University of Alberta

Through the Eyes of a Tree: Monitoring Environmental Change Using Stable Isotope Dendrochemistry

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

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Spring 2013
Edmonton, Alberta

Abstract

Stable isotope dendrochemistry of needles, twigs, and tree rings were used to identify the impacts of a changing global atmosphere in two separate environments with different anthropogenic loads: the boreal forest surrounding the Athabasca Oil Sands region (AOSR), Alberta, Canada, subject to a large point source of anthropogenic emissions, and the relatively isolated alpine environment of Grand Teton National Park (GTNP), Wyoming, USA. In the AOSR, neither $\delta^{13}C$, $\delta^{15}N$, or the C/N ratio in needles and twigs identified the spatial extent of emissions, but tree ring chronologies did highlight shifts in the nitrogen cycle due to increased anthropogenic nitrogen deposition since the beginning of economic development. Alternately, stable isotope geochemistry of tree ring chronologies in the GTNP indicated a greater impact on ecosystem dynamics from increased levels of CO_2 and climate change, reflecting water constraints and increased intrinsic water-use efficiency in trees. The ability of stable isotope dendrochemistry to record both nutrient cycle dynamics and physiological responses to the atmosphere makes it a valuable tool in monitoring the long-term effects of increasing anthropogenic emissions which will result in more pronounced impacts in the future.

Acknowledgements

I would like to thank my supervisor, Dr. Alexander P. Wolfe, for his knowledge and guidance, which has helped shape this dissertation into its current state. Also a big thanks to the field crews at both sites for their assistance. Sarah Spaulding, Megan Otu, and Julia Spencer for a memorable 3-week vacation and introduction to paleoecology in the beautiful Grand Teton National Park. Nathan Ballard and Kayla Stan for some remarkable adventures around northern Alberta, which brought full meaning to the saying 'if it doesn't work the first time, try, try again'.

For their various lab and technical abilities, I would have been sorely amiss without help from several people around campus. Allan Harms in the Natural Resources Analytical Laboratory for isotopic analysis and grinding facilities, Mark Labbe in the Earth and Atmospheric Thin Section Laboratory for making my core holder, Saulo Castro-Contreras for his ArcMap skills, and Steven Mamet for his introduction into tree ring analysis.

On a personal note, I could not have completed this degree without the contributions of my friends and family. My parents, Mark and Janet Mosher, and my family gave me their love and encouragement. Emma Jones brought some much-needed camaraderie to our lonely office, was a willing confidante and had invaluable advice whenever needed. Owen Davison's strength and support was critical in keeping my sanity and he abided my rollercoaster of emotions and, too often, thesis-tracked mind with endless patience.

Finally, I am grateful to all of my friends who have helped make Edmonton home for the past two years: Aleks, Meghan, Jamie, Set, Saulo, Megan, Libby, Liberty, Vanessa, Angus, Blenya, and Frisbeers/Late Night Sneaky Uncle.

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Chapter 1: Introduction

1.1. Global Environmental Change

Within the last two centuries, anthropogenic activities have greatly impacted global biogeochemical cycles, primarily through enhanced greenhouse gas emissions and widespread deployment of synthetic agricultural fertilizers. Atmospheric carbon dioxide has risen from 280 ppm before the industrial revolution to >390 ppm in 2012 (NOAA, 2012). Anthropogenic reactive nitrogen (Nr) production increased from 15 Tg yr^{-1} in 1860 to 160 Tg yr^{-1} in 1990 and now greatly exceeds all natural Nr fixation, estimated to be ~110 Tg N yr^{-1} (Galloway et al., 2008). Global environmental shifts in recent decades are unique within the last 200,000 years, even in regions far removed from proximal anthropogenic influences (Axford et al., 2010). Increases in industrial intensity and agricultural practices associated with the exponential growth of human population result in planetary boundary conditions unlike any before, collectively referred to as the Anthropocene (Crutzen 2002; Steffen et al. 2007). Although the acceleration of global change processes modulated by humanity is increasingly understood, the full scope of human impacts is not fully chronicled, particularly in remote regions.

Beyond the direct addition of vast amounts of CO_2 into the atmosphere from fossil fuel combustion (~9 Gt C yr^{-1}, NOAA 2012), agricultural fertilizers and industrial activities such as smelting and fossil fuel combustion also release large amounts of NO_x, NH_x, SO_x, and other pollutants, mainly as atmospheric emissions. The effect of these emissions is of particular concern as they can have far reaching effects on relatively isolated ecosystems that might otherwise be pristine. Depending on the ecosystem, these emissions can have significant ecological impacts. However, the biogeochemical processes that underpin ecosystem dynamics and their interactions with anthropogenic emissions are sufficiently nuanced between regions that uniform responses are not predicted.

Increasing CO_2 concentrations, for example, may have different regional effects, given that CO_2 also fuels plant growth and may stimulate tree growth. However, high levels of CO_2 in experimental studies have resulted in the exacerbation of nutrient and water limitation (Saurer et al., 2003; Luo et al., 2004). The addition of anthropogenic Nr, often a limiting nutrient, through atmospheric deposition may complement increased CO_2 concentrations and result in increased tree growth. Many nitrogen-limited ecosystems, including alpine and boreal forests, are highly sensitive to external inputs of nitrogen; deposition of NO_x and NH_x can drastically alter nitrogen cycling in soil, reduce species diversity, and ultimately induce ecosystem acidification (Townsend et al., 1996; Holland et al., 1997; Boucher et al., 1998; Rueth and Baron, 2002; Bukata and Kyser, 2007). Furthermore, SO_x and other pollutants associated with combustion and smelting emissions are phytotoxic and can be harmful to the plant if taken in through the stomata. Increased levels of these pollutants in the atmosphere may inhibit growth by constricting stomatal opening or inhibiting carboxylase enzyme activity (Rennenberg and Gessler, 1999; Siegwolf et al., 2001; Savard et al., 2002).

Isotope geochemistry can provide insights into attendant ecological and biogeochemical processes. Sediment, ice, and tree cores are natural archives that can record shifts in environmental geochemistry over time. Such records are critical to benchmark deviations of biogeochemical cycles relative to natural, pre-disturbance, conditions.

1.2. Stable Isotope Dendrochemistry

Stable isotope dendrochemistry has emerged as a potentially powerful proxy in reconstructing deviations of the C and N cycles. Unlike lake sediments and most ice cores, temperate and boreal trees form visible annual growth rings that provide unambiguous chronological control. The main bulk of the tree lies in its trunk and growth occurs from the center outward. Growth is fast during the spring and summer and slows down in the fall and winter. This results in a change in cell growth patterns: early in the growing season (the springwood or early-wood) cells are large and become smaller and more dense as the growing season progresses (the summerwood or latewood). This results in a visible difference between the heartwood of one year and the sapwood of the next.

Carbon and nitrogen are two of the main structural elements of tree tissues and their isotopes of ^{13}C and ^{15}N are commonly used in environmental studies. Nitrogen is present in lignin as well as sap (sugars, resins, and waxes) whereas carbon is present in all plant components. Nitrogen is taken up through the roots as either NO_3^- or NH_4^+, although NH_4^+ is most easily assimilated (Bassirirad et al., 1996). Carbon is taken in as CO_2 through stomata in the leaves or needles. Stable isotope dendrochemistry is based upon the kinetic fractionation of these isotopes through the many metabolic steps until they are incorporated as part of the tree tissue. Different tissue types have different practical purposes in environmental studies. For long-term records, the chronology afforded through tree core and ring analyses is ideal. Needles and new growth twig wood is useful for comparative studies between regions exposed to different environmental stressors or having different edaphic regimes.

Carbon isotopes in tree tissue have been used successfully to monitor both rising CO_2 levels and attendant climate shifts such as drought. The stable isotope dendrochemistry of carbon is based upon kinetic fractionation between the isotopes ^{13}C and ^{12}C during multiple metabolic steps, until they become incorporated as part of the tree tissue (Figure 1.1). In 1982, Francey and Farquhar defined an equation for the $\delta^{13}C$ of photosynthate inside a plant:

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a)(c_i / c_a) \qquad \text{(equation 1)}$$

where the carbon isotope value of the leaf photosynthate ($\delta^{13}C_p$) is based on atmospheric $\delta^{13}C$ ($\delta^{13}C_a$), the fractionation due to carboxylation (b), the fractionation due to diffusion (a), and the ratio of the internal CO_2 pressure (c_i) to the ambient CO_2 pressure (c_a). As *a* and *b* are constants, $\delta^{13}C_p$ is affected by $\delta^{13}C_a$ values and the c_i/c_a ratio, which is controlled by the rate of photosynthesis and the degree of stomatal opening. These last two can change during periods of high temperature, drought, increased pCO_2, or in the presence of phytotoxic emissions, and therefore $\delta^{13}C$ can be used to monitor physiological shifts in trees in response to changing environmental conditions.

Precipitation and temperature influence the c_i/c_a ratio during the incorporation of atmospheric C into the plant, and therefore can also influence the $\delta^{13}C$ of tree tissues (Farquhar et al., 1982; Francey and Farquhar, 1982). Increased temperature and drought conditions have resulted in decreasing $\delta^{13}C$ in some ecosystems (Saurer et al., 1997; Andreu et al., 2008). However, due to the many factors controlling isotopic discrimination against ^{13}C during uptake by the tree, $\delta^{13}C$ is not always easily interpretable as a proxy for climate change alone. Increasing atmospheric CO_2 concentrations and environmental stressors in the form of fossil fuel emissions are starting to influence $\delta^{13}C$ in trees, especially within the last 30 years (Treydte et al. (2009, Battipaglia et al., 2010).

Although climatic studies in dendrochemistry are complicated by the myriad of factors that regulate tree growth, dendrochemistry's use in environmental studies has emerged as a powerful tool to investigate the effects of changing atmospheric composition. CO_2 and pollutants such as NO_x, and SO_x are released to the atmosphere through fossil fuel combustion and industrial processes, whereas increased agriculture and land clearing release NH_4^+ to the environments shifting ecosystem dynamics.

Increased CO_2 concentrations may stimulate plant growth, as CO_2 is the major carbon source that is essential in plant metabolism. The release of isotopically depleted CO_2 from fossil fuel combustion over the last 150 years is reflected through decreased $\delta^{13}C$ detectable in tree cores (February and Stock, 1999; Dongarra and Varrica, 2002; Treydte et al., 2009). This must be taken into account when using $\delta^{13}C$ for paleoclimate reconstruction, through correction for the secular anthropogenic trend in atmospheric $\delta^{13}C$ (Treydte et al., 2009; McCarroll et al., 2009). However, any advantage to plant growth gained by increased CO_2 concentrations may be nullified by the associated exacerbation of nutrient limitation (Saurer et al., 2003). For example, increased atmospheric CO_2 concentrations may result in Nr limitation leading to shifts in soil dynamics that can be registered by decreasing $\delta^{15}N$ of the soil (Luo et al., 2004).

As Nr is limiting in many environments, the nitrogen cycle is sensitive to external changes or additions. The addition of anthropogenic reactive Nr species to ecosystems mainly occurs through atmospheric deposition of NO_x and NH_4^+, or through runoff of agricultural fertilizers contributing nitrogen in the form of NH_4^+ (Galloway et al., 1995). Nitrogen is taken up through the root system from the soil N pool and undergoes little fractionation as it is incorporated into tree tissue (Gebauer et al., 1994), making it an excellent recorder of soil $\delta^{15}N$. However, nitrogen does undergo extensive discrimination during microbial processes within the soil pool providing inherent complexities. The $^{15}N/^{14}N$ ratio of tree tissues can provide valuable insight into the history of changes in the N cycle as well as the role of anthropogenic pollutants in changing environments (Robinson, 2001; Solga, 2006).

The importance of studying the fate of anthropogenic Nr and its effect in ecosystems has been well acknowledged elsewhere (Hart and Classen, 2003; Savard 2010). Yet, due to the complexities of the nitrogen cycle, it is not fully understood how Nr is affecting the dynamics of individual ecosystems. A first step to understanding these changes is an assessment of the magnitude of Nr deposition effects across regions. It is estimated that 50-80% of the NO_x, NH_x, and SO_x that is deposited on land falls on non-agricultural, or natural vegetation (Dentener et al., 2006). N additions may be beneficial to an ecosystem by promoting plant growth. However, in an ecosystem that receives high anthropogenic Nr subsidies, the excess quantity of Nr above that which can be assimilated may alter nutrient dynamics to the point where there are fundamental and long-term changes in elemental cycling processes. The point above which this can occur is defined as the threshold (or critical) Nr load, and these changes have the potential to impact species survivorship (Nillson and Grennfelt, 1988). The global average amount of deposition required for an ecosystem to reach its threshold is estimated to be around 10 kg Nr $ha^{-1}yr^{-1}$ (Galloway et al., 2004; Bobbink et al., 1998). In 2006, Dentener et al. estimated that presently 10% of the natural terrestrial ecosystems receive nitrogen inputs above this amount.

Changing sources of atmospheric Nr are also reflected in the northern hemisphere $\delta^{15}N$ trend. A decline in $\delta^{15}N$ over the last century has been observed in ice from the summit of Greenland (Hastings et al. 2009), as well as in lake core sediments in Western North America (Wolfe et al. 2001) and elsewhere (Holtgrieve et al. 2011). Moreover, these trends have accelerated in the last 30 to 50 years, and furthermore have been captured by tree ring dendrochemical chronologies. Poulson et al. (1995) found a significant decrease in $\delta^{15}N$ values in eastern hemlock (*Tsuga canadensis*) within the last 30 years and Bukata and Kyser (2007) found a decrease of 1‰ in red and white oak (*Quercus rubra* and *Quercus alba*) cores in western Ontario since 1945. This decreasing trend in $\delta^{15}N$ values has been mostly attributed to less enriched atmospheric $\delta^{15}N$ values influenced by an increase in fossil fuel emissions. Fossil fuels tend to have $\delta^{15}N$ values around 0‰, similar to that of natural atmospheric N. However the $\delta^{15}N$ values of fossil fuel emissions can vary depending on combustion practices and source materials. Heaton (1990) found relatively depleted $\delta^{15}N$ values ranging from -13‰ to -2‰, whereas

Ammann et al. (1999) found positive values, as high as +5.7‰ adjacent to a motorway in Switzerland. Spatially, a decreasing trend in $\delta^{15}N$ has been found along rural-urban gradients: it has been documented from trees, soil, and mosses that the most depleted $\delta^{15}N$ values are proximal to urban areas, directly reflecting the effect of fossil fuel emissions (Liu et al., 2008; Kuang et al. 2011).

Stable isotope dendrochemistry can be applied to trees in a number of fashions. Comparative analyses can be undertaken on roots, the wood of the trunk, the leaves, or newly grown twigs. A project's intention determines the appropriate sample selection. Modern studies involving the effects of pollution or other treatments on growth in comparison to a control can be performed on leaves or newly grown twigs. For environmental reconstruction studies, chronologies determined through a series of tree rings are the most applicable method as they provide a long record of information in one particular locality (Figure 1.2).

The most time-consuming aspect of dendrochemistry is in the treatment of samples prior to isotope analysis. Wood is composed mainly cellulose and lignin (classified as the woody tissue) and mobile components (resins, waxes, sugars) containing C, H, O, and N. These mobile components can move laterally through the tree, potentially cross-contaminating years; therefore, they should be removed prior to isotopic measurements (Mead and Preston, 1994; Sheppard and Thompson, 2000). Cellulose is the most stable component of the wood, highly resistant to decomposition, and with stable elemental assimilation rates; it consists of C, H, and O. Lignin, a compound consisting of C,H,O, and N, is more labile than cellulose. Therefore, for C stable isotope determinations it is advised to analyze only cellulose and to analyze lignin-cellulose for N stable isotopes (Loader et al., 2003; Gaudinski et al., 2005; Savard, 2010). However, in many environmental studies, the additional extraction steps for cellulose are seen as unnecessary and it remains debated whether lignin-cellulose or even whole wood is adequate for analysis (Hart and Classen, 2003; Saurer et al., 2004; Ferrio and Voltas, 2005; Bukata and Kyser, 2007).

1.3. Thesis Organization

This thesis presents dendrochemical analyses of recent biogeochemical changes from two regions with contrasting biogeochemical regimes and levels of anthropogenic influence. The sites selected include one proximal to the Athabasca Oil Sands region (AOSR, Alberta, Canada), a large source of anthropogenic emissions. The second study is from the Grand Teton National Park (GTNP, Wyoming, USA), more than 160 km from industrial anthropogenic activities. The two very different anthropogenic and environmental context will allow a thorough examination of how different ecosystems are reacting to global environment change.

Chapter 2 focuses on the effects of anthropogenic emissions in the AOSR. Specifically, this chapter focuses on the signature of enhanced Nr deposition in the surrounding boreal forest over the last two decades. Needles and new growth twigs were analyzed to study the spatial extent of emissions and whether there is any relationship between $\delta^{13}C$, $\delta^{15}N$, and C/N ratios and wind direction or distance from processing facilities. A chronological study was performed on three tree cores to observe shifts within the local nutrient cycles throughout the economic development and expansion of the region.

Chapter 3 explores tree environmental and physiological shifts over the last several millennia in a remote, alpine environment situated in Grand Teton National Park. In this study, the effects of atmospheric Nr deposition as well influences associated with climate change are explored using tree cores collected from alpine sites near treeline, which are characteristically sensitive to shifts in nutrient supply.

Using stable isotopes of C and N from tree tissue I attempt to identify fingerprints of anthropogenic impacts on both ecosystems, grounded in the understanding of isotopic processes governed by plant physiology (Figure 1.2). I hope to identify and answer the following questions.

a) Are tree rings recording ecosystem-scale shifts in nitrogen cycling through their $\delta^{15}N$ signatures, and, if so, are the shifts in the nitrogen cycle reflected in the $\delta^{13}C$ as well?
b) Is there evidence of anthropogenic Nr deposition in the relatively isolated and protected GTNP?
c) What is the evidence of disruption in the N and C cycles from pollution associated with energy development in the AOSR and, if so, what is their spatial extent?
d) What are the dominant influences governing ecological shifts in both regions?

This thesis provides a expanded appreciation of recent biogeochemical changes as observed in two contrasting forests of northwestern North America. The study furthermore illuminates the potential and limitations of stable isotope dendrochemistry in paleoenvironmental studies.

