Characterization of Soil Spatial Heterogeneity and Improvement of Capping Materials for Oil Sands Mine Reclamation

by

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Abstract

Surface mining in the Athabasca Oil Sands Region (AOSR), Alberta, Canada creates a large-scale ecosystem disturbance requiring ‘land reclamation’. Mining approvals require that land reclamation returns the sites to an equivalent land capability class, but this goal has proven challenging to quantify. Restoring ‘ecosystem function’ might be more realistic and perhaps, quantifiable by examining spatial patterns of soil functional indices. Cover soil prescriptions that emulate close-to-nature conditions and recreate similar realized niches for the reestablishment of native flora and fauna can increase reclamation success. Surface applications of forest floor mineral-mix (FFM) sourced from upland forest ecosystems and peat mineral-mix (PM) sourced from lowland ecosystems are termed “cover soil” and used as a proxy for replacing native surface soils in upland forest reclamation; yet, there is a wide margin between these surface amendments and native surface soils. The focus of this research first was the spatial characterization of heterogeneity of key soil functions such as nutrient bioavailability, soil respiration, and microbial biomass. We compared reclaimed sites and natural benchmark sites in the AOSR. Secondly, methods for improvement of cover soil performance were tested.

In 2013 a field study was initiated that compared four different sites: 1) FFM reclaimed site; 2) PM reclaimed site; 3) type **b** ecosite recovering from fire; 4) type **ab** ecosite recovering from timber harvesting. We identified differences in spatial heterogeneity of nutrient profiles and soil respiration. Key bioavailable nutrients such as P were significantly different on PM and showed no heterogeneity. Seasonal patterns of respiration showed variability on natural reference sites and on FFM, indicating that disturbance had not removed belowground function completely. PM reclaimed sites showed no strong seasonal respiration patterns indicating homogeneous belowground function. During 2014 we measured soil microbial biomass (SMB)

and soil respiration on six different sites. Two natural benchmarks were added including a 5) mature type **a** ecosite forest stand and 6) a type **a** ecosite recovering from fire to further characterize spatial heterogeneity on type **a** ecosites. The data indicated that there were differences in the pattern of soil respiration. Reoccurring patterns of soil respiration on the Harvested **a/b** ecosite indicated a linkage of above- and belowground function. The PM reclaimed site showed large-scale spatial patterns in SMB, similar to the type **a** ecosite affected by severe fire. The FFM site showed smaller scale spatial pattern than sites disturbed by clear-cutting and severe forest fire. We demonstrated that the amount of SMB and its heterogeneity increased with time since disturbance, potentially indicating stages of site recovery on benchmark type **a** ecosites.

Boreal forest soils of by reclamation targeted ecosites are characterized by higher mineral soil fractions and notably they contain pyrogenic carbon (PyC) as a native soil component that affects biogeochemistry. Biochar is a humanmade analog for PyC and its amendment to cover soils used for reclamation might be a suitable method for reestablishing ecosystems that function more similarly to upland forest ecosystems recovering from fire. Observations indicated that tree growth on the tested PM reclaimed sites was lower in comparison to FFM reclaimed sites. Accordingly, methods for cover soil improvement were tested. The effect of admixing subsoil with peat and amendment of peat biochar on bioavailable nutrients, foliar nutrient concentration and stoichiometry, aspen (*Populus tremuloides* Michx.) growth, soil respiration, root exudation, and soil organic matter (SOM) stability was evaluated in two greenhouse studies. Seedling growth increased with admixing subsoil with and without biochar, and there was an overall positive effect of amendment with biochar in the first study and significant positive effect of biochar amendment on seedling growth and a reduction of soil respiration in the second study.

Our findings suggest that seedlings grown on PM and peat-subsoil mixes were potentially affected by nutrient deficiency as well as toxicity. Biochar improved seedling nutritional status and soil organic matter stability was positively correlated with tree growth and increased with biochar amendment.

