Effects of long-term nitrogen and sulfur deposition on soil microbial communities and soil properties in the Athabasca oil sands region in northern Alberta, Canada

by

Stephanie Michelle Chute Ibsen

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

Master of Science

in

Land Reclamation and Remediation

Department of Renewable Resources
University of Alberta

Abstract

Intensified oil sands activities have resulted in elevated levels of nitrogen (N) and sulfur (S) deposition in the mixedwood boreal forest in the Athabasca oil sands region (AOSR) of northern Alberta, Canada. The deposition of N and S can affect the surrounding ecosystem and it is important to monitor possible effects. To improve the understanding of the response of boreal forest ecosystems to increased N and S deposition in the AOSR, an experiment was established in 2006 with the following treatments: control, +N addition (30 kg N ha^{-1} yr^{-1} as NH_4NO_3), +S addition (30 kg S ha^{-1} yr^{-1} as Na_2SO_4), and +NS addition (30 kg N plus 30 kg S ha^{-1} yr^{-1}). Nine years of simulated N and S deposition did not affect soil bacterial diversity, community composition, abundance, functional profiles, and co-occurrence patterns, suggesting that soil bacteria were resistant or resilient to increases in N and S addition in the studied boreal forest. In contrast, increased N and S addition increased the abundance of soil fungal communities, suggesting fungi were more sensitive than soil bacteria. Additionally, ten years of simulated N and S deposition did not change soil chemical properties including soil pH, cation and N concentrations and leaching of N below the main rooting zone. Therefore, there was no evidence of N saturation or soil acidification in the experimental forest ecosystem in the AOSR after ten years of N and S addition. Continued long term research of N and S deposition in the AOSR is needed to enhance the current level of understanding and to quantify the collective ecosystem impacts.

Acknowledgements

I am incredibly grateful to my supervisor, Dr. Scott X. Chang. The knowledge, skills, and experience I have gained during my time with Scott will assuredly benefit me for the rest of my career. Scott has molded me into a better scientist and I am very thankful that he decided to take a chance on me. I would also like to express my sincere appreciation to my committee, Dr. Charles Driscoll, Dr. Ivan Fernandez, and Dr. Tariq Siddique whose constructive criticism, advice, and guidance helped considerably during this process.

Additionally I would like to thank Dr. Bin Ma and Dr. Allen Jobson for all of their support, assistance, and patience during my microbial research. I want to acknowledge and thank my parents, Dwayne Ibsen and Arliss Chute-Ibsen for all of their support, love, and encouragement. Thank you for always nurturing my creativity and love of science and for buying me my first microscope. I could not ask for better parents and the woman and scientist I am today is because of your love and guidance. I would also like to thank my three siblings, Kirsten Ibsen, Venessa Ibsen, and Justin Ibsen, for being my best friends, my support system, and for always making me laugh. The three of you are my world and I would not be where I am today without you.

I would also like to express my appreciation for my research colleagues, Prem Pokharel, Jin-hyeob Kwak, Kangyi Lou, Murtaza Jamro, Lijing Wang, Sophie Dang, Alison Wilson, Elizabeth Domreis, Gillian Mullan-Boudreau, and Sarah Ficko. My thesis research was supported by the Land Reclamation International Graduate School (LRIGS) and the NSERC Create program. The NSERC Discovery Grant also supported part of the research.

Dedication

This thesis is dedicated in loving memory of my cousin, Mackenzie Joshua May. And while your passion was music and not science, this is my way of making sure you will always be remembered. I will keep holding on. I love you. I miss you.

"Your life was a blessing,

Your memory a treasure.

You are loved beyond words

And missed beyond measure."

-Anonymous

Table of Contents

List of Tables

List of Figures

List of Symbols and Abbreviations

+N: nitrogen addition treatment

+S: sulfur addition treatment

+NS: nitrogen and sulfur addition treatment

AOSR: Athabasca oil sands region

Ca:Al: a molar ratio of calcium to aluminum

CEC: cation exchange capacity

CK: control

C:N: soil carbon to nitrogen ratio

GHG: greenhouse gas

MBC: microbial biomass carbon

MBN: microbial biomass nitrogen

TC: total carbon

TN: total nitrogen

Chapter 1: Introduction

1.1 Background

There has been a threefold increase in the amount of nitrogen (N) and sulfur (S) being emitted into the atmosphere globally over the last one hundred years (Denman et al. 2007, Detener et al. 2006). These increases are attributed to anthropogenic activities including the use of fertilizers in agricultural practices, fossil fuel combustion, and cultivation of N fixing plants (Galloway et al. 2004, Vitousek et al. 1997). Nitrogen and S are commonly emitted into the atmosphere in the form of nitrous oxide (NO_x) and sulfur dioxide (SO_2). NO_x emissions have increased by 29 percent in western Canada between 1985 and 2000 and are projected to increase by another 5 percent between 2000 and 2020 (Schindler et al. 2006). The Athabasca Oil Sands Region (AOSR) has the third largest oil reserves in the world and as of 2014 had daily production of 2.3 million barrels of oil (Alberta Energy 2014). The activities involved in oil sands mining and upgrading release large amounts of NO_x and SO_2 into the atmosphere and the surrounding area.

The S emissions in the AOSR peaked at approximately 400 Mg day^{-1} in the 1980s followed by a substantial reduction to between 250 and 300 Mg day^{-1} in the early 2000s (Hazewinkel et al. 2008). Sulfur emissions have stabilized due to the introduction of legislation to combat the effects of acid rain which is caused by the combination of SO_2 and water in the atmosphere. In the meantime, N emissions in the AOSR have increased steadily from 20 Mg day^{-1} in 1970 to approximately 300 Mg day^{-1} in the mid-2000s, likely due to increased fossil fuel combustion associated with the oil and gas activities and transportation, and the lack of legislation to cap the amount of emissions (Hazewinkel et al. 2008). There have been two major

oil sands plants that have been in operation for almost 30 years (Schindler et al. 2006). In the Oilsands Project Report released by the Alberta Government in the Fall of 2014, there are five major companies with six active mines operating in the AOSR. In addition, there are four projects under active construction and nine proposed projects that have either been approved and have yet to release a start date, or are within the application process. With the increased and projected increases in activities in the AOSR, the rate of N emissions are predicted to increase while S emissions are expected to remain stable (Aherne and Shaw 2010).

The effects of elevated N and S deposition are multifaceted and will depend on the deposition rates and the ecosystem type. The addition of S to the surrounding area in the AOSR can cause soil acidification, nutrient imbalances, and ultimately loss of biological diversity (Laxton et al. 2010, Ok et al. 2007). In the boreal forest ecosystem, high rates of N deposition can increase plant growth over the short term, but can eventually lead to changes in plant community composition and decreases in forest productivity (Aber et al. 1989). Chronic N enrichment can saturate forest soils after which excess N can leach into the groundwater or be emitted into the atmosphere as a gaseous form of N. The loss of N in the soil due to leaching will cause soil base cations including calcium and magnesium to be simultaneously leached. This loss of base cations can cause soil acidification, aluminum (Al) toxicity, plant nutrient imbalances, and eventually a decline in forest productivity (Aber et al. 1989, Laxton et al. 2010, Watmough et al. 2014). The progress of N saturation of forest ecosystems can be divided into four proposed stages based on the responses of ecosystems to chronic N deposition: N limitation, alleviation of N limitation, N saturation, and forest decline (Aber et al. 1989). In the first stage, N input will be taken up by plants therefore providing a beneficial effect on vegetation growth (Frey et al. 2004). However, N leaching and N emissions from the soil will increase as the ecosystem starts to shift

towards N saturation (Aber et al. 1998, Matson et al. 2002). Once the ecosystem has become N saturated and base cations have been lost from the soil the system responds by increasing aluminum solubility in the soil which causes a rapid decline in pH through aluminum hydrolization, and the culmination of all these effects will lead to forest decline.

The majority of the soils in the AOSR are considered to be acid sensitive due to their coarse soil texture, low exchangeable base cation content, and low buffering capacity (Holowaychuk and Fessenden 1987, Watmough et al. 2014, Whitfield et al. 2009). These soil characteristics in combination with increases in atmospheric deposition associated with the oil sands production are a threat to the structure and function of the N-limited boreal forest ecosystem (Galloway et al. 2004). Therefore, long-term impacts of N and S deposition on ecosystems are a major concern in the AOSR and should be a priority area for the government, industry and public.

In order to assess the impact of increases in N and S deposition by mining activities on forest ecosystems in the AOSR, a number of research projects have been conducted. There have been numerous studies examining the effects of N and S deposition on bogs, wetlands, surface and ground waters, and terrestrial ecosystems (Hazewinkel et al. 2008, Jung and Chang 2012, Laxton et al. 2010, Schindler et al. 2006, Whitfield et al. 2010, Wieder et al. 2010). However, there are currently no studies examining the effects of N and S deposition on soil microbial diversity and community composition in the AOSR. Additionally, while there has been long-term monitoring on N and S emissions there is a lack of long-term research being conducted on deposition and its effects on the terrestrial ecosystems in the AOSR. Given the sensitivity of microbes to elevated atmospheric N and S deposition, studying bacteria and fungi in the AOSR provides an opportunity for monitoring and detecting ecosystem changes. Here I report on nine years (2006-

2014) of simulated N and S deposition data for a site in the AOSR along with soil microbial response to assess potential ecosystems impacts of N and S deposition.

1.2 Site Description

Research plots were established in a boreal mixedwood forest stand (56.1° N 110.9° W) located about 100 km southeast of Fort McMurray in the AOSR in northern Alberta, Canada (Jung & Chang 2012). The majority of mining activities and upgrading facilities in the AOSR are located north of Fort McMurray; therefore, this site is minimally affected by deposition originated from oil sands activities. Nitrogen and S deposition rates, at sites 94 and 113 km away from the center of oil sands mining and upgrading activities, were 1.5 kg N ha^{-1} yr^{-1} and 1.2 kg S ha^{-1} yr^{-1}, respectively (Proemse et al. 2012, 2013). The values recorded by Proemse et al. (2012, 2013) are representative of the background deposition rates for my research site.

At the research site, the mean annual temperature and total precipitation measured from 1981 to 2010 were 0.96 °C, and 418.8 mm, respectively (Government of Canada 2010).The dominant tree species were *Populus tremuloides* (trembling aspen) and *Picea glauca* (white spruce) which constitute 71 and 22 % of the stem count, respectively (Jung and Chang 2012). Other tree species found in the plots were *Abies balsamea* (balsam fir), *Populus balsamifera* (balsam poplar), *Picea mariana* (black spruce), and *Betula papyrifera* (paper birch) (Jung and Chang 2012). The dominant shrub species were prickly rose (*Rosa acicularis*), lowbush cranberry (*Vaccinium oxycoccos*), and twinflower (*Linnaea borealis*) (Jung and Chang 2012). Soils were classified as Gray Luvisols based on the Canadian system of soil classification (Soil Classification Working Group 1998).

The experiment was established in 2006, with a 2 x 2 factorial design and in four blocks, for a total of 16 plots. Four treatments were set up: control (CK), N addition (+N, 30 kg N ha^{-1}

yr^{-1} as NH_4NO_3), S addition (+S, 30 kg S ha^{-1} yr^{-1} as Na_2SO_4), and N and S addition (+NS, 30 kg N ha^{-1} yr^{-1} as NH_4NO_3 and 30 kg S ha^{-1} yr^{-1} as Na_2SO_4). Four blocks were established based on topography and uniform soil and site conditions within the blocks; plots of 20 x 20 m were set up (Jung and Chang 2012). Treatments were randomly assigned to plots. The soil texture of the surface mineral soil (0-15 cm) was sandy loam in two blocks and silt loam in the other two blocks (Jung and Chang 2012). Soil that was deeper (15-45 cm) had higher clay content when compared to the surface mineral soil (Jung and Chang 2012).

1.3 Previous Research

The research site was previously established and a variety of studies have already been conducted. The first research objective was to examine changes in the tree and understory growth rates, in N cycling, and in the leaching loss of cationic nutrients from the soils and was conducted by Jung and Chang (2012). It was hypothesized that plots with the +N treatment would experience higher vegetation growth rates. It was also hypothesized that there would be no increase N and cation leaching. After 4 years of experimental +N and +S addition it was shown that tree growth increased in the +N and +NS plots, which confirmed the N limited status of the area. However, the understory vegetation growth and the soil microbial biomass were not affected by the treatments. In the mineral soil there was no effect on N leaching but the +N and +S treatments did significantly decrease the exchangeable calcium (Ca^{2+}) and magnesium (Mg^{2+}) cations. It was concluded that after 4 years the risk of N saturation was minimal, but the decrease in exchangeable cations suggested a nutrient imbalance and was an area of concern.

Following the results obtained by Jung and Chang (2012) the next research objective became to examine the changes in soil microbial biomass after an additional year of +N and +S

treatments and it was conducted by Hu et al. (2013). It was hypothesized that the soil community-level physiological profile would be altered because it is more sensitive to N and S addition when compared to soil microbial biomass. It was thought that soil urease and arylsulfatase enzyme activities would decrease because of the increases in soil availability of N and S. Additionally it was hypothesized that β-glucosidase enzyme activity would increase as soil microbes would no longer be carbon (C) limited. It was found that, after 5 years of N and S addition, the treatments did not significantly affect the soil organic C, total N, dissolved organic C and N, or soil microbial biomass C and N. However, soil microbial physiological profiles were affected by the +N and +S treatments, with the +NS treatment increasing β-glucosidase and the +S treatment decreasing soil arylsulfatase. It was concluded that +N and +S addition strongly affected soil microbial community functions and enzymatic activities without having any effect on soil microbial biomass.

While it had been established that tree growth was increased with +N addition, the response of other vegetation types in the site was lacking (Jung and Chang 2012). In unpublished data by Kangho Jung for thesis research, the effects of 7 years of+ N and +S treatment on understory vegetation was assessed. It was hypothesized that +N and +S addition would decrease the diversity and alter the species composition in the understory plant community by increasing the abundance of nitrophilic species. Additionally it was hypothesized that understory foliar cation concentration would decrease because of chronic N and S addition. It was found that the +N treatment increased dissolved organic carbon and the +S treatment decreased exchangeable cations in the mineral soil. In the shrub layer of the plots species evenness and overall shrub diversity was decreased by +N and +S addition. The total shrub cover was decreased by +S addition, while the herb layer was not affected by either treatment. In addition the foliar

phosphorus (P) and potassium (K) concentrations in some plant species decreased in response to the +N treatment. It was concluded that there was a significant risk to nutrient imbalances in the understory vegetation due to +N and +S addition.

1.4 Thesis Structure

There is currently little research being conducted on how N and S deposition affect the terrestrial ecosystem and soil microbes of the AOSR. Based on current knowledge gaps I conducted this thesis study (1) to assess long-term impacts of N and S addition on soil microbial communities in a boreal forest in the AOSR; (2) to assess long-term impacts of N and S addition on soil chemical properties; and (3) to determine the risk of N saturation in a boreal forest in the AOSR, based on the Aber et al. (1989) ecosystem N status assessment. For these objectives, research was conducted in the simulated N and S deposition experiment. For the first objective, I investigated bacterial diversity, community composition, abundance, and fungal community abundance (Chapter 2 and 3). For the second objective I assessed the N status of the soil through N emissions and leaching. I also measured other soil properties including soil pH, cation concentration, and total carbon and N (TC and TN respectively).

This thesis consists of five chapters. In chapter 1 I describe background information, previous research completed in the experimental N and S addition site, and a thesis overview of N and S deposition (this chapter). In chapter 2 I examine the effects of long-term N and S addition on soil bacterial community diversity, composition, metabolism profiles, and co-occurrence networks using sequencing of 16S rRNA genes. In chapter 3, further microbial analysis is described using plating and most probable number (MPN) methods to confirm bacterial results from chapter 2 and to obtain information on how N and S treatments affected

soil fungal abundance. And lastly, in chapter 4 the N status of the research site is determined by examining the amounts of N being leached and emitted from the soil. Additionally, other soil properties including TC, TN, pH, and exchangeable cation concentrations were measured to determine if N and S treatments had altered these soil properties. Each of the data chapters (2 to 4) constitute a manuscript that will be submitted for publication. In chapter 5 I provide a summary of key findings, general conclusions, and suggested future research in the experimental N and S addition site.

Chapter 2: Soil bacteria are resilient to long-term nitrogen and sulfur addition in a boreal forest in northern Alberta, Canada

2.1 Introduction

It has been estimated that due to human activities, including the use of fertilizers and fossil fuel combustion, the input of nitrogen (N) into the global N cycle has doubled in the last century (Galloway et al. 2008, Vitousek et al. 1997). In the last 150 years the northern hemisphere has shown a ten-fold increase in the rate of N deposition (Freedman and Zak 2014), which has started to plateau in Europe and the United States in the early 1990s because of legislation introduced to combat atmospheric pollution (Emmett 2007, Goulding et al. 1998). Sulfur dioxide ($SO_{2)}$ emissions in Canada peaked in the early 1980s at 400 Mg day^{-1} and have since decreased, while nitrous oxide (NO_x) emissions have been steadily increasing for the last 15 years (Aherne and Shaw 2010, Hazewinkel et al. 2008). Overall, emissions are expected to increase because of continued economic and industrial growth and expansion, which has been concentrated in western Canada due to expansion in transportation, oil sands mining, and urban centers (Aherne and Shaw 2010). Alberta has been reported to be Canada's largest emitter of SO_2 and NO_x largely due to the mining and refining of bitumen that occurs in the northern part of the province, with the most concentrated activities occurring in the Athabasca Oil Sands Region (AOSR) (Whitfield et al. 2009). Between 2003 and 2008 there were 274 -314 and 148-200 tons day^{-1} of SO_2 and NO_x, respectively, emitted in AOSR (Watmough et al. 2014).

Expansion of oil sands mining/extraction and upgrading activities have exposed the surrounding boreal forests to elevated levels of N and S deposition in the last forty years with

significant increases occurring in the last decade (Gosselin et al. 2010, Laxton et al. 2010, Whitfield et al. 2009). The increased N and S deposition (Environment Canada. 2004) have caused concerns as the effects are multifaceted and may have negative effects such as soil acidification, N saturation, altered plant health, and eutrophication of nearby water bodies (Laxton et al. 2010, Watmough et al. 2014). When the SO_4^{2-} is leached from the affected soil, it can cause the simultaneous loss of basic cations in the soil and release of aluminum (Al) which can alter the pH, cation exchange capacity (CEC), and charge composition of the soil (Jung and Chang 2012, Ok et al. 2007). While the addition of N can initially be favorable, as vegetation growth is typically limited by low levels of soil N availability in the boreal forest, the potential N saturation can lead to shifts in microbial species composition and nutrient imbalances; however, the impact on soil microbial communities is not fully understood (Chapin 1980, Lu et al. 2011).

Forest soils in the AOSR are sensitive to soil acidification because they have low exchangeable base cations and cation exchange capacity (Watmough et al. 2014, Whitfield et al. 2009). It is quite common for an ecosystem response to elevated N and S deposition to be measured by the changes in plants including foliar N concentration and net primary productivity (NPP), but few studies have explored soil microbial responses (Frey et al. 2004). Nitrogen and S deposition can affect an ecosystem in a variety of ways including reducing the biodiversity of ectomycorrhizal fungi, soil micro- and mesofauna and altering soil bacterial niches, enzyme function, rate of litter decay, and the N dynamics in the soil (Fransson et al. 2000, Frey et al. 2004, Lindberg and Persson 2004). Soil microbes are important because they exert control over the soil C and N cycles and influence the resilience of an ecosystem (Demoling et al. 2008, Hu et al. 2013). Soil bacterial communities are considered to be sensitive to the availability of nutrients in the soil; therefore, knowing the impact of N and S deposition on soil bacterial

communities could provide an early sign of ecosystem responses to acid deposition in boreal forests in the AOSR (Díaz-Ravina et al. 1993).

Soil microbial biomass and activity can decrease when forests are fertilized with N (Arnebrant et al. 1996, Bowden et al. 2004), but there is little information on how this will affect specific groups of soil microorganisms such as bacteria. Responses of microbial biomass, community structure and activities to N deposition have been found to be dependent on the ecosystem type, N deposition rate, and existing vegetation/soil nutrients (Compton et al. 2004, Demoling et al. 2008, Van Diepen et al. 2010, Zechmeister-Boltenstern et al. 2011). The ratio of bacterial to fungal species will shift with N enrichment and is a useful indicator of microbial response to N addition (Rousk et al. 2011). Unfortunately it was not possible to extract fungal DNA from the collected soil samples in this study, therefore only bacterial DNA was analyzed. Studies have addressed bacterial diversity (alpha diversity), phylum composition (beta diversity), functional and metabolism profiles, and the interactions among bacteria (co-occurrence networks) (Barberán et al. 2012, Faust and Raes 2012, Ma et al. 2016). While alpha and beta diversity indices are important to community analysis, it is possible to extrapolate further through the use of co-occurrence networks which help identify functional roles and environmental niches of bacteria (Barberán et al. 2012). Analyzing functional profiles in a bacterial community will help understanding in how soil bacteria participate in important biogeochemical processes (Sessitsch et al. 2001). It has been suggested that understanding microbial interactions will give more information on soil properties and processes when compared to more commonly used methods including diversity and biomass (Ma et al. 2016). Co-occurrence patterns will add to the knowledge of bacterial community structure and functions by showing interaction networks and exposing shared niches (Ma et al. 2016).

To improve the understanding of boreal forest ecosystem responses to increases in N and S deposition in the AOSR, a simulated N and S deposition experiment was established in 2006. It was found that after 4-5 years of treatment soil microbial biomass carbon and N were not affected, and that after 5 years the soil microbial functional diversity and enzymatic activities were strongly affected (Jung and Chang 2012, Hu et al. 2013). In this study, I used sequencing of 16S rRNA genes to determine the effects of N and S addition to experimental plots on soil bacterial community diversity, composition, metabolism profiles, and co-occurrence networks. I hypothesize that (1) the overall diversity of bacteria species will decrease after N and S addition because it will favour species conditioned to thrive in N and S rich environments creating a narrow niche for microbial life and this will cause a change in species composition; (2) bacteria in plots exposed to N and S treatments will have functional profiles that represent adaptation and metabolism profiles that will utilize N and S as a source of nutrition when compared to the control; and (3) the number of interactions in the co-occurrence network will be lower in the plots with N and S addition when compared to the CK because the chemical additions will create more confined niches for bacteria and thus limiting their intra- and interspecies interactions. Elevated N and S deposition have a wide range of effects that can include fertilization but also acidification; therefore, it is an important issue that is of concern not only to the government and industry but also to the general public (Aber et al. 1989, Jung and Chang 2012).

2.2 Materials and Methods

2.2.1 Research site

The research site (56.1°N 110.9°W) is located in a natural boreal forest in AOSR in northern Alberta, approximately 100 km southeast of the city of Fort McMurray (Jung and

Chang 2012). The majority of mining and upgrading facilities were located on the north side of Fort McMurray and therefore the research site can be considered to be not directly affected by the elevated levels of N and S deposition associated with mining. The mean annual temperature and total precipitation measured from 1981 to 2010 were 0.96 °C, and 418.8 mm respectively (Government of Canada 2010). The dominant tree species are trembling aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) with other less abundant species including balsam fir (*Abies balsamea*), balsam poplar (*Populus balsamifera*), black spruce (*Picea mariana*), and paper birch (*Betula papyrifera)* (Jung and Chang 2012). Based on the Canadian system of soil classification the soils in the site are classified as mainly Gray Luvisols (Soil Classification Working Group 1998).

2.2.2 Experimental design

The experiment was established in 2006, with a 2 x 2 factorial design and in four blocks, for a total of 16 plots. Four treatments were set up: control (CK), N addition (+N, 30 kg N ha^{-1} yr^{-1} as NH_4NO_3), S addition (+S, 30 kg S ha^{-1} yr^{-1} as Na_2SO_4), and N and S addition (+NS, 30 kg N ha^{-1} yr^{-1} as NH_4NO_3 and 30 kg S ha^{-1} yr^{-1} as Na_2SO_4). Four blocks were established based on topography and uniformity of soil and site conditions and within each block, plots of 20 x 20 m were set up (Jung and Chang 2012). Treatments were randomly assigned to plots within each block. Although the current N and S deposition rates in the AOSR are recorded at approximately 2 kg ha^{-1} yr^{-1}, the experimental deposition amounts were based on the expected increase in N and S deposition due to development and expansion of the oils sands industries in the AOSR and to accelerate the effect of acid deposition (Hu et al. 2013, Jung et al. 2011). The +N and +S addition first occurred in 2006 and have continued with applications in the growing season every year since then. From 2006 to 2008 the +N and +S were applied once in the beginning of the

summer. Starting in 2009, the +N and +S were applied in three equal splits over the growing season to better mimic the natural deposition process (Jung and Chang 2012). The NH_4NO_3 (granule) and Na_2SO_4 (powder) were broadcast applied below the canopy.