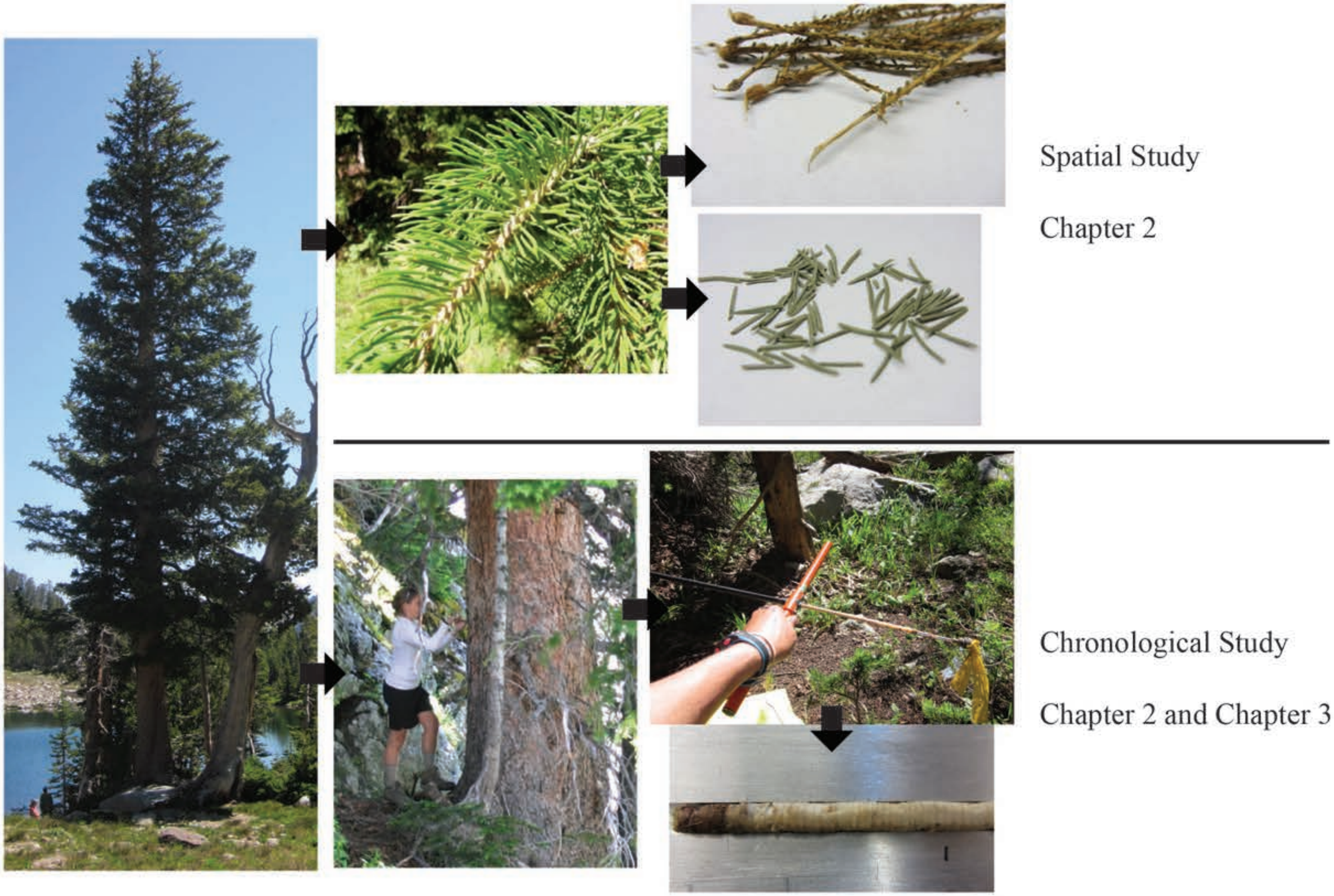

Figure 1.1. Tree tissue applicability in environmental studies.

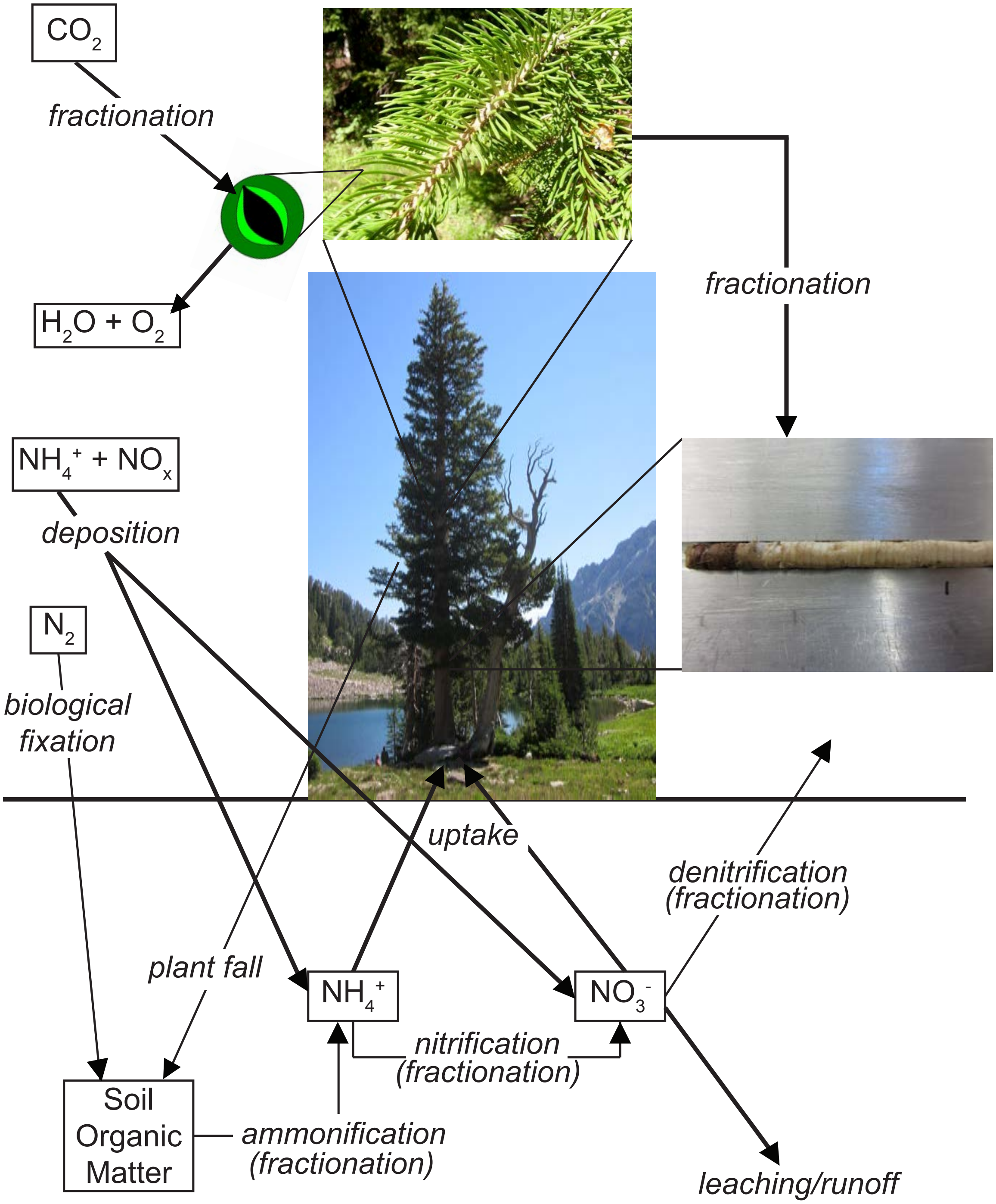

Figure 1.2. Biogeochemical flow chart between nitrogen (N) and carbon (C), and a Black Spruce (*Picea mariana*) tree. Bolded arrows represent relationships that dominate an alpine environment.

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Chapter 2: Stable Isotope Dendrochemistry of the Athabasca Oil Sands Region

2.1. Introduction

The Athabasca Oil Sands development in northeastern Alberta, Canada, is one of the largest mining operations on the planet. Through the extraction and processing of bitumen, an estimated 350 tons of emissions (NPRI, 2011) are released daily into the atmosphere and the surrounding boreal forest ecosystem. Of particular concern is the effect of the addition of reactive nitrogen (Nr) species (NO_x, NH_x) into the surrounding boreal forest as it may alter community structure and function (Aber et al., 1989; Zottl, 1990; Aber et al., 1998; Fenn et al., 1998). The addition of nitrogen may disrupt nutrient cycling, resulting in a variety of consequences including ecosystem acidification and reduced biodiversity (Galloway et al., 2004; 2008).

The Athabasca Oil Sands Region (AOSR) is a 420 000 km^2 deposit of bitumen, sandstone, and water with 663 km^2 currently disturbed by mining operations (Government of Alberta, 2010). The hydrocarbon is recovered by surface mining (55% of extraction) or by *in situ* steam injection (45% of extraction), followed by processing for transport prior to upgrading (Government of Alberta, 2010). This international hub produces a remarkable 1.49 million barrels of oil per day, up from 482,000 barrels per day 10 years ago, and will increase to around 3.2 million barrels per day in 2019 (Government of Alberta 2010). AOSR activity releases excessive emissions that result in an estimated 12 kg N ha^{-1} yr^{-1} to 24 N kg ha^{-1} yr^{-1} of atmospheric deposition (Laxton et al., 2011; Hemsley, 2012). Boreal forests have a critical load of approximately 6 kg ha^{-1} yr^{-1} (Nordin et al., 2005; Whitfield et al., 2010), beyond which ecological and stoichiometric equilibria begin to shift, a condition known as nitrogen saturation. This level is greatly exceeded by all estimates of atmospheric deposition in the AOSR.

In January 2012, the Canadian Government approved additional AOSR projects to come on-line in the next 5 years. With such an increase in activity, it is imperative that the effects of emissions become thoroughly understood. Measured nitrogen deposition in the AOSR, either wet or dry, is composed mainly of NH_3, however there is significant interaction between NO_3^- and NH_4^+ and the tree canopy, greatly affecting soil nitrogen dynamics (Hemsley, 2012). Lake, soil and foliar analyses are inconclusive with respect to shifts in ecosystem dynamics for the surrounding and down-wind areas despite measurable amounts of nitrogen deposition into these regions (Hazewinkel et al., 2008; Laxton et al., 2010; Hemsley, 2012; Wiklund et al., 2012). Although 60% of the deposition is thought to remain within a 50 km radius of the region (Kelly et al., 2009), it remains unknown how far the influence of oil sands emissions may be transmitted. Unfortunately, long-term records of atmospheric nitrogen and soil nitrogen dynamics are non-existent; dendrochemical studies may provide a powerful proxy tool to decode recent changes in the nitrogen cycle.

The use of clearly defined annual growth rings in tree cores combined with stable isotope geochemistry of wood tissue components and modern plant tissue in the form of needles and twigs can provide a natural chronology of nitrogen changes in the ecosystem. C/N, $\delta^{15}N$, and $\delta^{13}C$ in tree tissue can provide insight into disruptions of nutrient cycles in the surrounding ecosystem as well as any physiological shifts in the plant itself.

Dendrochemistry has been used successfully to monitor the impacts of anthropogenic pollution related to motorway emissions (Ammann et al., 1999; Saurer et al., 2004), smelting activity (Savard, 2002), and urban pollution (Bukata and Kyser, 2007; Powers and Collins, 2010).

The objectives of this study are to (A) explore the spatial distribution of carbon and nitrogen stable isotopes from modern tissues (needles and twig lignin + cellulose) of Black Spruce (*Picea mariana*) around the Athabasca Oil Sands, and (B) reconstruct time-series of these same parameters from tree-rings in order to assess ecological responses to industrial emissions in space and time. Dendrochemical chronologies paired with a spatial study of newly grown branches and needles will provide us with both a temporal and spatial understanding of anthropogenic nitrogen deposition in the Athabasca region.

2.2. Methods

2.2.1. Site description

The Athabasca Oil Sands region is part of the Regional Municipality of Wood Buffalo (population of ~104, 000) that includes the mining camps and the Urban Service Area of Fort McMurray (population of ~77, 000). The region is a boreal forest ecosystem with a mean annual temperature of +0.5°C. Winter mean temperature is -18.8 °C. The relatively short summer growing season has a mean temperature of +16.8 °C and receives the majority of the 455.5 mm mean annual precipitation (Environment Canada, 2012).

2.2.2. Sample collection

Black spruce needles and twigs were collected along transects developed by Kelly et al. (2009, 2010) during water and snow sampling along the Athabasca River and its tributary streams. New growth twigs and needles were collected from healthy Black Spruce (*Picea mariana*) in the summer of 2008 at sites around the Athabasca Oil Sands along the Athabasca River watershed (see Kelly et al. (2009) for site locations). The needles were separated from the twigs, placed in separate bags and frozen until further analysis.

Tree cores were sampled during July 2011 around the Athabasca Oil Sands (Figure 2.1). Cores were collected from Black Spruce (*Picea mariana*) using a 5.6 mm increment borer (SUUNTO, IML-Instrumenta Mechanik Labor Systme GmbH, Wiesloch, Germany) at breast height at least 100 m from the road. Of the 14 cores collected, three were selected for detailed isotopic analyses based on their age (older cores were preferred), the quality of rings, and their distal proximity to each other. One of these cores was collected adjacent to the open pit mining Aurora project (FM1311; N 57°17’49.9” W 111°31’49.6”), the second was taken 15 km NW (FM911; N 57°24’26.4” W 111°37’24.9”) of the Aurora project, and the third 30 km SW (FM411; N 57°12’46.6” W 111°02’07.8”).

2.2.3. Sample preparation and extraction

In September 2010, the bark and vascular cambium of the twigs was stripped to reveal the inside woody tissue. A portion of the wood tissue was cut and ground into chips approximately 1-2 mm squared in an agate mortar and pestle. The wood chips and the needles were then sealed in labeled filter pouches (F57, Ankom Technology, Macedon, New York, USA) for solvent extraction.

Tree cores were dated and ring widths measured using a stereomicroscope and Velmex slide stage micrometer with a digital encoder (Velmex Inc., Bloomfield, New York, USA). We used a stainless steel core holder with a 6 mm groove for dating without risking isotope contamination associated with conventional wood and glue core mounting methods. The cores fit snugly into the groove and a thin surficial layer of wood was shaved from the top with a steel scalpel to facilitate ring viewing. After the cores were dated and ring widths measured, they were separated into 3-year intervals, sliced into 1-2 mm sized pieces and sealed in labeled filter pouches (F57, Ankom Technology, Macedon, New York, USA).

In order to remove labile components from wood (polysaccharides and terpenoids) that may translocate laterally, potentially contaminating the geochemical signature, samples underwent extraction to isolate the lignin + cellulose fraction. Further extraction to beta-cellulose for carbon analyses was explored in a subset twig lignin + cellulose samples following a modified procedure by Loader et al. (1997). This extraction yielded $\delta^{13}C$ values with no significant difference from those of lignin + cellulose (Figure 2.2). A comparison of whole needles to needles extracted for lignin + cellulose also showed no significant difference in their $\delta^{13}C$ and $\delta^{15}N$ values; further needle analysis was performed on extracted needles.

Extraction followed a slightly modified version of the three step procedures outlined in Saurer et al. (2004) and Shepherd and Thompson (2000). Samples were first exposed to a 1:1 mixture of toluene and ethanol for 18 hours in a soxhlet apparatus.

Samples were thoroughly rinsed in deionized water and air dried for 2-3 hours before being exposed to 95% ethanol for 18 hours. Samples were rinsed again in deionized water before being soaked in boiling deionized water for 6 hours. Samples were dried overnight at 75°C and ground to <2 mm before isotope analysis.

2.2.4. Mass spectrometry

The $\delta^{15}N$ and $\delta^{13}C$ natural abundance and total %N and total %C analyses were conducted using a Costech ECS 4010 CHNS-O Elemental Combustion System (Costech Analytical Technologies Inc., Valencia, CA) coupled to a Conflo III interface and a continuous flow Finnigan Delta Plus Avantage IRMS (ThermoFinnigan, Bremen, Germany). All $\delta^{15}N$ values are expressed using the delta (δ) notation in per mille (‰) relative to atmospheric N_2,

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] * 1000$$

where R_{sample} and $R_{standard}$ are the $^{15}N/^{14}N$ ratios of the sample and atmospheric N_2, respectively. All $\delta^{13}C$ values are expressed using the delta (δ) notation in per mille (‰) on the international VPDB (Vienna Pee Dee Belemnite) scale for $\delta^{13}C$,

$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1] * 1000$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and VPBD C, respectively. NIST Standard Reference Materials (NBS22, LSVEC, NBS19 for ^{13}C and IAEA-N1, IAEA-N2, IAEA-N3 for ^{15}N) were used to calibrate and standardize the instrument with errors of ±0.05‰ for $\delta^{13}C$ and ±0.31‰ for $\delta^{15}N$.

2.2.5. Data processing and statistical analysis

The carbon isotopic values were corrected to remove the influence of decreasing atmospheric carbon $\delta^{13}C$ since the industrial revolution, also known as the Suess effect. Fossil fuel emissions have resulted in a decrease of approximately 1‰ in atmospheric $\delta^{13}C$ values. If not corrected, this atmospheric change in $\delta^{13}C$ may mask any physiological trends. Therefore, $\delta^{13}C$ values were corrected using the equation from McCarroll et al. (2009),

$$\delta^{13}C_{cor} = \delta^{13}C_{plant} - (\delta^{13}C_{atm} + 6.4) \qquad \text{(eqn. 1)}$$

where $\delta^{13}C_{plant}$ is the carbon isotopic signature of the tree ring in the year being corrected, $\delta^{13}C_{atm}$ is the carbon isotopic signature of the atmosphere in the year being corrected and -6.4 is an estimate for pre-industrial atmospheric $\delta^{13}C$. The pre-industrial estimate and the $\delta^{13}C_{atm}$ values are available for the last 150 years in McCarroll and Loader (2004) and were compiled from ice core records (Francey et al., 1999; Robertson et al., 2001) with more recent values from the NOAA (2012).

The carbon to nitrogen molar ratio was calculated from the percent nitrogen and percent carbon values. Descriptive statistics were performed on all data using the graphic and statistical program R (version 2.12.1, The R Foundation for Statistical Computing), including Pearson's correlations between individual time-series, and Student's *t*-tests to assess differences in the twig samples extracted for lignin and the twig samples extracted for cellulose.

2.3. Results

2.3.1. Isotopic variations between tissue types: needles, twigs, and modern tree rings

There are clear differences in nitrogen and carbon content and stable isotopes between the three different tissue types: needles, twigs, and tree rings (Table 2.1). Of all the measured variables ($\delta^{15}N$, $\delta^{13}C$, C/N, %N, and %C), $\delta^{15}N$ values showed the greatest variability between tissue types. Needle samples show the greatest variance in $\delta^{15}N$, followed by new growth twig samples, and finally modern tree ring samples. This suggests that trunk wood has the greatest consistency when assimilating carbon and nitrogen. The C/N ratio and the percent nitrogen values also showed large variability for all three tissue types. Among the three tissue types, twigs showed the greatest variability in $\delta^{13}C$, C/N, and %N values, suggesting that plant growth and nutrient assimilation is quite variable during the early stages of wood growth. Needle samples showed the greatest variability in $\delta^{15}N$ values, which may relate to spatial patterns within the soil nitrogen pool, variable stomatal nitrogen uptake, and within-canopy effects. It is surmised that trunk wood (*i.e.*, the year zero tree-ring at the time of sampling) provides the most conservative and, likely integrative, target for the application of stable isotope techniques in Black Spruce, validating applications of isotope dendrochemistry. On the other hand, twig and needle isotopic values more closely reflect localized processes and conditions. Both types of data are potentially of importance in assessing the fingerprint of atmospheric deposition.

2.3.2. Spatial patterns of needle and new-growth twigs

No obvious spatial trend was observed for $\delta^{13}C$ (Figure 2.3) and $\delta^{15}N$ (Figure 2.4) in needles or new-growth twigs in the AOSR based on geography relative to proximity of the nearest processing and extraction facility. There was large variation found in $\delta^{15}N$, $\delta^{13}C$, and C/N values in needles and twigs, but little evidence for trends with distance to processing facilities (Figures 2.5 and 2.6; Table 2.2). There appears to be little consistency in the spatial distribution of values relating to the oil sand developments at all, with sites having enriched isotopic values located proximal to sites with depleted isotopic values. Furthermore, there was no overall relationship between N and carbon isotopic ratios in twigs or needles (Figure 2.7; Table 2.2), implying some degree of decoupling of these elemental cycles in this highly polluted environment. Ultimately, this limits the ability to ascertain the isotopic signature of industrial pollution in twig and new-growth tissues from Black Spruce.

