Trembling aspen (*Populus tremuloides*) leaf litter decomposition under simulated nitrogen and sulfur deposition in a mixedwood boreal forest

by

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Abstract

As one of the largest oil sands deposits in the world, the Athabasca oil sands region (AOSR) has generated and released large amounts of nitrogen oxides (NO_x) and sulfur dioxide (SO_2) to the atmosphere and the surrounding area. Long-term N and S deposition at elevated rates can cause soil acidification, decrease forest productivity, and change plant community composition. Litter decomposition is an important component of nutrient and carbon (C) cycling in terrestrial ecosystems which relate to forest productivity and greenhouse gas emissions. Litter decomposition rates are affected by many factors such as climate, litter chemistry, N and S addition, soil properties, and litter enzyme activities. To better understand how N and S deposition impacts litter decomposition in a boreal forest, I conducted laboratory (100-day) and field (18-month) experiments in this study.

Litter and forest floor (F and H layers, after removing current litter layer) samples were collected from a mixedwood boreal forest located about 100 km southeast of Fort McMurray, northern Alberta. Litter C, N and S concentrations were determined using an elemental analyzer. Litter lignin concentration was analyzed by measuring absorbance at 280 nm following acetyl bromide digestion. Other elemental concentrations including calcium (Ca), magnesium (Mg), aluminum (Al), and manganese (Mn) were determined using an ICP-MS method. The MBC and MBN concentrations from the forest floor were determined using a fumigation-extraction method. Extracellular enzyme activities involved in C, N and S cycling were analyzed using fluorimetric and colorimetric methods: β-1, 4-glucosidase (GLU, enzyme classification (EC) EC 3.2.1.21) for C, β-1, 4-N-acetylglucosaminidase (NAG, EC 3.2.1.14) for N, and arylsulfatase (ARS, EC 3.1.6.1) for S.

In the laboratory study, 10 years of N and S deposition changed trembling aspen (*Populus tremuloides*) litter chemistry and forest floor microbial properties. Cumulative CO_2 emission (C_{cum}) from the litter was negatively correlated with C/N and lignin/N ratios in litter ($P < 0.05$), but not with forest floor microbial properties ($P > 0.05$). This laboratory study provided evidence that N and S addition can enhance litter decomposition through changed litter chemistry, suggesting that C cycling in the boreal forest ecosystem in the oil sands region can be significantly affected by long-term N and S deposition. However, these results need to be testified in the field. In the field study, N and S addition did not directly affect litter decomposition rates ($P > 0.05$) (Experiment I), and nutrient-induced changes in litter chemistry did not regulate litter decomposition rates under N and S addition ($P > 0.05$) (Experiment II). Litter N exhibited an immobilization phase followed by an initial release phase, while an initial leaching phase occurred followed by an immobilization phase until the end of study for S in both experiments. And extracellular enzyme activities in litter did not correlate with litter decay constants. To fully understand the effect of N and S deposition on litter decomposition, a long-term decomposition study is recommended.

Preface

This thesis is an original work by Qi Wang. No part of this thesis has been published previously. I was responsible for the sample collection, sample analysis, data analysis, and thesis writing. Jin-Hyeob Kwak assisted with sample collection and thesis edits. Woo-Jung Choi assisted with Chapter 2 experimental design and Chapter 2 and 3 edits. Scott X. Chang was the supervisor of this research project and contributed to the editorial corrections of the thesis.

Dedication

The price of success is hard work, dedication to the job at hand, and the determination that whether we win or lose, we have applied the best of ourselves to the task at hand.

– Vince Lombardi

In memory of my father-in-law, Stanley Ronald Meek, who was one of the finest, kindest, and greatest men I have ever known, and Miko Meek, we miss you deeply.

To my wonderful family from China, Canada, and America, especially my husband and my parents. Thank you all so much for the support and love.

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List of Symbols and Abbreviations

℃: degree Celsius

+N: nitrogen addition

+S: sulfur addition

+NS: nitrogen and sulfur additions

yr^{-1}: per year

ha^{-1}: per hectare

AET: annual actual evapotranspiration

Al: aluminum

AMP: acid monitoring program

ANOVA: analysis of variance

AOSR: Athabasca oil sands region

ARS: arylsulfatase

AUR: acid-unydrolyzable

Ca: calcium

C_{cum}: cumulative CO_2 emission

CH_4: methane

CK: control

cm: centimeter

CO_2: carbon dioxide

C-to-N ratio: C/N ratio

EC: enzyme classification

g: gram

GC: gas chromatography

GLU: β-1, 4-glucosidase

hr: hour

H_2O: dihydrogen oxide

F: fermentation

H: humified

K: potassium

km: kilometer

L: litter

Lignin-to-N ratio: lignin/N ratio

m: meter

MAP: mean annual precipitation

MAT: mean annual temperature

MBC: microbial biomass C

MBN: microbial biomass N

Mg: magnesium

Mg: megagram

mm: millimeter

Mn: manganese

Na: sodium

NAG: β-1, 4-N-acetylglucosaminidase

N_2O: nitrous oxide

NO_x: nitrogen oxides

P: phosphorous

SO_2: sulfur dioxide

WHC: water holding capacity

Chapter 1. General introduction

1. Nitrogen and sulfur deposition

1.1 Background

Since preindustrial era, the global emission of nitrogen (N) and sulfur (S) has increased three-fold due to increasing anthropogenic activities, such as the burning of fossil fuels and agriculture practices (Dentener et al., 2006). Regionally, especially for North America, Europe, and Asia, these emissions may have increased 10 times in the last century (Aardenne et al., 2001). The main forms of N and S emissions to the atmosphere are nitrogen oxides (NO_x), and sulfur dioxide (SO_2), and these can be removed from the atmosphere through dry and wet deposition on terrestrial and aquatic ecosystems (Dentener et al., 2006). The excess N and S deposition may reduce biodiversity (Phoenix et al., 2006), cause eutrophication (Bouwman et al., 2002), interrupt climate change and carbon (C) cycling relationships (Thornton et al., 2007), decrease plant growth (Savva and Berninger, 2010), cause soil acidification (Savva and Berninger, 2010), impair human health (World Health Organization, 2003), and may have potential adverse impacts on forest ecosystems, such as causing nutrient imbalance and disturbance to C cycling (Aber et al., 1989; Schimel et al., 1995; Sogn and Abrahamsen, 1998).

The Athabasca oil sands region (AOSR) in northern Alberta, Canada is one of the largest oil sands deposits in the world (Humphries, 2009; Alberta Energy, 2017; Canada's Oil Sands, 2017). Mining-related activities have resulted in the release of large amounts of NO_x and SO_2 into the atmosphere and deposited in the surrounding area. It has been reported that NO_x

emissions increased steadily from 20 Mg day^{-1} when the first oil sands operation began in the 1970s, to a maximum of 300 Mg day^{-1} in the mid-2000s (Hazewinkel et al., 2008; Clair and Percy, 2015). This is expected to increase further due to the expansion of current mining areas (Clair and Percy, 2015). Most SO_2 emissions, over 97%, in AOSR resulted from upgrading operations (NPRI, 2010, 2011).The initial SO_2 emission in AOSR was 132 Mg day^{-1} in the 1960s and peaked of 478 Mg day^{-1} in the mid-1990s, followed by a reduction in the late-1990, where it has remained at approximately 300 Mg day^{-1} (Hazewinkel et al., 2008; Clair and Percy, 2015). Proemse et al. (2013) estimated that yearly open field deposition (bulk deposition) ranged from 1.0 to 4.7 kg N ha^{-1} yr^{-1}, and 0.5 to 2.0 kg N ha^{-1} yr^{-1}for ammonium (NH_4^+) and nitrate (NO_3^-), respectively, around AOSR. Proemse et al. (2012) also estimated that bulk deposition for SO_4^{2-} varied between 1.4 and 11.7 kg S ha^{-1} yr^{-1}. Therefore, the forest ecosystems in AOSR have been experiencing relatively low but elevated N and S deposition and concerns have been raised about the increased N and S deposition effects on the boreal forest ecosystems in northern Alberta.

1.2 Effects of N and S deposition on forest ecosystems

In a N-limited boreal forest, short-term N deposition can increase plant growth (Jung and Chang, 2012), but over time it may cause N saturation and decrease forest productivity (Aber et al., 1989). Jung et al. (2017) reported that long-term N deposition has negative impacts on understory plant community diversity. Nitrogen deposition may cause excess NO_3^- leaching, which creates base cation leaching and soil acidification (Laxton et al., 2010; Watmough et al., 2014). Low soil pH will further restrict soil activities, resulting in the accumulation of litter on the forest floor (Aber et al., 1989). Friedland et al. (1984) reported that excess N concertation in

foliage resulting from N deposition caused winter frost damage to plant species in New England. Nitrogen deposition can increase soil N availability, which in turn will cause other essential nutrients to become limited, such as phosphorus (P). This nutrient imbalance will further limit microbial and plant growth (Aber et al., 1989).

Excess S deposition can decrease forest productivity and vegetation diversity through base cation leaching and soil acidification (Tamm, 1976; Jung et al., 2017). Jung et al. (2017) reported that S addition significantly decreased exchangeable cations, such as Ca^{2+} and K^{+} in the forest floor which may lead to decreased soil pH, and a decrease in the diversity of the shrub layer. Sulfur deposition may also increase nutrient leaching from the soil, thereby causing nutrients deficiency and further contributing to forest decline (Lee, 1998). It is also well documented that soil microorganisms are very sensitive to acidity, and many microorganisms become less active when pH drops below 5 (Williams and Gray, 1974). Sogn and Abrahamsen (1998) concluded that the combination of N and S deposition further increased NH_4^+ leaching and P concentration in Scots pine (*Pinus sylvestris L.*).

1.3 Previous studies on N and S deposition

Compared to historical emission rates, S deposition has declined due to the US Clean Air Act (Driscoll et al., 2001). However, N deposition remains elevated (approximately 7.7 kg N ha^{-1} yr^{-1} in the eastern US) and has doubled from human activity mainly from NO_x emission from burning fossil fuels (Baumgardner et al., 2002; Howarth et al., 2002; Du et al., 2014). Fenn et al. (2003) studied biological responses in the western US and demonstrated that some terrestrial and microbial communities were significantly negatively affected by N deposition. In contrast to data

from the US, N deposition rates in central Europe were even higher still, 17 kg N ha^{-1} yr^{-1} (EMEP, 2016). Stevens et al. (2004) reported that 23% of grasslands species have decreased due to chronic N deposition. In China, the average N deposition rate increased from 11 to 18 kg N ha^{-1} yr^{-1} from the 1990s to 2013, and become the largest creator and emitter of N globally (Liu et al., 2013; Jia et al., 2014; Zhu et al., 2015). In China, S deposition rate remained steady at 5.9 kg N ha^{-1} yr^{-1} between 2001 and 2005 (Kuribayashi et al., 2012). Liu et al. (2013) reported that this N deposition in China resulted in significantly increased plant tissue N concentration in non-agricultural ecosystems and increased crop N uptake from non-fertilized croplands.

In the AOSR, Canada, a long-term simulated N and S deposition has been examined experimentally since 2006, and a number of projects have been completed:

(1) Jung and Chang (2012) found that four years simulated N and S deposition resulted in increased tree growth since the forest was N limited. After 4 years of simulated N and S deposition, the boreal forest ecosystem did not have any evidence of N saturation. However, N deposition increased soil exchangeable cations leaching and tree growth increased;

(2) Hu et al. (2013) found that 5 years of simulated N and S deposition did not change soil organic C concentration, however, it did change soil microbial physiological profiles. This study also confirmed that soil microbial biomass was not sensitive to 5 years of simulated N and S deposition in a N-limited forest;

(3) Jung et al. (2017) concluded that 7 years of simulated N and S addition has decreased understory plant diversity. Results from this study indicated that long-term N and S deposition can negatively impact plant nutrition; and

(4) Another recent study demonstrated that 9 years of simulated N and S deposition did not change bacterial community composition but affected the fungi community, as fungi are more

sensitive to the N and S addition (Ibsen, 2016). Following 10 years of simulated N and S deposition, there was no evidence of N saturation or soil acidification in this boreal forest study area.

Since 1998, the Wood Buffalo Environmental Association has operated the Acid Monitoring Program (AMP) to access the potential effects of N and S deposition in the AOSR forest ecosystem. In the Wood Buffalo Environmental Association Technical Reports (WBEATR) (Clair and Percy, 2015), Fenn (2015) concluded that from the center of the industrial area to a distance 20 km, N deposition decreased by 88% from 22 kg ha^{-1} yr^{-1} to 3 kg ha^{-1} yr^{-1} and S deposition reduced by 56% from 24 kg ha^{-1} yr^{-1} to 11 kg ha^{-1} yr^{-1}. In the same WBEATR report, Masse et al. (2015) demonstrated a negative relationship between N and S deposition and soil microbial communities. Maynard (2015) found that after 15 years of monitoring, N and S concentration increased in both soil and foliar samples collected within 20 km from the source. In 2011 and 2012, Macdonald (2015) sampled understory vegetation from 19 sites in AOSR and concluded that N and S deposition from oil sands mining had a fertilization effect on plant growth.

In summary, N and S deposition, especially in the industrial countries, has become a major environmental issue due to its significant impact on the forest ecosystems. The AOSR in northern Alberta is one of the largest oil sands deposits, and with the increasing mining and upgrading activities, N and S deposition has been a huge concern to the surrounding area.

2. Litter decomposition

2.1 Importance of litter decomposition

Studying litter decomposition is very important for a variety of reasons. First, litter decomposition is an important component of the nutrient cycling process (Melillo et al., 1982). Swift et al. (1979) demonstrated that the majority of available nutrients in the forest soil is the result of litter decay. Second, litter decomposition can potentially increase soil pH by increasing base cations taken up by plants (Berg and McClaugherty, 2003). However, soil pH can also decrease through the release of carbon dioxide (CO_2) during litter decay. A significant amount of greenhouse gas (CO_2, CH_4, and N_2O) emissions are a product of litter decomposition which may potentially contribute to global climate change. Additionally, the last stage of litter decomposition is called humus. Humus can sequester C which provides a reservoir of nutrients for plants and microorganisms (Berg and McClaugherty, 2003). Finally, litter decomposition controls the balance between CO_2 returned to the atmosphere and long-term C sequestration in the forest soil (Swift et al., 1979). Therefore, studies of litter decomposition are essential to our ability to predict the consequences of climate change on the global C budget (Graça et al., 2005).

Rates of litter decomposition can strongly affect forest ecosystems in many ways. Slow litter decomposition rates can result in nutrient accumulation which can limit forest productivity (Florence, 1965; Heilman, 1966; Miller, 1969); while fast litter decomposition rates increase soil fertility and nutrient concentrations in the soil, as well as release a significant quantity of CO_2 which contributes to climate change (Berg and McClaugherty, 2003; Wood et al., 2006).

2.2 Definition of litter decomposition

In a forest ecosystem, the forest floor consists different plant parts, such as leaves, roots, branches, barks, and stems in various stages of decomposition present on the top of the mineral soil. Based on the different degradation stages of shed vegetative parts, the forest floor can be divided into litter (L) (newly fallen materials without much decomposition has taken place), fermentation (F) (partially decomposed materials), and humified (H) (very well decomposed materials) layers. The forest floor also consists a large number of organisms, such as fungi and bacteria. Litter accumulation can affect ecosystem functions in many ways, for example, it can prevent soil freezing (McKinney, 1929) and soil erosion (Dyksterhuis and Schmutz, 1947), reduce evaporation (Holland and Coleman, 1987), reduce weed infestation (Davies, 1988), and provide habitat for microorganisms (Bot et al., 2005). Litter includes foliar litter (leaves and needles), fine roots (<2mm), and woody litter (stems, stumps, branches, twigs, and coarse roots). Among the litter materials, leaf litter accounts for about 70% of the total litter fall in forest ecosystems (Bray and Gorham, 1964), and leaves are also the main source of annual aboveground litter production (Cornelissen et al., 2007).

In terrestrial ecosystems, forests cover about 30% of the earth's surface, and 90% of the net primary production enters the decomposition subsystem through litter fall (Swift et al., 1979; Graça et al., 2005). The amount of annual litter fall varies depending on the forest types, ranging from 1 to 15 Mg ha^{-1} yr^{-1} (Williams and Gray, 1974). Such a large amount of litter production suggests that decomposition is a vital process in the functioning of the forest ecosystem. Litter decomposition results essentially from the biological processes of microorganisms, mainly fungi and bacteria, which breakdown and transform organic matter into smaller compounds that can be up taken by plants and organisms (Swift et al., 1979).

2.3 Study methods of litter decomposition

Given the important role of litter decomposition, ecologists since the 18 century have developed a variety of methods to study litter decomposition rates (Graça et al., 2005). The most widely used method is called "litterbag" which has been used since the 1960s but was initially introduced in the 1930s. This method allows for accurate measurements of mass loss (Prescott, 2010). Litterbag techniques can be used either in laboratory microcosms or in field studies. In this method, a known quantity of newly shed litter is enclosed in a mesh bag of proper mesh size and either put on the top of the soil or buried under ground. At the beginning of the experiment, a large number of litterbags are installed and sampled periodically over time. This method is widely used because it is both simple and inexpensive (Berg et al., 1993; Aerts, 1997).

Recently ecologists have brought attention to the limitations of the litterbag method (Kurz et al., 2000). For example, the duration of the study has to be decided at the beginning prior to litter decomposition. In addition, the mesh size itself will have a dramatic effect on the total mass loss due to different fauna size (Hutchinson et al., 1990; De Santo et al., 1993). Furthermore, the process of confining litter within bags increases the moisture content which will lead to faster decomposition rates (Tanner, 1981; De Santo et al., 1993).

To overcome these limitations, Kendrick (1959) and Kurz et al. (2000) developed another method to study litter decomposition, which is the direct field observation method (litter traps). The advantages of this method are that it is done without any manipulation or microclimatic artifact and it is convenient because samples do not have to collect at the same time in order to be comparable (Kurz et al., 2005). The direct field observation method allows us to determine

the mass loss in situ and can build a predictive model over a long period of time. However, it is specific for needle litter and may not be suitable for broad-leaf litter (Kurz et al., 2000).

Litter decomposition is sometimes measured as CO_2 release. However, CO_2 release from litter respiration is only part of litter mass loss during litter decay (Berg and McClaugherty, 2003). Because litter mass loss is a combination of CO_2 release and compound leaching. Distinguishing litter respiration from other respiration (such as fine root and microbes) in the field is difficult. Therefore, using microcosms in the laboratory to study CO_2 efflux from litter is a simple and easy way to measure litter decomposition rates. In the microcosms, CO_2 efflux from the litter can be determined under favorable and standardized conditions of moisture and temperature. Two methods can be used to measure CO_2 efflux from litter, one is using gas chromatography (GC) (Wang and Wang, 2003) and the other one is using the alkali-absorption technique (Šesták et al., 1971; King and Harrison, 2007). The GC method is considered an easy and safe way to access CO_2 efflux and often used when additional trace gas fluxes are of interest (Castro et al., 1994). However, since CO_2 efflux is sensitive to temperature, moisture, and distance from collection to instrumental analysis, alkali-absorption is a better method to use in a field study (Keith and Wong, 2006).

2.4 Three-phase model of litter decomposition

Litter decomposition is a complex ecological process that involves physical, chemical, and biological processes (Graça et al., 2005). Litter decomposition can be described by a three-phase model developed by Berg and Matzner (1997). Early stage decomposition is carbohydrate-dominated in which N, P, or S concentrations influence the decomposition of water-soluble

substances, as well as unshielded cellulose and hemicellulose. At this stage, loss of organic matter from newly shed litter is primarily through leaching, consumption by microorganisms, or conversion to insoluble compounds (McClaugherty, 1983). At this early stage, positive relationships between litter decomposition rates and macronutrients, water-soluble substances concentration, and actual evapotranspiration (AET) (Waksman, 1924; Berg and Staaf, 1980; Berg et al., 1993; Berg, 2000). For broad-leaf litter, when litter mass loss reaches 40%, the decomposition stage shifts to late stage (Berg and McClaugherty, 2003). The late stage is lignin-/acid-unhydrolyzable residue (AUR)-dominated (Fogel and Cromack, 1977; Johansson et al., 1995). The AUR is resistant to decomposition and increasing AUR concentrations are negatively associated with litter decomposition rates. Within this late stage, Hobbie et al. (2012) found that cellulolytic and ligninolytic enzymes decreased after long-term N addition. Furthermore, increased N concentration suppressed lignin degradation within the decomposing litter, while manganese (Mn) appeared to stimulate lignin degradation. Finally, at the near-humus stage, lignin/AUR was constant, and litter decomposition rates fell close to zero.

2.5 Factors controlling litter decomposition

Research in the past several decades has illuminated several aspects of litter decomposition both at a specific field site and at a global scale. Swift et al. (1979) concluded three general components that determine decomposition processes including physical parameters such as climate and mineralogy of the parent material, the quality of the decomposing resources, and organisms. Lavelle et al. (1993) proposed a hierarchical model for litter decomposition in terrestrial ecosystems with the following factors regulating litter decomposition from high to low:

(1) climatic factors; (2) physical properties of soil, such as soil texture; (3) resource quality or litter quality of litter chemistry, such as N and lignin concentrations (Aber and Melillo, 1982; Yavitt and Fahey, 1986), and C to N (C/N) and lignin to N (lignin/N) ratios (Parton et al., 1987; Taylor et al., 1989; Aber et al., 1990); and (4) microorganisms and microorganisms (Swift et al., 1979). Subsequent research has identified mean annual temperature (MAT), mean annual precipitation (MAP) and annual actual evapotranspiration (AET) (Aerts, 1997; Berg et al., 2000). For example, studies reported that AET was a better predictor of litter decomposition rates when compared to temperature or precipitation alone (Aerts, 1997).

2.5.1 Climate

Climate remains the most important factor influencing litter decomposition rates on a global scale (Meentemeyer, 1984). Within a single site, litter decomposition rates varied among years and between different periods of the year due to differences in temperature and precipitation. The AET had previously been considered superior to climate index at broad, continental scales (Meentemeyer, 1978, 1984; Berg et al., 1993a, 1993b). More recently, Kang et al. (2009) reported from a regional experiment across Asia and Europe, a highly significant linear relationship between first-year litter mass loss, MAT, and MAP.

2.5.2 Litter chemistry

Litter chemistry dominates litter decomposition on a local level (Aerts, 1997). Emphasis has been placed on predicting the decomposition rate, and even more importantly, the rate of

nutrient release, based on the chemical composition of the resource. The C/N ratio is well established by 1920s as a general index of litter quality (Waksman, 1924). Carbon is used in plant cells as an energy source. During decomposition, C is used as an energy source by the decomposers while N is assimilated into cell proteins and other compounds. Thus a high N content in the original material promotes decomposition, at least in the early stages. Materials with C/N ratio <20 decompose rapidly. High N concentration may have a suppressive effect on lignin mass loss due to the formation of ligninase.