2.2.3 Soil sampling and DNA extraction and processing

Soil samples were taken at 0-15cm, which encompasses the A horizon, from three randomly selected points in each plot to form a composite sample and placed in a cooler with ice packs and transported back to laboratory and frozen. Soil DNA was extracted, after sieving with a 2 mm sieve to remove rocks, soil aggregates and plant debris, with the SoilMaster™ DNA Extraction Kit and KAPA2G Robust HotStart Readymix. Once the DNA was extracted, library construction was performed using a 2 PCR method. The PCR1 (locus specific amplification) amplifies a region of the 16s rRNA gene using the tagged primers Glenn-F515 (5'- CAG TCG GGC GTC ATC AGT GCC AGC MGC CGC GGT AA -3') and trp1-R806 (5'- CCT CTC TAT GGG CAG TCG GTG ATG GAC TAC VSG GGT ATC TAA T -3') where Glenn is a universal tag (Glenn 2011), trP1 is the Ion Torrent truncated P1 adaptor (Life Technologies), and F515 and R806 are previously published locus specific primers (Barberán et al. 2011). PCR2 (Barcode and Ion Torrent specific adaptor attachment) uses diluted PCR1 product as a template. Here the forward primer is, from the 5' end, Ion Torrent A adapter sequence including the 4bp key (Life Technologies), Barcode (Life Technologies), and Glenn tag. The reverse primer is trP1 which is the Ion Torrent truncated P1 adaptor sequence (Life Technologies). This amplification, which was verified using agarose gel electrophoresis, results in amplicons which contain Ion Torrent specific adaptors, barcode and the targeted region of the 16s rRNA gene.

Amplified samples were all individually barcoded and were then pooled in equal amounts and this pooled library was then gel purified using the QIAquick Gel Extraction kit (Qiagen)

following the manufacturer's instructions. Purified pooled library was then purified for a second time using QIAquick Gel Extraction kit (Qiagen) following the manufacturer's instructions. Quantitation of the second purified pooled library was performed using the Qubit dsDNA HS Assay Kit (Life Technologies) and the library was diluted to 20pM. Template preparation of the diluted pooled library was performed with an IonOneTouch2 Instrument using the Ion PGM Template OT2 400 Kit (Life Technologies). Sequencing of templated spheres was conducted using an Ion PGM400 Sequencing Kit and an Ion316 Chip on an IonTorrent Personal Genome Machine (PGM) System (Life Technologies), following the manufacturer's instructions. After sequencing, the individual sequence reads were filtered within the PGM software to remove low quality and polyclonal sequences. Sequences matching the PGM 3′ adaptor were also automatically trimmed. All PGM quality filtered data were exported as fastq files and subsequently analyzed using open reference operational taxonomic units (OTUs) pickup strategy in the QIIME pipeline (Caporaso et al. 2010).

2.2.4 Data analysis

Operational taxonomic units (OTUs) were selected by removing sequences shorter than 120 base pairs or mean quality lower than 32 and this was then used to make an OTU table and imported with the phyloseq package using R (Core Team 2015, McMurdie and Holmes 2013). The OTU table was normalized with the negative nominal model (McMurdie and Holmes 2013), which minimized the bias associated with sequencing coverage and allowed for comparison between samples. All of the data were analyzed with the R software (version 3.1.2). The diversity of the bacteria in the plots was determined with the Shannon-Wiener diversity index and dissimilarity in the bacterial community composition was found using principal coordinate

analysis (PCoA). Principal coordinate analysis is a dissimilarity matrix, meaning increasing distance between symbols indicates increasing dissimilarity in phylogenetic composition. A TukeyHSD comparison of the outputs was completed to detect significant differences between treatments. The significant level for all statistical tests was set at $\alpha = 0.1$ due to the high spatial variability of the measured parameters, limited replications and to reduce the possibility of a type II error.

This study employed PICRUSt (Version 1.0) to infer the metagenome of a sample from its phylogenetic composition (Langille et al. 2013). The OTUs table was used as the input file for metagenome imputation of individual soil samples. The gene content of 2590 KEGG (Kyoto Encyclopedia of Genes and Genomes) reference genomes were used to infer the approximate gene content of the detected phylotypes. Predicted gene family abundances were analyzed at KEGG Orthology group levels 3. The mean nearest sequenced taxon index, which indicates the quality of the prediction, was lower (0.16±0.05) than that reported for soil communities (0.17±0.02) (Langille et al. 2013).

Co-occurrence network was constructed based on the maximal information-based nonparametric exploration (MINE) using the *minerva* package in R. To reduce rare OTUs in the dataset, I removed OTUs with relative abundances less than 0.05%. The nodes in this network represent OTUs. All *P*-values were adjusted for multiple testing using the Benjamini and Hochberg FDR controlling procedure (Benjamini et al. 2006), as implemented in the *multtest* package in R (Pollard et al. 2005). The direct correlation dependencies were distinguished using the network deconvolution method (Fierer et al. 2013). Based on MINE coefficients and FDR adjusted p-values, I constructed co-occurrence networks. The links that connect nodes represent that the MINE were greater than 0.4 after deconvolution and the *P*-value was less than 0.05.

Network properties were calculated with the *igraph* package in R (Csardi and Nepusz 2006). I generated network images with Gephi (Bastian et al. 2009).

2.3 Results

2.3.1 Effects of N and S addition on bacterial composition characteristics

At the phylum level a total of 9 different dominating phyla were identified with *Verrucomicrobia* (the highest in the +NS treatment) having the largest number of OTUs followed by *Proteobacteria* (the highest in the +N treatment), *Planctomycetes* (the highest in the +N treatment), *Acidobacteria* (the highest in the CK), and *Actinobacteria* (the highest in the CK). There was no statistically significant difference between the number of OTUs of the major phyla, class and orders among the treatments (Fig. 2-1).

The mean Shannon-Wiener diversity indices were 7.74 ± 0.32 (mean±SD), 7.41 ± 0.29, 7.91 ± 0.22 and 7.56 ± 0.066, for the control, +N, +S and +NS, respectively (Fig. 2-2); they were not significantly affected by +N and +S treatments ($P > 0.1$). The PCoA showed no overtly distinct clusters, and there is still of overlap bacterial communities among the treatments and the CK, indicating that there were no significant differences in the bacterial community composition caused by +N and +S addition (Fig. 2-3). To look further into variation in species abundance and composition an ANOSIM was carried out. Some significant differences between community composition were detected ($P = 0.07$) but the R value was less than 0.2 ($R = 0.12$), indicating that N and S addition had minimal effects on microbial community composition. An important pattern to note is the CK and +NS plots exhibited some differences in phylogenetic composition, indicated by the lack of overlapping area with +N and +S bacterial communities.

I was able to create a bacterial community co-occurrence network using correlation relationships with 985 associations from 141 bacterial OTUs (Fig. 2-4). The co-occurrence network was non-random and was used to determine keystone bacterial species. In the experimental plots *Spartobacteria*, *Phycisphaerae*, and *Gammaproteobacteria* were the keystone bacterial species. The +N and +S treatments did not cause statistically significant changes in the degrees (the degree of a node in a network is the number of connections it has to other nodes, Fig. 2-5). The number of degrees was recorded for each phylum and the differences were not significant ($P > 0.1$). However, the *Chloroflexi* phylum had the highest number of degrees followed by *Verrucomicrobia, Actinobacteria*, and *Planctomycetes* (Fig. 2-6).

2.3.2 Effects of N and S addition on bacterial metabolism and function

Although the differences were not significant ($P > 0.1$), the bacterial functional profiles show that the CK tended to have higher abundances of profiles that contribute to translation, environmental adaptation, and cell motility when compared to the +N, +S, and +NS treatments (Fig. 2-7). The most abundant functional profiles in +N were related to metabolism, immune system, and cellular processes and signaling, while +S had the most abundant functional profiles associated with energy and carbohydrate metabolism. The +NS treatment had the highest abundance of profiles related to transcription and membrane transport.

The relative abundance of bacteria functional profiles for the metabolism of carbohydrates, nitrogen, phosphate, and sulfur in each of the treatments is shown in Fig. 2-8. There was no significant ($P > 0.1$) treatment effect on the bacteria functional profiles for metabolism. A pattern to make note of was that the abundance of bacterial profiles for carbohydrate and nitrogen metabolism were the highest in +N treatment.

2.3.3 Effects of N and S addition on soil chemistry

There were no statistical differences found among CK and +N, +S, and +NS treatments for soil properties including pH, total carbon (TC), total nitrogen (TN), C:N ratio, total available N leaching below 45 cm, and the Ca:Al ratio (Table 2-1).

2.4 Discussion

In this experiment, 9 years of experimental N and S addition did not significantly affect soil bacterial diversity, community composition, functional profiles, metabolism profiles, and the number of interactions in the co-occurrence network. This rejects all of the hypotheses that I outlined for this experiment and was unexpected, but the results are consistent. Changes found in the bacterial community composition are often associated with similar changes in corresponding functional capabilities (Fierer et al. 2012, Freedman and Zak 2015). Both community composition and functional profiles were unaffected by +N and +S addition in the experiment. Another consistent result was that there were no significant changes in the soil pH, TC and TN, Ca:Al ratio, and the amount of NO_3^- and NH_4^+ being leached. The composition of the soil microbial community is thought to be mainly controlled by soil pH and C:N ratio of the substrate, so once those parameters exhibit significant changes the microbes are thought to follow (Högberg et al. 2007). If conditions in the soil have not been affected by the addition of +N and +S then it supports the lack of changes in the soil bacterial community.

A forest ecosystem undergoes four stages when exposed to N deposition, (1) N limitation, (2) alleviation of N limitation, (3) N saturation, and (4) forest decline (Aber et al. 1989). Previous research conducted in 2012 found that this research site was likely still in stage 1

or early stage 2, so largely this site is still experiencing N limitation (Jung and Chang 2012). Because the vegetation in the boreal forest is N limited it is very efficient at N uptake and utilization, this results in low N availability for bacterial consumption (Laxton et al. 2010, Lovett and Goodale 2011). Previous research in this experimental site found that there was increased N uptake by trees followed by increased growth in the plots exposed to N deposition (Jung and Chang 2012). Also results from unpublished research from this site showed that the diversity of the understory plant community was decreased by the addition of +N and +S over five years (Jung et al. unpublished). The fact that understory vegetation exhibited decreased diversity while microbial communities did not, suggests that vegetation is more sensitive to the elevated levels of +N and +S when compared to the soil bacteria. It has also been shown that mosses can derive N from the soil or wet deposition, which may allow for increased competition for N in a wide range of ecosystems, if those environments are limited by N it could have further implications as well (Ayres et al. 2006). Because the mosses and vascular plants in my experimental site are likely efficient at utilizing the excess N it could explain why the soil bacteria did not exhibit any changes, due to limited changes in available N. The +N plots did not show any changes in the amount of TN in the soil or the amount of N leaching from the soil. Therefore, it might not be surprising that there was a lack of bacterial response to the +N treatment.

Additionally, the amount of N and S that was applied each year was quite modest in comparison to other experiments that reported significant bacterial responses to N and S addition. Fierer et al. (2011) used intermediate and high levels of N deposition spanning 29 years, to determine the effects on bacterial diversity and community composition. The intermediate levels for each site were 34 kg N ha^{-1} yr^{-1} and 101 kg N ha^{-1} yr^{-1} (Fierer et al. 2011). It was only in the sites with high N deposition (272 and 291 kg of N ha^{-1} yr^{-1}) that bacterial

communities were significantly different from the intermediate levels and the control (Fierer et al. 2011). Yao et al. (2014) also looked at soil bacterial diversity and community composition in a 15-year study using N treatment rates ranging from 0 to 280 kg N ha^{-1} yr^{-1} (Yao et al. 2014). They found the Shannon-Wiener diversity index and abundance of bacterial cells decreased when treatment rates exceeded 52.5 kg N ha^{-1} yr^{-1} while community structure changed significantly starting at 17.5 kg N ha^{-1} yr^{-1} (Yao et al. 2014). Another experiment used N addition rates ranging from 0 to 92 kg N ha^{-1} yr^{-1} for three years and investigators found differences in the Shannon-Wiener diversity index of soil bacteria at treatment rates higher than 46 kg N ha^{-1} yr^{-1} (Sun et al. 2014). Additionally in a 6 year study with treatment rates ranging from 0 to 280 kg N ha^{-1} yr^{-1} N addition decreased species richness and colonization of bacterial species, under application rates that exceeded 175 kg N ha^{-1} yr^{-1} (Zhang et al. 2011).

Studies that applied N treatment rates of 30 kg N ha^{-1} yr^{-1}, similar to rates used in my research, reported variable results concerning the effects to bacterial diversity and community composition (DeForest et al. 2004, Freedman et al. 2015, Freedman and Zak 2014). In an 8-year experiment, DeForest et al. (2004) did not find changes in the bacterial community composition. Another study by Freedman et al. (2015) explored microbial community responses to N deposition (20 years at 30 kg N ha^{-1} yr^{-1}) and found no significant effects to soil bacteria diversity and composition. And lastly, Freedman and Zak (2015) found that while the addition of 30 kg N ha^{-1} yr^{-1} for 20 years did not have an effect on the bacterial diversity in the mineral soil, future deposition could change the physiological potential in the soil microbial communities.

In addition to lower deposition rates, the duration of my treatments was quite short when compared to other studies which ran for more than 15 years (Fierer et al. 2011, Yao et al. 2014, Freedman et al. 2015, Freedman and Zak 2014). The studies that reported no changes in soil

bacterial communities ranged from 8 to 20 years, while studies with significant differences were as short as 3 years and as long as 29 years. These results suggest that perhaps the period of treatment is not as influential as previously thought or time is a factor that is site specific. This result is indicated by the studies of Freedman et al (2015) and Freedman and Zak (2014) where a rate of 30 kg N ha^{-1} yr^{-1} was applied for 20 years and no significant changes were reported for bacterial species diversity and community composition. While studies using higher deposition rates ranging between 0 to 280 kg N ha^{-1} yr^{-1} only found significant changes to these parameters well above the 30 kg N ha^{-1} yr^{-1} rate. This would suggest that the magnitude of deposition could have more of an effect on soil microbial populations when compared to the length of the elevated deposition.

It is also possible that soil bacteria were not affected by N and S addition in the boreal forest site because they were either resistant or resilient to such additions. The outcomes possible when soil microbes are exposed to a disturbance, such as N and S deposition, are either mortality or a change in the relative abundance (Rykiel 1985). Resistance and resilience are commonly measured by changes in community composition. Soil bacteria have numerous characteristics that can allow them to either be insensitive to disturbances, or allow them to be resilient and return to the pre-disturbance conditions after an initial change (Allison and Martiny 2008, Pimm 1984, Shade et al. 2012). Soil bacteria are highly abundant, have high growth rates, and have a wide dispersal, which allows them to be resistant (Fenchel and Finlay 2004). Even if the bacteria are initially sensitive to a change in their ecosystem, their physiological flexibility, ability to rapidly evolve through horizontal gene transfers and mutations, and quick generation times allows the bacteria to return to its prior composition and exhibit resilience (Allison and Martiny 2008).

Unfortunately, I do not have soil bacteria data from the beginning of this experiment, which would allow me to determine if the initial disturbance to the ecosystem caused a change in the soil bacteria. If there were significant changes to the soil bacteria in the initial stages of the +N and +S addition experiment, then the bacteria were resistant or resilient to elevated +N and +S. There was some microbial research in this research site conducted after 5 years of N and S treatments and it was found that soil microbial community level physiological profiles were strongly affected which caused changes to the community function and enzyme activity (Hu et al. 2013). While this previous research was not as detailed and did not explore the same bacterial parameters, as my research, it does show that initially soil bacteria responded to +N and +S addition, and now after an additional four years, there are no longer significant differences in the soil bacteria, indicating possible bacterial resilience. And while I hypothesized that the bacteria in these experimental sites are exhibiting resilience to increased +N and +S addition, unfortunately the evidence is not available to fully support these claims.

While the results were not statistically significant there were still some emerging patterns that should be considered. A common pattern found in long-term N addition experiments is a shift in the dominant taxa and bacterial community structure commonly referred to as the copiotrophic hypothesis (Yao et al. 2014). It predicts that adding N will cause an increase in enzyme production and cell growth which will increase the amount of soil organic carbon (SOC) being decomposed (Campbell et al. 2010). These conditions will create an ideal environment for bacteria that are able to utilize the SOC pool and are fast growing (r-selection) which will increase the abundance of these bacteria while decreasing slow growing bacteria (k-selection) that are able to grow in nutrient poor soils (Campbell et al. 2010, Fierer et al. 2011, Yao et al. 2014).

Bacteria classified in the copiotrophic group include *Proteobacteria* and oligotrophic groups including *Acidobacteria* (Derakshani et al. 2001, Fuerst 1995). My results showed that the number of OTUs in the phylum *Proteobacteria* was the highest in the +N plots and the largest number of *Acidobacteria* was in the CK plots, supporting the copiotrophic hypothesis. The differences in the abundances between these groups could be indicative that the addition of N and S caused a shift in the microbial community's life history strategy which ultimately could affect microbial diversity. This could be one of the explanations as to why there was an initial decrease in the Shannon-Wiener diversity index from the control to the +N treatment. After long-term exposure to +N and +S treatment, the copiotrophic bacteria will decrease available SOC and the quality of the plant material being contributed to the soil will decrease due to a shift in the composition of the vegetation (Campbell et al. 2010). This will cause another shift in the soil bacteria towards oligotrophic bacteria that are able to utilize the remaining recalcitrant components of the SOC, all of which will ultimately decrease the bacterial diversity and change the community structure of the soil bacteria (Campbell et al. 2010). These types of changes could have further implications for nutrient cycling and soil fertility by altering the C:N ratios of the microbial biomass and increasing N demands (Fierer et al. 2012).

2.5 Conclusions

The effects of nine years of experimental N and S addition on bacterial diversity, community composition, functional profiles, and co-occurrence patterns were not significant. My research suggests that soil bacteria were not susceptible to negative impacts from +N and +S treatments in the boreal forest in northern Alberta. Bacteria are just one facet of the entire soil microbial population, and while soil bacteria are shown to be resistant, further study is needed to

determine how other soil organisms such as soil fungal and protozoan communities respond to elevated N and S levels. Further research and continued long-term monitoring is needed to understand the long-term effect of N and S deposition on boreal forest soils in the AOSR.

Table 2-1 Soil properties of mineral soil in response to N and S treatments, in a mixedwood forest in the AOSR in northern Alberta, Canada. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition. Values are means with the standard error of the means in parentheses. There were no significant differences found among +N, +S, +NS and CK.

Treatment	**TC**	**TN**	**C:N ratio**	**pH**	**NO_3-N leached below 45 cm**	**NH_4-N leached below 45 cm**	**Ca:Al ratio**
	($g\ kg^{-1}$)				(mg per resin core)		
CK	7.0 (1.5)	0.4 (0.09)	17.5 (0.9)	4.8 (0.3)	0.206 (0.008)	0.846 (0.027)	63.9 (2.5)
+N	6.9 (0.9)	0.4 (0.06)	19.1 (0.5)	4.7 (0.2)	0.265 (0.031)	0.900 (0.034)	17.0 (1.6)
+S	5.6 (0.3)	0.3 (0.02)	17.6 (0.2)	4. 7 (0.2)	0.199 (0.007)	0.855 (0.030)	21.4 (0.56)
+NS	7.2 (1.4)	0.3 (0.05)	21.3 (0.6)	4.7 (0.07)	0.210 (0.019)	0.888 (0.033)	21.9 (0.58)

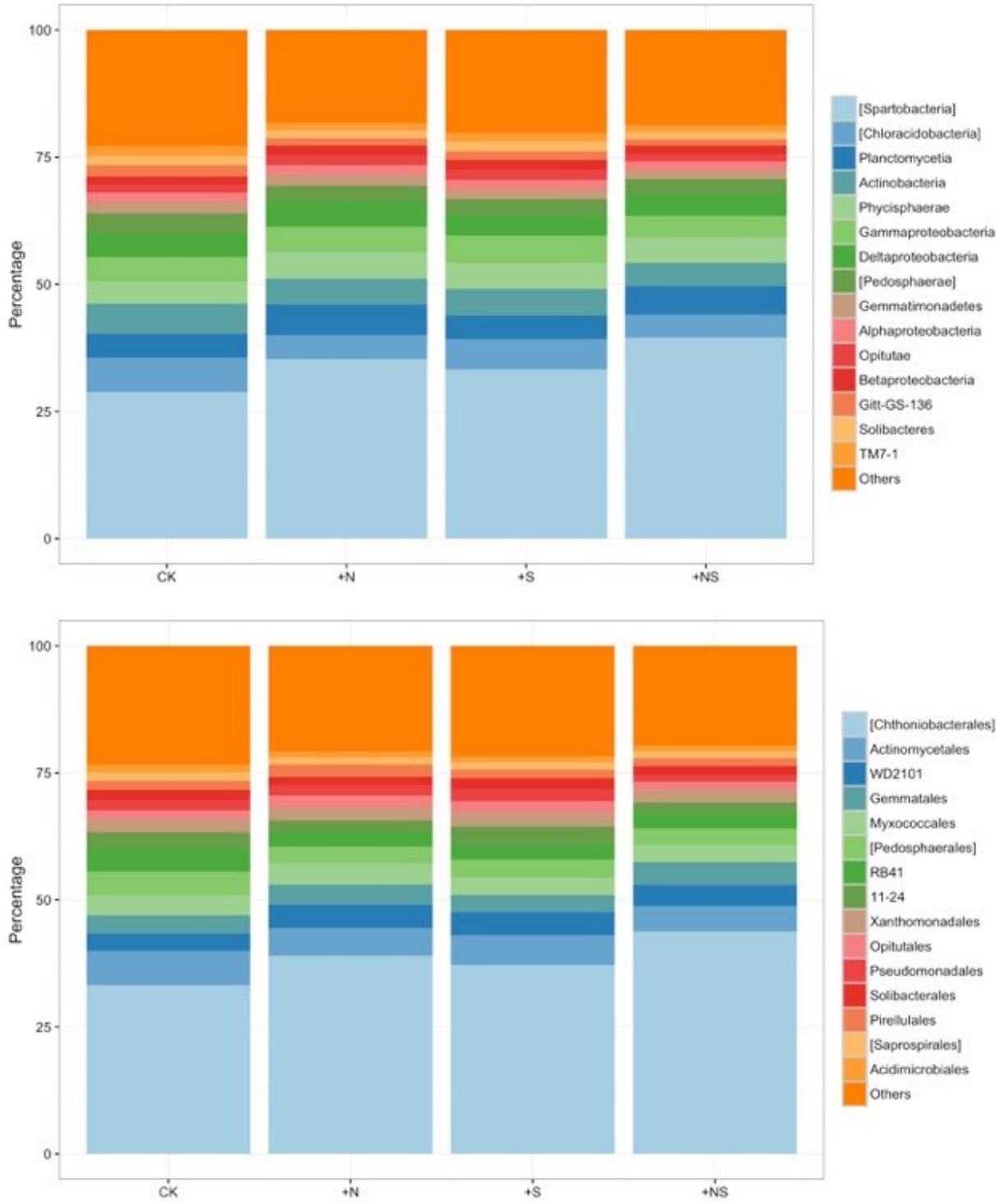


Figure 2-1 Composition of soil bacteria at the class (top) and order (bottom) level as affected by 9 years of experimental N and S addition in a boreal mixedwood forest in the Athabasca oil

sands region, Alberta, Canada. CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition.

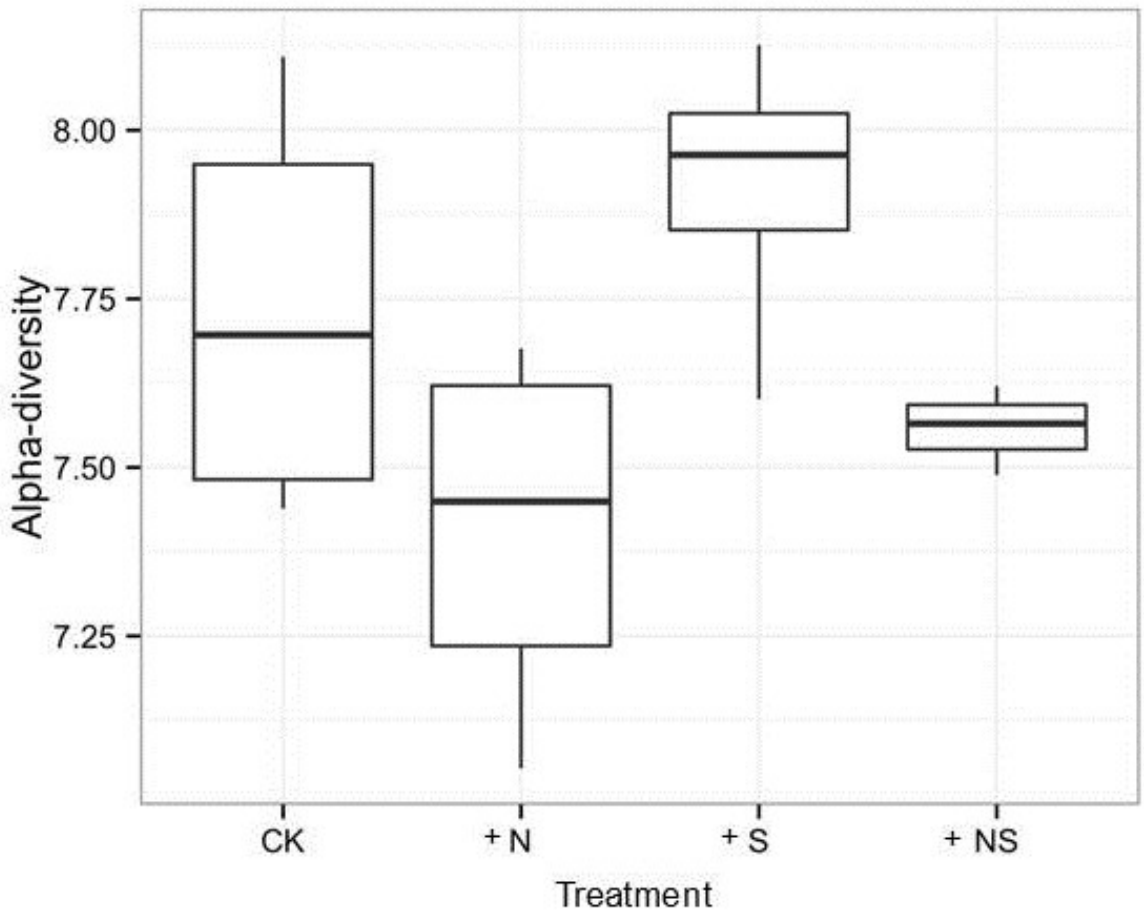


Figure 2-2 Shannon-Wiener diversity indices of soil bacteria communities in response to N and S addition, in a mixedwood boreal forest in the Athabasca oil sands region, Alberta, Canada. The boxes on the graph represent the values found between the 25th and 75th percentiles, the middle line is the median, and the extending lines represent the minimum and maximum values. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition.