Overall our studies demonstrate that by increasing heterogeneity in key soil functions, as well as assimilation of close-to-nature conditions, PM reclaimed sites can be improved. It is likely that this could be achieved by admixing of mineral subsoil, biochar amendment, and potentially targeted fertilizer application.

Preface

This thesis is an original work by Sebastian Thomas Dietrich. At the time of publishing this thesis, Chapter 4 has been published as "Building a better soil for upland surface mine reclamation in northern Alberta: Admixing peat, subsoil, and peat biochar in a greenhouse study with aspen" *Canadian Journal of Soil Science, 2017, 97(4): 592-605*. I was responsible for experimental design, conducting the experiment, data collection, and analysis as well as data interpretation and manuscript composition. M.D. MacKenzie was the supervisory author and was involved in concept formation and manuscript editing, J.P. Battigelli was involved with manuscript editing, J.R. Enterina supported method development for extrapolation of soil organic matter analysis using FT-IR.

Dedication

This dissertation is dedicated to my beloved wife Susann and our beloved daughter Mara.

I also dedicate this work to my parents who have always supported and encouraged me.

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I would like to express my gratitude to my advisor Prof. M. Derek MacKenzie for the support of my Ph.D. studies and related research and guiding my way. Derek thank you for your support throughout my studies. I know others would be happy to have a supervisor, mentor, and good friend as you are. Besides my advisor, I would like to acknowledge Prof. Thomas DeLuca and Prof. Brad Pinno for comments and encouragement, but also for the great time at scientific conferences, especially the Soil Science Society of America meetings. I would like to thank Prof. Anne Naeth for the great opportunities provided in line with the LRIGS program as well as the excellent advice and support in matters of professional and personal development. I am grateful to Marty Yarmuch of Syncrude Canada who mentored me and provided insight into operational needs and challenges in line with the oil sands business. Marty also provided comment on various manuscripts and presentations during the course of my dissertation. Thank you, Marty, for all your advice and support! Special thanks to Dr. Stefan G. Schreiber, Dr. Sanatan Das Gupta, Dr. Nduka Ipko, Mark Howell, Jamal Taghavimehr, Jeff Hogberg, Michelle Mausolf, Leah DeBertoli, and Adam Matthison for science talk and great conversations. Additionally, I would like to acknowledge Maksat Igdirov, Jennifer McGuinness, Sylyanne Foo, Monica Shandal, Peining Guan, William Kirby, and Patrick Neuberger for lab support. I would like to thank my family: my parents and my brothers, my wife and my daughter supporting me spiritually throughout writing this thesis and my life in general. Finally, I would like to thank our funding organizations the Land Reclamation International Graduate School (LRIGS) at the University of Alberta, the NSERC Collaborative Research and Training Experience (CREATE) Program, and Syncrude Canada.

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List of Symbols and Abbreviations

Abbreviation	**Definition**
Al	Aluminium
ANOVA	Analysis of variance
AOSR	Athabasca oil sands region
B	Boron
C	Carbon
Ca	Calcium
CO_2	Carbon dioxide
Cu	Copper
Cv	Coefficient of variation
DRIFT	Diffuse Reflectance Infrared Fourier Transform Spectroscopy
EC	Electric conductivity
Fe	Iron
FFM	Forest floor mineral-mix
FT-IR	Fourier-transform infrared spectroscopy
H_2O	Hydrogen dioxide

HCl	Hydrogen chloride
HSD	Honestly Significant Difference
K	Potassium
MBC	Microbial biomass carbon
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
NH_4^+	Ammonium
NO_3^-	Nitrate
OM	Organic matter
P	Phosphorus
PC	Principal component
PCA	Principal Component Analysis
PLSR	Partial least squared regression
PM	Peat mineral-mix