As decomposition proceeds, litter chemistry rapidly changes because the substrates which assimilate readily are rapidly metabolized and resistant compounds tend to accumulate. Lignin is one of the plant components most resistant to decomposition and its abundance is often inversely linked to decomposition rates (Fogel and Cromack, 1977; Berg et al., 1982). In some cases, the lignin to N ratio of decomposing material has a high predictive value for decomposition rates (Melillo et al., 1982; Parton et al., 1993). Lignin is the most recalcitrant and abundant aromatic compound on earth and is second only to cellulose in its contribution to living terrestrial biomass (Crawford, 1981). At the early stage, litter decomposition is mainly regulated by N and simple soluble carbohydrates. At the late stage, organic matters become more resistant to decay, polyphenols especially lignin become the main factor influencing litter decomposition. The element Mn is also involved in the regulation of other ligninolytic enzymes, including laccase (Archibald and Roy, 1992) and lignin peroxidase (Perez and Jeffries, 1992). Many studies have demonstrated that Mn is essential for the formation and activity of lignin-degrading enzymes and thus for degradation of lignin (Paszczynski et al., 1986; Bonnarme and Jeffries, 1990).

Nutrient concentrations, such as N, are known to positively correlate with litter mass remaining (Blair, 1988; Aerts and de Caluwe, 1997). Three phases of N release pattern during

litter decomposition was proposed by Berg and Staaf (1980), including an initial leaching phase, following by an accumulative phase, and finally a mineralization release phase. The release curve is controlled by initial and critical C/nutrient values (Manzoni et al., 2010). Although numerous studies have articulated the importance of N release during litter decomposition, little attention has been paid to S release.

2.5.3 Organisms

The primary decomposers in boreal forest soils are bacteria and fungi (Swift et al., 1979). Fungi, in general, produce a wider range of extracellular enzymes than bacteria (Kirk and Farrell, 1987). For example, white-rot fungi can completely mineralize lignin to CO_2 and H_2O (Cadisch and Giller, 1997).

To decompose plant litter, microorganisms need to produce extracellular enzymes to convert polymeric compounds, including cellulose, hemicellulose, and lignin (aromatic C polymers), into smaller molecules that can then be taken up by plants or other biological organisms (Sinsabaugh et al., 2002). The most relevant enzymes are those that break down the plant fibers (cellulases, hemicellulases, pectinases, and phenol oxidases) as well as enzymes important for the microbial acquisition of N, P, and S (peptidases, ureases, phosphatases, and sulfatase) (Sinsabaugh et al., 2002). Thus, enzymes can be directly used to estimate decomposition rates.

Litter decomposition is essentially the process of a microbial community's growth and succession over time (Sinsabaugh et al., 1991). To quantify the effect of soil microorganisms on litter decomposition, microbial biomass and microbial extracellular enzyme activity are widely

used in forest ecosystems (Allison et al., 2008). Many studies have shown that N deposition could directly or indirectly influence soil microbial community composition and function (Berg, 1986; Fog, 1988; DeForest et al., 2004). For example, Carreiro et al. (2000) demonstrated that N addition had a suppressive effect on lignin-degrading enzyme acidity in a high lignin litter type. In contrast, other studies have shown that N addition can have a positive effect on other C-, and N-acquiring enzyme activity (Waldrop et al., 2004a, 2004b; Sinsabaugh et al., 2005). Furthermore, microbes are sensitive to nutrient availability (Díaz-Raviña et al., 1993). For example, according to Swift et al. (1979) when the C/N is about 5-7:1 for bacterial growth is positively affected, and at 7-25:1 fungal growth is stimulated. It has reported that globally under N addition, soil microbial biomass declined over time (Treseder, 2008). However, the effect on soil microbial biomass is inconsistent and should be site-specific. For example, Hogberg (2007) concluded that in a N-limited forest, N addition can improve litter chemistry and may increase soil microbial biomass.

2.5.4 N and S addition

Finally, litter decomposition can be affected by N and S addition through changing litter chemistry and microbial and enzyme activities. Aber et al. (1991) concluded that the influence of N addition to litter chemistry may be one of the most important factors that affect litter decomposition rates. Nitrogen deposition to the forest floor may lead to increased soil N concentration, as a result, litter N concentration increased (Berg and Matzner, 1997). Perakis et al. (2012) suggested that high N concentration in the litter promotes decomposition, at least in the early stages (<3 yr). Added N can react with lignin or other phenolic compounds which will

increase litter's resistance to decomposition (Berg and Matzner, 1997). Studies also showed that N addition can change microbial community functions, and high N availability can suppress lignin-degrading enzymes activity (Sinsabaugh et al., 2002). Wang et al. (2010) reported that added S can suppress enzymatic activities, especially under low pH conditions. In contrast, it has also been reported that S can increase microbial biomass by supplying exogenous S (Donald et al., 1999). Since S is often a limiting macronutrient for microbial growth, site specific differences can significantly alter the results of S dynamics.

For the systematic study of the effect of N and S addition on litter decomposition, both laboratory and field studies are needed. Because field study is lack of control over important factors, and laboratory study is lack of realism. Litter decomposition rates can be influenced by many factors because litter decomposition is a complex process. Thus, to better understand the mechanisms within any specific ecosystem, there is a need to study the effect from several perspectives, such as abiotic (litter chemistry) and biotic (soil microbial properties and litter enzyme activities).

2.6 Effects of N and S deposition on litter decomposition

Nitrogen and S deposition not only can affect the plant, soil, and forest floor dynamics, but it can also influence litter decomposition processes (Berg and Matzner, 1997). Numerous studies have reported that N addition can significantly increase litter decomposition rates (Carreiro et al., 2000; Hobbie and Vitousek, 2000; van der Wal et al., 2007). Others found either suppressed (Magill and Aber, 1998; Carreiro et al., 2000; Ramirez et al., 2012) or no effect (Pastor et al., 1987; Hunt et al., 1988; Prescott, 1995) on litter decomposition rates. Similar to N

deposition, studies have also shown that S deposition inhibited (Traaen, 1980; McKinley and Vestal, 1982), enhanced (Ishac and Hovland, 1976; Roberts et al., 1980; Lee and Weber, 1983), or did not change (Killham et al., 1983) litter decomposition.

These inconsistent effects of N deposition on litter decomposition can be attributed to variation between different study sites, N deposition rates, N sources (NH_4^+, NO_3^-), litter types, litterbag mesh size, and experiment duration (Knorr et al., 2005; Janssens et al., 2010). Prescott and Parkinson (1985) concluded that the different effects of S deposition on litter decomposition may be explained by different experiment types (laboratory or field), S sources (H_2SO_4, SO_2), pH level, and duration of the study.

3. Synthesis

The significant impacts of N and S deposition on the forest ecosystems in the AOSR are of great concern since oil sands mining operations began in 1967 (Jung and Chang, 2012; Clair and Percy, 2015). Most of the nutrient return to the underground soil system is through litter decomposition (Swift et al., 1979), and litter decomposition is important to the global C budget (Coŭteaux et al., 1995). Thus, there is a strong need for studies to gain a better mechanistic understanding of N and S deposition on litter decomposition in AOSR. Considering the advantages and disadvantages of the various litter decomposition study methods, this study combined both laboratory and field experiments.

4. Research objectives and thesis structure

In the AOSR, significant amounts of N and S have released to the atmosphere and deposited to the surrounding area due to the intensive oil sands mining activities. These N and S deposition have a significant impact on the boreal forest ecosystems, such as plant growth, forest productivity, and soil microbial properties. On the other hand, litter plays a vital role in the nutrient and C cycling in the forest ecosystems. For example, fast litter decomposition rates can release large amounts of nutrients which will stimulate forest productivity.

In order to gain a better understanding of the impacts of N and S deposition on litter decomposition, I have conducted laboratory and field experiments on litter decomposition under long-term simulated N and S addition in a mixedwood boreal forest in northern Alberta, Canada. This study will contribute to our understanding of the mechanisms of C and nutrient cycling in a mixedwood boreal forest in the oil sands region. The specific objectives of this study are included below.

- To determine if 10 years of simulated N and S addition has changed litter chemistry, soil microbial biomass and enzymatic activities (Chapter 2).
- To assess the relationship between litter decomposition rate (measured as CO_2 emission) and initial litter chemistry and soil microbial properties with N and S addition under controlled laboratory conditions (Chapter 2).
- To determine the effect of 10 years N and S addition on litter decomposition in an 18-month field study (Chapter 3).
- To examine the combined effects of nutrient additions and nutrient-induced changes in litter chemistry on litter decomposition in an 18-month field study (Chapter 3).

- To study the effect of N and S addition and the combined effects of nutrient additions and changed litter chemistry on N and S release pattern in litter remaining during an 18-month field study (Chapter 3).
- To explore relationships between cumulative enzyme activities in litter and decay constants (Chapter 3).

This thesis consists of four chapters. Chapter 1 describes the background information of N and S deposition, the important role of litter decomposition in a forest ecosystem, previous studies in the AOSR, and study overview. Chapter 2 and 3 are data chapters (Fig. 1-1). Chapter 2 assesses the effect of 10 years of N and S addition on litter chemistry and forest floor microbial properties, and in turn, how the changed litter chemistry and forest floor microbial properties affect litter decomposition. Litter decomposition rates were expressed by litter respiration rates in a laboratory microcosm study. Chapter 3 examines the effect of nutrient additions and combined effects of nutrient additions and nutrient-induced changes in litter chemistry on litter decomposition (expressed as litter mass loss) and nutrient changes in litter remaining during the decomposition process, and determines the relationship between cumulative enzyme activities in litter and decay constants in a period of an 18-month field study. Chapter 4 summarizes the key findings, including the key results and highlights of this study and provides the recommendations for future work based on this research.

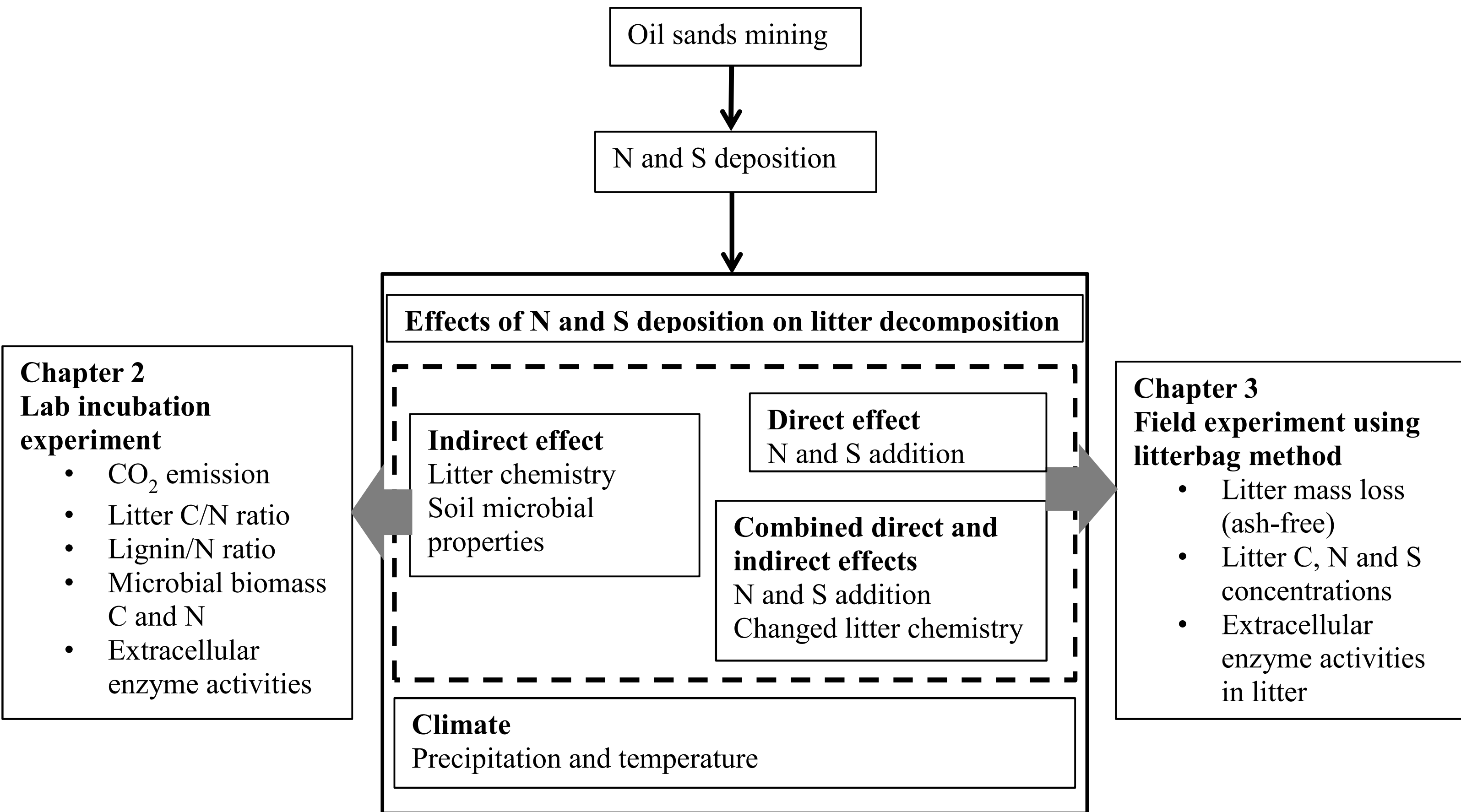

Fig. 1-1 Flow chart of this study.

Chapter 2. Decomposition of trembling aspen (*Populus tremuloides*) leaf litter under long-term nitrogen and sulfur deposition: Effects of litter chemistry

1. Introduction

Litter decomposition plays a critical role in nutrient and carbon (C) cycling in forest ecosystems (Melillo et al., 1982) as nutrients and carbon dioxide (CO_2) are released through litter decomposition in the soils (Berg and McClaugherty, 2003; Wood, 2006). Litter decomposition is affected by many abiotic and biotic factors that include climate, soil physico-chemical properties, litter chemistry, nitrogen (N) availability, and soil organisms (Tenney and Waksman, 1930; Fogel and Cromack, 1977; Swift et al., 1979).

Among those, at a given climate and site condition, the effect of N availability on litter decomposition has received special interest as increased N deposition alters litter decomposition pattern through changed litter chemistry by N supply (Berg and Matzner, 1997) as well as changed soil microbial community composition and enzyme activities (Berg, 1986; Fog, 1988; DeForest et al., 2004). For example, N deposition has been shown to increase N concentration of litter, leading to decreased C to N (C/N) and lignin to N (lignin/N) ratios, that are key parameters determining litter decomposability; i.e., litter with low lignin/N is known to decompose faster than those with high lignin/N ratios (Berg and Matzner, 1997; Carreiro et al., 2000). Studies have shown that N deposition can also increase soil N availability, leading to higher microbial biomass and enzyme activities and faster decomposition rates (Sinsabaugh et al., 2002; Lv et al., 2013).

Due to the lowered lignin/N, many studies reported that N addition increase litter decomposition rates (Hunt et al., 1988; Carreiro et al., 2000; Hobbie, 2000; Hobbie and Vitousek, 2000). However, no significant effect (Pastor et al., 1987; Hunt et al., 1988; Prescott, 1995) or a suppressive effect of N on litter decomposition (Magill and Aber, 1998; Carreiro et al., 2000) has also been reported. The inconsistent results are ascribed to the variations in litter type, the type of N added (NH_4^+, NO_3^-), N addition rate, and experiment duration (Thirukkumaran and Parkinson, 2000). In addition to the effect of N deposition, the effect of sulfur (S) deposition on litter decomposition has also been investigated, where S deposition has been found to inhibit (Traaen, 1980; McKinley and Vestal, 1982), enhance (Roberts et al., 1980; Lee and Weber, 1983), or has no effect (Killham et al., 1983) on litter decomposition. However, how the co-occurrence of N and S deposition might affect litter decomposition through altering litter chemistry and soil microbial activities (e.g., enzyme activities) is poorly understood. This is an important research issue as forests in the heavy industrial activity areas are often subject to N and S deposition.

To fill the knowledge gap identified above, changes in the litter chemistry and soil microbial properties by N and S addition and the effect of litter chemistry and soil microbial properties on litter decomposition were investigated in this study. I hypothesized that 1) external N and S addition will decrease litter C/N and lignin/N ratios by increasing litter N concentration; 2) external N and S addition will increase soil microbial activity as N and S are required macronutrients for microorganisms; and 3) lowered C/N and lignin/N ratios and increased forest floor microbial activity will result in enhanced litter decomposition.

2. Materials and methods

2.1 Research site and experimental design

To study the effect of N and S deposition on forest ecosystems in the oil sands region in northern Alberta, research plots were established in 2006 in a mixedwood boreal forest stand (56.1° N 110.9° W), located about 100 km southeast of Fort McMurray, a major city in the Athabasca oil sands region (AOSR) in western Canada (Jung and Chang, 2012). Bulk deposition rate in the forest sites >90 km away from the center of mining activities was estimated to be between 1-2 kg ha^{-1} yr^{-1} for N and S (Proemse et al., 2012, 2013). The climate of the region is continental boreal, with a mean annual temperature of 1 °C and mean annual precipitation of 419 mm from 1981 to 2010 (Environment Canada, 2010). The main canopy tree species were approximately 60-year old *Populus tremuloides* (trembling aspen, accounting for 71% of the total tree species) and 25-55-year old *Picea glauca* (white spruce, accounting for 22% of the total tree species) (Jung and Chang, 2012). Soils were classified as Gray Luvisols based on the Canadian system of soil classification (Soil Classification Working group, 1998) or Boralf in Soil Taxonomy (Soil Survey Staff, 1998).

The research plots were set up in a randomized complete block design with two factors. One factor was N addition (with two levels, 0 and 30 kg N ha^{-1} yr^{-1} as NH_4NO_3) and the other one was S addition (with two levels, 0 and 30 kg S ha^{-1} yr^{-1} as Na_2SO_4) to simulate elevated levels of N and S deposition in the oil sands region, resulting in, four treatments: control (CK), N addition (+N), S addition (+S), and N and S addition (+NS). Three blocks were set up and four plots of 20 × 20 m were established in each block and treatments were randomly assigned to each plot. The granule forms of ammonium nitrate (NH_4NO_3) and sodium sulfate (Na_2SO_4) were

applied once a year from 2006 to 2008 in early summer. From 2009, the N and S were applied three times each summer on an equal split.

2.2 Leaf litter and forest floor sampling and analysis

In September 2015, before sampling the forest floor, newly fallen aspen litter was collected from the surface of the research plots. The litter samples were then oven-dried. Litter samples from the same treatment plot were composited, crushed, and passed through a 2-mm sieve to obtain a homogenized sample. A portion (5 g) of the litter sample was further ground using a ball mill (MM 200, REtsch GmbH, Haan, Germany) and used for chemical analyses. Total C, N and S concentrations were determined using an elemental analyzer (Carlo Erba NA1500, Carlo Erba Instruments, Milano, Italy). Lignin concentration was analyzed by measuring absorbance at 280 nm following acetyl bromide digestion (Morrison, 1972 a, 1972b). Other elemental concentrations including calcium (Ca), magnesium (Mg), aluminum (Al), and manganese (Mn) were determined using an ICP-MS (Elan 6000 quadrupole, Perkin-Elmer, Inc., CT) after digestion with nitric and hydrogen peroxide (Jones, 2001).

Forest floor (F and H layers, after removing current litter layer) samples (approximately 10 kg) were then collected in July 2016 from three randomly selected locations in each plot approximately 10 days after N and S addition and the current-year litter was also removed from the surface. Three composited forest floor samples were collected from the same treatment. The forest floor was approximately 2-5 cm thick and had a 0.1 g cm^{-3} bulk density. Forest floor samples were then placed in a cooler, transported to the laboratory, and stored in a refrigerator at

4 °C. The samples were sieved through a 4-mm sieve to remove stones, roots, and debris. The forest floor samples were stored at 4 °C until analysis and incubation experiment.

The moisture content of the forest floor samples was determined with a portion of the sample (10 g) in a forced air oven at 60 °C for 48 h until constant weight. Another portion (50 g) of the forest floor sample was air-dried for chemical analysis. The pH (1:10 w/w soil to water ratio) was measured using a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA). Total C and N concentrations were analyzed using the elemental analyzer as described above. Exchangeable cations, including sodium (Na^{+}), potassium (K^{+}), Mg^{2+}, Ca^{2+}, and Al^{3+}, were determined using an ICP-MS after extraction of 2 g sample with 100 mL of 1 mol L^{-1} NH_4Cl (Shuman and Duncan, 1990).

Another subsample of each fresh forest floor sample was used to determine microbial biomass C (MBC) and N (MBN) and extracellular enzyme activities. The MBC and MBN concentrations were determined using a fumigation-extraction method (Brookes et al., 1985). The concentrations of C and N in the fumigated and un-fumigated samples extracted with 0.5 mol L^{-1} K_2SO_4 (Brookes et al., 1985) were determined with a TOC-V_{CSN} (Shimadzu, Kyoto, Japan). Extracellular enzyme activities involved in C, N, and S cycling were analyzed: β-1, 4-glucosidase (GLU, enzyme classification (EC) EC 3.2.1.21) for C, β-1, 4-N-acetylglucosaminidase (NAG, EC 3.2.1.14) for N, and arylsulfatase (ARS, EC 3.1.6.1) for S. The NAG and GLU were measured with a fluorimetric method (Sinsabaugh et al., 2002). Briefly, one gram of fresh soil was placed in a 250 mL Nalgene HDPE bottle, 125 mL of sodium acetate buffer (50 mmol L^{-1}, pH 5) was then added, and shook for 30 min on an end-over-end shaker at room temperature. Then 200 µl of soil suspension and 50 µl of 200 µmol L^{-1} of each substrate were pipetted into black 96 well plates. Reference standard and quench controls were added to

each reference and quench well in each plate. The plates were incubated at 20°C in the dark for 3 h. After incubation, a 20 µl of 0.5 mol L^{-1} sodium hydroxide solution was added to each well automatically to stop the enzyme reaction. Fluorescence was measured at 360 nm excitation and 460 nm emissions using a multi-detection microplate reader (Synergy HT, Bio-Tek Instruments, Winooski, VT, USA). The activity of ARS was assayed using the colorimetric determination of p-nitrophenol released when soils were incubated with p-nitrophenyl β-D-glucopyranoside (Tabatabai, 1994).

2.3 Laboratory incubation experiment

Emission of CO_2 from forest floor amended with or without litter was monitored in a laboratory incubation experiment. A total of 32 jars were prepared for the four N by S treatments, with or without litter, and with 4 replicates. Fresh forest floor (200 g in dry weight) was placed in a 1-L mason jar, and water content was adjusted to 40% of water holding capacity (WHC), which was determined using the method in Bernard (1963). The jars were then placed in the dark for 3 days at 25 °C to restore and stabilize the microbial activity. To prevent water loss, each jar was covered with aluminum foil with approximately 20 small holes to allow air exchange. After the 3-day pre-incubation, 100 g (in dry weight) of forest floor from each jar was collected and analyzed for the initial characteristics as described above. Then, for the litter treatments, a litter sample (1 g) was placed in the jars on the surface of the forest floor that was from the same field treatment plot. The amount of C added through litter addition (calculated using the C concentration of the litter) was similar across the treatments and ranged from 453 to 459 mg (Table 2-1). Deionized water was added to the jars to bring water content from 40 (in pre-

incubation) to 60% of WHC, and the weight of each jar was recorded for water content adjustment. Distilled water was added each week to restore the initial moisture level. All the jars were covered with the perforated aluminum foil and incubated in the dark at 25 °C.

