Tree Cores for Root Bores:
Exploring Tree Rooting Behaviour in Bituminous Soils

by

Marc La Flèche

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

Master of Science

In

Forest Biology and Management

Department of Renewable Resources
University of Alberta

Abstract

Surface mining in the Athabasca Oil Sands region has disturbed approximately 895 km^2 of boreal forest, all of which will need to be reclaimed to a state that is both representative of, and as productive of the surrounding boreal forest. However, much remains to be understood regarding the effects of different reclamation practices on restoring forests on mined landscapes. Reclamation practices, though varied, can involve the construction of landforms using overburden materials such as lean oil sand (LOS, sand containing concentrations < 8% hydrocarbons) with a layer of appropriate soil medium placed on top. While some early studies suggest that there may be negative effects of LOS on tree growth, forests have been growing on natural shallow bitumen deposits since the last ice age. The goal of this thesis is to explore whether using dendrochemical methods can allow us to better understand the relationship between tree rooting behaviour and shallow bitumen. Soil, soil pore water, and tree cores were taken from sites with natural shallow bitumen deposits and analyzed for trace metals enriched in bitumen (vanadium, nickel, molybdenum, and rhenium). Samples were also collected from sites without bitumen present. Significantly elevated concentrations of Ni were observed in trees growing on shallow bitumen deposits. Vanadium was also elevated in trees on bituminous sites though not significantly so. Molybdenum displayed the opposite trend and decreased in trees on bituminous sites. A baseline survey was also conducted on a reclaimed landform constructed using LOS to establish a proper reference against which future research can compare. My thesis establishes that with strong analytical methods and good reference conditions, dendrochemistry is a feasible methodology for monitoring root growth in bituminous soils with V and Ni acting as potential signal elements. This possibility presents reclamation practitioners and researchers with a useful tool to monitor plant growth across both constructed landforms and natural landscapes along with strong data against which to compare future values.

Preface

No data from this thesis has been published prior to thesis defense and publication.

Acknowledgements

First and foremost, I would like to thank my supervisor Dr. Justine Karst. I will be forever grateful she took a chance on me and I would not have been able to complete this project without her guidance, patience, and insights. It was not easy stepping back into the academic sphere after time away, but her open door made it much less daunting. Secondly, I would like to thank Dr. William Shotyk for his time as a committee member and for invaluable input on the chemistry behind this research. I would also like to thank Dr. Sylvie Quideau for chairing my examining committee.

A large thank you goes out to our industry partners from COSIA. I'd like to thank Rob Vassov for the time he took to show me around their reclamation projects up north and his excellent knowledge and ideas as my study plan took shape. This work was funded by an NSERC Industrial Research Chair awarded to Justine Karst in collaboration with Canada's Oil Sands Innovation Alliance.

To the Karst Lab: you folks rock. I couldn't imagine a more interesting group of people to slog through grad school with. It has been a true pleasure roping you all into weird activities. I would also like to thank Dr. Jon Cale for convincing me to pursue an MSc. His enthusiasm and passion for research proved just infectious enough to get me to apply and then, coupled with his advice, to help me through my degree. I am grateful for the incredibly skilled help, from DNA to trace metal chemistry, I received from Pak Chow, Dr. Chad Cuss, and Tommy Noernberg.

Lastly, I want to thank my friends and family for all their love and support over the seemingly endless years of school. To my parents Sharon and Gilles, and to my brother Paul; Thank you.

Table of Contents

List of Figures

List of Tables

Chapter 1: General Introduction

The Athabasca Oil Sands region spans 93,000 km^2 across north-eastern Alberta with 3% of that area (4,750 km^2) accessible through surface mining operations (Government of Alberta, 2017). Currently, an area of approximately 895 km^2 has been disturbed by mining operations, and this land will need to be reclaimed to a state of productivity equivalent to that of adjacent undisturbed land (Government of Alberta, 2014, 2017). In practice, this policy means that self-sustaining boreal forests and wetlands must be returned to the landscape following mining. Reclamation practices, though varied, can involve the construction of landforms using overburden such as lean oil sand (LOS, oil sand containing concentrations of hydrocarbons < 8% of bulk soil volume) under a layer of appropriate cover soil (Rowland, Prescott, Grayston, Quideau, & Bradfield, 2009; Visser, 2008). These upland landforms are subsequently revegetated to promote the establishment of forests typical of the region. Early studies show that plant growth, and ultimately reclamation success, may be negatively impacted by the presence of LOS in the rooting zone of plants. Previous studies show that LOS could:

1. Act as a physical barrier to root growth through the creation of a compacted textural break in the constructed soil profile (Jung, Duan, House, & Chang, 2014; Pernitsky, Hu, Si, & Barbour, 2016).
2. Alter the moisture regime either through textural discontinuities or hydrophobicity (Huang, Barbour, Elshorbagy, Zettl, & Cheng Si, 2011; Huang, Spies, Barbour, Si, & Zettl, 2013; Jung et al. 2014; Roy, McGill, Lowen, & Johnson, 2003).
3. Be toxic to plants and inhibit growth, though the mechanisms are poorly understood (Leskiw, 2005, 2006; Visser, 2008, 2011).

Much research has been conducted on the above-ground performance of plants used to revegetate reclaimed landforms across different soil profiles (Barber, Bockstette, Christensen, Tallon, & Landhäusser, 2015; Macdonald et al., 2015; Pinno, Landhäusser, MacKenzie, Quideau, & Chow, 2012; Rowland et al., 2009). However, little is known about tree root growth in the presence of shallow LOS. Understanding how roots develop in and around LOS can help inform reclamation practices to better promote root growth, which can promote forest establishment (Plourde, Krause, & Lord, 2009). With early research focusing on the potential negative effects LOS has on plant growth, it is important to note that shallow (< 50 cm) bitumen deposits also occur naturally in this region (Leskiw, 2005, 2006). Forests have been growing on these deposits since the last Ice Age (Leskiw, 2005, 2006) and could potentially provide a reference ecosystem for research on the effects of LOS on forest growth.

There are, however, some key differences between reclaimed LOS landforms and natural deposits of bitumen. Lean oil sand used in reclamation can originate from up to 70 m below the surface and therefore has not undergone the degree of weathering that natural shallow deposits have (Visser, 2008, 2011). After weathering, heavier petroleum hydrocarbon fractions are left, which are not relatively stable and not readily bioavailable (Brassington et al., 2007; Maletić, Dalmacija, Rončević, Agbaba, & Perović, 2011). Though total petroleum hydrocarbon concentrations may be similar between LOS and natural bitumen deposits, differences in the profile of compounds is ecologically relevant. Specifically, soils with a higher concentration of lighter hydrocarbon fractions may be more of a concern than soils with otherwise similar total hydrocarbon concentrations, but abundant in heavy fractions. In addition to differences in hydrocarbon profiles, the LOS on a reclaimed landform would present as a uniform layer at a given depth. Natural deposits are typically more variable in structure presenting as tar balls, blocks, or bands throughout the soil profile (Leskiw, 2005, 2006). This natural heterogeneity may create pockets of hydrocarbon-free soils allowing roots to avoid interacting directly with the bitumen present in the natural deposits should hydrocarbons have detrimental effects on growth. The challenge then rests in determining if tree roots are interacting with shallow bitumen deposits and understanding the outcome of these interactions on plant growth.

My thesis research explores dendrochemistry as a potential method of determining whether tree roots interact with bituminous soil. Dendrochemical techniques analyse the chemical composition of tree tissues to monitor local pollution events and ambient environmental conditions through time (Watmough, 1997; Watmough, 1999). These analyses are not without their challenges. Different elements can behave differently within different species of plants (Cutter & Guyette, 1993; Watmough, 1997, 1999). For instance, an element can behave differently (e.g., concentrate in certain tissues or absorb at different rates) in one tree species compared with another (Cutter & Guyette, 1993; Watmough, 1997, 1999). Similarly, within the same species of tree, different elements can also display contrasting behaviours. For example, Hutchinson, Watmough, Sager, and Karagatzides (1998) demonstrate positive correlations between wood and foliar concentration increases for some metals in sugar maple (*Acer saccharum*) and no correlation for others following the application of an acidifying fertilizer. These results suggest that some metals are more mobile within the boles of trees than others and different patterns of translocation may occur.

In my first data chapter, I explore the feasibility of using dendrochemistry to determine rooting behaviour in naturally occurring shallow bitumen deposits. I demonstrate that trees growing on soils containing bitumen display elevated concentrations of target elements compared to trees rooting in soil without bitumen present. In my second data chapter, I conduct a baseline survey of a reclamation landform constructed using LOS. The survey provides reclamation practitioners with baseline values of hydrocarbon and trace metal concentrations to monitor the rooting behaviour of trees over time.

Chapter 2: Trace Metals as Indicators of Tree Rooting Behaviour in Bituminous Soils

2.1 Introduction

The placement and distribution of roots may reflect resource availability (Campbell, Grime, & Mackey, 1991), competition (Bockstette, Pinno, Dyck, & Landhäusser, 2017), textural or chemical barriers (Jung et al., 2014), and even 'decision-making' by plants (McNickle & Cahill, 2009). Sophisticated methods to track or locate roots such as the use of tracers or rhizotrons exist, however, soil cores and excavations remain some of the most common ways to sample roots (Jackson et al., 1996; Schenk & Jackson, 2002). Locating roots of individual trees through excavation methods is an arduous, time-consuming, and destructive activity (Böhm, 1979). As plants have been shown to pass through, leave, or avoid substrates not conducive to growth (McNickle & Cahill, 2009), excavations and visual assessments may not provide an accurate representation of the substrates with which roots are interacting. Chemical analysis of aboveground tree tissue, i.e., dendrochemistry, may be a simple and effective method to determine whether tree roots are interacting with a target substrate within the soil profile.

Dendrochemistry is a growing branch of science using tree tissues to monitor changes in soil and atmospheric chemistry over time as trees can incorporate trace metals, organic pollutants, nutrients, and other elements of interest into their tissues at concentrations relative to those in its environment. (Cutter & Guyette, 1993; Märten, Berger, Köhler, & Merten, 2015; Watmough, 1997, 1999). There are, however, some challenges to using trees as bioindicators of some abiotic environmental conditions beyond climate. For example, the selection of tree species can be a major factor in the efficacy of dendrochemical studies (Cutter & Guyette, 1993; Watmough, 1997); physiological differences between species can result in different behaviours of different metals within individual trees. The physiological structure of heartwood versus the sapwood, xylem chemistry, and specific metal chemistry can affect the distribution of trace metals within a tree after absorption through the root system, foliage, or bark (Cutter & Guyette, 1993; Lepp, 1975; Watmough, 1997). With processes such as translocation within the tree making precise chronological record-keeping difficult, dendrochemical methods might be better used to observe coarse temporal trends, and as a surveying tool (Dunn, 2007; Pulford & Watson, 2003; Padilla & Anderson, 2002; Watmough, 1997). The successful use of these methods to survey for mineral deposits (Dunn, 2007) suggests it may be possible to utilize dendrochemistry to determine whether a tree interacts with a target substrate in the soil profile. Here, I use this approach to determine the potential of trace metals to act as indicators of tree rooting behavior in soils containing shallow, natural deposits of bitumen.

Northeastern Alberta, Canada, sits on one of the largest bitumen deposits in the world (~142,000 km^2) (Government of Alberta, 2017; Leskiw, 2006), which is mined for its ore. Within the region, natural shallow deposits of bitumen (< 3 m deep) exist within the soil profile in the form of tar balls, bands, and larger blocks (Leskiw, 2006). These deposits are believed to originate from a combination of glacial erosion and depositional events, including a major flood event from the release of glacial Lake Agassiz into the Clearwater and Athabasca waterways (Fisher & Smith, 1994; Leskiw, 2006). This flood event carved into the geology of the region exposing, and potentially depositing, portions of the McMurray formation at the surface. The bitumen itself originates from Mississippian and Jurassic age oil deposits which have been degraded by microbes into a semisolid mixture of oil, sand, clay, and trace elements (Zhou, Huang, & Liu, 2008). The organic fraction of bituminous sand is characteristically enriched in vanadium (V), nickel (Ni), molybdenum (Mo), and rhenium (Re) (Bicalho et al., 2017; Goldschmidt, 1937; Selby & Creaser, 2005). While all four metals can be mobile in plants, their relative availability can differ greatly depending on site conditions (Greger, 2004; Pendias & Kabata-Pendias, 2000).

Vanadium shows promise as an indicator of root behaviour in bituminous soils. Bicalho et al. (2017) confirmed early findings that of the four metals, V is the most enriched in bitumen (242 ppm for V compared to 83.3 ppm, 10.4 ppm, and 0.06 ppm for Ni, Mo, and Re, respectively). While not an essential element to plant growth, V can be taken up by plants in the form of vanadate (VO_3^-, a compound of V(V)) or more easily as vanadyl (VO^{2+}, a compound of V(IV)) when pH conditions are neutral to slightly acidic and in reducing conditions (Huang et al., 2015; Greger, 2004; Imtiaz et al., 2015; Schlesinger et al., 2017; Welch, 1973). Evidence suggests that the plants themselves can reduce V(V) to V(IV) during the uptake process (Morrell, Lepp, & Phipps, 1986). Hargesheimer, Apps, and Malhotra (1980) demonstrate that aerial deposition of V due to mining operations in the region was detectable when sampling the needles of jack pine (*Pinus banksiana*), though it is unclear if V was absorbed into the needles or was adhered to their surfaces. With aerial deposition affecting metal concentrations in or on the needles of trees, measuring internal wood tissue instead may be a viable way to infer if roots are taking up V from the soil (Dunn, 2001). However, its availability in soils for root uptake can be quite difficult to predict due to the ability of V to form diverse organic and inorganic compounds, its sensitivity to redox transformations, and the overall complexity of its chemistry (Huang et al., 2015). Further, Mikkonen and Tummavuori (1994) found that V can be immobilized by organic compounds in soils. These findings suggest that while V may be enriched in bitumen, its availability to plants may be limited by the bitumen itself.

Nickel also shows promise as an indicator of tree rooting. Nickel is mostly absorbed in its ionic (Ni^{2+}) form through roots of plants (Cataldo, Garland, & Wildung, 1978; Mishra & Kar, 1974; Nieminen, Ukonmaanaho, Rausch, & Shotyk, 2007). Within the soil, its solubility, and therefore availability to plants, is inversely related to soil pH (Pendias & Kabata-Pendias, 2000). It is a critical component to the compound urease in plants, which aids in the hydrolysis of urea and the incorporation of N (Brown, Welch, & Cary, 1987; Eskew, Welch, & Cary, 1983). While Ni is an essential element to plant growth due to its importance in N metabolism, it can also become toxic to many plants in high concentrations (Brown et al., 1987; Eskew et al., 1983; McIlveen & Negusanti, 1994). This may lead plants to actively regulate its uptake and incorporation into tissues. Thus, it is possible, though not definitive, that Ni could signal rooting in bituminous soils.

Molybdenum is an essential micronutrient for plants used as a component of nitrogenase and nitrate reductase (Greger, 2004; Pendias & Kabata-Pendias, 2000). It is absorbed most often in its anionic form as molybdate (MoO_4^{2-}) under oxidizing conditions. Concentrations of Mo in plants have been shown to be representative of the soluble Mo in soils, however, low soil pH (< 4.5) can greatly restrict its solubility (Pendias & Kabata-Pendias, 2000; Sigel & Sigel, 2002). Last of the enriched metals, Re is a rare-earth element occurring in low concentrations (0.196 ppb) in the Upper Continental Crust (UCC) (Greger, 2004; Pendias & Kabata-Pendias, 2000; Rudnick & Gao, 2014). Being a relatively rare element, Re is not well studied but appears to be readily available to plant uptake in its anionic form (ReO_4^-) and is moderately mobile once within the plant body (Greger, 2004; Pendias & Kabata-Pendias, 2000; Shinonaga, Pröhl, Müller, & Ambe, 1999).

In this study, I analyzed trace metal concentrations in soil, soil pore water, and tree cores to determine if they can indicate the rooting behaviour of trees in bituminous soils. If fine roots are produced within the bitumen and passively uptake trace metals upon water transport and wood growth, metals enriched in bitumen should be present in higher concentrations in the woody tissue of trees growing in bituminous soils than in trees growing in bitumen-free soils. To test this prediction, I addressed the following research questions: 1) Does the presence of bitumen in the soil profile affect root distribution? 2) Do soils with shallow bitumen deposits have elevated concentrations of V, Ni, Mo, and Re in both total soil and soil pore water concentrations than do similar soils without bitumen? 3) Does the wood of trees growing above shallow bitumen deposits contain higher concentrations of V, Ni, Mo, and Re than those growing above similar soils without bitumen? If a correlation exists between the presence of these trace metals in soils and within the tree, dendrochemical analysis could present a less destructive and more accurate method of determining whether tree roots interact with shallow bitumen deposits. The findings from this study will help expand our understanding of how trees root and interact with different soil substrates and may have implications for the reclamation and remediation of constructed landforms and contaminated sites.

2.2 Methods

2.2.1 Site Selection

Candidate field sites were located during the summer and fall of 2017 using a combination of GIS and field surveys. GIS data from the Alberta Vegetation Inventory (AVI) were used alongside GPS locations from earlier surveys by Leskiw (2005, 2006) to locate candidate stands of jack pine (*Pinus banksiana* Lamb.) where shallow bitumen deposits were likely to be present. I selected jack pine as the focal species because it is long-lived, maximizing the potential for root-bitumen interaction through time, maintains solid heartwood, and it is typically found on sandy soils, facilitating soil and root sampling.

Surveys of candidate sites were conducted to verify the presence of shallow bitumen deposits and of jack pines at least 15 cm in diameter at breast height (DBH). Surveys consisted of a minimum of four test pits (50 cm x 50 cm x 50 cm) dug at each site to confirm the presence of shallow bitumen (Fig. 2.1). From these surveys, I found five sites with shallow bitumen deposits (B1-5) north of Fort McMurray, Alberta, and two sites without bitumen (R1-2) ~200 km south of Fort McMurray, Alberta (Fig. 2.2). All sites are considered Mid-Boreal Upland ecotypes within the Boreal Plains ecozone (Agriculture Canada, 2019a). Soils with bitumen were classified as Eluviated Dystric Brunisols in the Mildred soil series (Turchenek & Lindsay, 1982). Soils without bitumen were also Brunisols, but of an unknown series (Agriculture Canada, 2019b). Average temperatures in the region range from 17.1˚C in July to -17.4 ˚C in January with an annual average of 1 ˚C. The region averages approximately 420 mm of precipitation annually (Environment Canada, 2019).

2.2.2 Field Survey

In 2017, a 10 m x 10 m plot was established at each site. Within the plot, five mature (DBH > 15 cm) trees were cored at 1.3 m of height using a 12 mm increment borer (Suunto, Vantaa, Finland) for metals analysis and a 4 mm borer (Haglöf, Sweden) for aging, analysing growth patterns, and cross-dating. Two sets of soil cores were collected a minimum of 5 m apart within the estimated shared rooting zones of the selected trees. Each set consisted of three separate cores with soil collected using a 6.5 cm x 15 cm slide hammer soil corer (AMS, American Falls, Idaho, USA) in four 10 cm depth increments to 40 cm total depth (i.e., approximate rooting zone). A plastic insert was used when sampling soil for trace metals and samples were quickly transferred to plastic bags to minimize contact with the metal soil corer. Soil collected for hydrocarbon analysis was packed tightly with minimal headspace into glass jars with Teflon-lined lids and stored in coolers on ice for transport to minimize hydrocarbon volatilization. The remaining soil core was collected for soil texture analysis, and root biomass and DNA identification with depth. A soil pit was dug at a central location within the site to obtain soil moisture, soil electrical conductivity, and temperature at 10 cm depth intervals along the soil profile (ProCheck GS3 Soil Moisture Probe, Decagon Devices, Pullman, Washington, USA). Plant species at each site were also recorded to provide a reference for the molecular identification of roots (see below).

To investigate dissolved (plant available) trace metal concentrations, custom lysimeters were used to collect soil pore water from two locations near the soil cores within each site in 2018. The lysimeters were constructed using surgical stainless steel (316 ss) and were approximately 15 cm x 2 cm with the upper 7.5 cm consisting of a 5 µm filter. Prior to deployment in the field, each lysimeter and all associated sampling equipment was thoroughly cleaned and underwent a bath in double-distilled (2D), trace-metal grade HNO_3 in the Soil, Water, Air, Manure, Peat (SWAMP) ultra-clean laboratory at the University of Alberta as described in Du et al. (manuscript in preparation). Complete sampling packages containing one lysimeter, two 0.45 µm polytetrafluoroethylene (PTFE) filters, two polypropylene syringes, and two polypropylene 50 mL centrifuge tubes were individually packaged within the ultra-clean lab. In the field, each lysimeter was installed by first collecting a soil plug to 30 cm of depth. The lysimeter was then inserted into the hole, a steel casing was installed around it, and 500 mL of Type I MilliQ water was added and allowed to soak for 30 minutes. A vacuum was then applied to the lysimeter for 20 minutes before the collected water was extracted and filtered through a 0.45 µm filter to collect the dissolved fraction for analysis. The detailed sampling protocol, modified from Du et al. (manuscript in preparation), is described in Appendix I.

2.2.3 Soil Texture and Chemistry

The second set of soil samples collected from the field sites were sieved through nested sieves (4 mm–2 mm) to remove roots. Samples were then homogenized and subsampled using a spinning riffler (SP- 230, Gilson Company Inc., Lewis Center, Ohio, USA). Particle size analysis was conducted by the Natural Resources Analytical Library (NRAL) at the University of Alberta using a Laser Diffraction Particle Size Analyzer (LS 13 320, Beckman Coulter, Brea, USA). I measured the pH of saturated soil pastes of 10 g of soil mixed with 10 mL of certified pH 7 HPLC grade water (Fisher Scientific, Hampton, New Hampshire, USA) using a portable pH meter (accumet AP110, Fisher Scientific, Hampton, New Hampshire, USA) to account for abiotic conditions affecting trace metal speciation.

2.2.4 Identification and Mass of Roots with Depth

Sieved roots were lyophilized for approximately 72 hours using a benchtop freeze dryer (Labconco FreeZone 2.5, Kansas City, USA), weighed, and then pulverized using a TissueLyser II (Qiagen Inc, Hilden, Germany) for two minutes at 30 rotations per second. Total genomic DNA was then extracted using DNeasy PowerSoil Kits (QIAGEN, Hilden, Germany). To purify the extracted DNA further, each sample underwent an additional ethanol precipitation step with the use of 125 mM ethylenediaminetetraacetic acid mixed with 3 M sodium acetate (EDTA/NaOAc) as a precipitating agent to remove impurities.

Three non-coding cpDNA regions (the *trn*T-*trn*L intergenetic spacer, the *trn*L intron, and the *trn*L-*trn*F intergenetic spacer) were then targeted using fluorescently labelled forward primers (A2_FAM; C_VIC; E_NED) established by Taberlet, Gielly, Pautou, and Bouvet (1991). Each region was amplified via polymerase chain reaction (PCR) in 25 μL volumes consisting of: 12.5 μL of EconoTaq PLUS 2X Master Mix (Lucigen Corp., Middleton, USA), 2.5 μL of each forward and reverse primer at 10 μM, 5.5 μL nuclease-free water, and 2 μL of diluted (5-10 ng μL^{-1}) DNA. Negative controls were added and included in each step from extraction to PCR along with positive controls to monitor for contamination and effectiveness of the PCR. Reaction conditions followed those used by Metzler, La Flèche, and Karst (2019): 94°C for five minutes, followed by 35 cycles of 94°C for 60 seconds, 60°C for 60 seconds, 72°C for 80 seconds and a final extension of 72°C for 30 minutes.

Samples were then prepared for fragment length analysis by diluting the product 200x for both the *trn*L intron and the *trn*L-*trn*F intergenetic spacer, and 100x the *trn*T-*trn*L intergenetic spacer. Diluted samples were then prepared for sequencing combining 2 μL of diluted product mixed with 8 μL of Hi-Di formamide and 0.15 μL of GeneScan 1200 LIZ Size Standard

(Applied Biosystems, Foster City, USA). Capillary electrophoresis was used to resolve the amplified fragment lengths (ABI 3730 DNA Analyzer, Applied Biosystems, Foster City, USA). Fragments were then sized to the nearest base pair using GeneMapper 4.0 software (Applied Biosystems, Foster City, USA) by noting prominent peaks in relative fluorescent units (RFUs). Resolved fragment lengths were compared to those developed by Metzler et al. (2019) to identify roots occurring with depth.

2.2.5 Tree Cores

The 4 mm tree cores were mounted and sanded using sand paper to 600 grit prior to scanning. Separate growth chronologies were constructed for the bituminous and reference sites using CDendro (Larsson, 2018) to calculate standardised ring width across all trees. These chronologies were then compared to assess growth rates of trees between sites.