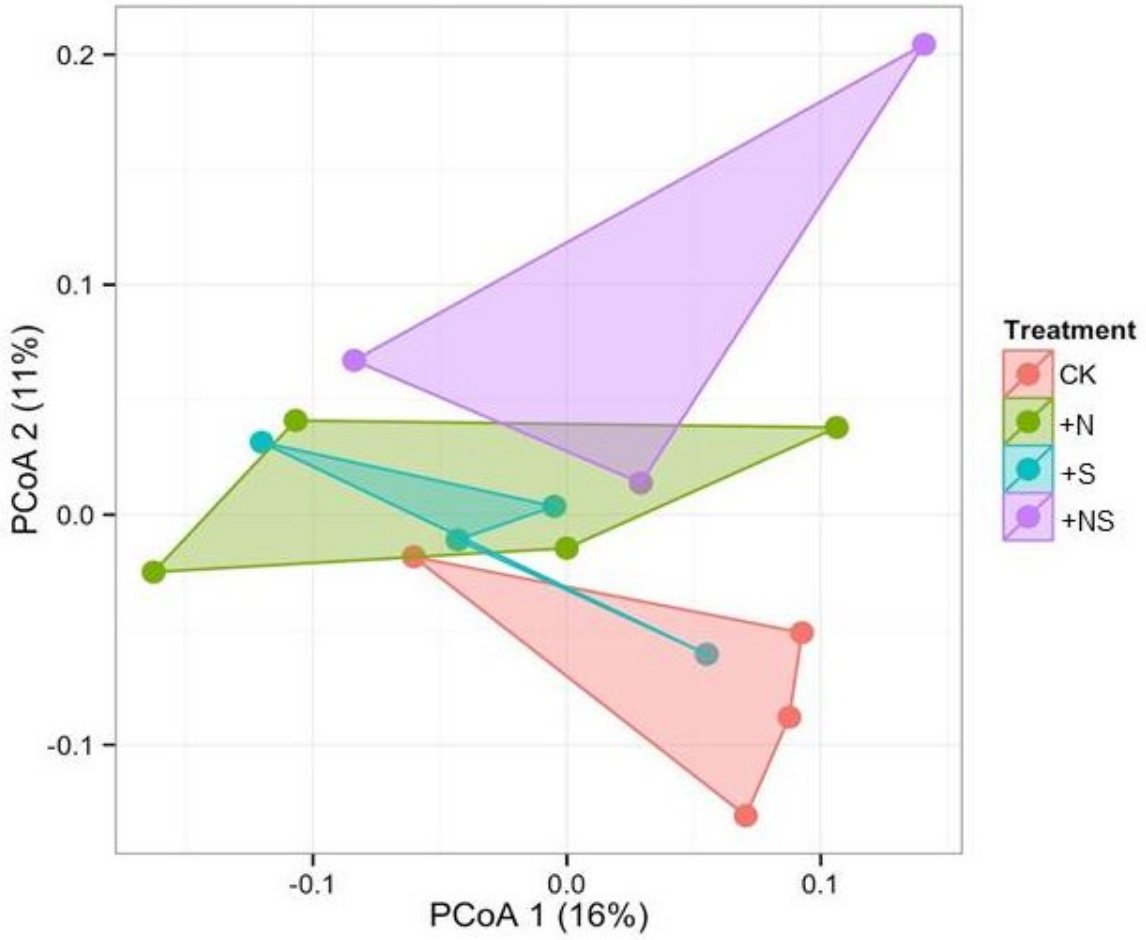


Figure 2-3 Principal component analysis of bacterial communities in response to N and S addition, in a mixedwood boreal forest soil in the Athabasca oil sands region, Alberta, Canada. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition.

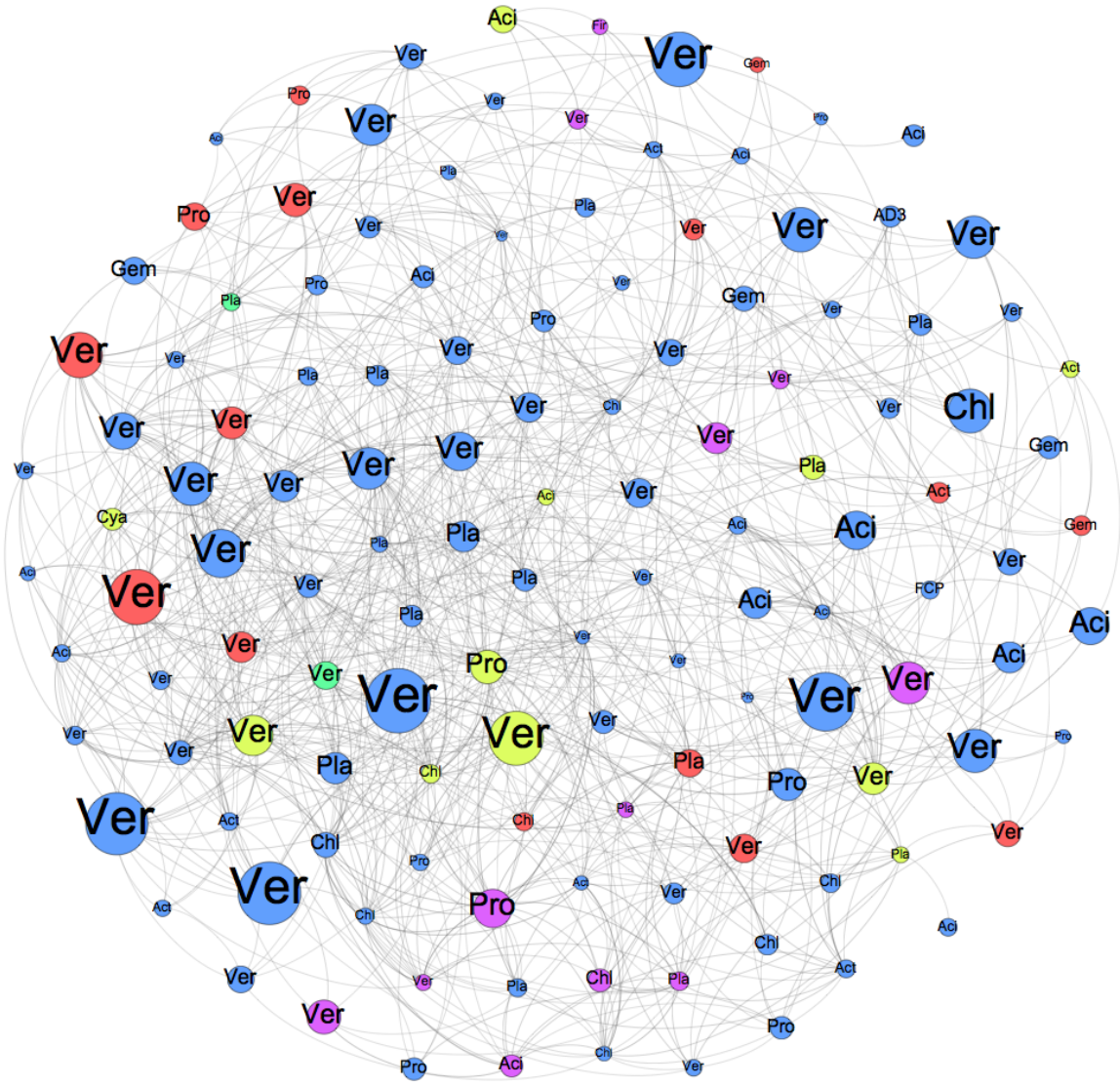


Figure 2-4 The co-occurrence network for dominant OTUs of soil bacteria in response to N and S addition, in a mixedwood boreal forest in the Athabasca oil sands region, Alberta, Canada (relative abundance >0.05%). The nodes represent unique sequences in the data sets and the size of each node is proportional to its relative abundance. .Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition. Abbreviations of Ver, Pro, Chl, Pla, Gem, Aci, and Act refer to *Verrucomicrobia*, *Proteobacteria*, *Chloroflexi*, *Planctomycetes*, *Gemmatimonadetes*, *Acidobacteria*, and *Actinobacteria* phyla.

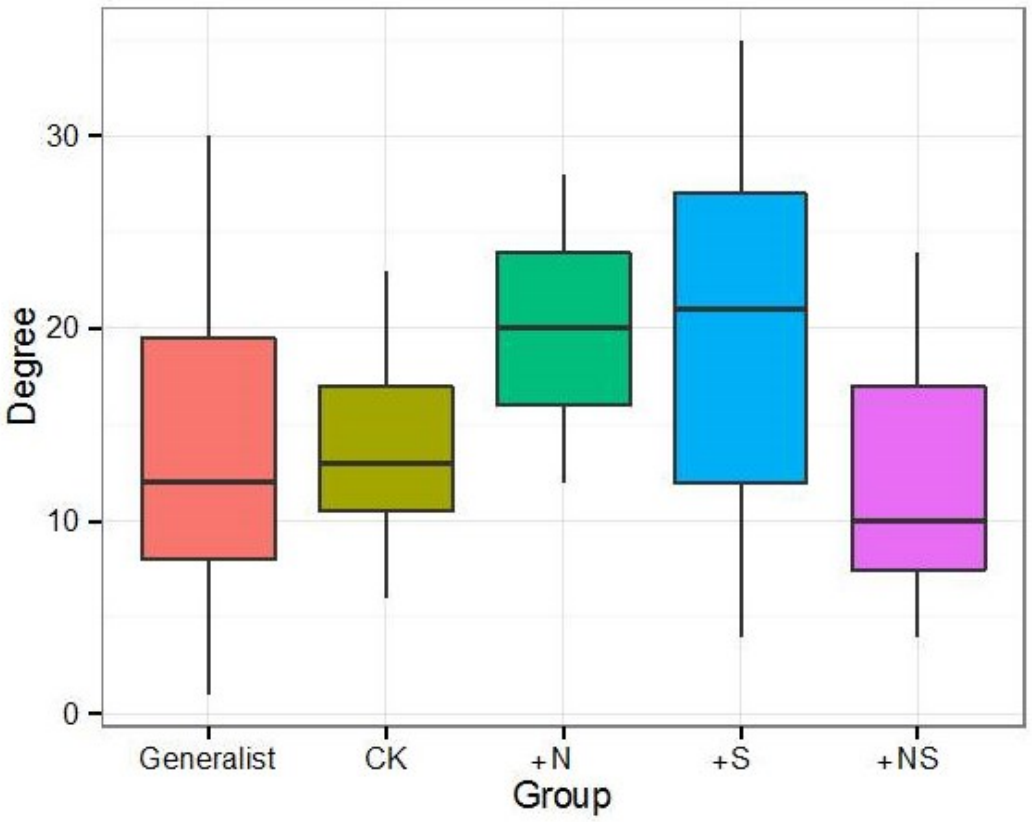


Figure 2-5 The Degree of OTUs of all soil bacteria samples in response to N and S addition, in a mixedwood boreal forest soil in the Athabasca oil sands region, Alberta, Canada. The boxes on the graph represent the values found between the 25th and 75th percentiles, the middle line is the median, and the extending lines represent the minimum and maximum values. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition.

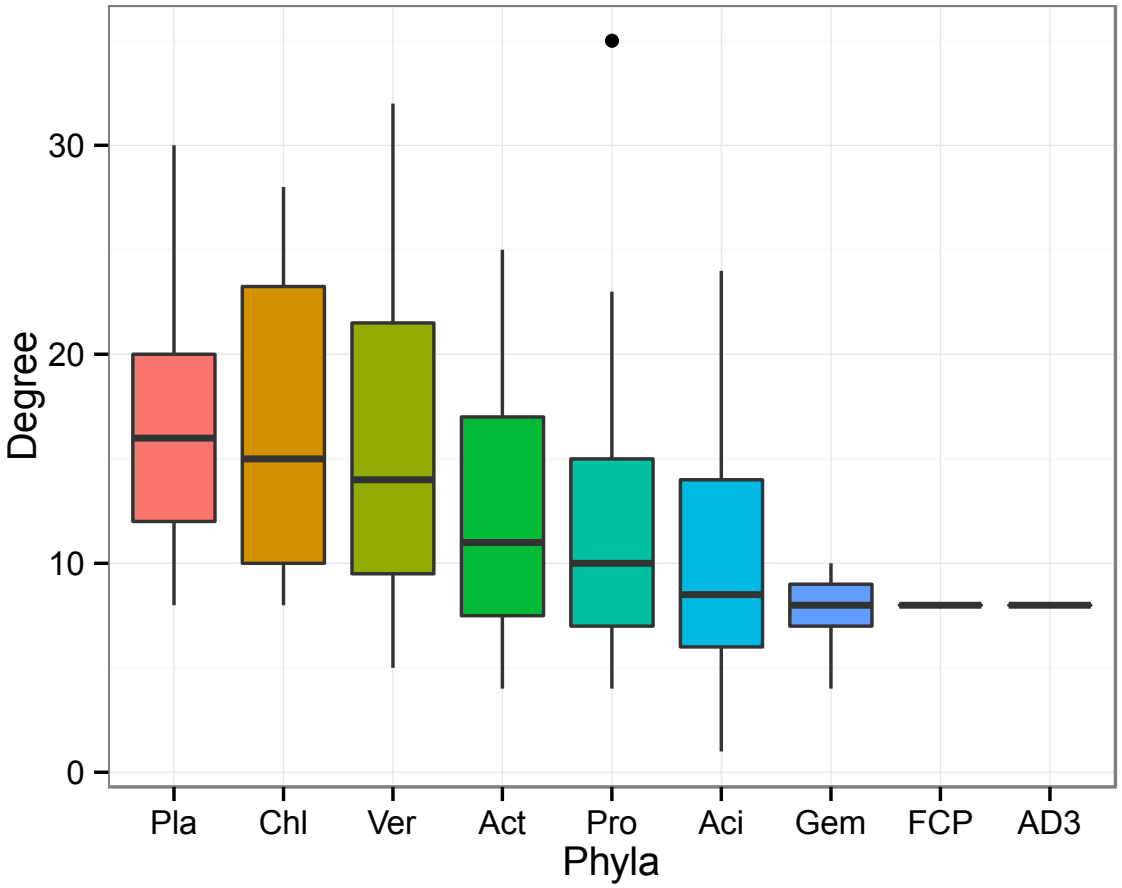


Figure 2-6 The Degree of OTUs of all soil bacteria samples belonging to different soil bacteria phyla in response to N and S addition, in a mixedwood boreal forest soil in the Athabasca oil sands region, Alberta, Canada. The boxes on the graph represent the values found between the 25th and 75th percentiles, the middle line is the median, and the extending lines represent the minimum and maximum values. The dots on the graph are outliers. Abbreviations of Pla, Chl, Ver, Act, Pro, Aco, and Gem stand for *Planctomycetes, Chloroflexi, Verrucomicrobia, Actinobacteria, Proteobacteria, Acidobacteria, Gemmatimonadetes* phyla.

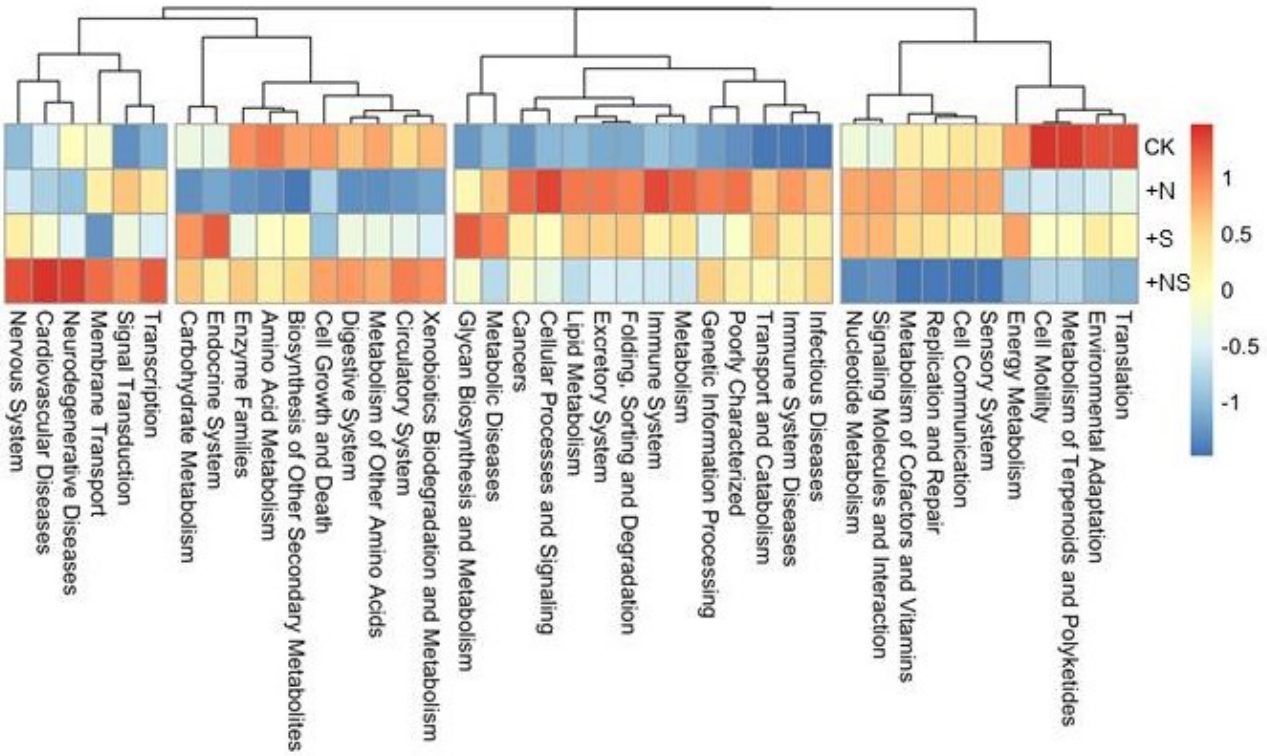


Figure 2-7 The functional profiles predicted with PICRUSt of soil bacteria in response to N and S addition, in a mixedwood boreal forest soil in the Athabasca oil sands region, Alberta, Canada. The values were centered and scaled in each column. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition.

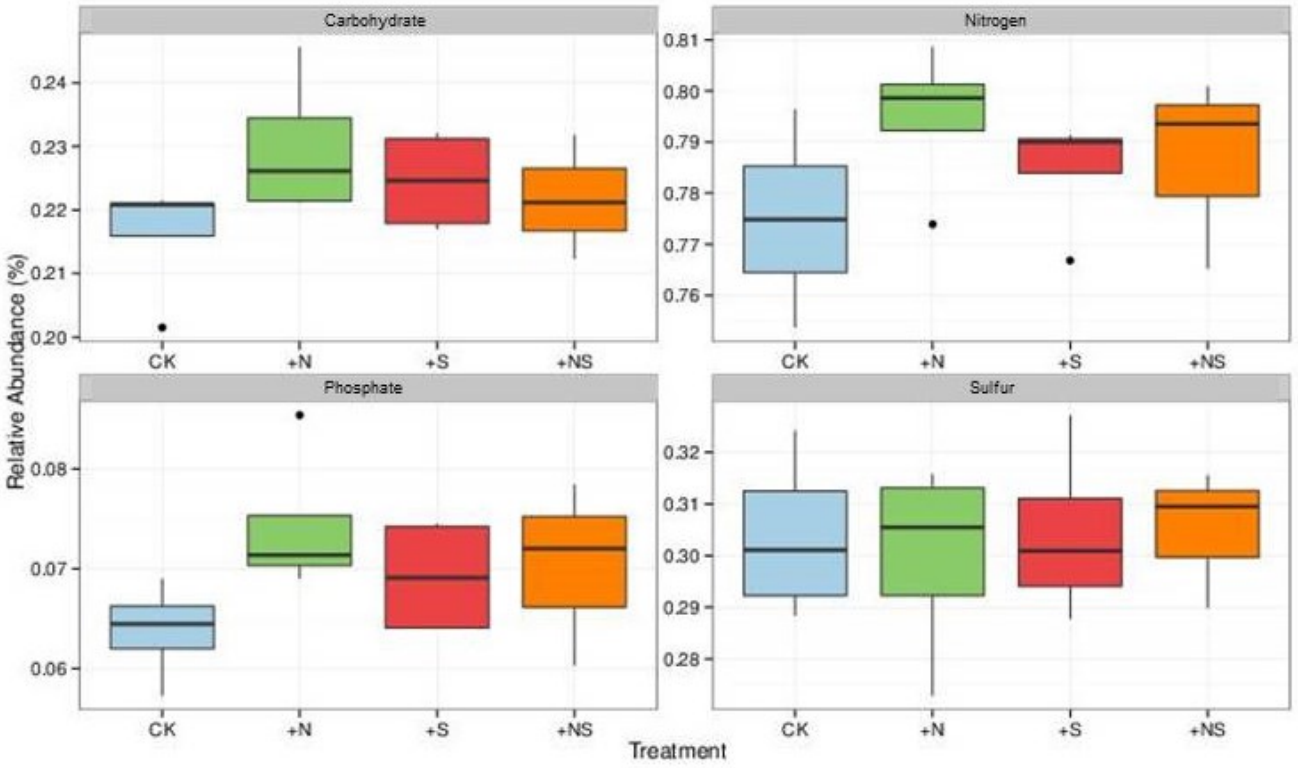


Figure 2-8 The relative abundance of metabolism profiles associated with carbohydrate, nitrogen, phosphate and sulfur metabolism in soil bacteria in response to N and S addition, in a mixedwood boreal forest soil in the Athabasca oil sands region, Alberta, Canada. The boxes on the graph represent the values found between the 25th and 75th percentiles, the middle line is the median, and the extending lines represent the minimum and maximum values. The dots on the graphs are outliers. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition.

Chapter 3: Soil fungal but not bacterial communities are sensitive to long-term nitrogen and sulfur addition in a boreal forest in northern Alberta, Canada

3.1 Introduction

Increased atmospheric emissions of nitrogen (N) and sulfur (S) since the Industrial Revolution mainly due to anthropogenic activities such as fertilizer use, fossil fuel combustion and biomass burning have been a significant environmental concern (Galloway et al. 2008, Matson et al. 2002); for example, the nitrogen cycle has doubled in the last 100 years (Vitousek et al. 1997). While S deposition has decreased in recent years in developed regions due to legislation to address concerns over acid rain, N emissions continue to increase and are causing major concerns due to their possible negative effects on ecosystems (Jung and Chang 2012, Laxton et al. 2010). Long term emissions of N and S can alter biogeochemical cycles, cause eutrophication of water bodies, affect plant health, lead to nutrient deficiencies and the loss of cations, alter species composition, and change biodiversity (Bowman et al. 2012, Emmett 2007, Jung and Chang 2012, Laxton et al. 2010, Watmough et al. 2014).

In western Canada, emissions of N and S have increased in the last several decades and are expected to continue to increase due to economic and industrial growth and expansion mainly in transportation, urban areas, and mining activities (Aherne and Shaw 2010). There is spatial variability associated with changes in N and S deposition, with some areas experiencing increasing rates of deposition; a special area of concern is the Athabasca Oil Sands Region (AOSR) (Aherne and Shaw 2010, Jung and Chang 2012). The AOSR contains the majority of

Alberta's oil sands, which on a global scale ranks as the third largest oil reserve (Alberta Energy 2015). These deposits underlie 140 200 km^2 of boreal forest. Major oil sands commercial developers and companies are found in a region roughly 30 km north of Fort McMurray, Alberta where mining and upgrading have exposed the surrounding boreal ecosystem to elevated N and S deposition stemming from NO_x and SO_2 emissions, respectively (Ferguson et al. 2009, Gosselin et al. 2010, King and Yetter 2011, Laxton et al. 2010). This northern region is especially susceptible to soil acidification because soils are characterized by low exchangeable base cations and a low cation exchange capacity (Watmough et al. 2014, Whitfield et al. 2009). And while currently the low oil price has caused oil production to slow down, there are major mining projects still under active construction, indicating that atmospheric deposition of N and S will only increase in the future (Alberta Energy 2015).