Gas samples were taken on days 1, 2, 4, 7, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, and 100 after the initiation of the incubation. At each sampling time, jars were sealed with a lid with a septum, and then the initial gas sample was collected immediately with a syringe. Then a gas sample was collected again after 24 hours to determine the gas emission rate. The jars were then covered with the perforated aluminum foil again until the next sampling time. The CO_2 concentration (ppm) was analyzed using a gas chromatography (Varian CP-3800, Mississauga, Canada).

The CO_2 emission flux (mg C kg^{-1} hr^{-1}) was calculated by the following formula (Hu et al., 2004):

$$CO_2 \text{ flux} = (dCO_2/dt) \times 10^x \times \rho \times \alpha \times (V/A) \times 10^y \times [273/(273+T)]$$

where: dCO_2/dt is the change in the CO_2 concentration for 24 hr (ppm hr^{-1}); 10^x is a conversion factor (ppm = mL m^{-3} = 10^{-6} m^3 m^{-3}); ρ is the density of CO_2 (44/22.4) under standard condition, and at standard temperature and pressure, one mole of gas accounts for 22.4 volumetric L of volume; α is the conversion factor for CO_2 to C (12/44); V is the volume of the jar (mL); A is the soil weight (g); 10^y is the conversion factor (g = 10^{-3} kg), T is the incubation temperature (°C). Rates of CO_2 emission from litter were calculated by subtracting the CO_2 emission of forest floor without litter from that of forest floor amended with litter. It was assumed that the priming effect, the change in indigenous soil organic C mineralization by adding litter, is negligible as the litter was not mixed thoroughly but placed on the surface of the forest floor (Kuzyakov et al., 2000).

At the end of the incubation, MBC, MBN and extracellular enzyme activities of the incubated forest floor with or without litter were analyzed again as described above. Cumulative enzyme activity was calculated by integrating enzyme activity over the 100-day laboratory incubation, using a cubic spline approximation approach (Mohanty et al., 2013).

2.4 Statistical analysis

Statistical analysis was conducted using the R software package for Windows (version 3.3.0, R Foundation for Statistical Computing, Vienna, Austria). Before conducting the analysis of variance (ANOVA), the data were explored by exploratory data analysis, including tests for homogeneity of variance and normality f distribution. Two-way ANOVA was conducted to determine the differences in initial leaf litter properties and forest floor properties with N and S deposition treatments in the field. Variations in the cumulative CO_2 emission (C_{cum}) and the changes in the concentrations of MBC, MBN, and extracellular enzyme activities during the incubation as affected by the field N and S deposition treatments were tested with two-way ANOVAs. The difference between initial and final values of MBC, MBN, and extracellular enzyme activities during the incubation was tested by one-way ANOVA.

Linear regression analysis was used to analyze the relationship between total C and N and MBC, MBN in the initial forest floor, between C_{cum} and initial litter chemistry, as well as between CO_2 emission and microbial biomass or enzyme activity. The significant level was set at $\alpha = 0.05$ for all statistical analysis.

3. Results

3.1 Chemical properties of litter and forest floor

Long-term addition of N and/or S did not change ($P > 0.05$) the C concentration of the litter, but increased N ($P = 0.031$ and 0.012 for N and S addition, respectively), and decreased lignin concentration ($P = 0.047$ for N addition and $P > 0.05$ for S addition), lead to lower C/N and lignin/N ratios (Table 2-1). When N and S were added together, lignin concentration did not change, but N concentration increased compared to controls, resulting in lower C/N and lignin/N ratios. Neither N nor S addition affected the concentrations of other elements including S and micronutrients.

Ten years of addition of N and/or S did not change forest floor pH, but increased total C and N concentrations ($P < 0.001$ for N addition and $P = 0.013$ for S addition for both total C and N), with a greater increase with N than with S addition (Table 2-2). As indicated by the interaction of N by S addition ($P = 0.026$), co-addition of N and S further increased total C concentration of the forest floor over N or S addition alone. Exchangeable Na^+ concentration was the highest in the +S treatment in which Na_2SO_4 was applied to the plots; meanwhile, other exchangeable cation concentrations were not affected by the treatments.

3.2 Microbial biomass C and N, and extracellular enzyme activities

Litter addition did not change microbial properties from the forest floor amended with or without litter samples (Table 2-3). Ten years of N addition with or without S addition, increased forest floor MBC and MBN ($P < 0.01$). Addition of N increased GLU ($P < 0.001$) and ARS ($P <$

0.01) but not NAG ($P > 0.05$) activities, while S addition increased GLU ($P < 0.01$), NAG ($P < 0.05$), and ARS ($P < 0.01$) activities. When N and S were co-added, the ARS activity decreased ($P < 0.01$) below N added alone and GLU and NAG activities did not change as compared to N or S addition alone.

Over the 100-day incubation, GLU and NAG activities increased ($P < 0.05$) while MBC, MBN, and ARS activity decreased ($P < 0.05$) as compared to the initial whether litter was added or not (Table 2-3). At the end of the incubation, though the absolute values of MBC, MBN and enzyme activities changed as mentioned above, the variations across the N and S treatments were similar to the initial.

After 10 years of N and S addition, forest floor total C was positively correlated with MBC ($R^2 = 0.76$, $P < 0.001$) and MBN ($R^2 = 0.38$, $P = 0.007$), and total N was also positively correlated with MBC ($R^2 = 0.71$, $P < 0.001$) and MBN ($R^2 = 0.37$, $P = 0.008$) (Fig. 2-1).

3.3 CO_2 emission and its relationship with litter chemistry and forest floor microbial properties

In the forest floor with and without litter addition, the rate of CO_2 emissions varied between 200 and 700 mg C kg^{-1} day^{-1} and peaked at day 20, then gradually deceased until the end of the study (Fig. 2-2A and B). The daily rate of CO_2 emission from litter peaked at day 40 followed by a significant decrease until day 50; thereafter it fluctuated without a clear trend (Fig. 2-2C).

During the 100-day incubation, C_{cum} from the forest floor with or without litter was increased by S addition ($P = 0.008$) but not by N addition and there was an interaction ($P < 0.001$)

between N and S addition (Table 2-4). Co-addition of N and S decreased C_{cum} from forest floor without litter more than N or S addition alone. The C_{cum} from litter only was higher in the +S and +NS treatments ($P < 0.001$ and $P = 0.010$, respectively) than in the CK or the +N treatment (Table 2-4). Between the +S and +NS treatments, N addition decreased C_{cum} resulting in the interaction between the N and S treatments ($P = 0.010$).

For the C_{cum} from litters only, when all the data were pooled across treatments, the C_{cum} was positively correlated with litter C/N ($R^2 = 0.39$, $P = 0.006$) and lignin/N ratios ($R^2 = 0.24$, $P = 0.03$) (Fig. 2-3). However, there was no correlation between C_{cum} and mean MBC, or between mean MBN and cumulative enzyme activities (Appendix A and B).

4. Discussion

4.1 Litter and forest floor chemistry and microbial properties after 10 years of N and S addition

The results showed that 10 years of N and S addition decreased litter C/N and lignin/N ratios through increased N and decreased lignin concentrations, which supports the first hypothesis. Litter N concentration is affected not only by the duration of N addition but also by the rate of N addition (Tamm et al., 1974; Tamm, 1991; Bergkvist and Folkeson, 1992; Jung and Chang, 2012; Jung et al., 2017). In previous studies that were conducted at the study site, 4 years of N addition did not cause any change in litter N concentration (Jung and Chang, 2012); however, after 7 years, N addition increased foliar N concentration in some understory species (Jung et al., 2017). Though the effect of the rate of N addition was not tested in this study, N

addition rate is a critical factor in changing N concentration of litter. For example, Tamm et al. (1974) and Tamm (1991) showed that litter N concentration was increased by 6 years of N addition at 80 kg N ha^{-1} yr^{-1} but not at 40 kg N ha^{-1} yr^{-1}. In this study, N concentration in the litter was also increased under the +S and +NS treatments, which may suggest that this study site is also S-limited, and the enzymes that are involved in N cycling are sensitive to S deficiency, such as nitrogenase and ferredoxin (Duke and Reisenauer, 1986). The previous study also reported that in a S-deficiency site, S addition increased N concentration in ryegrass (*Lolium*) leaves and stems (Zhao et al., 1999). Downs et al. (1996) also reported that N concentration in litter increased under high S addition during the first year of their study, and O'Connell (1994) reported that under N addition, S concentration in litter increased in association with increased N concentration. The higher N concentration than S concentration in litter may be an indication of a stronger N limitation in the forest ecosystem in this study. Decreased C/N and lignin/N ratios are believed to favor litter decomposition and N mineralization, which suggests that long-term N and S addition may change forest C and N dynamics.

Ten years of N and S addition did not change forest floor pH; this is consistent with Jung et al. (2017) findings. Several earlier studies conducted at the study site (Cheng et al., 2011; Jung and Chang, 2012; Hu et al., 2013) also found no treatment effects on soil pH. My data suggest that more time may be needed to affect soil pH with the current N and S addition rates in the studied site. For example, Bergkvist and Folkeson (1992) reported that after 5.5 years of simulated N addition (as NH_4NO_3) at 66 and 198 kg N ha^{-1} yr^{-1} soil pH decreased to 4.07 and 3.81, respectively, from 4.51. It is well established that S addition can increase base cation leaching to decrease soil pH (Likens and Bormann, 1995; Jung and Chang, 2012). In this study,

neither N nor S addition affected forest floor exchangeable cations except Na^+ due to the addition of Na_2SO_4.

Ten years of N addition has increased total C and N in the forest floor, as litterfall amount increased under N addition (Jung and Chang, 2012), and added litter can increase C pools in the soil (Rinnan et al., 2008). Berg and Matzner (1997) concluded that added N can increase soil N concentration. The previous study did not find increased total C under S addition, this may be due to the amount of S applied which did not change soil microbial activities in this study. Xu et al. (2016) observed that under high concentrations of S addition both soil fungal activity and litter decomposition rates increased. This may lead to higher forest productivity and greater litterfall input, leading to higher total C on the forest floor.

The results also showed that 10 years of N and/or S addition increased MBC, MBN, and enzyme activities in the forest floor as soil microbes are sensitive to nutrient availability (Díaz-Raviña et al., 1993), and this result supports the second hypothesis. Several studies have also found positive effects of N and S addition on microbial biomass (Beare et al., 1990; Gallardo and Schlesinger, 1994). However, my results and others (Beare et al., 1990; Gallardo and Schlesinger, 1994) are in contrast with reports of N and S addition decreasing (Ohtonen et al., 1992), or having no effect on microbial biomass (Prescott et al., 1992; Hu et al., 2013). Such variable results are related to the experimental conditions, such as the time since N addition to the ecosystems and sampling time (Gallardo and Schlesinger, 1994; Treseder, 2008; Janssens et al., 2010; Ramirez et al., 2010). The sampling time in a growing season in relation to the time of N and S addition may be also important for microbial biomass measurement as microbial response to N addition is quick and the response may diminish with time (Ohtonen et al., 1992). In this study, the forest floor samples were collected shortly after N and S addition in the field, which

results in significant difference in microbial biomass C and N across the treatment. The positive relationship between C and N concentration and MBC and MBN in the forest floor suggested that N and S deposition may affect microbial biomass both directly and indirectly; the indirect effect via influences on vegetation growth that produce litter is a source of organic C.

The increased GLU activity by 10 years of N and S addition is consistent with Hu et al. (2013) who investigated enzyme activities after 5 years of N and S addition at the same site. Sinsabaugh and Moorhead (1994) suggested that fertilization (such as N and S addition) could shift microbial resource allocation from nutrient acquisition to C acquisition, thereby increasing the GLU activity, which is involved in C cycling. Similar to Hu et al. (2013), N addition did not suppress NAG activity, suggesting that N is still a limiting factor in the study site and thus N concentrations are not high enough to reduce the production of N-degrading enzymes by soil microbes even after 10 years of N addition. The increased ARS activity by N addition coupled with the decreased ARS activity by S addition suggests again that N is still a limiting element and reduced demand for S-degrading enzymes is due to increased S availability in the soil (Hu et al., 2013). In this study, S was applied as Na_2SO_4, suggesting that sodium may have potential suppressive effects on ARS activity.

4.2 Litter decomposition as affected by litter chemistry and microbial and enzyme properties

The difference in C_{cum} from the forest floor exposed to N and S addition with or without litter was directly associated with greater C concentration in the forest floor as soil organic C is the substrate of microbial respiration (Xu et al., 2016). When C_{cum} from litter only was compared,

the negative correlation between C_{cum} and litter C/N and lignin/N ratios suggest that changed litter chemistry by N and S addition also affected litter decomposition as hypothesized. Many studies have reported that litter with high N and low lignin decompose faster than that with low N or high lignin (Wang and Yang, 2007; Chodak et al., 2016). In this study, litter C/N and lignin/N was decreased after 10 years of N and S addition, which can stimulate microbial activities leading to higher litter decomposition rates (Carreiro et al., 2000). For example, N addition can accelerate the degradation of cellulose by increasing exocellulase enzyme activities (Sinsabaugh et al., 2002), and cellulose is one of the main components of plant litter.

Many studies have demonstrated that microbial biomass and enzyme activities are important factors that affect litter decomposition (Carriero et al., 2000; Thirukkumaran and Parkinson, 2000; Iqbal et al., 2010). However, in this study, the lack of correlation between C_{cum} from litter only and the microbial and enzyme attributes indicates that litter decomposition is more likely to be affected by litter chemistry than by microbial and enzyme activities. Keeler et al. (2009) also reported no correlation between enzyme activities and litter decomposition rates, suggesting that the response of microbial activities to N and S deposition occur in a very short time period that they are hard to detect when the sampling intensity is low (Sinsabaugh et al., 2008), or factors other than microbial activities were responsible for the variation in the litter decomposition rates. In a controlled environment, litter chemistry becomes the predominant factor in regulating litter decomposition rates (Aerts, 1997). During the laboratory incubation experiment, litter addition did not change microbial properties in the forest floor, however, microbial activity was significantly different between the N and S addition and control treatments, suggesting that aboveground litter does not play as significant a role as the added N and S to microbial and enzyme activities. This also implies that placing litter on the forest floor

was responsible for the lack of correlation between microbial activities in the forest floor and litter decomposition rates. For example, in a laboratory study, Holland and Coleman (1987) reported that cumulative CO_2 emission correlated with microbial biomass when litter incorporated with soil not placed on the surface.

5. Conclusions

Ten years of N and S addition changed litter chemistry by decreasing C/N and lignin/N ratios via increased N concentration in the litter, and the changed litter chemistry, in turn, affected litter decomposition. Though N and S addition also altered microbial biomass and enzyme activities in the forest floor, such changes were not related to litter decomposition. I concluded that litter chemistry rather than microbial and enzyme activities were the driver of litter decomposition at this study site. The increased decomposition rate of litter from plots under N and S deposition suggest that increased atmospheric N and S deposition may potentially increase CO_2 emissions from the forest floor, which will contribute net C loss from ecosystems or increase primary productivity (net C uptake). In this study, experiments were conducted in a controlled incubation environment and the results need to be verified in field studies. In spite of the limitations of an incubation experiment, this study provides novel insight into the potential impact of N and S deposition on litter decomposition through changed litter chemistry.

Table 2-1. Chemistry of trembling aspen litter collected from plots of an N and S deposition experiment in the Athabasca oil sands region.

	Concentrations (g kg^{-1})								C/N	Lignin/N
Treatment[a]	C	N	S	Lignin	Ca	Mg	Al	Mn		
CK	453	6.5b	1.4	208a	21.7	3.00	0.11	0.25	69.7a	32.1a
	(5.2)	(0.11)	(0.15)	(1.6)	(0.48)	(0.10)	(0.03)	(0.01)	(0.72)	(0.55)
+N	455	7.5a	1.4	190b	19.2	2.90	0.06	0.17	60.5b	25.3b
	(2.4)	(0.27)	(0.22)	(1.8)	(1.16)	(0.27)	(0.02)	(0.01)	(2.05)	(1.18)
+S	459	7.7a	1.2	195b	18.1	2.74	0.08	0.25	59.9b	25.5b
	(0.6)	(0.42)	(0.20)	(0.17)	(1.16)	(0.17)	(0.01)	(0.05)	(3.29)	(1.45)
+NS	454	8.1a	1.3	205a	19.6	3.05	0.13	0.21	56.4b	25.4b
	(1.8)	(0.14)	(0.02)	(2.9)	(0.72)	(0.04)	(0.02)	(0.03)	(1.05)	(0.41)
ANOVA[b]										
N	ns	0.031	ns	0.047	ns	ns	ns	ns	0.014	0.009
S	ns	0.012	ns	ns	ns	ns	ns	ns	0.010	0.012
N × S	ns	ns	ns	<0.001	ns	ns	ns	ns	ns	0.009

Values reported are means (n=4) with standard errors in parentheses.

Different letters indicate significant differences between treatments at $P < 0.05$.

[a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

[b] ns, not significant ($P > 0.05$).

Table 2-2. Chemical properties of forest floor samples collected from plots of an N and S deposition experiment in the Athabasca oil sands region.

Treatment[a]	pH	Total C	Total N	C/N	Exchangeable cations				
					Na^+	K^+	Mg^{2+}	Ca^{2+}	Al^{3+}
		(g kg^{-1})			(cmol kg^{-1})				
CK	5.2	246.8c	11.5b	21.6ab	0.18c	2.5	91	56	0.08
	(0.22)	(4.10)	(0.37)	(0.55)	(0.04)	(0.60)	(3.71)	(1.89)	(0.01)
+N	4.9	305.0a	14.9a	20.5ab	0.18c	2.9	106	64	0.09
	(0.17)	(6.19)	(0.25)	(0.36)	(0.02)	(0.33)	(3.01)	(3.80)	(0.02)
+S	4.9	280.8b	12.7b	22.1a	1.29a	2.4	78	51	0.10
	(0.09)	(4.46)	(0.46)	(0.49)	(0.19)	(0.19)	(8.42)	(1.89)	(0.02)
+NS	5.2	316.6a	15.6a	20.3b	0.81b	2.1	82	51	0.07
	(0.11)	(1.11)	(0.20)	(0.30)	(0.16)	(0.52)	(26.9)	(14.6)	(0.01)
ANOVA[b]									
N	ns	<0.001	<0.001	0.006	0.017	ns	ns	ns	ns
S	ns	<0.001	0.013	ns	<0.001	ns	ns	ns	ns
N × S	ns	0.026	ns	ns	0.020	ns	ns	ns	ns

Values reported are means (n=4) with standard errors in parentheses.

Different letters indicate significant differences between treatments at $P < 0.05$.

[a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

[b] ns, not significant ($P > 0.05$).

Table 2-3. Microbial properties from forest floor amended without and with litter samples.

	MBC		MBN		GLU		NAG		ARS	
	(mg kg-1)				(μg p-nitrophenol·g^{-1}·h^{-1})					
					Without litter[x]					
Treatment[a]	Initial[A]	Final[B]	Initial[A]	Final[B]	Initial[A]	Final[B]	Initial[A]	Final[B]	Initial[A]	Final[B]
CK	2928b	1215d	583b	164b	21b	206c	63b	168c	1388b	937c
	(105)	(28)	(57)	(12)	(3)	(11.0)	(1)	(11.5)	(34)	(12)
+N	3740a	2468a	778a	417a	50a	289b	74a	204bc	1837a	1559a
	(11)	(68)	(24)	(7)	(6)	(11.4)	(2)	(7.3)	(14)	(49)
+S	3371ab	1717c	635ab	223b	50a	287b	76a	223b	1360b	1284b
	(148)	(87)	(64)	(21)	(1)	(17.4)	(3)	(14.9)	(23)	(34)
+NS	3775a	2151b	779a	173b	50a	383a	70a	282a	1396b	1377ab
	(178)	(93)	(29)	(41)	(1)	(20.9)	(1)	(5.2)	(109)	(69)
ANOVA[b]										
N	<0.001	<0.001	0.003	0.001	<0.001	<0.001	ns	<0.001	0.001	<0.001
S	ns	ns	ns	0.002	0.001	<0.001	0.046	<0.001	0.002	ns

N × S	ns	<0.001	ns	<0.001	0.001	ns	<0.001	ns	0.004	<0.001
	With litter[x]									
	Initial[A]	Final[B]	Initial[A]	Final[B]	Initial[A]	Final[B]	Initial[A]	Final[B]	Initial[A]	Final[B]
CK	2688b	1468c	586b	238	23b	192b	62b	171b	1358b	913c
	(279)	(41)	(53)	(18)	(1)	(11.3)	(1)	(12.4)	(39)	(48)
+N	3538a	2264b	718ab	326	48a	349a	74a	255a	1849a	1661a
	(186)	(68)	(36)	(32)	(4)	(18.1)	(2)	(10.0)	(46)	(84)
+S	3050ab	2076b	633b	233	48a	285a	77a	226ab	1402b	1189c
	(62)	(78)	(25)	(42)	(3)	(25.6)	(1)	(21.4)	(26)	(39)
+NS	3634a	2695a	786a	288	49a	327a	70ab	277a	1429b	1390b
	(117)	(91)	(19)	(35)	(2)	(9.0)	(3)	(12.0)	(26)	(33)
ANOVA[b]										
N	0.002	<0.001	0.002	ns	<0.001	<0.001	ns	<0.001	<0.001	<0.001
S	ns	<0.001	ns	ns	<0.001	ns	0.013	0.024	<0.001	ns
N × S	ns	ns	ns	ns	0.001	0.006	<0.001	ns	<0.001	<0.001

Values reported are means (n=4) with standard errors in parentheses.

Different capital letters indicate significant differences between initial and final values at $P < 0.05$.

Different lowercase letters indicate significant differences between treatments at $P < 0.05$.

[x] significant differences between with and without litter samples at $P < 0.05$.

Initial, values at day 0; Final, values at day 100.

[a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

[b] ns, not significant ($P > 0.05$).

Initial, initial values are slightly different due to dilution effects.

Table 2-4. Cumulative CO_2 emission (C_{cum}) from forest floor without and with litter addition and from litter only.

	C_{cum} (g C kg^{-1})		
Treatment [a]	Forest floor without litter	Forest floor amended with litter	Litter only [c]
CK	32.7b	41.3c	8.91b
	(0.42)	(1.08)	(0.10)
+N	40.7a	48.1b	10.63b
	(0.76)	(0.85)	(0.87)
+S	41.3a	52.0a	14.52a
	(1.02)	(0.38)	(0.36)
+NS	40.4a	48.2b	11.79ab
	(0.45)	(0.41)	(0.53)
ANOVA [b]			
N	ns	ns	ns
S	0.008	<0.001	<0.001
N × S	<0.001	<0.001	0.010

Values reported are means (n=4) with standard errors in the parentheses.

Different letters indicate significant differences between treatments at $P < 0.05$.

[a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

[b] ns, not significant ($P > 0.05$).

[c] litter only was calculated by subtracting the values of forest floor without litter from forest floor with litter addition.