2.2.6 Hydrocarbon Analysis

To confirm the presence of bitumen, soil samples (7 sites x 2 cores x 4 depths = 56 samples) collected for hydrocarbon analysis were sent to Maxxam Analytics (Edmonton, Canada) for testing of F2, F3, and F4 fraction concentrations using the CCME Tier 1 Reference Method (CCME, 2001). These fractions represent hydrocarbons containing between 10 and 50 carbon atoms which are less easily weathered and volatilized than the F1 (nC6-10) fraction (CCME, 2001).

2.2.7 Trace Metal Analysis

Tree cores collected with the 12 mm borer were processed using methods similar to those described in Kirchner, Biondi, Edwards, and McConnell (2008). Each core was first separated into pre- and post-1967 segments to account for a potential increase in trace metal availability to tree tissue with the expansion of mining operations after 1967 (Mullan-Boudreau et al., 2017;

Shotyk et al., 2016). Exterior surfaces contaminated with V from the increment borer of each tissue segment were trimmed using a Kershaw Pro-Grade Breaking Knife (Model 1280GE, Tualatin, Oregon) previously tested to not contaminate samples with trace metals (Table I.1, Appendix I). Samples were processed on a polycarbonate cutting board with plastic wrap changed between each sample, and the board cleaned with a 10% ethanol solution between samples of each site to reduce the risk of cross-contamination. Tree core samples were dried at 105˚C for 36 hours before being analyzed at the SWAMP lab at the University of Alberta. There, samples were digested in double-distilled (2D) HNO_3 in a high-pressure microwave (Ultraclave, MLS, Leutkirch, Germany) and analyzed on an Inductively-Coupled Plasma Mass Spectrometer (iCAP Qc ICP-MS, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Soils samples (both cores and lysimeter plugs) were homogenized, randomly subsampled using a scoopula, and dried at 105˚C for 36 hours. Dried samples were then ground in a boron carbide mortar to pulverize the soil prior to digestion in nitric acid and analysis in the ICP-MS following the same methods for the tree core samples. The mortar and pestle were cleaned with Milli-Q water and 10% ethanol solution between each sample to reduce the risk of cross-contamination. Ultraclave digestions and ICP-MS analyses were conducted according to US EPA Method 3051A for soil and wood samples, and US EPA Method 200.8 for pore water samples (Campisano et al., 2007).

Procedural blanks were run with each batch in the ICP-MS (n = 3 per digestion batch). Standard Reference Materials with certified trace metals concentrations (NIST 1640a – Trace Elements in Natural Water, NIST 1547 – Peach Leaves, and NIST 2711 – Montana Soil) were used to assess analytical performance (n = 3 per digestion batch, recoveries averaged between 85-115%). Limits of detection and lysimeter blank values are documented in Appendix I.

2.3 Statistical Analysis

All statistical analysis was conducted in R (R Core Team, 2019) using the tidyverse packages tidyr, dplyr, ggplot2, and car (Wickam, 2019) along with the NADA package for censored data analyses (Lee, 2019).

2.3.1 Rooting Distribution

I compared the vertical root distribution between sites using the non-linear model $Y = 1 - \beta^d$ where Y represents the cumulative root fraction observed to depth d and β is the estimated parameter (Gale & Grigal, 1987). High values of β represent a rooting profile with roots distributed throughout depths, and lower values represent profiles where roots are concentrated closer to the surface. I then compared the β values between site type using a Welch two-sample t-test. To determine the relationship between root mass and hydrocarbon concentrations, I conducted a partial correlation test with depth as a controlling factor.

2.3.2 Trace Metals – Soil

To compare total metal concentrations across sites, concentrations of V, Ni, Mo, and Re were first standardised to chromium (Cr), a conservative lithophile element associated with mineral matter in soils, and which did not differ significantly between site type, by dividing each by Cr. Standardisation to a lithophile element was done to account for inter-site variation in total mineral concentrations. These ratios were subsequently divided by the average ratio of each metal to Cr in the UCC using values from Rudnick and Gao (2014) (UCC ratios are 1.054, 0.511, 0.012, and 2.15e-6 for V, Ni, Mo, and Re respectively) to obtain their enrichment factors ($EF = ([M]/[Cr]_{soil}) / ([M]/[Cr]_{UCC})$, where M represents the metal of interest) in the study soils. These EFs were compared across sites using a one-way ANOVA. Raw metal concentrations were also compared to total hydrocarbon concentrations (the sum of F2-4 fractions) using Kendall's Tau correlations for non-normal data to determine the strength of the relationship between the presence of bitumen and the concentrations of V, Ni, Mo, and Re in soil.

2.3.3 Trace Metals – Pore Water

Since the dissolved fraction of metals (< 0.45 μm filtered) was measured, these data were not standardised to another element to compare concentrations across sites. Differences in raw concentrations between the bituminous and reference sites were determined using one-way ANOVA. Vanadium concentrations were log transformed for improved normality. Relative availability of trace metals for plant uptake (soluble fraction) compared to their total concentrations in soil were calculated using the equation $\log(([M]/[Cr]_{water}) / ([M]/[Cr]_{soil}))$ as presented in Shotyk, Krachler, Aeschbach-Hertig, Hillier, and Zheng (2010).

2.3.4 Trace Metals – Wood

Two-way ANOVAs testing main and interactive effects of site and wood tissue age were conducted with a variety of transformations and data manipulations (log and rank transformations, raw [M], [M]/[Cr], substitution of LOD/sqrt(2), list-wise deletion of <LOD values) failed to display an interaction effect between wood age and soil type. Therefore, a one-way ANOVA was used to compare raw concentrations of Ni, and Wilcoxon Rank-Sum Tests were used for V and Mo (due to values below the limit of detection (LOD) present in the data) to compare values between sites. To complete the rank-sum tests, the LOD was substituted for the censored values for V and Mo. Rhenium was not detected in any wood sample and is therefore excluded from this portion of the analysis.

2.4 Results

2.4.1 Site Characteristics

Soil texture analysis confirmed the sandy nature of the study sites with the bituminous ('B') sites along with reference site, R1 classified as a loamy sand (80% sand and < 10% clay) and the second reference site, R2 as a sandy loam (60% sand and < 20% clay) (Table III.1, Appendix III). The bituminous sites were significantly more acidic than the reference sites with an average pH of 4.6 (±0.47) compared to 5.4 (±0.28)) (F (1,54) = 44, p = 1.62e^{-8}).

All sites were mature, jack pine-dominated forest stands with moss (B1, B4, B5, R1, and R2) or lichen (B2, B3) dominated forest floor cover. Understory vegetation communities were comprised predominantly of the shrub species *Vaccinium oxycoccos*, *Vaccinium myrtilloides*, *Arctostaphylos uva-ursi*, *Cornus canadensis*, and to a lesser extent *Linnea borealis*, *Rhododendron groenlandicum*, and *Amelanchier alnifolia* (Appendix II). The cored trees at the bituminous sites averaged 26 cm in diameter at breast height (DBH) and 69 years of age and 25 cm in DBH and 81 years of age at the reference sites (Table III.1, Appendix III). Analysis of the standardized ring widths showed a significant correlation (CorrC = 0.47, TT = 4.7) in tree growth rates and responses to climate between the bituminous and reference sites. Due to the correlation in growth rates, the rate of uptake of nutrients and trace metals is also assumed to be similar for trees across the study sites.

2.4.2 Bitumen Presence in Soils

To test my main prediction, I first confirmed the presence/absence of shallow bitumen deposits. Across the bituminous sites, each fraction of hydrocarbons (F2-F4) was detected and increased in concentration with depth (Fig. 2.3, Table 2.1). The F2 fraction occurred in low concentrations increasing with depth from a mean of 2 (± 5) mg kg^{-1} (maximum of 15 mg kg^{-1}) at the soil surface to 23 (± 14) mg kg^{-1} (maximum of 43 mg kg^{-1}) at 40 cm of depth. The F3 fraction likewise increased with depth from a mean of 279 (± 435) mg kg^{-1} (maximum of 1200 mg kg^{-1}) at the surface and a mean of 2430 (± 1290) mg kg^{-1} (maximum of 4400 mg kg^{-1}) at 40 cm of depth. The F4 fraction, which had the highest concentrations overall, increased from a mean of 358 (± 558) (maximum of 1500 mg kg^{-1}) to 2744 (± 1245) mg kg^{-1} (maximum of 4900 mg kg^{-1}). Note that both F3 and F4 fractions frequently exceeded CCME guidelines for hydrocarbons in parklands with coarse-textured soils (300 and 2800 mg kg^{-1} for F3 and F4, respectively) (CCME, 2008). The F3 fraction exceeded guidelines at all depths, and the F4 fraction at depths > 10 cm. No hydrocarbons were detected at any depths in soils of the reference sites.

2.4.3 Root Distribution with Soil Depth

Across the sites, roots were present to 40 cm depth and total root mass per core did not differ significantly between bituminous and reference sites (0.6421 (± 1.22) g versus 0.3635 (±0.45) g; t (53.86) = -1.24, p = 0.22) (Fig. 2.4). However, at 10 cm depth root mass was four times higher in the bituminous than the reference sites (Table 2.2). This pattern was also demonstrated in the analysis of cumulative root fraction with depth; reference sites had a significantly more even vertical root distribution with depth than bituminous sites ($\beta = 0.95$ versus β of 0.88; t (10.3) = 2.35, p = 0.04) (Fig. 2.5). When controlled for depth, root biomass was not significantly correlated with the concentration of hydrocarbons in the soil (*Pearson's* r (55) = -0.077, p = 0.58).

Molecular identification of roots returned a total of 29 unique species across all sites with 24 species detected at the bituminous sites and 15 species at the reference sites. Of the 29 species observed across all sites, 25 occurred in the top 10 cm, 17 in the 10–20 cm depth, 17 in the 20-30 cm depth, and 5 at 40 cm depth (Fig. 4). The reference sites had on average 6.25 unique species rooting near the surface, and bituminous sites had on average 5.44 species (Fig. 2.6). Importantly, I was able to identify jack pine roots growing at all depths in four of seven sites and to a minimum of 30 cm depth in all sites (Fig. 2.6). This latter result confirms that roots of jack pine grow at depths where bitumen deposits are present and can potentially be growing through bitumen. The occurrence of all species throughout the soil profiles is presented in Appendix II.

2.4.4 Trace Metals – Soil

Sites in the study were poor in nearly all metals sampled (Table I.4, Appendix I). Specifically, only two metals on the reference sites (Ag and Ga) and four metals (Ag, Ga, Mo, and Re) on the bituminous sites were above average values of the UCC (Rudnick & Gao, 2014) and 15 metals were found at concentrations over 6x lower than UCC values. All four target metals, V, Ni, Mo, and Re, were significantly more enriched in soils of the bituminous sites compared to the reference sites (V: $F\,(1,68) = 26.38$, $p = 2.54e^{-6}$; Ni: $F\,(1,68) = 13.67$, $p = 0.00044$; Mo: $F\,(1,68) = 151.8$, $p = <2e^{-16}$; Re: $F\,(1,68) = 9.5$, $p = 0.0029$). Both V and Ni were about three times more enriched in the bituminous sites, and Mo by a factor of 11.5 (Fig. 2.7). Rhenium was only detected in 29 of 70 total soil samples when including the lysimeter soil plugs, 25 from bituminous sites and 4 from reference sites. In these samples, Re was found to have an average enrichment factor of 309 on bituminous sites and 7.6 on the reference sites.

All four metals of interest correlated well to the concentration of hydrocarbons in the soil (Fig. 2.8). Kendall's τ correlation coefficients ranged from 0.46 for V to 0.65 for Mo ($p < 0.001$ for both). Interestingly, Li, Th, and Y were also correlated with total hydrocarbon concentrations ($r_\tau = 0.478$, 0.343, and 0.306, respectively). The remaining 18 metals had neutral to strong negative correlations with the concentrations of hydrocarbons in soil.

2.4.5 Trace Metals – Soil Pore Water

Raw concentrations, i.e., those not standardized to concentrations of a lithophile element in the UCC, of the dissolved fraction (< 0.45 μm) of target metals increased in soil pore water in the bituminous sites when compared with the reference sites (Fig. 2.9), though only V and Re did so significantly (F (1,12) = 24.2, p = 0.00035 and F (1,12) = 10.4, p = 0.0073, respectively). However, all 42 metals analyzed had higher concentrations in the soil pore water on the bituminous sites compared with those of the reference sites. Soil pH was significantly lower in the bituminous sites (4.6 (±0.47)) than in reference sites (5.4 (±0.28)) (F (1,54) = 44, p = 1.62e^{-8}). This acidity may increase the water solubility for many of the metals in soils of the bituminous sites, and may be a driver of the higher observed concentrations (Watmough, 2008). When pH was included as a covariate, V and Re were no longer significantly elevated (F (1,11) = 4.4, p = 0.059 for each) in soils of the bituminous sites. Interestingly there is a weak, though significant, negative correlation (*Pearson's* r (37) = -0.38, p = 0.017) between soil pH and total hydrocarbon concentration. This relationship raises the possibility that the increased acidity and increased metal solubility may be related to the concentration of bitumen in the soil.

The relative availability (i.e., solubility) for plant uptake from the total soil concentrations showed a higher metal solubility in the reference sites for Ni, Mo, and Re and a higher solubility for V in the bituminous sites (Fig. 2.10). This difference was only statistically significant for Mo (F(12) = 28.48, p = 0.00018) and Re (F(12) = 19.55, p = 0.00083).

2.4.6 Trace Metals – Wood

Concentrations of Ni and V were both higher in trees growing on the bituminous sites, though only Ni was significantly so (Ni: F(68) = 4.31, p = 0.042; V: W = 437.5, p = 0.39) (Fig. 2.11). Additionally, both Ni and V showed significantly higher concentrations in the newer wood compared to the older (Ni: *F*(68) = 15.7, p = 0.00018; V: W = 264, p = $1.09e^{-5}$). Molybdenum was reversed; it was present in significantly lower concentrations in trees growing on the bituminous sites (W = 672, p = 0.018) and higher concentrations in the older wood versus the new (W = 695.5, p = 0.30). Both V and Mo were difficult to detect in wood samples and the data were 51% and 48% censored (<LOD), respectively.

2.5 Discussion

Concentrations of nickel (Ni) and vanadium (V) were higher in trees growing in bituminous soils, while concentrations of molybdenum (Mo) were lower. Soils containing shallow bitumen deposits were significantly enriched in all four of the target trace metals and this pattern translated into the soil pore water concentrations. With the DNA analysis of collected root samples confirming the presence of jack pine roots at depths with elevated, available trace metal concentrations, it is likely the increase in Ni and V within the woody tissue can be attributed to the presence of bitumen.

The enrichment of V, Ni, Mo, and Re in bulk soil samples containing bitumen is consistent with the findings of Bicalho et al. (2017) regarding their association with bitumen. However, the soil pore water concentrations are more relevant in addressing the differences in metal concentrations in wood across sites as they represent what is available to plant uptake (Sauvé, Hendershot, & Allen, 2000; Watmough, 2008). Soil pH has been shown to significantly affect the availability of trace metals in the soil and their resulting concentrations in trees (Watmough, 2008; Pendias & Kabata-Pendias, 2000). The increased acidity at the bituminous sites is likely playing a significant role in the increased availability of many elements in the soil pore water. However, the fact that Mo, which should decrease in solubility at a lower pH (Sigel & Sigel, 2002), was also elevated at these more acidic sites suggests that the input from the bitumen is still a major player. With the elevated concentrations of Mo in the soil pore water at the bituminous sites, the significant decrease in the wood tissue concentrations was unexpected. Previous research has observed a similarity in V and Mo behaviours in plants, and it could be that the decrease in the soil pH and the significant increase in V availability has created competition between these two metals favouring V (Pendias & Kabata-Pendias, 2000; Welch, 1973). Another possibility is that, as a component of the N incorporation process, the effect on Mo concentrations within the tree may be restricted to their roots, which were not sampled in this study (Pendias & Kabata-Pendias, 2000; Sigel & Sigel, 2002).

The difference in trace metal concentrations between tree ring age classes may indicate an acidification event of forests soils in the region during from 1980–2000 when SO_2 and NO_x emissions were at their highest (Jung, Chang, Ok, & Arshad, 2013; Vitousek et al., 1997). The decrease in Mo along with the increase in both Ni and V in the more recent tree rings may be an indicator of increased acidic conditions in the forest soils resulting in their respective decreased or increased mobility in soil. These trends, along with the trends displayed by Rb and Sr, agree with the findings in Watmough (2008). The increase in atmospheric deposition in the region due to increased mining operations could provide another possible explanation for the differences in metal concentrations between wood age classes (Hargesheimer et al., 1980; Kelly et al., 2010; Shotyk et al., 2016). However, I do not think this is likely. As Shotyk et al. (2016) demonstrate, deposition in the region occurs in the form of large particulate matter and incorporation of aerial deposition through leaf stomata is not thought to be a major pathway of intake (Cutter & Guyette, 1993; Lepp, 1975; Watmough, 1997). The poor mobility of dust into the woody tissue is well illustrated in Shcherbenko, Koptsik, Groenenberg, Lukina, and Livantsova, (2008). In this study the concentrations of trace metals analyzed on needles and twigs increased significantly with increased proximity to industry. However, this trend failed to materialize in the internal tree wood tissue. In addition, my data for trace elements in the top 10 cm of the soil profile show no consistent trend of accumulation among elements associated with industrial activity in the region (e.g., Ag, Cd, Sb, Tl, Al, or Pb) (Table I.4, Appendix I). It is important to note that while my study illustrated a relative enrichment of certain trace elements on sites containing natural bitumen deposits and near mining operations, their concentrations were still quite low when compared to other values in the literature. For example, the highest recorded concentration in wood for Ni in this study was 252 µg kg^{-1}, nearly two and a half times lower than a mean wood concentration of 653 µg kg^{-1} across control sites in seven studies of various coniferous species using comparable methods to my own (Chun & Hui-yi, 1992; Kirchner et al., 2008; Odabasi et al., 2016; Panyushkina et al., 2016; Schmidt, Leybourne, & Kyser, 2017; Shcherbenko et al., 2008; Watmough & Hutchinson, 1999).

While the differences in rooting distribution between the reference and bituminous sites were marginally significant, it would be tenuous to attribute these differences to the presence of bitumen, given the low number of reference sites. The deeper mean rooting of the reference sites appears to be driven largely by site R2 with R1 being quite similar to the bituminous sites. Interestingly, when considering the textural differences between the sites, we'd expect the rooting patterns to be reversed. Being sandier (80% sand of R1 versus the 60% sand of R2), we'd expect the rooting distributions of the bituminous sites and R1 to be deeper (Jackson et al. 1996; Schenk & Jackson, 2002; Strong & Roi, 1983). This slight reversal of expected trends could potentially be a result of the hydrophobic properties of hydrocarbons slowing drainage during certain periods of the year and concentrating roots near the surface (Jung et al. 2014, Roy et al., 2003). The higher degree of shrub cover at R2, as seen in the site photographs in Appendix III, may provide another explanation for the larger amount of deeply occurring roots.

By investigating multiple lines of evidence, I was able to show the enrichment of two target trace metals in trees growing on similarly enriched soils. This research helps support the use of dendrochemistry to investigate and monitor tree rooting behaviour in locations where a known anomaly in the soil profile is present. It also further stresses the importance of site conditions such as soil pH, in understanding and predicting metal behaviour as it relates to the uptake and translocation by individual species of plants.

The potential for Ni and V to act as indicators for rooting behaviour in natural bituminous soils has implications for reclamation efforts in the oil sands region. While only Ni displayed a statistically significant increase in concentration in the tree wood, V may be a more appropriate indicator for rooting in bitumen. Comparing the Ni/V ratios in the tree wood (22:1) versus the soil (0.24:1) suggest that the tree is actively accumulating Ni while its uptake of V is more passive, which is a preferred attribute for a metal in dendrochemistry (Cutter & Guyette, 1993; Watmough, 1997). Future research expanding our understanding of how these metals are taken up and translocated through reclamation species such as jack pine, white spruce (*Picea glauca*), and trembling aspen (*Populus tremuloides*) could enable reclamation practitioners to monitor tree root growth on landforms constructed with lean oil sand (LOS) overburden.

While the results of this study relate to bitumen, they support applications of dendrochemical methods outside the oil sands region. With the selection of an appropriate temporal or spatial reference (e.g., pre-disturbance conditions at a location or a nearby site with similar chemical properties), dendrochemistry could be used to monitor rooting behaviour in other reclamation, remediation, or natural scenarios. For example, as roots grow in soils with distinct chemical signatures, tree wood could be analyzed for target metals to infer rooting depth. The extent of tree roots could help monitor the size of spill and contamination events and help remediation efforts in natural and urban settings. Tree wood chemistry could also help understand long-term environmental trends in a given area. With careful consideration of target chemical signals and local environmental conditions, dendrochemistry could provide researchers with a useful tool in determining where and how roots grow.

Figures

Figure 2.1 Presence of bitumen within the rooting zone. Bitumen was characterized by banding, or the presence of hard clumps, along with hydrocarbon odour. (57° 9'57.18"N, 111°32'28.29"W, Alberta, Canada)

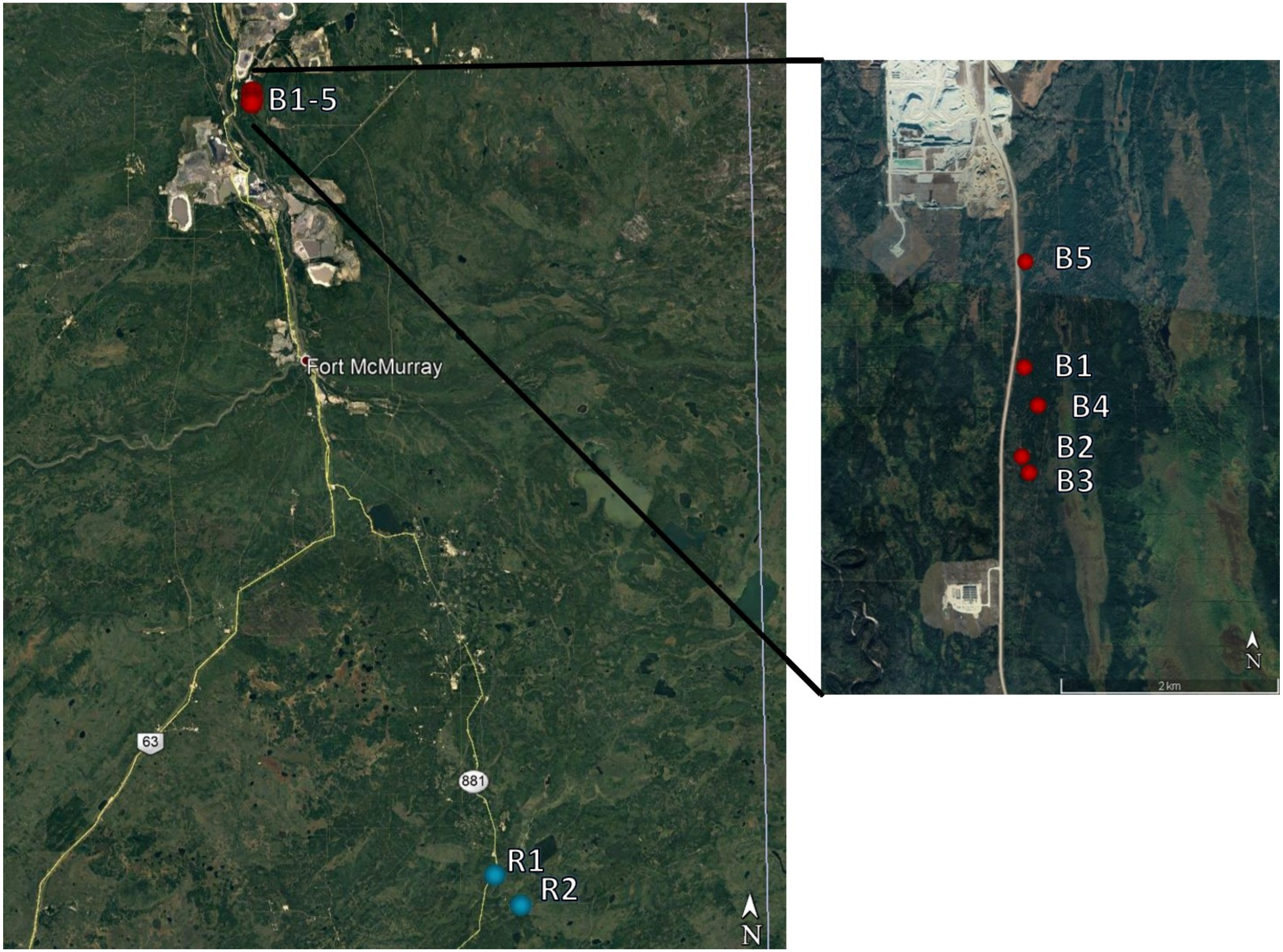

Figure 2.2 Location of field sites with the reference sites visible in blue on the left near Janvier, Alberta, Canada. Close-up on the right shows the bituminous sites in red near the Hammerstone Quarry north of Fort MacKay, Alberta.