Microbes have a strong influence over soil system processes; they mediate the soil C, N, S and P cycles, and are responsible for a majority of litter decomposition (Allison and Martiny 2008, Högberg et al. 2007, Hu et al. 2013). There is no lack of research on how N and S deposition affect soil microbial populations, in terms of diversity, composition, biomass, respiration and enzymatic activities, but the results are variable and dependent on the ecosystem type, the deposition rate, and the length of the study (De Boer et al. 1992, Freedman et al. 2015, Frey et al. 2004, Gupta et al. 1961, Krumins et al. 2009, Liu et al. 2011, Sun et al. 2014, Zhang et al. 2011).

Quantitative estimation of the number of viable microbes has been a foundational method in microbiology since Robert Koch first described the technique in 1883. While it is a well-established microbiological technique, there are fewer and fewer environmental studies that use plating and most probable number (MPN) techniques because these methods are labour and time

(Marshall et al. 2011). There is a focus on using newer microbiology methods including phospholipid fatty acid analysis (PLFA) and polymerase chain reaction (PCR). However, approximately 80% of cells and 50% of the operational taxonomic units (OTUs) found using these two methods may be inactive (Lennon and Jones 2011). After bacterial cells have lost their viability, their DNA will persist in the environment which causes DNA-based quantification methods to overestimate the size of microbial populations (Nocker and Camper 2006). Therefore, MPN and plating methods can be useful methods in determining viable cell counts in the soil. Soil health is vital to the proper functioning of ecosystems. Microbial counts are one indicator of the soil health because it shows a proportion of the living microbes in the soil and gives information on ecosystem dynamics (Hayat et al. 2002).

To improve understanding of how elevated N and S deposition can influence soil microbial communities in the boreal forest in AOSR of northern Alberta, a simulated N and S deposition experiment was established in 2006 using rates of 30 kg N ha^{-1} yr^{-1} and 30 kg S ha^{-1} yr^{-1}. Previous microbe related research in this site showed that after 4 years soil microbial biomass C (MBC) and N (MBN) were not affected, but after 5 years the soil microbial community-level physiological profiles, community function, and enzyme activity were affected by N and S addition (Hu et al. 2013, Jung and Chang 2012). Besides this previous research, there have been no studies on the effects of elevated N and S deposition on soil microbes in the AOSR. In this study, I want to further explore the effects these treatments have on soil microbes after 9 years of N and S addition. I have already conducted research on this site using sequencing of 16S rRNA genes of soil bacteria, but unfortunately was not able examine the response of soil fungi. This experiment was designed to obtain information on how elevated N and S deposition affect soil fungal communities and to confirm the results obtained for soil bacteria described in

the previous chapter (Chapter 2). The microbial methods of plating and MPN for bacteria enabled me to gain a more comprehensive understanding on the bacterial communities at the AOSR study site, including what species can be cultured and their viable counts. Note that I was not able to find published research using these methods to study N and S treatment effects on microbial communities in boreal forest ecosystems. Additionally, this work provides an opportunity to corroborate the results from my previous microbial research.

More specifically the research goal was to determine if soils experiencing elevated inputs of N and S are exhibiting lower numbers of culturable bacteria and fungi when compared to the control. I wanted to gain a better understanding of how soil microbial communities, specifically fungi, are affected by elevated levels of N and S deposition. I hypothesized that (1) the number of bacterial and fungal colonies will decrease after N and S addition because these treatments will change the soil pH and alter the soil chemical properties; (2) denitrifying bacteria will be the highest in plots treated with N as the added N will provide a nutrient for the bacteria to metabolize and thus promote bacterial growth; and (3) iron-reducing bacteria will be the highest in plots treated with N because N fertilization promotes iron reduction.

3.2 Materials and Methods

3.2.1 Research site

The research site is located in a natural boreal forest in the AOSR in northern Alberta (56.1°N 110.9°W), approximately 100 km southeast of the city of Fort McMurray and away from the concentrated area of major oil sands surface mining activities (Jung and Chang 2012). The mean annual temperature and total precipitation measured from 1981 to 2010 were 0.96 °C

and 418.8 mm, respectively (Government of Canada 2010). Based on the Canadian system of soil classification the soils are mainly Gray Luvisols (Soil Classification Working Group 1998). The dominant tree species are trembling aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) (Jung and Chang 2012).

3.2.2 Experimental design

The experiment was established in 2006, with a 2 x 2 factorial design, replicated in four blocks for a total of 16 plots. Four treatments were set up: control (CK), N addition (+N, 30 kg N ha^{-1} yr^{-1} as NH_4NO_3), S addition (+S, 30 kg S ha^{-1} yr^{-1} as Na_2SO_4), and NS addition (+NS, 30 kg N ha^{-1} yr^{-1} as NH_4NO_3 plus 30 kg S ha^{-1} yr^{-1} as Na_2SO_4). Four blocks were established based on topography and uniformity of soil and site conditions and within each block, plots of 20 x 20 m were set up (Jung and Chang 2012). Treatments were randomly assigned to plots. The experimental treatments were based on the expected increase in N and S deposition due to development and expansion of the oils sands industries in the AOSR and to accelerate the effects of acid deposition (Hu et al. 2013, Jung et al. 2011). The N and S addition first occurred in 2006 and have continued with applications in the growing season every year since then. From 2006 to 2008 the N and S were applied once in the beginning of the summer. Starting in 2009, the N and S were applied in three equal splits over the growing season to better mimic the natural deposition process (Jung and Chang 2012). The NH_4NO_3 (granule) and Na_2SO_4 (powder) were broadcast applied below the canopy.

3.2.3 Soil sampling

Mineral soil samples were collected in October 2014 using a stainless steel soil corer. The forest floor was collected by hand followed by 0-15cm of the mineral soil from three randomly selected points in each plot. Samples were combined and homogenized to form a composite sample, placed in a cooler with ice packs and transported back to laboratory and frozen until the start of this experiment in January 2015.

3.2.4 Plating procedure

The composite samples were passed through a 2 mm sieve to homogenize the soil. Subsamples were dried in an oven for 24 hours at 110 °C to obtain the soil water content (Black 1965). Then 10 g of soil was weighed and added to 90 mL of phosphate buffered water and shaken for 20 minutes using a mechanical shaker to create the stock solution. Following this, 10 mL of the stock solution was added to 90 mL of phosphate buffered water and inverted 10 times. The technique used to make a single dilution was repeated sequentially using increasingly dilute solutions as the "stock" solution to create a serial dilution.

Agar plates for bacteria culture were made by mixing 23.5 g of Difco plate count agar with 1000 mL of distilled water. Rose bengal agar for fungi was made by adding 17 g of laboratory standard agar, 10 g of malt extract and 10 mL of 1:10,000 dilution rose bengal to 1,000 mL of distilled water. Recipes were according to Difco Laboratories (1984). All agar solutions for this analysis were autoclaved, poured into individual petri dishes and stored for no more than two weeks. Each serial dilution had 0.1 mL pipetted onto four plates of both the rose bengal agar and plate count agar. Using a sterilized glass spreader while rotating the petri dish, the sample was spread evenly over the agar. Each of the four replicates of inoculated plates was

secured with elastic bands and incubated in sealed Ziploc© bags at room temperature for two weeks.

After the incubation, plates that contained between 30 and 300 colonies were selected for counting (Fig. 3-1). Plates were assessed for the total number of morphologically distinct bacterial or fungal colonies (Table 3-1). Colonies were allowed to mature for another two weeks and then selected morphologically distinct isolates, based on unique colony morphology, were streaked onto new plates in order to obtain single colonies. Following another week of incubation the single colonies were gram stained. One individual colony was selected on a sterilized loop and spread onto a microscope slide containing one drop of distilled water. The slide was passed through a flame in order to fix the sample to the slide. Gram staining was performed on each sample and viewed with a light microscope using immersion oil on the slide with a 970× magnification (Davies et al. 1983, Beveridge and Davies 1983). The morphology of the colony, including form, elevation, margin, surface, opacity, and chromogenesis, was recorded as well as the status of the gram staining (Tables 3-2, 3-3). Further, gram negative bacteria were identified to the species level using the Biomerieux API 20 NE strip following the manufacturer's instructions (Biomerieux Canada Inc. 2015). The strips were left for one week and then read using the strip result indicator colours (Table 3-3).

3.2.5 Most probable number procedure

The population size of denitrifying and iron-reducing bacteria capable of being cultured was determined using the most probable number (MPN) method (Cochrane 1950). Denitrifying bacteria cultural media were made by dissolving 5 g of potassium nitrate (KNO_3) and peptone into 1 L deionized water (Aaronson 1970). The pH of the solution was 7 and a Durham tube was

added to each test tube. Iron-reducing bacteria growth media was prepared by dissolving 0.5 g of ammonium sulphate (NH_4SO_4), 0.5 g of sodium sulphate (Na_2SO_4), 0.1 g of dipotassium phosphate (K_2HPO_4), 1.0 g of magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), 5 g of ferric ammonium phosphate and 5 g of nutrient broth into 1 L deionized water (Aaronson 1970). Sodium hydroxide (0.1 M NaOH) was added to the iron-reducing medium to ensure the pH was approximately 7. The denitrifying and iron-reducing bacteria media were dispensed into 16 x 150 mm culture tubes, autoclaved, and capped.

One milliliter of each of the solutions from the soil dilution series were pipetted into media tubes with five replicates. These tubes were incubated for 3 weeks at room temperature and assessed to determine the MPN number. The denitrifiers were positive if gas bubbles formed in the small glass tubes; and the iron reducers were positive if the clear ferric solution was precipitated as ferrous salts (Fig. 3-2).

3.2.6 Statistical analysis

All statistical analysis was completed with the R software (version 3.2.3). The means and standard errors of the mean (SEM) were calculated for the plating colonies and MPN. Shapiro-Wilk and Bartlett tests were performed to test the assumptions of normality and heterogeneity of variance. Analysis of variance (ANOVA) was used to determine the differences in the number of bacterial colonies, fungal colonies, dentrifiers, and iron reducers between the treatments (CK, +N, +S, +NS). When a significant difference between treatments was found the Tukey's HSD was used as a post hoc test. The significant level for all statistical tests was set at $\alpha = 0.1$ due to the high spatial variability of the measured parameters, limited replications and to reduce the possibility of a type II error.

3.3 Results

3.3.1 Effects of N and S addition on bacterial and fungal colonies

The number of culturable soil bacteria colonies was not significantly affected following 9 years of N and S treatments ($P > 0.1$) when compared to the CK (Fig. 3-3). The gram positive/negative status and colony morphology were determined for distinct colonies (Tables 3-2 and 3-3). The API strips showed that *Photobacterium damselae* was a bacterial species found in all experiment plots, *Chrysobacterium indologenes* was found in +N and +S plots, *Sphingomonas paucimobilis* only in +S, *Psychrobacter phenylpyruvicus*, *Pseudomonas aeruginosa* and *Vibrio alginolyticus* in the +NS plots. Because not every bacterial colony was examined for the gram staining and API strips, it was not possible to do statistical analysis on individual bacterial species, and this is presented as qualitative information. In contrast, the number of culturable soil fungal colonies were significantly increased by N and S addition ($P = 0.013$) when compared to the CK (Fig. 3-4). The addition of S caused the largest increase in fungal colonies followed by +N and +NS (Table 3-1). The number of morphologically distinct fungal colonies were not affected by +N and +S treatments ($P > 0.1$).

3.3.2 Effects of N and S addition on iron-reducing and denitrifying bacteria

The N and S treatments did not have a significant effect on the number of iron-reducing bacteria in the treatment plots when compared to the CK ($P > 0.1$). However, note that there was a general pattern of +N and +S causing a decrease in the number iron-reducing bacteria across all treatments. In comparison there were significant differences in the number of denitrifying bacteria between the treatments and CK ($P = 0.026$) except between the +S and +NS plots. The

+N plot had the highest number of denitrifying bacteria followed by the CK (Fig. 3-5). The lowest number of denitrifying bacteria was recorded in the +NS plots.

3.4 Discussion

3.4.1 Bacterial colonies

My results showed that the number of culturable bacterial colonies was not affected by 9 years of N and S addition. As a result, I reject the hypothesis. The various soil properties, including pH, total carbon (TC), total nitrogen (TN), available N (NO_3^- and NH_4^+) , and exchangeable cations including calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), potassium (K^+), and aluminum (Al^{3+}) were not significantly different between the CK and the treatments. Changes are anticipated to start to occur in the soil bacterial community when the environmental conditions including soil properties start to change, because these changes will affect an organism's fitness which in turn will alter their abundance (Zhang et al. 2011). The results of the soil properties support the results of no significant differences between the bacterial plate counts.

It is possible that I did not see changes to the number of soil bacteria colonies because either the rates of +N and +S were too low, or the treatment needs more time for changes to become apparent, or the bacteria were exhibiting resilience to this type of disturbance. Numerous studies that explored the effects of N and S deposition and reported changes to soil bacteria, were utilizing much higher deposition rates and had been running for more than 15 years (Campbell et al. 2010, Fierer et al. 2011, Freedman et al. 2015, Freedman and Zak 2014, Liu et al. 2011, Peciulyte et al. 2009, Sun et al. 2014, Xu et al. 2012, Zhang and Han 2012). Studies by DeForest et al. (2004) and Freedman et al. (2015) both used a N deposition rate of 30 kg N ha^{-1} yr^{-1} except

that the studies ran for 8 and 20 years, respectively. Interestingly both studies reported no changes to the soil microbes in their experimental site suggesting that maybe time is not as influential in the development of a bacterial response as previously thought. This lack of response would indicate that the rate of deposition has more of an effect on soil microbial populations when compared to the length of the study.

It is also possible that the number of bacteria colony forming units were not affected by +N and +S because the bacteria were either resistant or resilient to changes, as previously discussed in Chapter 2. A plating and MPN study on the effects of zinc application to soil microbial communities found that soil microbes had significantly decreased counts and biomass after 15 days of addition but after 420 days these differences were no longer significant (Kelly et al. 1999). The soil microbes had time to adjust to the disturbance, stresses, and chemical additions. The researchers concluded that adaptation was occurring in the soil microbial communities (Kelly et al. 1999). Because my N and S experiment has been running for 9 years, and I do not have data from the first year of application, it is impossible to determine if the bacteria would be considered resistant or resilient. It is possible that throughout the study the bacteria have been resistant to the N and S disturbance, or it could be that there was an initial change in the number of bacterial colonies, but they were able to adapt to the disturbance and return to pre-disturbance numbers.

3.4.2 Fungal colonies

While the bacterial colonies showed no changes, there were significant differences in the number of fungal plate colony counts between treatments. The +N and +S treatments caused an

increase in the number of fungal plate colonies which rejects the hypothesis that a decrease would be observed. It has been suggested that fungi are more susceptible and sensitive to N and S addition possibly because fungi traditionally have lower individual numbers and this makes them more vulnerable to disturbances when compared to soil bacteria (Emmett 2007, Liu et al. 2011). Freedman et al. (2015) found that active fungi composition was altered and diversity was decreased by N deposition, while the bacterial community was unaffected. Another study on the response of protozoan and microbial communities in coniferous forest soils to atmospheric pollution (including N and S) reported a change in the structure of microbial communities from bacterial to fungal dominance (Coûteaux et al. 1998). This pattern would suggest that a change in fungal communities and not soil bacteria is not an unusual result. It is important to note that when fungi are grown on agar there is mycelial extension which will increase the number of colonies on the plate that actually belong to the same individual (Dursun et al. 1996). This could have inflated the number of fungal colony counts; however, I used the exact same counting technique for all of the plates and had replicates so this decreases the possibility of experimental error. These results only represent a portion of the fungal colonies in the research site. Further research is needed to determine if the significant changes to culturable fungal colonies are consistent and applicable to *in situ* fungal species.

The +N and +NS treatments increased the number of culturable fungal colonies. Vegetation of northern Alberta's boreal forest is generally limited by N. Therefore it is reasonable to observe an increase in fungal colonies when exposed to elevated levels of N and S. Nitrogen is essential to fungi nutrition and diet, so being exposed to higher levels would allow for abundance to increase (Tandon and Grewal 1956). However, there is no consensus in the literature about how N affects soil fungi as some results suggest that N amendment will suppress

fungal biomass while others saw no changes (Chen et al. 2014, Freedman et al. 2015, Frey et al. 2014, Hesse et al. 2015, Liu et al. 2014). Declines in the abundance of soil fungi were more evident in studies with higher N addition and longer duration (Treseder 2008). These preliminary results suggest that the N treatments affected the soil fungal community by increasing its abundance; this is in contradiction to other research results. However, this was a plated experiment and only represents a small fraction of the soil fungal community. It is possible with continued N and S addition fungal colonies will continue to increase until resources and nutrients are exhausted, competition and niche displacement increases, which would ultimately lead to a sharp decrease in the number of fungal colonies. While there are higher plate counts among the plots when compared to the CK, further data including diversity and composition are needed before strong conclusions can be made on the susceptibility and changes occurring to the fungal communities in this study site.

The largest increase in the number of culturable fungal colonies however was in the +S treatment. This was an unexpected result especially considering the +S treatment had the lowest number of bacterial colonies. A study on the effects of SO_2 pollution from a "sour gas" plant on various sites within the vicinity (2.8 km, 6.0 km, and 9.6 km from) of the pollution source found that there were significant decreases in bacterial and increases in fungal contributions to the total soil microbial respiration in the site that was closest to the plant (Bewley and Parkinson 1985). This study shows that S addition could cause different effects on soil fungal communities as compared to soil bacteria.

Soil mycorrhizal fungi are capable of oxidizing sulfur into thiosulfate and sulphate and the rates at which these processes can be carried out will depend on the amount of available carbon in the soil (Grayston and Wainwright 1988, Kertesz and Mirleay 2004). It is possible that

the increased vegetation growth in the plots increased the amount of litter fall to the forest floor providing the fungi a large source of available carbon (Jung and Chang 2012, Wieder et al. 2010). This increase, accompanied by the increased amount of S to the soil creates ideal conditions for fungi capable of oxidizing sulfur so this could explain the higher number of fungal colonies that were found in the +S plots. Also S in the soil is used by plants to synthesize proteins, some essential vitamins, and is needed for cysteine biosynthesis (Kertesz and Mirleay 2004, Schnug and Haneklaus 1998). It is possible that the demand for available S by plants, due to their increased growth, has created a microbial selection pressure. Microbes are responsible for the S cycle in soil and therefore microbes that can facilitate this increased need for S will be favoured. This offers a potential explanation for the observed increase in the abundance of fungal colonies in the +S treatment.

Another explanation could be that certain species of fungi have their growth, abundance, and respiration negatively affected when exposed to SO_4^{2-} (Dursun et al. 1996, McLeod 1988). *Phoma exigua* and *Phoma macrostona* had their respiration inhibited by S while *Cladosporium cladosporioides* and *Coniothyrium quercinum* were not affected by the same S exposure (Dursun et al. 1996). When fungi are exposed to S there is selective inhibition occurring which results in higher abundance of fungi that are less sensitive to S addition. This effect would decrease competition among fungal species and make new environmental niches and nutrients available within the soil, creating an opportunity for fungi that are not inhibited by S to rapidly increase their numbers and abundance. It is possible that the high number of culturable fungal colonies recorded all belonged to fungal species that were not inhibited by S and were able to increase their abundance. Unfortunately, I do not have species information on the fungal communities found in the soil so I am unable to determine if there are fungal species that would be sensitive or

resistant to S addition in the experimental plots. To make strong conclusions for or against S inhibition on fungal species sequencing of fungal genes in the soil would be required.

3.4.3 Irion-reducing and denitrifying bacteria

There were no significant differences in the number of iron-reducing bacteria among the treatments. It has been shown that long-term N fertilization promotes iron reduction and has a strong influence on iron-reducing bacterial communities. In an iron-reducing system elemental S is required which means when S is added to a system there should be an increase in iron-reducing bacteria, which is contrary to my results (Ding et al. 2015, Sugio et al. 1985). It is possible, that similar to my results from the bacterial plates, the treatment application rates were not high enough to evoke changes and the duration of N and S addition was not long enough. A study that showed that long-term N fertilization caused significant increases to the number iron-reducing bacteria was established in 1990 and samples were not collected until 2010 (Ding et al. 2015). Perhaps, given additional time there could be increases in the iron-reducing bacteria population size.

The +N caused a significant increase in the number of denitrifying bacteria while +S and +NS caused a significant decrease, when compared to the CK. These results confirmed the hypothesis that there would be an increase in this type of bacteria in the +N treatment. This pattern suggests that when N and S were added simultaneously, the response of denitrifying bacteria was different than under separate elevated inputs. It has been shown that S can increase or suppress the effects of N, when terrestrially deposited in combination. My results indicate that the addition of S suppressed the effects of N on denitrifying bacteria (Sogn and Abrahamsen 1998). The decrease in denitrifying bacteria in the +S treatment is expected as without the

presence of N it is unlikely there would be a bacterial response in this highly N limited environment.

The process of denitrification is the transformation of nitrate (NO_3^-) to either N_2 or nitrous oxide (N_2O). It is mediated by microbes, mainly bacteria, but can also include some species of archaea and fungi (Philippot et al. 2007). The majority of denitrifying bacteria are found in the upper 5 cm of the mineral soil (Mergel et al. 2001). The rates of denitrification have been found to significantly increase with NO_3^- availability (Merrill and Zak 1992). The form of N used for +N to the plots was NH_4NO_3, which when combined with water will dissociate into ammonium (NH_4^+) and NO_3^- ions. This increase in NO_3^- concentration in the +N plots would explain why I am seeing significant increases in the number of denitrifying bacteria. The denitrifying bacterial species have greater N availability and have increased their abundance in the treatment plot.

3.5 Conclusions

My study confirmed that soil bacteria, including iron-reducing bacteria, were resistant to N and S addition in the study site in the N limited boreal forest ecosystem, even after 9 years of addition. It was also confirmed that increasing available NO_3^- in a soil will increase the abundance of denitrifying bacteria. The number of soil fungal colonies was significantly affected, indicating that fungi were more sensitive to N and S treatments. Note that my results were based on plating and MPN techniques which only represent one aspect of the soil microbial community. I would be able to make stronger conclusions if DNA sequencing could be conducted on the fungi in the site and on the MPN counts. Further research would allow a greater understanding of the impact of N and S addition on the soil microbial community. Additionally,

the analysis only involved a single soil type in a boreal forest ecosystem; therefore these findings need to be tested in additional soil types and other ecosystems.

Table 3-1 Number of bacterial and fungal colony forming units in 1 gram of dry weight soil (CFU/g) and morphologically distinct isolates, in response to N and S addition, in a mixedwood forest in the Athabasca oil sands region in northern Alberta, Canada. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition. Values found in parentheses are standard errors of the means.

Treatment		Mean Colonies (CFU/g)	Mean isolates
CK	Bacteria	8.52×10^7 (2.50×10^7)	6.2
	Fungi	1.68×10^5 (3.55×10^4)	5.6
+N	Bacteria	3.11×10^8 (1.37×10^8)	7.2
	Fungi	3.38×10^5 (2.55×10^4)	5.9
+S	Bacteria	1.07×10^7 (1.59×10^6)	7.0
	Fungi	6.11×10^5 (1.85×10^5)	4.9
+NS	Bacteria	1.46×10^8 (4.23×10^7)	6.9
	Fungi	3.49×10^5 (2.50×10^4)	6.0

Table 3-2 Gram positive soil bacteria colony morphology obtained by gram staining and plating, in response to N and S addition, in a mixedwood forest in the Athabasca oil sands region in northern Alberta, Canada. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition.

Treatment	Colony description
CK	Gram positive tetracocci; punctiform, convex, entire margins, milky white
	Gram positive rods; small, circular, convex, entire margins, milky white
	Gram positive cocci; medium, circular, flat, entire margins, butter yellow
	Gram positive rods; circular, convex, entire margins, butter yellow
	Gram positive double rods; filamentous, flat, filamentous, clear
	Gram positive rods; punctiform, convex, entire margins, white
	Gram positive diplococci; irregular, convex, entire margins, white with sheen
	Gram positive rods; punctiform, convex, entire margins, off white
	Gram positive rods; small, convex, circular, entire margins, white
+N	Gram positive cocci; circular, flat, entire margins, butter yellow
	Gram positive rods; filamentous, raised, filamentous, off white
	Gram positive tetracocci; punctiform, convex, entire margins, tinge of yellow
	Gram positive rods; circular, raised, entire margins, off white, small to medium
	Gram positive diplococci; irregular, raised, lobate, shiny white
	Gram positive rods; large, filamentous, flat, filamentous, white with

	projections
	Gram positive cocci; circular, convex, entire margins, off white
	Gram positive rods; medium, circular, umbonate, entire margins, off white
+S	Gram positive rods; medium, irregular, raised, undulate, opaque
	Gram positive rods; circular, flat, entire margins, dark orange
	Gram positive rods; large, filamentous, flat, filamentous, white with projections
	Gram positive diplorods; punctiform, raised, entire margins, opaque
	Gram positive rods; medium, circular, convex, entire margins, off white
	Gram positive rods; irregular, raised, lobate, large, opaque and off white
	Gram positive rods; medium, circular, flat, curled, white, opaque
	Gram positive rods; circular, convex, entire margins, small, opaque
+NS	Gram positive cocci; circular, flat, entire margins, yellow
	Gram positive rods; filamentous, flat, filamentous, white halo, clear center
	Gram positive rods; medium, circular, convex, entire margins, bright yellow
	Gram positive rods; small, irregular, flat, lobate, opaque and off white
	Gram positive rods; circular, convex, entire margins, milk white
	Gram positive rods; punctiform, convex, entire margins, clear/pale white
	Gram positive diplorods; irregular, raised, lobate, shiny and white
	Gram positive rods; convex, small circular, entire margins, yellow, opaque
	Gram positive diplococci; white, small, circular, convex, entire margins

Table 3-3 Gram negative colony morphology and species identification, obtained by plating, gram staining and API strips, in response to N and S addition, in a mixedwood forest in the Athabasca oil sands region in northern Alberta, Canada. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition.