2.3.3. Temporal trends from dendrochemical time-series

The dendrochemical chronologies reveal an increase in total N content in tree core lignin + cellulose (p=0.014, df=19, r^2=0.27; Figure 2.8). This increase appears to begin around 1975, after which all three cores show a steady and coherent increase in nitrogen content. This inflection corresponds closely to the initial expansion of development in the oil sands and is consistent with increased nitrogen deposition in the area due to this activity.

The carbon content value of the tree rings remains fairly consistent over time with only a couple of extreme values from two separate rings in two separate trees (p=0.2415, df=19, r^2=0.033; Figure 2.9). There are slight oscillations in all tree cores, mostly likely due to environmental or climatic variables. The carbon content does however start to vary amongst tree cores within the last 8 years, suggesting variation in plant physiology due to ecosystem disturbances.

A commensurate declining trend was observed in the C/N molar ratio of tree rings, by about 500 units (mole/mole) since the early 1980s (p=0.032, df=19, r^2=0.19; Figure 2.10). This decline is driven by increases in nitrogen content, and is most likely the result of increased nitrogen assimilation. Given the consistency of trends among trees, the declining C/N ratio provides direct evidence of ecological shifts in the nutrient balance.

The δ^{15}N record shows a significant increasing trend (p-value=0.0012, df=19, r^2=0.44; Figure 2.11) over time, rising from an average of -5.17 ± 1.11‰ in 1970-1972 to -3.29 ± 0.32‰ in 2006-2008. This change in isotopic signature may suggest the assimilation of nitrogen from a different source than natural background Nr.

The averaged values for C/N, percent N, and δ^{15}N show a clearer picture of the changes over time (Figure 2.12). All three trends show a significant change over time beginning in the mid 1970s. This corresponds to a few years after the initial oil sand project began (Suncor's Mildred Lake in 1967) and a few years before the opening of Syncrude's Millenium project, during which construction and development in the area would be underway and the environment would be experiencing significant perturbations. These three trends provide strong evidence of changes in the nitrogen cycle within the last 30 years for this region in response to oil sands development.

The corrected carbon isotopic signature shows a convoluted trend over time. Two of the cores show increases over time; however only one of these is significant (*p*=0.056, df=19, r^2=0.15; Figure 2.13). The third core shows an insignificant decreasing trend. However, this core is less than 30 years old and does not provide a sufficiently lengthy record to allow further inference.

2.4. Discussion

2.4.1. Spatial distribution of Black Spruce tissue stable isotopic values

2.4.1.1. Heterogeneity

No spatial trend was obvious in our data, despite evidence of greater deposition of Oil Sand emissions nearer the processing facilities existing in both pine needles and snow cover studies (Kelly et al., 2009; Proemse, 2012). There was no relationship between the geochemical data in needles and twigs, and distance from the processing facilities. The variability in our spatial data shows large differences between values of neighboring sites with no correlation to distance or direction from processing facilities. The variability in our data suggests that there is large heterogeneity in either soil microbial processes or in the physiological response of trees to anthropogenic pollution in the AOSR.

2.4.1.2. Soil nitrogen availability

The spatial variability in isotopic data from tree tissues may be due to heterogeneity in soil nitrogen values. $\delta^{15}N$ in trees reflect the isotopic composition of mineral forms of the soil N pool (Amundson et al., 2003). Hemsley (2012) reported highly heterogeneous soil N composition in the AOSR. Although significant relationships were not observed, the amount of soil inorganic Nr is, for the most part, proportional to wet N deposition. An increasing flux of Nr to the ecosystem may result in the acceleration of the N cycle, ultimately increasing Nr availability (Gunderson, 1991; Fang et al., 2011), and potentially resulting in highly variable isotopic values. Therefore, our data reflects either variable N deposition in relation to atmospheric composition surrounding the region, or variable rates of Nr deposition to local soils.

Our $\delta^{13}C$ values also show great variability. As trees obtain C from atmospheric CO_2, a variable $\delta^{13}C$ may reflect varying concentrations of emissions in the atmosphere. However, $\delta^{13}C$ may also reflect physiological shifts in the tree in response to local changes in available soil N due to different amounts of atmospheric N depositions. Although Hemsley (2012) identified elevated Nr deposition regionally, between 12 to 24 kg N ha^{-1} yr^{-1}, the standard deviation of these values is also large, in the order of ±20 kg N ha^{-1} yr^{-1}. Variability in Nr deposition will cause variable microbial responses and ultimately a range of available N species in the soil pool. The magnitude of deposition is greatly affected by canopy cover (Friedland et al., 1991; Fenn et al., 2003; Nowak et al., 2004; Hemsley, 2012), which is probably strongly impacting deposition rates in the AOSR. Therefore the lack of spatial trend present in our data may be caused by differing deposition rates due to differences in the canopy cover.

2.4.1.3. Physiological shifts

The highly variable spatial distribution of needle and new-growth twig stable isotopic values may alternately be the result of shifting tree physiology. The $\delta^{13}C$ trend suggests that the physiological processes of the tree are different throughout the region in regards to carbon uptake and discrimination. Therefore, the trees may also be altering their physiological functioning in regards to Nr uptake, namely through an increase in foliar nitrogen uptake. Trees generally take up nitrogen from the soil nitrogen pool through their root system, preferentially as NH_4^+. However, stomatal uptake of NO_x has been found to occur in highly polluted environments (Gebauer and Schulze, 1991). Up to 25% of total foliar nitrogen can be sourced from NO_x in environments proximal to large sources of anthropogenic emissions (Ammann et al., 1999; Saurer et al., 2004; Fang et al., 2011). In the AOSR, Nr deposition is greater in areas with reduced canopy cover (Hemsley, 2012), which support the importance of foliar Nr uptake. Increased foliar uptake of Nr results in reduced uptake by the root system (Rennenberg and Gessler, 1999).

N deposition in the AOSR consists mainly of NH_4^+ with an average $\delta^{15}N$ of 9.6±1.2‰ (Hemsley, 2012). Atmospheric NO_x, which is less prevalent in N deposition, has a much lower $\delta^{15}N$ of -1.8±2.7‰ (Hemsley, 2012). Modern average needle $\delta^{15}N$ (-3.11±2.05‰) is lower than year-zero tree ring $\delta^{15}N$ (-2.17±2.46‰). Thus, needles appear to be exploiting lower $\delta^{15}N$ sources, such as atmospheric NO_x, while tree rings are incorporating N with a higher $\delta^{15}N$, such as NH_4^+ deposition. Although this is mainly speculative, variable apportionment of available Nr species between foliage and roots can help explain the spatial array and between-tissue $\delta^{15}N$ trends in this study.

2.4.1.4. Cause of variability

In this environment, with such pervasive anthropogenic influence, more than one factor is likely causing the variable spatial trends presented in this study and others from the region (Laxton et al., 2010; Proemse, 2012). Complexities in the N cycle combined with differences in microhabitats are resulting in variable atmospheric deposition and stomatal uptake of Nr. Yet it remains unknown what the exact mechanisms are that modulate the apparent variability of needle and twig geochemistry. Our spatial data for $\delta^{15}N$, $\delta^{13}C$, and C/N suggest that this variability is not restricted to either atmospheric Nr composition or physiological responses of forest trees, but rather a complex interaction of factors.

2.4.2. Chronological shifts in tree-ring stable isotope geochemistry

2.4.2.1. Nitrogen contents and $\delta^{15}N$

Both nitrogen content and $\delta^{15}N$ show similar increases over time beginning around 1975. The increasing trend of lignin + cellulose N and decreasing C/N suggest a disruption in the nitrogen cycle in the AOSR. The C/N decline likely reflects an acceleration of N cycling resulting from increases soil N availability (Gundersen, 1991; Fang et al., 2011). Such a disruption can be related directly to atmospheric Nr deposition, as seen in other regions (Tietema et al., 1998; Rueth and Baron, 2002; Power and Collins, 2010). Indeed, similar trends have been observed worldwide in dendrochemical studies (Jung et al., 1997; Ammann et al., 1999; Hietz et al., 2010; Powers and Collins, 2010). The paralleled increase in $\delta^{15}N$ over time is indicative that the increase in nitrogen is coming from a new, external source, increasing the baseline

$\delta^{15}N$ of the ecosystem. In the tree cores, $\delta^{15}N$ increases from -5.17 ± 1.11‰ in 1970-1972 to -3.29 ± 0.32‰ in 2006-2008. This increase of approximately 2‰ over 36 years suggests that the nitrogen deposition occurring has a $\delta^{15}N$ greater than the background signature of ~-5‰.

2.4.2.2. Anthropogenic inputs into the AOSR nitrogen cycle

The reduced C/N ratio and increasing $\delta^{15}N$ suggests an input of anthropogenic N into ecosystem N cycling. How much of this Nr is being incorporated into plant tissues can inform to what degree the N cycle is currently influenced by anthropogenic Nr from atmospheric deposition. An analysis of aerosol $PM_{2.5}$ from a smoke stack of one of the primary processing plants reveals highly enriched $\delta^{15}N$ values (Proemse, 2012) and, in the same study, lichen $\delta^{15}N$ suggests a source value for atmospherically-derived Nr in the order of 20‰. Hemsley (2012) found NH_4^+, the dominant form of Nr in deposition, to have a $\delta^{15}N$ around 9‰. Using both of these $\delta^{15}N$ values, 20‰ and 9‰, as representative for Oil Sands Nr emissions, and -5‰ as the historical background $\delta^{15}N$ (taken from before 1960 in the tree core records) of the ecosystems surrounding the AOSR, we can obtain a rough estimate the percentage of anthropogenic Nr used by the trees by developing a two-component isotope mixing calculations (Phillips and Gregg, 2001):

$$\delta^{15}N_{tree} = (f_{nat} \cdot \delta^{15}N_{nat}) + (f_{anthro} \cdot \delta^{15}N_{anthro}) \qquad \text{(eqn. 1)}$$

$$f_{nat} + f_{anthro} = 1 \qquad \text{(eqn. 2)}$$

The $\delta^{15}N$ of the tree is therefore represented by the contribution of background ($\delta^{15}N_{nat}$) and Oil Sands ($\delta^{15}N_{anthro}$) nitrogen based on their respective fractions (f_{nat} and f_{anthro}) in the plant's δ^{15}N. Using this approach, we estimate that during 2008-2011, assuming AOSR emissions having a δ^{15}N of 20‰, approximately 12% of the Nr assimilated by the tree originated from industrial activity. Using an end-member $\delta^{15}N_{anthro}$ value of 9‰ this amount rises to 20% (Table 2.3). Hemsley's (2012) δ^{15}N value reflects Nr deposited onto soil and thus seems realistic for Nr assimilated through the root system. As demonstrably there is foliar Nr uptake occurring at the same time, this is likely an overestimate. Alternately, the δ^{15}N value from Proemse (2012), derived from lichens that obtain Nr solely from the atmosphere by foliar uptake, is likely an underestimate. These values are simple estimates, and the actual value is probably intermediate, on the order of 15% N_{anthro}. In a nutrient limited boreal forest, a contribution of 15% into the tree's N budget represents a significant impact and is evidence of the magnitude of disruption in the N cycle.

2.4.3. Implications

This study provides evidence that anthropogenic Nr deposition affects the forest ecosystem surrounding the AOSR and possibly induces physiological shifts in tree and soil N cycling. However, natural archives have to this point not chronicled the impacts of this deposition. Hazewinkel et al. (2009) and Curtis et al. (2010) both failed to observe evidence of acidification in lake-sediment diatom assemblages, although shifts owed to other processes are clearly evident. Similarly, lake sediments fail to record anthropogenic influence in the Peace River watershed, 200 km away (Wiklund et al., 2012). Unfortunately, trees used in this study were too young for representative tree-ring growth analyses, which may indicate whether trees are reacting positively or negatively to anthropogenic Nr emissions. Nonetheless, the tree-ring chronologies reveal a significant increase in C/N and $\delta^{15}N$, evidencing significant shifts in the regional N cycle, beginning in the mid 1970s, but with the greatest inflections occurring in the last decade. This suggests that Nr deposition from the AOSR is only beginning to alter ecosystem dynamics regionally. It is thus possible that the previous studies simply were conducted too early to register a significant ecological impact. The predicted increase in production from the AOSR in the next decade will unquestionably exacerbate the trends identified herein, with consequences for the structure and function of the entire ecosystem, potentially over areas of thousands of km^2 or more.

Table 2.2. Statistical values for needle and twig $\delta^{15}N$, $\delta^{13}C$, and C/N over distance to nearest processing facility from Black Spruce (*Picea mariana*) collected around the Athabasca Oil Sands region, Alberta, Canada.

	p-value	df	r^2
Needles			
$\delta^{15}N$ over distance	0.019	28	0.16
$\delta^{13}C$ over distance	0.069	28	0.088
C/N over distance	0.26	28	0.012
$\delta^{15}N$ vs. $\delta^{13}C$	0.27	28	0.0096
Twigs			
$\delta^{15}N$ over distance	0.46	28	-0.016
$\delta^{13}C$ over distance	0.49	28	-0.019
C/N over distance	0.43	28	-0.013
$\delta^{15}N$ vs. $\delta^{13}C$	0.19	28	0.022

Table 2.1. Average needle, new growth twig, and modern tree ring values for δ^{15}N, δ^{15}N, C/N, %C, and %N with standard deviation from Black Spruce (*Picea mariana*) collected around the Athabasca Oil Sands region, Alberta, Canada.

	Needles	New Growth Twigs	Tree Ring
δ^{13}C (‰)	-28.38 ± 1.29	- 23.82 ± 1.79	-25.95 ± 2.23
δ^{15}N (‰)	-3.11 ± 2.05	-4.36 ± 2.07	-2.17 ± 2.46
C/N	37.48 ± 5.48	137.35 ± 30.04	1011.24 ± 144.68
%C	46.83 ± 0.70	45.02 ± 1.64	46.87 ± 0.04
%N	1.49 ± 0.24	0.40 ± 0.11	0.055 ± 0.0079

Table 2.3. Estimates of anthropogenic Nr input into Black Spruce (*Picea mariana*) tissue around the Athabasca Oil Sands region, Alberta, Canada, using anthropogenic $\delta^{15}N$ values from Hemsley (2012) and Proemse (2012).

	$\delta^{15}N_{tree}$ (‰)	$\delta^{15}N_{nat}$ (‰)	$\delta^{15}N_{anthro}$ (‰)	f_{nat}	f_{anthro}
Proemse, 2012	-2.17	-5	20	0.88	0.12
Hemsley, 2012	-2.17	-5	9	0.80	0.20

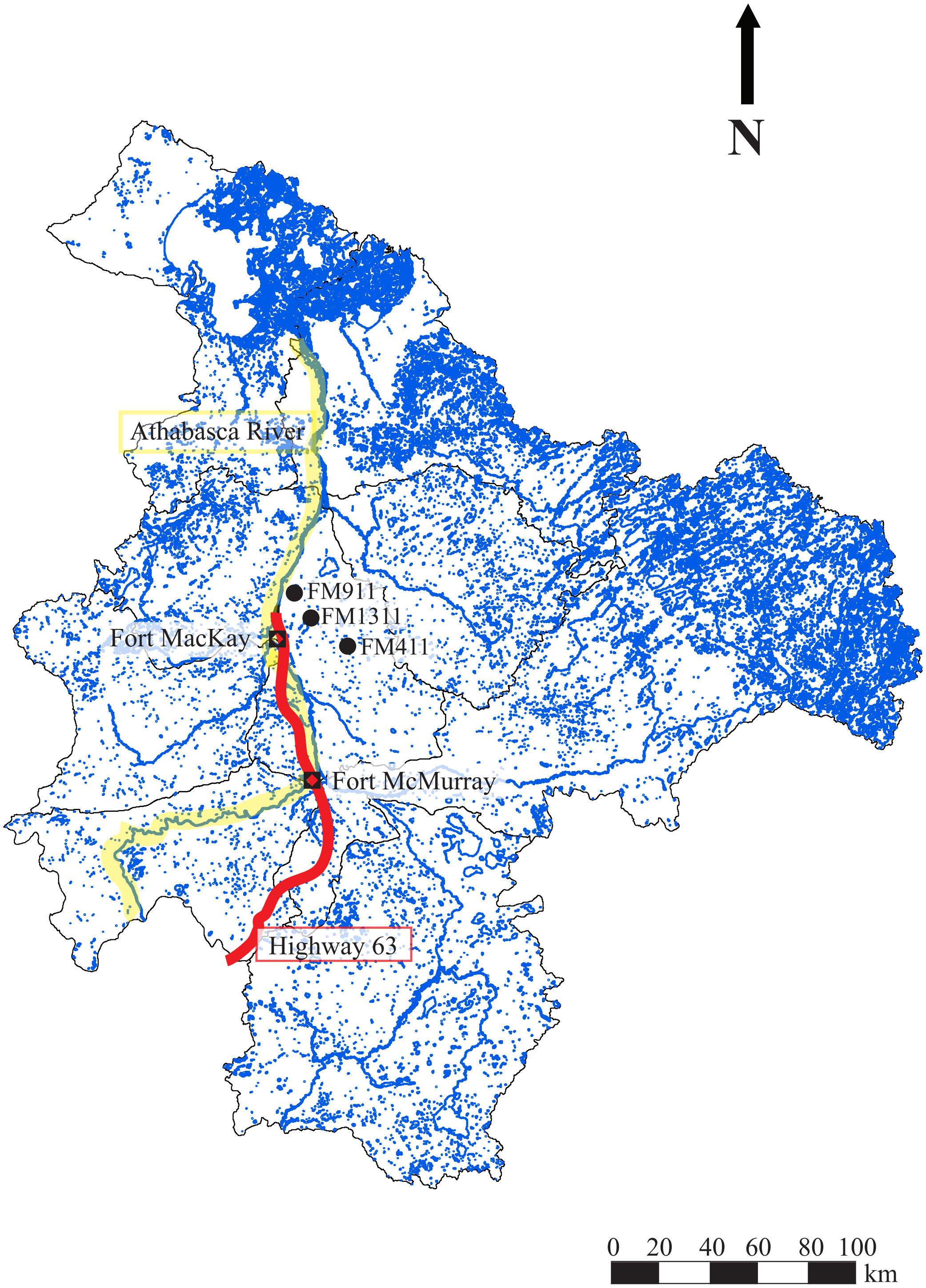

Figure 2.1. Site map for the three cores collected Black Spruce (*Picea mariana*) around the Athabasca Oil Sands region.