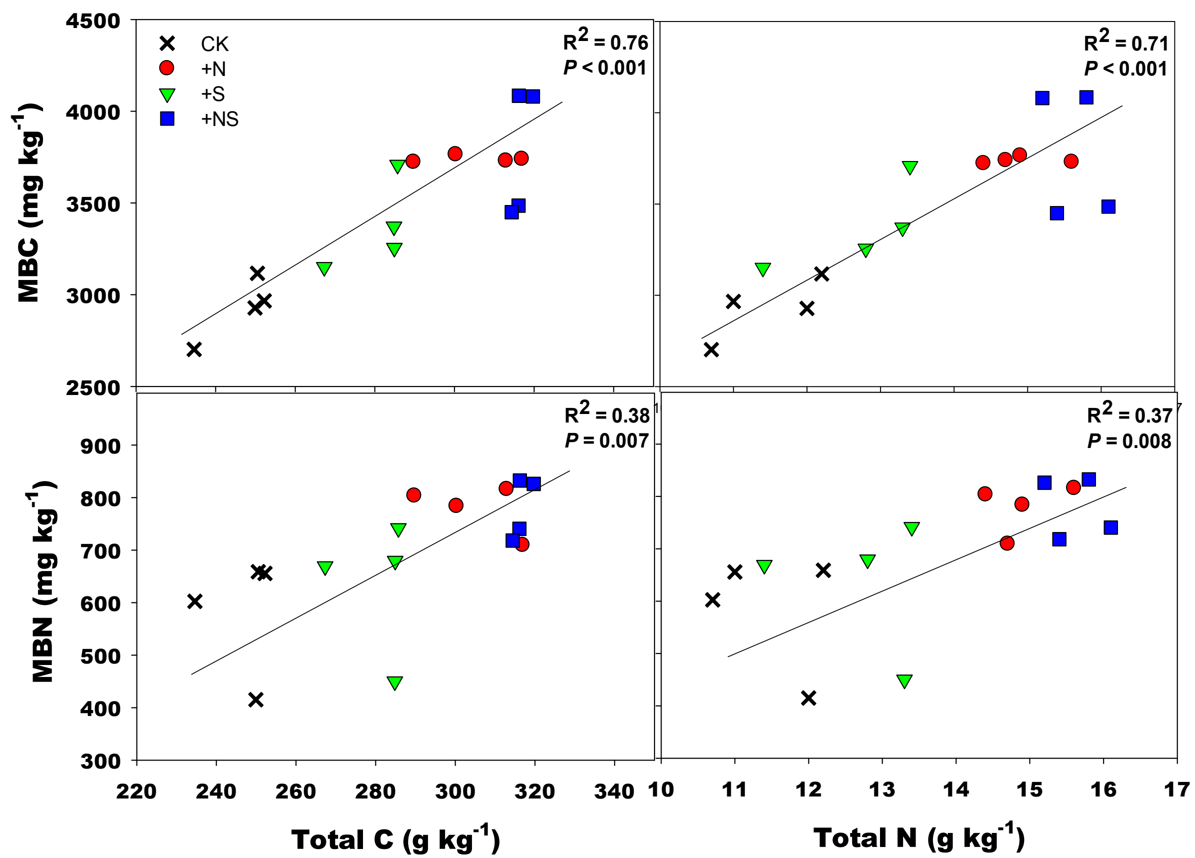

Fig. 2-1 Relationships between total C and N and MBC and, MBN in the forest floor.

CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

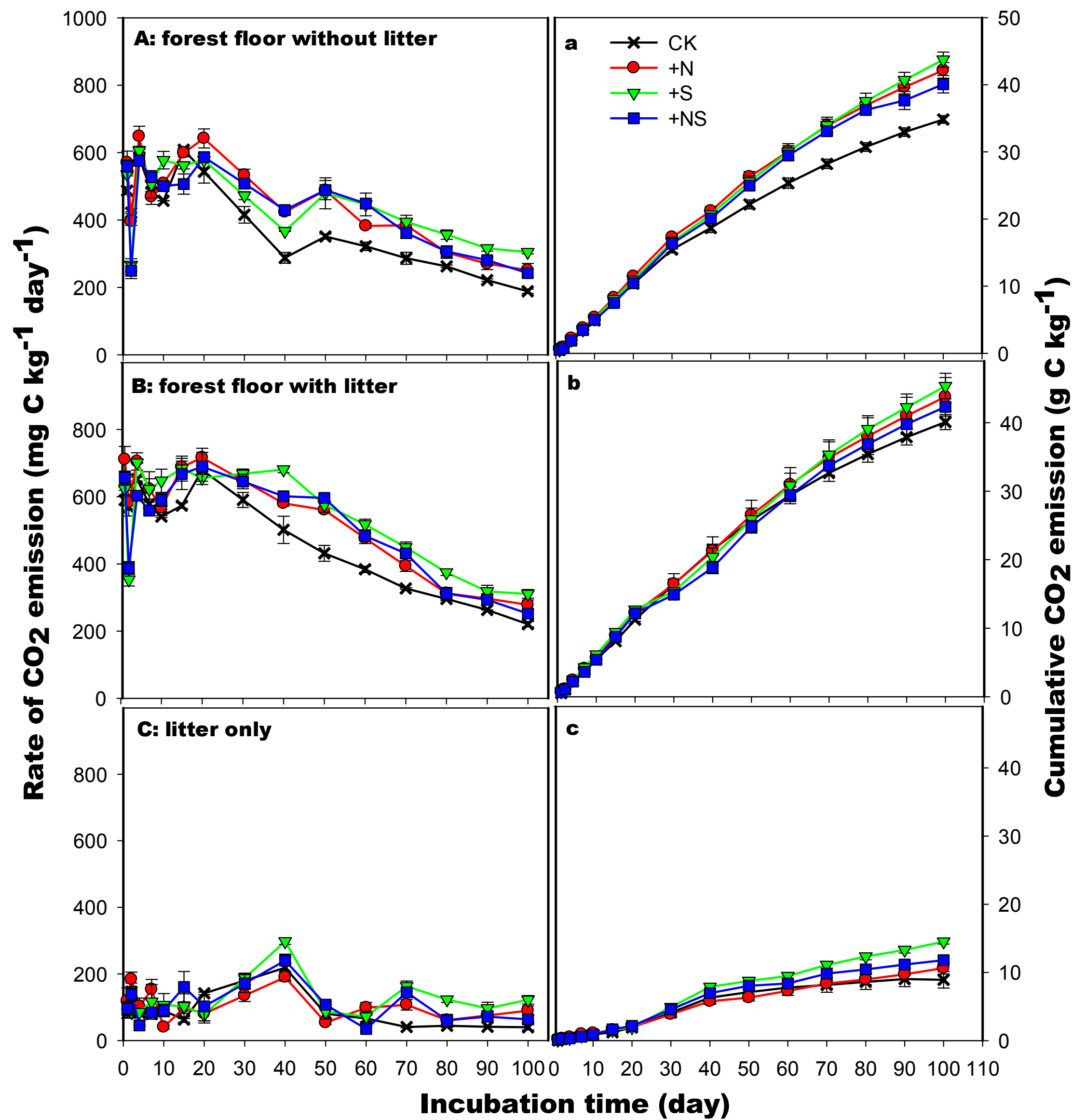

Fig. 2-2 Daily CO_2 release rate (left) and cumulative CO_2 emission (right) during the 100-day incubation. CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition. A and a, forest floor amended without litter; B and b, forest floor amended with litter; C and c, litter only. Litter only, calculated by subtracting the values of forest floor without litter from forest floor with litter.

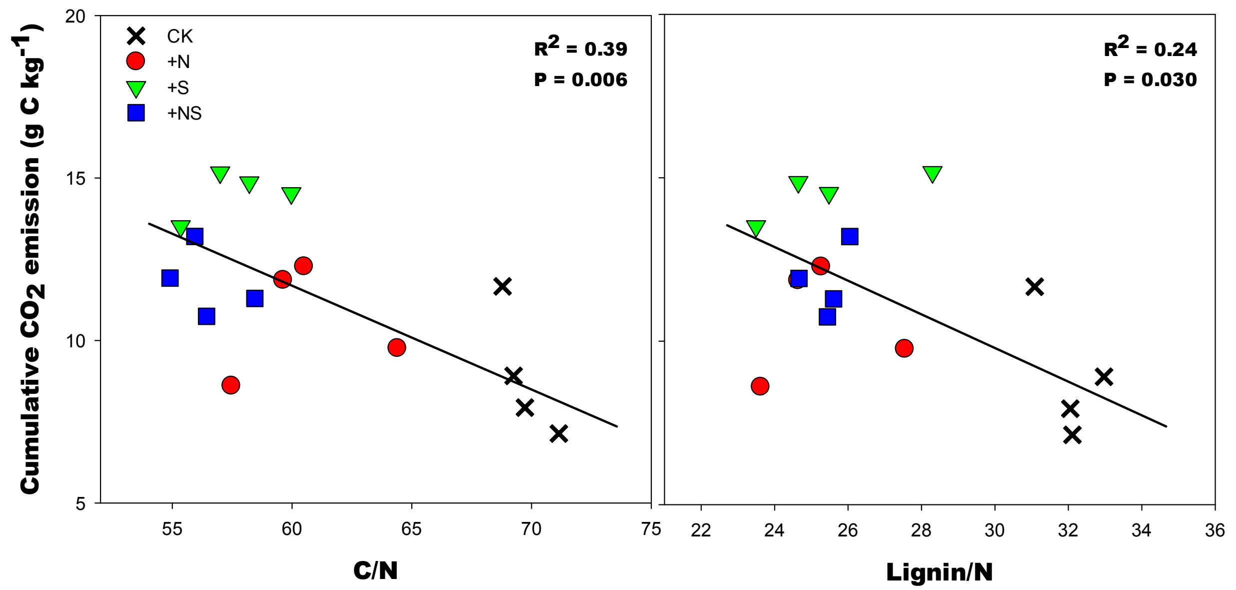

Fig. 2-3 Linear relationship between litter chemistry and cumulative litter CO_2 emission (mg C kg^{-1}). CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

Chapter 3. Nutrient-induced changes in litter chemistry did not regulate the effect of long-term nitrogen and sulfur deposition on trembling aspen (*Populus tremuloides*) leaf litter decomposition in a boreal forest

1. Introduction

Nutrient release through litter decomposition is a critical process that supplies nutrients to plants in ecosystems such as forests with limited artificial nutrient addition (Swift et al., 1979; Allison and Vitousek, 2004). Litter decomposition and nutrient release are affected by many biotic (e.g., enzyme activities) and abiotic (e.g., climate, litter chemistry, and nutrient availability) factors (Swift et al., 1979; Sinsabaugh et al., 1994; Berg and McClaugherty, 2003; Hobbie, 2005). Anthropogenic activities can also influence litter decomposition through changing litter chemistry (Berg and Matzner, 1997; Knorr et al., 2005) and enzyme activities (Carreiro et al., 2000) by deposition of nitrogen (N) and sulfur (S) (Fog, 1988; Dentener et al., 2006; Fang et al., 2007). The Athabasca oil sands region (AOSR) in western Canada is one of the largest oil sands reservoirs in the world (Humphries, 2009). Activities related to oil sands mining have released vast amounts of N and S to the atmosphere and deposited to the surrounding area (Hazewinkel et al., 2008). The literature suggests that N deposition may increase (Carreiro et al., 2000; Hobbie and Vitousek, 2000; van der Wal et al., 2007), suppress (Magill and Aber, 1998; Carreiro et al., 2000; Ramirez et al., 2012), or have no effect (Pastor et al., 1987; Hunt et al., 1988; Prescott, 1995) on litter decomposition. The inconsistent results are explained by N addition rate, duration of N addition, and litter type (leaf, root, or branch) (Knorr et al., 2005; Janssens et al., 2010). For example, Knorr et al. (2005) demonstrated that N addition increased litter decomposition when

between 75 and 125 kg N ha^{-1} yr^{-1} was added, but inhibited litter decomposition when N addition rate was below or above the range. Thus, there is a need for gaining a mechanistic understanding of the effect of N addition on litter decomposition. In addition, most past studies have been focused on N addition and few studies have looked at the role of S addition or both N and S addition on litter decomposition.

Litter chemistry including N and lignin concentrations, the ratio of carbon (C) to N (C/N), and the ratio of lignin to N (lignin/N) are also important factors affecting litter decomposition and nutrient release (Aber and Melillo, 1982; Berg, 1986; Parton et al., 1987; Blair, 1988; Taylor et al., 1989; Aber et al., 1990). Litter decomposition rates are often positively correlated with N concentration in the litter (Aber and Melillo, 1982; Taylor et al., 1989; Talbot and Treseder, 2012; Tu et al., 2014) with some exception due to suppressive effects of N on ligninolytic enzyme (Fog, 1988; Magill and Aber, 1998), and negatively correlated with C/N and lignin/N ratios (Parton et al., 1987; Taylor et al., 1989; Aber et al., 1990). Tamm et al. (1974) and Tamm (1991) reported that high N addition increased litter N concentration, and Downs et al. (1996) concluded that litter N concentration increased under high S addition in the first year. Nitrogen and/or S addition increased C/N and lignin/N ratios mainly through increased N concentration of litter (Data from Chapter 2). These findings indicate that N and S addition may alter litter decomposition indirectly by changing litter chemistry. However, no study has investigated how changed litter chemistry by N and S addition affects litter decomposition; only a few study investigated the interactive effect of N addition (but nothing on S addition) and litter chemistry on litter decomposition (van Diepen et al., 2015; Zhang et al., 2016). Parton et al. (2007) reported that litter N release during decomposition was mainly driven by initial litter chemistry, such as N concentration. Carbon to nutrient ratios are often used as important determinants if an

element will be released as litter decomposes (Gosz et al., 1973; Berg and Staaf, 1981; Parton et al., 2007). Berg and Staaf (1980) proposed a three-phase litter N release pattern, an initial leaching phase, an accumulation phase, and a release phase. Studies often found that litter mass remaining correlated with N concentration in litter residue (Berg and Staaf, 1981; Aerts and de Caluwe, 1997). However, few studies have focused on S release pattern under N and S addition.

Studies often found that litter enzyme activity correlates with litter decomposition rates (Carreiro et al., 2000). Because of the complex chemical composition of litter, decomposition requires many classes of enzymes (Eriksson and Wood, 1985; Zeikus, 1982). The effect of N addition on litter decomposition rates has been found to be closely related to changes in phenol oxidase activity (Carreiro et al., 2000). On the other hand, N addition can decrease litter decomposition by suppressing the production of ligninolytic enzymes by white rot fungi (Fog, 1988; Magill and Aber, 1998). Some studies concluded that fungi only produced ligninolytic enzymes in N limited environment, and N addition suppressed the activities of these enzymes, lignin breakdown, and litter decomposition (Keyser et al., 1978; Kirk, 1987). However, many studies reported contrasting findings (Swift et al., 1979; Aber and Melillo, 1982; Carreiro et al., 2000). Because most terrestrial ecosystems are N limited, and also including its limitation for microbial litter decay; therefore, N addition should accelerate litter decay by reliving the N limitation for microbial activities (Carreiro et al., 2000). Thus, more studies are needed in order to gain a better understanding of the effect of N addition on litter decomposition by changing enzyme activities. Although many studies have been focused on N addition, the effect of S and NS addition is lack of study.

This study was conducted to address the questions of 1) how does N and S addition change decomposition and associated nutrient dynamics and enzyme activities in a common litter

(direct effect of N and S addition), and 2) how changed litter chemistry (i.e., decreased C/N and lignin/N) by long-term N and S addition and the direct effect of N and S addition combined affect litter decomposition and associated nutrient dynamics and enzyme activities (combined direct and indirect effects). I tested four hypotheses in this study: (1) N addition would stimulate litter decomposition in a N-limited forest, and S addition would also increase litter decomposition rates because S is also a secondary limitation in boreal forests (Experiment I); (2) N and S induced changes (e.g., decreased lignin/N ratio) in litter chemistry would further enhance the positive effect of N and S addition on litter decomposition (Experiment II); (3) an earlier release phase will occur under N and S addition in Experiment I, while changed litter chemistry by long-term N and S addition would further accelerate the release phase to occur due to the lower initial litter C/N ratio in litter, and (4) extracellular enzyme activities would be increased by N and S addition, and enzyme activities correlate with litter decay constants.

2. Materials and methods

2.1 Research site and experimental design

This study was conducted in conjunction with previous study that investigated litter chemistry and decomposition (Chapter 2) in experimental plots established in a mixedwood boreal forest stand (56.1° N, 110.9° W) designed to simulate the effect of N and S deposition on forest ecosystems in the oil sands region in northern Alberta. The research plots were established in 2006 and located about 100 km southeast of Fort McMurray (Jung and Chang, 2012). The background N and S bulk deposition were between 1 and 2 kg ha^{-1} yr^{-1} (Proemse et al., 2012,

2013). This area is characterized as a continental boreal, with a mean annual temperature of 1 °C and mean annual precipitation of 419 mm from 1981 to 2010 (Environment Canada, 2010). Approximately 71% of the main canopy trees were *Populus tremuloides* (trembling aspen) and approximately 22% of the main canopy trees were *Picea glauca* (white spruce) (Jung and Chang, 2012). Soils were classified as Gray Luvisols based on the Canadian system of soil classification (Soil Classification Working group, 1998) or Boralf in Soil Taxonomy (Soil Survey Staff, 1998).

With the experiment had four treatments (Control or CK: 0 kg N or S ha^{-1} yr^{-1}; +N: 30 kg N ha^{-1} yr^{-1} applied as NH_4NO_3; +S: 30 kg S ha^{-1} yr^{-1} as Na_2SO_4; +NS: 30 kg N ha^{-1} yr^{-1} + 30 kg S ha^{-1} yr^{-1}) were conducted in twelve 20 m × 20 m plots in the mixedwood boreal forest described above. Each treatment was replicated three times and in a randomized complete block design. The N and S were applied once a year from 2006 to 2008 in early summer. From 2009 to 2017, the N and S were applied three times each summer on an equal split directly to the forest floor.

2.2 Leaf litter and forest floor analysis

The amount of litterfall was approximately 3500 kg ha^{-1} yr^{-1} under CK and +S treatments, while it was approximately 4100 kg ha^{-1} yr^{-1} under +N and +NS treatments (Jung and Chang, 2012). Newly fallen aspen leaf litter was collected from the 12 experimental plots in September 2015 (9 years after the first N and S addition). A portion (5 g) of oven-dried litter sample was ground using a ball mill (MM 200, REtsch GmbH, Haan, Germany) and used for the analysis of the concentrations of lignin, total C, N and S, some mineral (calcium (Ca), magnesium (Mg), aluminum (Al), and manganese (Mn)), and litter ash-free content. Lignin concentration was analyzed by measuring absorbance at 280 nm following acetyl bromide digestion (Morrison,

1972 a, 1972b). Total C and N concentrations were determined using an elemental analyzer (Carlo Erba NA1500, Carlo Erba Instruments, Milano, Italy). Other elemental concentrations including S, Ca, Mg, Al, and Mn were determined using an ICP-MS (Elan 6000 quadrupole, Perkin-Elmer, Inc., CT) after digestion with nitric and hydrogen peroxide (Jones, 2001). The ash content in litter samples was determined using approximately 0.1 g of ground litter and combusted in a 20-mL high temperature-tolerant glass vial in a muffle furnace at 550 °C for 4 hours.

Extracellular enzyme activities were determined with fresh leaf litter samples. For the analysis of enzyme activities, litter samples were cut into about 2 mm × 2 mm size in order to provide homogenous samples. Extracellular enzyme activities involved in C, N and S cycling were analyzed: β-1, 4-glucosidase (GLU, enzyme classification (EC) EC 3.2.1.21) for C, β-1, 4-N-acetylglucosaminidase (NAG, EC 3.2.1.14) for N, and arylsulfatase (ARS, EC 3.1.6.1) for S. The NAG and GLU were measured with fluorimetric methods (Sinsabaugh et al., 2002) by placing one gram of fresh soil was placed in a 250 mL Nalgene HDPE bottle, then added 125 mL of sodium acetate buffer (50 mmol L^{-1}, pH 5), and on an end-over-end shaker at room temperature shook for 30 min. For each substrate, 200 µl of soil suspension and 50 µl of 200 µmol L^{-1} of were pipetted into black 96 well plates. In each plate, reference standard and quench controls were added to each reference and quench well. The plates were incubated at 20°C in the dark for 3 h. After incubation, a 20 µl of 0.5 mol L^{-1} sodium hydroxide solution was added to each well automatically to stop the enzyme reaction. Fluorescence was measured at 360 nm excitation and 460 nm emissions using a multi-detection microplate reader (Synergy HT, Bio-Tek Instruments, Winooski, VT, USA). The activity of ARS was assayed by the colorimetric determination of p-nitrophenol released when soils were incubated with p-nitrophenyl β-D-

glucopyranoside (Tabatabai, 1994). For the litter sample before the initiation of the experiment (at month 0), it was assumed that there was no enzyme activity due to air-drying as air-dried litters were used for the field experiment (Sherman and Steinberger, 2012).

Forest floor samples were collected in July 2016 from the same experimental plots approximately 10 days after N and S addition and the current-year litter was also removed from the surface. Oven-dried forest floor samples were analyzed for pH, total C and N concentrations, and exchangeable cations. The pH (1:10 w/w soil to water ratio) was measured using a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA). Total C and N concentrations were analyzed using the elemental analyzer as described above. Exchangeable cations, including sodium (Na^+), potassium (K^+), Mg^{2+}, Ca^{2+}, and Al^{3+}, were determined using an ICP-MS after extraction of 2 g sample with 100 mL of 1 mol L^{-1} NH_4Cl (Shuman and Duncan, 1990).

2.3 Litterbag experiments

Two experiments (Experiments I and II) were conducted for18-months in the field that had received N and S addition for 10 years. Experiment I was conducted to investigate the direct effect of N and S addition on litter decomposition by placing a common leaf litter that was collected from plots that had not received N and S addition into the four N×S treatment plots. Experiment II was conducted to examine the combined effects of N and S addition (direct effect) and changed litter chemistry by the N and S addition (indirect effect) by placing litters collected from N and/or S addition plots into the corresponding plots.

Approximately 5 g of air-dried litter sample was placed in each litterbag (20 × 20 cm) which was made of fiberglass mesh (1 × 1 mesh size) and then sealed with an impulse sealer. For

Experiments I and II, 120 litterbags were prepared for each experiment: two litterbags (one for measurement of mass loss and elemental composition after drying and the other for the determination of enzyme activities) × five sampling times × four N and S treatments × three replicates. Each bag was labeled by attaching a numbered aluminum tag. The litterbags were placed on the forest floor in the field in early November 2015 by pinning them to the ground.

For each experiment, two litterbags were randomly selected from each plot 6, 8, 10, 12, and 18 months (in May, July, September, November 2016, and May 2017, respectively) after litterbag placement. Samples were placed on ice in a cooler immediately to preserve the samples. Litterbags were then transported to the laboratory and stored at 4 °C until analyses. All the analyses were completed within 3 days. Before analysis, any visible live vegetation and forest floor materials adhering to the litterbags were removed carefully. Litter samples were then removed from the bags. Forest floor materials were gently brushed off from the litter surface if there was any. One set of samples from each experiment was oven-dried to determine water content, ash-free mass loss rate, as well as total C, N, and S concentrations following the procedures described earlier. The other set of samples was used to determine enzyme activities in the litter as described above.