PRS	Plant-Root-Simulator
PyC	Pyrogenic carbon
R^2	Coefficient of determination
RMSE	Root mean square error
RSS	Residual sum of squares
sCFE	Simultaneous chloroform fumigation extraction
SE	Standard error
SMB	Soil microbial biomass
SOM	Soil organic matter
TC	Total carbon
TOC	Total organic carbon
TN	Total nitrogen
TIN	Total inorganic nitrogen
Zn	Zinc

Chapter 1 Introduction

1.1 Surface mining and natural disturbance in Alberta's boreal forest

Technical progress and human kinds desire for resources in the context of increasing global demand have created undesirable legacies of large disturbed areas. This is especially true in open cast mining operations, which leads to disturbance of ecosystem processes and structures, which cannot be reestablished to productive function without human improvement (Urbanska et al. 1997). Reclamation of these large disturbed areas requires the implementation of sustainable best practices (Horowitz 2006). Good management of environmental and social issues results in better operational quality and lower risk for companies in the context of legal action or regulatory changes, which relates to a reduction of potential financial and reputational risks and social responsibility (Horowitz 2006).

In Alberta, Canada, mining of Bitumen in open cast mines leads to a large-scale disturbance, which requires *"reclamation of land to its equivalent capability"* (Alberta 2014a: 99). The conservation and reclamation regulations for the province currently defines this term as *"the ability of the land to support various land uses after conservation and reclamation is similar to the ability that existed prior to an activity being conducted on the land, but that the individual land uses will not necessarily be identical"*(Alberta 2014b: 2&3). Important in this context is also the definition of land capability: *"the ability of land to support a given land use, based on an evaluation of the physical, chemical and biological characteristics of the land, including topography, drainage, hydrology, soils and vegetation"* (Alberta 2014b: 3). These definitions imply that reestablishment of functional sites on a local and regional scale is required. Up to date, 940.95 km^2 have been disturbed by oil sands surface mining and 6,163 ha have been permanently reclaimed (Environment&Parks 2015), but have to be monitored 15 years or more before they can be certified if ecological trends are achieved (Alberta 2013). Currently, only a small area of 104 ha is certified reclaimed, which equals less than 0.01 % of the total disturbed area (Environment&Parks 2015). Local plant species have to be used to achieve the target of native boreal forest (Alberta 2013; Environment&Parks 2015). Surface mining bitumen requires the movement of vast amounts of overburden and results in a change of the distribution of natural ecosystems to comparatively higher amounts of upland sites and an end pit lake on the anthropogenic shaped landscape (COSIA 2016; Rooney et al. 2012).

The oil sands mining-affected areas in western Canada's boreal mixed wood are structured by wildfire, which is a major natural disturbance in this area (Cumming 2001). Fire exerts an influence on vegetation and biogeochemical cycling that is dependent upon the fire severity, type (crown vs ground, prescribed vs wildfire), and prevalence (return interval) (Maynard et al. 2014). The forests of this region of boreal mixedwood are includes the common presence of wetlands and stands of coniferous and deciduous species (Cumming 2001). The diversity of forest composition is an important factor for the conservation of biodiversity, forest productivity, resistance and resilience (Bergeron et al. 2014). Upland forest ecosystems observed in this study lie within Alberta's boreal forest natural region, in the Central Mixedwood Natural Subregion (Natural Regions Committee 2006). The Central Mixedwood Natural Subregion is characterized by upland forests of aspen mixedwood and white spruce, but also with areas of treed fens or jack pine stands on coarser materials in the east, where this study was conducted (Natural Regions Committee 2006). Summers in the Central Mixedwood Natural Subregion are short and warm while winters are long and cold (Natural Regions Committee 2006).

Most modeling approaches for future climatic conditions in the Athabasca Oil Sand Region (AOSR) predict less available moisture, even though total precipitation is predicted to increase. This effect relates to warmer temperatures and an increase in evapotranspiration from soils and vegetation, and shorter periods with snow cover that would reduce evapotranspiration losses (Schneider 2013). Summers are expected to have less precipitation and increased water stress above current conditions (Schneider 2013). Research conducted in the western United States suggests that a change similar to the ones predicted for the AOSR will lead to an increase in forest wildfire activity (Westerling et al. 2006).