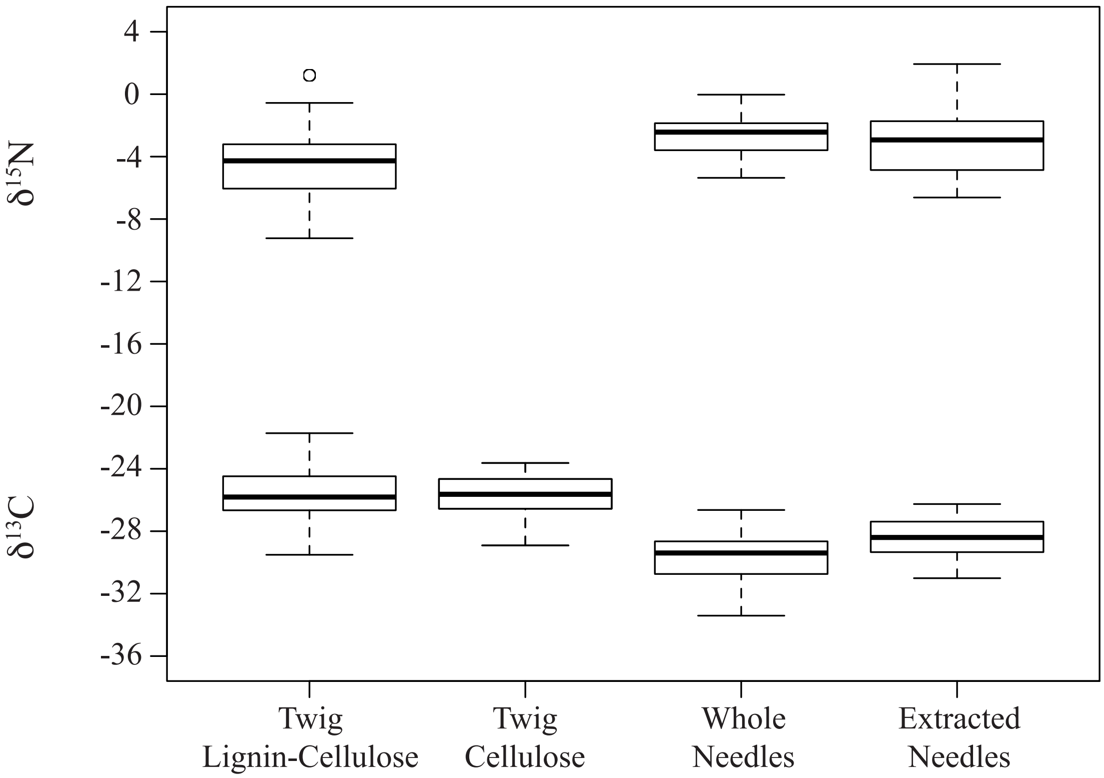

Figure 2.2. $\delta^{15}N$ and $\delta^{13}C$ for twig lignin+cellulose, twig cellulose, whole needles and needle cellulose from Black spruce (*Picea mariana*) around the Athabasca Oil Sands region, Alberta, Canada.

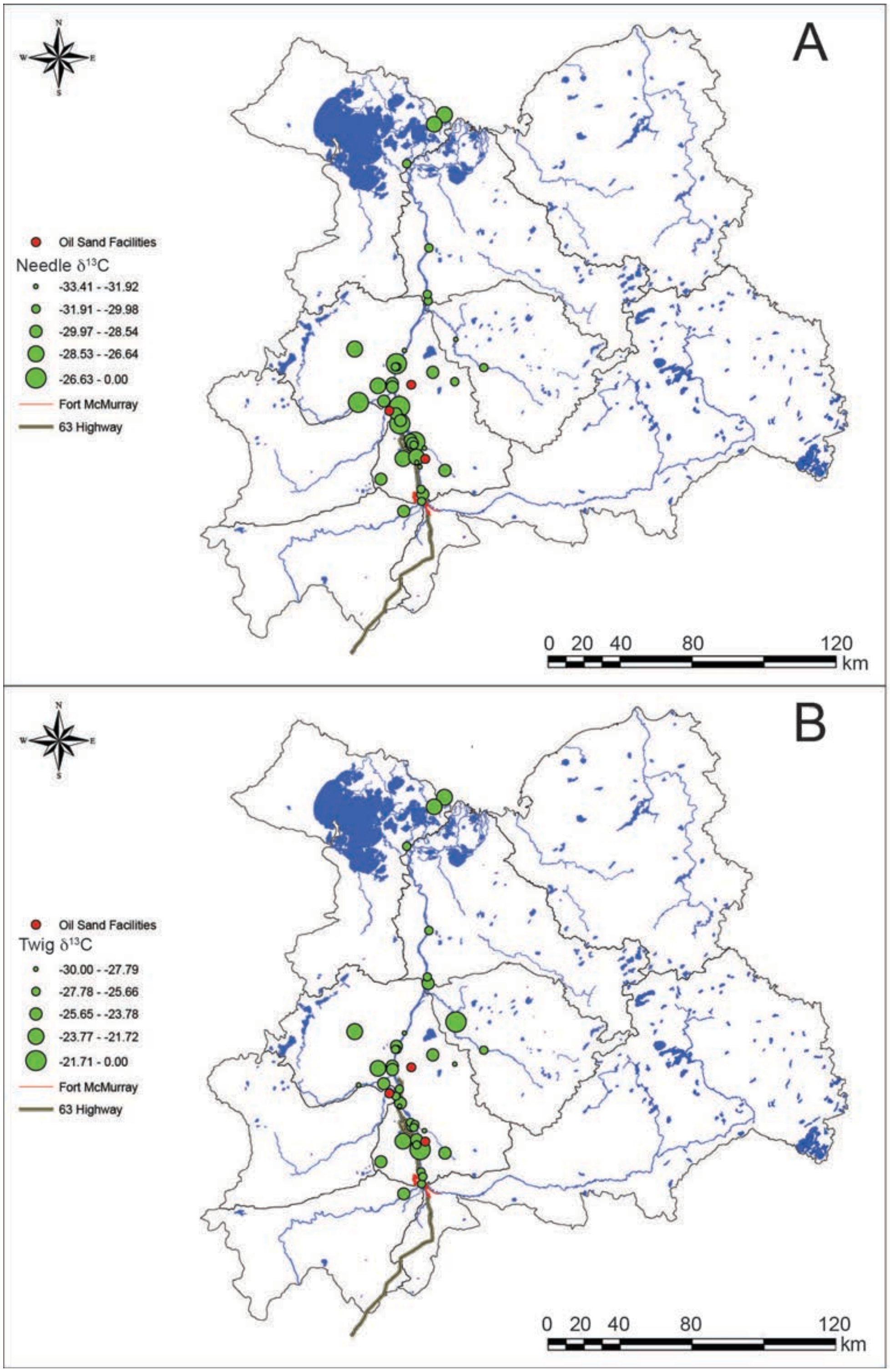

Figure 2.3. Spatial distribution of δ^{13}C in needles (A) and twigs (B) sampled from Black spruce (*Picea mariana*) around the Athabasca Oil Sands region, Alberta, Canada.

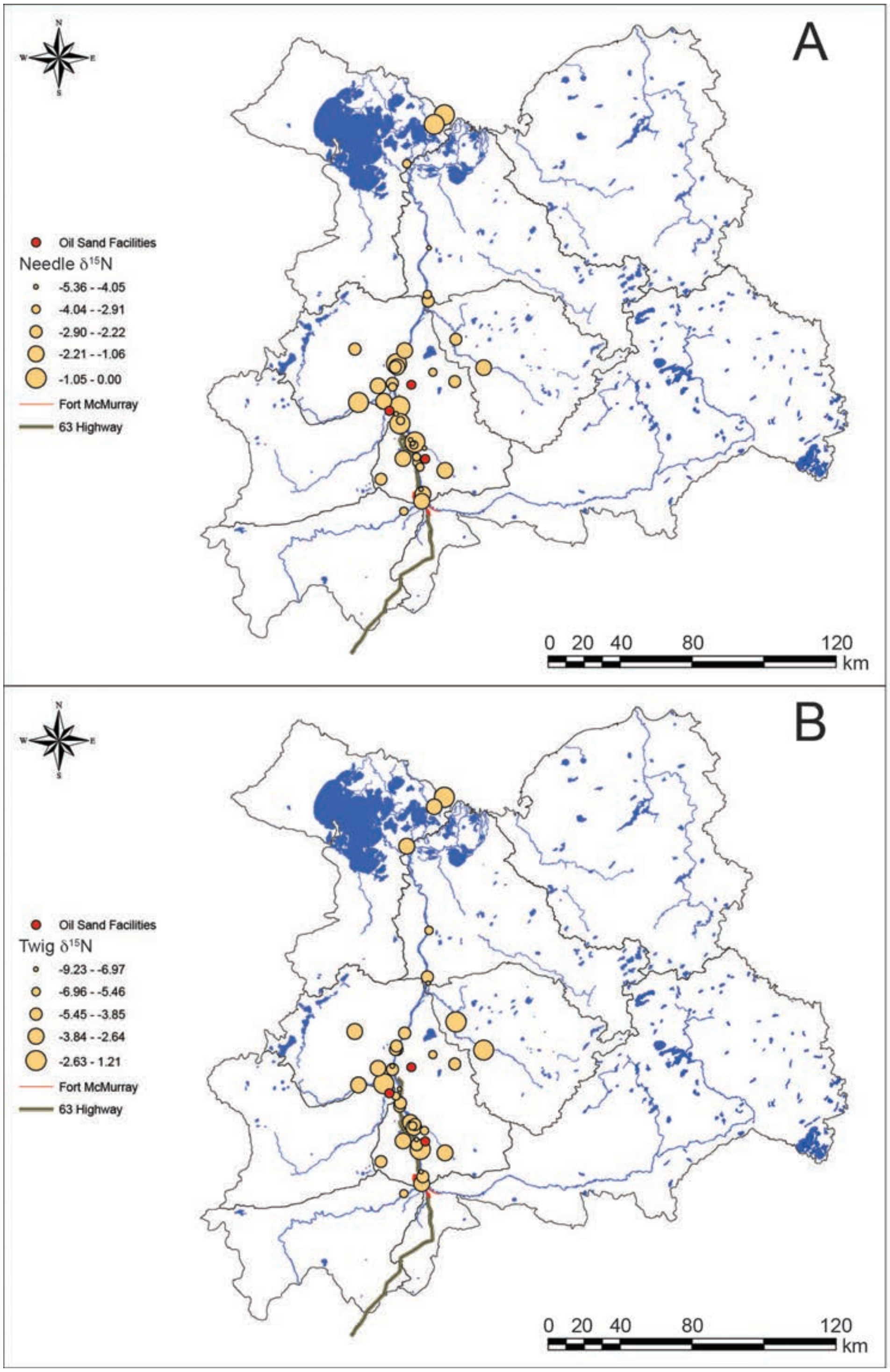

Figure 2.4. Spatial distribution of $\delta^{15}N$ in needles (A) and twigs (B) sampled from Black spruce (*Picea mariana*) around the Athabasca Oil Sands region, Alberta, Canada.

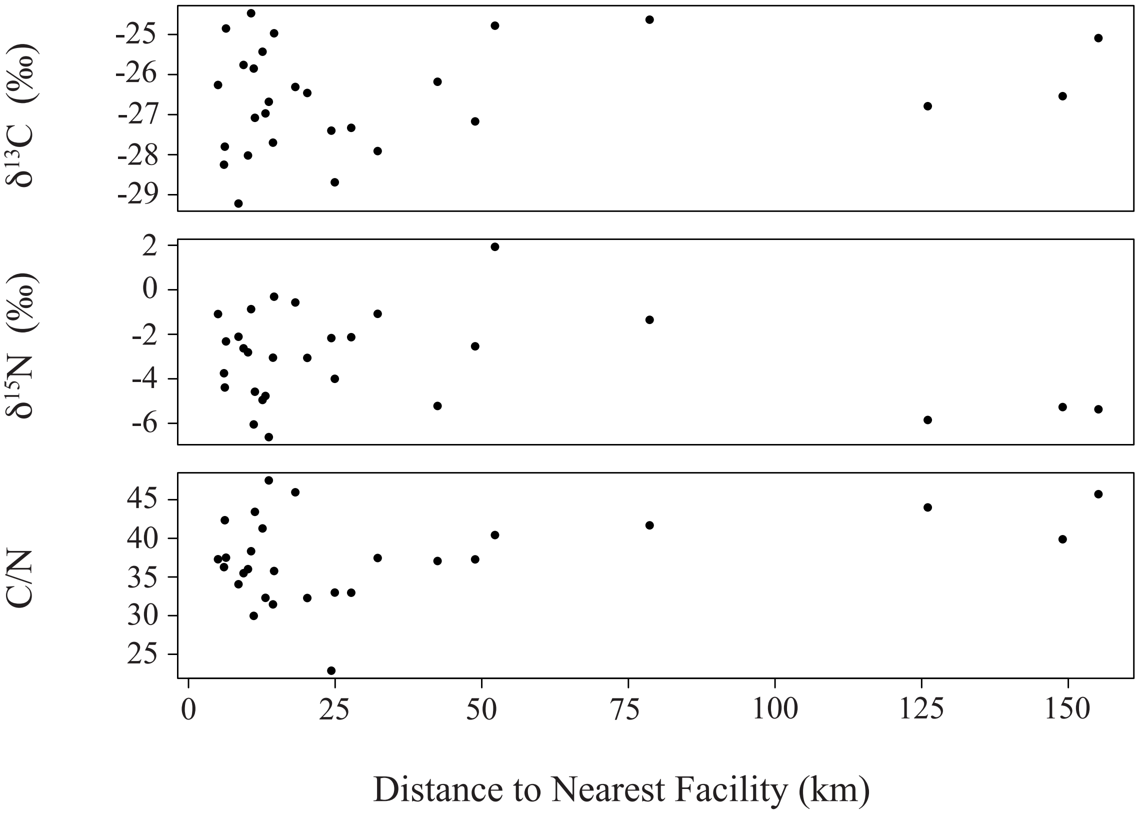

Figure 2.5. Needle δ^{15}N, δ^{13}C, C/N over distance to nearest processing facility from Black Spruce (*Picea mariana*) around the Athabasca Oil Sands region, Alberta, Canada.

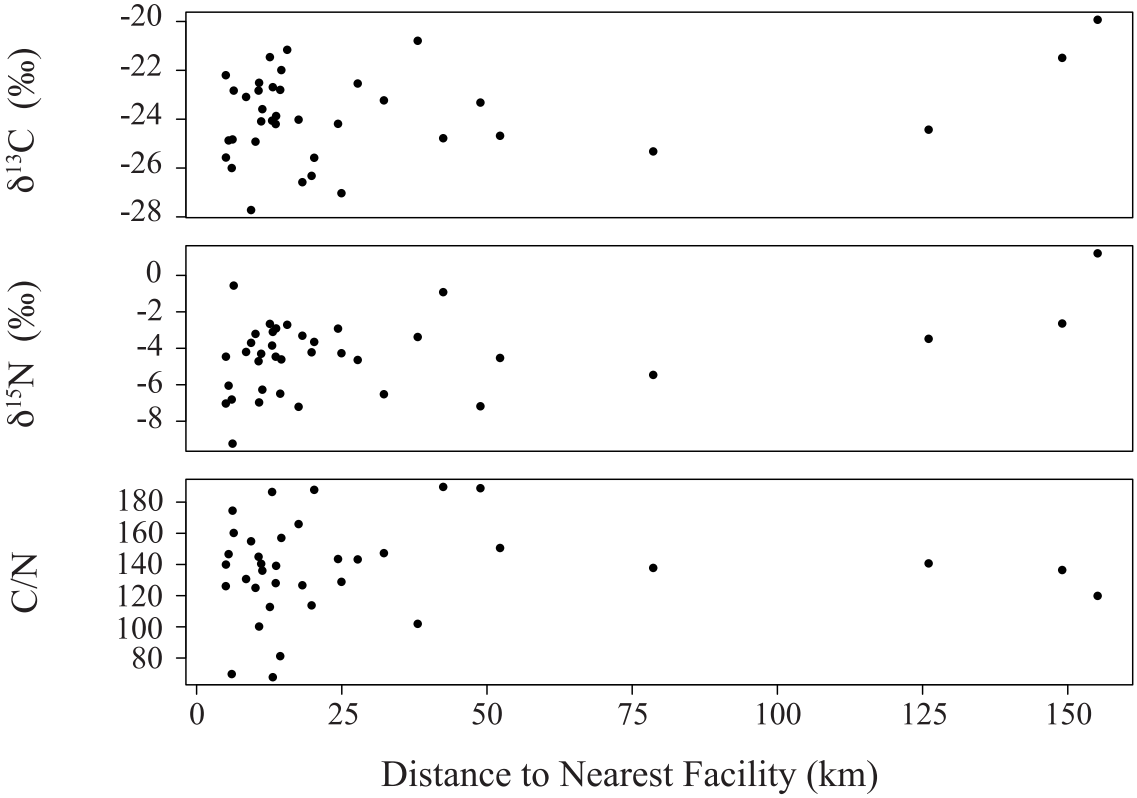

Figure 2.6. Twig δ^{15}N, δ^{13}C, C/N over distance to nearest processing facility from Black Spruce (*Picea mariana*) around the Athabasca Oil Sands region, Alberta, Canada.

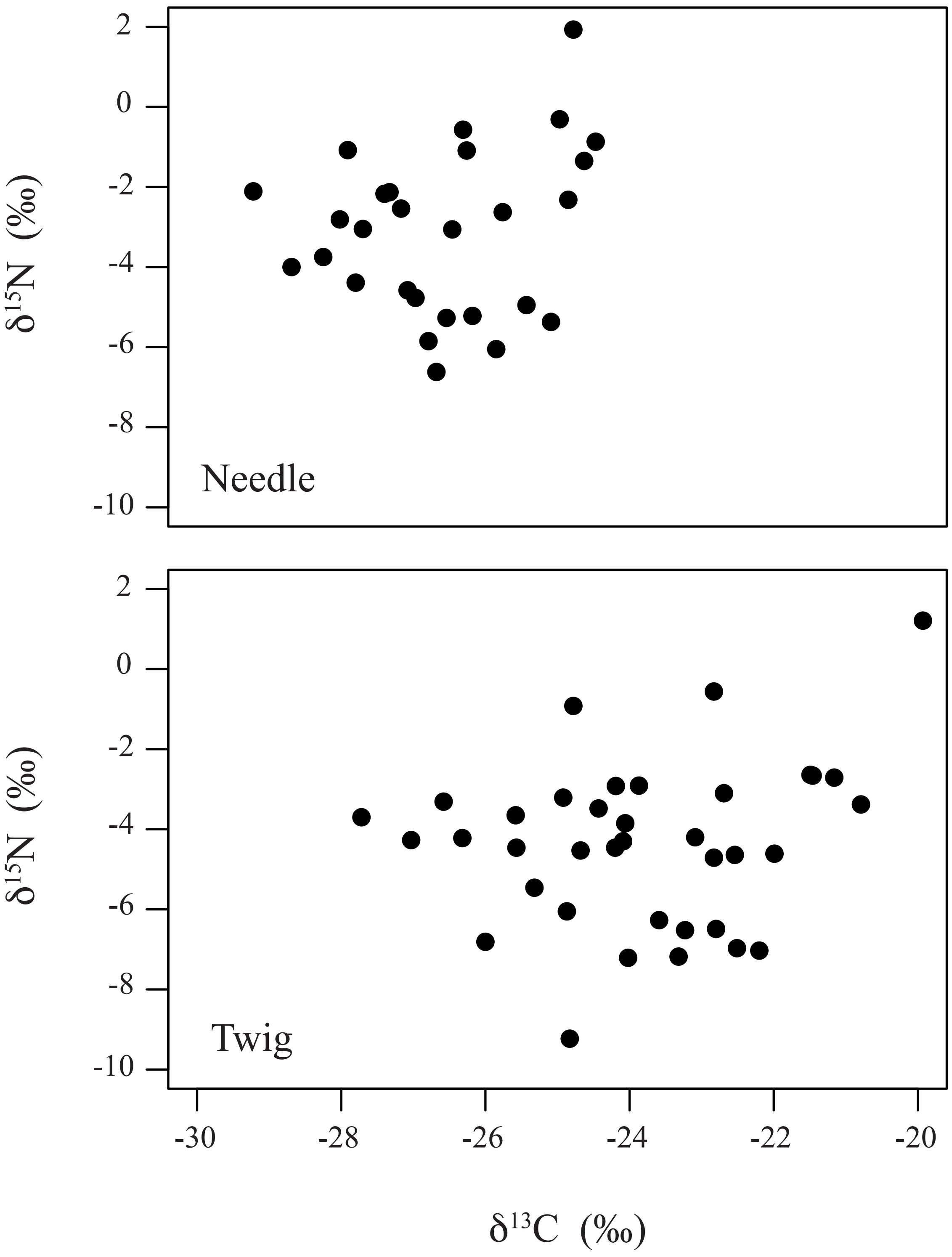

Figure 2.7. Needle and twig $\delta^{13}C$ versus $\delta^{15}N$ from Black Spruce (*Picea mariana*) around the Athabasca Oil Sands region, Alberta, Canada.