2.4 Calculations and statistical analysis

The same measurements on litter samples were conducted in Experiments I and II. All measured parameters were tested for homogeneity of variance and normality of distribution with Kolmogorov-Smirnov and Levene’s tests, respectively. No heterogeneity was detected and the

distribution was normal. Litter mass loss was fitted to the single-pool exponential model as proposed by Wider and Lang (1982):

$$M_t = M_0 e^{-kt}$$

where: M_t is the ash-free litter mass remaining at time t (in years), M_0 is the initial ash-free litter mass, and k is the decay constant (yr^{-1}). Litter decay constants were examined at different study periods: 0 to 6 months (first winter season), 6 to 12 months (first growing season), 12 to 18 months (second winter season), and 0 to 18 months (the entire study period). In order to calculate decay constants for the period from 6th to 12th months and from 12th to 18th months, the initial mass remaining was assumed to be the predicted percentage mass remaining at t = 0.5 month and t = 1 month, respectively, based on first 6 and 12 months (Blair, 1988).

Statistical analyses were performed using the R software package for Windows (version 3.3.0, R foundation for Statistical Computing, Vienna, Austria). Figures were plotted using Sigmaplot v.12 (Systat Software Inc., San Joese, CA).

Initial litter and forest floor properties were analyzed using two-way (N and S parameters) analysis of variance (ANOVA). Cumulative GLU, NAG, and ARS activities were approximated for each treatment at 0 to 6 months (first non-growing season), 6 to 12 months (first growing season), 12 to 18 months (second non-growing season), and 0 to 18 months (the entire study period) periods in the field (Sinsabaugh et al., 2002; Allison and Vitousek, 2004). A cubic spline approximation was used to integrate enzyme activities to ensure accuracy (Mohanty et al., 2013) using the R software.

Litter mass remaining over time was analyzed using a two-way ANOVA. Linear relationships between percent litter mass remaining and nutrient (N and S) concentrations in the remaining litter showed as slope, coefficients of determination (R^2), and P value at times 0, 6, 8,

10, 12, and 18 months for N, at times 6, 8, 10, 12, and 18 months for S. It should be noted that the nutrient concentrations used in the regression analysis were calculated after excluding any initial rapid loss in the nutrient concentration due to leaching (Aber and Melillo, 1980). Litter decay constant at the 0 to 6 months, 6 to 12 months, 12 to 18 months, and 0 to 18 months were analyzed by a two-way ANOVA. In each decomposition period, cumulative enzyme activities were performed by a two-way ANOVA for each enzyme type.

3. Results

3.1 Characteristics of initial leaf litter and the forest floor

Addition of N did not change ($P > 0.05$) C concentration but increased ($P = 0.031$) N concentration of litter while decreased ($P = 0.047$) lignin concentration, resulting in a decreased C/N and lignin/N ratios (Table 2-1). Addition of S also did not change ($P > 0.05$) C concentration but increased ($P = 0.012$) N concentration with no effect ($P > 0.05$) on lignin concentration, and thus also led to decreased C/N and lignin/N ratios. Other elements including S and micronutrients were not affected by N and S addition ($P > 0.05$).

Ten years of N and S addition did not change forest floor pH ($P > 0.05$), but increased total C and N concentrations ($P < 0.05$) (Table 2-2). Exchangeable Na^+ concentration was affected by S addition ($P < 0.001$), while other exchangeable cation concentrations were not affected by the treatments

3.2 Effects of N and S addition on litter mass loss

Overall, N and S addition did not directly affect litter decomposition rates (Experiment I), and changed litter chemistry did not result in different litter decomposition rates under N and S addition (Experiment II) (Fig. 3-1, Table 3-1, Table 3-2).

Decomposition of litter followed a single-pool negative exponential pattern over the 18-month study period ($R^2 > 0.90$) (Fig. 3-1). In Experiment I, there was a general trend of litter decomposing the slowest under the +N treatment. However, in Experiment II, it appears that litter under the +S treatment decomposed the slowest.

Litter mass remaining was not affected by N and S addition ($P > 0.05$), but was affected by time since litterbag placement ($P < 0.001$, Table 3-1). For both experiments, during the first 6 months and 6 to 12 months, litter samples lost approximately 20% of its original mass in each of those periods, while approximately 5% of its original mass was lost from 12 to 18 months (Fig. 3-1 and Table 3-1). Over the 18-month study period, litter lost approximately 45% of its original mass. Litter decay constants were not affected by N and S addition or the combined effects of nutrient addition and changed litter chemistry (Table 3-2).

3.3 Effects of N and S addition on litter C, N and S concentrations during decomposition

In Experiment I, litter C concentration peaked at month 8, and did not significantly change among the rest of the sampling dates (Fig. 3-2a, Table 3-3). Litter C concentration was increased by S addition ($P = 0.002$) not by N or NS additions ($P > 0.05$) at month 8. The co-addition of N and S decreased litter C concentration ($P = 0.015$) as compared to the N or S added alone at month 10. In Experiment II, litter C concentration peaked at month 6, and remained at a

relatively stable level for the rest of the study period (Fig. 3-2b, Table 3-3); however, litter C decreased (P = 0.028) under S addition compared to control at month 8, while N addition decreased (P = 0.003) litter C concentration at month 10. The co-addition of N and S decreased (P = 0.041) litter C concentration at month 10 as compared to S added alone, however, when N and S co-added, litter C concentration increased (P = 0.025) as compared to N or S added alone at month 12. In the two experiments, C concentration increased by approximately 104% for the four treatments at the end of the study.

In Experiment I, litter N concentration peaked at month 10 and remained at a relatively stable level thereafter (Fig. 3-2c, Table 3-3). Litter N concentration was increased (P= 0.033) by N addition at month 8. While in Experiment II, litter N concentration also peaked at month 10 and remained at a relatively stable level thereafter (Fig. 3-2d, Table 3-3). Litter N concentration was also increased by N addition at month 8 and10 (P= 0.025 and P = 0.002, respectively). In Experiment I, N concentration in CK, +N, +S, and +NS increased by 108%, 134%, 131%, and 144%, respectively at the end of the study as compared to the initial concentrations. While in Experiment II, N concentration increased by 110%, 88%, 70%, and 76%, respectively at the end of the study as compared to the initial concentrations.

In Experiment I, litter S concentration significantly decreased in the first 6 months, then reached to peak at month 12 and remained at a stable level until the end of the study (Fig. 3-2e, Table 3-3). Litter S concentration was not affected by N and S addition in Experiment I at any sampling time. While in Experiment II, litter S concentration also decreased significantly in the first 6 months, then reached to peak at month 10 and remained at a stable level until the end of the study (Fig. 3-2f, Table 3-3). Litter S concentration was increased (P = 0.038) by S addition at month 10 in Experiment II. In Experiment I, S concentration decreased by approximately15% at

the end of study for all treatments. While in Experiment II, S concentration in CK, +S, and +NS increased by 111%, 22%, and 24%, respectively at the end of study and S concentrations decreased in +N treatment.

Regressions of the percentage of mass remaining over N and S concentrations in the residual litter indicated a linear relationship between mass remaining and N retention in litter (Table 3-4). The slope of the relationship was a measure of the increase in N concentration per unit C mineralized. This negative linear relationship also existed between the percentage of mass remaining and increases in S concentration.

3.4 Effects of N and S addition on the litter C, N and S remaining during decomposition

In Experiment I, litter C remaining decreased significantly overtime and reached the lowest level at month 12 then remained the same until the end of the study (Fig. 3-3a, Table 3-5). When N and S co-added, the litter C remaining increased compare to N or S added alone at month 6 and month 8 ($P= 0.003$ and $P= 0.026$, respectively). Litter C remaining also increased ($P = 0.025$) by S addition at month 8. While in Experiment II, litter C remaining significantly decreased and reached a stable level from month 12 (Fig. 3-3b, Table 3-5). Litter C remaining was not affected by N and S addition in Experiment II at any sampling time.

In Experiment I, litter N remaining increased significantly and peaked at month 8 and remained at a relatively stable level thereafter (Fig. 3-3c, Table 3-5). Litter N remaining increased ($P = 0.026$) under N addition at month 8 in Experiment I. While in Experiment II, litter N remaining significantly increased and peaked at month 8 and remained at a relatively stable level thereafter (Fig. 3-3d, Table 3-5). Litter N remaining decreased by S addition at month 6, 8,

10, and 12 (P = 0.004, P = 0.003, P = 0.045, and P = 0.080, respectively), while N addition increased (P = 0.046) litter N remaining at month 10. When N and S co-added, litter N remaining increased compared to S addition alone but decreased compared to N added alone (P= 0.037).

In Experiment I, litter S remaining significantly decreased during the first 6 months and remained at the same level during the study period (Fig. 3-3e, Table 3-5). Litter S remaining was not affected by N and S addition. While in Experiment II, litter S remaining also decreased significantly in the first 6 months and remained at a stable level until the end of the study (Fig. 3-3f, Table 3-5). Litter S remaining was not affected by N and S addition at any sampling time either in Experiment II.

3.5 Effects of N and S addition on litter C/N and C/S ratios during decomposition

Litter C/N ratio decreased significantly in the first 6 months, and reached a stable level at month 10 then remained the same thereafter (Fig. 3-4a, Table 3-6). Litter C/N ratio decreased (P = 0.009) under N addition compared to control. While in Experiment II, litter C/N ratio significantly decreased and reached to a relatively stable level from month 10 (Fig. 3-4b, Table 3-6). Litter C/N ratio was decreased (P = 0.001) by N addition in Experiment II.

Litter C/S ratio first increased and peaked at month 6, then decreased from month 8, and reached a relatively stable level from month 10 (Fig. 3-4c, Table 3-6). Litter C/S ratios were not affected by N and S addition during the study period. While in Experiment II, litter C/S ratio was also first decreased and peaked at month 6, then decreased from month 8, and remained at a relatively stable level from month 10 (Fig. 3-4d, Table 3-6). Litter C/S ratio was decreased by N addition at month 10 and month 12 (P = 0.027 and P = 0.037, respectively) in Experiment II.

3.6 Effects of N and S addition on cumulative enzyme activities

In Experiment I, the cumulative GLU and ARS activities in the litter were not affected by N and S addition (Fig. 3-5, Table 3-7). While cumulative NAG decreased when N and S co-added at 12 to 18 months and 0 to 18 months ($P = 0.025$ and $P = 0.023$, respectively). In Experiment II, the cumulative GLU and ARS activities in the litter were also not affected by the combined effects of N and S addition and changed litter chemistry. However, N addition decreased ($P = 0.048$) cumulative NAG activity at 0 to 6 months, while increased ($P = 0.019$) at 0 to 18 months. Cumulative NAG activity was increased by S addition at 6 to 12 months and 0 to 18 months ($P = 0.001$ and $P = 0.014$, respectively). When N and S co-added, cumulative NAG activity increased ($P = 0.008$) compared to N or S added alone at 0 to 6 months, while decreased at 12 to 18 months and 0 to 18 months ($P = 0.029$ and $P = 0.008$, respectively).

While NAG activity was the highest in +S treatment and lowest under +N treatment at the first winter and growing season, as well as over the 18-month study period In Experiment I and II, ARS activity appeared to be the predominant one in the first winter season; NAG activity was higher in the growing season; and GLU activity seemed to be the dominant one at the second winter season and over the whole study period. Enzyme activities were higher in the second winter season than the first in Experiment I and II. However, one thing needs to be noticed that at time 0, enzyme activities in the litter were assumed to be 0. There was no correlation between cumulative enzyme activities and litter decay constants (Appendix C and D).

4. Discussion

4.1 Direct effect of N and S addition on litter mass loss

The results showed that litter mass loss was not directly affected by N and S addition in Experiment I, and this rejects the first hypothesis. Previous studies in forest ecosystems have shown diverse effect of N addition on litter mass loss, which was dependent on site-specific soil nutrient availability, litter types, N addition rates, and duration of the study (Knorr et al., 2005; Mo et al., 2006, 2008; Janssens et al., 2010). Berg et al. (1987) and Prescott (1995) reported that N addition had no effect on a pine needle mass loss, due to the lack of different species composition (different litter types) with high N concentration and microclimate. Zheng et al. (2017) concluded that in phosphorus (P)-limited forest, N addition did not change litter decomposition due to the site-specific nutrient availability. Where N addition inhibited litter decomposition when N oversaturated (Chen et al., 2013) and stimulated litter decomposition when N was limited (Hobbie, 2000). Xu et al. (2016) also reported that in a 420-day laboratory incubation, low N application did not alter litter decomposition rate, but high N did stimulate decomposition. And N addition also exhibited contrast effect on litter decomposition at different stages (early and late), suggesting long-term study is needed to fully evaluate the overall impacts of N additions on litter decomposition (Xu et al., 2016).

It is commonly considered that external nutrient additions would stimulate litter decomposition by supplying to microbes (Hobbie and Vitousek, 2000). Thus litter decomposition under S addition would be expected to increase. However, in this study, +S and +NS treatment did not change litter mass loss which inconsistent with the negative or positive responses to S addition on litter mass loss in previous studies (Lv et al., 2014; Xu et al., 2016) due to the pH

level. Many microorganism activities in the soil are sensitive to the pH of their environment (Will et al., 1986; Williams and Gray, 1974), which are responsible for litter decomposition. However, in this study, soil pH was not affected by treatments after long-term S addition. My results were consistent with Jung et al. (2017) findings that soil pH was not affected by N and S addition which was conducted at the same site.

However, litter appeared a clear trend that litter under +N treatment tended to decompose the slowest. Many studies (Magill and Aber, 1998; Carreiro et al., 2000) have shown that N addition may have a negative impact on the ligninolytic activity and perhaps the abundance of white rot fungi which mainly produce ligninolytic enzyme and this enzyme is essential for degrading lignin completely. And lignin is a major compound in the litter. Over more extended periods, decay rates were determined by the breakdown of the resistant component of litter, which was significantly slower on N treated than untreated plots (O'Connell, 1994). Studies have also shown that microbial decomposers synthesize phenolic compounds and/or break down litter lignin and other polyphenolic compounds into compounds that react with inorganic N to form additional compounds that are resistant to decomposition (Fog 1988, Davidson et al., 2003). The formation of these resistant compounds would be stimulated by NH_4NO_3 addition, and their greater abundance could slow decomposition in N-fertilized plots compared to control plots (Stevenson, 1994; Berg and Matzner, 1997).

4.2 Combined direct and indirect effects of N and S addition on litter mass loss

Nutrient-induced changes in litter chemistry could interact with N and S addition, either decreasing or increasing the effect of nutrient addition on litter decomposition (Hobbie, 2005;

van Diepen et al., 2015; Zhang et al., 2016; Zheng et al., 2016). However, the results from Experiment II showed that N and S induced changes in litter chemistry did not have an impact on nutrient additions on litter decomposition, and the combined effects did not change litter mass loss, this rejects the second hypothesis.

This implied that soil nutrient availability was not affected by N and S addition. Because studies suggested that nutrient-induced changes in litter chemistry enhance or alleviate the impact of nutrient addition on litter decomposition rates by interacting with changed soil nutrient availability by nutrient addition (Zheng et al., 2017). It may also indicate that litter chemistry may not be robust predictors of the decomposition rates in this study because of N was still a limit factor of litter decomposition in the forest ecosystems (Schlesinger and Hasey, 1981; Moore, 1984; McClaugherty et al., 1985; Schaefer et al., 1985). Additionally, the absence of the significant relationship may be due to the small range of lignin concentrations in the litter (Moore, 1984), in this study, the lignin only range between 19.0-20.8%. Berg and Staaf (1980) have developed a three-stage of decomposition, and they concluded that lignin only regulated litter decomposition at the later stage. And in the later stage, N concentrations appear a suppressive effect on litter decomposition rates (Berg and Staaf, 1980). However, through the 18-month study, it appeared that N is still the limiting factor to the ecosystems, which suggests that litter decomposition in this study is still in the early phase.

In Experiment II, it appeared that litter decomposed the slowest under +S treatment, which suggests that this study site may be S-limited as well. Weedon et al. (2009) demonstrated that when one nutrient became limited in the forest, decomposers prefer to use the external nutrient with low energy cost instead of mineralizing organic matter in litter, which result in the reduction of litter mass loss.

4.3 Effects of N and S addition on litter C, N and S dynamics

Nitrogen concentration in litter increased during the 18-month field study due to immobilization of N from soil (Anderson, 1973; Melillo et al., 1982; McClaugherty et al., 1985). Studies have concluded that the early stage of litter decomposition is controlled by N availability, and when N increases it will increase microbial activity and litter decay constants (Magill and Aber, 1998). However, litter studies have proved that accumulated N is used by microbes (Fog, 1988) and some studies have shown that imported N by microbes may be transferred to resistant compounds (such as lignin) instead of maintaining in microbial biomass (Berg, 1988; Gallardo and Merino, 1992). Berg and Staaf (1980) concluded three phases of N dynamics during litter decomposition, that is: (i) an initial leaching phase; (ii) an accumulation phase (or net immobilization); and (iii) a final net mineralization phase. The nutrient dynamics curve is controlled by initial and critical C/nutrient ratios (Manzoni et al., 2010). In this study, N concentration did not exhibit an initial leaching phase because the initial C/N ratio (Table 2-1) was higher than the critical value (Berg and Staaf, 1981; Manzoni et al., 2010). Sulfur concentration in litter also exhibited an immobilization phase. However, S concentration decreased rapidly in the first 6 months presumably through a leaching process, and this macronutrient should be rapidly returned to the soil and plant root systems (Moore, 1984). The ready loss of S from litter may suggest that S is not a critical element in controlling litter decomposition.

In this study, litter N occurred release phase from month 10 for both experiments, and no significant difference among treatments, this rejects the third hypothesis. However, these results

are consistent with the litter mass results. Parton et al. (2007) reported that N release occurred when C/N ratio in the litter was less than 40, and my study paralleled with this conclusion. In forest floor, the main form of N release is mineralization to NH_4^+ and may be further nitrified to NO_3^-, but nitrification is limited (Pajares and Bohannan, 2016). However, S did not exhibit initial release phase in this study because C/S ratio did not reach to a critical value. Gosz et al. (1973) and Blair (1988) reported that when C/S ratio reached to approximately 300, S release occurred by the end of year 2 which may suggest that longer term decomposition is needed.

4.4 Effects of N and S addition on cumulative enzyme activities

Cumulative enzyme activities were not correlated with litter decay constant in both Experiments I and II, and this rejects the forth hypothesis. Litter enzyme activities and decomposition rates were not correlated; however, this result was not entirely unexpected because weak differences in litter chemistry in this study, which compare to other studies strong differences in different litter species, can have a direct effect on the relationship between enzyme activities and decay constant. Allison and Vitousek (2004) pointed that in recalcitrant aspen litter, enzymes may be present but unable to catalyze decomposition because they cannot physically interact with their substrates. Lignin may shield enzyme substrates, thereby reducing enzyme efficiency and shifting control of mass loss rates toward other mechanisms, such as leaching (Allison and Vitousek, 2004). In the early stage, most of the soluble compounds lost from litter through leaching. In a later stage, enzymes targeting lignin and cellulose, two major compounds in the litter. This may suggest that GLU activity at 12 to 18 months was higher than 0 to 6 months and 6 to 12 months. Among these three enzymes, NAG responded to the treatment

significantly difference at four study periods in Experiment II. The ecosystem-specific response in NAG activity may indicate that there is a change in fungal litter inputs to soil, a change in the capacity of the microbial community to degrade fungal litter, or both (Waldrop et al., 2004b).

Additionally, Sinsabaugh et al. (2008) concluded that extracellular enzymes respond quickly to the climate change or external nutrient availability and if sampling happens over the response period may not capture the seasonal and inter-seasonal dynamics of the enzyme activities. In this study, samples were collected 2-6 months after N and S addition which may lose the chance to capture the real dynamics of enzyme activities. For lignocellulase enzymes appeared to have relationships with mass loss, however, other types of enzymes, such as arylsulfatase, usually do not correlate with mass loss or exhibited site-specific relationships (Sinsabaugh et al., 1994). The GLU did not have any linear relationship with mass loss. And GLU (involved in lignocellulose degradation) reflects the relationship with mass loss. The NAG and ARS only give site-specific characteristics.

5. Conclusions

By assessing the effect of N and S addition, I did not find any significant difference between treatments, suggesting that higher nutrient applications and longer experiment durations are needed. The interaction effect of nutrient additions and nutrient-induced changes in litter chemistry did not change litter mass loss in this study, which indicates that litter chemistry was not robust predictors in litter decomposition. Litter N release occurred at the same collection time (month 10) for both experiments and was not affected by treatments, suggesting changed litter chemistry did not affect litter N release during decomposition. Litter S did not exhibit an

initial release phase, which may suggest longer time study is needed. Cumulative enzyme activities were not correlated with litter decomposition rates due to the weak differences in litter chemistry and the sampling time. To fully understand the impact of N and S deposition on litter decomposition in the oil sands region, long-term decomposition study is recommended.

Table 3-1. Litter mass remaining at different sampling time.

		Sample collection time (month)					
Experiment	Treatment[a]	0 mo[A]	6 mo[B]	8 mo[C]	10 mo[D]	12 mo[D]	18 mo[E]
I	CK	100	80 (1.4)	73 (1.3)	63 (1.6)	61 (1.4)	55 (2.2)
	+N	100	81 (1.1)	74 (1.7)	66 (0.7)	65 (1.2)	58 (2.3)
	+S	100	78 (1.1)	73 (0.1)	63 (1.2)	62 (1.6)	55 (1.2)
	+NS	100	79 (1.5)	71 (0.7)	63 (2.0)	61 (2.1)	55 (1.3)
	ANOVA[b]						
	N	-	0.481	0.594	0.516	0.337	0.267
	S	-	0.178	0.300	0.290	0.337	0.349
	N × S	-	1.000	0.300	0.152	0.141	0.449
II	CK	100	78 (1.5)	73 (2.0)	63 (4.3)	59 (3.9)	57 (1.8)
	+N	100	80 (1.7)	73 (0.7)	63 (2.2)	59 (3.4)	56 (1.3)
	+S	100	80 (2.5)	75 (2.9)	67 (4.1)	62 (3.8)	54 (1.9)
	+NS	100	79 (1.8)	70 (0.7)	64 (1.5)	60 (1.7)	54 (0.0)
	ANOVA[b]						
	N	-	0.864	0.198	0.624	0.802	0.577
	S	-	0.732	0.735	0.438	0.653	0.169
	N × S	-	0.401	0.198	0.496	0.802	0.736

Values reported are means (n=4) with standard errors in parentheses. Different capital letters indicate significant differences between sample collection times at $P < 0.05$. [a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

[b] -, no value.

Table 3-2. Litter decay constants at study periods: 0 to 6 months, 6 to 12 months, 12 to 18 months, and 0 to 18 months.