Treatment	Colony description	Species
CK	Gram negative rods; punctiform, convex, entire margins, yellowish tinge	*Photobacterium damselae*
+N	Gram negative diplococci; circular, convex, entire margins, small, deep yellow/orange	*Chryseobacterium indologenes*
	Gram negative tetracocci; circular, convex, entire margins, small, off white and slimy	*Photobacterium damselae*
	Gram negative rods; circular, convex, entire margins, yellow	*Photobacterium damselae*
+S	Gram negative rods; large, circular, flat, entire margins, pale white/brown	*Photobacterium damselae*
	Gram negative rods; convex, circular, entire margins, opaque, white	*Photobacterium damselae*
	Gram negative rods; convex, circular,	*Chrysobacterium*

	entire margins, opaque, white	*indologenes*
	Gram negative rods; medium sized, convex, circular, entire margins, white and shiny	*Photobacterium damselae*
	Gram negative rods; irregular, flat, lobate, off white	*Sphingomonas paucimobilis*
	Gram negative cocci; punctiform, convex, entire margins, bright yellow	*Ochrobactrum anthropic or Aphingomonas paucimobilis*
+NS	Gram negative rods; filamentous, flat, filamentous, white halo and clear center	*Photobacterium damselae*
	Gram negative rods; large, circular, off white slim, convex, entire margins	*Photobacterium damselae*
	Gram negative cocci; filamentous, raised, filamentous, off white	*Psychrobacter phenylpyruvicus*
	Gram negative rods; medium, irregular, flat, curled, opaque, off white	*Pseudomonas aeruginosa*
	Gram negative diplococci; small, circular, convex, entire margins, milky white	*Vibrio alginolyticus*

Table 3-4 Most probable number (MPN) of iron-reducing and denitrifying bacteria, in response to N and S addition, in a mixedwood forest in the Athabasca oil sands region in northern Alberta, Canada. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition. Values found in parentheses are standard errors of the means.

Treatment	Bacteria Type	MPN
CK	Iron reducers	359.75 (145.30)
	Denitrifiers	105.00 (12.75)
+N	Iron reducers	102.00 (48.79)
	Denitrifiers	30.00 (5.76)
+S	Iron reducers	39.25 (8.87)
	Denitrifiers	54.25 (13.99)
+NS	Iron reducers	177.75 (47.66)
	Denitrifiers	590.00 (251.10)

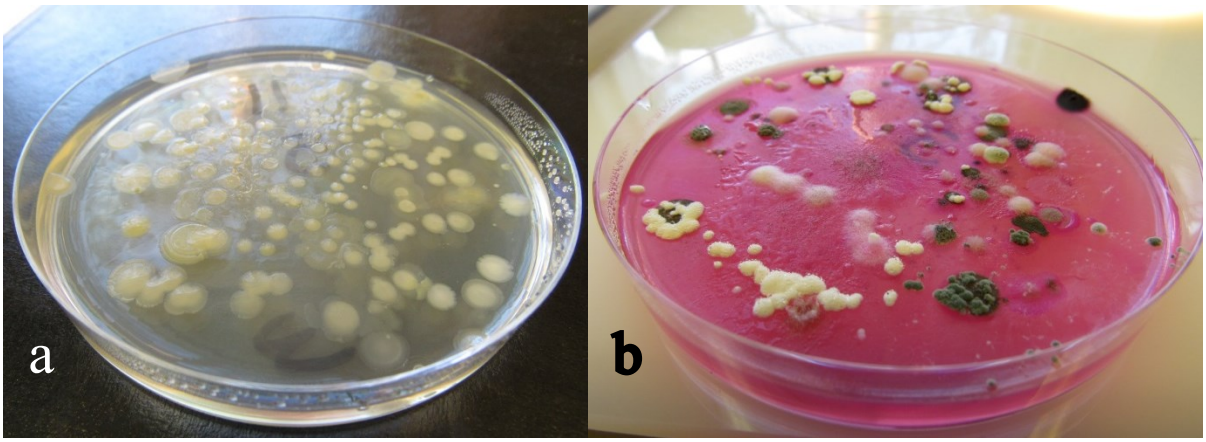


Figure 3-1 Colony growth of a) bacterial species on plate count agar and b) fungal species on rose bengal agar.

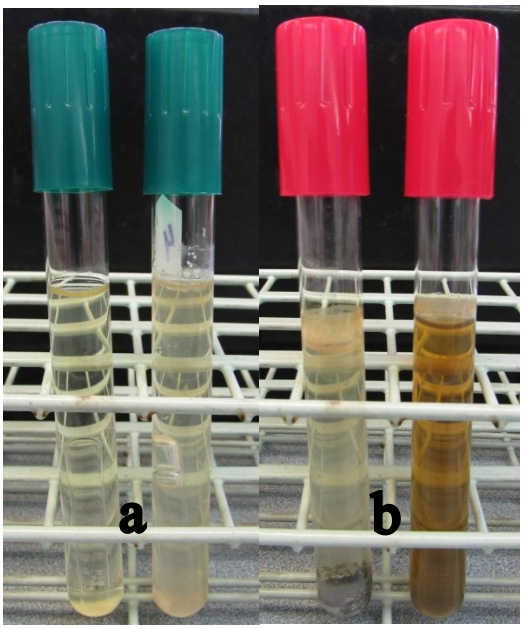


Figure 3-2. Negative (left tube) and positive results (right tube) for MPN (most probable number) for a) denitrifying bacteria and b) iron-reducing bacteria.

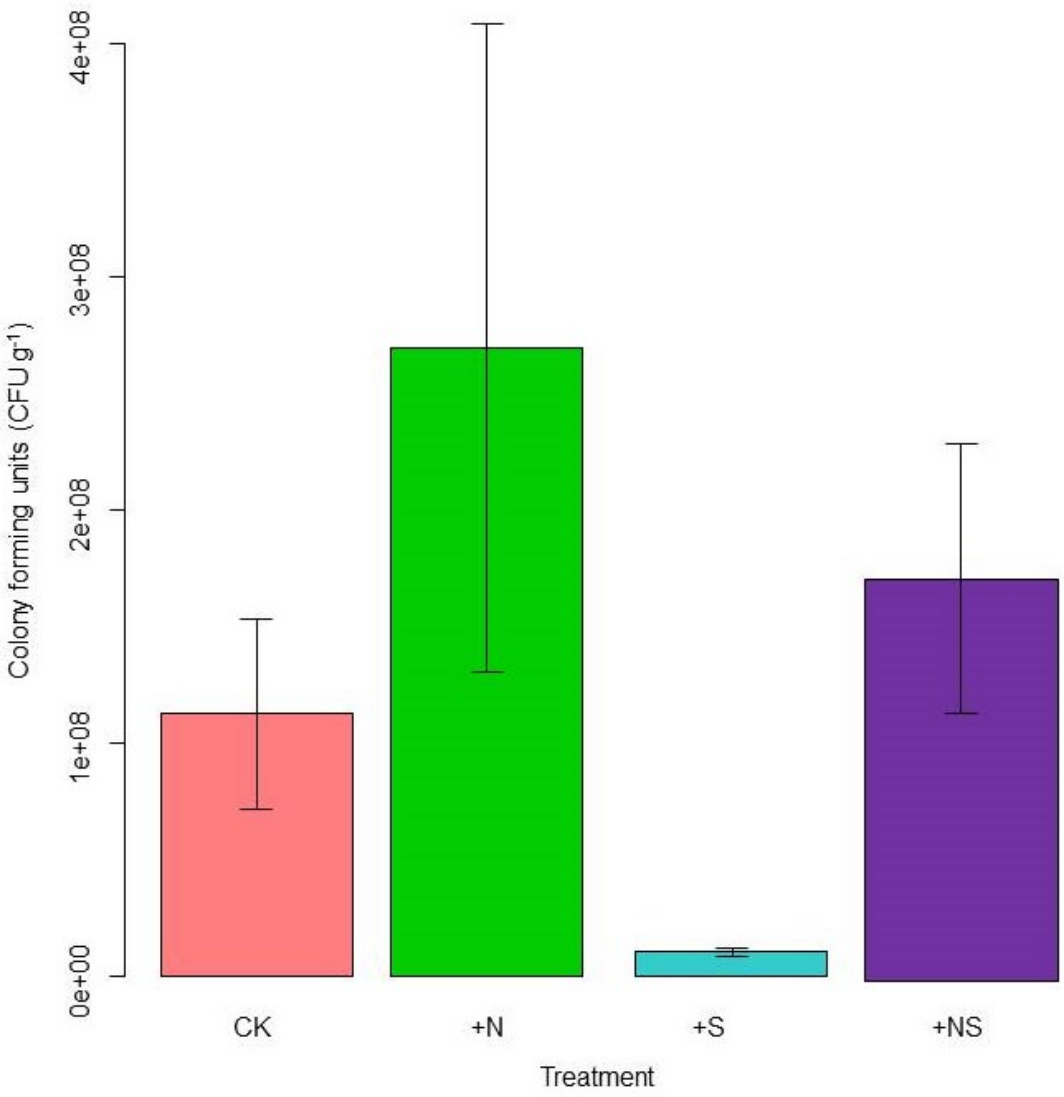


Figure 3-3 Soil bacteria colony forming units per gram of soil (CFU g^{-1}) in response to N and S addition, in a mixedwood forest in the Athabasca oil sands region in northern Alberta, Canada, obtained from plate counts. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition.

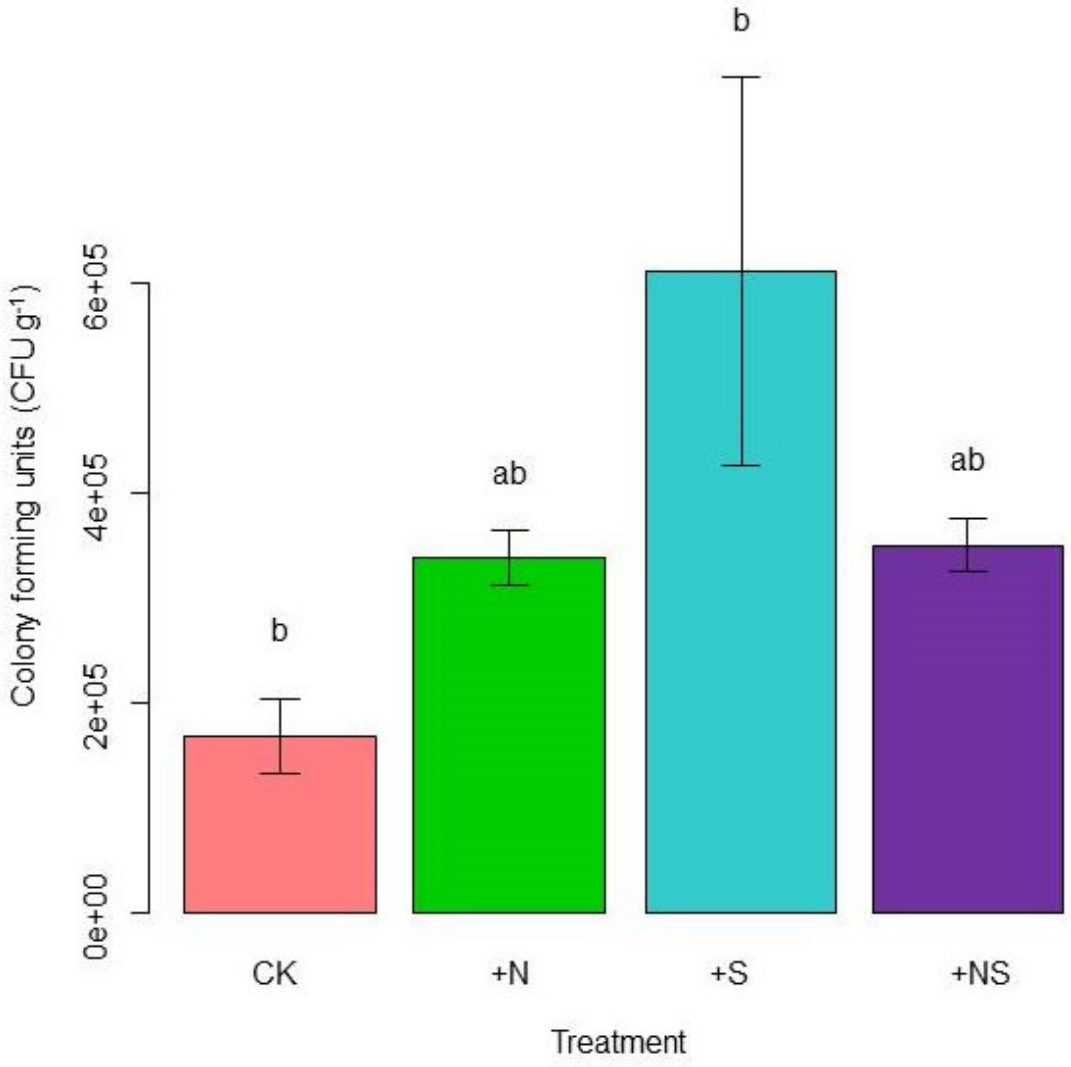


Figure 3-4 Soil fungal colony forming units per gram of soil (CFU g^{-1}) in response to N and S addition, in a mixedwood forest in the Athabasca oil sands region in northern Alberta, Canada, obtained from plate counts. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition. Lowercase letters indicate a significant difference at α=0.1.

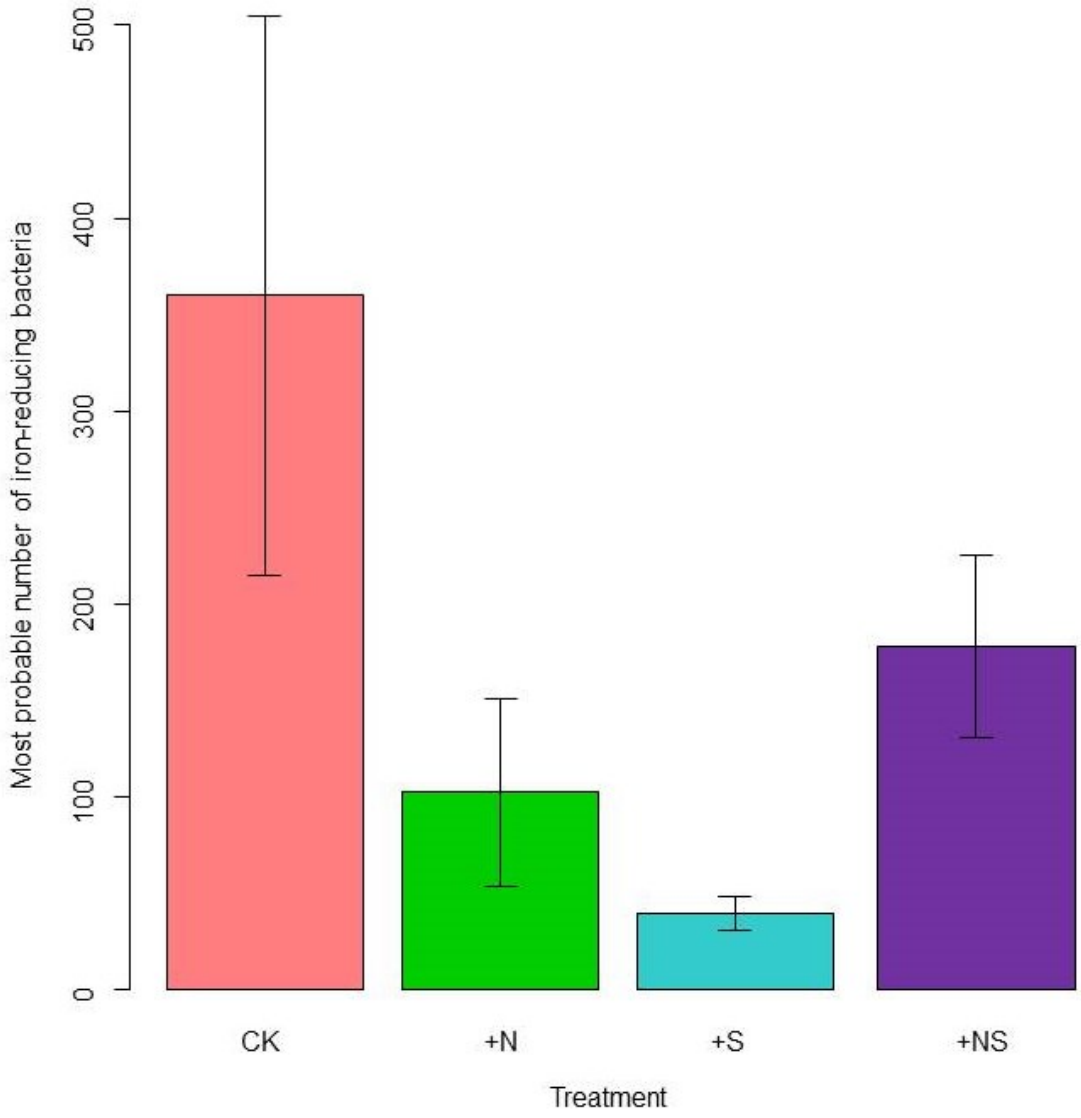


Figure 3-5 Most probable number of soil iron-reducing bacteria in response to N and S addition, in a mixedwood forest in the Athabasca oil sands region in northern Alberta, Canada. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition.

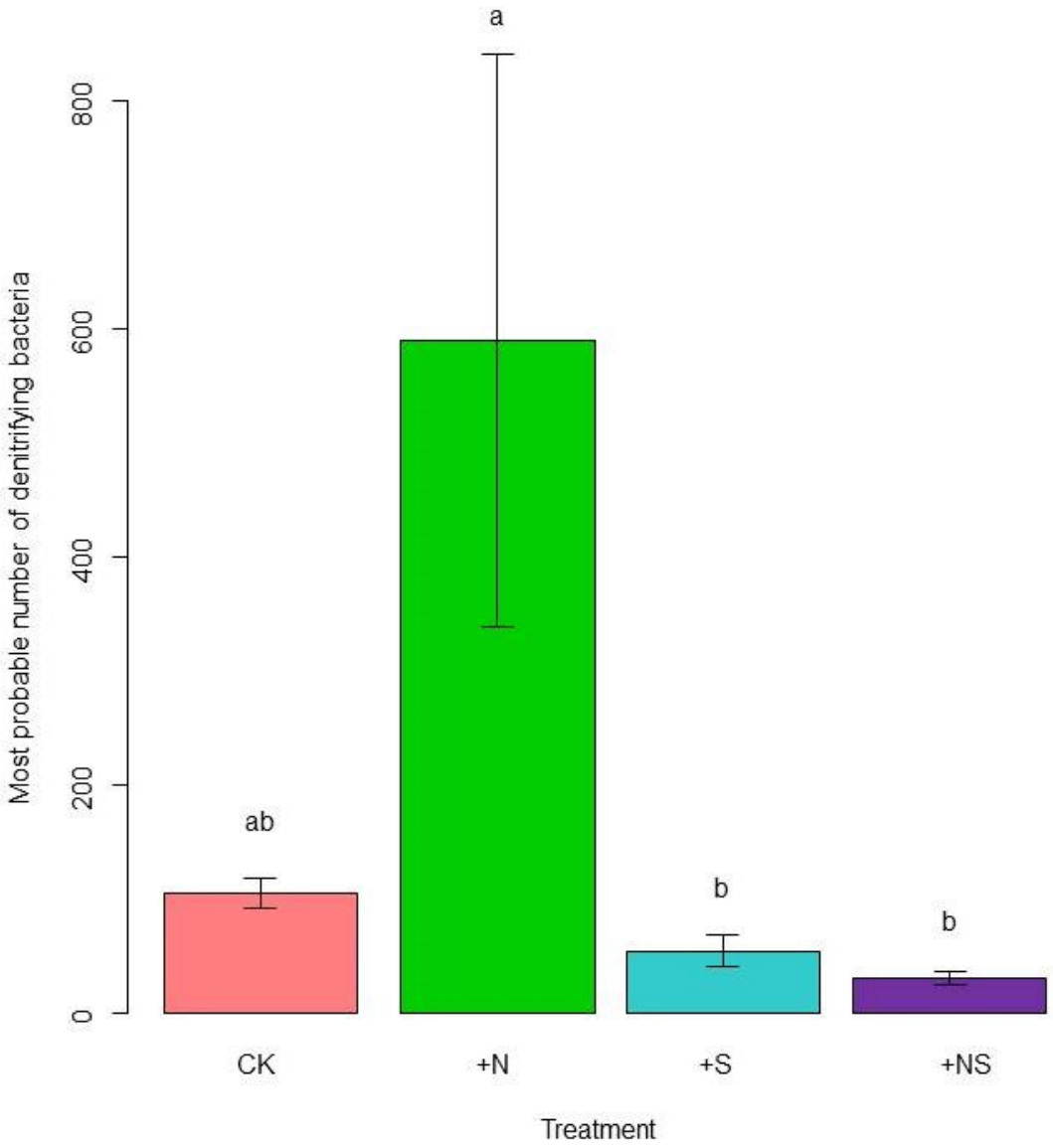


Figure 3-6 Most probable number of soil denitrifying bacteria in response to N and S addition, in a mixedwood forest in the Athabasca oil sands region in northern Alberta, Canada. Codes of CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition. Lowercase letters indicate a significant difference at α=0.1.

Chapter 4: Ten years of experimental nitrogen and sulfur addition do not cause nitrogen saturation in a boreal forest in northern Alberta, Canada

4.1 Introduction

The global amount of reactive nitrogen (N) emitted into the atmosphere has increased three to five times in the last one hundred years (Denman et al. 2007). These increases are attributed to anthropogenic activities including the use of N fertilizers, fossil fuel combustion, and cultivation of N-fixing plants, all of which have caused disruption to the global N cycle (Galloway et al. 2004, Vitousek et al. 1997). Similarly the global emission of sulfur dioxide (SO_2) has tripled since the preindustrial era (Dentener et al. 2006). The increased emissions of N and S have been exacerbated by anthropogenic activities in large areas of North America, Europe and Asia and it has been suggested that global emissions will continue to increase until 2050 (Galloway et al. 2004). Alberta is the largest emitter of SO_2 and nitrous oxide (NO_2 and NO, generically referred to as NO_x) in Canada, largely due to the mining and refining of bitumen that occurs in the northern part of the province, particularly in the Athabasca Oil Sands Region (AOSR) (Whitfield et al. 2009). The SO_2 emissions were highest in the early 1980s but since have decreased due to legislation to address atmospheric pollution and acid rain (Hazewinkel et al. 2008). Conversely NO_x emissions in the AOSR have seen a steady increase in the last 15 years (Aherne and Shaw 2010, Hazewinkel et al. 2008). The amounts of SO_2 and NO_x being emitted in the AOSR between 2003 and 2008 were 274-314 Mg S day^{-1} and 148-200 Mg N day^{-1} (Watmough et al. 2014).

High rates of emissions of NO_x and SO_2 in the oil sands have exposed the surrounding boreal forests to significantly elevated levels of N and S deposition in the last ten years (Gosselin

et al. 2010, Laxton et al. 2010, Whitfield et al. 2009). While the addition of N can initially be favorable, as vegetation growth is limited by low levels of soil N in the boreal forest (Jung and Chang 2012), there is cause for concern as N and S deposition can eventually cause soil acidification, loss of basic cations, nutrient imbalances, aluminum (Al) toxicity, and changes in cation exchange capacity (CEC) and accelerate loss of biological diversity (Laxton et al. 2010, Ok et al. 2007, Watmough et al. 2014, Whitfield et al. 2009, Vitousek et al. 1997). Some forest soils in the AOSR are sensitive to soil acidification due to coarse-texture, low exchangeable base cations, cation exchange capacity and low buffering capacity (Watmough et al. 2014, Whitfield et al. 2009). While there has been considerable research in Canada and Alberta involving N deposition, there are no long-term deposition experiments currently being conducted in the AOSR, with the exception of this study. There are still major knowledge gaps in fully understanding cumulative long-term ecosystem impacts of elevated N and S deposition and defining critical loads in the AOSR (Laxton et al. 2010, Liu et al. 2011). There is a lack of understanding on the rate of deposition associated with alleviation of N limitation, N saturation, and forest decline. It is vital to enhance understanding of the ecosystem responses and carrying capacity in the AOSR to long-term N and S deposition, so we can avoid N saturation, acidification and forest decline in the northern boreal forest ecosystem. This information can only be gathered through long term experiments such as my research.

Aber et al. (1989) established a model for determining the status of an ecosystem in response to long term N deposition that has four stages: N limitation, alleviation of N limitation, N saturation, and forest decline. Nitrogen saturation was defined by Fenn et al. (2003) as "the long-term removal of N limitations on biotic activity, accompanied by a decrease in the capacity to retain N". It is important to monitor indicators in the ecosystem that are sensitive to changes

caused by N and S deposition as previous research has shown that after 4 years of deposition a boreal forest stand in northern Alberta was still in the initial stages of N limitation and alleviation of N limitation (Jung and Chang 2012). Nitrogen is stored in the soil in the form of soil organic matter (SOM), by being immobilized by microbes and plants, and by being incorporated into the CEC (Silver et al. 2008). These forms of stored N account for 90% of the total storage in a forest ecosystem (Silver et al. 2008, Templer et al. 2008). However, there is a limit to the amount that can be accommodated by soil storage, and when N inputs exceed that threshold the system will become N saturated. It is important to understand the capacity of an ecosystem to store N in order to avoid potential N saturation and the associated negative effects.