Fires in boreal forests convert a portion of vegetation exposed to fire into charcoal, which is a stable form of C and for this reason is considered as a long-term C sink (Ohlson et al. 2009). The spatial variation of charcoal in the soil in boreal forests in Norway ranges from 0 to 222 g C m^{-2}, and it is assumed that globally boreal forests soils contain about 1 Pg of carbon (C) (Ohlson et al. 2009). Charcoal or biochar has been used as an amendment for forest restoration as early as in the 1820's and is known to increase the growth of trees (Thomas and Gale 2015). Thomas and Gale (2015) suggest that biochar has the potential of replacing other forms of organic matter or liming agents in the field of forest restoration.

Upland reclamation of surface mines in the AOSR has traditionally been carried out by adding a "cover soil", that is mostly peat mineral mix (PM), over suitable subsoil or overburden. Since the early 2000's operators are legally required to salvage all available topsoil, which to some degree is salvaged from uplands and contains native seed propagules, and to use it for reclamation, this material is called forest floor mineral mix (FFM). Communications with professionals suggested that historically forest soils were buried under overburden resulting in a lack of this material today and requiring that future sites be reclaimed with PM.

That is why it is of great importance to achieve a more completeunderstanding if current reclamation strategies for upland sites (i.e., establishment of upland forests on sites with PM or FFM placement) will lead to sustainable, functioning ecosystems, which can cope with challenges like climate change and are capable of recovering from disturbance like insect outbreaks or forest fires. It is imperative that we improve our knowledge about how these reclaimed ecosystems function and if they are capable of providing comparable services and functions similar to the undisturbed boreal upland forest (e.g., habitat, water and air filtration, carbon sequestration). A key for this is understanding soil-plant interactions as the phenotypical response of plants to site characteristics or aboveground-belowground processes, which govern nutrient cycling and the identification of appropriate measures to set targets and certify reclamation. This research is focusing on the characterization of soils of type **a** and **b** ecosites as well as the reclamation of type **a** and **b** ecosites.

1.2 Using wildfire disturbed, logged, and FFM reclaimed sites as a reference

Forest fire is the main and most important natural disturbance in boreal forests (Armstrong 1999). Natural disturbance is fundamental to the development of structure and function of forest ecosystems (Attiwill 1994). Habitat heterogeneity in boreal forests is a consequence of disturbances on various scales (populations, communities, ecosystems) and has been considered a useful measure for the development of guidelines for forest management (Niemelä 1999). Successful forest ecosystem recovery following fire (Attiwill 1994) and forest harvesting (Marshall 2000) has been observed in boreal forest ecosystems, and for this reason, those kinds of disturbance are used as a reference for field studies when evaluating reclamation sites. Recreating reclamation site conditions that rather emulate the realized niches of targeted species versus reestablishing fundamental niches of targeted species or ecosites is advisable. Species will only be able to persist in their realized niche (Wiens and Graham 2005). For that reason, we suggest mimicking soil conditions on reclamation sites that are similar to post-fire conditions, as organisms are likely adapted to those. Vegetation recovery using FFM or LFH donor material as cover soil is more successful (Mackenzie and Naeth 2010). Close-to-natural biogeochemical conditions of FFM might also play a key role in the cover soils potential to quickly reestablish stands similar to natural forest ecosystems.