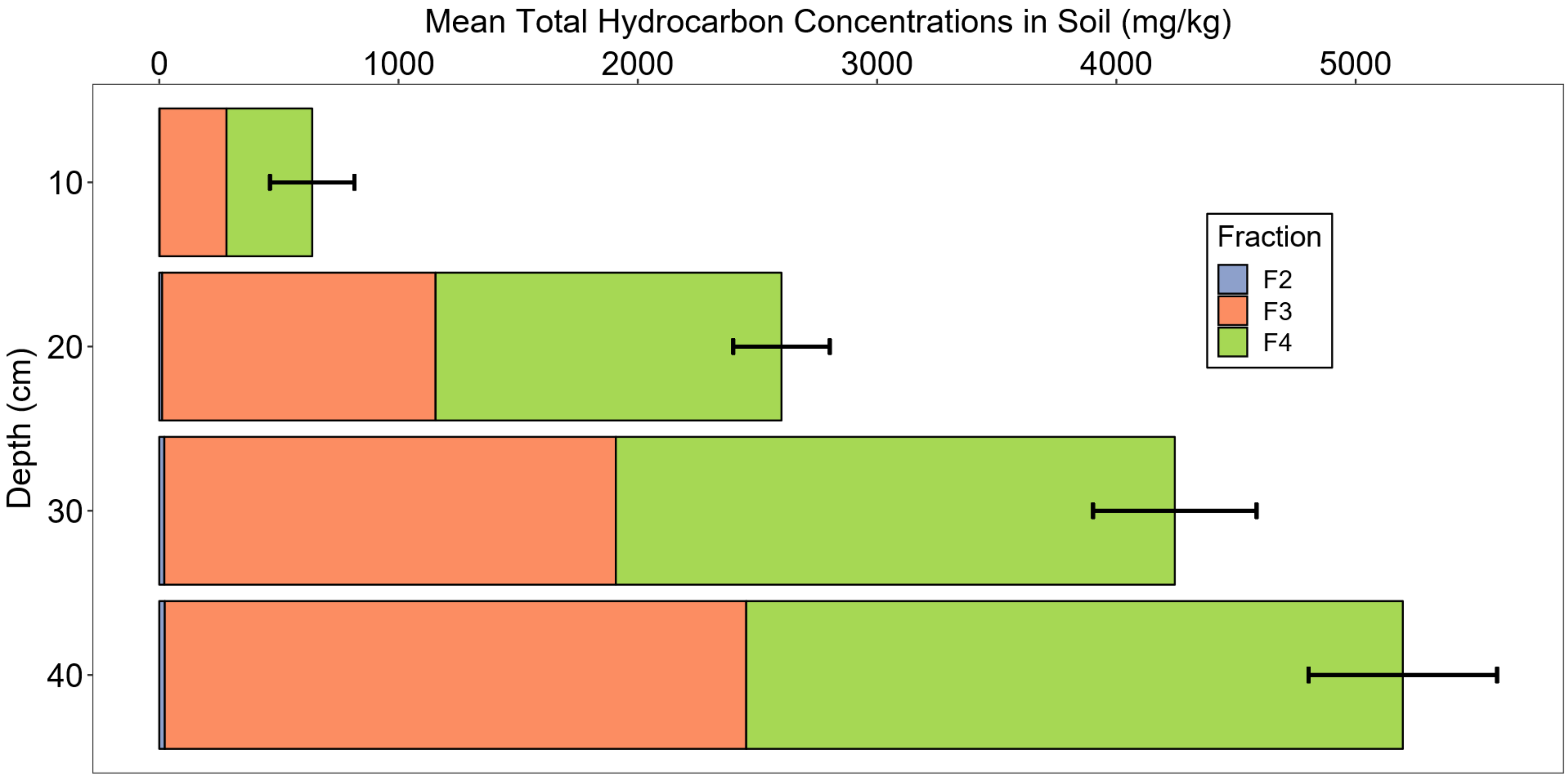

Figure 2.3 Total hydrocarbon concentrations in soil of bituminous sites by depth and F fraction. Error bars represent standard error.

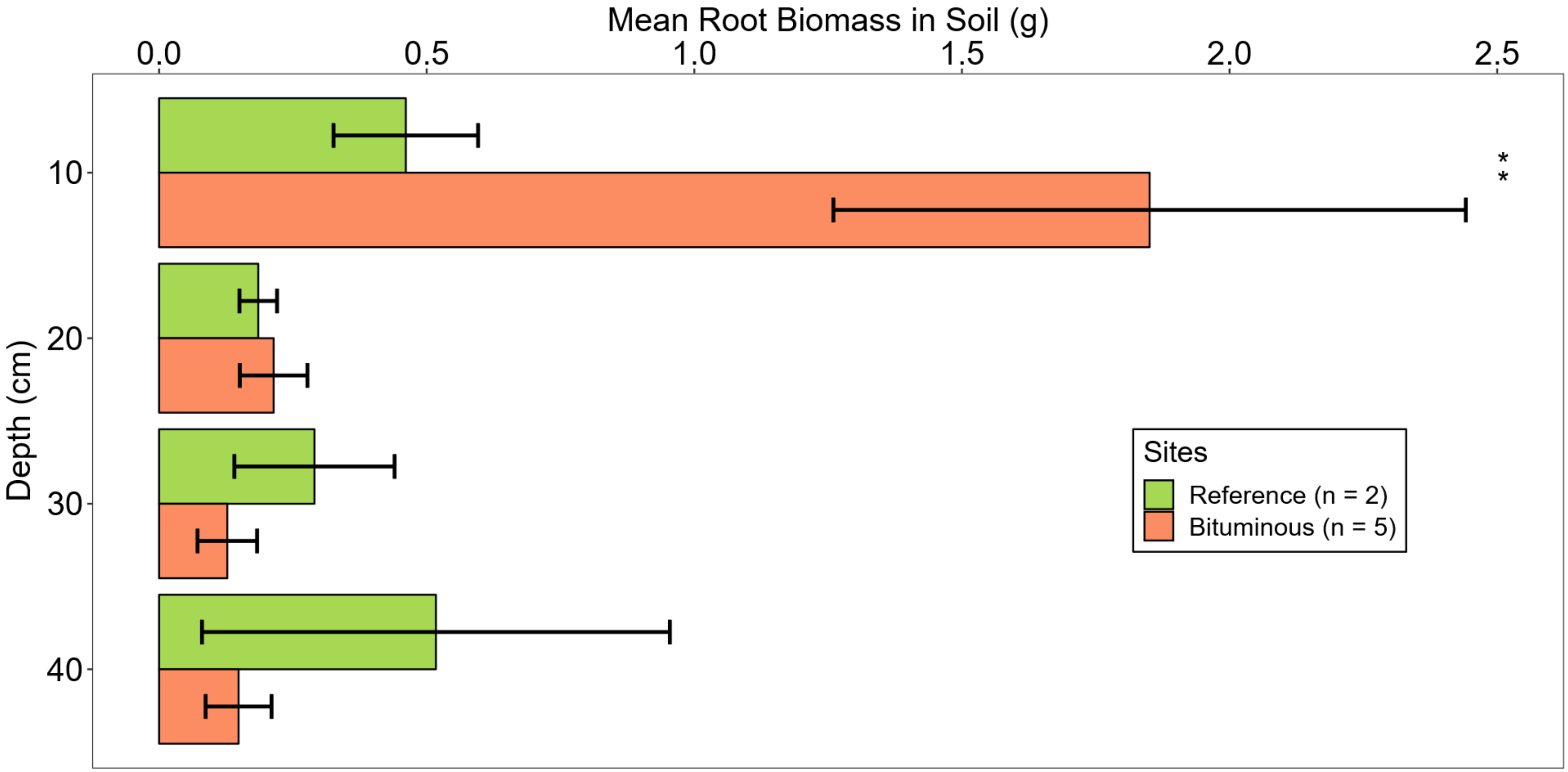

Figure 2.4 Mean root biomass (g dry weight) by depth between the two site types. Error bars represent standard error of the mean. Significant differences at a depth between site types as determined by Tukey's HSD post-hoc test for pairwise-comparisons (at 10 cm depth: *F*(47) = 2.95, p = 0.005) denoted. Sites are *Pinus banksiana* stands in northeastern Alberta, Canada.

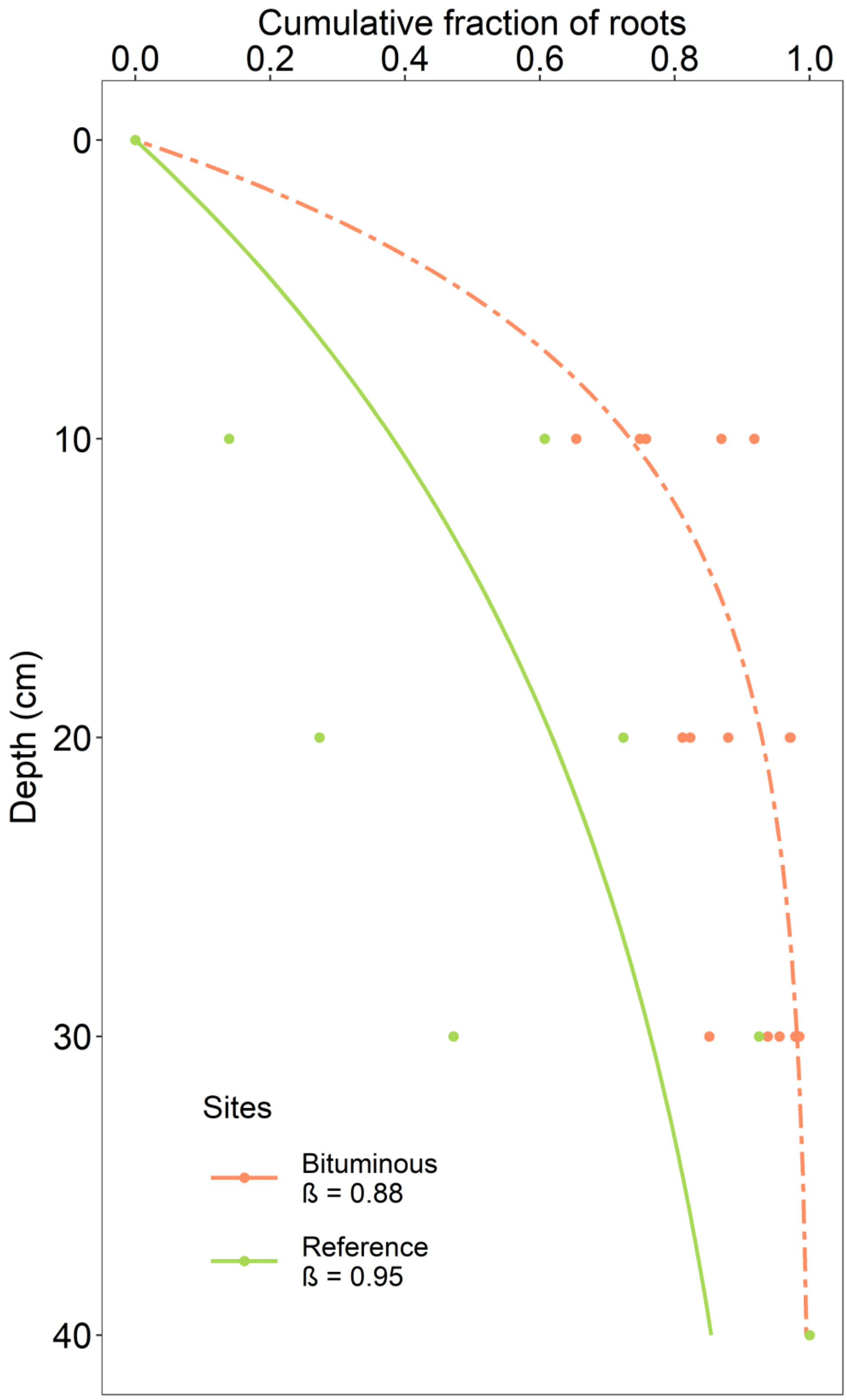

Figure 2.5 Cumulative root distribution with depth. Bituminous sites display a significantly shallower rooting distribution than the reference sites (t (10.3) = 2.35, p = 0.04).

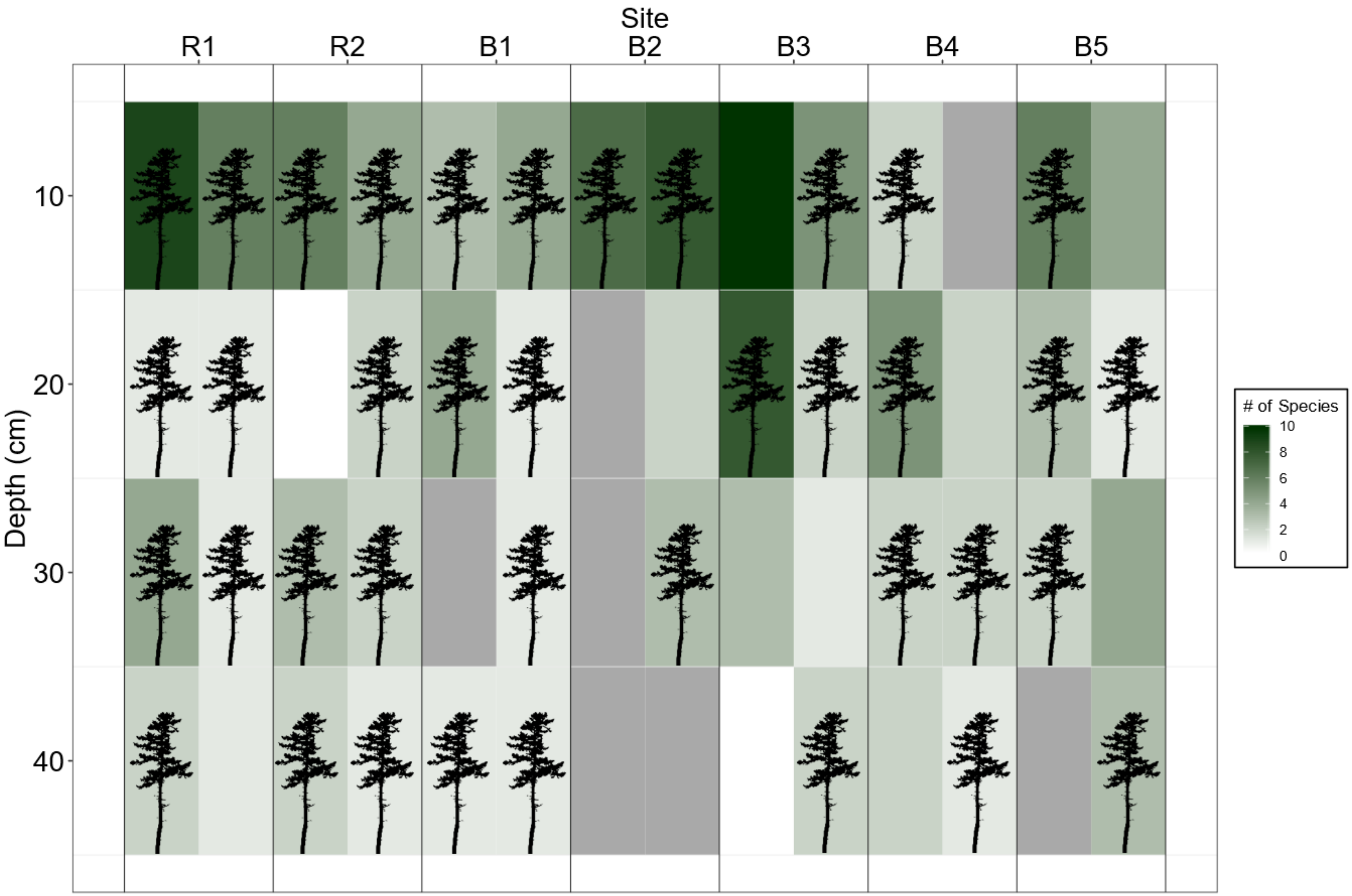

Figure 2.6 Number of plant species detected in each soil sample. Sites R1 and R2 represent the reference sites and sites B1-B5 represent bituminous sites. Gray squares represent samples where root DNA did not amplify. Squares with trees represent the detection of jack pine (*Pinus banksiana* Lamb.) in the samples.

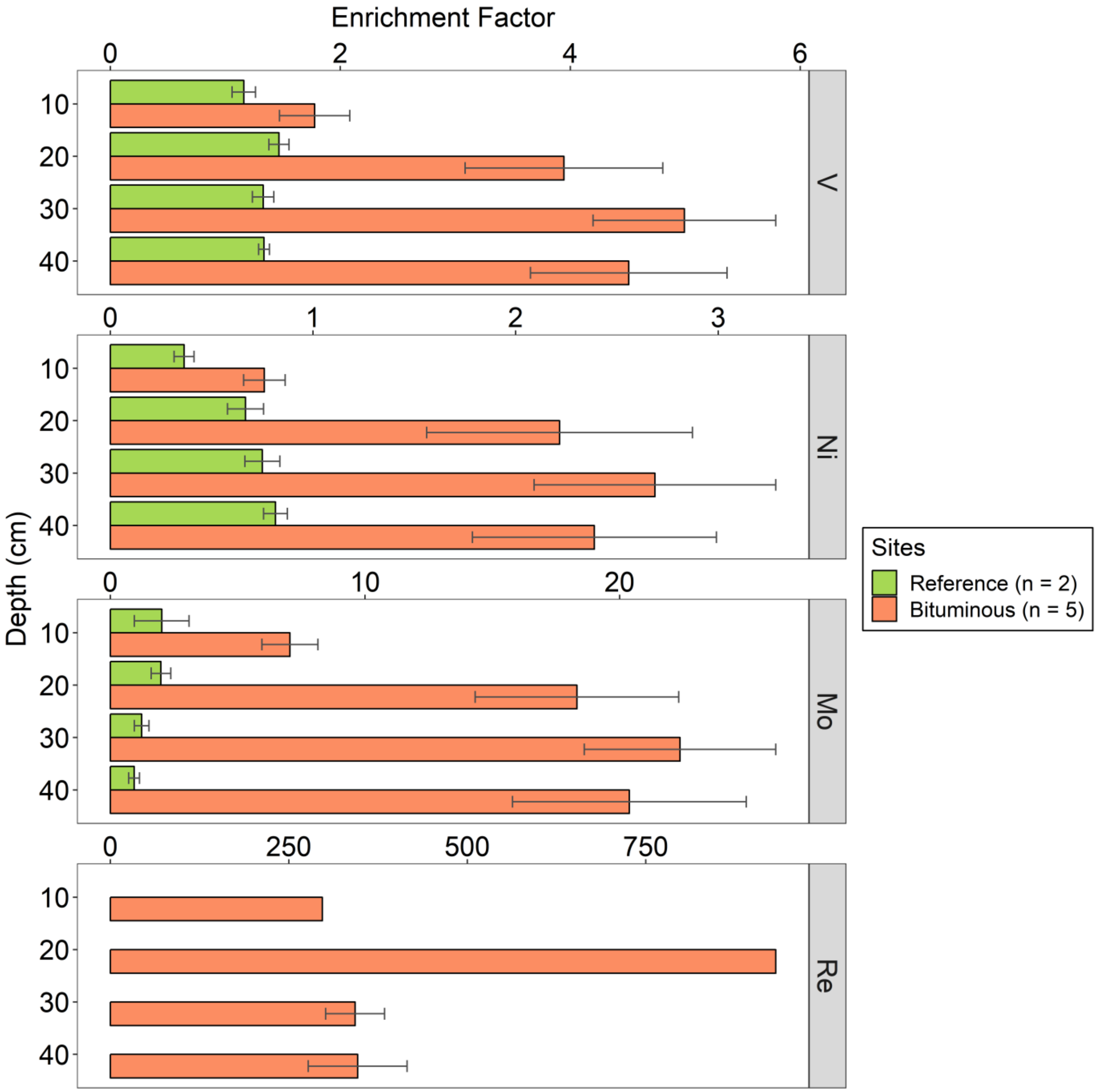

Figure 2.7 Enrichment factors $(([M]/[Cr])_{soil}/([M]/[Cr])_{UCC})$ of V, Ni, Mo, and Re with depth across both site types. Error bars represent standard error of the mean. Samples <LOD for Re were excluded for the purpose of graphical representation.

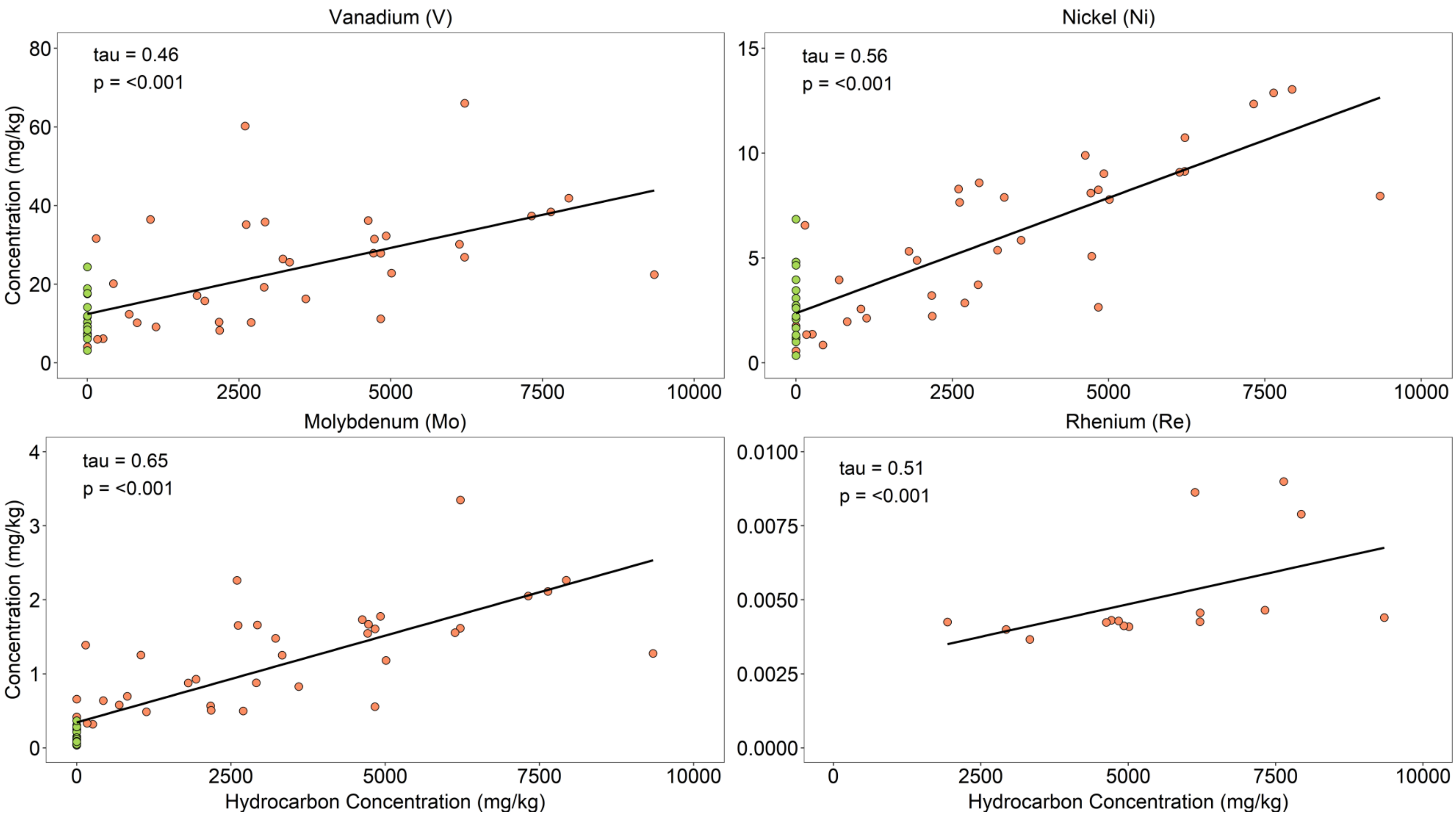

Figure 2.8 Kendall’s Tau correlation between hydrocarbon and total trace metal concentrations in soil. Green dots represent reference sites and orange represent the bituminous sites.