		Decay constant (k)			
Experiment	Treatment[a]	0 to 6 mo[A]	6 to 12 mo[A]	12 to 18 mo[B]	0 to 18 mo[A]
I	CK	0.45 (0.04)	0.56 (0.06)	0.20 (0.03)	0.40 (0.03)
	+N	0.42 (0.03)	0.44 (0.01)	0.15 (0.01)	0.36 (0.03)
	+S	0.50 (0.03)	0.46 (0.04)	0.25 (0.07)	0.40 (0.01)
	+NS	0.48 (0.04)	0.52 (0.06)	0.15 (0.00)	0.40 (0.02)
	ANOVA				
	N	0.473	0.525	0.093	0.310
	S	0.133	0.926	0.490	0.380
	N × S	0.957	0.103	0.539	0.380
II	CK	0.50 (0.04)	0.54 (0.11)	0.24 (0.05)	0.37 (0.02)
	+N	0.44 (0.04)	0.52 (0.01)	0.19 (0.02)	0.39 (0.02)
	+S	0.44 (0.06)	0.53 (0.06)	0.20 (0.01)	0.41 (0.02)
	+NS	0.47 (0.05)	0.55 (0.08)	0.22 (0.06)	0.41 (0.00)
	ANOVA				
	N	0.785	0.967	0.607	0.660
	S	0.734	0.887	0.870	0.121
	N × S	0.353	0.780	0.388	0.791

Values reported are means (n=4) with standard errors in parentheses. Different capital letters indicate significant differences between different study periods at $P < 0.05$. [a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

Table 3-3. Litter C, N and S concentrations (g kg^{-1}) at different sampling time.

Element (g kg^{-1})	Treatment[a]	Experiment I						Experiment II					
		0 mo[B]	6 mo[AB]	8 mo[A]	10 mo[B]	12 mo[B]	18 mo[AB]	0 mo[B]	6 mo[AB]	8 mo[A]	10 mo[A]	12 mo[A]	18 mo[A]
Carbon	CK	455 (3.2)	477 (6.0)	475 (1.5)	461 (1.4)	459 (3.7)	468 (1.4)	453 (5.2)	465 (3.1)	476 (9.0)	495 (6.8)	481 (2.5)	474 (0.6)
	+N	456 (1.9)	457 (8.1)	463 (0.8)	470 (4.0)	463 (4.6)	473 (2.8)	455 (2.4)	466 (4.3)	488 (7.7)	456 (4.5)	463 (5.0)	472 (3.6)
	+S	459 (0.8)	468 (8.7)	495 (8.9)	467 (8.4)	466 (2.7)	471 (1.1)	459 (0.6)	472 (1.2)	463 (8.6)	469 (5.2)	464 (8.5)	476 (2.8)
	+NS	455 (0.2)	480 (5.7)	494 (6.8)	446 (3.8)	465 (4.2)	473 (3.5)	454 (1.8)	466 (0.9)	459 (5.3)	459 (6.3)	479 (6.1)	470 (4.9)
	ANOVA[b]												
	N	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.003**	ns	ns
	S	ns	ns	**0.002**	ns	ns	ns	ns	ns	**0.028**	ns	ns	ns
	N × S	ns	ns	ns	**0.015**	ns	ns	ns	ns	ns	**0.041**	**0.025**	ns

		0 mo^{D}	6 mo^{C}	8 mo^{B}	10 mo^{A}	12 mo^{A}	18 mo^{AB}	0 mo^{C}	6 mo^{BC}	8 mo^{B}	10 mo^{A}	12 mo^{A}	18 mo^{A}
		6.1	8.4	10.4	12.6	13.4	12.7	6.5	8.9	10.5	13.3	12.8	13.6
Nitrogen	CK	(0.31)	(0.33)	(0.27)	(0.55)	(0.38)	(0.72)	(0.11)	(0.32)	(0.45)	(0.61)	(0.35)	(0.27)
		5.7	7.7	10.5	12.7	13.1	13.4	7.5	8.9	11.5	16.0	14.3	14.2
	+N	(0.15)	(0.64)	(0.30)	(0.32)	(1.2)	(0.95)	(0.27)	(0.35)	(0.55)	(0.17)	(1.2)	(1.4)
		5.8	8.7	10.4	12.8	12.6	13.3	7.7	9.0	9.7	11.4	12.6	13.1
	+S	(0.17)	(0.29)	(0.43)	(0.60)	(0.33)	(0.79)	(0.42)	(0.39)	(0.88)	(0.88)	(0.84)	(0.79)
		5.9	8.7	12.1	14.9	15.2	14.4	8.1	8.8	11.3	15.5	14.4	14.2
	+NS	(0.09)	(0.40)	(0.43)	(1.3)	(1.5)	(0.74)	(0.14)	(0.44)	(0.60)	(0.95)	(1.4)	(1.3)
	ANOVA[b]												
	N	ns	ns	**0.033**	ns	ns	ns	ns	ns	**0.025**	**0.002**	ns	ns
	S	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	N × S	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		0 mo^{A}	6 mo^{B}	8 mo^{B}	10 mo^{AB}	12 mo^{A}	18 mo^{AB}	0 mo^{A}	6 mo^{C}	8 mo^{BC}	10 mo^{A}	12 mo^{AB}	18 mo^{A}
Sulfur	CK	1.3	0.9	0.8	1.1	1.4	1.2	1.4	0.9	0.9	1.3	1.1	0.16

	(0.15)	(0.03)	(0.12)	(0.07)	(0.13)	(0.06)	(0.15)	(0.11)	(0.13)	(0.06)	(0.02)	(0.20)
	1.2	0.9	1.0	1.1	1.2	1.3	1.4	0.7	0.9	1.3	1.2	1.3
+N	(0.07)	(0.04)	(0.09)	(0.05)	(0.08)	(0.15)	(0.22)	(0.07)	(0.13)	(0.11)	(0.06)	(0.12)
	1.1	0.9	0.9	1.2	1.3	1.4	1.2	0.8	0.9	1.1	1.1	1.4
+S	(0.06)	(0.08)	(0.12)	(0.03)	(0.08)	(0.21)	(0.20)	(0.04)	(0.07)	(0.06)	(0.04)	(0.09)
	1.3	0.8	1.1	1.2	1.5	1.3	1.3	0.8	1.0	1.3	1.3	1.6
+NS	(0.05)	(0.25)	(0.23)	(0.01)	(0.05)	(0.09)	(0.02)	(0.04)	(0.03)	(0.05)	(0.11)	(0.03)
ANOVA[b]												
N	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.038**	ns	ns
N × S	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Values reported are means (n=4) with standard errors in parentheses.

Different capital letters indicate significant differences between sample collection times for each element concentration at each experiment at $P < 0.05$.

[a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

[b] ns, not significant ($P > 0.05$).

Table 3-4. Correlations between percentage of litter mass remaining and nutrients (nitrogen and sulfur) concentration in the remaining residual material showed as slope, coefficients of determination (R^2), and P value.

		Nitrogen			Sulfur		
Experiment	Treatment[a]	Slope	R^2	P	Slope	R^2	P
I	CK	-54	0.83	<0.001	-155	0.33	0.014
	+N	-43	0.81	<0.001	-303	0.58	<0.001
	+S	-55	0.91	<0.001	-241	0.51	0.002
	+NS	-39	0.84	<0.001	-326	0.66	<0.001
II	CK	-53	0.87	<0.001	-143	0.24	0.040
	+N	-41	0.76	<0.001	-123	0.70	<0.001
	+S	-67	0.87	<0.001	-264	0.64	<0.001
	+NS	-39	0.62	<0.001	-278	0.82	<0.001

[a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

Table 3-5. Litter C, N and S remaining (%) at different sampling time.

Element		Experiment I						Experiment II					
(%)	Treatment[a]	0 mo^{A}	6 mo^{B}	8 mo^{B}	10 mo^{C}	12 mo^{CD}	18 mo^{D}	0 mo^{A}	6 mo^{B}	8 mo^{B}	10 mo^{C}	12 mo^{CD}	18 mo^{D}
Carbon	CK	100	85 (1.6)	77 (1.6)	65 (1.7)	64 (2.3)	58 (3.2)	100	81 (2.6)	75 (3.2)	68 (4.5)	64 (4.9)	54 (4.3)
	+N	100	80 (1.8)	73 (0.4)	68 (1.7)	64 (0.6)	60 (1.8)	100	81 (1.2)	79 (0.6)	65 (3.3)	63 (2.8)	61 (1.3)
	+S	100	78 (1.3)	77 (2.0)	62 (1.3)	61 (1.8)	55 (1.7)	100	81 (1.6)	71 (2.2)	64 (1.5)	59 (1.3)	55 (1.0)
	+NS	100	85 (1.6)	80 (0.6)	64 (1.5)	63 (2.2)	57 (0.7)	100	82 (3.1)	77 (3.3)	68 (5.4)	65 (5.8)	55 (0.6)
	ANOVA[b]												
	N	-	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
	S	-	ns	**0.025**	ns	ns	ns	-	ns	ns	ns	ns	ns
	N × S	-	**0.003**	**0.026**	ns	ns	ns	-	ns	ns	ns	ns	ns
		0 mo^{C}	6 mo^{BC}	8 mo^{AB}	10 mo^{A}	12 mo^{A}	18 mo^{AB}	0 mo^{B}	6 mo^{B}	8 mo^{AB}	10 mo^{A}	12 mo^{AB}	18 mo^{B}
Nitrogen	CK	100	113 (9.0)	126 (9.9)	132 (1.8)	139 (9.1)	118 (14.4)	100	108 (2.0)	115 (4.2)	129 (13.8)	118 (7.4)	109 (11.5)
	+N	100	106 (10.3)	132 (1.8)	146 (8.4)	145 (11.7)	137 (15.5)	100	94 (2.8)	112 (3.5)	138 (6.1)	117 (8.3)	109 (7.8)
	+S	100	117 (6.1)	128 (1.4)	136 (10.7)	131 (10.3)	124 (5.5)	100	93 (6.7)	90 (4.9)	93 (6.6)	96 (7.1)	90 (0.3)

	+NS	100	118 (6.0)	150 (1.7)	163 (17.6)	160 (21.3)	134 (5.9)	100	87 (5.2)	105 (1.9)	129 (9.9)	108 (7.8)	94 (8.4)
	ANOVA[b]												
	N	-	ns	**0.026**	ns	ns	ns	-	ns	ns	**0.046**	ns	ns
	S	-	ns	ns	ns	ns	ns	-	**0.004**	**0.003**	**0.045**	**0.080**	ns
	N × S	-	ns	ns	ns	ns	ns	-	ns	**0.037**	ns	ns	ns
		0 mo[A]	6 mo[B]	8 mo[B]	10 mo[B]	12 mo[B]	18 mo[B]	0 mo[A]	6 mo[B]	8 mo[B]	10 mo[B]	12 mo[B]	18 mo[B]
Sulfur	CK	100	57 (3.8)	44 (7.7)	55 (6.4)	59 (0.5)	49 (2.3)	100	52 (4.7)	44 (8.7)	59 (4.4)	46 (3.2)	48 (3.3)
	+N	100	57 (3.6)	58 (2.8)	56 (1.3)	67 (4.1)	64 (10.5)	100	45 (8.0)	52 (14.2)	68 (9.8)	58 (12.4)	66 (12.8)
	+S	100	64 (7.0)	60 (7.8)	63 (2.7)	71 (4.8)	66 (8.5)	100	54 (9.0)	57 (14.4)	64 (11.1)	57 (11.2)	68 (10.3)
	+NS	100	52 (1.3)	59 (3.8)	59 (2.7)	68 (4.0)	54 (2.1)	100	47 (4.2)	63 (3.6)	65 (4.5)	55 (4.2)	67 (1.1)
	ANOVA[b]												
	N	-	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
	S	-	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
	N × S	-	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns

Values reported are means (n=4) with standard errors in parentheses.

Different capital letters indicate significant differences between sample collection time for each element concentration at each experiment at $P < 0.05$.

[a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

[b] -, no value; ns, not significant ($P > 0.05$).

Table 3-6. Litter C/N and C/S ratios at different sampling time.

		Experiment I						Experiment II					
Ratio	Treatment[a]	0 mo[A]	6 mo[B]	8 mo[C]	10 mo[D]	12 mo[D]	18 mo[D]	0 mo[A]	6 mo[B]	8 mo[C]	10 mo[D]	12 mo[D]	18 mo[D]
C/N	CK	70.9 (3.1)	56.6 (1.6)	45.9 (1.3)	36.6 (1.6)	34.4 (0.7)	37.1 (2.1)	69.7 (0.7)	52.2 (1.9)	45.7 (2.9)	37.3 (1.5)	37.6 (1.1)	34.8 (0.8)
	+N	69.6 (1.7)	60.7 (6.7)	44.0 (1.3)	37.1 (1.0)	35.9 (3.5)	35.6 (2.5)	60.5 (2.1)	52.5 (1.9)	42.7 (2.8)	28.5 (0.4)	32.7 (2.5)	33.9 (3.2)
	+S	69.8 (2.4)	53.6 (1.4)	47.9 (1.4)	36.5 (1.1)	37.2 (0.8)	35.7 (2.2)	59.9 (3.3)	52.8 (2.3)	47.6 (1.3)	41.7 (3.2)	37.1 (3.0)	36.8 (2.5)
	+NS	68.9 (1.2)	55.3 (1.9)	41.0 (1.2)	30.4 (2.2)	31.1 (2.7)	32.9 (1.7)	56.4 (1.1)	53.4 (2.9)	41.0 (2.6)	29.9 (1.6)	33.8 (3.3)	33.6 (2.8)
	ANOVA[b]												
	N	ns	ns	**0.009**	ns	ns	ns	**0.014**	ns	ns	**0.001**	ns	ns
	S	ns	ns	ns	ns	ns	ns	**0.010**	ns	ns	ns	ns	ns
	N × S	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

		0 mo^{C}	6 mo^{A}	8 mo^{AB}	10 mo^{C}	12 mo^{BC}	18 mo^{C}	0 mo^{C}	6 mo^{A}	8 mo^{AB}	10 mo^{C}	12 mo^{BC}	18 mo^{C}
C/S	CK	345 (35.0)	513 (16.6)	635 (85.6)	405 (25.6)	330 (33.9)	401 (21.4)	329 (31.2)	524 (69.0)	586 (84.7)	381 (22.1)	454 (11.2)	314 (43.0)
	+N	370 (18.6)	513 (26.9)	471 (42.0)	450 (24.4)	376 (28.2)	361 (34.8)	356 (61.3)	655 (61.6)	592 (108.6)	341 (4.6)	395 (17.4)	363 (33.3)
	+S	414 (22.6)	539 (43.8)	547 (69.3)	404 (10.1)	355 (23.0)	361 (55.3)	416 (83.3)	618 (31.5)	531 (39.9)	413 (18.2)	431 (23.7)	333 (20.2)
	+NS	344 (12.3)	568 (10.7)	469 (14.4)	368 (4.4)	321 (10.7)	368 (29.9)	352 (5.0)	617 (36.0)	443 (14.9)	368 (13.2)	385 (28.4)	295 (4.8)
	ANOVA[b]												
	N	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.027**	**0.037**	ns
	S	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	N × S	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Values reported are means (n=4) with standard errors in parentheses.

Different capital letters indicate significant differences between sample collection time for each C/nutrient ratio concentration at each experiment at $P < 0.05$.

[a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

[b] ns, not significant ($P > 0.05$).

Table 3-7. Cumulative enzyme activities at different study periods.

Enzyme	Treatment[a]	Experiment I				Experiment II			
		0 to 6 mo[D]	6 to 12 mo[C]	12 to 18 mo[B]	0 to 18 mo[A]	0 to 6 mo[C]	6 to 12 mo[C]	12 to 18 mo[B]	0 to 18 mo[A]
GLU	CK	0.53 (0.02)	2.01 (0.11)	7.98 (0.70)	13.0 (0.86)	0.49 (0.03)	1.93 (0.17)	7.65 (0.54)	12.66 (1.37)
	+N	0.67 (0.08)	2.01 (0.14)	7.34 (0.39)	13.0 (0.87)	0.54 (0.08)	1.81 (0.05)	7.25 (0.24)	12.28 (0.44)
	+S	0.56 (0.07)	2.01 (0.15)	7.11 (0.40)	12.4 (0.85)	0.49 (0.05)	1.94 (0.12)	6.52 (0.56)	11.47 (0.95)
	+NS	0.54 (0.07)	2.19 (0.06)	8.07 (0.24)	13.9 (0.38)	0.51 (0.16)	1.88 (0.15)	7.69 (0.48)	13.11 (1.18)
	ANOVA[b]								
	N	ns	ns	ns	ns	ns	ns	ns	ns
	S	ns	ns	ns	ns	ns	ns	ns	ns
	N × S	ns	ns	ns	ns	ns	ns	ns	ns
		0 to 6 mo[C]	6 to 12 mo[AB]	12 to 18 mo[B]	0 to 18 mo[A]	0 to 6 mo[C]	6 to 12 mo[AB]	12 to 18 mo[B]	0 to 18 mo[A]
NAG	CK	0.19 (0.00)	4.04 (1.19)	2.65 (0.32)	4.40 (0.25)	0.29 (0.05)	3.71 (0.49)	2.39 (0.04)	4.40 (0.29)
	+N	0.22 (0.03)	4.63 (1.07)	4.01 (0.74)	7.27 (1.60)	0.11 (0.03)	3.11 (0.27)	2.80 (0.58)	4.68 (0.94)
	+S	0.17 (0.05)	6.72 (0.22)	4.31 (0.62)	8.19 (1.43)	0.19 (0.01)	5.20 (0.19)	3.81 (0.16)	7.72 (0.14)
	+NS	0.18 (0.05)	5.10 (0.05)	2.87 (0.08)	4.96 (0.26)	0.23 (0.01)	4.48 (0.02)	2.55 (0.18)	4.51 (0.10)

	ANOVA[b]								
	N	ns	ns	ns	ns	**0.048**	ns	ns	**0.019**
	S	ns	ns	ns	ns	ns	**0.001**	ns	**0.014**
	N × S	ns	ns	**0.025**	**0.023**	**0.008**	ns	**0.029**	**0.008**
		0 to 6 mo[C]	6 to 12 mo[B]	12 to 18 mo[B]	0 to 18 mo[A]	0 to 6 mo[C]	6 to 12 mo[B]	12 to 18 mo[B]	0 to 18 mo[A]
ARS	CK	1.31 (0.17)	3.36 (0.14)	4.26 (0.56)	9.34 (0.93)	1.29 (0.10)	3.43 (0.14)	4.13 (0.55)	9.18 (1.16)
	+N	1.17 (0.16)	3.19 (0.08)	4.33 (0.47)	9.38 (1.02)	1.26 (0.33)	3.57 (0.29)	4.80 (0.34)	10.11 (0.57)
	+S	1.26 (0.23)	4.44 (1.28)	3.84 (0.39)	9.03 (1.03)	1.03 (0.09)	3.52 (0.18)	4.79 (0.16)	9.87 (0.39)
	+NS	0.97 (0.07)	2.95 (0.19)	4.68 (0.65)	9.51 (1.12)	1.46 (0.30)	3.86 (0.84)	4.00 (0.52)	9.51 (0.46)
	ANOVA[b]								
	N	ns	ns	ns	ns	ns	ns	ns	ns
	S	ns	ns	ns	ns	ns	ns	ns	ns
	N × S	ns	ns	ns	ns	ns	ns	ns	ns

Values reported are means (n=4) with standard errors in parentheses.

Different capital letters indicate significant differences between different study periods for each cumulative enzyme activity at each experiment at $P < 0.05$.

[a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

[b] ns, not significant ($P > 0.05$).

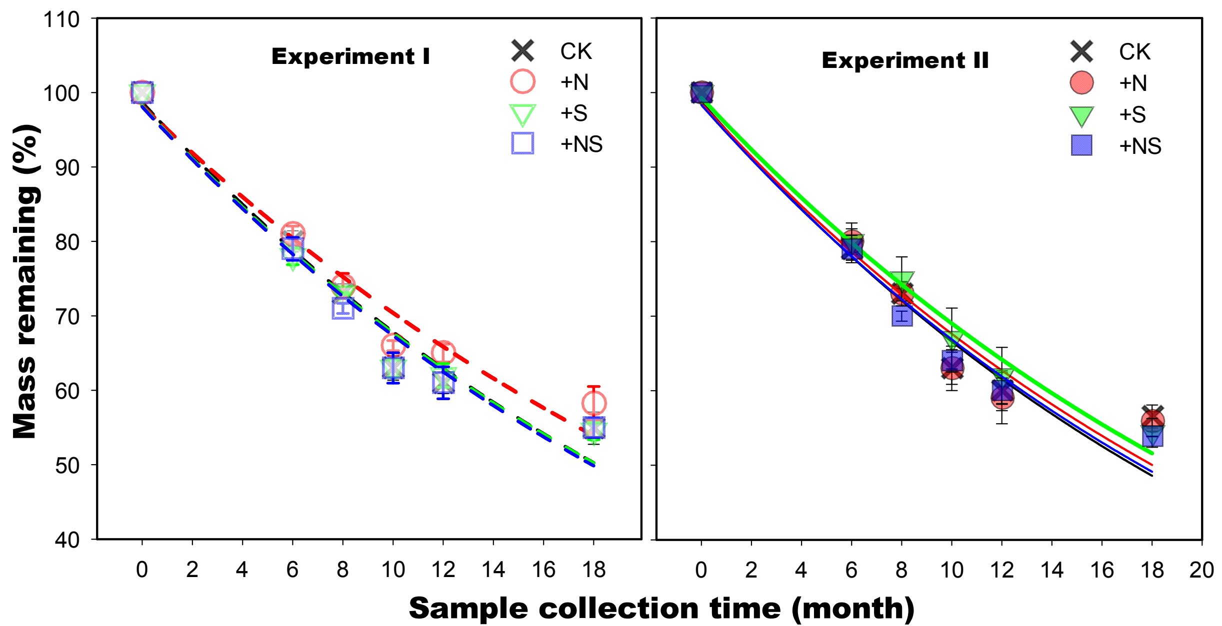

Fig. 3-1 Ash-free litter dry mass remaining (%) at each sampling time for the duration of 18-month field study. Decay curves were fitted for each dataset using single-pool exponential decay function. No significant difference between treatments.

CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

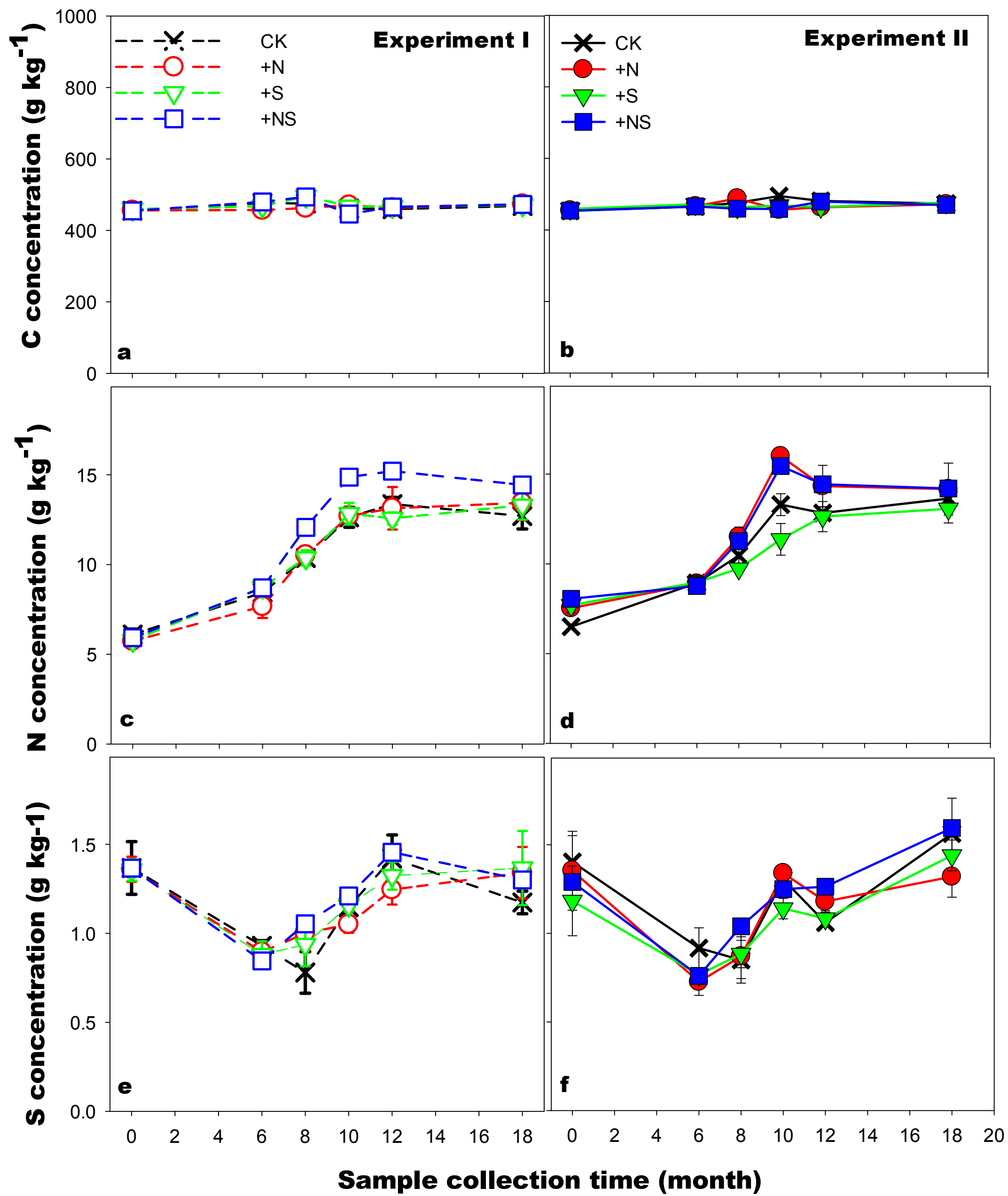

Fig. 3-2 Changes in the concentrations of C, N and S (g kg^{-1}) in litter during the 18-month decomposition.

CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

a and b, litter C concentration; c and d, litter N concentration; e and f, litter S concentration.

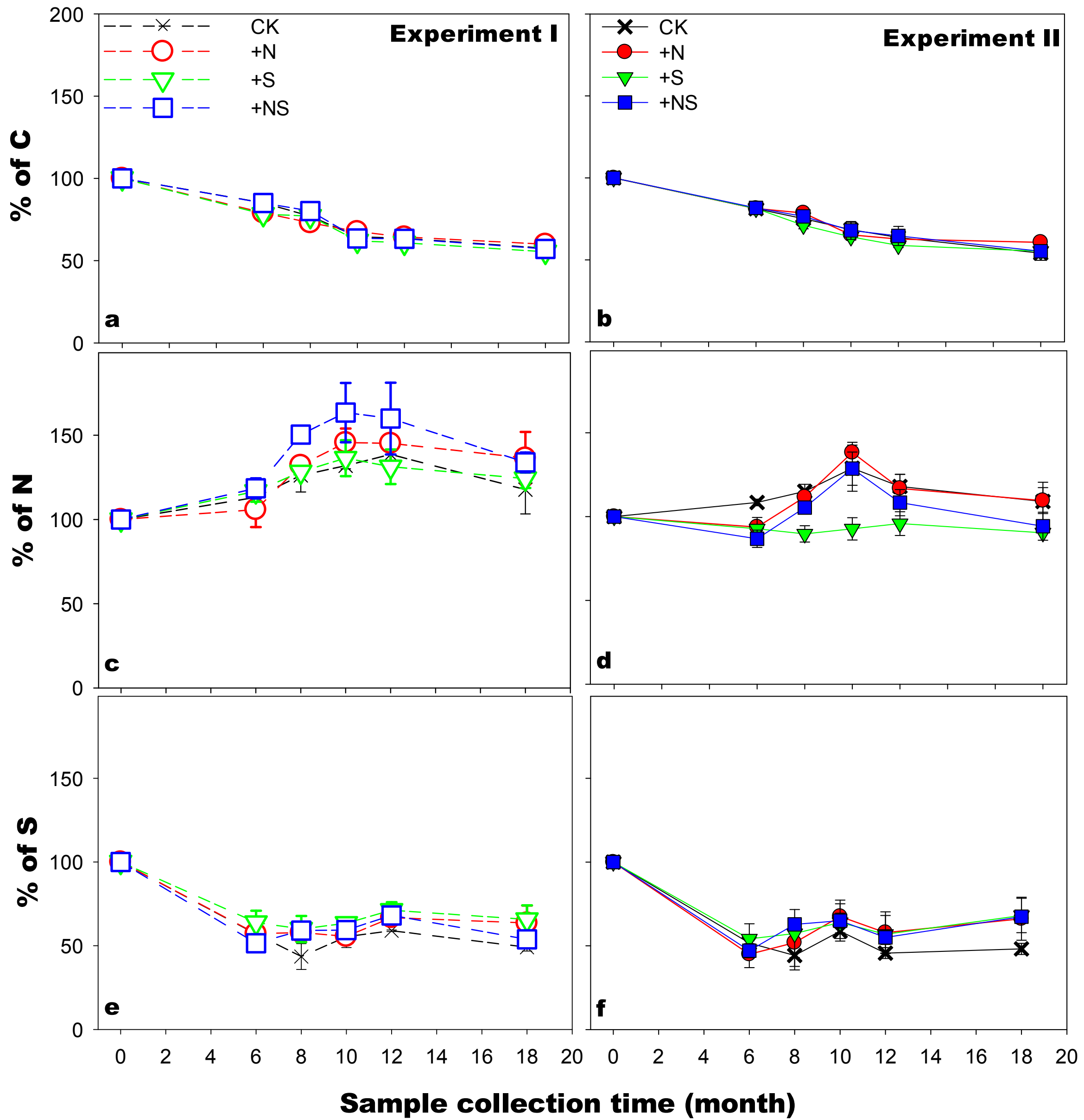

Fig. 3-3 Changes in the C, N and S remaining (% of initial) in litter during the 18-month decomposition.

CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition. a and b, percent of litter C remaining; c and d, percent of litter N remaining; e and f, percent of litter S remaining.

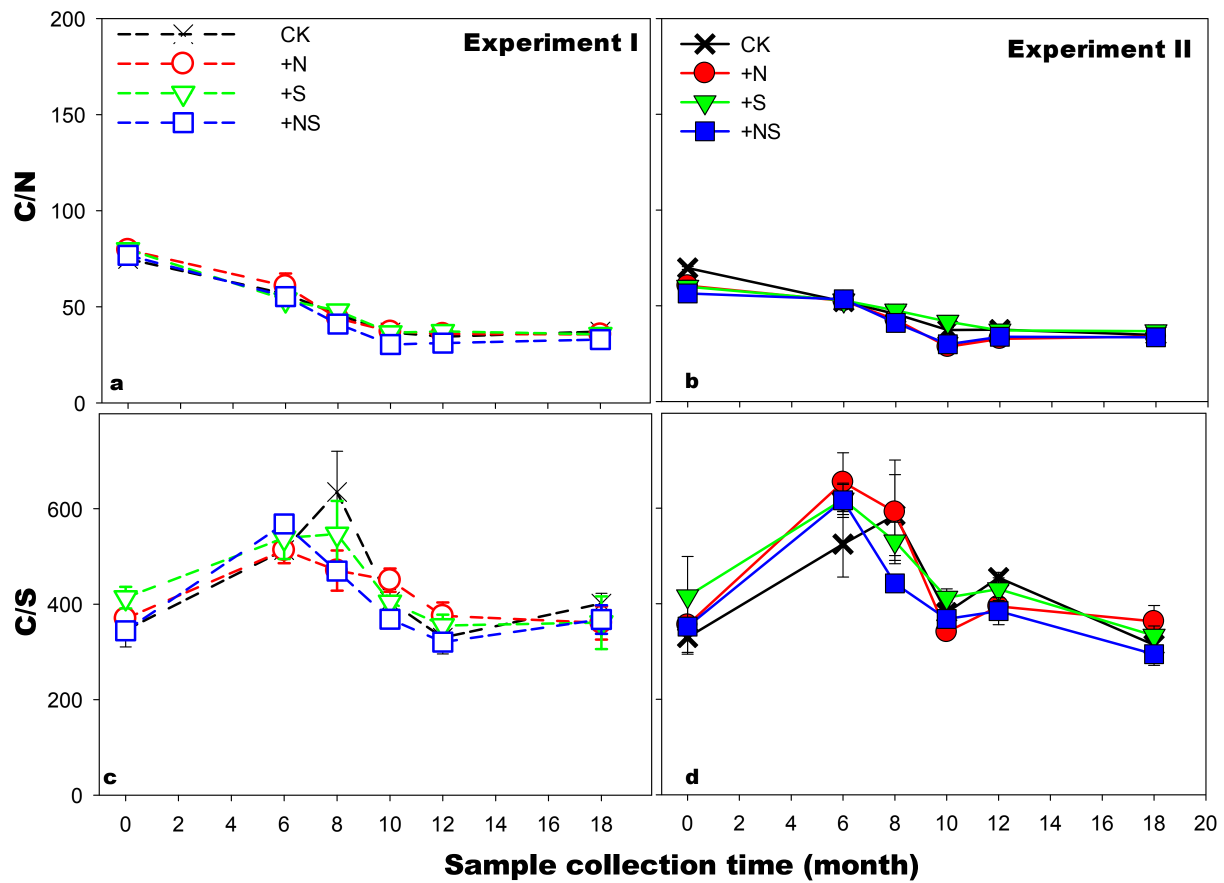

Fig. 3-4 Changes in the C/N and C/S ratios in litter remaining during the 18-month decomposition.

CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition. a and b, litter C/N ratio; c and d, litter C/S ratio.

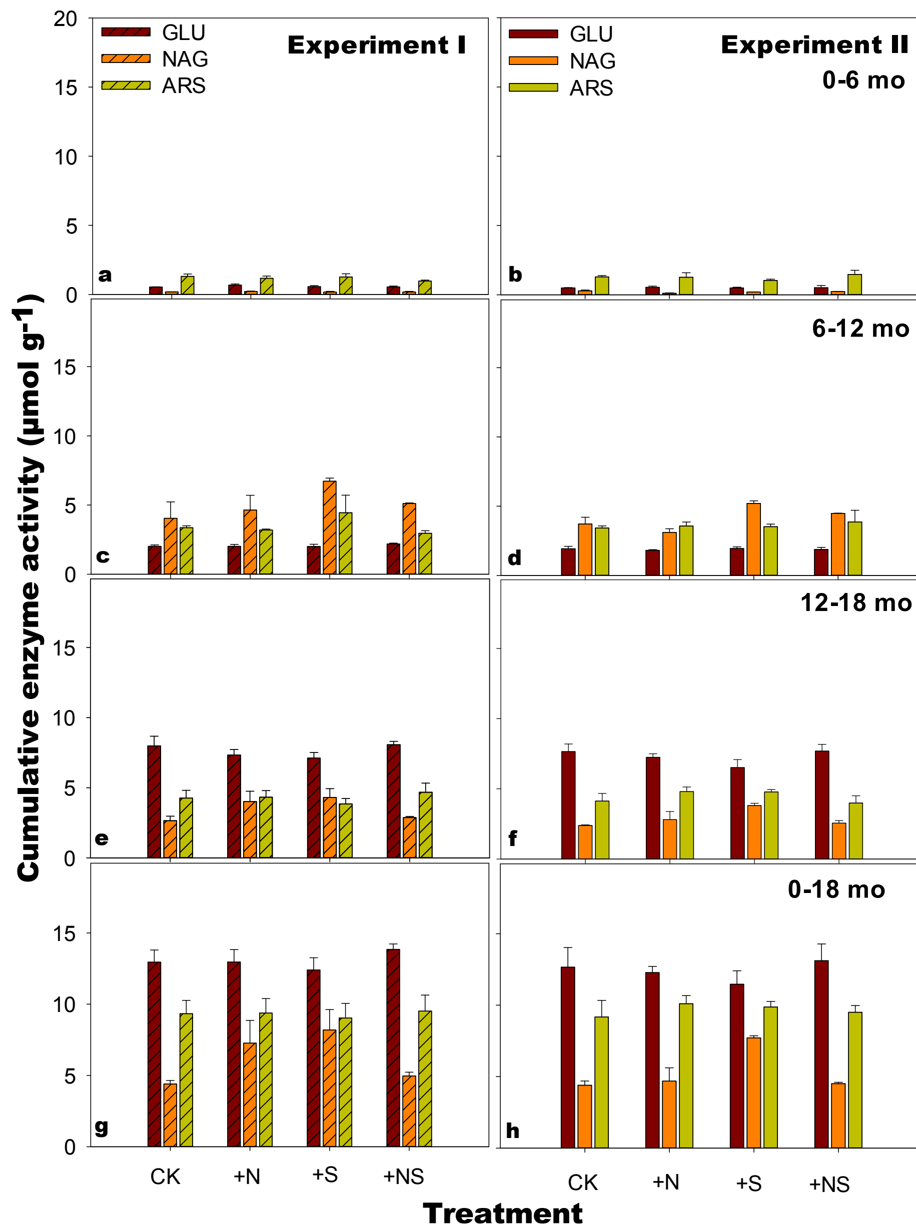

Fig. 3-5 Cumulative enzyme activities (GLU, NAG, and ARS) in litters at different study period. CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition. a and b, 0 to 6 months study period; c and d, 6 to 12 months study periods; e and f, 12 to 18 months study periods; g and h, 0 to 18 months study periods.

Chapter 4. Conclusions and future research

1. Overview of the study objectives

The overall objective of this research was to assess the effect of simulated nitrogen (N) and sulfur (S) deposition on trembling-aspen leaf litter decomposition in order to provide another way to assess the health of a boreal forest in northern Alberta. In this research, I studied many factors that affected litter decomposition rates including litter chemistry, soil microbial properties, litter enzyme activities, and external nutrient availability. A laboratory incubation experiment was conducted for 100 days to assess whether litter chemistry and forest floor (F and H layers, after removing litter layer) microbial properties were affected by 10 years of N and S addition, and how the changed litter chemistry and forest floor microbial properties, and this in turn, affected litter decomposition rates. A field experiment was also conducted for 18 months to determine whether N and S addition can affect litter decomposition rates, and how the combined effect of nutrient additions and nutrient-induced changes in litter chemistry alter litter decomposition rates. In the field study, I also examined nutrient changes in litter remaining and the relationship between litter enzyme activities and litter decay constants.

2. Summary and synthesis of the research results

2.1 Laboratory experiment

The results showed that 10 years of N and S addition have significantly affected litter chemistry and forest floor microbial properties. Long-term N and S addition increased N and

decreased lignin concentrations, resulting in lower C (carbon) to N (C/N) and lignin to N (lignin/N) ratios. Ten years of N and S addition increased forest floor microbial biomass C (MBC) and N (MBN). Addition of S increased all three enzyme activities (β-1, 4-glucosidase (GLU) for C, β-1, 4-N-acetylglucosaminidase (NAG) for N, and arylsulfatase (ARS) for S) in the forest floor, while N addition increased GLU and ARS not NAG.

An analysis of the forest floor amended with or without litter, over the 100-day incubation, indicating that forest floor GLU and NAG activities increased, while MBC, MBN, and ARS activity decreased. Without litter addition, cumulative CO_2 emission (C_{cum}) from forest floor increased with S but not N addition, and no interaction effect was detected. When litter was added on the forest floor, C_{cum} was not affected by N and S addition. The C_{cum} from litter only was increased by +S and +NS treatments. Linear regressions showed that the C_{cum} from litter significantly correlated with litter C/N and lignin/N ratios, but there were no correlations between litter C_{cum} and mean MBC, MBN, or cumulative enzyme activities in the forest floor.

2.2 Field experiment

The 18-month field study results revealed that N and S addition did not affect litter decomposition rates (Experiment I), and changed litter chemistry did not result in different litter decomposition rates under N and S addition (Experiment II). But litter decay constants were affected by time. In Experiment I, litter decay constant appeared to be the lowest under +N treatment and may have a significant effect over the longer study period. However, litter under the +S treatment decomposed the slowest in Experiment II.

Litter N did not exhibit an initial leaching phase but an apparent accumulation phase from 0 to 10 months, N release occurred after month 10. Compare to N release pattern, litter S

exhibited an initial leaching phase then accumulation phase until the end of the study. Regressions revealed that litter mass remaining strongly correlated with litter N and S remaining in the residue.

The cumulative GLU and ARS activities in the litter were not affected by N and S addition in Experiment I, while cumulative NAG activity was negatively affected by N and S co-addition at 12 to 18 months and 0 to 18 months study periods. Litter chemistry did not change cumulative GLU and ARS activities in the litter under N and S addition in Experiment II, while NAG activity increased. There was no correlation between cumulative enzyme activities and litter decay constants at any study period.

2.3 Conclusions of laboratory and field experiments

Ten years of N and S addition decreased litter C/N and lignin/N ratios through increased N concentrations in litter, and in turn, changed litter chemistry which significantly affected litter decomposition rates. Although long-term N and S addition changed forest floor microbial properties, it did not affect litter decomposition rates. This laboratory incubation experiment concluded that litter chemistry instead of forest floor microbial properties were the main factor regulating litter decomposition. This study provides insight into the current conditions in the oil sands region in northern Alberta, which has intensive oil sands mining activity which generates a significant amount of N and S emissions. As in a consequence, litter decomposition rates accelerated and more CO_2 was released from forest floor into the atmosphere. However, limitations of a laboratory incubation study should be recognized, and results should be verified in a field setting.

The field study concluded that 18-month of N and S addition did not affect litter decomposition rates, which suggest that higher applications rates or a longer study period may be needed to observe measurable effects. The combined effects of nutrient additions and nutrient-induced changes in litter chemistry also did not affect litter decomposition rates, which indicate that litter chemistry is not a robust predictor of litter decomposition.

2.4 Contrasts of laboratory and field experiments

Litter decomposition rates were expressed as CO_2 emission (mineralization) in the lab incubation, while it was expressed as litter mass loss in the field study. Litter mass loss is a combination of mineralization (CO_2 emission) and nutrient leaching. In the lab incubation experiment, changed litter chemistry had significant impacts on CO_2 emission; however, litter decay constants were not affected directly (N and S addition) or when combining direct and indirect (changed litter chemistry) effects in the field experiment. The inconsistent results from laboratory and field experiments may due to the measurement of litter decomposition rates, either by using mineralization rates or mass loss. Other factors may also contribute to the inconsistent results, such as nutrients input to the environment (ambient N deposition), soil fauna presence (earthworms), and periodic dynamics of temperature and moisture (Teuben and Verhoef, 1992). However, the lab incubation experiment gives the opportunity to study the important factors which may not be able to observe in the field due to the complexity of the field environment. And field study is more realistic.

3. Future research and implications

3.1 Longer term study is needed

In the context of ongoing oil sands mining (especially in northern Alberta), increasing levels of N and S deposition, and concerns over climate change, my study provides a better understanding of nutrient and C cycling in the boreal forest. To gain further understanding, longer-term evaluation is needed. Studies have shown that complete decomposition may take weeks to decades. For example, in a coniferous forest in Britain decomposition was showed to take 3-5 years (Ovington, 1962), and as many as 28 years may be required for decomposition in a high mountain oak forest (Jenny et al., 1949).

3.2 Soil microbial properties in a field experiment

For a better understanding of microbial community impacts on litter decomposition, it would be valuable to test soil microbial properties over time in addition to litter microbial properties, because microbial activities react differently to nutrient additions in litter and soil (Saiya-Cork et al., 2002). In this study, even though the laboratory experiment did not show significant relationships between microbial activity and litter CO_2 release rates may be affected by measurement frequency which may have been insufficient to reflect the fluctuations of microbial biomass. In addition, a greater number of enzymes might improve the correlative resutles. On the other hand, prior research suggests a linear relationship between litter decomposition rates and cumulative extracellular enzyme activities (Sinsabaugh et al., 1992; Waring, 2013). In addition, external N and S can increase overall soil microbial abundance and

result in the production of specific enzymes (Weand et al., 2010). In another word, when N is added, it can shift microbial resource allocation from nutrient acquisition to C acquisition. This can, in turn, result in increasing enzyme activities and a shift to C cycling (Sinsabaugh and Moorhead, 1994). Therefore, incorporating additional enzyme assays may be beneficial for subsequent studies involving soil and litter. The enzymes β-1, 4-endoglucanase (endocellulase, EC 3.2.1.4), β-xylosidase (EC 3.2.2.37), phenol oxidase (EC 1.10.3.2), and peroxidae (EC 1.11.1.7) would be a productive place to start.

3.3 Different litter type

This study was limited to the leaf litter of a single tree species, trembling aspen, from 2015 to 2017. In my study site, trembling aspen accounted for 70% of the total tree population (Jung and Chang, 2012). The research site, however, is a mixedwood boreal forest, and white spruce is also an important tree species representing 20% of forest tree diversity. The nature diversity of leaf litter can be an important instrumental factor in plant establishment, growth, and community development (Nilsson et al., 1999). Gartner and Cardon (2004) concluded from a mega-analysis of 30 research papers that decomposition rates of mixed-species litter were higher than predicted by single species and overall nutrient concentrations. Studies also showed that microbial activity can be influenced by different plant species mixtures (Taylor et al., 1989; Rustad, 1994).

Furthermore, most studies of litter decomposition in forest ecosystems have focused on foliar litter due to the large amount and relatively high nutrient content. However, woody and fine roots litter can also contribute large amounts of nutrients to forest soil (Berg and

McClaugherty, 2003). Vogt et al. (1990) concluded that the biomass of fine roots may account for less than 2% of a forest ecosystem, but they can contribute as much as 40% of the annual productivity. Harmon et al. (1986) demonstrated that woody litter (stems, stumps, branches, twigs, and bigger roots) plays an important role but is poorly studied in forest ecosystems. Woody litter often results from extreme weather conditions, such as strong wind or heavy snowfall. Because woody litter contains very low nutrient contents, it is suitable decomposition matter for organisms that require low nutrients (Berg and McClaugherty, 2003). In addition, because of the low nutrient content, N addition will be less likely to influence lignin degradation (Berg and McClaugherty, 2003).

3.4 Implications

Oil sands mining activities, especially in the AOSR which is one of the largest oil sands reservoirs in the world, have released significant amounts of N and S to the atmosphere and deposited to the surrounding area. Nitrogen and S deposition can have significant impacts on litter decomposition, and litter decomposition is a very important component of nutrient and C cycling in the forest ecosystems. Although the lab incubation results showed that changed litter chemistry by long-term N and S addition affected CO_2 emission, this was studied in a controlled environment. The field experiment suggested that N and S addition did not affect the boreal forest productivity as it did not change the litter decomposition. However, the field study has implications for potential risk of reducing forest productivity in the boreal forest in AOSR by N and S addition emitted through oil sands mining over long-term. Therefore, continuing monitoring of the impacts of N and S deposition on litter decomposition in the AOSR is needed.