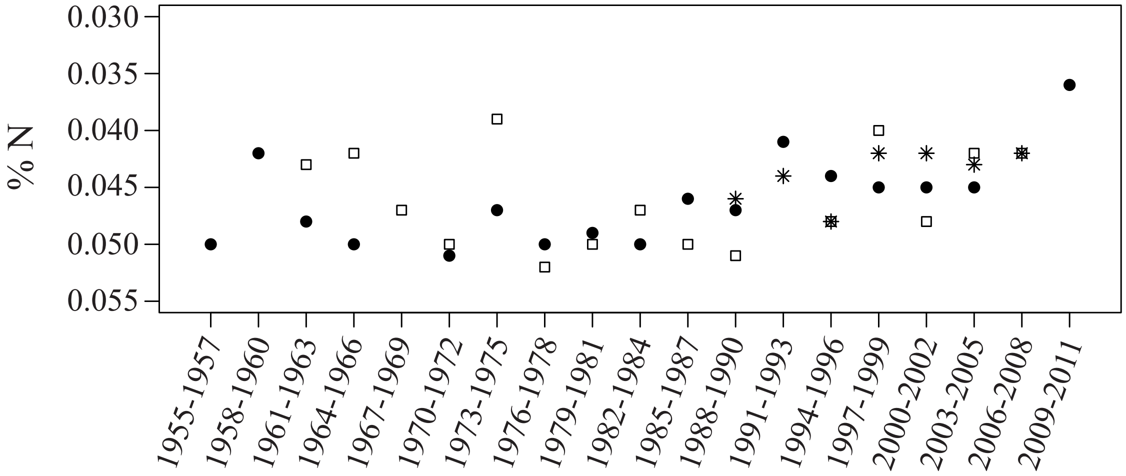

Figure 2.8. Percent nitrogen content over time of three tree cores, FM4 (●), FM9 (☐), FM13 (✳), from Black Spruce (*Picea mariana*) around the Athabasca Oil Sands region, Alberta, Canada.

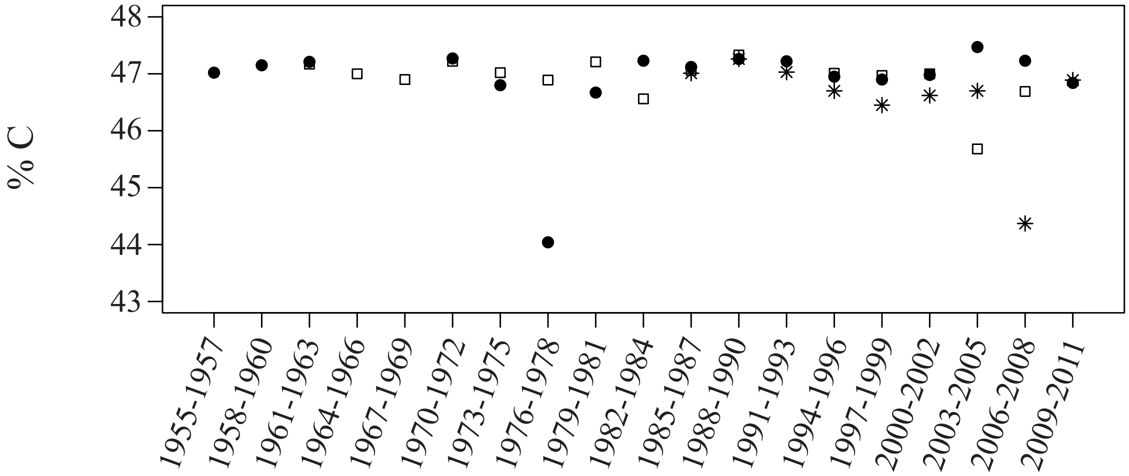

Figure 2.9. Percent carbon content over time of three tree cores, FM4 (●), FM9 (□), FM13 (✳), from Black Spruce (*Picea mariana*) around the Athabasca Oil Sands region, Alberta, Canada.

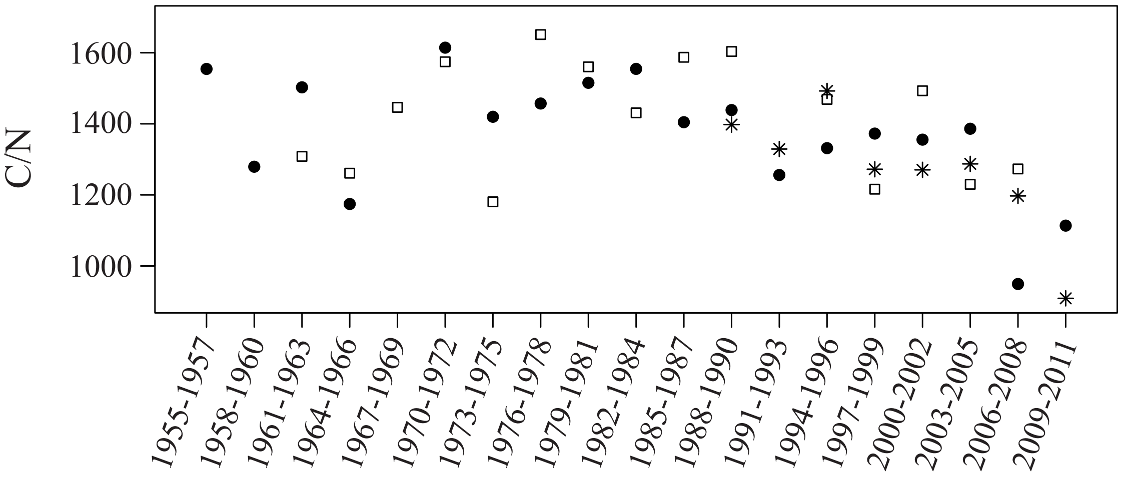

Figure 2.10. Carbon to nitrogen ratio over time of three tree cores, FM4 (●), FM9 (☐), FM13 (✳), from Black Spruce (*Picea mariana*) around the Athabasca Oil Sands region, Alberta, Canada.

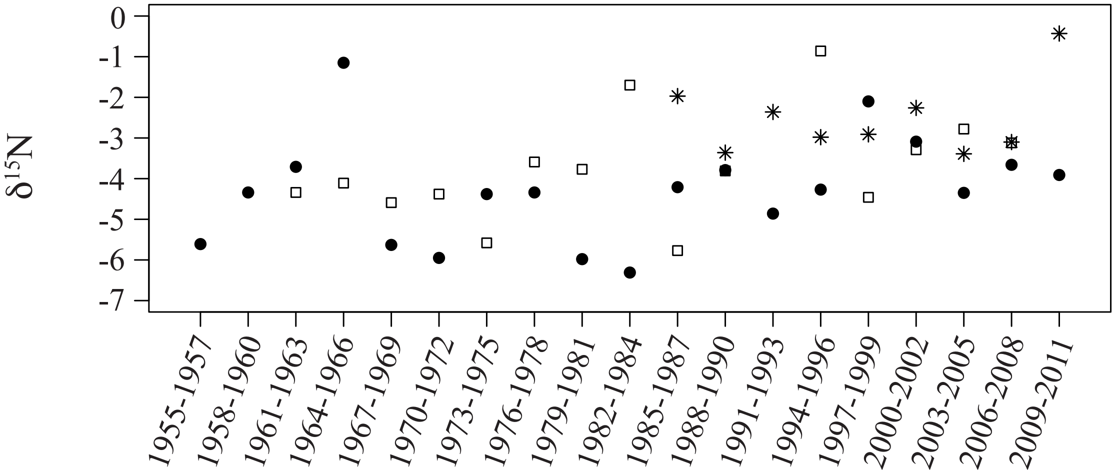

Figure 2.11. $\delta^{15}N$ over time of three tree cores, FM4 (●), FM9 (☐), FM13 (✳), from Black Spruce (*Picea mariana*) around the Athabasca Oil Sands region, Alberta, Canada.

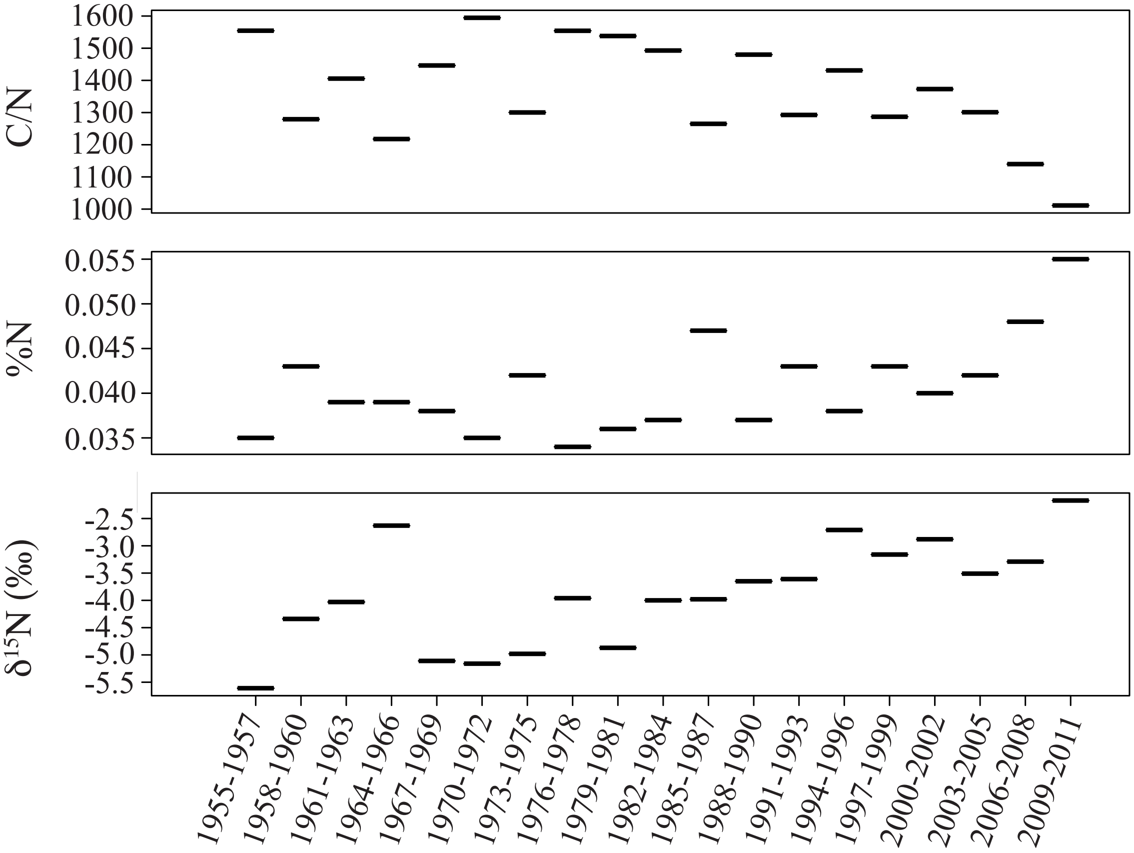

Figure 2.12. Mean C/N ratio, mean percent nitrogen content, and mean $\delta^{15}N$ of three tree cores from Black Spruce (*Picea mariana*) around the Athabasca Oil Sands region, Alberta, Canada.

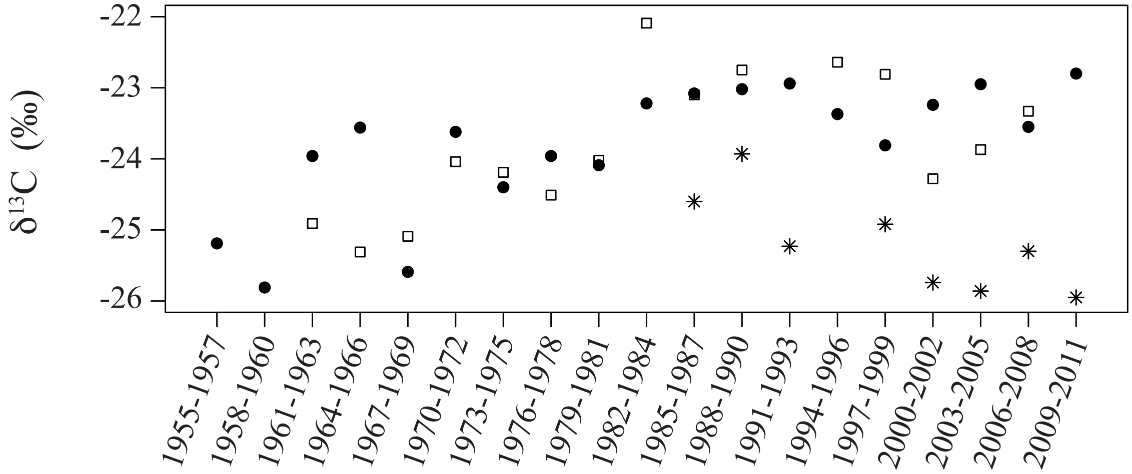

Figure 2.13. $\delta^{13}C$ over time of three tree cores, FM4 (●), FM9 (☐), FM13 (✳), from Black Spruce (*Picea mariana*) around the Athabasca Oil Sands region, Alberta, Canada.

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Chapter 3: A Dendrochemical Assessment of Recent Environmental Change in Alpine Catchments of Grand Teton National Park, Wyoming, USA

3.1. Introduction

Alpine environments contain unique biological communities. Steep altitudinal gradients combined with high topoclimatic variability result in the spatial compression of several ecosystems. Climates separated by thousands of kilometers at sea level may be present on a single mountain slope, in general enhancing local biodiversity (Körner, 2004). Variable exposure associated with topographical irregularities further enhance species richness and diversity, and lead to considerable heterogeneity with respect to ecological structure and function. Consequently, ecological adaptations within alpine plant communities are typically pronounced. At the same time, this uniqueness confers a considerable amount of ecological sensitivity to environmental change, including the effects of climate change and atmospheric deposition, particularly of reactive nitrogen (Nr).

High mountain ecosystems are sensitive to climate change due to cold conditions, thin soils, sparse vegetation, and a snowmelt-dominated hydrology (Baron, 1992; Baron et al., 2000; Fenn et al., 2003). Despite their remoteness, there is mounting evidence for a suite of recent environmental changes in many alpine areas, ultimately stemming from the combined effects of climate change and atmospheric deposition. For example, many regions exhibit a low capacity to sequester nitrogen derived from atmospheric deposition of Nr. Decreased soil and foliar C/N ratios have been identified in regions with deposition levels as low as 3-5 kg N ha^{-1} yr^{-1} (Rueth and Baron, 2002). Recent changes in sediment diatom communities and nitrogen stable isotopes in lakes from the Rocky Mountains of the Colorado Front Range are unique in the last 14,000 years (Wolfe et al., 2001), with similar 20th-century shifts arising in alpine lakes most proximal to anthropogenic activity (Baron et al., 2000; Parker et al., 2008; Hobbs et al., 2010). However, responses to environmental change in alpine environments with low anthropogenic Nr emissions, in the order of 1-2 kg N ha^{-1} yr^{-1}, remain poorly understood.

Grand Teton National Park (Wyoming, USA, hereafter GTNP) contains some of the most pristine alpine environments in western North America, and as such is ideal for studying ecological changes over time. The first European settlers did not arrive in the area until the 1880s, and due to harsh climate and difficult access, human population was sparse until creation of a National Park in 1929. Using a dendrochemical approach, I create a chronological record of geochemical shifts occurring in several alpine catchments of GTNP using $\delta^{13}C$, $\delta^{15}N$, %C, %N, C/N of tree rings in 5-year subgroups. Tree ring $\delta^{15}N$ has been used successfully to monitor atmospheric Nr deposition (Ammann et al., 1999; Saurer et al., 2004; Bukata and Kyser, 2007), whereas $\delta^{13}C$ can reflect changes in plant physiological function in response to both climatic and atmospheric vectors (Savard et al., 2002; Waterhouse et al., 2004; Gagen et al., 2009). Although some ecological shifts have been observed in conifer population density under fire management (Jacobs and Whitlock, 2008) and devastation of Whitebark pine populations by Mountain Pine Beetle is underway, geochemical data from tree rings can show an additional dimension of ecological shifts occurring in both plant physiology and ecosystem nutrient cycling.

3.2. Methods

3.2.1. Site description

Grand Teton National Park is located in the southern Rocky Mountains immediately north of Jackson Hole, Wyoming, USA. The Teton Range consists mainly of uplifted Precambrian metamorphosed granite overlain by sedimentary Paleozoic rocks that have been denuded by glaciation along the mountain crest (National Park Service, U.S. Department of the Interior). The mean annual precipitation recorded at the Moran climate station is 600 mm and mean annual temperature is 2°C (WRCC, 2012). The Tetons receive a large amount of winter precipitation (400 mm, average) resulting in snowmelt-dominated hydrology. They have a short summer growing season, primarily during the months of July and August.

Six high altitude lake catchments were selected for the collection of tree cores surrounding the three highest peaks in the Park: Grand Teton, Middle Teton, and Lower Teton (Figure 3.1; Table 3.1). Delta Lake is isolated from hiking trails and has extensive colluvium (avalanche and rockfall) in the basin. Amphitheatre Lake and its slightly smaller sister, Surprise Lake, are both on the popular Leigh Lake trail. Holly Lake and Whitebark Moraine Pool, in Paintbrush Canyon, are also popular hiking destinations with Whitebark Moraine Pool sitting unimposing off the trail and Holly Lake ~1 km further, in an alpine meadow. Grizzly Bear Lake is far more remote, and can be accessed only by crossing the ridge beyond Holly Lake and descending into the upper Leigh Canyon.

3.2.2. Sample collection

Tree cores were collected at breast height (1.4 m) with a 5.6 mm increment borer (SUUNTO, IML-Instrumenta Mechanik Labor System GmbH, Wiesloch, Germany) from healthy mature Engelmann spruce (*Picea engelmannii*) trees surrounding the 6 lakes Trees selected for sampling were chosen on the basis of relative health and size; I targeted older trees in order to yield the longest tree ring records possible. Tree height and circumference were also measured. Engelmann spruce was selected as it is abundant and healthy in the alpine ecosystem. Cores were stored in a -4°C freezer until further preparation.