1.3 Native upland soils in the study area and reclamation materials for upland reclamation

The Alberta oil sands environmental research program identified mineral soils of the Gray Luvisol, Brunisolic Gray Luvisol, and Brunisolic Great Groups in the study area (Turchenek and Lindsay 1982). Native upland soils considered in both field studies conducted in line with this PhD research were classified as Orthic Dystric Brunisol and Orthic Gray Luvisol. The genesis of Brunisols is associated with perhumdid to semiarid moisture and mesic to arctic temperature regimes (Turchenek and Lindsay 1982). Brunisolic soils are rapidly to imperfectly drained mineral soils; characterized by a brownish Bm horizons, which results from hydrolysis or in situ oxidation, leading to changes in colour, composition, and structure allowing a differentiation from A and C horizons (Turchenek and Lindsay 1982). Processes that lead to the diagnostic horizons of podzols and luvisols have not been expressed substantially in the Brunisols in this area, but they are likely transitioning to these soil orders with more time.. Soils of the Luvisolic order are well to imperfectly drained and developed in mild to cold climates, with Luvsiols of the Gray Great Group being associated to cool climates (Turchenek and Lindsay 1982). Luvisolic soils are characterized by the movement of silicate clay minerals from the A to the B horizon leading to diagnostic Ae and Bt horizons (Turchenek and Lindsay 1982). Besides the two mineral upland soils, soils of the organic order are releavant in context of this research. Organic soils are being used as basis for peat or PM cover soils used in upland forest reclamation. Soils of the organic order developed from decomposition of hydrophytic or mesohydrophytic vegetation (Turchenek and Lindsay 1982). Organic C contents of of those soils must be 17 % or greater to meet the minimum classification requirements and a certain horizon thickness (Turchenek and Lindsay 1982). The peat types identified in the Alberta oil sands environmental research program are bog, fen, and forest peat (Turchenek and Lindsay 1982).

Reclamation targeting upland forests is realized by placing cover soil over suitable subsoil or overburden. Cover soil materials used for reclamation are forest floor mineral-mix (FFM) when salvaged from upland forest ecosystems (Alberta Environment and Water 2012) or peat or peat mineral-mix (PM), when salvaged from bogs and fens, which are characterized by higher contents of organic materials (Alberta Environment and Water 2012). Early vegetation recovery using FFM as cover soil has been demonstrated to be more successful (Mackenzie and Naeth 2010). However, the material is less abundant than PM within the mine footprint, and thus PM will be used in most reclamation sites targeting upland forest (Alberta Environment and Water 2012; Pinno and Hawkes 2015). Forest floor-mineral mix in this study was salvaged from the LFH horizon and the top 10-15 cm of A/B horizon from coarse-textured upland type **a** or **b** ecosites. Type **a** ecosites are characterized by xeric to subxeric moisture regimes and poor to very poor nutrient regimes; type **b** ecosites are characterized by submesic to subxeric moisture regimes and medium to poor nutrient regimes (Beckingham and Archibald, 1996). The peat mineral-mix used in this study was salvaged from a **j** ecosite (poor fen), which is characterized by an intermediate nutrient regime and a subhydric moisture regime, as well as from a **k** ecosite (rich fen), which is characterized by a rich nutrient regime and a subhydric moisture regime within Aurora North mine. Soils of both ecosite types are composed of OM derived from sedges, golden, tufted and brown moss, and peat moss (Beckingham and Archibald 1996). The peat of fens is primariliy derived from sedges with inclusions of decayed stems of shrubs and developed under eutrophic conditions under the influence of mineral rich groundwater; sphagnum cover is usually low or not present (Turchenek and Lindsay 1982). The pH of fens in the study area commonly ranges from 5.5. to 7.5 (Turchenek and Lindsay 1982), at the specific study site peat was characterized by a pH of 7. Increased mineral components of PM are a result of including underlying mineral horizons when salvaging the material. Methods for the characterization of PM should as a minimum include the analysis total organic carbon content (TOC), as well as pH, salinity, and mineral nutrient content. Characterization of cover soils in the AOSR has also been conducted with advanced methods like nuclear magneticresonance spectroscopy (Norris et al. 2013). Other techniques as the evaluation of thermal SOM stability may be relevant for further improvement and characterization of PM cover soils with the goal to increase reclamation success.