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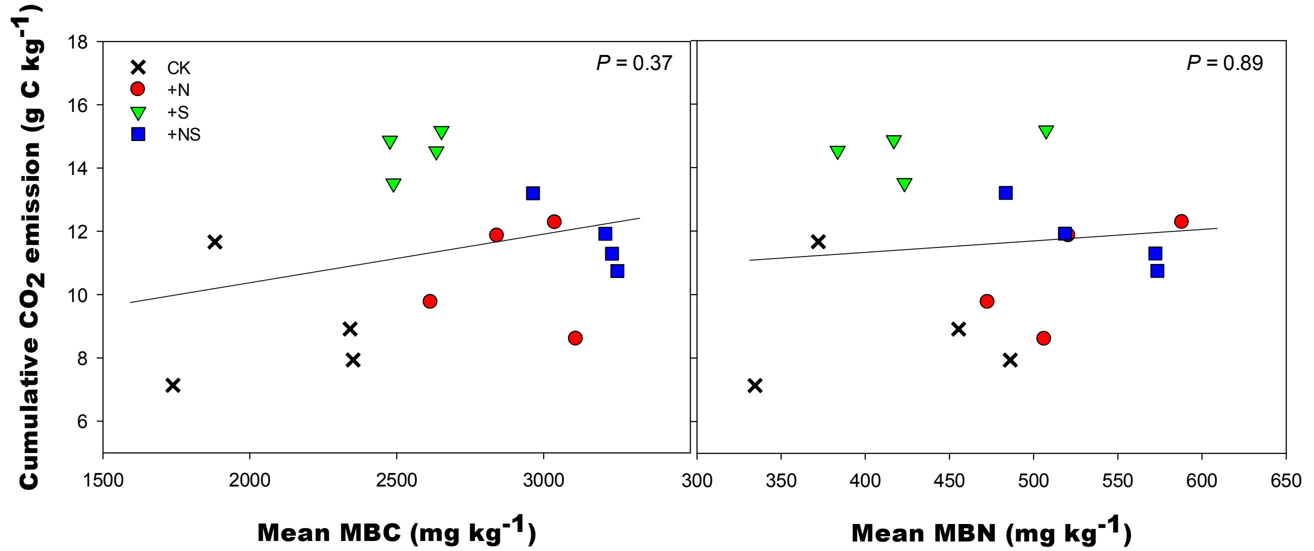
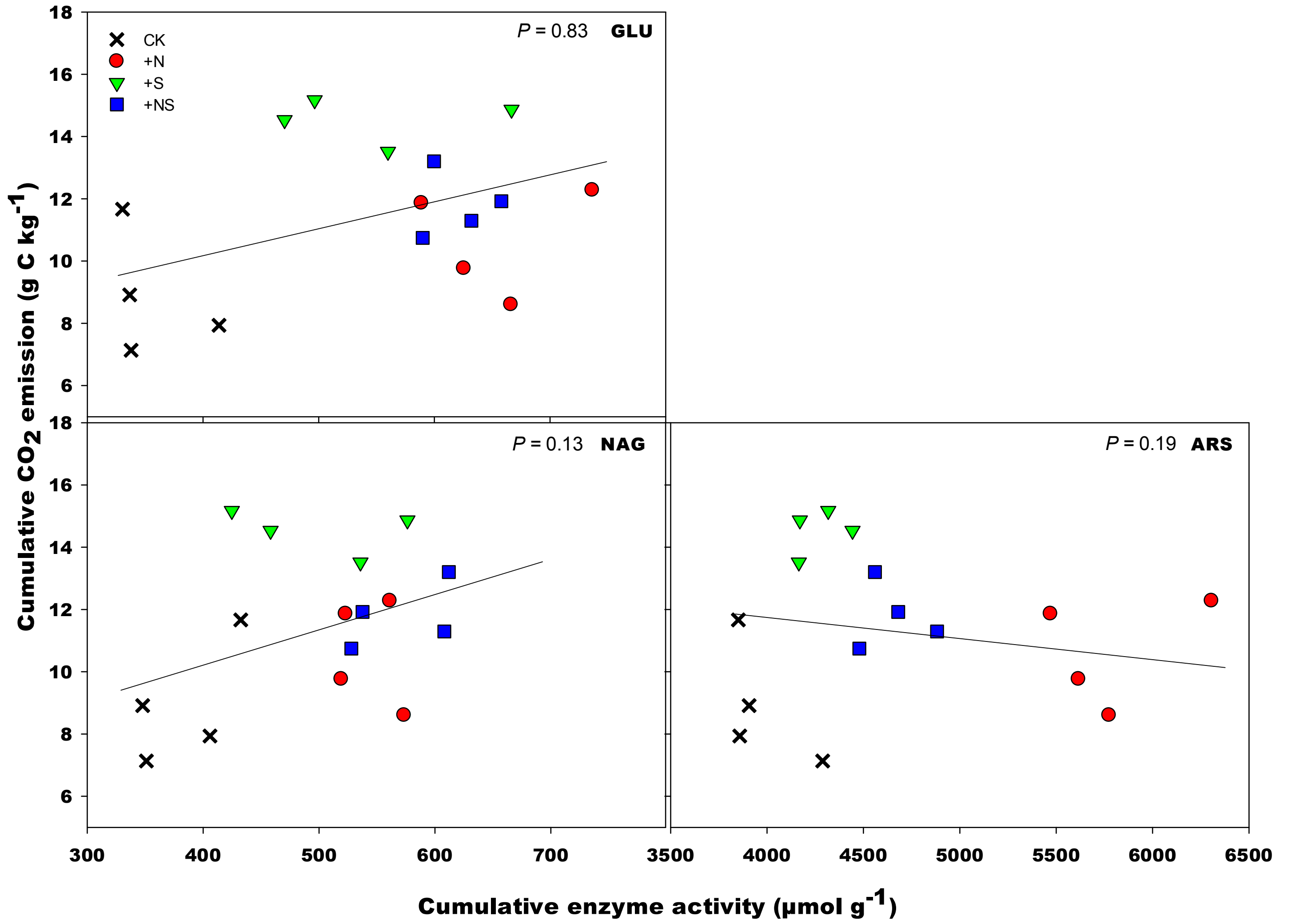
Appendices

Appendix A: Linear relationship between mean microbial biomass (mg kg^{-1}) and cumulative litter CO_2 emission (mg C kg^{-1}).



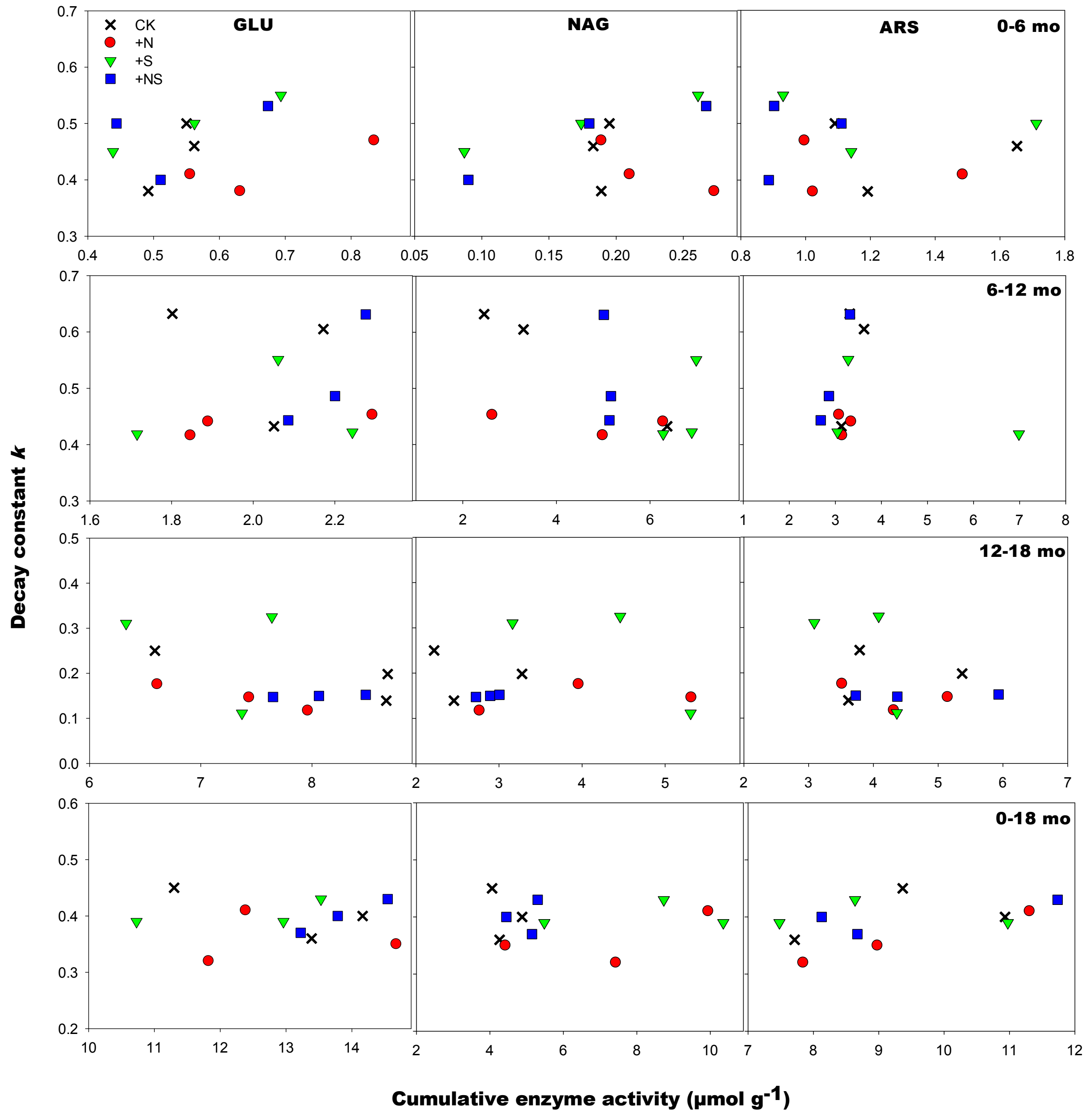
CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

Appendix B: Linear relationship between cumulative enzyme activities (μmol g^{-1}) and cumulative litter CO_2 emission (mg C kg^{-1}).



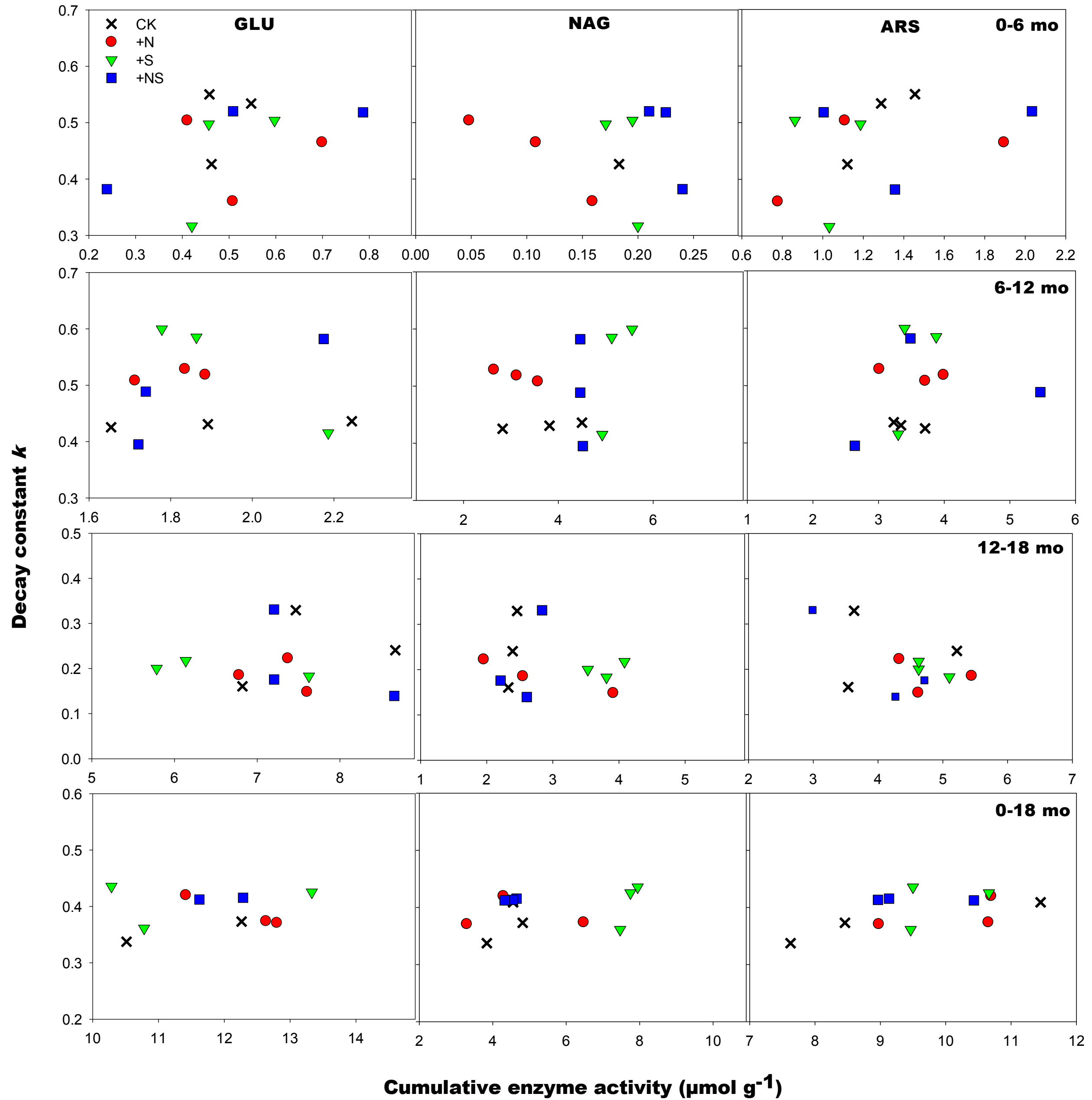
CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

Appendix C: Correlations between cumulative enzyme activities (μmol g^{-1}) and litter decay constants (k) at different study periods in Experiment I.



CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

Appendix D: Correlations between cumulative enzyme activities (μmol g^{-1}) and litter decay constants (k) at different study periods in Experiment II.



CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

Appendix E: Litter ash-free mass remaining at different study periods.

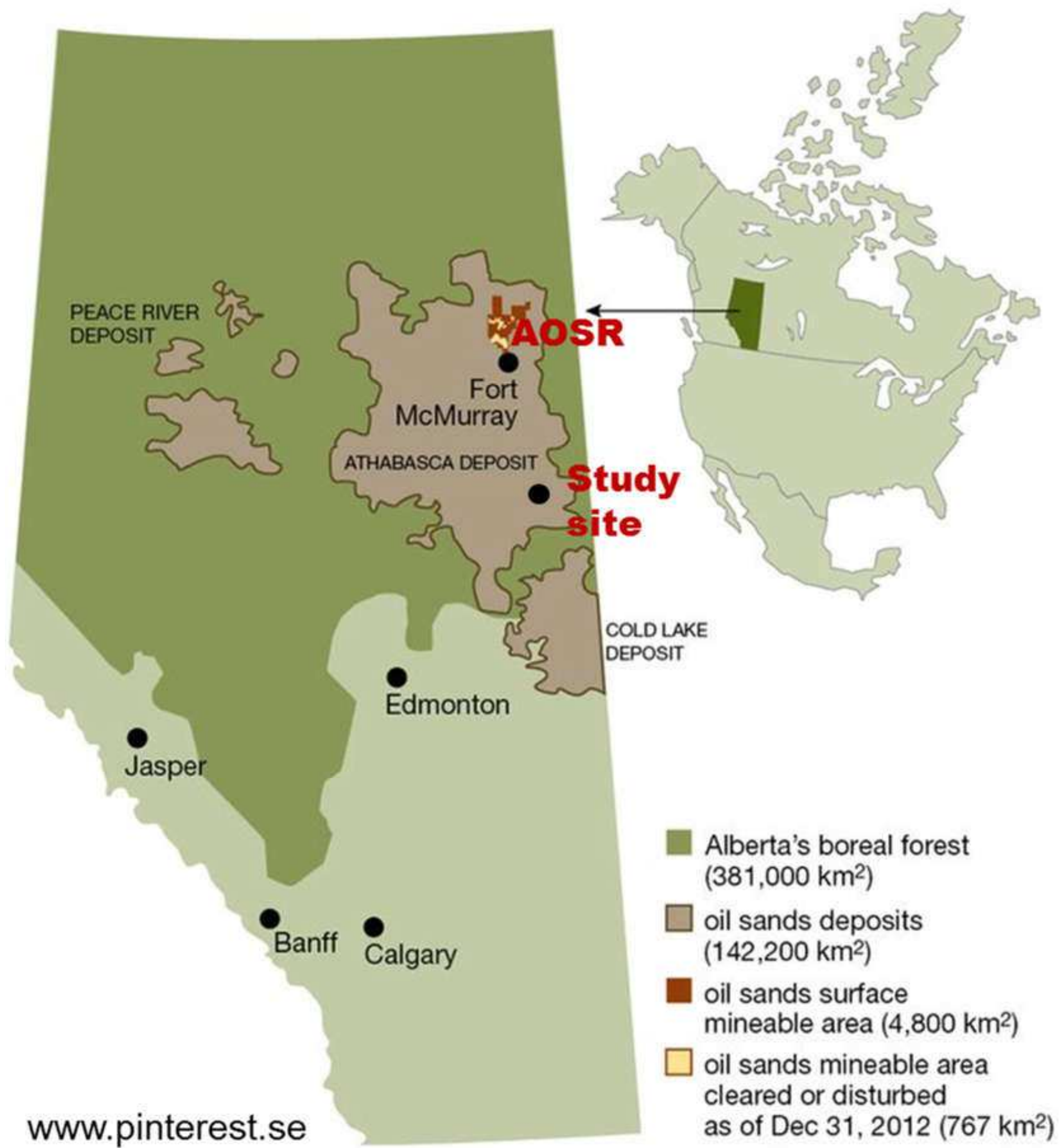
		0-6 moB	6-12 moB	12-18 moA	0-18 moC
Experiment	Treatment[a]	Decay constant (k)			
I	CK	80 (1.4)	76 (2.4)	91 (1.5)	55 (2.2)
	+N	81 (1.1)	80 (0.4)	89 (3.6)	58 (2.3)
	+S	78 (1.1)	79 (1.7)	88 (3.1)	55 (1.2)
	+NS	79 (1.5)	77 (2.2)	91 (2.2)	55 (1.3)
	ANOVA				
	N	0.481	0.529	0.868	0.267
	S	0.178	0.931	0.833	0.349
	N × S	1.000	0.103	0.548	0.449
II	CK	78 (1.5)	76 (4.2)	89 (2.2)	57 (1.8)
	+N	80 (1.7)	74 (3.1)	94 (3.4)	56 (1.3)
	+S	80 (2.5)	77 (2.3)	94 (3.4)	54 (1.9)
	+NS	79 (1.8)	76 (3.2)	90 (2.6)	54 (0.0)
	ANOVA				
	N	0.864	0.656	0.763	0.577
	S	0.732	0.745	0.907	0.169
	N × S	0.401	0.799	0.134	0.736

Values reported are means (n=4) with standard errors in parentheses.

[a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

Different capital letters indicate significant differences among study periods at $P < 0.05$.

Appendix F: Map of study site.



Appendix G: Litter enzyme activities at each sampling time during the 18-month field study.

			Sample collection time[b]				
Experiment	Enzymes	Treatment[a]	6 month	8 month	10 month	12 month	18 month
I	GLU	CK	0.18 (0.01)	0.28 (0.04)	0.09 (0.03)	1.30 (0.09)	1.36 (0.17)
		+N	0.22 (0.03)	0.28 (0.01)	0.06 (0.01)	1.36 (0.10)	1.09 (0.08)
		+S	0.19 (0.02)	0.21 (0.10)	0.06 (0.00)	1.33 (0.09)	1.16 (0.13)
		+NS	0.18 (0.02)	0.33 (0.01)	0.05 (0.02)	1.50 (0.08)	1.35 (0.16)
	NAG	CK	0.09 (0.03)	0.19 (0.04)	1.47 (0.57)	0.66 (0.24)	0.46 (0.11)
		+N	0.07 (0.01)	0.15 (0.04)	1.62 (0.37)	0.83 (0.23)	0.50 (0.02)
		+S	0.11 (0.06)	0.16 (0.04)	2.91 (0.34)	1.01 (0.21)	0.54 (0.12)
		+NS	0.06 (0.02)	0.17 (0.04)	2.31 (0.37)	0.51 (0.04)	0.45 (0.12)
	ARS	CK	0.44 (0.06)	0.58 (0.06)	0.53 (0.05)	0.73 (0.12)	0.69 (0.07)
		+N	0.39 (0.05)	0.55 (0.07)	0.46 (0.09)	0.80 (0.08)	0.64 (0.08)
		+S	0.42 (0.08)	0.45 (0.05)	0.55 (0.07)	0.77 (0.09)	0.52 (0.04)
		+NS	0.32 (0.02)	0.46 (0.01)	0.44 (0.03)	0.87 (0.11)	0.68 (0.12)
II	GLU	CK	0.16 (0.01)	0.29 (0.01)	0.04 (0.01)	1.32 (0.23)	1.23 (0.05)
		+N	0.18 (0.03)	0.26 (0.03)	0.03 (0.01)	1.28 (0.06)	1.13 (0.09)
		+S	0.16 (0.02)	0.32 (0.03)	0.05 (0.00)	1.25 (0.16)	0.92 (0.12)
		+NS	0.17 (0.05)	0.23 (0.01)	0.05 (0.01)	1.42 (0.12)	1.15 (0.05)
	NAG	CK	0.10 (0.02)	0.16 (0.04)	1.38 (0.21)	0.63 (0.20)	0.47 (0.10)
		+N	0.04 (0.01)	0.12 (0.01)	1.74 (0.66)	0.51 (0.12)	0.42 (0.07)
		+S	0.06 (0.00)	0.11 (0.01)	1.88 (0.08)	0.79 (0.21)	0.46 (0.19)
		+NS	0.09 (0.02)	0.14 (0.02)	1.96 (0.24)	0.89 (0.44)	0.40 (0.07)

ARS	CK	0.43 (0.03)	0.40 (0.03)	0.73 (0.09)	0.73 (0.13)	0.65 (0.06)
	+N	0.42 (0.11)	0.56 (0.04)	0.61 (0.07)	0.84 (0.08)	0.76 (0.12)
	+S	0.34 (0.03)	0.46 (0.05)	0.68 (0.09)	0.91 (0.08)	0.68 (0.06)
	+NS	0.49 (0.10)	0.45 (0.04)	0.87 (0.34)	0.74 (0.09)	0.59 (0.08)

Values reported are means (n=4) with standard errors in parentheses.

[a] CK, control; +N, nitrogen addition; +S, sulfur addition; and +NS, nitrogen and sulfur addition.

[b] Litter enzyme activities were assumed to be 0 at sample collection time 0.