3.2.3. Sample preparation and extraction

The cores were first dated: ring widths were measured using a stereomicroscope and Velmex slide stage micrometer with a digital encoder at 0.001 mm resolution (Velmex Inc., Bloomfield, New York, USA). A stainless steel mount slotted with a 6 mm wide and 3 mm deep groove was used to count the cores without the use of adhesives, avoiding the possibility of contamination. The cores fit into the groove and a thin layer was sliced from the top to facilite easy ring viewing (Figure 3.2). After they were dated and measured, the cores were subdivided into 5-year increments, cut further into ~1x1 mm pieces, and heat-sealed in filter bags (F57, Ankom Technology, Macedon, New York). I settled on five-year increments for analytical reasons; single rings were frequently insufficient to yield enough material for analysis, and furthermore multi-year increments reduce the influence of inter-annual variability without masking general, decadal to sub-decadal scale trends.

One master core from each lake basin was selected for isotopic analysis based on the age and quality of the core, and the health of the tree. Mobile components of xylem (resins and other fluids) were first extracted to avoid lateral cross-contamination between years. I performed a lignin-cellulose extraction that isolates only the more stable structural components of the wood, and still contains nitrogen and cellulose for a thorough biogeochemical analysis. The extraction was done following a slightly modified version of the three-step procedure outlined by Sheppard and Thompson (2000) and Saurer et al. (2004). In a soxhlet apparatus, samples were exposed to a 1:1 mixture of toluene and ethanol (95%) for 18 hours, followed by 18 hours in ethanol (95%). They were then soaked for 6 hours in boiling deionized water, and finally rinsed with cold deionized water. Samples were dried in a 70°C oven for at least 24 hours before being ground to pass through 2 mm mesh. Samples were analyzed for nitrogen and carbon isotopic signatures, as well as carbon and nitrogen content.

3.2.4. Mass spectrometry

The δ^{15}N and δ^{13}C natural abundance and total %N and total %C analyses were conducted using a Costech ECS 4010 CHNS-O Elemental Combustion System (Costech Analytical Technologies Inc., Valencia, CA) coupled to a Conflo III interface and a continuous flow Finnigan Delta Plus Avantage IRMS (ThermoFinnigan, Bremen, Germany). All $\delta^{15}N$ values are expressed using the delta (δ) notation in per mille (‰) relative to the N_2 of air:

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] * 1000$$

where R_{sample} and $R_{standard}$ are the $^{15}N/^{14}N$ ratios of the sample and atmospheric N_2, respectively. $\delta^{13}C$ values are also expressed using the delta (δ) notation in per mille (‰), relative to the international VPDB (Vienna Pee Dee Belemnite) standard:

$$\delta^{13}C = [(^{13}R_{sample}/R_{standard}) - 1] * 1000$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and VPBD, respectively. NIST Standard Reference Materials (NBS22, LSVEC, NBS19 for ^{13}C and IAEA-N1, IAEA-N2, IAEA-N3 for ^{15}N) were used to calibrate and standardize measurements, yielding errors of ±0.3‰ for $\delta^{15}N$ and ±0.05‰ for $\delta^{13}C$.

3.2.5. Data transformation and statistical analysis

The record for $\delta^{13}C$ over time was detrended for secular changes in atmospheric $\delta^{13}C$ values due to additions of ^{13}C-depleted carbon from fossil fuel combustion, also known as the Suess effect. $\delta^{13}C$ values were corrected using the equation of McCarroll et al. (2009):

$$\delta^{13}C_{cor} = \delta^{13}C_{plant} - (\delta^{13}C_{atm} + 6.4) \qquad \text{(eqn. 1)}$$

where $\delta^{13}C_{plant}$ and $\delta^{13}C_{atm}$ are the carbon isotopic signatures of the tree and atmosphere, respectively, in the year being corrected and -6.4 is an estimate for pre-industrial $\delta^{13}C$ in the global atmosphere. This pre-industrial estimate and the $\delta^{13}C_{atm}$ values are available for the last 150 years in McCarroll and Loader (2004), constrained by ice-core measurements.

The carbon to nitrogen molar ratio was calculated for each 5-year wood increment using percent carbon and nitrogen values. The C/N ratio provides an index of relative changes in elemental stoichiometry in plant tissues, and hence is a proxy of ecophysiological shifts.

Conifers assimilate carbon (C) through stomata in the needles as described by the following equation of Farquhar et al. (1989):

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a)\frac{c_i}{c_a} \qquad \text{(eqn. 2)}$$

where $\delta^{13}C_p$ is the $\delta^{13}C$ of the plant, $\delta^{13}C_a$ is the $\delta^{13}C$ of the atmosphere, *a* is the fractionation due to diffusion, *b* is the fractionation due to carboxylation, and c_i and c_a are the internal and ambient concentration of CO_2. As *a* and *b* are constants (~4.4‰ and ~27‰ respectively; Farquhar et al., 1982), $\delta^{13}C_p$ is controlled by $\delta^{13}C_a$ and the c_i/c_a ratio. Thus a shift in $\delta^{13}C_p$ captures either changing stomatal conductance or photosynthetic rate, both of which influence c_i/c_a.

The intrinsic water-use efficiency (iWUE), the unit of carbon gained per unit of water lost, was calculated to further explore deviations in $\delta^{13}C$ and the c_i/c_a ratio over time. iWUE is defined by Ehleringer et al. (1993) as:

$$\text{iWUE} = \frac{A}{g_w} = \frac{c_a - c_i}{1.6} \qquad \text{(eqn. 3)}$$

where A is the assimilation rate for the uptake of CO_2 and g_w is leaf conductance to water vapor. In calculating iWUE, c_i was first calculated by rearranging equation 2, with $\delta^{13}C_a$

and atmospheric CO_2 concentrations (c_a) taken from the compilations of Loader et al. (2004) and NOAA (2012).

The statistical program R was used to perform Pearson correlations in order to ascertain relationships between dendrochemical trends and time. The mean trend from all five sites was computed for each variable, with corresponding standard errors for each 5-year bin. Annual precipitation and temperature values were also averaged to compute time-series matching temporally each five-year bin captured by the tree rings. Further linear regressions were performed on the tree-ring derived parameters and temperature, atmospheric [CO_2] and %N in order to explore causal relationships.

In order to summarize and reduce the dimensionality of these multivariate data, principal components analysis (PCA) was performed on a matrix comprising of five climatic and dendrochemical variables from 16 5-year temporal bins. PCA is a unimodal indirect ordination technique that defines a number of orthogonal axes (or principal components) for which variable responses are modeled as linear, and which explain incremental and independent fractions of the total variance within the matrix (ter Braak and Prentice, 1988). Data were first centered and standardized to uniform variance, and PCA was performed using CANOCO v.4.5 (ter Braak and Šmilauer, 2002).

3.3. Results

3.3.1. General characteristics

Average tree trunk diameter was 0.6 m but varied from 0.25 to 1.10 m. Tree height varied from ~15 m to ~45 m. The oldest tree core analyzed originated from Whitebark Moraine Pool and dated to 1661 AD. All trees were healthy with no evidence of insect infestation or disease, however many had some obvious physical damage from ice and snow. All trees had an initial period of elevated growth rates for about 20-25 years in their youth before settling into consistent growth thereafter.

3.3.2. Carbon and nitrogen content and molar ratios

Percent carbon remains relatively constant over time despite high values from one site (Whitebark Pine Moraine) during the 18th century (Figure 3.3). On the other hand, an increasing trend in percent nitrogen over time was detected in the binned 5-year means of all cores (p-value < 0.0005; $n = 29$; $r^2 = 0.52$; Figure 3.4). The increase is slight, beginning in the late 1880s, but increases sharply between the 1996-2000 and 2001-2005 bins. The C/N shows a slight decreasing trend since the 1700s with an accelerated decline beginning in the mid 1900s (p-value < 0.0005; $n = 29$; $r^2 = 0.66$; Figure 3.5), reflecting the attendant increases in percent nitrogen. However, the sharpest decreases of C/N occur between 1996-2000 and 2001-2005 in all but one core, Delta Lake, where the shift occurs in the 1991-1995 rings.

3.3.3. Time-series of tree-ring $\delta^{15}N$ and $\delta^{13}C$

The δ^{15}N time-series from individual tree cores do not show any discernible trends over time. Despite some degree of variability between 5-year bins, the overall trend is remarkably flat, suggesting little long-term change in local N cycling due to atmospheric deposition of Nr (p-value = 0.64; n = 29; r^2 = 0.028; Figure 3.6). The bins with significant differences may be a result of climate and other environmental parameters controlling soil microbial activity or run-off.

The uncorrected δ^{13}C record showed a strong decrease that parallels the global secular atmospheric δ^{13}C trend. This trend begins around the mid 19th century, decreasing by about 2‰ until the present. Correcting the tree ring δ^{13}C values reduces the δ^{13}C trend considerably, but there is still a clear trend towards lighter isotopic values that is statistically significant in the mean data for 5-year bins (p-value < 0.0005; n=29; r^2 = 0.50; Figure 3.7).

3.3.4. iWUE

The computed iWUE values from GTNP tree cores show a significant increase over time at all sites, especially within the last century (p-value < 0.0005; n = 29; r^2 = 0.81; Figure 3.8). The mean iWUE values increase from 88.84 ± 4.60 μmol mol^{-1} in 1901-1915 to 129.54 ± 5.02 μmol mol^{-1} in 2006-2010 (i.e., 46%). Although the individual tree cores show variable trends before 1950, in recent decades all records converge and reveal a conspicuous increasing trend in iWUE.

3.4. Discussion

3.4.1. Nitrogen

3.4.1.1. δ^{15}N, %N, and C/N over time

The dendrochemical data show a decrease in C/N over time driven by an increase in %N. Although there is a decline beginning in the 1700s, a shift to a steeper decline begins around the mid 1900s and is greatest in the 2001-2005 tree rings. At the ecosystem level, decreasing C/N driven by increases in N indicates enhanced Nr mineralization and availability (Tietema et al., 1998; Rueth and Baron, 2002; Power and Collins, 2010). Alpine ecosystems are generally nutrient-poor (Baron et al., 1994; 2000), so an increase in N availability may indicate an increased Nr subsidies from atmospheric deposition, which should be reflected as declining δ^{15}N.

Samples from lake sediments, tree cores, and ice cores have shown a decline in δ^{15}N since the industrial revolution due to the deposition of atmospheric Nr species, which are generally isotopically depleted relative to pre-industrial samples (Poulson et al., 1995; Wolfe et al., 2001; Hastings et al., 2009). In tree-rings, δ^{15}N appears to record faithfully the isotopic composition of Nr in atmospheric deposition (Bukata and Kyser, 2007). However, the δ^{15}N of tree rings in the GTNP do not show any significant trend over time, whereas sediments from the lakes adjacent to the trees studied here indeed record a significant decline in δ^{15}N (1-3‰) since AD 1950 in keeping with other studies in the Rocky Mountains, and indeed the northern hemisphere as a whole (Holtgrieve et al., 2011). Taken together, the lake-sediment δ^{15}N and tree ring C/N represent evidence of increased Nr availability.

It then remains mysterious why the tree-rings fail to incorporate the isotopic fingerprint of anthropogenic Nr sources. It may be that the complexities of the N cycle are the reason we see no response in tree ring $\delta^{15}N$ to N deposition (Aber et al., 1998). The lack of $\delta^{15}N$ trend may also be explained by the low capacity for soil N retention in alpine ecosystems due to shallow soil layers, steep slopes and melt-water driven hydrology (Baron et al., 1994; Fenn et al., 2003). Surface runoff may remove excess deposited N from alpine forests to local lake catchments before it can be greatly incorporated in the N cycle and shift $\delta^{15}N$. However, it is also possible that the tree-ring %N and lake sediment $\delta^{15}N$ are recording separate processes not wholly related to the $\delta^{15}N$ fingerprint of atmospheric Nr inputs to the ecosystem.

Doucet et al. (2012) cautions against using percent nitrogen in environmental and climatic studies. As trees age, nitrogen is transferred from heartwood to sapwood in ways that may preclude any meaningful insights into ecosystem-scale changes in nitrogen biogeochemistry. As my data show a slightly decreasing %N since the 1700's that is uncorrelated with $\delta^{15}N$, we can assume that similar processes are occurring. Furthermore, diatom populations in adjacent lake sediment cores do not show shifts over time to more nitrophilous taxa concurrent with shifts towards lighter sediment $\delta^{15}N$ values. Therefore, it is possible that the isotopes are simply reflecting physiological processes within the water column, such as intensified fractionation by algae during assimilation, as opposed to a source signature of atmospheric deposition (Goericke et al., 1994). A $\delta^{15}N$ signature reflecting the physiological processes of the lake could explain the lack of a $\delta^{15}N$ in the tree rings as well. From this study, there appears to be inconclusive evidence for enhanced atmospheric Nr deposition in the GTNP alpine ecosystem.

3.4.2. Carbon isotopic time-series

The $\delta^{13}C$ of tree rings show a decrease of about 1‰ since the late 1800s, even after correcting for the Suess effect. Similar trends have also been observed in urban and arid environments (Dongarra and Varrica, 2002; Sakata and Suzuki, 2002). As shifts in $\delta^{13}C_p$ captures either changing stomatal conductance or photosynthetic rate, both of which influence c_i/c_a. Such alterations in physiological function can be linked to global processes; climate change and atmospheric emissions both influence plant metabolism. The relationships can be further explored by deriving isotopic indices for intrinsic water use efficiency (iWUE)

3.4.3. Intrinsic water use efficiency (iWUE)

The Engelmann Spruce in GTNP show a decreasing trend, indicating physiological shifts within the tree to maintain a constant c_i/c_a ratio. This decline in plant $\delta^{13}C$ can therefore clearly be interpreted as an improved iWUE since the mid 20th century, as has been observed in other regions but, to my knowledge, is the first report that extends to the alpine environment (Bert et al., 1997; Duquesnay et al., 1998).

3.4.3.2 Climate

The reconstructed iWUE trends represent the balance between carbon and water budgets. Therefore, iWUE often reflects underlying climatic factors such as precipitation and temperature. In times of elevated temperatures and/or drought, increasing iWUE reduces canopy water loss through evapotranspiration (Gagen et al., 2011). Tree ring $\delta^{13}C$ and iWUE records have been successfully correlated to local precipitation, relative humidity and temperature. In alpine environments, iWUE tends to more closely relate to temperature as opposed to precipitation (Sakata and Suzuki, 2002; Treydte et al., 2009). Precipitation has a stronger influence than temperature over iWUE in water-limited environments, such as in the Mediterranean (Saurer et al., 1997; Ferrio et al., 2003; Battapaglia et al., 2009; Leavitt et al., 2010).

Records from the Moran climate station in GTNP did not show a significant change in annual precipitation since the beginning of the record in 1911 (p-value = 0.11; n = 16; $r^2 = 0.11$). However, mean annual temperatures in 5-year bins, corresponding with the analyzed tree ring increments, increase significantly since 1911 (p-value = 0.0027; n = 16; $r^2 = 0.45$). When compared with corresponding iWUE values from tree rings mean annual temperature shows a weak positive correlation (p-value = 0.019; n = 16; $r^2 = 0.29$; Figure 3.9).

Our data agree with other studies relating iWUE with climatic factors at high altitude sites. The correlation between iWUE and temperature suggests that the c_i/c_a ratio is controlled by the carboxylation efficiency of the photosynthetically active enzyme Rubisco instead of by stomatal conductance (Treydte et al., 2009). This can be explained by the short growing season in alpine ecosystems where temperature would be a predominant control over plant growth, as opposed to water limitation. Although temperature does appear to affect iWUE, it is a weak relationship and is not deemed the principal cause of the $\delta^{13}C$ trend reported in this study.

3.4.3.3. *Nitrogen*

The increase in iWUE present in this study may alternately relate to an increase in Nr supply (Duquesnay et al., 1998). As discussed in section 3.4.1, there appears to be increasing nitrogen availability due to the deposition of Nr from the atmosphere, even in the absence of an isotopic fingerprint. Indeed, the increase of tree-ring %N correlates positively with iWUE (p-value<0.005; n=29; r^2=0.81). N fertilization studies have not shown consistent results with respect to establishing linkages with C metabolism. Balster et al. (2009) found no effect on $\delta^{13}C$ with increased Nr availability. However, Rippulone et al. (2004) and Choi et al. (2005) found an increase in the iWUE due to increased Nr. The strong relationship between iWUE and %N in GTNP tree-rings suggests that such a fertilization effect is occurring in the GTNP, concomitantly to a secondary climatic influence over water-use efficiency.

3.4.3.4. Rising atmospheric CO_2 concentrations

Rising atmospheric CO_2 levels can also influence the $\delta^{13}C$ of plants by shifting the c_i/c_a ratio in the same direction, as is borne out by the results of this study. Since 1850, atmospheric CO_2 concentrations have increased from ~285 ppm to ~390 ppm in 2010 (NOAA, 2012). CO_2 is the main food source for plants, so that increasing levels may lead to a possible ‘fertilization’ effect, enhancing plant growth if not limited by temperature, precipitation, or soil nutrients. Laboratory studies investigating plant reaction to higher levels of CO_2 exposure note a decrease in the $\delta^{13}C$ values of plants through decreasing stomatal constriction and increasing the assimilation rate (Morrison, 1993; Polley et al., 1993; Picon et al., 1996). As iWUE values from the present study do show a significant positive correlation with atmospheric CO_2 levels (p-value<0.005; df=28; r^2=0.95), atmospheric CO_2 is also influencing the $\delta^{13}C$ of Engelmann Spruce in GTNP, resulting in an improved iWUE.

Plants respond to increasing CO_2 levels in one of two ways. A passive response occurs when plants do not alter either stomatal conductance or photosynthetic rate. As atmospheric CO_2 concentrations (c_a) increase, so do internal CO_2 concentrations (c_i) so the c_a-c_i remains constant. In this response, there is little physiological adaptation to changing atmospheric conditions. With an active response, the plant works to maintain a constant c_i/c_a ratio, which is achieved through the reduction of evapotranspiration by closing or reducing the number of stomatal apertures during exposure to high levels of CO_2 (Woodward, 1987; Woodward and Kelly, 1995; Gray et al., 2000; Lake et al., 2001). As our data shows a change in iWUE, c_a-c_I does not remain constant. Therefore, the trees in GTNP are displaying an active response to changing atmospheric CO_2 levels, adjusting their physiological functioning to maintain a constant c_i/c_a ratio.

An active response is typical among plants. However, Waterhouse et al. (2004) found a decrease in the rate of increase in iWUE at higher values of c_a. They suggests that this indicates a lower sensitivity in iWUE to increasing levels of CO_2. Similar results were found by Gagen et al. (2001) in tree cores from Finland, where iWUE ceased rising after 1970. This is direct evidence of a shift from an active to a passive response to rising CO_2 levels, indicating that the ability of trees to maintain a constant c_i/c_a ratio through stomatal conductance becomes limited at higher atmospheric CO_2 concentrations. The data from GTNP show the greatest rate of increase in iWUE values within the last 30 years, with no signs of a plateau being reached.