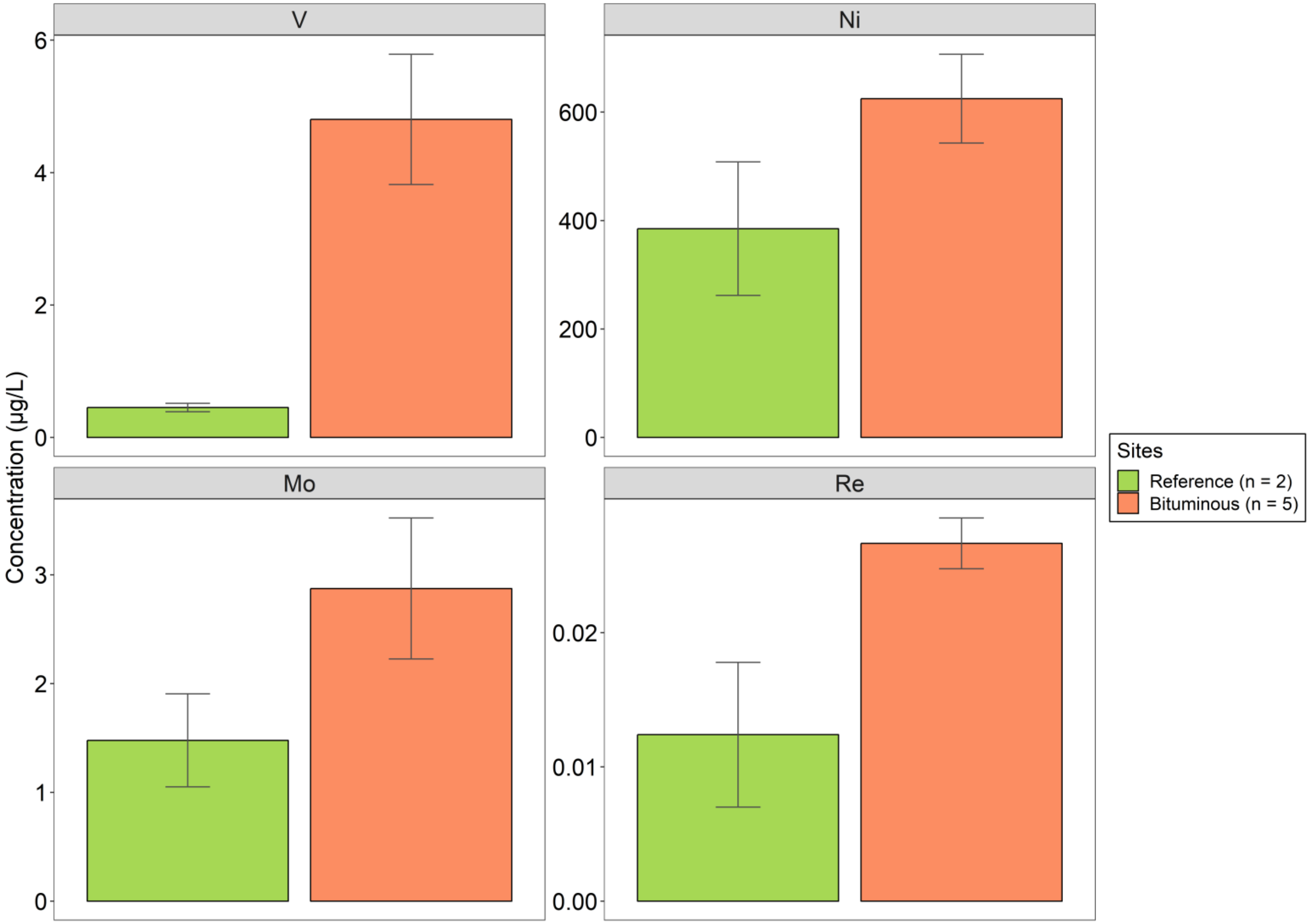

Figure 2.9 Dissolved (<0.45 µm) trace metal concentrations in soil pore water between site types. While all four metal concentrations were higher in pore waters of the bituminous sites, only V and Re were significantly so (F (1,12) = 24.2, p = 0.00035 and F (1,12) = 10.4, p = 0.0073, respectively). Error bars represent standard error of the mean.

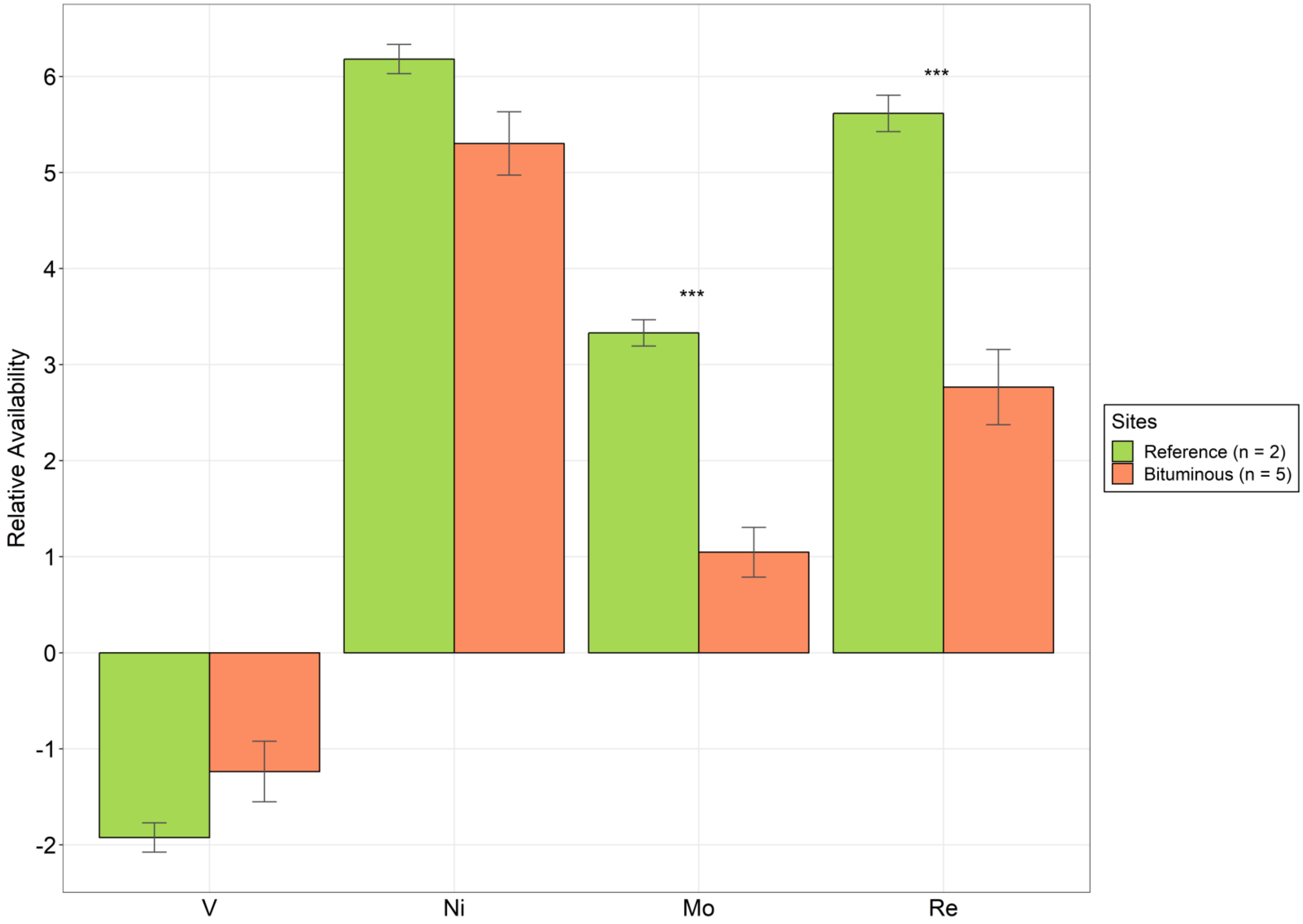

Figure 2.10 Relative availability (log{([M]/[Cr])water/([M]/[Cr])soil)}) of V, Ni, Mo, and Re across both site types. Error bars represent standard error of the mean. Significant differences are denoted by *** as tested by one-way ANOVA. Molybdenum (F(12) = 28.48, p = 0.00018) and Rhenium (*F*(12) = 19.55, p = 0.00083) were both more elevated in pore water relative to their concentrations in the soil at the reference sites than the bituminous.

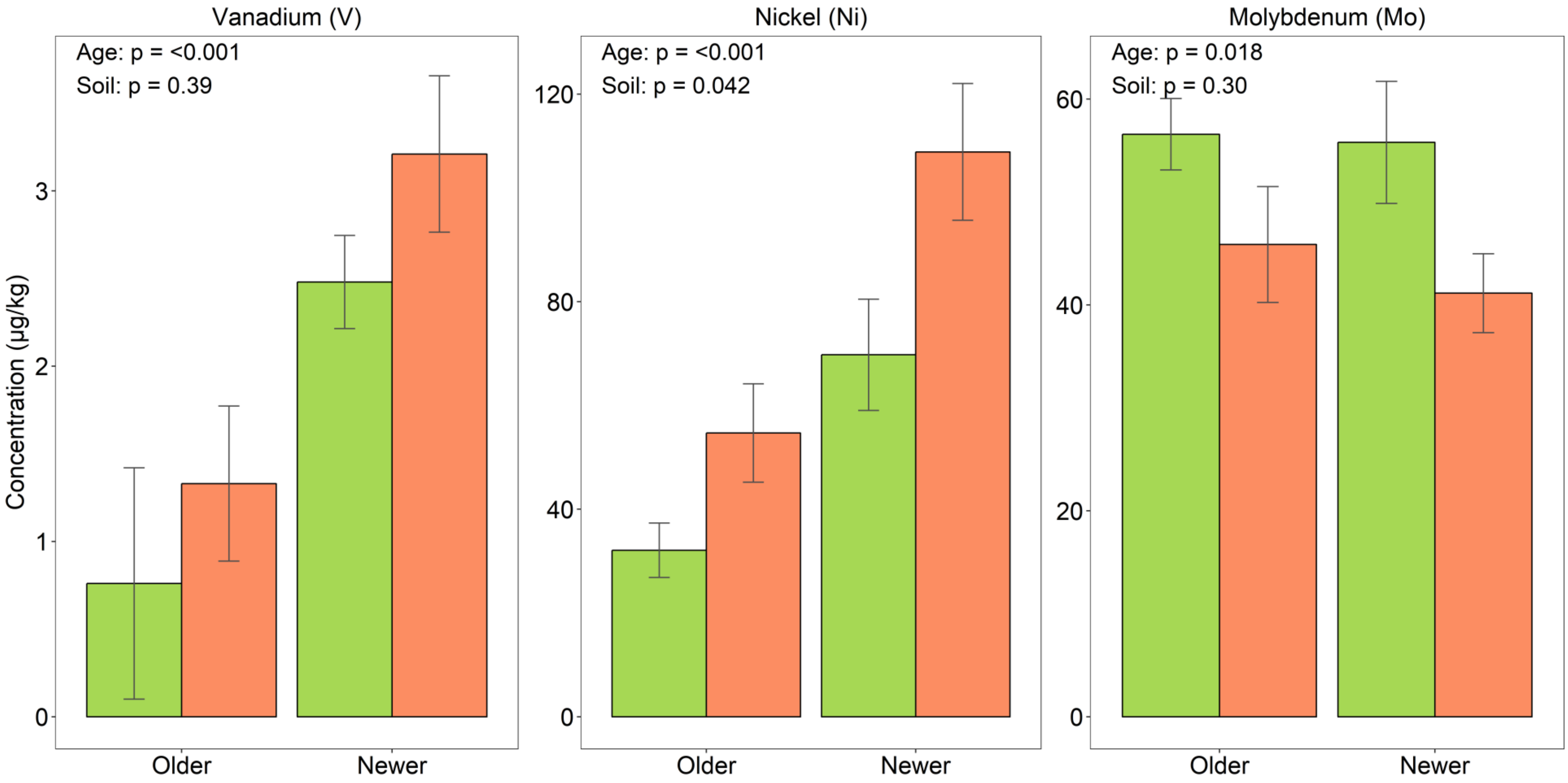

Figure 2.11 Trace metal concentrations in tree wood by wood age and by site (green = reference sites and orange = bituminous sites). Owing to the presence of <LOD values, bars for V and Mo were created using summary statistics calculated by maximum likelihood estimation with the NADA package in R (Lee, 2019). P-values calculated using raw concentrations by Wilcoxon rank-sum tests for V and Mo, and one-way ANOVA for Ni. Error bars represent standard error of the mean.

Tables

Table 2.1 Hydrocarbon concentrations in natural bituminous soils with depth. All units in mg kg^{-1}. Canadian Council of Ministers of the Environment (CCME, 2008) limits for coarse-textured, natural parklands provided for reference.

	F2			F3			F4			Total		
Depth	Mean (±SD)	Min	Max	Mean (±SD)	Min	Max	Mean (±SD)	Min	Max	Mean (±SD)	Min	Max
10	1.7 (±5.0)	0	15	279.4 (±435.1)	0	1200	358.1 (±558.3)	0	1500	639.2 (±995.5)	0	2700
20	12.0 (±10.4)	0	35	1142.9 (±781.5)	0	2600	1445.6 (±902.3)	0	2993	2600.5 (±1676.5)	0	5496
30	21.2 (±13.5)	0	38	1887.0 (±1081.7)	170	3700	2336.0 (±1080.6)	260	4100	4244.2 (±2141.5)	430	7638
40	23.2 (±14.2)	0	43	2430.0 (1290.2)	400	4400	2744.0 (±1245.2)	640	4900	5197.2 (±2521.3)	1040	9343
CCME			150			300			2800			3460

Table 2.2 Root biomass data (g $_{\text{dry weight}}$) with depth across sites. Bolded values represent significant differences at a depth between site types as determined by Tukey's HSD post-hoc test for pairwise-comparisons (α = 0.05).

	Reference Sites (n = 2)			Bituminous Sites (n = 5)		
Depth (cm)	Mean (±SD)	Min	Max	Mean (±SD)	Min	Max
10	0.4610 (±0.27)	0.2393	0.8155	**2.0783 (±1.82)**	0.4689	5.4806
20	0.1853 (±0.07)	0.0965	0.2466	0.2141 (±0.20)	0.0336	0.6993
30	0.2903 (±0.30)	0.0286	0.6544	0.1275 (±0.18)	0.0049	0.5561
40	0.5172 (±0.87)	0.0745	1.8284	0.1485 (±0.19)	0.0036	0.6448

Chapter 3: Baseline Survey of a Reclaimed Lean Oil Sand Landform

3.1 Introduction

Understanding how roots are distributed in response to lean oil sand (LOS) is integral to developing future reclamation practices to better promote root growth, which can lead to self-sustaining forests (Plourde et al., 2009). When root growth is inhibited it can lead to issues for long term tree growth such as reduced access to nutrients and resources (Bockstette et al., 2017), and anchorage (Plourde et al., 2009). While the literature is sparse regarding the effects of LOS specifically, much research has been done evaluating other types of reclamation in the oil sands region on aboveground performance. This research concentrates on the influence of physical characteristics (e.g., nutrient availability, organic matter content, bulk density) of soil cover types and depths (Howell & MacKenzie, 2017; MacKenzie & Quideau, 2010; Turcotte, Quideau, & Oh, 2009) on above-ground performance of vegetation (Dhar et al., 2018; Macdonald et al., 2015; Pinno et al., 2012).

The belowground picture of how tree roots are growing in reclaimed areas in the region, however, remains poorly resolved. Jung et al. (2014) found that textural (compaction) and chemical (salinity) interfaces between soil amendments and overburden material restricted fine root growth. Above-ground tree growth was also positively correlated to fine root biomass in their study. Bockstette et al. (2017) however, describes how belowground competition with grasses may be the largest factor affecting aspen root and shoot growth rather than compaction of the subsoil and overburden layers. However, he also noted that the shallow LOS treatment was significantly more compacted than the saline-sodic overburden used in other types of reclamation and no roots were observed growing within the LOS (personal communication, 2018).

Whether the lack of roots in the LOS observed by Bockstette et al. (2017) was due to the compaction/textural differences, the hydrophobicity of the bitumen affecting site drainage and water storage, the potential presence of toxic compounds, or simply the young age of the site is difficult to tell (Jung et al., 2014; Pernitsky et al., 2016; Rowland et al., 2009; Roy et al., 2003). While isolating these mechanisms is outside the scope of my research, it is important first to be able to reliably and definitively determine if roots are interacting with LOS.

This chapter of my thesis establishes baseline values for trace metal concentrations in the soils and seedlings of a reclaimed landform constructed with LOS. The findings in my second chapter that Ni, and to a lesser extent V, may act as indicators of tree rooting behaviour in bituminous soils could help monitor the growth of tree roots in proximity to LOS. The second chapter also demonstrates the importance of understanding the background or baseline conditions of a study site, such as soil pH and metal concentrations, prior to analyzing dendrochemical data. Due to the heterogenous nature of the soils and the young age of the seedlings on the study site, this chapter does not seek to draw comparisons with results of my second chapter or within the study site itself. Rather, it establishes a baseline condition against which future measurements can be compared to monitor root distribution through time.

3.2 Methods

3.2.1 Site Description

The study site was located on the south-facing slope of a lean oil sand (LOS) overburden constructed landform located on an oil sand mining lease north of Fort McMurray. This landform was capped with 35 cm of peat-mineral mix (PMM) over LOS and three contouring treatments were then applied across the slope (Fig. 3.1). A thorough description of site construction and details regarding the different contouring treatments are available in Melnik, Landhäusser, and Devito (2017). One of the contouring treatments was constructed by pushing mounds of PMM or forest floor material (FFM) (1.5 x 3.5 x 1.5 m in size) down the slope over the 35 cm PMM cap. Study plots were located within three replicates of the mounding treatment in the space between the mounds as it provided the most uniform depth to the LOS overburden. Within each replicate, a plot was selected at the top and bottom of the slope for a total of six plots. Seedlings of jack pine (*Pinus banksiana*) were planted on the site in 2015 and were four years of age at time of sampling.

3.2.2 Field Survey

In 2018, field surveys were conducted using similar methods to those described in Chapter 2.0 (Section 2.2.2) with the following adjustments: Within the 10 x 10 m plot, five jack pine seedlings were selected and their height and diameter above the first whorl of branches were recorded. A section of stem between the first and second whorl was collected for trace metal analysis. Seedling roots were then excavated to estimate rooting depth and to visually assess if they had penetrated the LOS overburden. Any roots observed growing in or along the LOS interface were collected for molecular identification. Two sets of three soil cores (one core each for hydrocarbon, trace metal, and texture/root mass and identification) were collected using the slide hammer corer in 10 cm increments to only 30 cm in depth as the LOS was too difficult to penetrate. As a result, samples for trace metal and hydrocarbon analysis of the LOS were collected using a shovel from the bottom of the tree excavation pits. Soil pore water was extracted using the lysimeters as described in section 2.2.2

3.2.3 Sample Processing

Soil texture and pH were analyzed as described in section 2.2.3 of Chapter 2.0. Roots from cores were collected and identified as described in section 2.2.4 of Chapter 2.0. Soil samples were sent to Maxxam Analytics for hydrocarbon analysis as described in 2.2.6. The bark and sapwood were trimmed from the tree segments using the Kershaw Pro-Grade Breaking Knife (Model 1280GE, Tualatin, Oregon) tested to be contaminant-free (Table I.1, Appendix I). Samples of soils, LOS, tree tissue and pore water were then processed and analyzed for trace metals as described in 2.2.7.

3.3 Data Analysis

Summary statistics were calculated in R (R Core Team, 2019) using the tidyverse packages tidyr, dplyr, ggplot2, and car (Wickam, 2019) along with the NADA package for censored data analyses (Lee, 2019). The data for root distribution, hydrocarbon concentrations, and trace metals were summarised following the methods described in sections 2.3.1 – 2.3.4. Enrichment factors in soil for V, Ni, Mo, and Re were calculated using yttrium (Y) as a reference in the place of Cr as it did not vary in concentration between the PMM or LOS.

3.4 Results

3.4.1 Site Characteristics

The peat-mineral mix (PMM) used as the capping material on the study site was classified as a loam (41% sand, 12% clay) and the LOS as a sandy loam (57% sand, 15% clay) (Table III.2, Appendix III). Particle size analysis classified both the PMM and the LOS as fine-grained soils (median particle size <75 µm). The PMM and the LOS were similar in pH averaging 7.3 (± 0.2) and 7.8 (± 0.2), respectively. The depth of the PMM ranged from 30 cm to 40 cm in between the mounding treatments. Jack pine seedlings had a mean diameter of 1.78 cm (± 0.49) and a mean height of 1.14 m (± 0.30) (Table III.2, Appendix III).

3.4.2 Hydrocarbon Concentrations

Hydrocarbons were present throughout the soil profile (Fig. 3.2, Table 3.1). Concentrations in the PMM of each fraction (F2-F4) varied with depth. The F2 fraction was detected in all samples except four within the PMM. Values ranged from 0 to 720 mg kg^{-1} and were variable across the three depths. Concentrations of hydrocarbons in the LOS were much higher, averaging 961 (± 439) mg kg^{-1} to a maximum of 1900 mg kg^{-1}. The F3 fraction followed a similar pattern of variability in the PMM ranging from 360 mg kg^{-1} to 6000 mg kg^{-1}. The LOS overburden had an average F3 concentration of 6800 (± 2254) mg kg^{-1} and reached a maximum concentration of 11000 mg kg^{-1}. F4 concentrations ranged from 180 to 3000 mg kg^{-1} within the PMM and increased to an average of 3418 (± 951, maximum 5100) mg kg^{-1}) in the LOS. Interestingly, within the PMM all three fractions were highest on average at 20 cm of depth (F2: 130 (± 128); F3: 1515 (± 923); F4: 932 (± 479) mg kg^{-1}) though the maximum observed values all occurred at 30 cm depth. Both the F2 and the F3 fractions frequently exceeded the CCME guidelines for hydrocarbons in fine-grained parkland soils (150 and 1300 mg kg^{-1} for F2 and F3, respectively) (CCME, 2008). The F4 fraction remained below the maximum guideline values for fine-grained parkland soils (5600 mg kg^{-1}) in both the PMM and LOS.

3.4.3 Root Distribution with Soil Depth

Root mass among plots was an average of 0.2244 (±0.21) g $_{\text{dry mass}}$ in the top 10 cm and decreased to an average of 0.0880 (±0.12) g $_{\text{dry mass}}$ at 30 cm (Fig. 3.3, Table 3.2). Roots were rarely observed to penetrate the LOS overburden. Analysis of the cumulative root fraction with depth established an average β of 0.92. While this represents a moderately shallow rooting trend, samples were only collected to 30 cm of depth on the five-year-old site.

Molecular identification of roots returned a total of 19 unique species occurring throughout the PMM. Species included two trees, one shrub, six forbs, eight grasses and sedges, and two orchids. *Symphyotrichum boreale* and *S. lanceolatum* were recorded the most frequently, both occurring in six samples (of 36 total samples). Trees were not identified with great success. *Populus tremuloides* was recorded in three samples and *Pinus banksiana* in only two (Fig. 3.4). Roots of species occurring deepest in the constructed soil profile included *Carex concinna*, *Carex bebbii*, *S. boreale*, *S. lanceolatum*, *Calamogrostis canadensis*, *Poa compressa*, *Corallorhiza trifida*, and *P. tremuloides*. Of the six roots collected from the LOS interface, two resolved in PCR and were identified as *P. tremuloides* and a *Salix* species. A full list of species recorded is presented in Appendix II.

3.4.4 Trace Metals – Soil

Both the PMM and the LOS were relatively poor in total trace metal concentrations when compared to average values in the upper continental crust (UCC) (Rudnick & Gao, 2014) with only six metals (As, Cd, Ga, Li, Mo, and Re) out of 42 present at elevated concentrations. The LOS contained higher concentrations of 32 of 42 metals analyzed than the PMM (Table I.5,

Appendix I). Three of the target metals (V, Mo, and Re) were enriched in both the PMM and LOS when standardized to Y and the M/Y ratio in the UCC (Fig. 3.5). Rhenium was enriched by a factor of 95 in the PMM and 89 in the LOS and Mo by a factor of ~3.5 in both. Vanadium was significantly more enriched in the LOS than the PMM (factors of 2 versus 1.5 respectively; *F*(10) = 7.025, p = 0.024). The Ni/Y ratio on the study site was similar to the average Ni/Y ratio in the UCC.

3.4.5 Trace Metals – Soil Pore Water

Trace metal concentrations in the soil pore water varied across plots (Fig. 3.6). Mean concentration of target metals were: V = 0.20 µg L^{-1} (±0.09) (maximum of 0.28 µg L^{-1}); Ni = 868.67 µg L^{-1} (±713.05) (maximum of 1501.78 µg L^{-1}); Mo = 22.81 µg L^{-1} (±10.24) (maximum of 30.90 µg L^{-1}); Re = 0.43 µg L^{-1} (±0.39) (maximum of 0.85 µg L^{-1}). There was no significant correlation for any metal with soil pH, nor any significant differences depending on slope position. The relative availability (i.e., solubility) of V was poor across all plots while Ni, Mo, and Re appeared to be relatively soluble (Fig. 3.7).

3.4.6 Trace Metals – Wood

In tree wood sampled, Ni and Mo were both difficult to detect. Nickel was detected in 18 of 30 trees and had an estimated mean concentration of 5.51 (± 7.4) µg kg^{-1} using the Kaplan-Meier method (Helsel, 2010). Molybdenum was detected in only 8 of 30 trees and had an estimated mean concentration of 2.96 (± 1.4) µg kg^{-1}. Vanadium was detected in 29 of 30 trees and had a mean of 4.02 (± 3.2) µg kg^{-1} (Fig. 3.8).

3.5 Discussion

Baseline conditions for hydrocarbon concentrations in soil, root mass and species with depth, and trace metal concentrations in soil, water, and wood were established for a newly reclaimed site with LOS overburden. This information is critical for monitoring root growth on this site as it establishes reference values against which future studies can compare.