The effects of N and S deposition on ecosystems can be assessed by examining the base saturation and molar base cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) to Al^{3+} ratio in the mineral soil (Watmough et al. 2014). When elevated N input ceases to be immobilized by plants and soil microbes, the nitrate (NO_3^-) transport will lead to leaching of base cations, an increase in soluble Al, and soil acidification which will have effects on growth and diversity in the ecosystem on a broad scale (Bowman et al. 2008). Also N addition will eventually lead to leaching of NO^{3-} and N_2O being emitted from the soil into the atmosphere, so these are useful indicators of N saturation (Laxton et al. 2010). The production of NO_3^- through net nitrification is a critical process because the dynamics of this anion, including its mobility and loss, is what is driving the negative effects associated with N saturation (Aber et al. 1992). Nitrogen deposition will increase the rates of nitrification and denitrification leading to increased emissions of N_2O, which is a concern as N_2O has 298 times the global warming potential as that of carbon dioxide (CO_2) (Zhu et al. 2015).

To improve the understanding of boreal forest ecosystem responses to N and S deposition in AOSR, its potential for N saturation, and advancing the understanding of potential critical loads, a simulated deposition experiment was established in 2006. It was found that after 4-5 years of treatment, there was no difference in the amounts of NO_3^- being leached between the N deposition and control treatments, exchangeable Ca^{2+} and Mg^{2+} in the soil decreased with the N and S addition, but there were no changes in the pH, total organic carbon (C) and N, dissolved organic C and N (Jung and Chang 2012, Hu et al. 2013). It was concluded that this experimental site was between the first and second stages of the Aber et al. (1989) model for N deposition. In this study, I will examine parameters including base cation concentrations, leaching of available N, and emissions of N_2O, to determine if an additional 4-5 years of simulated deposition since Jung and Chang's (2012) research, has changed the status of the ecosystem and caused N saturation. I hypothesize that after 10 years of N and S treatments (1) the soil concentration of basic cations will decrease because elevated concentrations of SO_4^{2-} and NO_3^- facilitate leaching from the soil leading to an increase in Al concentration; (2) the amount of available N being leached below the rooting zone in the soil will increase because the soil will be N saturated and no longer be able to accommodate this nitrification product; and (3) the amount of N_2O emissions will increase. Nitrogen and S addition have a wide range of effects on an ecosystem but the larger concern is the eventual soil acidification and N saturation. It is important to understand how the AOSR will be affected so we can define critical loads and advise possible regulatory framework for air emissions of N and develop best management practices for affected industries.

4.2 Materials and Methods

4.2.1 Research site

The majority of surface mining and upgrading facilities are located within 100 km of the city of Fort McMurray, Alberta, so the research site (56.1°N 110.9°W) was established 100 km south of the city in an area not considered to be directly influenced by N and S emissions (Bytnerowicz et al. 2010, Jung and Chang 2012). The site is located in a natural boreal forest in the AOSR with mean annual temperature, relative humidity, and precipitation of 0.7 °C, 68%, and 456 mm, respectively (Environment Canada 2010). The two dominant tree species are trembling aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) and the dominant shrub species are prickly rose (*Rosa acicularis*) and lowbush cranberry (*Vaccinium oxycoccos*) (Jung and Chang 2012, Jung et al. unpublished). Based on the Canadian system of soil classification the soil is classified as mainly Gray Luvisols (Soil Classification Working Group 1998).

4.2.2 Experimental design

The experiment began in 2006, with a 2 x 2 factorial design with blocking for a total of 16 plots of 20 x 20 m. Four treatments were set up and assigned at random within each block: control (CK), N addition (+N, 30 kg N ha^{-1} yr^{-1} as NH_4NO_3), S addition (+S, 30 kg S ha^{-1} yr^{-1} as Na_2SO_4), and NS addition (+NS, 30 kg N ha^{-1} yr^{-1} as NH_4NO_3 and 30 kg S ha^{-1} yr^{-1} as Na_2SO_4). The experimental application rates were based on the projected increases in N and S deposition due to development and expansion of the oil sands industries in the AOSR and to accelerate the effects of acid deposition (Hu et al. 2013, Jung et al. 2012). The N and S addition first occurred in 2006 and have continued with applications in the growing season every year since then. From

2006 to 2008 the N and S were applied once in the beginning of the summer. Starting in 2009, the N and S were applied in three equal splits over the growing season to better depict the natural deposition process (Jung and Chang 2012). The NH_4NO_3 (granule) and Na_2SO_4 (powder) were broadcast applied below the canopy.

4.2.3 Soil sampling and chemical analysis

Soil sampling was conducted on October 23, 2015. The forest floor (LFH) was collected by hand (roughly 50 g) and mineral soil samples in 15 cm increments from 0 to 60 cm were collected using a soil core auger from three different locations in each plot. The soil samples from the same depth within each plot were combined to form a composite sample. Soil temperature and soil moisture were recorded at the time of collection using a thermometer and the Fieldscout TDR 300 Soil Moisture Meter (Spectrum Technologies Inc.). Samples were transported back to the laboratory in a cooler with ice packs. Fresh soil samples were homogenized, passed through a 2 mm sieve, and stored in a freezer until analysis. All analysis was completed within 2 weeks of sampling. A sub-sample of each sample was used to analyze ammonium (NH_4^+) and nitrate (NO_3^-) as described below.

The remainder of the samples were air-dried at room temperature for 96 hours and used for pH analysis. Soil pH was measured with a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, Ma, USA) using 10 g of air-dried soil in 40 mL of deionized water for forest floor samples or 20 mL of deionized water for mineral soil samples. Each forest floor and soil sample was further ground with a ball mill and used for total C and N analysis on a Carlo Erba NA 1500 elemental analyzer (Carlo Erba Instruments, Milano, Italy). Exchangeable cations, including Ca^{2+}, Mg^{2+}, K^+, Na^+ and Al^{3+} were determined after the forest floor and soil samples were extracted with 1 mol L^{-1} NH_4Cl at a ratio of 5 g of mineral soil samples or 10 g of forest floor

samples, to 100 mL extractant. After filtration using Whatman No. 42 filter paper, the filtrates were analyzed using a Perkin-Elmer Elan 6000 quadrupole ICP-MS (Shelton, CT). The Ca/Al ratio was calculated with exchangeable Ca^{2+} and Al^{3+} on a molar basis.

4.2.4 Soil solution sampling and analysis

In 2007, zero-tension lysimeters made of stainless steel were installed at 15 cm and 45 cm soil depths to collect soil solution (Jung and Chang 2012). Each plot had two soil pits that each contained one lysimeter at 15 cm and 45 cm. A resin based lysimeter (Fig.4-1) was used based on the design in Susfalk and Johnson (2002). Soil lysimeters were placed in the plots on June 4, 2015 using the existing soil pits that had been excavated for the zero-tension lysimeters. The lysimeters were placed at 15 cm and 45 cm as to be within the main rooting zone. They were removed on December 9, 2015. Lysimeters were placed in a cooler and transported back to the laboratory. They were then placed in the freezer until January 6, 2016 for storage until extraction could be performed. A 2 M KCl was used to extract (50 mL added to the ion exchange resin (IER) and shaken for an hour, repeated once) available ammonium (NH_4-N) and nitrate (NO_3-N) from the lysimeters. Nitrate concentrations were determined using the colorimetric method with vanadium, sulfanilamide, and N-(1-naphthyl) ethylenediamine (Miranda et al. 2001). The Indophenol blue colorimetric method was used for determining NH_4^+ concentrations (Keeney and Nelson 1982). To quantify the NH_4-N and NO_3-N colorimetric concentrations a Genesys 10 UV-Vis Spectrophotometer machine was used.

4.2.5 Gas sampling and analysis

Plastic collars that were fitted to gas chambers were placed in each plot at three random locations on May 22, 2014 to allow the soil to recover from the disturbance prior to the first gas sampling. A total of 48 collars (4.3cm height, 10.9 cm inner diameter) were installed about 3 cm into the soil and then kept in place for the duration of the study in order to avoid soil disturbance. Gas efflux was measured using static chambers (Hutchinson and Mosier 1981, headspace 10 cm, 0.00104 m^3 volume) on June 7, July 6, August 10, and September 5 in 2014 and on June 5, July 9, August 5, and September 3 in 2015. Gas samples (20 mL) were collected at 0 (ambient condition), 10, 20 and 30 minutes after position of the static chambers on the collars through a rubber septum. Samples were stored in pre-evacuated 10 mL soda glass Isomass Exetainers®. Gas samples were collected on fair weather days to avoid confounding effects of soil moisture and temperature. The N_2O concentrations in the collected gas samples were analyzed using a Varian CP-3800 gas chromatograph (GC, Varian Canada, Mississauga, Canada) within 2 days of collection.

The N_2O fluxes were calculated based on N_2O concentrations using equations 1 and 2 (Nakayama 1990). A mole of an ideal gas at standard temperature (0 °C) and pressure (101.3 kPa) has a volume of 22.4 L, which can be converted to 44.6 moles m^{-3}. This ideal volume is then corrected for the actual air temperature at the time of sampling and used to calculate the efflux. Flux rates were calculated from the slope of the linear regression lines.

$$\text{Efflux} = \Delta C \times T \times V / \Delta t \times A = \Delta C \times T \times h / \Delta t \qquad (1)$$

$$T = 44.6 \text{ mol } m^{-3} \times 273.15 / (273.15 + T_A) \qquad (2)$$

Where ΔC is the change in N_2O concentration between two sampling intervals (μmol mol^{-1}), T is temperature adjustment for molecular volume of gas (mol m^{-3}), V is the volume of the static gas chamber (0.00104 m^3), A is the area of ground covered by the Hutchinson chamber (0.0092 m^2), h is the height of the static chamber (0.1 m), Δt is the time interval between samplings (1800 s), and T_A is actual air temperature (°C). Soil temperature and volumetric water content were collected at the time of each sampling (Thermometer, Fieldscout TDR 300 Soil Moisture Meter by Spectrum Technologies Inc.).

4.2.6 Statistical analysis

All statistical analyses were conducted with the R software (version 3.2.3). Assumptions of normality of distribution and homogeneity of variance were checked using Kolmogorov-Smirnov and Levene's tests. All assumptions were met. The means and standard error of the mean (SEM) were calculated for available NH_4 and NO_3, TC, TN, exchangeable cations, and pH. Analysis of variance (ANOVA) was used to determine the treatment (+N vs +S addition) effects on these soil parameters. The month of each gas sampling was considered a repeated measures variable for determining seasonal variation in 2014 and 2015. The significant level for all statistical tests was set at $\alpha = 0.1$ due to the high spatial variability of the measured parameters, limited replications and to reduce the possibility of a type II error.

4.3 Results

4.3.1 Effects of N and S addition on soil properties

In the forest floor and mineral soil, ten years of N and S treatments did not cause significant changes ($P > 0.1$) to concentrations of soil exchangeable cations when compared to the CK (Table 4-1). The TC and TN in the forest floor and at various depths in the mineral soil were also not significantly affected ($P > 0.1$) by ten years of +N and +S addition (Table 4-2). There was a general pattern that the treatments tended to cause a decrease in TC and TN in the forest floor and mineral soil when compared to the CK. In addition, another pattern was that the +N and +S treatments tended to cause a decrease in pH of the forest floor and mineral soil (Table 4-2).

4.3.2 Effects of N and S addition on N leaching

The concentration of NH_4-N in the soil at 15 cm and 45 cm in all the plots was much higher when compared to the NO_3-N concentrations ($P < 0.05$), indicating that there were higher concentrations of ammonium compared to nitrate in the studied soil (Table 4-3). However, the treatments did not affect the amount of NO_3-N and NH_4-N leached at 15 cm or 45 cm ($P > 0.1$). And while the results were not significant, there was a general pattern that +N tended to increase the concentrations of NH_4-N and NO_3-N below 45 cm in the soil.

4.3.3 Effects of N and S addition on nitrous oxide emission rates

Both positive and negative values for nitrous oxide (N_2O) efflux were found in all plots in 2014 and 2015, indicating that there was both uptake and emission occurring within the same plot (Fig. 4-2). This result indicates that there was a high degree of spatial variability within individual plots. The standard error of the mean (SEM) was larger than the mean soil N_2O

emissions, which again shows the high degree of variability in the recorded soil emissions (Table 4-4). Statistical analysis showed that there were no significant differences found between the treatments and the CK ($P > 0.1$). However, because of the high degree of variability, strong conclusions cannot be made on how +N, +S, and +NS treatments affected the soil N_2O efflux.

4.4 Discussion

In this experiment, 10 years of N and S treatments did not significantly affect soil cation concentrations, pH, and amount of N leaching. This rejects all of the hypotheses and while it was unexpected, the results are consistent. These results suggest that the experimental site was not N saturated and therefore inputs did not exceed the amounts required by vegetation and soil microbes for growth (Aber et al. 1989). Previous research conducted after 4 years of N and S treatments concluded that this experimental site was likely still in stage 1 or the early beginnings of stage 2, so largely it was still experiencing N limitation (Jung and Chang 2012). My results indicate that an additional 5 years of N addition did not change the N status. Because the vegetation in the boreal forest is N limited it is adapted to be very efficient at N uptake and utilization, leaving low amounts of available N in the soil pool (Laxton et al. 2010, Lovett and Goodale 2011). Previous research in this study site found that there was increased N uptake by trees followed by increased growth when exposed to N deposition, showing that the vegetation in my site was utilizing the N being added (Jung and Chang 2012). It has also been shown that mosses can derive N from the soil or wet deposition, which may allow for increased competition for N in a wide range of ecosystems; if environments are limited by N it could have further increased competition for N (Ayres et al. 2006). The utilization of excess N by mosses and

vascular plants in the treatment plots is a possible explanation as to why I did not observe significant effects of N leaching and emissions from elevated N and S addition.

Many factors, including elevation, stand age and composition, and soil properties can cause an ecosystem to be more susceptible to N deposition (Fenn et al. 1998). A forest with higher elevation will be prone to higher deposition rates through occult deposition when compared to low lying sites, and mature forests have lower nutrient requirements which will reduce the amount of N that can be immobilized and stored (Lovett et al. 1982, Nilsson et al. 1998). The experimental site does not have any characteristics that would cause a predisposition to accelerated N saturation. In addition, forests soils found in the boreal forest ecosystem in Alberta are formed from sedimentary parent material and are underlain by glacial deposits, sandstones, shales, and bituminous sands; this causes the soil to be characterized as calcareous and well drained (Holowaychuk and Fessenden 1987). The soil types commonly found in the AOSR were evaluated for their sensitivity to acidification. It was found that Brunisols and Organic soils were most at risk, followed by Luvisols and Solonetzic classes which had an intermediate sensitivity, and the most resistant soil series were the Organic Cryosols (Holowaychuk and Fessenden 1987). The soils in my experimental site are Gray Luvisols with calcareous parent material and calcium carbonates in the C horizons, which have an intermediate level of risk for acidification (Holowaychuk and Fessenden 1987).

In addition to N and S treatments, base cations are being emitted and deposited into the surrounding soil, mainly in the form of dust from mining activities in the AOSR (Fenn et al. 2015, Watmough et al. 2014). Base cation deposition was similar to and sometimes exceeded the combined inputs of N and S in bulk deposition in the oil sands, particularly during the summer months (Fenn et al. 2015, Watmough et al. 2014). The risk for soil acidification for a site within

3 km of the largest mine was moderated by high base cation deposition even though it had a low base cation weathering rate and high rates of N and S deposition (Watmough et al. 2014). Atmospheric deposition, including base cation deposition, was roughly 100 to 150 $mol_c\ ha^{-1}\ yr^{-1}$ in the AOSR (Aherne 2008). Because base cation deposition has the potential to neutralize soil acidification effects of N and S deposition, the greater concern and focus for future research should be excess N deposition causing eutrophication, and perhaps the focus needs to shift away from terrestrial to wetland ecosystems (Fenn et al. 2015). The addition of base cations from the dust of mining activities in the AOSR will help buffer the potential negative effects from N and S emissions in the AOSR, and could be a possible explanation for the lack of significant changes in soil properties 10 years after experimental N and S treatments.

Soil responses to N deposition depended on the N status of seven European sites with deposition ranging from 11 to 59 kg $N\ ha^{-1}\ yr^{-1}$; sites with low N status retained a majority of the N deposited and NO_3^- leaching was minimal, whereas sites with a high N status retained less of the N deposited (Tietema et al. 1998). Shifting the N status of an ecosystem from low to high can decrease the C:N ratio of the forest floor, which in turn promotes organic matter decomposition, mineralization, and nitrification (Falkengren-Grerup and Diekmann 2002, Gundersen 1998). It was also found that the litter C:N ratio was inversely related to N leaching rates, suggesting that the C:N ratio may be a possible indicator of N saturation. It has been found that when C:N ratios are high there is a low rate of N mineralization, when the ratio is greater than 25 the microbes will immobilize N because they are limited by N, and conversely when the ratio is lower than 25, mineralization will occur because the microbes are limited by C and the amount of N is sufficient (Zhu et al. 2015). My results showed that C:N ratios were below 25 but there were no significant differences between treatments, which may help explain why I did not see higher concentrations

of N leaching in the +N treatment. The results indicate that N and S treatments did not yet cause acidification or N saturation at my study site.

4.4.1 Nitrate leaching

The amount of N being leached showed that there were no significant effects caused by the +N and +S treatments. Similarly, Emmett et al. (1995) used deposition rates of 35 and 70 kg N ha^{-1} yr^{-1} for two years and also reported no significant treatment effects on N leaching. There are, however, other experimental N manipulation studies that reported significant N leaching. Gundersen (1998) used N treatment rates of 10 to 35 kg N ha^{-1} yr^{-1} for 4 years and found that increasing the amount of N input significantly increased the amount of NO_3^- leaching. Stuanes et al. (1995) found that 2 years of N addition, using rates of 12.5 and 34.5 kg N ha^{-1} yr^{-1}, significantly increased NO_3^-leaching in the upper portion of the soil but not below the rooting zone. Note that these studies were conducted in Europe, and many European soils have igneous parent materials and soils that already have a low pH and are more susceptible to N induced changes when compared to soils in the AOSR (Dise and Wright 1992, Falkengren-Grerup and Diekmann 2002). While the ecosystem type and N input rate were variable in these studies, it has been shown that the amount of N input and the soil pH will directly influence the amount of mineral N that was leached. The pH in the experimental site was unaffected by +N and +S treatments and the rates were moderate. This may further help explain the lack of treatment effects on the leaching of mineral N at the research site.

The lysimeters that were used to collect leachate from the soil pits rely on a moderate amount of precipitation input into the soil so there will be movement of soil water through the soil profile. The AOSR is located in the moist subregion of the Boreal Mixedwood ecoregion with a mean annual precipitation of ~400 mm, with a monthly maximum in July (Alberta Environment

and Parks 2015). The lysimeters were installed in 2015 and this happened to be a very dry year in Alberta (Fig. 4-3) (Agriculture and Agri-Food Canada 2015) with the experimental site only receiving between 100-150 mm of precipitation, which was reflected in lower soil moisture content in the 2015 field season (Alberta Environment and Parks 2015). It is possible higher rates of NO_3^- and NH_4^+ leaching would have been observed in the experimental site had the soil been at field capacity and received more precipitation. However, lack of N addition effects on N leaching supports the conclusion that the site had not reached N saturation.

4.4.2 Nitrous oxide emissions

Nitrous oxide soil emissions are one of the largest concerns associated with elevated N deposition, because N_2O is a highly potent greenhouse gas (GHG). This becomes especially problematic as there are global attempts to decrease GHG emissions in order to counteract climate change (Denman et al. 2007). The influence that N deposition has on soil N_2O emissions is variable. However, the prevalent relationship observed is that soil N_2O emissions are positively correlated with the rates of N deposition. Increasing the amount of N being deposited would increase the amount of soil N_2O emissions (Ambus and Robertson 2006, Bowden et al. 2000, Carnol and Ineson 1999, Du et al. 2016, Oura et al. 2001). A study in Japan examining the effects of N treatments, using rates of 10 to 30 kg N ha^{-1} yr^{-1}, found that soil N_2O emissions were variable but ultimately increased (Oura et al. 2001). It was also found that the increases in the soil N_2O emissions were significantly correlated with the soil temperature and moisture (Oura et al. 2001). A laboratory experiment with N inputs of 0 to 75 kg N ha^{-1} yr^{-1} and temperatures of 4, 12, or 20 °C that ran for 112 days, found that 59% of the variability in N_2O emissions was explained by soil temperature and moisture (Carnol and Ineson 1999). They also reported high variability of N_2O emissions within the same treatment and that when soil temperature and

moisture were low the N_2O fluxes were negative (Carnol and Ineson 1999). When the temperature was above 12 °C the simulated fluxes were positive and increased with increasing temperature (Carnol and Ineson 1999). In an alpine ecosystem it was found that increased N_2O emissions were correlated with the rate of N deposition (20 kg N ha^{-1} yr^{-1}) and precipitation (Du et al. 2016). Following N input there was a 68.8% increase in N_2O emission when compared to the control, also increasing the soil moisture led to higher levels of emissions as well (Du et al. 2016). While emission results are variable they have been shown to be dependent on the soil temperature, moisture, the N status of the ecosystem, and the N distribution within the soil profile.

Similar to N leaching, N_2O gas emissions are found to be correlated to the amount of soil moisture, and therefore it is possible that I saw low emission levels because there was a very dry field season in 2014 and 2015 (Fig. 4-3). The annual reviews of agroclimate conditions across Canada reported drought conditions in northern Alberta in 2014 and severe drought conditions in 2015 (Agriculture and Agri-Food Canada 2014 and 2015). The N_2O emission rates showed so much variability in my individual treatment plots and there are some potential factors that could explain these results. Ambus and Robertson (2006) conducted *in vivo* (in the field) and *in vitro* (in the lab) experiments and found that in the field N inputs had no effect on the soil N_2O emissions while in the lab there was a better response. They attributed their lack of response to poor N distribution in the soil profile because of variable infiltration depths (Ambus and Robertson 2006). In a lab setting uniform N distribution can be easily controlled which will ensure faster N diffusion and peak nitrification and denitrification activity (Ambus and Robertson 2006). In my 20 m x 20 m experimental plots there was considerable spatial and topographical variation which would create individual microsites that could have inconsistent

infiltration depths of the +N and +S treatments. This would explain the high amount of variability reported in the soil N_2O emissions.

Inorganic N pools are very dynamic and can exhibit high rates of variability, which could explain my soil N_2O emissions results (Harrison and Maynard 2014). Variability in N_2O emission rates is a reoccurring issue in research and there have been adjustments made to try and combat this issue. Oura et al. (2001) tried to reduce sampling error and spatial variability by installing permanent gas sampling units 10 cm into the soil and they were able to obtain consistent results. Other studies used water as a transport agent for the N addition, by dissolving the chemicals in water and then applying them to sites with a sprayer, to ensure infiltration and a more even distribution (Du et al. 2016, Carnol and Ineson 1999). Adjusting the experimental methods to include these parameters could have reduced the variability reported in the results. Also N_2O is not the only gas that can be emitted from the soil when N inputs are elevated and/or the system has reached a threshold of N saturation. Nitric oxide (NO) losses can exceed N_2O in ecosystems experiencing elevated N input, especially in conifer forests (Butterbach-Bahl et al. 2002, Skiba et al. 1998). Not measuring NO flux rates could underestimate the amount of N that is being emitted from the soil.

4.5 Conclusions

There was no evidence of N saturation or soil acidification in the study forest ecosystem in the AOSR after ten years of experimental N and S treatments. There was no increase of inorganic N concentrations in the soil and leaching of N in the soil profile was not affected by the N and S treatments. These results, in conjunction with unaltered soil pH and cation concentrations in the soil, indicate that N limitation is still the dominant stage in this forest stand. There is still research needed, including the effects of long term N and S addition on N cycling

and quantifying NO_x emissions, in order to have a complete understanding of the effects N and S deposition. My results suggest that currently there is a very low risk of N saturation occurring in the boreal forests of the AOSR by long-term chronic N and S deposition. Continued long term research, however, is needed in the AOSR to help define critical loads of N and S deposition, and to fully understand potential ecosystem impacts. Additionally, this research only examined one soil type found in the boreal forest ecosystem; therefore the effects of N and S deposition need to be tested on other soil types.

Table 4-1 Exchangeable cations in the forest floor and mineral soil layers, in experimental plots, in a mixedwood forest in the AOSR in northern Alberta, Canada after 9 years of experimental N and S addition. The CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition. Values in parentheses are standard errors of the means.