3.4.3.5. Drivers of environmental change in GTNP alpine forests

There is direct dendrochemical evidence for enhanced water-use efficiency in GTNP Engelmann spruce, which is ultimately related to diffuse anthropogenic influences that may assume three forms: (1) increasing ambient temperature; (2) atmospheric Nr subsidies; and (3) increasing atmospheric CO_2 concentrations. Although it remains difficult to apportion variance among these various influences, it appears that Nr deposition and atmospheric CO_2 have the greatest overall influence. Both encourage the trees to reduce stomatal conductance in order to maintain a constant c_i/c_a ratio as environmental conditions change. The relationship between iWUE and temperature is weaker, but this is not to say climate change is benign in GTNP alpine forests, as evidenced independently by the expansion of the Mountain pine beetle's range in relation to warming winter temperatures (Carroll et al., 2006; Kurz et al., 2008). There is possible evidence for Nr deposition in the tree-ring %N values and in the $\delta^{15}N$ of adjacent lacustrine sediments. However, the absence of diatom assemblage shifts parallel to the decline in $\delta^{15}N$ of lake sediments suggests a somewhat muted signature. The lack of a $\delta^{15}N$ trend in tree rings suggests that Nr deposition may not be influencing tree growth to the extent that the statistical correlations imply. Therefore, it can be surmised that rising atmospheric CO_2 levels are resulting in the greatest change in the physiology of trees in the GTNP.

These complex relationships are efficiently captured by the PCA of 5-year bins that integrate climatic and dendrochemical data over the 20th century (Figure 3.10). The leading PCA axis, which captures nearly 90% of cumulative variance, is strongly driven by precipitation and CO_2, and to a lesser extent by iWUE. Sample scores along this axis inflect beginning around 1960, and this trend accelerates markedly after 1995. The second axis of PCA (10.6% variance explained) confirms the correlation between CO_2 and iWUE. The PCA supports the contention made above that direct temperature effects are secondary to those associated with moisture availability and ambient CO_2. Summarily, the recent increase in iWUE provides evidence of tree physiological adaptations to rising atmospheric CO_2 concentrations. However, with continued rising CO_2 levels, trees may become unable to maintain constant c_i/c_a and shift towards a passive response (Waterhouse et al., 2004; McCarroll et al., 2009; Silva et al., 2010). Increased water limitation, as shown through the increasing iWUE, may limit possible CO_2 induced growth stimulation and result in decreased growth rates instead (Andreu-Hayles et al., 2011).

Although GTNP tree-rings provide a geochemical framework that is consistent with recent environmental changes, the record is complex and involves many competing causative factors, including nitrogen availibility, climatic influences, and CO_2 supply. In this ecosystem, dendrochemistry does not appear sufficient, on its own, to determine the full extent of anthropogenically induced ecological change occurring in GTNP. Nonetheless, the reported results identify key ecophysiological responses among alpine tree populations to a changing global environment, while underscoring several elements of their inherent ecological resilience.

Table 3.1. Latitude, longitude and elevation of the six lakes around which tree cores were collected from Engelmann Spruce (*Picea engelmanii*) in the Grand Teton National Park, Wyoming, USA.

Site	Latitude	Longitude	Elevation (m)
Delta Lake	43 43 57.15	110 46 22.26	2770
Surprise Lake	43 43 42. 73	110 46 38.04	2870
Amphitheatre Lake	43 43 47.05	110 46 52.57	2960
Holly Lake	43 47 33.56	110 47 52.95	2870
Whitebark Morraine Pool	43 47 18.13	110 47 39.46	2800
Grizzly Bear Lake	43 48 10.81	110 48 37.84	2800

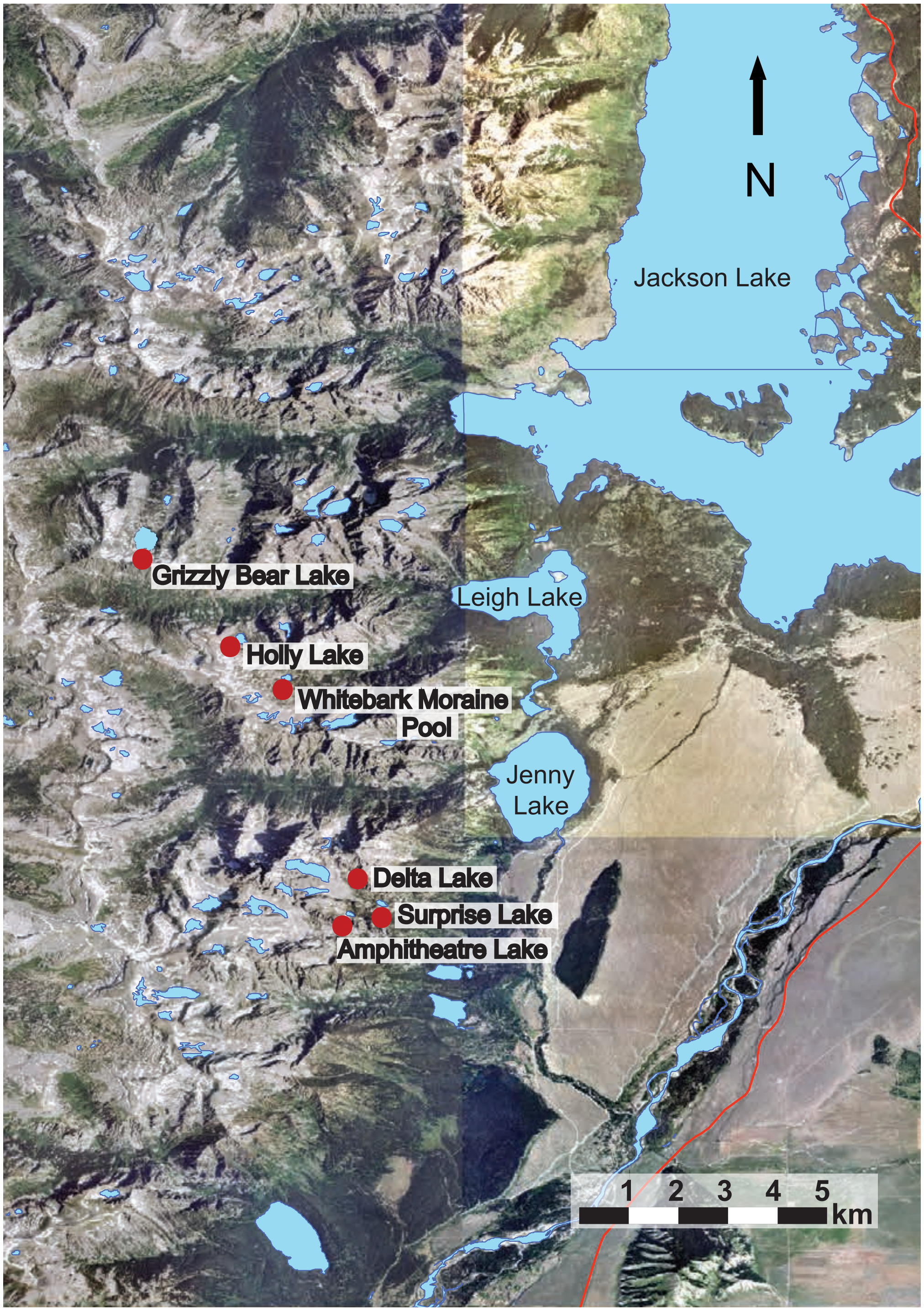


Figure 3.1. Site map of the 6 lakes around which tree cores were collected from Engelmann Spruce (*Picea engelmanii*) in the Grand Teton National Park, Wyoming, USA.

Figure 3.2. Core holder (A) and a magnification of the first section of a core (B). This core holder was used to avoid cross contamination associated with traditional wood and glue core mounting.

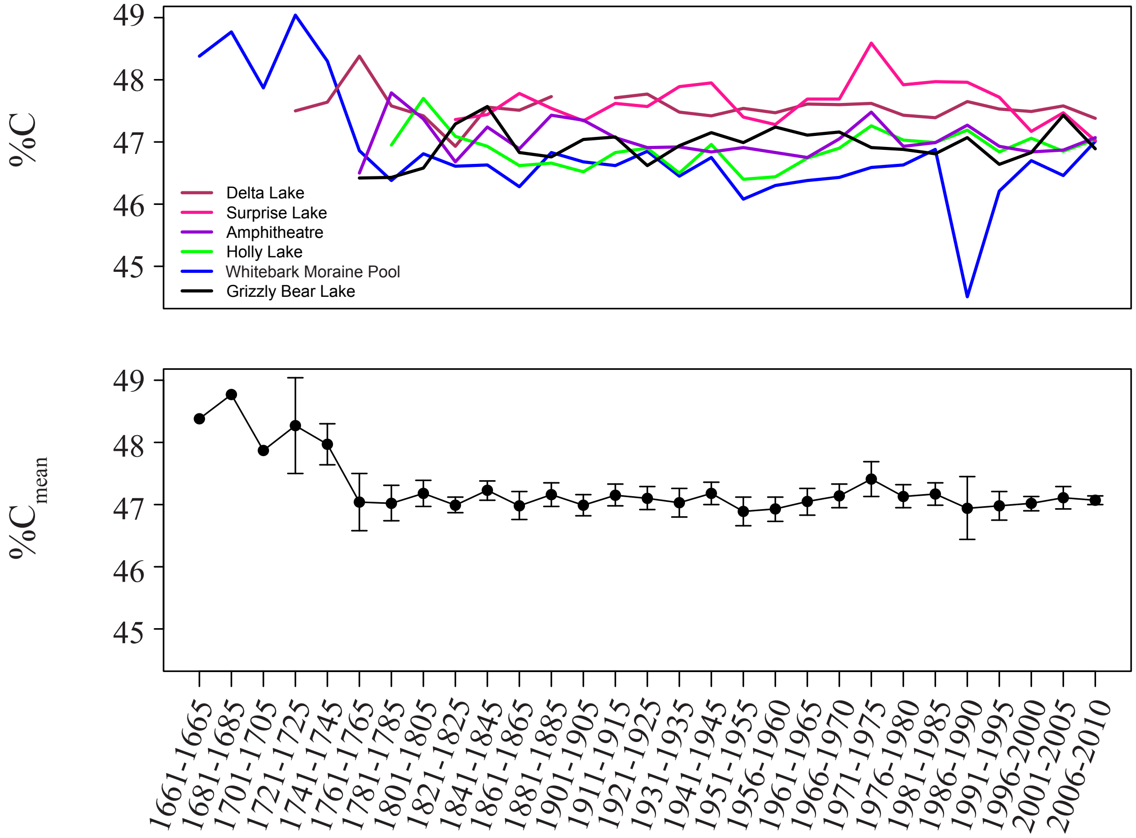


Figure 3.3. Percent carbon content and mean percent carbon content with standard error bars of tree ring lignin-cellulose collected from Engelmann Spruce (*Picea engelmanii*) around 6 lakes in the Grand Teton National Park, Wyoming, USA.

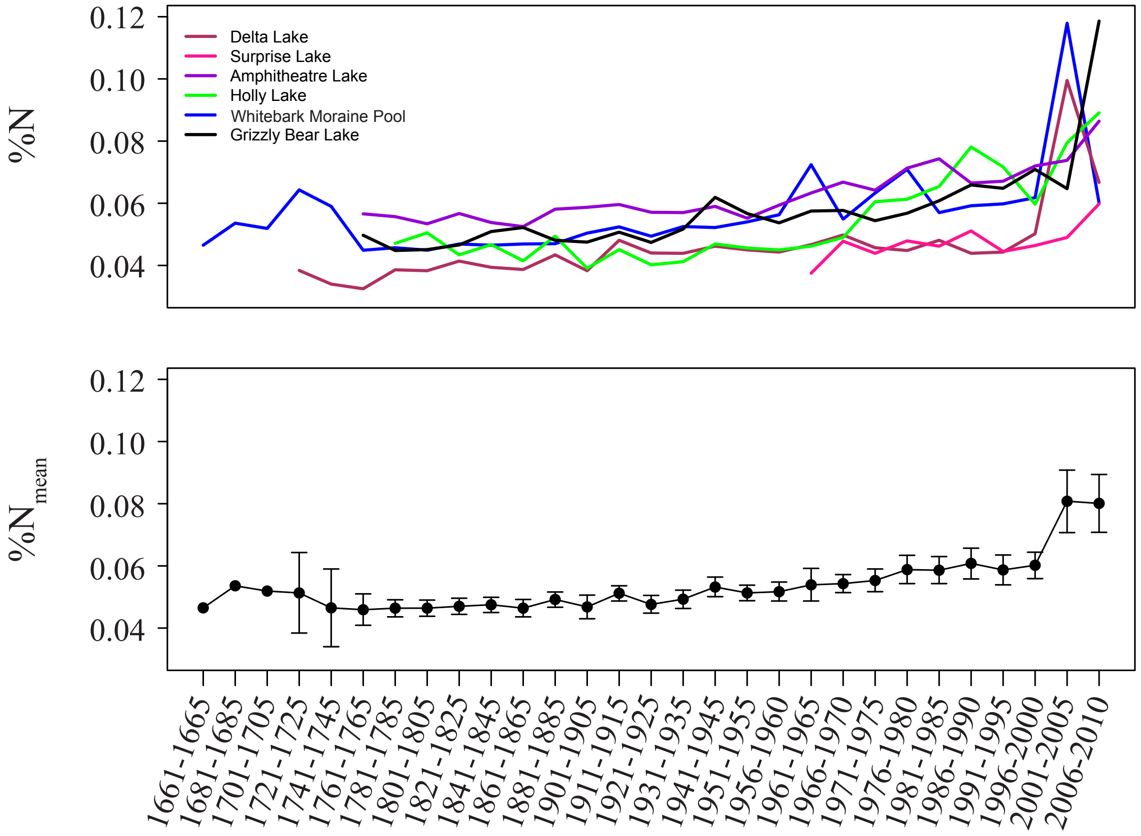


Figure 3.4. Percent nitrogen content and mean percent nitrogen content with standard error bars of tree ring lignin-cellulose collected from Engelmann Spruce (*Picea engelmanii*) around 6 lakes in the Grand Teton National Park, Wyoming, USA.

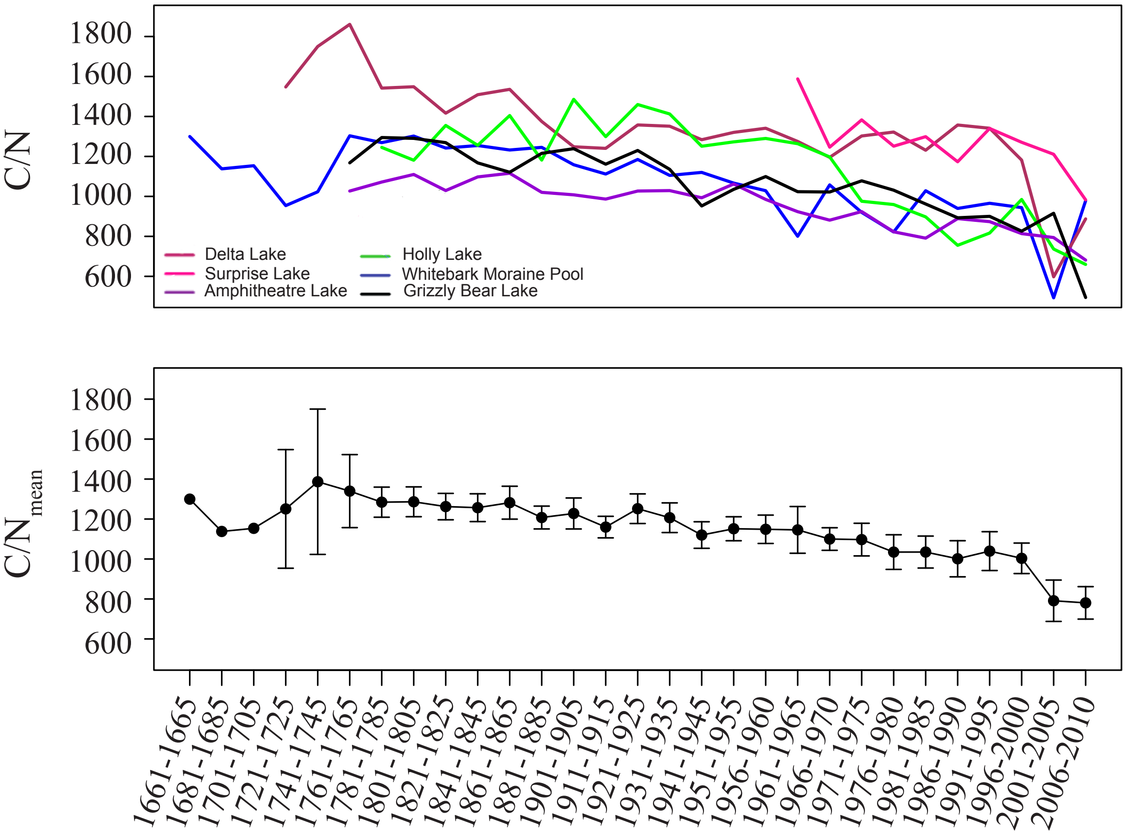


Figure 3.5. Molar carbon to nitrogen ratio (C/N) and mean C/N with standard error bars of tree ring lignin-cellulose collected from Engelmann Spruce (*Picea engelmanii*) around 6 lakes in the Grand Teton National Park, Wyoming, USA.

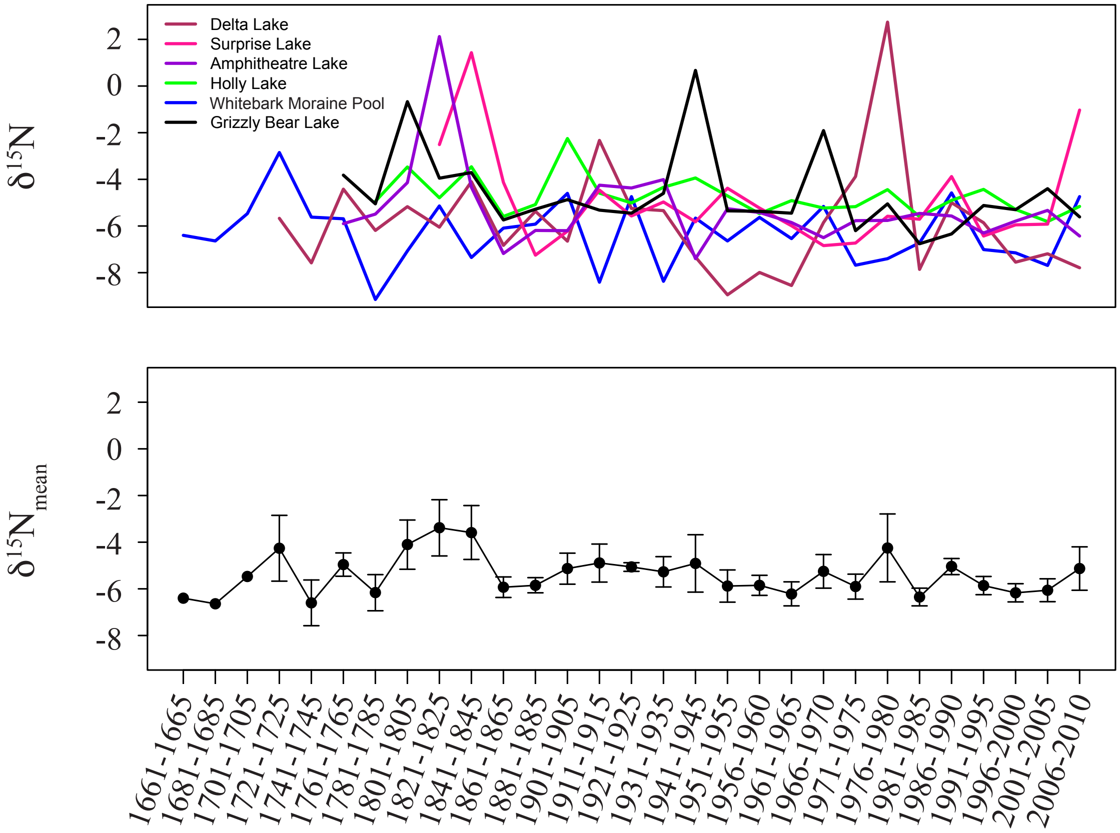


Figure 3.6. $\delta^{15}N$ and mean $\delta^{15}N$ with standard error bars of tree ring lignin-cellulose collected from Engelmann Spruce (*Picea engelmanii*) around 6 lakes in the Grand Teton National Park, Wyoming, USA.