This survey found hydrocarbons throughout the PMM capping material in concentrations often exceeding the CCME (2008) guidelines for coarse-textured parkland soils, and less frequently for fine-textured parkland soils. Trace metal concentrations of Ni, Mo, and Re were not significantly different between the PMM and the LOS, though Ni and Mo were marginally more enriched in the LOS while Re was marginally more enriched in the PMM. Vanadium was the only metal of interest that was significantly more enriched in the LOS than the PMM. In the soil pore water, Ni, Mo, and Re appear to be relatively soluble across the study plots appearing in higher concentrations relative to those in the soil. Vanadium displayed poorer relative availability in soil pore water. Nickel and molybdenum proved difficult to detect in tree wood while V was detected in nearly every sample.

The elevated concentrations of hydrocarbons throughout the PMM were unanticipated and may complicate the use of the study metals in determining when roots begin interacting with the LOS. The total hydrocarbon concentrations (THCs) in the PMM were largely driven by the F3 fraction and it is possible that the peat in the soil mixture may be contributing biogenic organic compounds (BOCs) and inflating the measured F3 fraction. The CCME Tier 1 Method for Analyzing Hydrocarbons in Soil (2001) utilizes a silica gel matrix, which can remove biogenic hydrocarbons prior to analyzing samples for petroleum hydrocarbons to account for this BOC contribution (Brassington et al., 2007). However, the gel can become oversaturated and the excess BOCs will then be included in the final THC (Kelly-Hooper et al., 2013). Regardless of the potential BOC input to the THCs, the presence of both the F2 and F4 fractions suggest bitumen from the LOS has been incorporated in the PMM. Future research characterizing the degree of BOC contribution to the THCs will be important before reclaimed landforms begin to move forward in the certification process. As seen in this survey, THC values in the PMM vary around the CCME (2008) guideline limits for petroleum hydrocarbons in soil. If the biogenic contribution from the peat is significant, it could cause some plots to appear to exceed guidelines when they do not.

The potential incorporation of bitumen into the PMM may explain the similarity in Ni, Mo, and Re enrichment between the PMM and LOS, and may complicate their use as indicator metals moving forward. Vanadium was significantly more enriched in the LOS than the PMM and had a good detection rate in the pine seedlings. As such, it may be possible to use V as an indicator of when tree roots begin interacting with the LOS overburden. If concentrations of V in the tree reflect the concentrations in soil surrounding fine roots, a significant increase in V concentrations in wood in a given year compared to the baselines established in this survey may indicate that roots have grown into the LOS.

Going forward, to better understand how roots interact with LOS, researchers should include measurements of soil organic matter and dissolved organic matter concentrations in the soil pore water. With the variable mixture of peat and mineral matter in the capping material, these measurements should be included in future analysis as both can affect the movement and availability of metals through sorption/desorption to organic matter (Sauvé et al., 2000). In addition, I chose to analyse jack pine to remain consistent with chapter 2. Extending the analysis to other reclamation species could prove beneficial. Trembling aspen, a faster growing species whose roots were detected at the LOS interface, may show a more immediate relationship between tree and soil metal concentrations while white spruce, the slowest growing species on the site, may be the least likely to have already reached the LOS and may provide a clearer signal. Care should be taken, however, when comparing trace metal concentrations between species as they may uptake and translocate elements differently.

Figures

Figure 3.1 Replicated contouring treatments (red squares) and location of plots (blue dots) on the reclaimed lean oil sands overburden landform. Note the series of mounds down the slope.

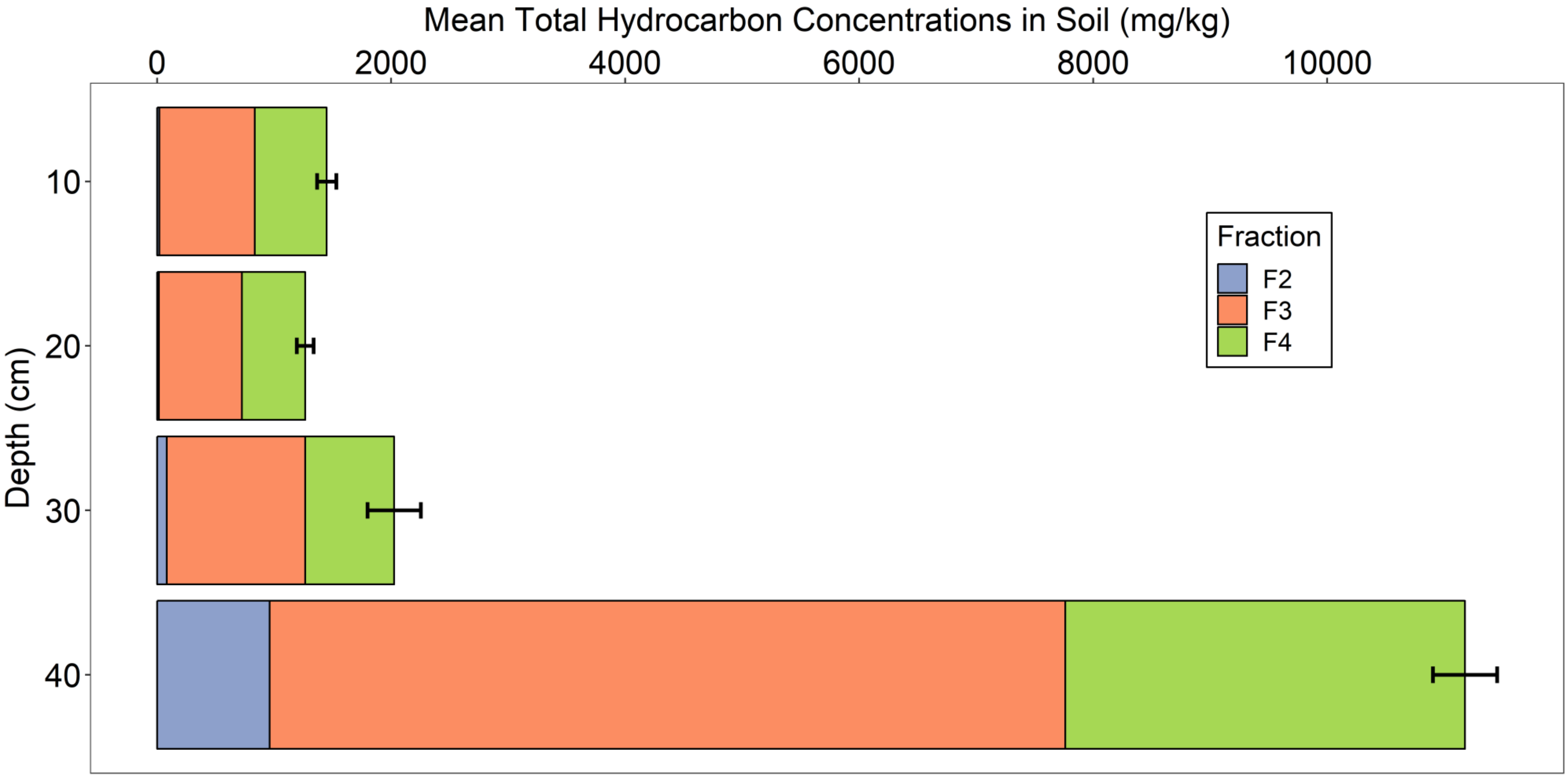

Figure 3.2 Total hydrocarbon concentrations in soils placed over reclaimed lean oil sands overburden by depth and F fraction. The 0-30 cm depths comprise the peat-mineral-mix capping material and the 40 cm depth is the lean oil sands overburden. Error bars represent standard error of the mean of the total concentration.

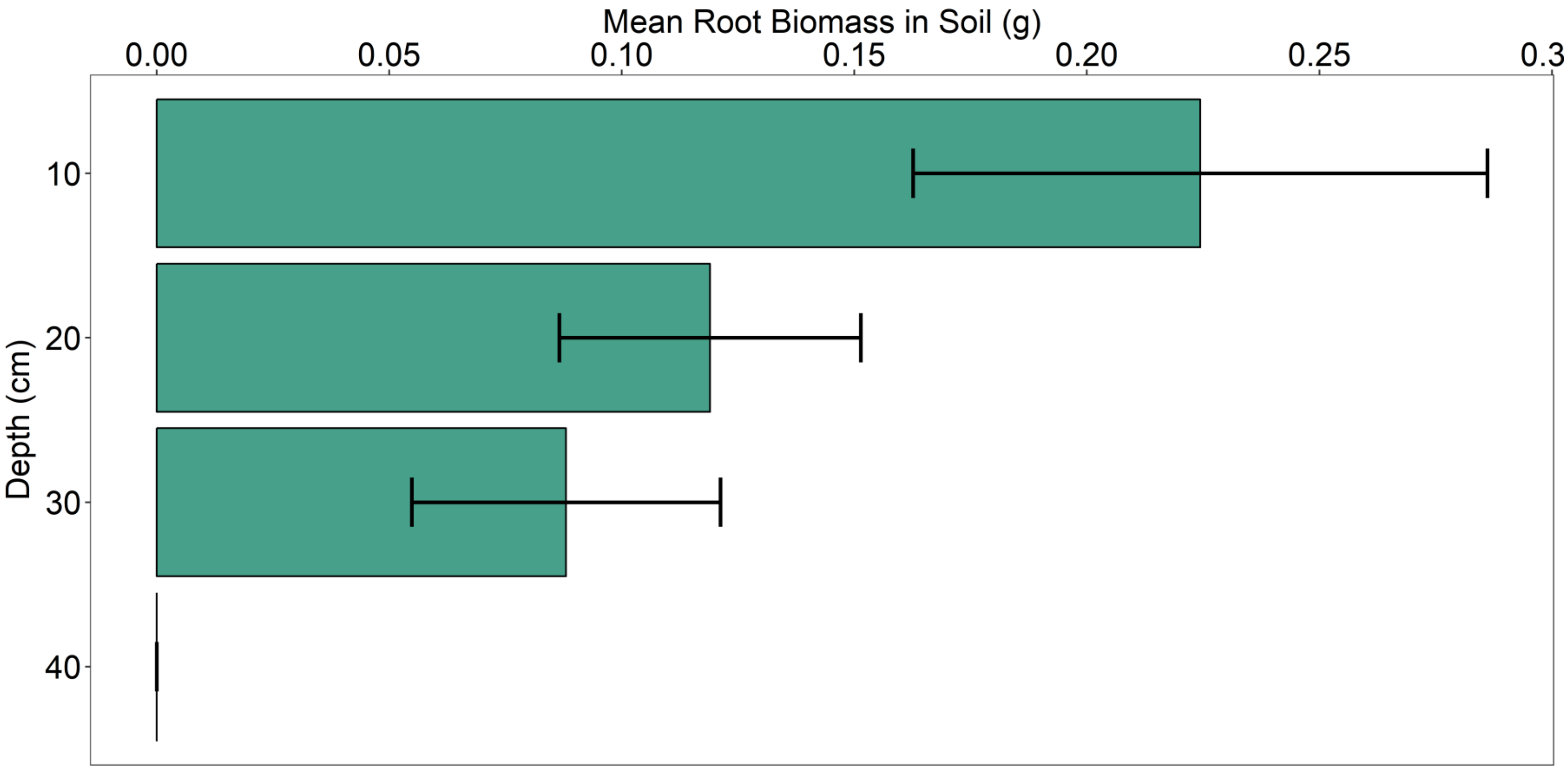

Figure 3.3 Mean root biomass (g dry mass) by depth. The 0-30 cm depths comprise the peat-mineral-mix capping material and the 40 cm depth is the lean oil sands overburden. Error bars represent standard error of the mean.

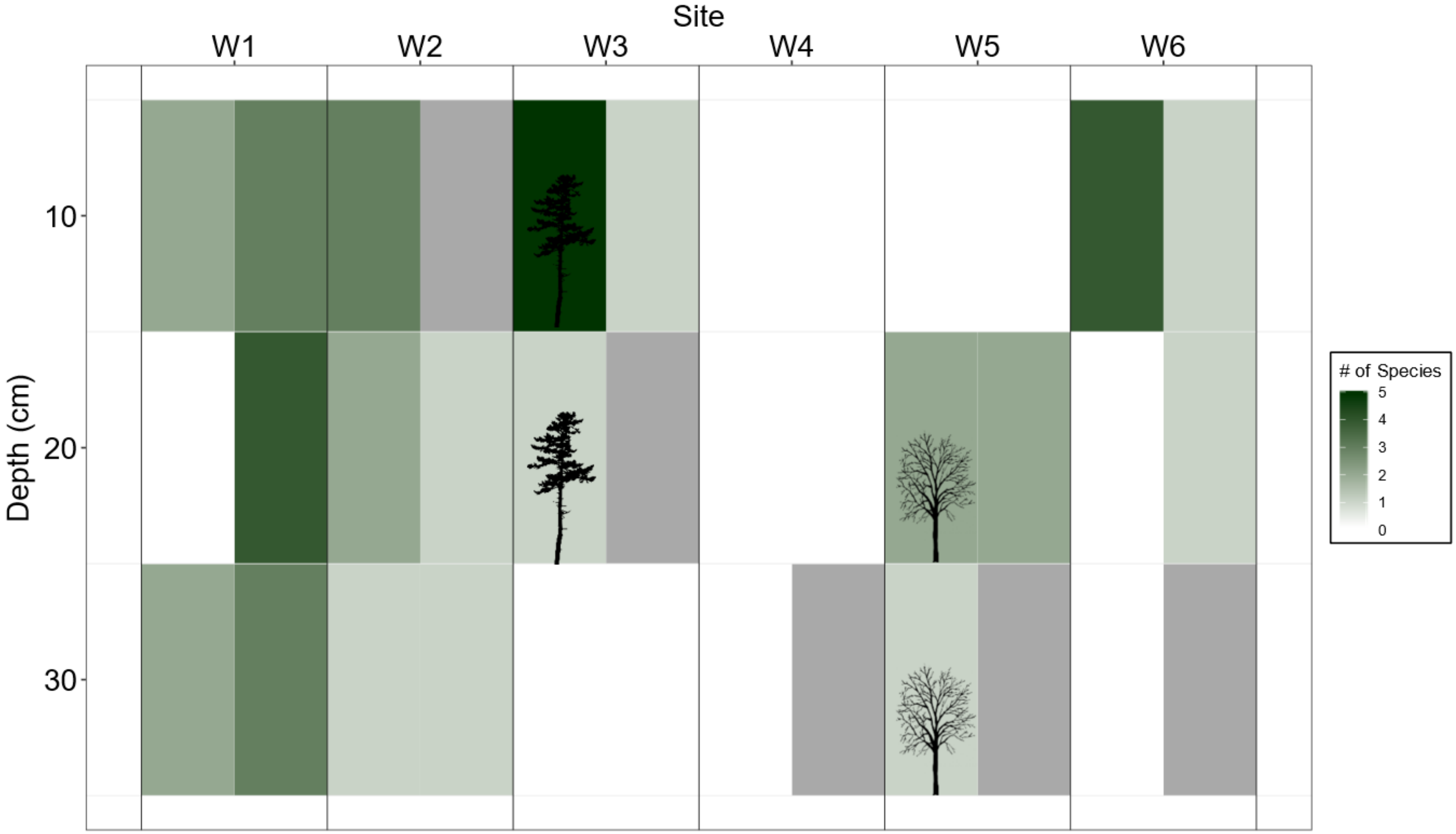

Figure 3.4 Number of plant species detected in each soil sample across study plots. Gray squares represent samples where root DNA did not amplify. Squares with trees represent the detection of jack pine (*Pinus banksiana* Lamb.) and trembling aspen (*Populus tremuloides)* in the samples.

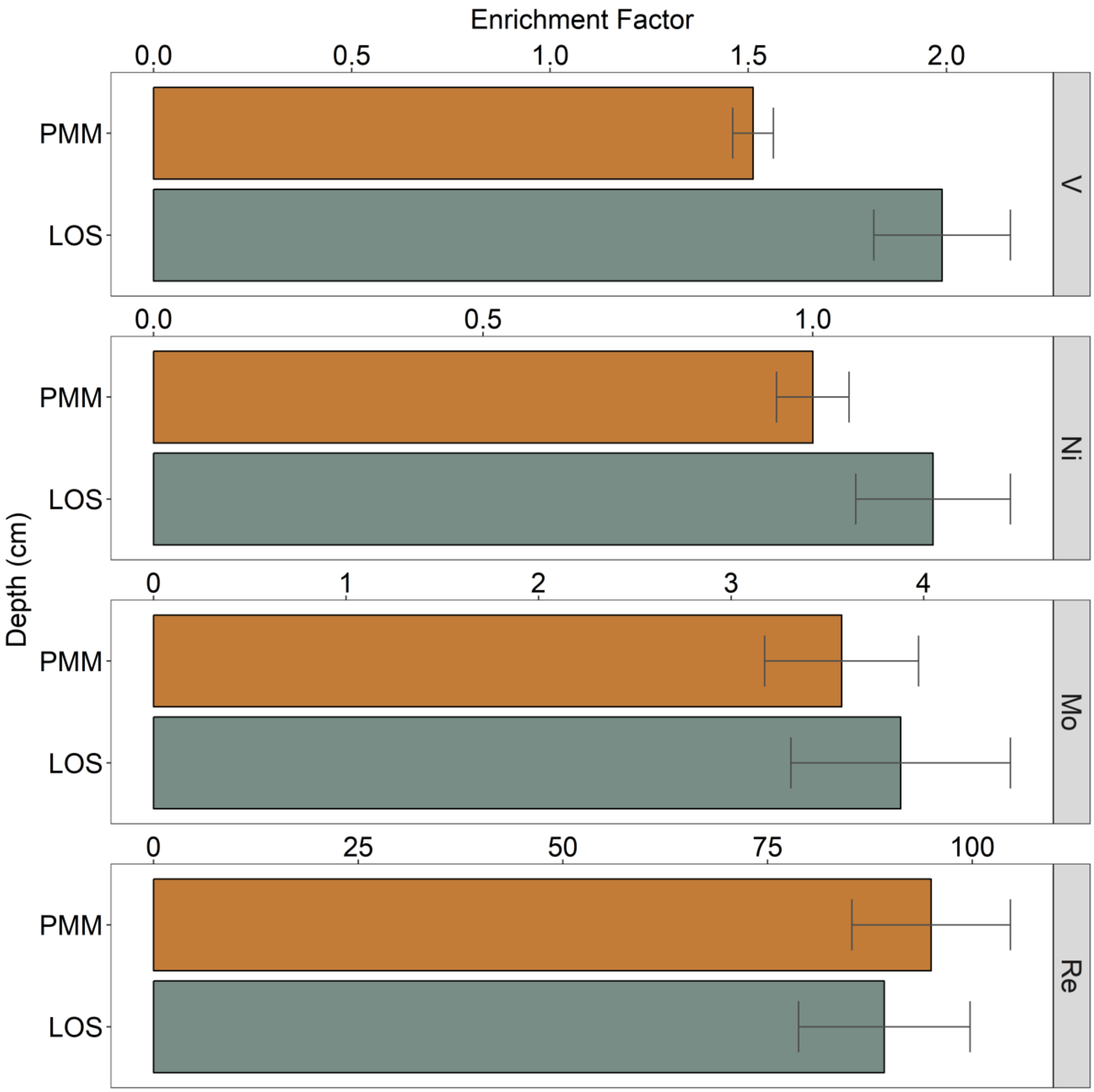

Figure 3.5 Enrichment factors (([M]/[Y])$_{soil}$/([M]/[Y])$_{UCC}$)) of V, Ni, Mo, and Re with depth. Error bars represent standard error of the mean.

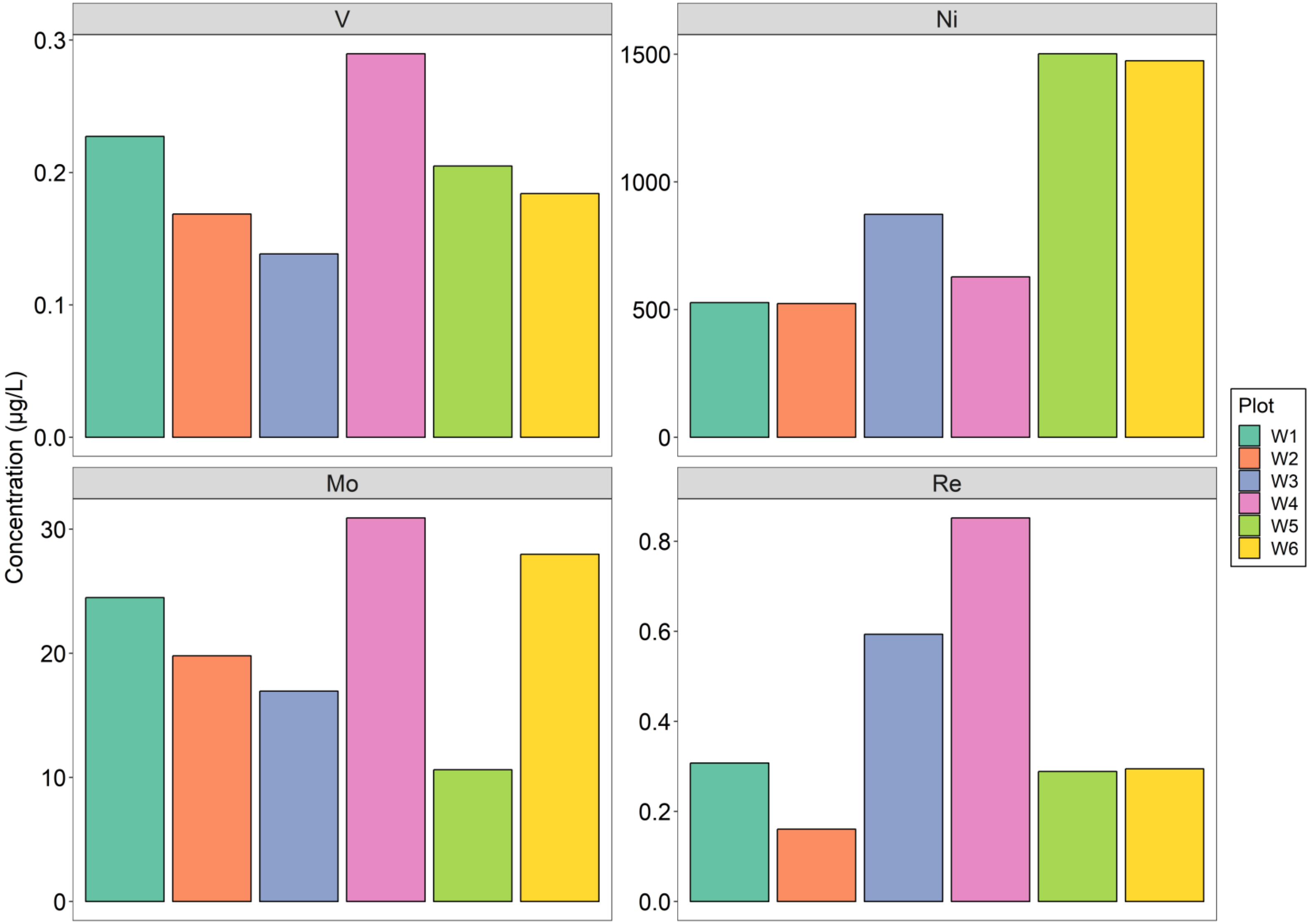

Figure 3.6 Trace metal concentrations in soil pore water among plots.

