^{15}\text{N}-\text{NH}_4^+$  was added,  $\text{NH}_4^+$  in the extract solution was volatilized to  $\text{NH}_3$  by adding 0.2 g MgO to the extract solution and collecting volatilized  $\text{NH}_3$  on a  $\text{H}_2\text{SO}_4$ -acidified filter paper within a closed container (Fig. 3.2). For samples to which  $^{15}\text{N}-\text{NO}_3^-$  was added,  $\text{NH}_4^+$  was volatilized from the extract solution using 0.2 g MgO and allowed to

escape from an uncapped container. After 6 days, 0.4 g of Devarda's alloy was added and the container capped to induce volatilization of remaining  $\text{NO}_3^-$  to  $\text{NH}_3$  gas, which was captured on a small piece of  $\text{H}_2\text{SO}_4$ -acidified filter paper, as above (Hart *et al.* 1994, Fig. 3.2). Total N and atom%  $^{15}\text{N}$  of the N recovered on the filter papers was determined at the UC Davis Stable Isotope Facility.



**Figure 3.2** Method for collecting  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N from extract solution. The top half indicates the procedure for samples that received  $^{15}\text{N}-\text{NH}_4^+$  and the bottom half those that received  $^{15}\text{N}-\text{NO}_3^-$ . Filter papers represented above are acidified with  $\text{H}_2\text{SO}_4$ .

To calculate gross N mineralization and consumption, I used the following equations, developed by Kirkham and Bartholomew (1954) and subsequently used and reported by Davidson (1991):

$$m = \frac{M_0 - M_1}{t} \times \frac{\log (H_0 M_1 / H_1 M_0)}{\log (M_0 / M_1)}$$

(3.1)

where

$m$ =gross mineralization rate,  $\mu\text{g N g}^{-1} \text{ d}^{-1}$

$t$ =time, days

$M_0$ = total  $^{14+15}\text{NH}_4^+$  initial concentration,  $\mu\text{g N g}^{-1}$

$M_1$ =total  $^{14+15}\text{NH}_4^+$  concentration at time  $t$ ,  $\mu\text{g N g}^{-1}$

$H_0$ =initial  $^{15}\text{NH}_4^+$  pool,  $\mu\text{g N g}^{-1}$

$H_1$ =  $^{15}\text{NH}_4^+$  pool at time  $t$ ,  $\mu\text{g N g}^{-1}$

After the mineralization rate was calculated, gross consumption was quantified as:

$$c = m - \frac{M_1 - M_0}{t} \times \frac{\log (H_0 / H_1)}{\log (M_0 / M_1)}$$

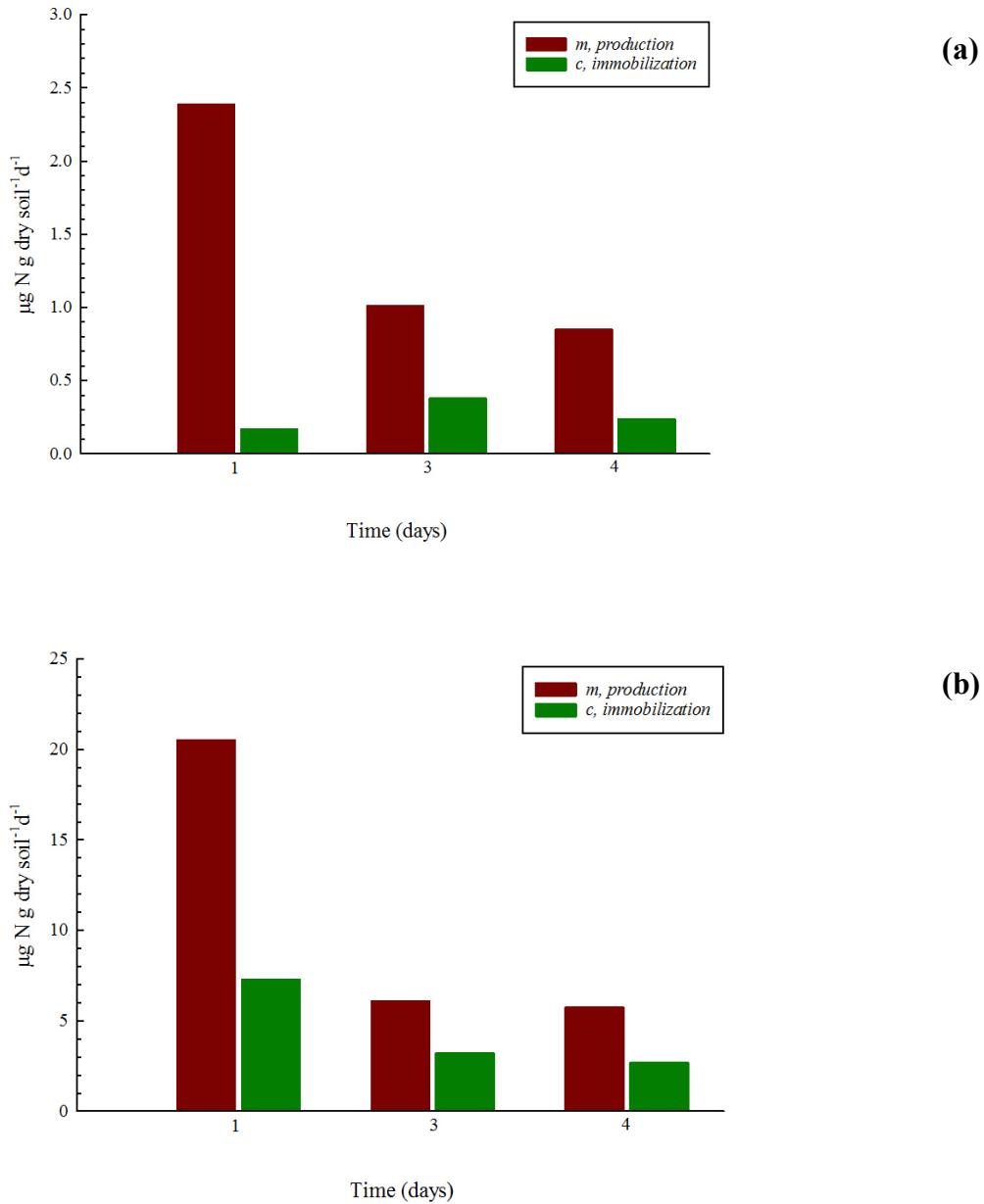
(3.2)

To calculate rates of nitrification and nitrate consumption, I replaced all instances of  $\text{NH}_4^+$  with  $\text{NO}_3^-$  in the above formulas. These equations are not always perfect, and it is possible to calculate a negative gross rate, even though a negative gross rate is impossible. Calculating a negative rate indicates that an error occurred in the experiment.

Generation of negative numbers is possible due to the equation relying upon the ratio of  $^{14}\text{N} : ^{15}\text{N}$ ; for example, if enough isotopic tracer is not measured, either through natural loss or human error, the equation will not be accurate. Some experiments have used Bayesian analysis to avoid this error, as this allows them to estimate underlying distribution based on observed distribution (Mary *et al.* 1998; Zhang *et al.* 2012).

## Results

For each time point, the four replicate samples that received the same treatment were averaged together to calculate gross mineralization and consumption (**Fig. 3.3**). For both ammonification and nitrification, the highest rates were observed at t=1, and subsequently decreased at t=3 and t=4.  $\text{NO}_3^-$  consumption followed a similar pattern, but  $\text{NH}_4^+$  consumption rates were similar for all time points. For each time point and treatment, gross consumption was less than gross mineralization. The obtained gross mineralization rates are generally small, though gross nitrification was higher than ammonification at all times.



**Figure 3.3** Gross (a) ammonification and (b) nitrification rates obtained in pilot study. Time  $t$  indicates days of incubation, with each day being precisely 24 hours.

## *Discussion*

The objective of this project was to explore the possibility of using isotopic pool dilution to measure rates of gross N mineralization and nitrification in peat from boreal bogs. Unlike most prior gross N mineralization experiments, the samples are comprised only of peat, and not mixed with any mineral soil (Davidson 1991; Westbrook and Devito 2004; Kwak 2015). Adjustments, such as the amount and type of  $^{15}\text{N}$  and incubation periods, have been implemented to attempt to compensate for potential experimental error.