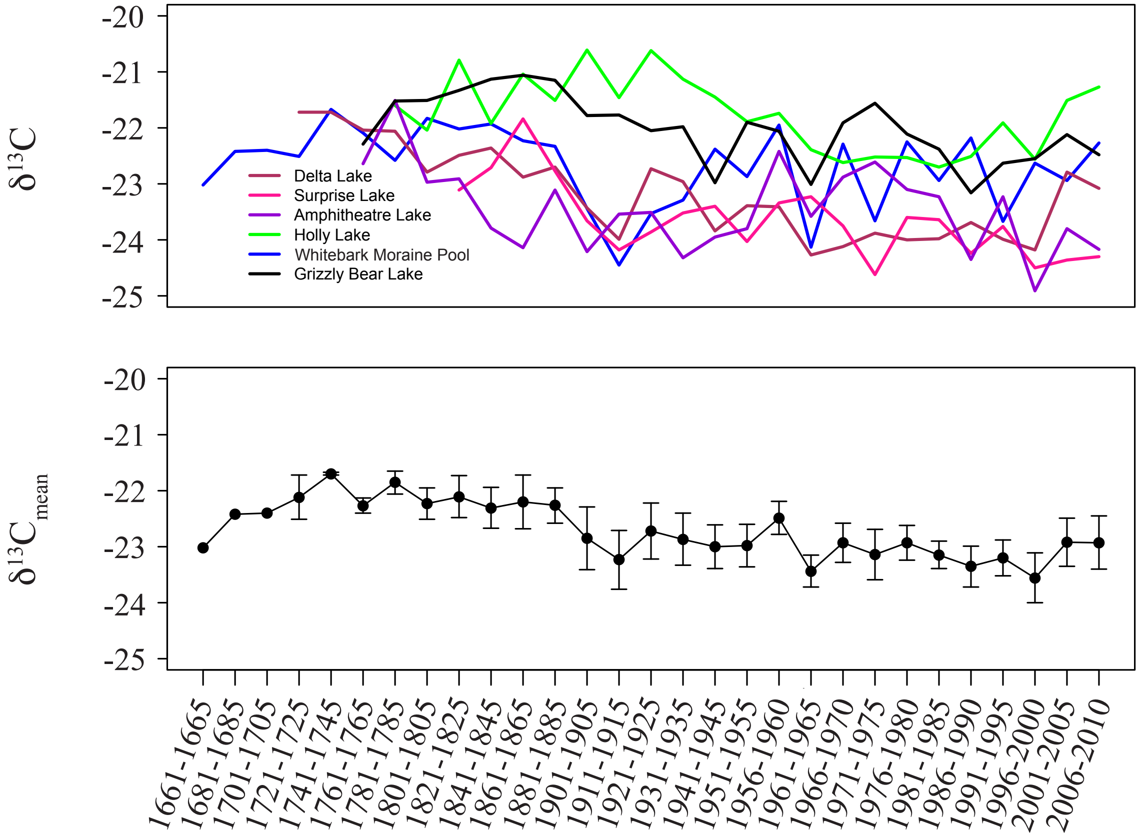


Figure 6. $\delta^{13}C$ of tree ring lignin-cellulose collected around 6 lakes in the Grand Teton National Park, Wyoming, USA, and mean $\delta^{13}C$ with standard error bars.

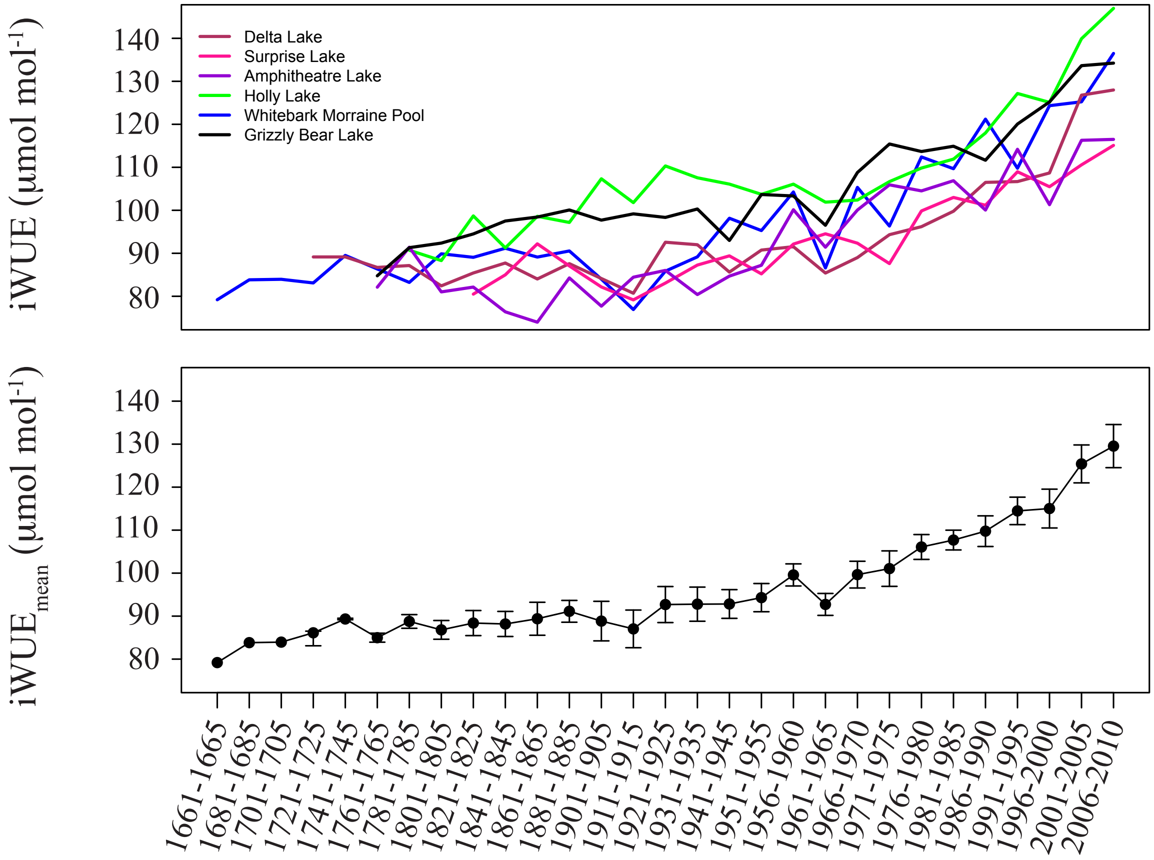


Figure 3.8. Intrinsic water-use efficiency (iWUE) and mean iWUE with standard error bars measured in µmol mol^{-1} of tree ring lignin-cellulose collected from Engelmann Spruce (*Picea engelmanii*) around 6 lakes in the Grand Teton National Park, Wyoming, USA.

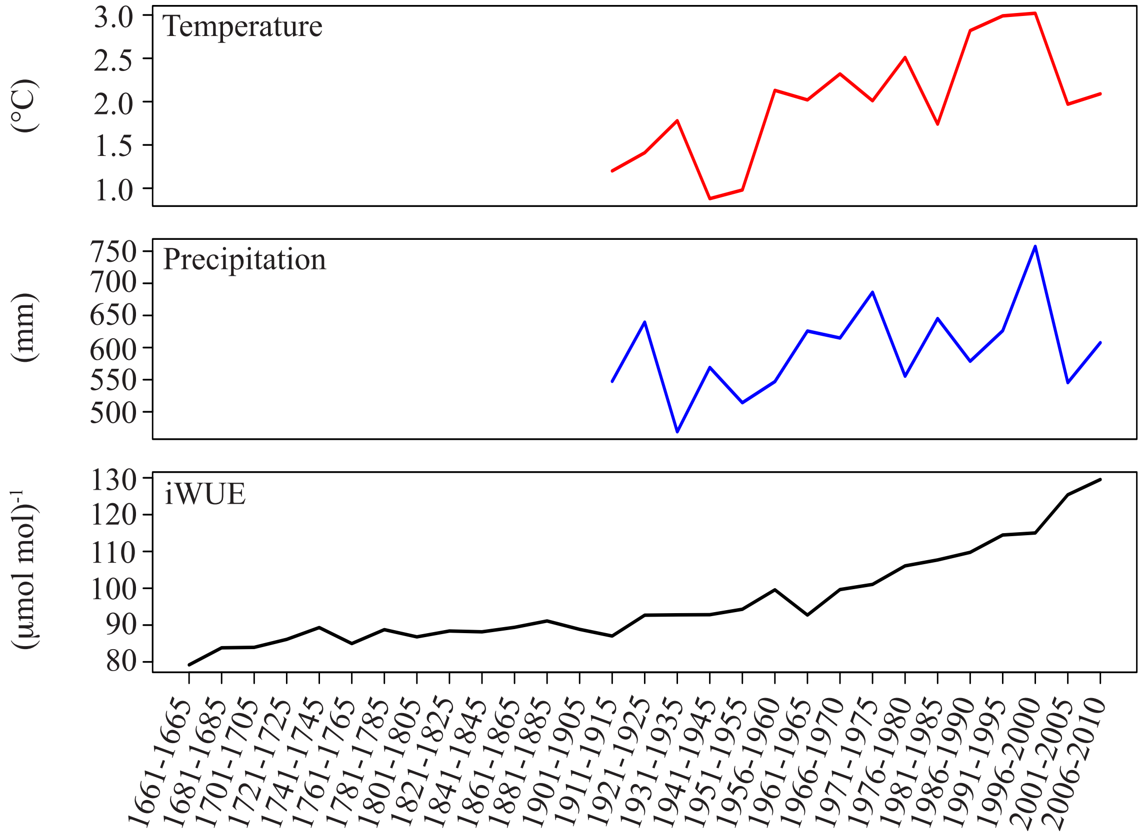
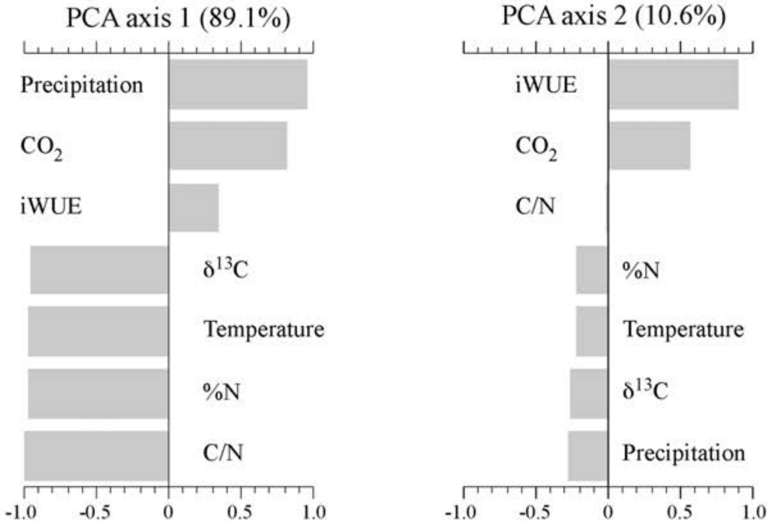


Figure 3.9. Mean annual temperature measured in degrees Celsius and mean annual precipitation measured in millimeters collected at the Moran climate station in the Grand Teton National Park, and mean water-use efficiency measured in μmol mol^{-1} from tree ring lignin-cellulose collected from Engelmann Spruce (*Picea engelmanii*) around 6 alpine lakes in the Grand Teton National Park.

PCA parameter loadings



PCA sample scores

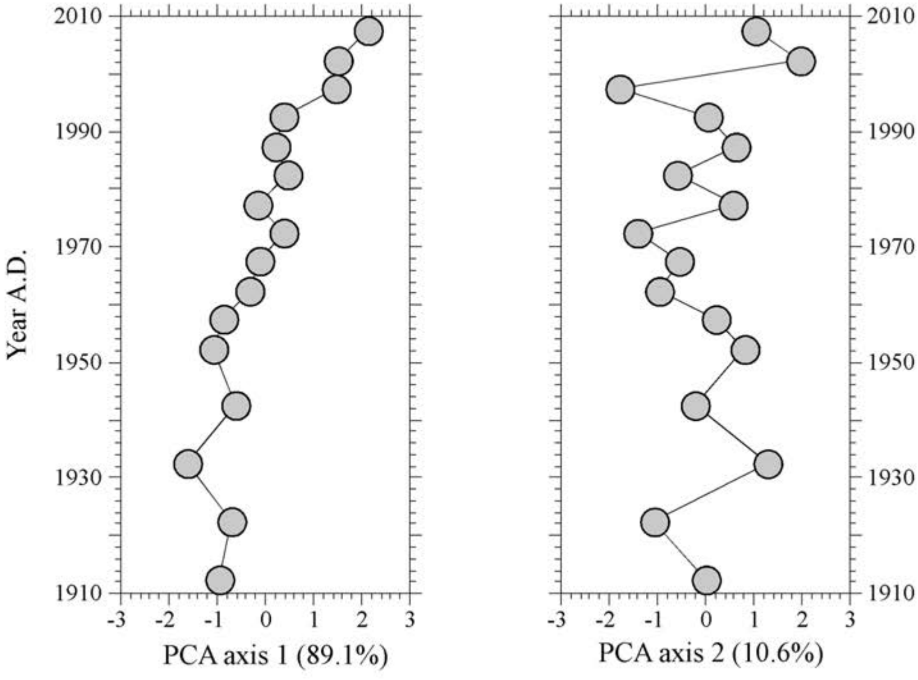


Figure 3.10. Principle component analysis (PCA) for mean iWUE, [CO_2], C/N, Temperature, Precipitation, %N, and $\delta^{13}C$ in 5-years bins through time from Engelmann Spruce (*Picea engelmanii*) trees collected around the Grand Teton National Park, Wyoming, USA.

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Chapter 4: Conclusions

4.1. Summary of Work

The data presented in Chapters 2 and 3 depict contrasting results with respect to the cumulative effects of anthropogenic influences on atmospheric composition. This thesis was successful in answering several of the objectives put forth at the outset, namely:

a) Are tree rings reflecting ecosystem Nr shifts in their $\delta^{15}N$ signatures, and, if so, are the shifts in the nitrogen cycle reflected in the $\delta^{13}C$ as well?
b) Is there evidence of anthropogenic Nr deposition in the relatively isolated and protected GTNP?
c) What is the evidence of disruption in the N and C cycles from pollution associated with energy development in the AOSR and, if so, what is their spatial extent?
d) What are the dominant influences governing ecological shifts in both regions?

However, the results also draw attention to some of the inherent limitations associated with stable isotope dendrochemistry as an environmental proxy. The data presented in Chapters 2 and 3 reveal contrasting results concerning the cumulative effects of anthropogenic atmospheric emissions, which is perhaps not surprising given their contrasting environmental contexts, as defined from the outset.

4.1.1. Atmospheric Nr deposition in the AOSR

Tree cores from the AOSR did show changes in the percent nitrogen and $\delta^{15}N$ since the beginning of oil sands activity in the 1960s. The changes present in the C/N ratio and the $\delta^{15}N$ is evidence of shifting ecosystem nutrient dynamics due to the magnitude of regional Nr deposition. The increasing C/N may be indicative of nearing ecosystem N saturation. However, as there was no evidence of physiological shifts in the tree due to nitrogen deposition, I was unable to determine how trees are reacting to the biogeochemical shifts occurring around them. Unfortunately, the needle and twig studies were unsuccessful in delimiting the spatial extent of N deposition. Yet they did highlight heterogeneity in nutrient cycle dynamics within subregions. This may be due to background landscape heterogeneity present in the area or due to uneven canopy interactions with atmospheric deposition, variable soil development, and other influences including local hydrology and groundwater dynamics.

4.1.2. Rising atmospheric [CO_2] and climate change in the GTNP

Dendrochemical data from the Grand Teton National Park show evidence of increasing moisture constraints in association with increasing atmospheric CO_2 concentrations. Quantitative increases of intrinsic water-use efficiency derived from C stable isotopes, especially within the second half of the 20th century, provide evidence of ecophysiological changes within forest trees in relation to changing atmospheric composition. However, tree-ring data show inconclusive evidence of the direct effects of atmospheric Nr deposition. Although the percent nitrogen value of tree rings increased with time, a similar trend was not present in the $\delta^{15}N$ values as would be expected from external input into the nitrogen cycle. In this ecosystem, tree ring $\delta^{15}N$ was not capable of successfully identifying changes in the nitrogen cycle. This provides important constraints on the interpretation of recent N-isotopic excursions in the lake-sediment record, which reveal marked excursions (~2 ‰) towards lighter values since the mid-20th century.

4.2. Recommendations for Future Dendrogeochemical Studies

For future studies, a more thorough ecological evaluation would provide more conclusive answers into shifts in nutrient cycling borne out by isotopic measurements. Tree ring width analyses may provide additional insights into the response of tree growth to the shifts observed in the geochemistry and would represent a valuable comparison to the inferences from the isotopic record. In the AOSR, tree ring width analyses may provide additional evidence as to whether the increase in nitrogen in the ecosystem is stimulating tree growth, or whether additional compounds associated with oil sands emissions are having detrimental effects on tree growth. In the GTNP, tree ring width analyses may prove helpful in understanding whether physiological shifts of intrinsic water-use efficiency may compensate for water stress associated with increased atmospheric CO_2. Soil samples would provide useful comparative values for source $\delta^{15}N$ and C/N. In the AOSR, this might help explain the spatial variability evident in the data from twigs and needles. In the GTNP, a similar approach would help in determining the ultimate effects of subtle enhancement of Nr deposition in the region.

4.3. Dendrochemical Insights for the Planetary Future

Both of the case studies considered here show evidence of the influence of atmospheric emissions with respect to ecosystem dynamics and nutrient cycling. Increasing industrial activity and agricultural intensity, and their associated atmospheric emissions, are likely to amplify the interactions inferred from the tree-ring record of the last ~50 years. The dendrogeochemical data presented suggest that in areas proximal to anthropogenic activities, such as the AOSR, Nr deposition has the greatest effect on ecosystem dynamics, shifting soil C/N and δ^{15}N. The influence of Nr overrides the impact of other environmental changes, such as atmospheric CO_2 and attendant climate shifts. In regions more distal to point-source anthropogenic emission, ecosystems appear to be more sensitive to the secular, long-term changes in atmospheric CO_2 and temperature. However, Nr deposition may also be linked to declining water availability, implying that iWUE will continue to increase in the GTNP, perhaps foreshadowing synergistic effects in the AOSR should water availability be impaired directly or indirectly in the future.

Dendrogeochemistry can be a valuable tool in long-term environmental monitoring. Used alone, its limitations prevent a fully rounded appreciation of changes in ecosystem dynamics, but when combined with soil, lake-sediment, and/or tree ring width analyses, the information in dendrogeochemical time-series become more powerful, especially given their tight chronological dimension. Futhermore, dendrogeochemistry records both nutrient dynamics within the soil and physiological responses to the atmosphere, and as such should be considered a powerful tool in paleoenvironmental reconstruction.