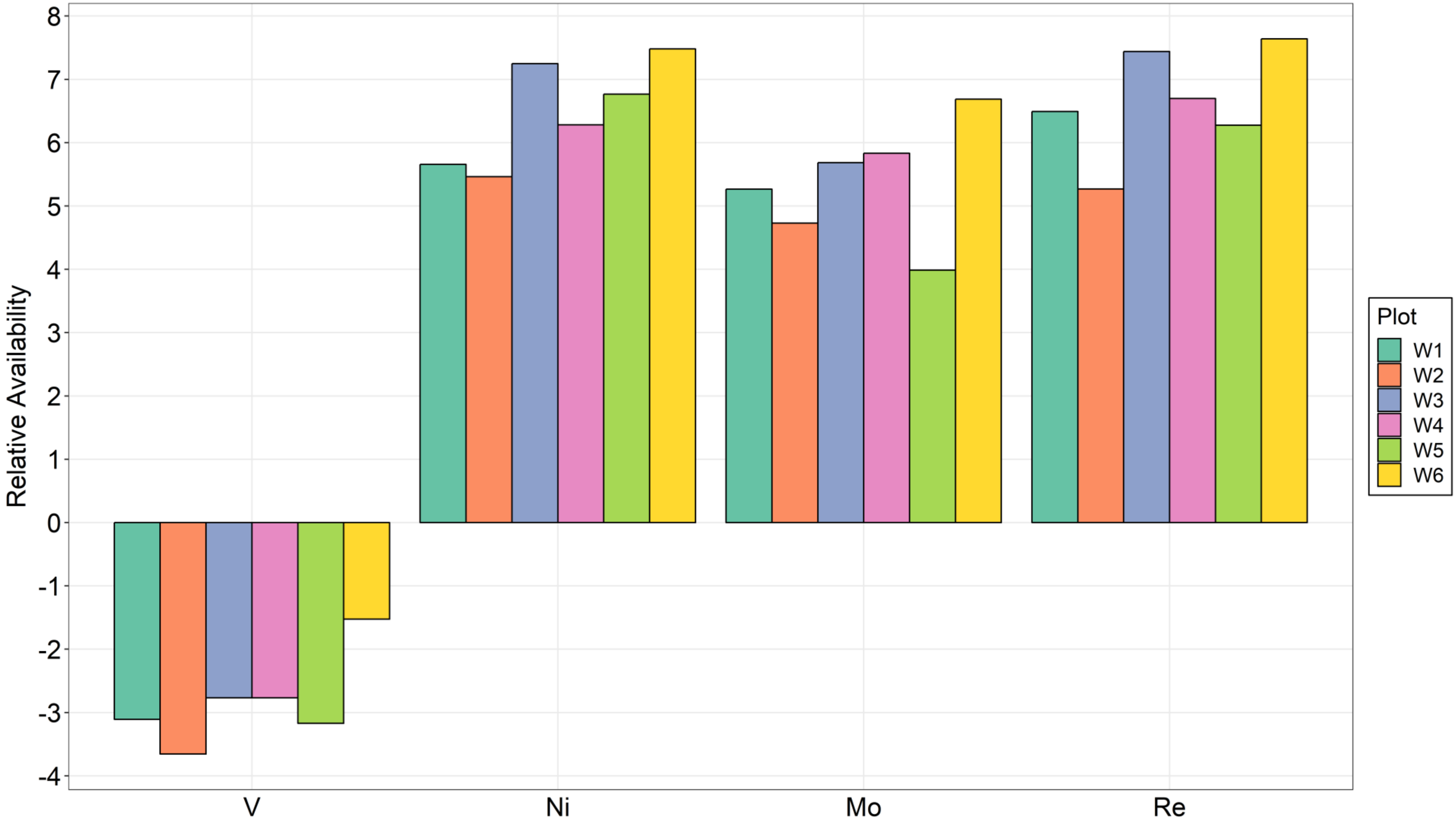

Figure 3.7 Relative availability (log{([M]/[Cr])water/([M]/[Cr])soil)}) of V, Ni, Mo, and Re across plots.

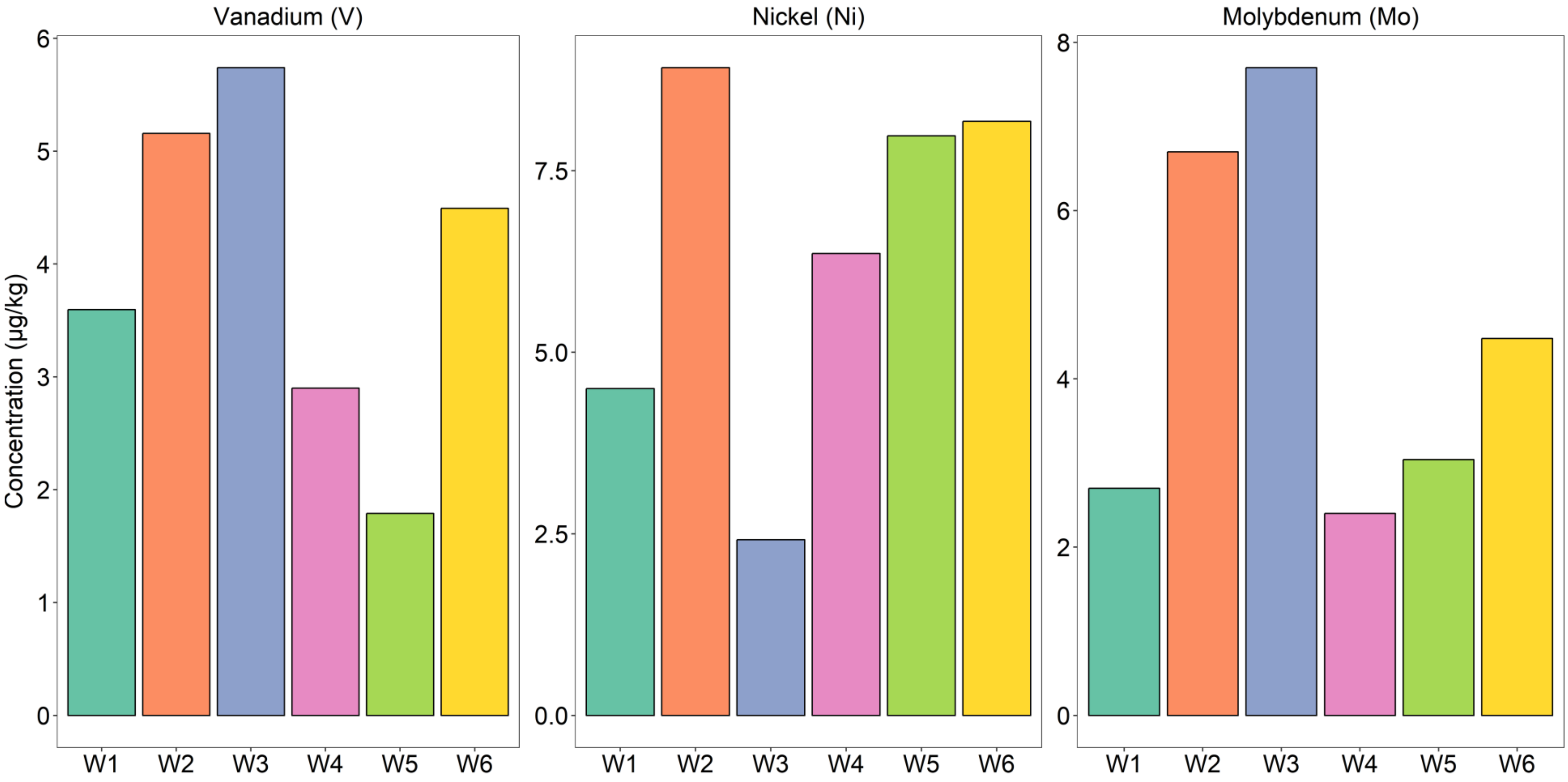

Figure 3.8 Trace metal concentrations in tree wood by plot. Owing to the presence of <LOD values, bars for Ni and Mo were created using summary statistics calculated by the Kaplan-Meier method with the NADA package in R (Lee, 2019).

Tables

Table 3.1 Hydrocarbon concentrations with depth. All units in mg kg^{-1}. Canadian Council of Ministers of the Environment (CCME, 2008) limits for coarse- and fine-textured natural parkland soils provided for reference.

	F2			F3			F4			Total		
Depth	Mean (±SD)	Min	Max	Mean (±SD)	Min	Max	Mean (±SD)	Min	Max	Mean (±SD)	Min	Max
10	19.8 (±13.0)	0	51	814.2 (±474.7)	540	2300	615.0 (±284.1)	450	1500	1448.9 (±766.6)	1001	3851
20	130.4 (±127.7)	0	359	1515.4 (±922.5)	360	3068	932.2 (±471.8)	230	1692	2578.0 (±1507.9)	610	5043
30	83.0 (±201.5)	0	720	1183.3 (±1565.9)	390	6000	759.2 (±789.2)	180	3000	2025.5 (±2538.7)	586	9720
40	960.8 (±438.9)	400	1900	6800.0 (±2254.3)	400	11000	3416.7 (±951.4)	2200	5100	11177.5(±3620.1)	7260	18000
CCME	Coarse		150			300			2800			3460
	Fine		150			1300			5600			7050

Table 3.2 Root biomass data (g dry weight) with depth across study plots

	WODA Root Mass (g) (n = 6)		
Depth (cm)	Mean (±SD)	Min	Max
10	0.2244 (±0.21)	0.0359	0.7552
20	0.1190 (±0.11)	0.0199	0.3620
30	0.0880 (±0.12)	0.0068	0.4115

Chapter 4: General Synthesis

This thesis sought to explore dendrochemistry as a potential way to monitor the rooting behaviour of trees growing in proximity to bitumen. My first data chapter (chapter 2) compared the concentrations of vanadium (V), nickel (Ni), molybdenum (Mo), and rhenium (Re) in jack pine (*Pinus banksiana*) wood from trees growing on sites containing natural shallow deposits of bitumen to those growing on similar soils without bitumen. I found that Ni concentrations, and to a lesser extent V, were higher in trees growing on shallow bitumen deposits. Chapter 3 established baseline values for hydrocarbon and trace metal concentrations in soil, root biomass with depth, and trace metal concentrations in jack pine seedling for a reclamation site constructed with lean oil sands (LOS). These values will enable reclamation practitioners to monitor changes in root behaviour and trace metal concentrations over time.

4.1 Contrasts Across Sites, Limitations, and Future Directions:

4.1.1 Hydrocarbons in Soils

The existence of natural shallow deposits of bitumen provides an opportunity to study plant relationships with elevated concentrations of hydrocarbons in an intact forest system. They may also provide long-term reference sites for reclamation efforts as these forests have established on soils with hydrocarbon concentrations often in excess of CCME recommended guideline values for parklands with coarse-textured soils. However, further research characterizing the nature of the hydrocarbon compounds present is necessary before direct comparisons can be made. While total hydrocarbon concentrations in the natural soils (chapter 2) are comparable to those in the peat-mineral mix (PMM) on the reclamation site (chapter 3), the PMM appears to have more F2 (nC10-16) and F3 (nC16-34) fractions where the natural soils are mostly F3 and F4. This difference could be very important in the future as shorter chain hydrocarbons (nC6-25) may have greater ecological significance due to their bioavailability (Brassington et al., 2007; Maletić et al., 2011). It is also worth noting that the total hydrocarbon concentration of the LOS on the reclaimed site was on average double that of the natural soils at 40 cm of depth.

4.1.2 Trace Metals and Dendrochemistry

In Chapter 2, I showed that concentrations of V, Ni, Mo, and Re were enriched in soils and their pore waters on sites with shallow bitumen deposits and that this enrichment translated to elevated concentrations of Ni and V in the trees. The decrease in Mo concentrations in trees, though marginal, demonstrates the importance of individual metal chemistry in dendrochemical studies. The acidic and mineral natures of the study soils can explain the differing trends between the target metals, but the picture could still be clearer with additional data. Metal mobility in soil, water, and wood is heavily influenced by many factors including total organic mater in soil and dissolved organic mater in soil pore water (Sauvé et al., 2000), which were not measured in my research. Including the analysis of macro-nutrients in future research could also allow researchers to compare metal concentrations between trees standardised to their rates of nutrient uptake by using a nutrient/metal ratio such as Ca/Sr or K/Rb (Watmough, 2008). Longer-term monitoring of the reclamation site could benefit from taking these measurements to help predict metal mobility due to the variable mixture of organic peat and mineral matter in the capping material.

My thesis stresses the importance of robust sample collection and processing methods and properly understanding background reference conditions prior to drawing conclusions from trace metal data. As many concentrations of trace metals in this study were in the ppb (µg kg $^{-1}$ or µg L $^{-1}$) range, any contamination from sampling or processing can significantly influence the results. Properly identifying and controlling for sources of metal contamination, such the increment borer used in this study, enables researchers to be confident in the data collected. I was able to correlate the concentrations of the target metals to the presence of bitumen due to the similarity in non-target trace metal concentrations between the bituminous and reference soils. Soil pH, soil type, and tree age are all significantly different between the natural and reclaimed sites, and it is for this reason that I do not draw direct comparisons in trace metal concentrations between them.

4.2 Conclusion

In all, with strong analytical methods and good reference conditions, dendrochemistry can be used to monitor root growth in soils with characteristic substrates. My findings show the potential to use Ni and V as indicators of tree rooting behaviour in soils containing shallow deposits of bitumen. This possibility presents reclamation practitioners and researchers with a useful tool to monitor plant growth across both constructed landforms and natural landscapes along with strong data against which to compare future values.

Literature Cited

Agriculture Canada (2019a). National Ecological Framework of Canada. Government of Canada. http://www.agr.gc.ca/atlas/agpv?webmap-en=302f656cd80c42af85e9b411e7202c8f&webmap-fr=b595bef91d8d4ec3817eae5b5dfd4d61. Accessed 10 April 2019.

Agriculture Canada (2019b). Soils of Canada. Government of Canada. http://www.agr.gc.ca/atlas/agpv?webmap-en=c225cc78d5b142d58eacefae91cc535b&webmap-fr=ad0b6822a33e411683f99979a1167efa . Accessed 10 April 2019.

Turchenek, L.W., & Lindsay, J.D. (1982). Soils inventory of the Alberta Oil Sands Environmental Research Program by Alberta Research Council. AOSERP Report 122. 240.

Barber, L. A., Bockstette, J., Christensen, D. O., Tallon, L. K., & Landhäusser, S. M. (2015). Effect of soil cover system design on cover system performance and early tree establishment. *Mine Closure. InfoMine Inc., Vancouver, BC, Canada*, 1–9.

Bicalho, B., Grant-Weaver, I., Sinn, C., Donner, M. W., Woodland, S., Pearson, G., … Shotyk, W. (2017). Determination of ultratrace (<0.1 mg/kg) elements in Athabasca Bituminous Sands mineral and bitumen fractions using inductively coupled plasma sector field mass spectrometry (ICP-SFMS). *Fuel*, *206*, 248–257. doi:10.1016/j.fuel.2017.05.095

Bockstette, S. W., Pinno, B. D., Dyck, M. F., & Landhäusser, S. M. (2017). Root competition, not soil compaction, restricts access to soil resources for aspen on a reclaimed mine soil. *Botany*, *95*(7), 685–695. doi: 10.1139/cjb-2016-0301

Böhm, W. (2012). Methods of Studying Root Systems. *Ecological Studies*. 33. Springer Science & Business Media. 188.

Brassington, K. J., Hough, R. L., Paton, G. I., Semple, K. T., Risdon, G. C., Crossley, J., … Pollard, S. J. T. (2007). Weathered Hydrocarbon Wastes: A Risk Management Primer. *Critical Reviews in Environmental Science and Technology*, *37*(3), 199–232. doi: 10.1080/10643380600819625

Brown, P. H., Welch, R. M., & Cary, E. E. (1987). Nickel: A Micronutrient Essential for Higher Plants. *Plant Physiology*, *85*(3), 801–803. doi:10.1104/pp.85.3.801

Campbell, B. D., Grime, J. P., & Mackey, J. M. L. (1991). A trade-off between scale and precision in resource foraging. *Oecologia*, *87*(4), 532–538. doi: 10.1007/BF00320417

Campisano, R., K. Hall, J. Griggs, S. Willison, S. Reimer, H. Mash, M. Magnuson, L. Boczek, & E. Rhodes. Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2017. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-17/356, 2017

Cataldo, D. A., Garland, T. R., & Wildung, R. E. (1978). Nickel in Plants: I. Uptake Kinetics Using Intact Soybean Seedlings. *Plant Physiology*, *62*(4), 563–565. doi:10.1104/pp.62.4.563

CCME (2008). *Canada-wide Standards for Petroleum Hydrocarbons (PHC) in Soil*. Canadian Council of Ministers of the Environment. 8.

CCME (2001). Reference Method for the Canada-Wide Standard for Petroleum Hydrocarbons in Soil - Tier 1 Method. Canadian Council of Ministers of the Environment. 44.

Chun, L., & Hui-yi, H. (1992). Tree-ring element analysis of Korean pine (Pinus koraiensis Sieb. et Zucc.) and Mongolian oak (Quercus mongolica Fisch. ex Turcz.) from Changbai Mountain, north-east China. *Trees*, *6*(2). doi:10.1007/BF00226588

Cutter, B. E., & Guyette, R. P. (1993). Anatomical, Chemical, and Ecological Factors Affecting Tree Species Choice in Dendrochemistry Studies. *Journal of Environmental Quality*, *22*(3), 611–619. doi:10.2134/jeq1993.00472425002200030028x

Dhar, A., Comeau, P. G., Karst, J., Pinno, B. D., Chang, S. X., Naeth, A. M., … Bampfylde, C. (2018). Plant community development following reclamation of oil sands mine sites in the boreal forest: a review. *Environmental Reviews*, *26*(3), 286–298. doi: 10.1139/er-2017-0091

Du, L., Cuss, C.W., Dyck, M., Noernberg, T. and Shotyk, W. Bioaccessibility and bioavailability of trace elements in soils: size based analysis of the chemical composition of molecular species in soil solutions using AF4 ICP MS. Canadian Journal of Soil Science (manuscript in preparation)

Dunn, C. E. (2001). Biogeochemical exploration methods in the Canadian Shield and Cordillera. *Geological Society, London, Special Publications*, *185*(1), 151–164. doi:10.1144/GSL.SP.2001.185.01.07

Dunn, C. E. (2007). Biogeochemistry in Mineral Exploration. In C. E. Dunn (Ed.), *Handbook of Exploration and Environmental Geochemistry* (Vol. 9, pp. xiii–460). Elsevier Science B.V. doi:10.1016/S1874-2734(07)09001-8

Environment Canada (2019). Canadian Climate Normals 1981-2010 Station Data - Fort McMurray. http://climate.weather.gc.ca/climate_normals/results_1981_2010_e.html?stnID=2519&month1=0&month2=12. Accessed 10 April 2019.

Eskew, D. L., Welch, R. M., & Cary, E. E. (1983). Nickel: An Essential Micronutrient for Legumes and Possibly All Higher Plants. *Science*, *222*(4624), 621–623. doi:10.1126/science.222.4624.621

Fisher, T. G., & Smith, D. G. (1994). Glacial Lake Agassiz: Its northwest maximum extent and outlet in Saskatchewan (Emerson Phase). *Quaternary Science Reviews*, *13*(9), 845–858. doi:10.1016/0277-3791(94)90005-1

Government of Alberta. (2017). *Oil Sands Facts and Stats*. https://open.alberta.ca/publications/oil-sands-facts-and-stats . Accessed on 10 April 2019

Government of Alberta (2014) Environmental protection and enhancement act. Revised Statutes of Alberta 2000 Chapter E-12. Alberta Queen's Printer. http://environment.alberta.ca/01530.html. Accessed on 10 April 2019.

Gale, M. R., & Grigal, D. F. (1987). Vertical root distributions of northern tree species in relation to successional status. *Canadian Journal of Forest Research*, *17*(8), 829–834. doi:10.1139/x87-131

Goldschmidt, V. M. (1937). The principles of distribution of chemical elements in minerals and rocks. The seventh Hugo Müller Lecture, delivered before the Chemical Society on March 17th, 1937. *Journal of the Chemical Society (Resumed)*, 655–673.

Greger, M. (2004). Uptake of nuclides by plants. Technical Report TR-04-14. SKB. 66

Hargesheimer, E. E., Apps, M. J., & Malhotra, S. S. (1980). Determination of vanadium in jack pine needles using neutron activation analysis. *Environmental Technology Letters*, *1*(6), 273–282. doi:10.1080/09593338009383977

Helsel, D. R. (2010). Summing nondetects: Incorporating low-level contaminants in risk assessment. *Integrated Environmental Assessment and Management*, *6*(3), 361–366. doi: 10.1002/ieam.31

Howell, D. M., & MacKenzie, M. D. (2017). Using bioavailable nutrients and microbial dynamics to assess soil type and placement depth in reclamation. *Applied Soil Ecology*, *116*, 87–95. doi: 10.1016/j.apsoil.2017.03.023

Huang, M., Barbour, S. L., Elshorbagy, A., Zettl, J. D., & Cheng Si, B. (2011). Infiltration and drainage processes in multi-layered coarse soils. *Canadian Journal of Soil Science*, *91*(2), 169–183. doi: 10.1139/CJSS09118

Huang, M., Spies, J., Barbour, S. L., Si, B. C., & Zettl, J. (2013). Impact of Textural Layering on Water Retention Within Drained Sand Profiles: *Soil Science*, *178*(9), 496–504. doi: 10.1097/SS.0000000000000014

Huang, J.-H., Huang, F., Evans, L., & Glasauer, S. (2015). Vanadium: Global (bio)geochemistry. *Chemical Geology*, *417*, 68–89. doi:10.1016/j.chemgeo.2015.09.019

Hutchinson, T. C., Watmough, S. A., Sager, E. P., & Karagatzides, J. D. (1998). Effects of excess nitrogen deposition and soil acidification on sugar maple (Acer saccharum) in Ontario, Canada: an experimental study. *Canadian Journal of Forest Research*, *28*(2), 299–310. doi: 10.1139/x97-215

Imtiaz, M., Rizwan, M. S., Xiong, S., Li, H., Ashraf, M., Shahzad, S. M., … Tu, S. (2015). Vanadium, recent advancements and research prospects: A review. *Environment International*, *80*, 79–88. doi:10.1016/j.envint.2015.03.018

Jackson, R. B., Canadell, J., Ehleringer, J. R., Mooney, H. A., Sala, O. E., & Schulze, E. D. (1996). A global analysis of root distributions for terrestrial biomes. *Oecologia*, *108*(3), 389–411. doi:10.1007/BF00333714

Jung, K., Duan, M., House, J., & Chang, S. X. (2014). Textural interfaces affected the distribution of roots, water, and nutrients in some reconstructed forest soils in the Athabasca oil sands region. *Ecological Engineering*, *64*, 240–249. doi:10.1016/j.ecoleng.2013.12.037

Jung, K., Chang, S. X., Ok, Y. S., & Arshad, M. A. (2013). Critical loads and H+ budgets of forest soils affected by air pollution from oil sands mining in Alberta, Canada. *Atmospheric Environment*, *69*, 56–64. doi: 10.1016/j.atmosenv.2012.12.010

Kelly, E. N., Schindler, D. W., Hodson, P. V., Short, J. W., Radmanovich, R., & Nielsen, C. C. (2010). Oil sands development contributes elements toxic at low concentrations to the Athabasca River and its tributaries. *Proceedings of the National Academy of Sciences*, *107*(37), 16178–16183. doi:10.1073/pnas.1008754107

Kelly-Hooper, F., Farwell, A. J., Pike, G., Kennedy, J., Wang, Z., Grunsky, E. C., & Dixon, D. G. (2013). Is it clean or contaminated soil? Using petrogenic versus biogenic GC-FID chromatogram patterns to mathematically resolve false petroleum hydrocarbon detections in clean organic soils: A crude oil–spiked peat microcosm experiment. *Environmental Toxicology and Chemistry*, *32*(10), 2197–2206. doi: 10.1002/etc.2285

Kirchner, P., Biondi, F., Edwards, R., & McConnell, J. R. (2008). Variability of trace metal concentrations in Jeffrey pine (Pinus jeffreyi) tree rings from the Tahoe Basin, California, USA. *Journal of Forest Research*, *13*(6), 347–356. doi:10.1007/s10310-008-0093-5

Larsson, L. Å. (2018). Coorecorder. Cybis Elektronick & Data AB, Saltsjobaden, Sweden.

Lee, T. (2019). NADA: Nondetects and Data Analysis for Environmental Data. R package version 1.6-1. https://CRAN.R-project.org/package=NADA. Accessed 10 April 2019.

Lepp, N. W. (1975). The potential of tree-ring analysis for monitoring heavy metal pollution patterns. *Environmental Pollution (1970)*, *9*(1), 49–61. doi:10.1016/0013-9327(75)90055-5

Leskiw, LA. (2005). Hydrocarbons in Natural Soils: Literature Review. Report prepared for the Cumulative Environmental Management Association. 30.

Leskiw, LA. (2006). Hydrocarbons in Natural Oil Sands Soils: Field Survey. Report prepared for the

Cumulative Environmental Management Association. 56.