Layer	Treatment	Exchangeable Cations					Ca:Al
		Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	Al^{3+}	
		($mol_c\ kg^{-1}$)					
Forest floor	CK	0.85	0.16	0.077	0.023	0.0037	230
		(0.040)	(0.017)	(0.012)	(0.00035)	(0.00099)	(9.2)
	+N	0.87	0.16	0.070	0.027	0.0040	220
		(0.073)	(0.024)	(0.0097)	(0.027)	(0.0013)	(16.1)
	+S	0.85	0.15	0.075	0.045	0.0047	183
		(0.076)	(0.015)	(0.014)	(0.0052)	(0.001)	(13.9)
	+NS	0.90	0.15	0.092	0.041	0.0032	279
		(0.057)	(0.020)	(0.0072)	(0.0078)	(0.0015)	(15.8)
Mineral soil (0-60cm)	CK	0.34	0.14	0.016	0.023	0.0052	63.9
		(0.040)	(0.032)	(0.0031)	(0.00041)	(0.0024)	(2.5)
	+N	0.40	0.15	0.022	0.033	0.024	17.0
		(0.094)	(0.028)	(0.0077)	(0.0067)	(0.0098)	(1.6)
	+S	0.26	0.12	0.014	0.029	0.012	21.4
		(0.025)	(0.022)	(0.0011)	(0.00093)	(0.0065)	(0.56)

+NS	0.27	0.11	0.014215	0.029724	0.012	21.9
	(0.026)	(0.016)	(0.0027)	(0.00051)	(0.0048)	(0.58)

Table 4-2 TC, TN, and pH in the forest floor and in the 0-60 cm mineral soil in a mixedwood forest in the AOSR in northern Alberta, Canada after 9 years of experimental N and S addition. The CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition. Values in parentheses are standard errors of the means.

Treatment	Layer and depth	TC	TN	C:N	pH
		($g\ kg^{-1}$)			
CK	LFH	378.5	17.56	21.5	5.6
		(27.5)	(1.3)	(1.3)	(0.2)
	0-15 cm	7.0	0.4	17.5	4.8
		(1.5)	(0.09)	(0.9)	(0.3)
	15-30 cm	4.3	0.2	17.8	5.0
		(0.7)	(0.04)	(0.3)	(0.3)
	30-45 cm	4.3	0.3	17.0	5.1
		(0.3)	(0.03)	(0.3)	(0.3)
	45-60 cm	6.1	0.3	21.00	5.4
		(2.2)	(0.07)	(0.1)	(0.4)
+N	LFH	327.0	15.7	20.8	5.4
		(29.0)	(1.3)	(1.4)	(0.2)
	0-15 cm	6.9	0.4	19.1	4.7
		(0.9)	(0.06)	(0.5)	(0.2)
	15-30 cm	3.2	0.2	16.0	4.9
		(0.3)	(0.02)	(0.2)	(0.2)
	30-45 cm	4.7	0.3	18.1	4.9
		(0.4)	(0.03)	(0.3)	(0.2)
	45-60 cm	4.0	0.2	16.8	4.9
		(0.9)	(0.05)	(0.5)	(0.2)
+S	LFH	316.6	14.3	22.2	5.3
		(36.0)	(1.8)	(1.6)	(0.2)
	0-15 cm	5.6	0.3	17.6	4.7
		(0.3)	(0.02)	(0.2)	(0.2)
	15-30 cm	2.8	0.2	15.3	4.9
		(0.4)	(0.03)	(0.3)	(0.2)
	30-45 cm	3.8	0.2	16.5	5.0
		(0.2)	(0.008)	(0.1)	(0.3)
	45-60 cm	3.9	0.2	18.6	5.2
		(0.6)	(0.03)	(0.3)	(0.4)
+NS	LFH	339.9	16.1	21.1	5.3
		(29.9)	(1.3)	(1.4)	(0.1)
	0-15 cm	7.2	0.3	21.3	4.7
		(1.4)	(0.05)	(0.6)	(0.07)
	15-30 cm	3.4	0.2	17.2	4.7
		(0.8)	(0.03)	(0.5)	(0.09)

30-45 cm	3.9 (0.4)	0.2 (0.03)	17.7 (0.2)	4.6 (0.2)
45-60 cm	3.4 (0.4)	0.2 (0.02)	17.7 (0.2)	4.9 (0.2)

Table 4-3 Leaching loss of NO_3^- and NH_4^+ from May to December 2015, in response to N and S addition, in a mixedwood forest in the AOSR in northern Alberta, Canada. The CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition. Values in parentheses are standard errors of the means.

Depth	Treatment	NO_3^- (mg per resin core)	NH_4^+ (mg per resin core)
Below 15 cm soil depth	CK	0.238 (0.025)	0.929 (0.042)
	+N	0.233 (0.013)	0.993 (0.106)
	+S	0.221 (0.006)	1.25 (0.242)
	+NS	0.239 (0.030)	0.891 (0.030)
Below 45 cm soil depth	CK	0.206 (0.008)	0.846 (0.027)
	+N	0.265 (0.031)	0.900 (0.034)
	+S	0.199 (0.007)	0.855 (0.030)
	+NS	0.210 (0.019)	0.888 (0.033)

Table 4-4 N_2O emission rates from a mixedwood forest soil in the AOSR in northern Alberta, Canada in 2014 and 2015, after 9 and 10 years of N and S addition, respectively. The CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition. Values in parentheses are standard errors of the means.

Year	Treatment	June	July	August	September
		Emissions measured in mg N_2O m^{-2} h^{-1}			
2014	CK	-0.0009 (0.003)	-0.006 (0.003)	0.003 (0.004)	-0.004 (0.004)
	+N	-0.001 (0.003)	0.007 (0.003)	-0.002 (0.004)	0.02 (0.01)
	+S	0.007 (0.003)	-0.0001 (0.002)	0.0005 (0.002)	-0.009 (0.002)
	+NS	-0.003 (0.003)	-0.005 (0.002)	0.0002 (0.002)	-0.008 (0.0003)
2015	CK	-0.008 (0.003)	0.002 (0.003)	0.003 (0.003)	0.006 (0.004)
	+N	-0.0005 (0.003)	0.003 (0.003)	0.0001 (0.003)	0.01 (0.002)
	+S	-0.007 (0.002)	0.003 (0.003)	0.002 (0.003)	0.003 (0.001)
	+NS	0.008 (0.002)	0.0001 (0.002)	0.003 (0.002)	-0.0005 (0.002)

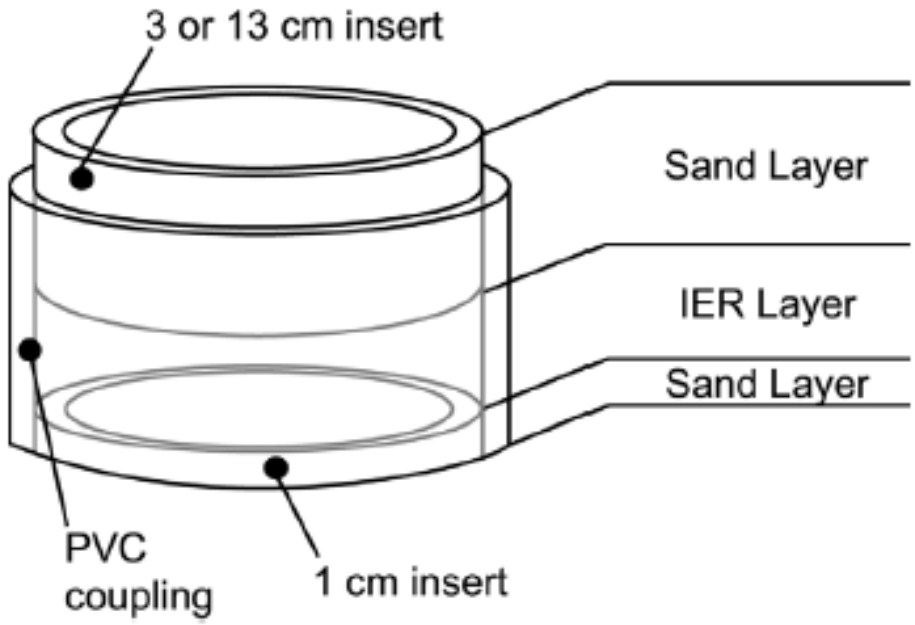
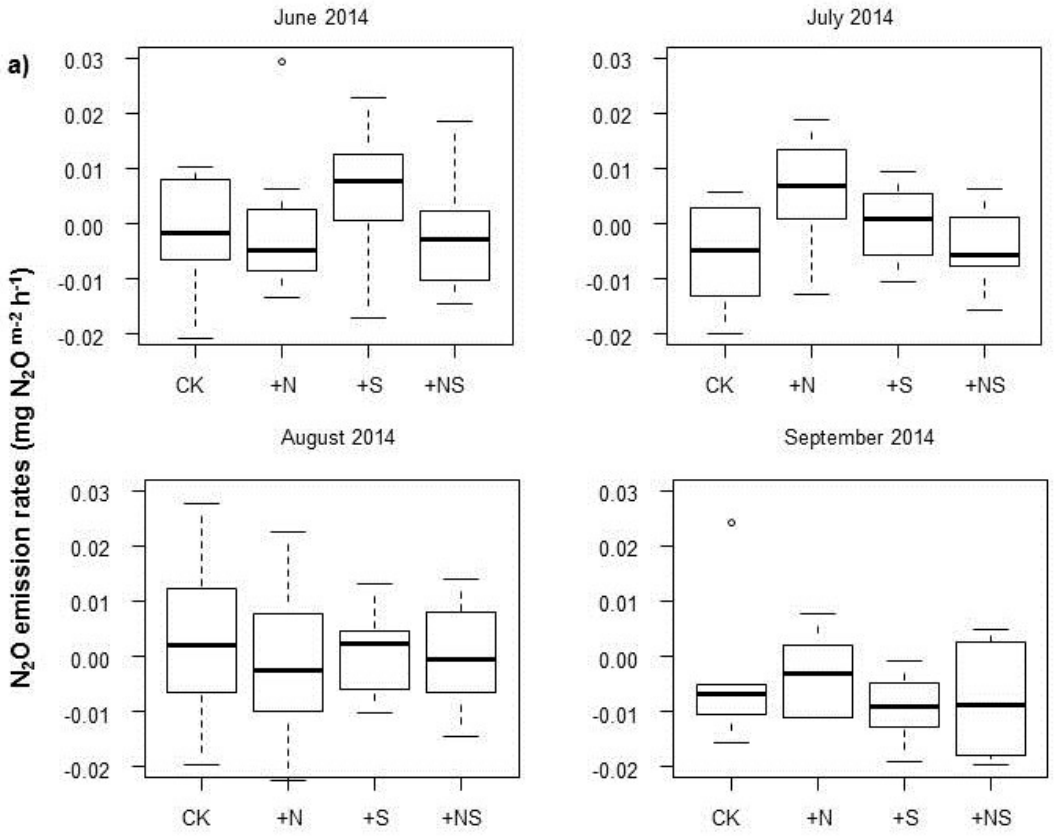


Figure 4-1 Schematic of resin-based soil lysimeters, using 3 cm and 1 cm inserts and Rexyn™ ion exchange resin (IER) from Fisher Scientific (Susfalk and Johnson 2002).


a)
June 2014
July 2014
August 2014
September 2014
N_2O emission rates (mg N_2O m^{-2} h^{-1})
0.03
0.02
0.01
0.00
-0.01
-0.02
CK
+N
+S
+NS

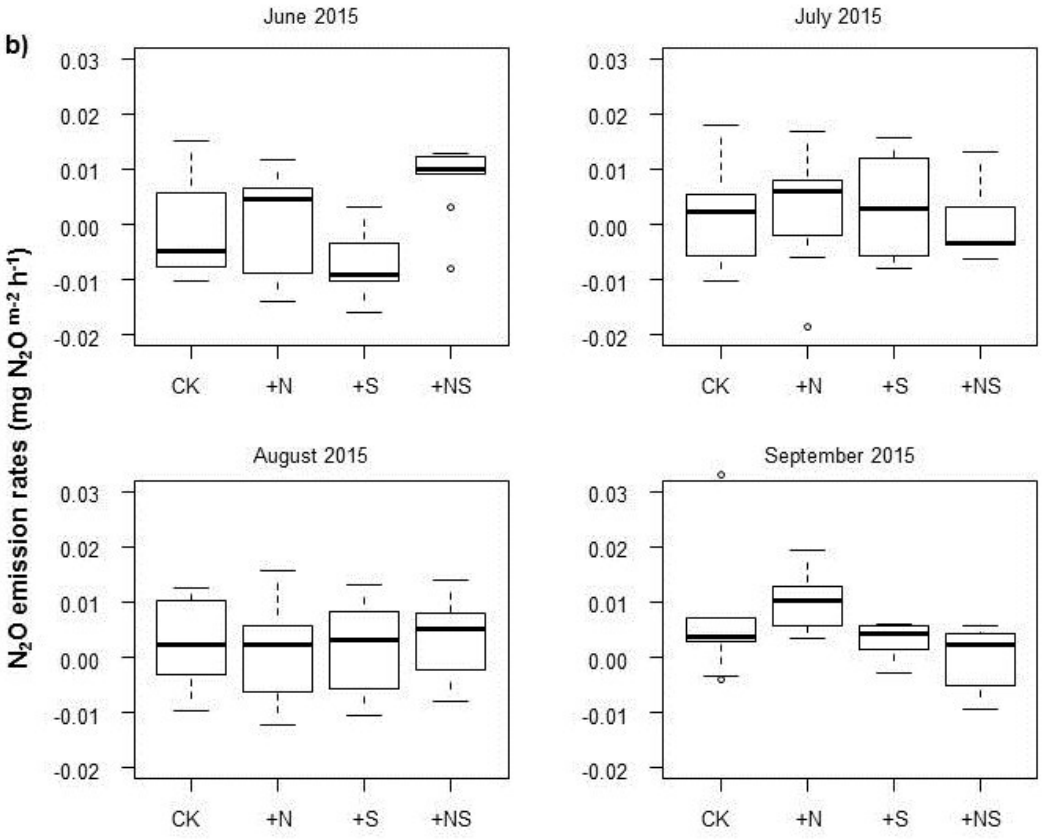


Figure 4-2 N_2O emission rates from a mixedwood forest in the AOSR in northern Alberta, Canada in a) 2014 and b) 2015, after 9 and 10 years of N and S addition, respectively. The boxes on the graph represent the values found between the 25th and 75th percentiles, the middle line is the median, and the extending lines represent the minimum and maximum values. The CK, +N, +S, and +NS refer to control, N addition, S addition, and N+S addition.

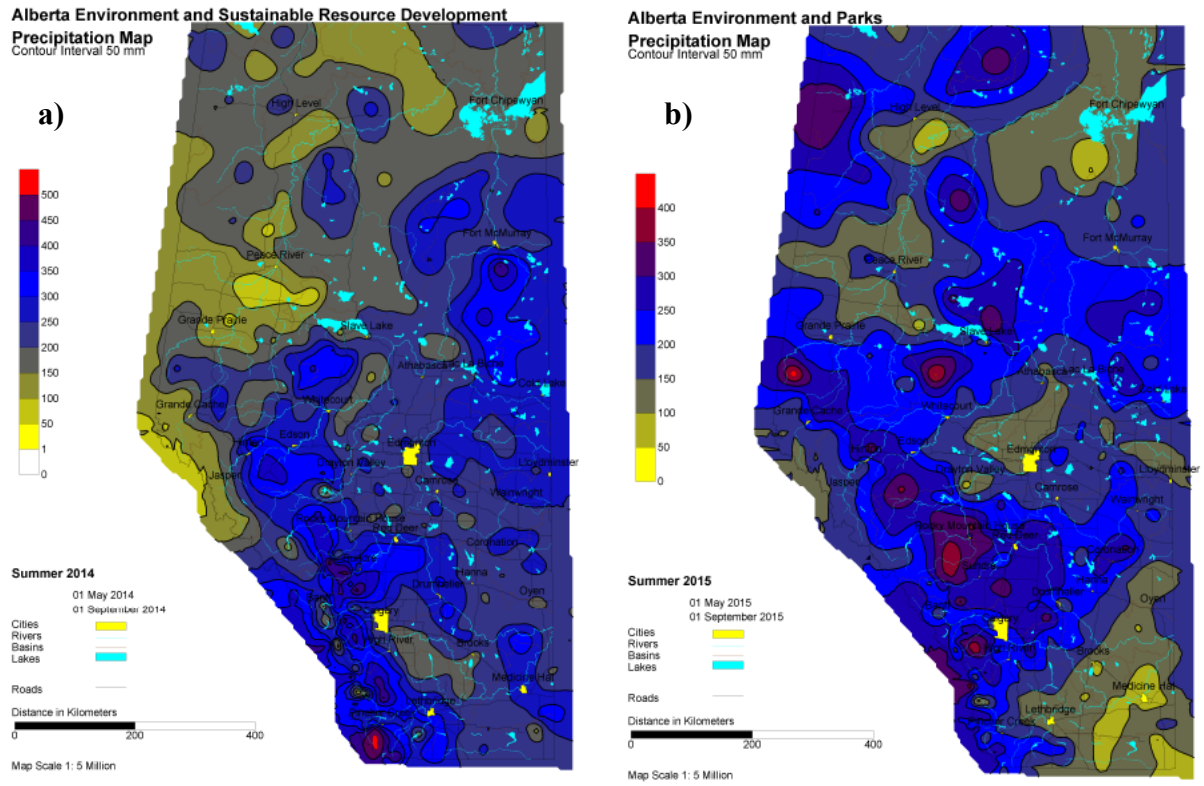


Figure 4-3 Precipitation contour interval map for Alberta for the summer of a) 2014 and b) 2015 (May to September) with the AOSR experiencing between 50 and 200 mm depending on the location. (Source: Alberta Environment and Parks 2015).

Chapter 5: Conclusions and future research

5.1 Overview and study objectives

The objectives of the study were to assess long-term impact of N and S addition on (1) soil microbial communities in a boreal forest in the Athabasca oil sands region (AOSR); (2) soil chemical properties; and (3) the risk of N saturation in a boreal forest in the AOSR, based on the Aber et al. (1989) ecosystem N status assessment. To address these objectives research was conducted in an experimental N and S treatment site in the AOSR. Studies in chapters 2 and 3 were conducted to answer the first objective. I examined soil bacterial diversity, community composition, abundance, functional gene profiles, and co-occurrence network patterns. In addition the abundance of soil fungal colonies were also examined. For the second objective I assessed the soil pH, cation concentrations, total carbon (C) and N, and soil moisture (Chapter 4). And for the last objective I evaluated the N status of the research site through quantifying the amounts of N leaching and being emitted in gaseous form from the soil (Chapter 4).

5.2 Summary and synthesis of the research results

5.2.1 Impacts of N and S addition on soil microbes

The rates of N and S atmospheric emissions increased in the AOSR ever since commercial oil sands mining started in the area in 1967 (Hazewinkel et al. 2008). And while there is research exploring how these increased emissions will affect the vegetation and soil of the N limited boreal forest in the AOSR, there have been no studies on soil microbes in this region. Soil bacteria in the AOSR have shown to be resilient to long-term N and S addition. In

this research it was shown that nine years of N and S addition did not affect soil bacterial diversity, community composition, functional gene profiles, and co-occurrence patterns. While the majority of bacterial characteristics were not affected by the N and S treatments, the number of denitrifying bacteria did respond to N addition, by increasing their numbers. Increasing available N in the soil in the AOSR will increase the abundance of denitrifying bacteria. The abundance of soil fungi was significantly affected, indicating that fungi are sensitive to N and S addition. The addition of N and S to the research plots caused significant increases in the number of soil fungal colonies.

5.2.2 Impacts of N and S addition on soil properties and N status

The increasing levels of N emission in the AOSR have caused concern about possible N saturation in the surrounding boreal forest. However, there was no evidence of N saturation or soil acidification in the studied forest ecosystem after ten years of experimental N and S treatments. Although there was a pattern of changes in N leaching, calcium to aluminum ratios, and total C and N, the results did not present any trend over time among the treatment plots. The soil pH remained unaltered along with the cation concentrations in the soil, which indicates that acidification is not occurring. Additionally, the lack of increase in the amount of N being leached in the treatment plots concludes that N limitation is still the dominant status in this forest stand. The results suggest that currently there is a very low risk of N saturation occurring in the boreal forests of the AOSR by long-term chronic N and S deposition.

5.3 Conclusions

The soil bacterial communities and soil properties of the boreal forest research site in the AOSR have not been negatively affected by long-term N and S addition. Interestingly after five years of addition there were changes to the soil chemistry and the soil bacterial community functions. An additional 4 years has allowed this site to acclimate and adapt to the disturbance of N and S treatments, and return to a state of homeostasis. This could be due to base cation deposition that is driving trajectories of change that is opposite to soil acidification. The soil bacterial communities and the soil properties are resilient to the effects of N and S addition. While the soil fungal communities were significantly increased by N and S treatments, note, our soil fungi results were based on plating microbial techniques which only represent one aspect of the soil fungal community. There was no data collected on the soil fungal diversity, community composition, functional gene profiles, and co-occurrence network patterns. Therefore, the conclusions on fungal communities are not as definitive when compared to the soil bacteria, which exhibited resilience. Based on this research, the risk of soil acidification and N saturation caused by N and S deposition is low in this boreal mixedwood forest stand in the AOSR. However, further research is needed to help enhance the current understanding of N and S deposition in the AOSR.

5.4 Suggestions for future research

Even though the boreal forest in the AOSR is N limited, the region has some characteristics that are susceptible to acidification and N saturation including coarse-textured soils with low exchangeable base cations, cation exchange capacity and low buffering capacity (Aherne and Shaw 2010, Watmough et al. 2014,Whitfield et al. 2009). Therefore, continued monitoring in

the AOSR and this research site is recommended. It is suggested that yearly soil pH, C:N ratio, N emissions and leaching be measured in order to continually assess the N status of the site (Aber 1992, Bowman et al. 2008,Falkengren-Grerup and Diekman 2002, Gundersen 1998, Laxton et al. 2010). This ongoing research would help determine critical loads of N and S and the tipping point at which N saturation occurs in the AOSR. Additionally, there is a lack of long-term acidification and deposition studies in the AOSR, and with N emission rates being predicted to increase; this type of research is needed.

While it has been concluded that soil bacteria are resilient to N and S addition, further information is needed on how soil fungal, archaea, and protozoan communities will respond to these elevated N and S levels. Stronger conclusions about overall microbial resilience to N and S addition could be made if DNA sequencing were conducted on the soil fungi and protozoans in these experimental plots. If possible a new deposition experiment should be established in the same area, in order to test the initial microbial response to the addition of N and S. If microbial response could be tracked on a yearly basis in response to N and S addition it would be possible to conclude if the microbes are exhibiting resilience or resistance.

Further research would allow a greater understanding of the impact of N and S deposition on the soil microbial community. It would also be beneficial to once again examine the vegetative response to additional years of N and S addition. While the soil in the experimental site is still N limited, it is possible that the vegetation has transitioned into alleviation of N limitation after six additional years of N and S addition. Additionally, my analysis only involved a gray Luvisol soil in a boreal forest ecosystem; therefore these findings need to be tested in other soil types in the AOSR and in different ecosystems. There is still some research needed, including the effects of long term N and S addition on N cycling and NO_x emissions, in order to have a complete

understanding of the effects of N and S deposition. The biggest priority should be continuing long-term monitoring of this site in order to determine the critical N and S loads for the AOSR.

Literature Cited

Aaronson S. 1970. Experimental Microbial Ecology. Academic Press, New York.

Aber JD. 1992. Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. Trends in Ecology and Evolution 7: 220- 223.

Aber JD, Nadelhoffer KJ, Steudler P, Melillo TM. 1989. Nitrogen saturation in northern forest ecosystems. Bioscience 39: 378-386.

Agriculture and Agri-Food Canada. 2014. Annual review of agroclimate conditions across Canada. Government of Canada. http://www.agr.gc.ca/eng/?id=1399580648022

Agriculture and Agri-Food Canada. 2015. Annual review of agroclimate conditions across Canada. Government of Canada. http://www.agr.gc.ca/eng/?id=1456343985043

Aherne J. 2008. Calculating critical loads of acid deposition for forest soils in Alberta: critical load, exceedance and limitations. Final Report. Canadian Council of Ministers of the Environment, 14pp.

Aherne J and Shaw DP. 2010. Impacts of sulphur and nitrogen deposition in western Canada. Journal of Limnology 69 (Suppl. 1): 1-3.

Alberta Energy. 2015. About Oil sands: facts and statistics. http://www.energy.alberta.ca/OilSands/791.asp

Alberta Environment and Parks. 2015. http://www.environment.alberta.ca/forecasting/reports/index.html

Allison SD and Martiny JBH. 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences 105 (suppl. 1): 11512-11519.

Ambus P and Robertson GP. 2006. The effect of increased N deposition on nitrous oxide, methane and carbon dioxide fluxes from unmanaged forest and grassland communities in Michigan. 2006. Biogeochemistry 79: 315-337.

Arnebrant K, Bååth E, Söderström B, Nhorsted HO, 1996. Soil microbial activity in eleven Swedish coniferous forests in relation to site fertility and nitrogen fertilization. Scandanavian Journal for Forest Research 11: 1-6.

Ayres E, Van der Val R, Sommerkorn M, Bardgett RD. 2006. Direct uptake of soil nitrogen by mosses. Biology Letters 2(2): 286-288.

Barberán A, Bates ST, Casamayor EO, Fierer N. 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. The ISME Journal 6: 343-351.

Bastian M, Heymann S, Jacomy M. 2009. Gephi: an open source software for exploring and manipulating networks. Proceedings of the Third International ICWSM Conference: 361-362.