One of the challenges associated with isotope pool dilution is adding enough  $^{15}\text{N}$ -labeled substrate to recover and measure differences between initial and final time points without artificially augmenting the substrate pool. In order for this technique to be effective and reliable, experiments will be performed to test the viability of different label amounts. The equations developed by Kirkham and Bartholomew (1954) allow both production and consumption to be estimated; the difference between measured gross N mineralization and N consumption rates should be comparable to net N mineralization rates, although may not be exact due to consumption possibly being attributable to several different pathways. The calculated gross rates in this experiment were low compared to average net daily mineralization rates, but were within the range of data presented in Part II.

Very few gross N mineralization measurements have been made in peat; the results of those that have been done are summarized in Table 1.1 in Chapter 1. Wray and Bayley (2008) recorded gross ammonification rates 4 to 10 times greater than net

ammonification, and gross nitrification rates 500 to 800 times greater than net rates. In that study, stimulation of nitrification may have been induced due to the choice of isotopically labeled ammonium nitrate. Kwak (2015) reported higher rates of gross ammonification than net ammonification and higher rates of gross nitrification than net nitrification, though not to the scale indicated by Wray and Bayley.

I predicted that isotope pool dilution would reveal high inorganic N production rates and high inorganic N consumption rates, which may help explain why net rates have traditionally been low even as it is hypothesized that N cycling in ombrotrophic bogs is more rapid than previously predicted. Additionally, given the generally positive rates of net ammonification, I predicted that gross nitrification rates would be higher than net nitrification rates have suggested.

The rates obtained, albeit from a small pilot study, do seem to show higher gross nitrification rates, indicating that net nitrification studies may be underestimating  $\text{NO}_3^-$  inputs. For future studies, 24 hours does seem to be an appropriate time window for the measurement of ammonification and nitrification. The decline in the rate of N ammonification and mineralization after the first time point may indicate turnover of N resources. By comparing the initial  $\text{NH}_4^+$ -N or  $\text{NO}_3^-$ -N, the relative speed of the turnover rate can be assessed. In this study, the initial concentrations of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N were  $1.5 \mu\text{g g peat}^{-1}$  and  $4.7 \mu\text{g g peat}^{-1}$ , respectively. The turnover rate for ammonification was  $0.6$  to  $1.6 \text{ d}^{-1}$ , which means that, for  $t_1$ , the entire  $\text{NH}_4^+$ -N pool was replaced  $1.6$  times per day. The turnover time range for nitrification was even higher, ranging from  $1.2$  to  $4.4 \text{ d}^{-1}$ . Turnover rates calculated from the data presented in Part II indicate a slower turnover rate for all measured variables. Average  $\text{NH}_4^+$ -N pool turnover was  $0.07 \text{ d}^{-1}$ ,

$\text{NO}_3^-$  pool turnover was  $0.24 \text{ d}^{-1}$ , and DON turnover was  $0.01 \text{ d}^{-1}$ . As in the gross mineralization data presented,  $\text{NO}_3^-$  turnover was fastest, but still much slower when utilizing the buried bag method. The fast N turnover rates suggest that net ammonification and nitrification measurements may not be appropriate in peat.

The exploration of gross N mineralization rates in this ecosystem may be able to address lingering questions about the nature and rapidity of N cycling in ombrotrophic bogs. The isotopically labeled signatures were measurable in most gross N mineralization samples, while still abiding by the recommendation set forth by Hart (1994) of not augmenting the available N pool by more than  $2 \text{ mg N kg dry soil}^{-1}$ . The average dry weight of samples collected for net N mineralization and nitrification measurements in 2014 was used as the metric to evaluate appropriate N amendments. While quite preliminary, the results of this pilot study indicate that net nitrification rates in particular may be underestimated. Additionally, being able to assign gross rates of inorganic N production to a chronosequence of time since fire or N treatment may illuminate processes obscured by net measures of the same processes. As the biological paradigm of plant DON uptake gains favor, particularly for plants found in bogs with mycorrhizal associations, decreasing gross N mineralization may provide a mechanism for vascular plant competition.

### *Conclusion*

The recoverability of the isotopic tracer in peat samples indicates that it may be possible to perform accurate gross N mineralization experiments in the field and within experimental sites. Doing so may be able to shed further light on the somewhat

counterintuitive age effects observed in the net N ammonification and nitrification rates presented in this thesis, or to explain the absence of the predicted N treatment effect. The amount, form, and turnover rate of organic and inorganic rhizosphere N in bogs may help predict future species shifts and C storage capabilities of this critical ecosystem.

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