Macdonald, S. E., Landhäusser, S. M., Skousen, J., Franklin, J., Frouz, J., Hall, S., … Quideau, S. (2015). Forest restoration following surface mining disturbance: challenges and solutions. *New Forests*, *46*(5), 703–732. doi: 10.1007/s11056-015-9506-4

MacKenzie, M. D., & Quideau, S. A. (2010). Microbial community structure and nutrient availability in oil sands reclaimed boreal soils. *Applied Soil Ecology*, *44*(1), 32–41. doi: 10.1016/j.apsoil.2009.09.002

Maletić, S. P., Dalmacija, B. D., Rončević, S. D., Agbaba, J. R., & Perović, S. D. U. (2011). Impact of hydrocarbon type, concentration and weathering on its biodegradability in soil. *Journal of Environmental Science and Health, Part A*, *46*(10), 1042–1049. doi: 10.1080/10934529.2011.590380

Märten, A., Berger, D., Köhler, M., & Merten, D. (2015). The dendroanalysis of oak trees as a method of biomonitoring past and recent contamination in an area influenced by uranium mining. *Environmental Science and Pollution Research*, *22*(24), 19417–19425. doi:10.1007/s11356-015-4902-z

McIlveen, W. D., & Negusanti, J. J. (1994). Nickel in the terrestrial environment. *Science of The Total Environment*, *148*(2), 109–138. doi:10.1016/0048-9697(94)90390-5

McNickle, G. G., & Cahill, J. F. (2009). Plant root growth and the marginal value theorem. *Proceedings of the National Academy of Sciences*, *106*(12), 4747–4751. doi: 10.1073/pnas.0807971106

Metzler, P., La Flèche, M., & Karst, J. (n.d.). Expanding and testing fluorescent amplified fragment length polymorphisms for identifying roots of boreal forest plant species. *Applications in Plant Sciences*. doi: 10.1002/aps3.1236

Melnik, K., Landhäusser, S. M., & Devito, K. (2018). Role of microtopography in the expression of soil propagule banks on reclamation sites. *Restoration Ecology*, *26*(S2), S200–S210. doi: 10.1111/rec.12587

Mikkonen, A., & Tummavuori, J. (1994). Retention of vanadium (V) by three Finnish mineral soils. *European Journal of Soil Science*, *45*(3), 361–368. doi:10.1111/j.1365-2389.1994.tb00520.x

Mishra, D., & Kar, M. (1974). Nickel in plant growth and metabolism. *The Botanical Review*, *40*(4), 395–452. doi:10.1007/BF02860020

Morrell, B. G., Lepp, N. W., & Phipps, D. A. (1986). Vanadium uptake by higher plants: Some recent developments. *Environmental Geochemistry and Health*, *8*(1), 14–18. doi:10.1007/BF02280116

Mullan-Boudreau, G., Davies, L., Devito, K., Froese, D., Noernberg, T., Pelletier, R., & Shotyk, W. (2017). Reconstructing Past Rates of Atmospheric Dust Deposition in the Athabasca Bituminous Sands Region Using Peat Cores from Bogs. *Land Degradation & Development*, *28*(8), 2468–2481. doi: 10.1002/ldr.2782

Nieminen, T. M., Ukonmaanaho, L., Rausch, N., & Shotyk, W. (2007). Biogeochemistry of Nickel and Its Release into the Environment. *Metal Ions in Life Sciences, 2,* 1-30.

Odabasi, M., Tolunay, D., Kara, M., Falay, E. O., Tuna, G., Altiok, H., … Elbir, T. (2016). Investigation of spatial and historical variations of air pollution around an industrial region using trace and macro elements in tree components. *Science of the Total Environment*, *550*, 1010–1021. doi:10.1016/j.scitotenv.2016.01.197

Padilla, K. L., & Anderson, K. A. (2002). Trace element concentration in tree-rings biomonitoring centuries of environmental change. *Chemosphere*, *49*(6), 575–585. doi:10.1016/S0045-6535(02)00402-2

Panyushkina, I. P., Shishov, V. V., Grachev, A. M., Knorre, A. A., Kirdyanov, A. V., Leavitt, S. W., … Hughes, M. K. (2016). Trends In Elemental Concentrations of Tree Rings From the Siberian Arctic. *Tree-Ring Research*, *72*(2), 67–77. doi:10.3959/1536-1098-72.02.67

Pendias, H., & Kabata-Pendias, A. (2000). *Trace Elements in Soils and Plants, Third Edition*. CRC Press. doi:10.1201/9781420039900

Pernitsky, T., Hu, W., Si, B. C., & Barbour, L. (2016). Effects of petroleum hydrocarbon concentration and bulk density on the hydraulic properties of lean oil sand overburden. *Canadian Journal of Soil Science*, *96*(4), 435–446. doi: 10.1139/cjss-2015-0126

Pinno, B. D., Landhäusser, S. M., MacKenzie, M. D., Quideau, S. A., & Chow, P. S. (2012). Trembling aspen seedling establishment, growth and response to fertilization on contrasting soils used in oil sands reclamation. *Canadian Journal of Soil Science*, *92*(1), 143–151.

Plourde, A., Krause, C., & Lord, D. (2009). Spatial distribution, architecture, and development of the root system of Pinus banksiana Lamb. in natural and planted stands. *Forest Ecology and Management*, *258*(9), 2143–2152. doi: 10.1016/j.foreco.2009.08.016

Pulford, I. D., & Watson, C. (2003). Phytoremediation of heavy metal-contaminated land by trees—a review. *Environment International*, *29*(4), 529–540. doi:10.1016/S0160-4120(02)00152-6

Rowland, S. M., Prescott, C. E., Grayston, S. J., Quideau, S. A., & Bradfield, G. E. (2009). Recreating a Functioning Forest Soil in Reclaimed Oil Sands in Northern Alberta: An Approach for Measuring Success in Ecological Restoration. *Journal of Environmental Quality*, *38*(4), 1580–1590. doi: 10.2134/jeq2008.0317

R Core Team. (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website https://www.R-project.org/. Accessed 10 April 2019.

Roy, J. L., McGill, W. B., Lowen, H. A., & Johnson, R. L. (2003). Relationship between Water Repellency and Native and Petroleum-Derived Organic Carbon in Soils. *Journal of Environmental Quality*, *32*(2), 583–590. doi:10.2134/jeq2003.5830

Rudnick, R. L., & Gao, S. (2014). Composition of the Continental Crust. In *Treatise on Geochemistry* (pp. 1–51). Elsevier. doi:10.1016/B978-0-08-095975-7.00301-6

Sauvé, S., Hendershot, W., & Allen, H. E. (2000). Solid-Solution Partitioning of Metals in Contaminated Soils: Dependence on pH, Total Metal Burden, and Organic Matter. *Environmental Science & Technology*, *34*(7), 1125–1131. doi:10.1021/es9907764

Schenk, H. J., & Jackson, R. B. (2002). The Global Biogeography of Roots. *Ecological Monographs*, *72*(3), 311–328. doi:10.1890/0012-9615(2002)072[0311:TGBOR]2.0.CO;2

Schlesinger, W. H., Klein, E. M., & Vengosh, A. (2017). Global biogeochemical cycle of vanadium. *Proceedings of the National Academy of Sciences*, *114*(52), E11092–E11100. doi:10.1073/pnas.1715500114

Schmidt, M., Leybourne, M. I., & Kyser, T. K. (2017). Dendrochronology in mineral exploration: developing tools to see through anthropogenic impacts. *Geochemistry: Exploration, Environment, Analysis*, geochem2017-027. doi:10.1144/geochem2017-027

Selby, D., & Creaser, R. A. (2005). Direct Radiometric Dating of Hydrocarbon Deposits Using Rhenium-Osmium Isotopes. *Science*, *308*(5726), 1293–1295. doi:10.1126/science.1111081

Shcherbenko, T. A., Koptsik, G. N., Groenenberg, B.-J., Lukina, N. V., & Livantsova, S. Y. (2008). Uptake of nutrients and heavy metals by pine trees under atmospheric pollution. *Moscow University Soil Science Bulletin*, *63*(2), 51–59. doi:10.3103/S0147687408020026

Shinonaga, T., Pröhl, G., Müller, H., & Ambe, S. (1999). Experimentally determined mobility of trace elements in soybean plants. *Science of The Total Environment*, *225*(3), 241–248. doi:10.1016/S0048-9697(98)00368-4

Shotyk, W., Bicalho, B., Cuss, C. W., Duke, M. J. M., Noernberg, T., Pelletier, R., … Zaccone, C. (2016). Dust is the dominant source of “heavy metals” to peat moss (Sphagnum fuscum) in the bogs of the Athabasca Bituminous Sands region of northern Alberta. *Environment International*, *92–93*, 494–506. doi: 10.1016/j.envint.2016.03.018

Shotyk, W., Krachler, M., Aeschbach-Hertig, W., Hillier, S., & Zheng, J. (James). (2010). Trace elements in recent groundwater of an artesian flow system and comparison with snow: enrichments, depletions, and chemical evolution of the water. *Journal of Environmental Monitoring*, *12*(1), 208–217. doi:10.1039/B909723F

Sigel, A., & Sigel, H. (2002). Molybdenum and Tungsten: Their Roles in Biological Processes. *Metals Ions in Biological System: Volume 39*. CRC Press. 856.

Strong, W. L., & Roi, G. H. L. (1983). Rooting depths and successional development of selected boreal forest communities. *Canadian Journal of Forest Research*, *13*(4), 577–588. doi:10.1139/x83-084

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, *17*(5), 1105–1109.

Turcotte, I., Quideau, S. A., & Oh, S.-W. (2009). Organic matter quality in reclaimed boreal forest soils following oil sands mining. *Organic Geochemistry*, *40*(4), 510–519. doi: 10.1016/j.orggeochem.2009.01.003

Visser, S. (2008). Petroleum Hydrocarbons (PHCs) in Lean Oil Sand (LOS): Degradation Potential and Toxicity to Ecological Receptors. Report prepared for Cumulative Environmental Management Association. 121.

Visser, S. (2011). Petroleum Hydrocarbons (PHCs) in Mineral Soil (Cg) Located Beneath Peat Deposits in the Athabasca Oil Sands Region: Their Potential for Degradation and Effects on Plants and Soil Fauna. Report prepared for Cumulative Environmental Management Association. 79

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., … Tilman, D. G. (1997). Human Alteration of the Global Nitrogen Cycle: Sources and Consequences. *Ecological Applications*, *7*(3), 737–750. doi:10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2

Watmough, S. A. (1999). Monitoring historical changes in soil and atmospheric trace metal levels by dendrochemical analysis. *Environmental Pollution*, *106*(3), 391–403. doi:10.1016/S0269-7491(99)00102-5

Watmough, S. A., & Hutchinson, T. C. (1999). Change in the dendrochemistry of sacred fir close to Mexico City over the past 100 years. *Environmental Pollution*, *104*(1), 79–88. doi:10.1016/S0269-7491(98)00150-X

Watmough, Shaun A. (1997). An evaluation of the use of dendrochemical analyses in environmental monitoring. *Environmental Reviews*, *5*(3–4), 181–201. doi:10.1139/a97-010

Watmough, Shaun A. (2008). Element mobility and partitioning along a soil acidity gradient in central Ontario forests, Canada. *Environmental Geochemistry and Health*, *30*(5), 431–444. doi:10.1007/s10653-007-9127-8

Welch, R. M. (1973). Vanadium Uptake by Plants: Absorption Kinetics and the Effects of pH, Metabolic Inhibitors, and Other Anions and Cations. *Plant Physiology*, *51*(5), 828–832. doi:10.1104/pp.51.5.828

Wichard, T., Mishra, B., Myneni, S. C. B., Bellenger, J.-P., & Kraepiel, A. M. L. (2009). Storage and bioavailability of molybdenum in soils increased by organic matter complexation. *Nature Geoscience*, *2*(9), 625–629. doi:10.1038/ngeo589

Wickham, H. (2019). Tidyverse: Easily Install and Load the ‘Tidyverse’. R package version 1.2.1. https://CRAN.R-project.org/package=tidyverse. Accessed 10 April 2019.

Zhou, S., Huang, H., & Liu, Y. (2008). Biodegradation and origin of oil sands in the Western Canada Sedimentary Basin. *Petroleum Science*, *5*(2), 87–94. doi:10.1007/s12182-008-0015-3

Appendix I – Trace Metals

Lysimeter Sampling Procedure

At each sampling site, select two pre-packaged lysimeter sampling kits, rinse two SS casings and the soil probe with Type I MilliQ water, and fill two 500 mL polypropylene sample bottles with Type I MilliQ water. Each lysimeter kit, assembled in the Class 100 room at the SWAMP lab, contains:

- 1 lysimeter
- 2 acid washed hydrophilic 0.45 μm PTFE filters in separate Ziploc bag
- 2 acid washed 50 mL polypropylene centrifuge tubes in separate Ziploc bag
- Extra Ziploc bag for collected samples.

Field Installation

1. Locate appropriate location, having sparse vegetation cover and relatively flat surface.
2. Remove any vegetation from sampling area (circle roughly 30 cm in diameter).
3. Remove ~30 cm of soil using stainless steel (SS) coring tool, and scrape into a Ziploc bag using the SS scraping tool.
4. Place the soil samples in a cooler.
5. Assemble the lysimeter and place it into the empty core hole using shoulder-length polypropylene (PP) gloves. Ensure that the lysimeter is touching the bottom of the hole by placing a plastic bag over the end of the open lysimeter rod and lightly tapping the end with a PP mallet.
6. Install the 40 cm x 10 cm SS casing by placing it around the lysimeter with the lysimeter centered, placing the PP cap over top, and tapping it into the soil using the PP mallet.
7. Pour 250mL type II MilliQ water on the soil inside the lysimeter casing.

8. Wait for 30 minutes. Ideally, all the water will have infiltrated into the soil. The wet surface should be lightly tamped with a gloved hand to remove air passages between the surface and lysimeter pores that may interfere with the vacuum.
9. Remove the bag from the end of the lysimeter, attach the vacuum hose, and apply a vacuum for at least 15 minutes and up to 30 minutes (pressures will vary depending on soil type/saturation, but they should be in the range of 300–450 mm Hg if the vacuum is holding).

Sampling

1. Assemble the sampling device using shoulder-length PP gloves: attach the PEEK sampling tubing assembly to the 20-mL PP syringe using a PEEK micro-fitting and female Luer lock.
2. Remove the vacuum hose and carefully place the sampling tubing all the way into the lysimeter through the open rod. Be sure that the tubing is touching the bottom of the lysimeter and remains there during sampling.
3. Fill the syringe with the sample (~ 24 mL).
4. Place a hydrophilic 0.45 μm PTFE filter onto the end of the syringe to prevent accidental expulsion of the sample during the next steps.
5. Expel ~ 3 mL of sample from the syringe into the CT, cap and shake, and discard liquid.
6. Expel 2 mL (~ 10 drops) of sample through the filter assembly to condition the filters and discard this sample.
7. Filter ~ 25 mL of sample through the filter directly into the CT, cap the CT, and place it in a Ziploc bag.
8. Repeat step 7, replacing for the second filter if needed, into the second CT.

9. Remove the filtration assembly and place it back into the original Ziploc bag marked with the appropriate identifier (i.e. #1, #2, or #3).
10. Place all filters and CTs in a Ziploc bag marked with the site ID and place the bag into a cooler.

Increment Borer Contamination

Table I.1 Test samples to determine contamination from the increment borer (Suunto, Vantaa, Finland). Clean 1 and 2 represent samples trimmed using a Kershaw Pro-Grade Breaking Knife (Model 1280GE, Tualatin, Oregon), and Full 1 and 2 represent non-trimmed.

Analyte	Mo	Ni	Re	V
Concentration	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}
LOD	0.03	0.79	0.01	0.13
LOQ	0.10	7.30	0.01	0.30
Clean 1	7.51	70.82	< LOQ	2.87
Clean 2	3.79	36.92	< LOQ	1.40
Full 1	7.06	63.97	< LOQ	9.82
Full 2	9.01	207.16	< LOQ	12.59

Limits of Detection and Lysimeter Blank Values

Table I.2 Limits of detection for trace metal analysis across sample type and ICP-MS runs (iCAP Qc ICP-MS, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

	Ch. 2 Soil	Ch. 2 Wood		Pore Water	Ch.3 Soil and Lysimeter Soil	Ch. 3 Wood
		Batch 1	Batch 2			
Metal	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg L^{-1}	μg kg^{-1}	μg kg^{-1}
Ag	5.5	0.6	0.5	0.01	0.0091	0.010
Al	16526.7	89.8	102.7	1.1	0.8010	0.890
As				0.01	0.0065	0.007
Ba	23.4	1.5	3.2	0.03	0.0247	0.027
Be	13.6	1.3	1.2	0.01	0.0050	0.006
Bi	1.4	0.2	0.1			
Cd	17.7	1.4	1.8	0.005	0.0037	0.004
Ce				0.01	0.0047	0.005
Co	4.0	0.5	0.4	0.01	0.0104	0.012
Cr	14.3	2.2	1.6	0.02	0.0181	0.020
Cs	4.3	0.4	0.5			
Cu	168.8	14.2	16.6	0.1	0.1122	0.125
Dy				0.0005	0.0004	0.0004
Er				0.001	0.0005	0.001
Eu				0.001	0.0006	0.001
Fe		64.5	32.6	1.6	1.2399	1.378
Ga	13.5	0.8	0.9	0.003	0.0024	0.003
Gd				0.002	0.0013	0.001
Ho				0.0004	0.0003	0.0004
La				0.01	0.0038	0.004
Li	38.7	4.8	6.9	0.01	0.0075	0.008
Lu				0.0005	0.0003	0.0004
Mn	15.6	3.1	2.4	0.03	0.0204	0.023
Mo	537.5	40.3	52.5	0.01	0.0052	0.006
Nd				0.002	0.0014	0.002
Ni	50.7	7.7	8.3	0.04	0.0292	0.032
Pb	5.0	0.6	0.7	0.001	0.0009	0.001
Pr				0.002	0.0012	0.001
Rb	8.5	1.3	0.9	0.01	0.0070	0.008
Re	1.1	0.2	0.1	0.0004	0.0003	0.0003
Sb	12	6.0	3.5	0.01	0.0088	0.010
Sc		0.5	0.4	0.02	0.0163	0.018
Sm				0.002	0.0012	0.001
Sr	15.6	1.4	1.4	0.03	0.0224	0.025
Tb				0.0004	0.0003	0.0003
Th	1.4	0.2	0.1	0.001	0.0008	0.001
Tl	0.8	0.1	0.1	0.001	0.0008	0.001
Tm				0.0003	0.0002	0.0002
U	2.0	0.2	0.2	0.001	0.0005	0.001
V	6.3	1.5	1.2	0.02	0.0188	0.021
Y	1.9	0.3	0.2	0.002	0.0019	0.002
Yb				0.001	0.0006	0.001
Zn	1122.4	128.8	113.2	1	0.5888	0.654

Table I.3 Mean lysimeter blank values measured after passing Type I MilliQ water through three lysimeters and analysed in triplicate on an ICP-MS (iCAP Qc ICP-MS, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Metal	Units	LOD	Mean Blank Value	SD
Ag	ng/L	0.37	2.99	1.35
Al	µg/L	0.14	1.88	1.00
As	ng/L	1.32	1.75	1.10
Ba	µg/L	0.001	0.17	0.04
Be	ng/L	0.56	0.93	0.23
Cd	ng/L	0.35	0.82	0.39
Ce	ng/L	0.04	2.59	0.99
Co	µg/L	0.0002	3.05	2.52
Cr	µg/L	0.001	1.76	0.13
Cu	µg/L	0.004	1.54	0.77
Dy	ng/L	0.06	0.14	0.08
Er	ng/L	0.04	0.06	0.07
Eu	ng/L	0.06	0.06	0.05
Fe	µg/L	0.01	83.08	30.82
Ga	ng/L	0.40	1.32	0.49
Gd	ng/L	0.12	0.21	0.09
Ho	ng/L	0.02	0.04	0.08
La	ng/L	0.04	1.28	0.52
Li	ng/L	0.14	111.13	70.49
Ly	ng/L	0.03	0.03	0.07
Mn	µg/L	0.002	36.55	4.56
Mo	ng/L	0.36	87.91	34.46
Nd	ng/L	0.11	0.98	0.28
Ni	µg/L	0.002	378.25	281.87
Pb	ng/L	0.04	3.22	1.28
Pr	ng/L	0.07	0.28	0.08
Rb	ng/L	1.62	2.43	5.18
Re	ng/L	0.01	14.42	6.73
Sb	ng/L	0.76	24.73	3.60
Sc	ng/L	1.53	0.56	0.62
Sm	ng/L	0.10	0.19	0.08
Sr	µg/L	0.001	0.15	0.05
Tb	ng/L	0.04	0.04	0.06
Th	ng/L	0.02	0.72	0.21
Tl	ng/L	0.10	0.18	0.18
Tm	ng/L	0.05	0.04	0.09
U	ng/L	0.05	0.24	0.10
V	ng/L	0.74	8.83	2.89
Y	ng/L	0.07	0.59	0.23
Yb	ng/L	0.05	0.06	0.09
Zn	µg/L	0.01	0.80	0.38

Trace Metal Concentrations

Table I.4 Mean trace metal concentrations in soils from the reference and bituminous soils described in Chapter 2.0. All values in mg kg $^{-1}$.

	Reference Sites				Bituminous Sites			
	Depth (cm)				Depth (cm)			
	10	20	30	40	10	20	30	40
Ag	0.104	0.078	0.125	0.104	0.126	0.088	0.151	0.172
Al	653.037	982.218	802.946	718.554	587.948	537.774	526.277	555.239
As	NA	2.225	NA	NA	NA	2.429	NA	NA
Ba	175.178	279.279	209.280	181.420	120.861	212.141	120.862	131.277
Be	0.232	0.332	0.320	0.277	0.054	0.117	0.097	0.165
Bi	0.027	0.041	0.036	0.032	0.019	0.016	0.019	0.029
Cd	0.099	0.038	0.033	0.024	0.034	0.020	0.022	0.023
Ce	NA	11.445	NA	NA	NA	21.662	NA	NA
Co	0.897	1.887	1.743	1.430	0.377	0.930	0.757	1.217
Cr	5.321	12.847	10.165	8.098	6.069	11.395	7.288	11.259
Cu	1.359	2.056	1.806	1.532	1.460	1.774	1.354	1.588
Dy	NA	0.591	NA	NA	NA	0.774	NA	NA
Er	NA	0.360	NA	NA	NA	0.433	NA	NA
Eu	NA	0.187	NA	NA	NA	0.202	NA	NA
Fe	NA	9339.039	NA	NA	NA	12570.462	NA	NA
Ga	2.137	64.435	3.112	2.693	0.830	46.718	1.401	1.839
Gd	NA	0.703	NA	NA	NA	1.112	NA	NA
Ho	NA	0.118	NA	NA	NA	0.147	NA	NA
La	NA	4.671	NA	NA	NA	10.116	NA	NA
Li	1.376	3.463	1.946	1.777	1.926	3.610	3.149	3.732
Lu	NA	0.051	NA	NA	NA	0.060	NA	NA
Mn	128.869	154.208	77.127	44.469	20.305	65.075	56.319	107.619
Mo	0.114	0.264	0.163	0.089	0.449	1.477	1.484	1.875
Nd	NA	4.986	NA	NA	NA	8.681	NA	NA
Ni	0.966	4.101	3.995	3.305	2.255	6.209	7.427	8.831
Pb	4.463	6.644	5.124	4.888	2.663	4.628	3.157	4.401
Pr	NA	1.316	NA	NA	NA	2.337	NA	NA
Rb	14.184	17.500	14.049	11.811	6.492	11.783	8.454	9.678
Re	<LOD	0.00025	<LOD	<LOD	0.004	0.006	0.005	0.005
Sb	0.058	0.065	0.078	0.065	0.064	0.065	0.068	0.075
Sc	NA	0.606	NA	NA	NA	0.513	NA	NA
Sm	NA	0.917	NA	NA	NA	1.500	NA	NA
Sr	41.132	66.902	54.219	49.532	18.893	27.131	18.591	20.751
Tb	NA	0.100	NA	NA	NA	0.142	NA	NA
Th	0.427	0.641	0.609	0.462	0.929	1.845	1.295	1.216
Tl	0.094	0.171	0.122	0.085	0.046	0.103	0.065	0.077
Tm	NA	0.053	NA	NA	NA	0.062	NA	NA
U	0.408	0.317	0.509	0.557	0.356	0.332	0.451	0.580
V	6.091	18.079	14.529	11.433	9.879	27.492	28.654	38.835
Y	0.887	1.086	1.091	0.755	1.369	1.863	1.278	1.854
Yb	NA	0.363	NA	NA	NA	0.423	NA	NA
Zn	11.787	21.490	20.564	11.322	2.447	3.877	2.449	3.671

Table I.5 Mean trace metal concentrations in soil pore water and in tree wood from the reference and bituminous sites described in Chapter 2.0. All values in µg L^{-1} for pore water, and in µg kg^{-1} for tree wood. Values presented are the mean concentrations with <LOD values omitted.