Benjamini Y, Krieger AM, Yekutieli D. 2006. Adaptive linear step-up procedures that control the false discovery rate. Biometrika 93(3): 491-507.

Beveridge TJ, Davies JA. 1983. Cellular response of *Bacillus subtilis* and *Escherichia coli* to gram stain. Journal of Bacteriology 156: 846-858.

Bewley R, Parkinson D. 1984. Bacterial and fungi activity in sulphur dioxide polluted soils. Canadian Journal of Microbiology 31: 13-15.

Black C.A. 1965. "Methods of Soil Analysis: Part I Physical and mineralogical properties. American Society of Agronomy, Madison, Wisconsin, USA.

Bowden RD, Davidson E, Savage K, Arabia C, Steudler P. 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the

Harvard Forest. Forest Ecology and Management 196: 43-56.

Bowden RD, Rullo G, Stevens GR, Steudler PA. 2000. Soil fluxes of carbon dioxide, nitrous oxide, and methane at a productive temperate deciduous forest. Journal of Environmental Quality 29: 268-276.

Bowman WD, Cleveland CC, Halada L, Hresko J, Baron JS. 2008. Negative impact of nitrogen deposition on soil buffering capacity. Nature Geoscience 1: 767-770.

Bowman WD, Murgel J, Blett T, Porter E. 2012. Nitrogen critical loads for alpine vegetation and oils in Rocky Mountain National Park. Journal of Environmental Management 103: 165-171.

Butterbach-Bahl K, Breuer L, Gasche R, Willibald G, Papen H. 2002. Exchange of trace gases between soils and the atmosphere in Scots pine forest ecosystems of the northeastern German lowlands 1. Fluxes of N2O, NO/NO2 and CH4 at forest sites with different N-deposition. Forest Ecology and Management 167: 123–134.

Bytnerowicz A, Fraczek W, Schilling S, Alexander D. 2010. Spatial and temporal distribution of ambient nitric acid and ammonia in the Athabasca Oil Sands Region, Alberta. Journal of Limnology 69 (Suppl. 1): 11e21.

Campbell BJ, Polson SW, Hanson TE, Mack MC, Schuur EA. 2010. The effect of nutrient deposition on bacterial communities in Arctic tundra soil. Environmental Microbiology 12(7): 1842-1854.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J,Knight R, 2010.

QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7(5): 335-336.

Carnol M and Ineson P. 1999. Environmental factors controlling NO_3^- leaching, N_2O emissions and numbers of NH_4^+ oxidisers in a coniferous forest soil. Soil Biology and Biochemistry 31(7): 979-990.

Chapin F, 1980. The mineral nutrition of wild plants. Annual Review of Ecology, Evolution, and Systematics 11:233-260.

Chen Y, Zhang X, Ye J, Han H, Wan S, Chen B. 2014. Six year fertilization modifies the biodiversity of arbuscular mycorrhizal fungi in a temperate steppe in Inner Mongolia. Soil Biology and Biochemistry 69: 371-381.

Cochrane WG. 1950. Estimation of bacterial densities by means of the "most probable number". Biometrics 6(2): 105-116.

Compton JE, Watrud LS, Porteous A, DeGrood S, 2004. Response of soil microbial biomass and community composition to chronic nitrogen additions at Harvard forest. Forest Ecology and Management 196: 143-158.

Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http:// www.R-project.org/

Coûteaux MM, Raubuch M, Berg M. 1998. Response of protozoan and microbial communities in various coniferous forest soils after transfer to forests with different levels of atmospheric pollution. Biology and Fertility of Soils 27: 179-188.

Csardi G, Nepusz T. 2006. The igraph software package for complex network research. InterJournal, Complex Systems 1695.

Davies JA, Anderson GK, Beveridge TJ, Clark HC. 1983. Chemical mechanism of the gram

stain and synthesis of a new electron-opaque marker for electron microscopy which replaces the iodine mordant of the stain. Journal of Bacteriology 156(2): 837-845.

De Boer W, Tietema A, Gunnewiek PJA, Laanbroek HJ, 1992. The chemolithtrophic ammonium-oxidizing community in a nitrogen saturated acid forest soil in relation to pH dependent nitrifying activity. Soil Biology and Biochemistry 24(3): 229-234.

DeForest JL, Zak DR, Pregitzer KS, Burton AJ. 2004. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Science Society of America Journal 68: 132-138.

Demoling F, Nilsson L, Baath E, 2008. Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. Soil Biology and Biochemistry 40: 370-379.

Denman K et al. in IPCC Climate Change 2007: The Physical Science Basis (eds Solomon, S. et al.), 499–587 (Cambridge Univ. Press, 2007).

Dentener F et al. 2006 Nitrogen and sulfur deposition on regional and global scales: A multimodel evaluation. Global Biogeochemical Cycles 20: GB4003.

Derakshani M, Lukow T, Liesack W, 2001. Novel bacterial lineages at the (sub)division level as detected by signature nucleotide-targeted recovery of 16S rRNA genes from bulk soil and roots of flooded rice microcosms. Applied and Environmental Microbiology 67: 623-631.

Díaz-Ravina M, Acea MJ, Carballas T. 1993. Seasonal fluctuations in microbial populations and available nutrients in forest soils. Biology and Fertility of Soils 16: 205–210.

Ding, L., J. Su, H. Xu, Z. Jia, and Y. Zhu. Long-term nitrogen fertilization of paddy soil shifts iron-reducing microbial community revealed by RNA-^{13}C-acetate probing coupled with pyrosequencing. The ISME Journal 9: 721-734.

Dise NB and Wright RW. 1992. The NITREX project (Nitrogen saturation experiments). Commission of the European Communities (Environmental Research Programme), Brussels, Belgium.

Du Y, Guo X, Cao G, Li Y. 2016. Increased nitrous oxide emissions resulting from nitrogen addition and increased precipitation in an alpine meadow ecosystem. Polish Journal of Environmental Studies 25(1): 447-451.

Dursun S, Ineson P, Frankland JC, Boddy L. 1996. Sulphur dioxide effects fungi growing on leaf litter and agar media. New Phytology 134: 167-176.

Emmett BA. 2007. Nitrogen saturation of terrestrial ecosystems: some recent findings and their implications for our conceptual framework. Water, Air, and Soil Pollution: Focus 7: 99-109.

Emmett BA, Brittain SA, Hughes A, Kennedy V. 1995. Nitrogen additions ($NaNO_3$ and NH_4NO_3) at Abert forest, Wales: II. Response of trees and soil nitrogen transformations. Forest Ecology and Management 71:61-73.

Energy Alberta. 2014. About oil sands: facts and statistics. Accessed January 5, 2015. http://www.energy.alberta.ca/oilsands/791.asp

Environment Canada, 2004. Canadian acid deposition: science assessment. Environment Canada Meteorological Service.

Falkengren-Grerup U, Diekmann M. 2002. Use of a gradient of N-deposition to calculate effect-related soil and vegetation measures in deciduous forests. Forest Ecology and Management 6187: 1-12.

Faust K, Raes J. 2012. Microbial interactions: from networks to models. Nature Reviews 10: 538-550.

Fenchel T and Finlay BJ. 2004. The ubiquity of small species: patterns of local and global diversity. BioScience 54: 777-784.

Fenn ME, Baron JS, Allen EB, Rueth HM, Nydick KR, Geiser L, Bowman WD, Sickman JO, Meixner T, Johnson DW, Neitlich P. 2003. Ecological effects of nitrogen deposition in the Western United States. BioScience 53(4): 404-420.

Fenn ME, Bytnerowicz A, Schilling SL, Ross CS. 2015. Atmospheric deposition of nitrogen, sulfur, and base cations in jack pine stands in the Athabasca Oil Sands Region, Alberta, Canada. Environmental Pollution 196: 497-510.

Fenn ME, Poth MA, Aber JD, Baron JS, Bormann BT, Johnson DW, Lemly AD, McNulty SG, Ryan DF, Stottlemyer R. 1998. Nitrogen excess in North American ecosystems: predisposing factors, ecosystem responses, and management strategies. Ecological Applications 8:706-733.

Ferguson GP, Rudolph DL, Barker JF. 2009. Hydrodynamics of a large oil sand tailings impoundment and related environmental implications: Canadian Geotechnical Journal 46: 1446–1460.

Fierer N, Ladau J, Clemente JC, Leff JW, Owens SM, Pollard KS, Knight R, Gilbert JA, McCulley RL. 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. Science 342(6158): 621-624.

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R, 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal 6:1007-1017.

Fierer N, McCain CM, Meir P, Zimmermann M, Rapp JM. Silaman MR. 2011. Microbes do not follow the elevational diversity patterns of plants and animals. Ecology 92:797-

804.

Fransson PMA, Taylor AFS, Finlay RD. 2000. Effects of continuous optimal fertilization on belowground ectomycorrhizal community structure in a Norway spruce forest. Tree Physiology 20: 599–606.

Freedman ZB, Romanowicz KJ, Upchurch RA, Zak DR. 2015. Differential responses of total active soil microbial communities to long-term experimental N deposition. Soil Biology and Biochemistry 90: 275-282.

Freedman ZB, Zak DR. 2014. Atmospheric N deposition increases bacterial laccase-like multicopper oxidases: implications for organic matter decay. Applied and Environmental Microbiology 80(14): 4460-4468.

Freedman ZB, Zak DR. 2015. Atmospheric N deposition alters connectance, but not functional potential among saprotrophic bacterial communities. Molecular Ecology 24(12): 3170-3180.

Frey SD, Knorr M, Parrent JL, Simpson RT, 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196: 159-171.

Frey SD, Ollinger S, Nadelhoffer K, Bowden R, Brzostek E, Burton A, Wickings K. 2014. Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. Biogeochemistry 121(2): 305-316.

Fuerst JA, 1995. The Planctomycetes – emerging models for microbial ecology, evolution and cell biology. Microbiology 141: 1493-1506.

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend

AR, Voosmarty CJ. 2004. Nitrogen cycles: Past, present and future. Biogeochemistry 70(2): 153-226.

Glenn TC. 2011. Field guide to next-generation DNA sequences. Molecular Ecology Resources 11: 759-769.

Gosselin P, Hrudey S, Naeth M, Plourde A, Therrien R, Van Der Kraak G, Xu Z, 2010. Environmental and health impacts of Canada's oil sands industry. Royal Society of Canada, Ottawa, ON, Canada.

Goulding KWT, Bailey NJ, Bradbury NJ, Hargreaves P, Howe M, Murphy DV, Poulton PR, Willison TW. 1998. Nitrogen deposition and its contribution to nitrogen cycling and associated soil processes. New Phytologist 139(1): 49-58.

Government of Canada. 2010. 1981 to 2010 Canadian Climate Normals station data. Fort McMurray A, Alberta, 56°39'N, 111°13'W, 369.10m, 3062693. http://climate.weather.gc.ca/climate_normals/index_e.html

Grayston SJ, Wainwright M. 1988. Sulphur oxidation by soil fungi including some species of mycorrhizae and wood-rotting Basidiomycetes. FEMS Microbiology Ecology 53:1-8.

Gundersen P. 1998. Effects of enhanced nitrogen deposition in a spruce forest at Klosterhede, Denmark, examined by moderate NH_4NO_3 addition. Forest Ecology and Management 101:251-268.

Gupta VV, Lawrence JR, Germida JJ. 1961. Factors limiting microbial activities in soil. Archiv für Mikrobiologie 40(4): 370-382.

Harrison DJ, Maynard DG. 2014. Nitrogen mineralization assessment using PRSTM probes (ion exchange membranes) and soil extractions in fertilized and unfertilized pine and spruce soils. Canadian Journal of Soil Science 94: 21-34.

Hayat S, Ahmad I, Azam A, Ahmad A, Inam A, Samiullah A. 2002. Effect of long-term application of oil refinery wastewater on soil health with special reference to microbiological characteristics. Biosource Technology 84: 159-163.

Hazewinkel RRO, Wolfe AP, Pla S, Hadley CC. 2008. Have atmospheric emissions from the Athabasca Oil Sands impacted lakes in northeastern Alberta, Canada? Canadian Journal of Fisheries and Aquatic Sciences 65: 1554-1567.

Hesse CN, Mueller RC, Vuyisich M, Gallegos-Graves LV, Gleasner CD, Zak DR, Kuske CR. 2015. Forest floor community metatranscriptomes identify fungal and bacterial responses to N deposition in two maple forests. Frontiers in Microbiology 6(337): 1-15.

Högberg MN, Högberg P, Myrold DD. 2007. Is microbial community composition in Boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150: 590-601.

Holowaychuk N, Fessenden RJ. 1987. Soil sensitivity to acid deposition and the potential of soils and geology in Alberta to reduce the acidity of acid inputs. Alberta Environment, Edmonton, Alberta.

Hu Y, Jung K, Zeng D, Chang SX. 2013. Nitrogen and sulfur deposition altered soil microbial community functions and enzyme activities in a boreal mixedwood forest in western Canada. Canadian Journal of Forest Research 28: 799-807.

Hutchinson GL, Mosier AR. 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Science Society of America Journal 45: 311-316.

Jung K, Chang SX. 2012. Four years of simulated N and S depositions did not cause N saturation in a mixedwood boreal forest ecosystem in the oil sands region in northern Alberta, Canada. Forest Ecology and Management 280: 62-70.

Jung K, Ok YS, SX Cahng. 2011. Sulfate adsorption properties of acid-sensitive soil in the Athabasca oil sands region in Alberta, Canada. Chemosphere 84: 457-463.

Keeney DR, Nelson DW. 1982. Nitrogen-inorganic forms. *In* Methods of Soil Analysis, Part 2-Chemical and microbiological properties, 2nd ed., eds A.L. Page et al., p.672-676.

Kelly J, Häggblom M, Tate R. 1999. Changes in the soil microbial communities over time resulting from one time application of zinc: a laboratory microcosm study. Soil Biology and Biochemistry 31: 1455-1465.

Kertesz MA, Mirleau P. 2004. The of soil microbes in plant sulphur nutrition. Journal of Experimental Botany 55(44): 1939-1945.

King KS, Yetter J. 2011. Groundwater and Alberta's oil sands. National Ground Water Association 49: 316-318.

Krumins JA, Dighton J, Gray D, Franklin RB, Morin PJ, Roberts MS. 2009. Soil microbial community response to nitrogen enrichment in two oak scrub forests. Forest Ecology and Management 258: 1383-1390.

Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C. 2013. predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology 31: 814-821.

Laxton DL, Watmough SA, Aherne J, Straker J. 2010. An assessment of nitrogen saturation in *Pinkus banksiana* plots in the Athabasca oil sands region, Alberta. Journal of Limnology 69: 171-180.

Lennon JT, Jones SE. 2011. Microbial seed banks: the ecological and evolutionary implications of dormancy. Nature Reviews Microbiology 9: 119-130.

Lindberg N and Persson T. 2004. Effects of long-term nutrient fertilisation and irrigation on the microarthropod community in a boreal Norway spruce stand. Forest Ecology and Management 188: 125-135.

Liu X, Duan L, Mo J, Du E, Shen J, Lu X, Zhang Y, Zhou X, He C, Zhang F. 2011. Nitrogen deposition and its ecological impacts in China: an overview. Environmental Pollution 159(10): 2251-2264.

Liu Y, Mao L, Li J, Shi G, Jiang S, Ma X, Feng H. 2014. Resource availability differentially drives community assemblages of plants and their root associated arbuscular mycorrhizal fungi. Plant Soil 386: 341-355.

Lovett GM and Goodale CL. 2011. A new conceptual model of nitrogen saturation based on experimental nitrogen addition to an oak forest. Ecosystems 14: 615-631.

Lovett GM, Reiners WA, Olson RK. 1982. Cloud droplet deposition in subalpine balsam fir forests: hydrological chemical inputs. Science 218:1303-1304.

Lu M, Yang Y, Luo Y, Fang C, Zhou X, Chen J, Yang X, Li B, 2011. Responses of ecosystems nitrogen cycle to nitrogen addition: a meta-analysis. New Phytologist 189:1040-1050.

Ma B, Wang H, Dsouza M, Lou J, He Y, Dai Z, Brookes PC, Xu J, Gilbert JA. 2016. Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. The ISME Journal 10: 1-11.

Marshall K, Nguyen C, Tong KMR, Wee T. 2011. Bacterial plating is a suitable method for determining the effect of alginate lyase on *Pseudomonas aeruginosa* PAO1. Journal of Experimental Microbiology and Immunology 15: 90-95.

Matson P, Lohse KA, Hall SJ. 2002. The globalization of nitrogen deposition: consequences for terrestrial ecosystem. Ambio 31: 113-119.

McLeod AR. 1988. Effects of open-air fumigation with sulphur dioxide on the occurrence of fungal pathogens in winter cereals. Phytopathology 78: 88-94.

McMurdie, Holmes, 2013. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome Consensus data. PLoS ONE 8(4): e61217.

Mergel A, Kloos K, Bothe H. 2001. Seasonal fluctuations in the population of denitrifying and N_2-fixing bacteria in an acid soil of a Norway spruce forest. Plant and Soil 230: 145-160.

Merrill AG, Zak DR. 1992. Factors controlling denitrification rates in upland and swamp forests. Canadian Journal of Forest Research 22: 1597-1604.

Miranda KM, Espey MG, Wink DA. 2001. A rapid, simple pectrophotometric method for simultaneous determination of nitrate and nitrite. Nitric Oxide: Biology and Chemistry 5: 62-71.

Nakayama FS, 1990. Soil respiration. Remote Sensing Revelations 5: 311-321.

Nilsson SI, Berggren D, Westling O. 1998. Retention of deposited NH_4^+ N and NO_3^- N in coniferous forest ecosystems in Southern Sweden. Scandinavian Journal of Forest Research 13:393-401.

Nocker A, Camper AK. 2006. Selective removal of DNA from dead cells of mixed bacterial communities by use of ethidium monoazide. Applied Environmental Microbiology 72(3): 1997- 2004.

Ok YS, Chang SX, Feng YS, 2007. The role of atmospheric N deposition in soil acidification in forest ecosystems. Ecological Research Progress. Nova Science Publishers, USA, pp47-77.

Oura N, Shindo J, Fumoto T, Toda H, Kawashima H. 2001. Effects of nitrogen deposition in nitrous oxide emissions from the forest floor. Water, Air, and Soil Pollution 130(1): 673-678.

Peciulyte D, Volodkiene VD. 2009. Effect of long-term industrial pollution on soil microorganisms in deciduous forests situated along a pollution gradient next to a fertilizer factory. Ekologija 55(1): 67-77.

Philippot L, Hallin S, Schloter M. 2007. Ecology of denitrifying prokaryotes in agricultural soil. Advances in Agronomy 96: 249-305.

Pimm SL. 1984. The complexity and stability of ecosystems. Nature 307: 321-326.

Pollard KS, Dudoit S, Laan MJ. 2005. Multiple Testing Procedures: R multtest Package and Applications to Genomics, in Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Springer.

Proemse BC, Mayer B, Fenn ME. 2012. Tracing industrial sulfur contributions to atmospheric sulfate deposition in the Athabasca oil sands region, Alberta, Canada. Applied Geochemistry 35: 507-522.

Proemse BC, Mayer B, Fenn ME, Ross CS. 2013. A multi-isotope approach for estimating industrial contributions to atmospheric nitrogen in the Athabasca oil sands region in Alberta , Canada. Environmental Pollution 182: 80-91.

Rousk J, Brookes PC, Bååth E. 2011. Fungal and bacterial growth responses to N fertilization and pH in the 150-year "Park Grass" UK grassland experiment. FEMS Microbiology Ecology 76: 89-99.

Rykiel EJ. 1985. Towards a definition of ecological disturbance. Australian Journal of Ecology 10: 361-365.

Schindler DW, Dillon PJ, Schreier H. 2006. A review of anthropogenic sources of nitrogen and their effects on Canadian aquatic systems. Biogeochemistry 79: 25-44.

Schnug E, Haneklaus S. 1998. Diagnois of sulphur nutrition, In: Schnug E, ed. Sulphur in agroecosystems. Dordrecht: Kluwer, 1-38.

Sessitsch A, Weilharter A, Gerzabek MH, Kirchmann H, Kandeler E. 2001. Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. Applied and Environmental Microbiology 67: 4215-4224.

Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, Huber DH, Langenheder S, Lennon JT, Martiny JBH, Matulich KL, Schmidt TM, Handelsman J. 2012. Fundamentals of microbial community resistance and resilience. Frontiers in Microbiology 3:1-19.

Silver WL, Thompson AW, Reich A, Ewel JJ, Firestone MK. 2005. Nitrogen cycling in tropical plantation forests: potential controls on nitrogen retention. Ecology Applied 15(5): 1604-1614.

Skiba UM., Sheppard LJ, Macdonald J, Fowler D. 1998. Some key environmental variables controlling nitrous oxide emissions from agricultural and semi-natural soils in Scotland. *Atmospheric Environment* 32: 3311-3320.

Sogn TA, Abrahamsen G. 1998. Effects of N and S deposition on leaching from an acid forest soil and growth of Scots pine (*Pinus sylvestris*) after 5 years of treatment. Forest Ecology and Management 103(2-3): 177-190.

Stuanes AO, Kjonaas OJ, van Miegroet H. 1995. Soil solution response to experimental addition of nitrogen to a forested catchment at Gardsjon, Sweden. Forest Ecology and Management 71:99-110.

Sugio T, Domatsu C, Munakata O, Tano T, Imai K. 1985. Role of ferric iron-reducing system in sulfur oxidation of *Thiobacillus ferrooxidans*. Applied Environmental Microbiology 49(6): 1401-1406.

Sun S, Xing F, Zhao H, Gao Y, Bai Z, Dong Y. 2014. Response of bacterial community to simulated nitrogen deposition in soils and a unique relationship between plant species and soil bacteria in the Songnen grassland in Northeastern China. Journal of Soil Science and Plant Nutrition 14(3): 565-580.

Susfalk RB, Johnson DW. 2002. Ion exchange resin based soil solution lysimeters and snowmelt solution collectors. Communications in Soil Science and Plant Analysis, 33: 1261-1275.

Tandon R, Grewal J. 1956. Effect of nitrogen nutrition on growth and sporulation on some pathogenic fungi. Proceedings of the Indian Academy of Sciences 44(2): 61-67.

Templer PH, Silver WL, Rett-Ridge J, Deangelis KM, Firestone MK. 2008. Plant and microbial controls on nitrogen retention and loss in a humid tropical forest. Ecology 89(11): 3030-3040.

Tietema A, Boxman AW, Bredemeier M, Emmett BA, Moldan F, Gundersen P, Schleppi P, Wright RF. 1998. Nitrogen saturation experiments (NITREX) in coniferous forest ecosystems in Europe: a summary of results. Environmental Pollution 102:433-437.

Treseder KK. 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters 11(10): 1111-1120.

Van Diepen LTA, Lilleskov EA, Pregitzer KS, Miller RM, 2010. Simulated nitrogen deposition causes a decline of intra- and extraradical abundance of arbuscular mycorrhizal fungi and changes in microbial community structure in northern hardwood forests. Ecosystems 13:683-695.

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tillman DG. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications 7(3): 737 – 750.

Watmough SA, Whitfield CJ, Fenn ME. 2014. The importance of atmospheric base cation deposition for preventing soil acidification in the Athabasca Oil Sands Region of Canada. Science of the Total Environment 493: 1-11.

Whitfield CJ, Aherne J, Watmough SA. 2009. Modeling soil acidification in the Athabasca Oil Sands Region, Alberta, Canada. Environmental Science and Technology 43(15): 5844-50.

Whitfield CJ, Atkinson A, Eimers MC, Watmough SA. 2010. The adsorption and release of sulfur in mineral and organic soils of the Athabasca oil sands region, Alberta, Canada. Journal of Environmental Quality 39: 1108-1112.

Wieder RK, Vitt DH, Burke-Soll M, Scott KD, House M, Vile MA. 2010. Nitrogen and sulphur deposition and the growth of *Sphagnum fuscum* in bogs of the Athabasca oil sands region, Alberta. Journal of Limnology 69: 161-170.

Xu Y, Yu W, Ma Q, Zhou H. 2012. Responses of bacterial and archaeal ammonia oxidisers of an acidic luvisols soil to different nitrogen fertilization rates after 9 years. Biology and Fertility of Soils 48: 827-837.

Yao M, Rui J, Li J, Dai Y, Bai Y, Hedenec P, Wang J, Zhang S, Pei K, Liu C, Wang Y, He Z, Frouz J, Li X. 2014. Rate-specific responses of prokaryotic diversity and structure to nitrogen deposition in the *Leymus chinensis* steppe. Soil Biology and Biochemistry 79: 81-90.

Zechmeister-Boltenstern S, Michel K, Pfeffer M. 2011. Soil microbial community structure in

European forests in relation to forest type and atmospheric nitrogen deposition. Plant and Soil 343 :37-50.

Zhang X, Han X. 2012. Nitrogen deposition alters soil chemical properties and bacterial communities in the Inner Mongolia grassland. Journal of Environmental Sciences (China) 24(8): 1483-1491.

Zhang X, Liu W, Bai Y, Zhang G, Han X. 2011. Nitrogen deposition mediates the effects and importance of chance in changing biodiversity. Molecular Ecology 20: 429-438.

Zhu X, Zhang W, Chen H, Mo J. 2015. Impacts of nitrogen deposition on soil nitrogen cycle in forest ecosystems: A review. Acta Ecologica Sinica 35: 35-43.