	Reference Sites			Bituminous Sites		
	Pore Water	Wood		Pore Water	Wood	
		Old	New		Old	New
Ag	<LOD	45.140	56.460	<LOD	31.368	58.632
Al	374.358	2572.730	3737.714	497.418	2184.288	2933.839
As	0.152	NA	NA	0.387	NA	NA
Ba	22.119	6489.512	3478.895	35.558	2101.588	1263.668
Be	0.048	<LOD	<LOD	0.089	<LOD	<LOD
Cd	0.041	91.430	72.300	0.057	76.212	70.408
Ce	0.558	NA	NA	0.747	NA	NA
Co	6.406	12.780	12.900	12.330	7.280	8.524
Cr	2.430	64.890	122.830	6.028	113.352	97.524
Cu	4.604	333.926	1171.684	11.094	352.421	342.479
Dy	0.031	NA	NA	0.042	NA	NA
Er	0.016	NA	NA	0.024	NA	NA
Eu	0.009	NA	NA	0.014	NA	NA
Fe	299.678	1043.186	3396.731	531.769	1506.609	2612.106
Ga	0.081	<LOD	<LOD	0.140	<LOD	<LOD
Gd	0.034	NA	NA	0.054	NA	NA
Ho	0.006	NA	NA	0.009	NA	NA
La	0.247	NA	NA	0.364	NA	NA
Li	0.311	<LOD	<LOD	2.213	<LOD	<LOD
Lu	0.002	NA	NA	0.004	NA	NA
Mn	59.484	49276.114	48408.616	95.598	53034.089	38098.214
Mo	1.478	58.944	72.300	2.874	68.945	59.620
Nd	0.188	NA	NA	0.300	NA	NA
Ni	385.070	32.078	69.758	624.784	54.690	108.860
Pb	0.073	2.057	6.122	0.142	2.439	3.505
Pr	0.053	NA	NA	0.087	NA	NA
Rb	2.188	306.749	361.148	6.708	564.569	582.470
Re	0.012	<LOD	<LOD	0.027	<LOD	<LOD
Sb	0.384	<LOD	<LOD	0.463	<LOD	<LOD
Sc	0.174	<LOD	<LOD	0.323	<LOD	<LOD
Sm	0.036	NA	NA	0.059	NA	NA
Sr	10.741	3501.642	2164.467	11.523	1668.004	977.244
Tb	0.005	NA	NA	0.008	NA	NA
Th	0.108	<LOD	<LOD	0.111	<LOD	0.900
Tl	<LOD	0.550	1.478	0.022	1.021	3.342
Tm	0.003	NA	NA	0.004	NA	NA
U	0.022	0.550	1.300	0.058	4.100	0.900
V	0.451	3.900	2.813	4.803	3.433	3.779
Y	0.157	1.160	0.890	0.219	1.383	0.996
Yb	0.013	NA	NA	0.020	NA	NA
Zn	<LOD	9125.019	9696.096	9.628	9399.262	7402.305

Table I.6 Mean trace metal concentrations from the reclamation site described in Chapter 3.0. Values presented are the mean concentrations with <LOD values omitted.

	Soil		Pore Water	Wood
Depth:	20 cm	LOS		
	mg kg^{-1}	mg kg^{-1}	µg L^{-1}	µg kg^{-1}
Ag	0.046	0.047	<LOD	10.361
Al	3249.147	2510.542	6.227	3153.547
As	4.948	5.129	0.422	4.950
Ba	485.334	361.266	54.293	119.160
Be	0.689	0.947	0.040	2.256
Cd	0.306	0.105	0.084	10.390
Ce	37.334	41.096	0.050	4.570
Co	5.811	7.646	9.688	3.253
Cr	31.431	38.635	2.585	33.287
Cu	8.114	8.972	5.090	1169.277
Dy	2.047	2.404	0.006	0.228
Er	1.188	1.397	0.006	0.145
Eu	0.581	0.658	0.006	0.070
Fe	22424.271	20888.699	115.597	2623.367
Ga	168.258	123.664	0.015	43.363
Gd	2.569	2.930	0.010	0.267
Ho	0.400	0.470	0.006	0.082
La	15.559	16.496	0.035	2.640
Li	21.440	30.642	58.081	37.952
Lu	0.162	0.191	0.006	<LOD
Mn	741.065	323.072	51.343	4651.653
Mo	1.338	1.422	22.806	4.513
Nd	16.116	18.173	0.024	1.540
Ni	16.163	19.235	868.673	8.844
Pb	9.561	10.950	0.018	1.667
Pr	4.192	4.660	0.032	0.400
Rb	11.004	15.666	1.833	242.180
Re	0.006	0.006	0.428	<LOD
Sb	0.118	0.143	0.443	<LOD
Sc	2.360	2.284	0.190	3.700
Sm	3.048	3.547	0.012	0.303
Sr	147.349	106.682	846.598	2387.227
Tb	0.356	0.415	0.006	0.055
Th	2.831	2.274	0.034	0.900
Tl	0.305	0.359	0.011	0.914
Tm	0.170	0.200	0.008	0.600
U	0.465	0.472	0.524	<LOD
V	50.082	68.103	0.204	3.884
Y	7.201	7.575	0.038	1.693
Yb	1.154	1.358	0.007	0.121
Zn	37.126	33.623	6.910	11515.757

Appendix II – DNA

Plant species observed aboveground

Reference Sites:

Alnus spp.
Arctostaphylos uva-ursi
Cladina mitis
Dicranum polysetum
Diphasiastrum digitatum
Hylocomnium splendens
Linnaea borealis
Maianthemum canadense
Pinus banksiana
Picea glauca
Pleurozium schreberi
Populus tremuloides
Ptilium crista-castrensis
Shepherdia canadensis
Trientalis borealis
Usnea lapponica
Vaccinium myrtilloides
Vaccinium oxycoccos
Viburnum edule
Viola adunca

Bituminous Sites:

Alnus spp.
Amelanchier alnifolia
Arctostaphylos uva-ursi
Betula papyrifera
Ceratodon purpureus
Chamaenerion angustifolium
Cladina mitis
Cladonia sp.
Cladonia stellaris
Cornus canadensis
Cyperipedium acaule
Dicranum polysetum
Dicranum spp.
Galium triflorum
Hylocomnium splendens
Linnaea borealis
Maianthemum canadense
Peltigera sp.
Pinus banksiana
Picea glauca
Pleurozium schreberi
Populus tremuloides
Ptilium crista-castrensis
Rhododendron groenlandicum
Rosa acicularis
Vaccinium myrtilloides
Vaccinium oxycoccos

Plant species occurring belowground resolved via root DNA

Chapter 2

Reference Sites:

Trees:
Pinus banksiana Lambert

Shrubs:
Alnus alnobetula subsp. crispa (Aiton) Raus
Alnus incana subsp. *tenuifolia* (Nuttall) Breitung
Linnaea borealis Linnaeus
Lysimachia borealis (Rafinesque) U. Manns & Anderberg
Sibbaldia tridentata (Aiton) Paule & Soják
Vaccinium vitis-idaea Linnaeus

Sedges:
Carex concinna R. Brown

Forbs:
Arabis ssp. Linnaeus
Maianthemum canadense Desfontaines
Potentilla norvegica Linnaeus
Solidago canadensis Linnaeus
Symphyotrichum boreale (Torrey & A. Gray) A. Love & D. Love
Symphyotrichum lanceolatum subsp. *hesperium* (A. Gray) G.L. Nesom

Ferns:
Dryopteris carthusiana (Villars) H.P. Fuchs

Bituminous Sites:

Trees:
Abies balsamea (Linnaeus) Miller
Picea glauca (Moench) Voss
Picea mariana (Miller) Britton, Sterns & Poggenburgh
Pinus banksiana Lambert

Shrubs:
Alnus alnobetula subsp. crispa (Aiton) Raus
Alnus incana subsp. *tenuifolia* (Nuttall) Breitung
Cornus canadensis Linnaeus
Rhododendron groenlandicum (Oeder) Kron & Judd
Vaccinium myrtilloides Michaux
Vaccinium vitis-idaea Linnaeus

Sedges:
Carex concinna R. Brown
Carex magellanica subsp. irrigua (Wahlenberg) Hiitonen
Carex utriculata Boott

Forbs:
Arabis ssp. Linnaeus
Galeopsis tetrahit Linnaeus
Maianthemum canadense Desfontaines
Mentha arvensis Linnaeus
Potentilla norvegica Linnaeus
Ranunculus sceleratus Linnaeus
Streptopus amplexifolius (Linnaeus) de Candolle
Symphyotrichum boreale (Torrey & A. Gray) A. Love & D. Love
Symphyotrichum lanceolatum subsp. *hesperium* (A. Gray) G.L. Nesom
Trifolium pratense Linnaeus

Ferns:
Botrypus virginianus (Linnaeus) Michaux

Chapter 3

Trees:
Pinus banksiana Lambert
Populus tremuloides Michaux

Shrub:
Salix spp. Linnaeus

Sedges:
Carex bebbii (L.H. Bailey) Olney ex Fernald
Carex brunnescens (Persoon) Poiret
Carex concinna R. Brown

Grasses:
Calamagrostis canadensis (Michaux) Palisot de Beauvois
Elymus trachycaulus subsp. *trachycaulus* (Link) Gould ex Shinners
Poa compressa Linnaeus
Typha latifolia Linnaeus

Orchids:
Corallorhiza trifida Châtelain
Platanthera orbiculata (Pursh) Lindley

Forbs:
Eurybia conspicua (Lindley) G.L. Nesom
Fragaria virginiana Miller
Potentilla norvegica Linnaeus
Symphyotrichum boreale (Torrey & A. Gray) A. Love & D. Love

Symphyotrichum ciliolatum (Lindley) A. Love & D. Love
Symphyotrichum lanceolatum subsp. *hesperium* (A. Gray) G.L. Nesom

Species Detection Rate

Chapter 2

Table II.1 Species detection rate by site and depth in Chapter 2. R1 and R2 represent reference sites and B1-B5 represent sites with natural shallow bitumen. Green squares represent successful detection and grey squares represent samples that did not amplify in the PCR process.

Group	Species	Depth (cm)	R1		R2		B1		B2		B3		B4		B5	
Tree	*Abies balsamea* (Linnaeus) Miller	10								🟩				⬛		
		20							⬛							
		30					⬛		⬛							
		40							⬛	⬛					⬛	
	Picea glauca (Moench) Voss	10								🟩		🟩		⬛	🟩	🟩
		20							⬛			🟩				
		30					⬛		⬛							🟩
		40							⬛	⬛					⬛	
	Picea mariana (Miller) Britton, Sterns & Poggenburgh	10								🟩		🟩		⬛	🟩	🟩
		20							⬛			🟩				
		30					⬛		⬛							🟩
		40							⬛	⬛					⬛	
	Pinus banksiana Lambert	10	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	⬛	🟩	
		20	🟩	🟩		🟩	🟩	🟩	⬛		🟩	🟩	🟩		🟩	🟩
		30	🟩	🟩	🟩	🟩	⬛	🟩	⬛	🟩			🟩	🟩	🟩	
		40	🟩		🟩	🟩	🟩	🟩	⬛	⬛		🟩		🟩	⬛	🟩
Shrub	*Alnus alnobetula* subsp. *crispa* (Aiton) Raus	10	🟩	🟩					🟩		🟩			⬛		
		20							⬛		🟩					
		30			🟩		⬛		⬛							
		40							⬛	⬛					⬛	
	Alnus incana subsp. *tenuifolia* (Nuttall) Breitung	10		🟩					🟩		🟩			⬛		
		20							⬛							
		30			🟩		⬛		⬛							
		40							⬛	⬛					⬛	

Group	Species	Depth (cm)	R1		R2		B1		B2		B3		B4		B5	
Shrub	*Cornus canadensis* Linnaeus	10						🟩						⬛		
		20							⬛							
		30					⬛		⬛	🟩						
		40							⬛	⬛					⬛	
	Linnaea borealis Linnaeus	10			🟩									⬛		
		20							⬛							
		30					⬛		⬛							
		40							⬛	⬛					⬛	
	Lysimachia borealis (Rafinesque) U. Manns & Anderberg	10			🟩									⬛		
		20							⬛							
		30					⬛		⬛							
		40							⬛	⬛					⬛	
	Rhododendron groenlandicum (Oeder) Kron & Judd	10												⬛		
		20							⬛					🟩		
		30					⬛		⬛							
		40							⬛	⬛					⬛	
	Sibbaldia tridentata (Aiton) Paule & Soják	10			🟩									⬛		
		20							⬛							
		30					⬛		⬛							
		40							⬛	⬛					⬛	
	Vaccinium myrtilloides Michaux	10					🟩			🟩	🟩	🟩	🟩	⬛	🟩	
		20					🟩		⬛	🟩		🟩	🟩		🟩	
		30					⬛		⬛			🟩	🟩		🟩	
		40							⬛	⬛		🟩	🟩		⬛	🟩

Group	Species	Depth (cm)	R1		R2		B1		B2		B3		B4		B5	
Shrub	*Vaccinium vitis-idaea* Linnaeus	10			🟩	🟩					🟩			⬛	🟩	
		20							⬛				🟩			
		30	🟩				⬛		⬛							
		40							⬛	⬛					⬛	
Sedge	*Carex concinna* R. Brown	10	🟩				🟩							⬛		
		20							⬛				🟩		🟩	
		30	🟩				⬛		⬛							
		40							⬛	⬛					⬛	🟩
	Carex magellanica subsp. *irrigua* (Wahlenberg) Hiitonen	10												⬛		
		20					🟩		⬛							
		30					⬛		⬛							
		40							⬛	⬛					⬛	
	Carex utriculata Boott	10												⬛		
		20					🟩		⬛							
		30					⬛		⬛							
		40							⬛	⬛					⬛	
Forb	*Arabis* ssp. Linnaeus	10	🟩			🟩								⬛		
		20				🟩			⬛							
		30	🟩			🟩	⬛		⬛							
		40	🟩	🟩	🟩				⬛	⬛					⬛	
	Galeopsis tetrahit Linnaeus	10												⬛		🟩
		20							⬛							
		30					⬛		⬛							🟩
		40							⬛	⬛					⬛	

Group	Species	Depth (cm)	R1		R2		B1		B2		B3		B4		B5	
Forb	*Maianthemum canadense* Desfontaines	10	🟩		🟩				🟩		🟩	🟩		⬛		
		20							⬛		🟩					
		30					⬛		⬛		🟩					
		40							⬛	⬛					⬛	
	Mentha arvensis Linnaeus	10							🟩					⬛		
		20							⬛							
		30					⬛		⬛							
		40							⬛	⬛					⬛	
	Potentilla norvegica Linnaeus	10	🟩											⬛		
		20							⬛		🟩					
		30					⬛		⬛							
		40							⬛	⬛					⬛	
	Ranunculus sceleratus Linnaeus	10								🟩				⬛		
		20							⬛							
		30					⬛		⬛							
		40							⬛	⬛					⬛	
	Solidago canadensis Linnaeus	10			🟩									⬛		
		20							⬛							
		30					⬛		⬛							
		40							⬛	⬛					⬛	
	Streptopus amplexifolius (Linnaeus) de Candolle	10						🟩						⬛	🟩	
		20							⬛							
		30					⬛		⬛							🟩
		40							⬛	⬛					⬛	

Group	Species	Depth (cm)	R1		R2		B1		B2		B3		B4		B5	
Forb	*Symphyotrichum boreale* (Torrey & A. Gray) A. Love & D. Love	10	🟩	🟩					🟩		🟩	🟩		⬛		
		20							⬛		🟩					
		30					⬛		⬛		🟩					
		40							⬛	⬛					⬛	
	Symphyotrichum lanceolatum subsp. *hesperium* (A. Gray) G.L. Nesom	10	🟩	🟩					🟩		🟩	🟩		⬛		
		20							⬛		🟩					
		30					⬛		⬛		🟩					
		40							⬛	⬛					⬛	
	Trifolium pratense Linnaeus	10						🟩			🟩			⬛	🟩	
		20							⬛	🟩			🟩			
		30					⬛		⬛	🟩						
		40							⬛	⬛			🟩		⬛	
Fern	*Botrypus virginianus* (Linnaeus) Michaux	10												⬛		
		20							⬛					🟩		
		30					⬛		⬛					🟩		
		40							⬛	⬛					⬛	
	Dryopteris carthusiana (Villars) H.P. Fuchs	10	🟩			🟩								⬛		
		20							⬛							
		30					⬛		⬛							
		40							⬛	⬛					⬛	

Table II.2 Species detection rate by plot and depth in Chapter 3. Green squares represent successful detection and grey squares represent samples that did not amplify in the PCR process.

Group	Species	Depth (cm)	W1	W2	W3	W4	W5	W6
Tree	*Pinus banksiana* Lambert	10		⬛	🟩			
		20			🟩			
		30				⬛	⬛	⬛
		LOS Root						
	Populus tremuloides Michaux	10		⬛				
		20					🟩	
		30				⬛	🟩⬛	⬛
		LOS Root					🟩 T1	
Shrub	*Salix* spp. Linnaeus	10		⬛				
		20						
		30				⬛	⬛	⬛
		LOS Root	🟩 T3					
Sedge	*Carex bebbii* (L.H. Bailey) Olney ex Fernald	10		⬛	🟩			
		20		🟩			🟩	
		30		🟩		⬛	⬛	⬛
		LOS Root						
	Carex brunnescens (Persoon) Poiret	10		⬛				
		20					🟩	
		30				⬛	⬛	⬛
		LOS Root						
	Carex concinna R. Brown	10	🟩	⬛				
		20						
		30	🟩			⬛	⬛	⬛
		LOS Root						

Group	Species	Depth (cm)	W1		W2		W3		W4		W5		W6	
Grass	*Calamagrostis canadensis* (Michaux) Palisot de Beauvois	10			🟩	⬛								
		20												
		30			🟩				⬛			⬛		⬛
		LOS Root												
	Elymus trachycaulus subsp. *trachycaulus* (Link) Gould ex Shinners	10				⬛	🟩							🟩
		20		🟩										🟩
		30							⬛			⬛		⬛
		LOS Root												
	Poa compressa Linnaeus	10		🟩		⬛								
		20		🟩										
		30		🟩					⬛			⬛		⬛
		LOS Root												
	Typha latifolia Linnaeus	10			🟩	⬛		🟩						
		20			🟩						🟩			
		30							⬛			⬛		⬛
		LOS Root												
Orchid	*Corallorhiza trifida* Châtelain	10	🟩			⬛								
		20												
		30	🟩						⬛			⬛		⬛
		LOS Root												
	Platanthera orbiculata (Pursh) Lindley	10				⬛	🟩							
		20												
		30							⬛			⬛		⬛
		LOS Root												

Group	Species	Depth (cm)	W1		W2		W3		W4		W5		W6	
Forb	*Eurybia conspicua* (Lindley) G.L. Nesom	10				⬛							🟩	
		20												
		30							⬛			⬛		⬛
		LOS Root												
	Fragaria virginiana Miller	10			🟩	⬛								
		20			🟩									
		30							⬛			⬛		⬛
		LOS Root												
	Potentilla norvegica Linnaeus	10				⬛	🟩							
		20												
		30							⬛			⬛		⬛
		LOS Root												
	Symphyotrichum boreale (Torrey & A. Gray) A. Love & D. Love	10		🟩		⬛							🟩	
		20		🟩										
		30		🟩					⬛			⬛		⬛
		LOS Root	🟩 T3											
	Symphyotrichum ciliolatum (Lindley) A. Love & D. Love	10				⬛							🟩	
		20												
		30							⬛			⬛		⬛
		LOS Root												
	Symphyotrichum lanceolatum subsp. *hesperium* (A. Gray) G.L. Nesom	10		🟩		⬛							🟩	
		20		🟩										
		30		🟩					⬛			⬛		⬛
		LOS Root	🟩 T3											

Appendix III – Site Characteristics

Table III.1 Soil and tree characteristics by site. B1-B5 represent the bituminous sites located near the Hammerstone Quarry north of Fort MacKay, Alberta, and R1 & R2 the reference sites near Janvier, Alberta, Canada.

	Depth	Volumetric Water Content	Soil pH	Electrical Conductivity	Sand	Silt	Clay	Tree	DBH	Age
Site	cm	%		(mS/cm)	%				cm	Years
B1	10	25.2	4.3	0.012	86.63	9.25	4.13	1	23.9	67
	20	23.1	4.1	0.01	83.55	10.51	5.95	2	19.15	68
	30	18.3	4.0	0.004	75.64	16.07	8.29	3	27	62
	40	13	4.1	0.004	79.16	14.12	6.72	4	25.4	57
								5	19.5	60
B2	10	24.1	4.7	0.003	87.72	9.24	3.05	1	29	73
	20	23.2	4.3	0.002	84.59	9.81	5.60	2	31.9	77
	30	23.8	4.2	0.006	82.62	10.26	7.12	3	22.8	75
	40	21.2	4.0	0.004	70.79	16.74	12.48	4	23.1	77
								5	29	75
B3	10	21.6	5.0	0.004	90.26	7.41	2.33	1	27.8	73
	20	14.3	4.7	0.001	78.26	13.92	7.83	2	27.5	77
	30	11.3	4.6	0.002	80.02	11.94	8.05	3	29.1	69
	40	10.6	4.7	0.001	71.22	19.03	9.75	4	21.4	76
								5	37.8	72

Table III.1 Cont'd

Site	Depth	Volumetric Water Content	Soil pH	Electrical Conductivity	Sand	Silt	Clay	Tree	DBH	Age
	cm	%		(mS/cm)	%				cm	Years
B4	10	22.2	4.9	0.004	82.48	11.32	6.20	1	24.1	73
	20	21.9	5.3	0.003	77.48	12.09	10.44	2	22.7	65
	30	24.2	5.1	0.005	69.38	16.70	13.92	3	34.9	76
	40	17.5	5.1	0.003	83.70	9.98	6.32	4	29.5	81
								5	23	73
B5	10	13.2	5.4	0.009	81.78	13.25	4.97	1	29.1	50
	20	20	4.6	0.009	77.50	14.77	7.74	2	26.7	57
	30	21.3	4.5	0.009	79.34	13.41	7.26	3	29.1	61
	40	8.9	4.6	0.003	85.00	10.66	4.35	4	20.8	59
								5	26	59
R1	10	22.9	5.4	0.01	83.85	11.38	4.77	1	29.9	87
	20	15.4	5.4	0.005	83.35	8.44	8.21	2	25.9	84
	30	18.3	5.7	0.003	88.94	5.31	5.75	3	29.7	84
	40	18	5.6	0.004	91.14	3.73	5.14	4	26.7	84
								5	25	85
R2	10	25.5	5.5	0.009	68.52	23.36	8.12	1	25.9	81
	20	24.6	5.5	0.003	58.30	25.99	15.71	2	25.4	79
	30	22.8	5.3	0.004	60.01	25.02	14.97	3	22.3	76
	40	16.6	5.1	0.008	60.83	23.36	15.81	4	23.5	73
								5	17.1	76

Table III.2 Plot characteristics from a reclaimed landform in the Fort McMurray region. Depths 10-30 represent the peat-mineral mix and 40 cm represents the lean oil sand overburden. All trees were Pinus banksiana seedlings four years of age. Diameter was measured between the first and second whorl of branches and the depth to LOS was measured from the top of the root collar to the interface.

Plot	Depth	Volumetric Water Content	Soil pH	Sand	Silt	Clay	Tree	Diameter	Height	Depth to LOS
	cm	%		%				cm	cm	cm
W1	10	25.50	7.3	45.53	39.07	15.41	1	2.4	1.74	35
	20	25.00	7.6	39.81	47.71	12.49	2	1.5	1.45	34
	30	24.85	7.7	39.20	47.84	12.97	3	2.6	1.87	43
	40	8.00	7.6	47.83	36.62	15.56	4	2.8	1.7	32
							5	2.4	1.56	35
W2	10	24.00	7.2	46.17	42.33	11.50	1	1.3	1	47
	20	24.50	7.1	37.14	51.52	11.34	2	1.2	0.95	25
	30	26.00	7.2	38.06	51.14	10.81	3	1.4	1.15	18
	40	6.65	7.8	61.79	23.62	14.61	4	1.4	1.15	39
							5	1.9	1.15	38
W3	10	23.00	7.4	31.85	53.33	14.83	1	1.5	0.95	44
	20	24.50	7.4	36.13	50.95	12.92	2	1.3	0.9	30
	30	27.00	7.5	50.40	37.70	11.90	3	2	1.15	40
	40	7.15	7.7	59.51	25.52	14.98	4	1.5	1.05	31
							5	1.2	0.8	67

Table III.2 Cont'd

Plot	Depth	Volumetric Water Content	Soil pH	Sand	Silt	Clay	Tree	DBH	Height	Depth to LOS
	cm	%		%				cm	cm	cm
W4	10	28.00	7.6	44.56	41.56	13.88	1	1.4	0.8	68
	20	29.50	7.6	51.54	37.20	11.26	2	2.1	1.05	65
	30	23.00	7.5	41.11	46.14	12.75	3	1.7	1.2	48
	40	10.50	8.0	57.48	26.51	16.02	4	1.8	1.1	40
							5	1.4	0.75	46
W5	10	27.00	7.1	43.09	46.40	10.52	1	2.8	1.55	44
	20	23.50	7.2	45.48	44.13	10.40	2	1	0.8	39
	30	22.50	7.3	41.08	47.68	11.25	3	2.1	1.3	39
	40	10.80	7.6	59.51	25.52	14.98	4	1.3	0.9	46
							5	1.9	1.1	45
W6	10	21.00	7.4	46.41	41.46	12.13	1	1.6	0.92	47
	20	19.00	7.5	33.46	54.14	12.41	2	1.6	0.83	31
	30	26.50	7.4	31.24	54.71	14.05	3	1.9	1.12	57
	40	8.70	7.8	59.26	26.43	15.41	4	2.4	1.32	40
							5	1.6	1.03	50

Site Photographs

Figure III.1 R2 -- Reference site located near Janvier, Alberta, Canada.

Figure III.2 B2 -- Bituminous site near the Hammerstone Quarry north of Fort McMurray, Alberta, Canada.

Figure III.3 Example of bitumen "tar balls" and banding in natural soils. B3 -- Bituminous site near the Hammerstone Quarry north of Fort McMurray, Alberta, Canada.

Figure III.4 Reclaimed landform constructed with lean oil sand overburden.

Figure III.5 Example study plot with the mounded soil amendment.