1.4 Vegetation of type a and b ecosites of northern Alberta

The xeric to subxeric and nutrient-poor type **a** ecosites are characterized by jack pine, bearberry, bog cranberry, blueberry, twin-flower, sand heather, wild lily-of-the-valley, Schreber's moss, awned hair-cap, and reindeer lichen (Beckingham and Archibald 1996). The for type **a** ecosites typical Jack pine stands are commonly open canopied and lichen covers the forest floor, which is characterized by a thin organic layer (Beckingham and Archibald 1996).

Type **b** ecosites are charaterized by subxeric to submesic moisture regimes and poor to to medium nutrient regimes (Beckingham and Archibald 1996). Vegetation typical for type **b** ecosites includes jack pine, aspen, white birch, bog cranberry, blueberry, green alder, bearberry, labrador tea, twin-flower, buffalo-berry, prickly rose, white spruce, bunchberry, cream-colored vetchling, Schreiber's moss, stair-step moss, and reideer lichen (Beckingham and Archibald 1996).

1.5 Spatial heterogeneity: a target for reclamation sites?

Spatial statistics provides the benefit of using the variable of space to account for other factors that are not measured or unmeasurable and allows a closer look at the relationship between those variables (McIntire and Fajardo 2009). As ecological data is mostly characterized by spatial structures due to spatial autocorrelation, which is linked to spatial properties, this results in a distinct spatial pattern (Fortin et al. 2006). This statistical prediction is based on Tobler's first law of geography: *"everything is related to everything else, but near things are more related than distant things"* (Tobler 1970: 236). This fundamental statute supports the claim of a positive or negative correlation between both entities. However, spatial associations do not necessarily imply causality, as observed correlation might result out of a non-causal relationship (Miller 2004). Measures that are associated can involve a causal relationship, or there can be hidden variables that cause the association (Miller 2004). Spatial patterns of soil respiration have been suggested to link above and belowground function in boreal forest ecosystems (Das Gupta and MacKenzie 2016). Establishing a linkage of above and belowground function might be of importance for successful reclamation.

Another ecologically important measure is spatial heterogeneity, which provides information on the intensity and pattern of spatial associations (Miller 2004). For surface patterns that are spatially continuous such as soil, spatial heterogeneity refers to the variability of values among sub-regions or to the distribution of those parameters in space (Dutilleul 1993). Spatial heterogeneity is a key factor dictating the ecological resilience of complex adaptive systems at various scales (Desjardins et al. 2015). This relates to its effect on genetic diversity and intraspecific genetic polymorphism (Bell and Lechowicz 1991), habitat partitioning (Weider 1984), competition (Powell and Richerson 1985), species diversity (Pringle 1990), parasitism (Nachman 1981), dynamics of populations (Cantrell and Cosner 1991), predation (Gilinsky 1984), and population stability (Lodge et al. 1988) of an ecosystem. Especially, peat mineral-mix reclaimed sites in the oil sands have been shown to have different scale spatial patterns of nutrient availability in comparison to natural benchmark sites (Sorenson et al. 2017). It is likely that by reestablishing reclamation sites with close-to-nature spatial heterogeneity following disturbance boreal forest ecosystems can recover faster. This research aims to characterize spatial heterogeneity of soil function on reclamation and reference sites and to evaluate differences to allow further improvement of reclamation practice.

1.6 Research objectives, outline, and toolbox

The research objective of this work is to improve our knowledge on how reclamation materials can be used to create cover soils that allow the reestablishment of ecosystems with close to natural conditions, which allow native organisms adapted to these conditions to reestablish on reclamation sites. This includes suggestions of methods for improving reclamation cover soils and identification of cover soil related measures for determination of reclamation success. An essential component of this work is the characterization of natural ecosystems, which have similar characteristics to the ones targeted in line with the reclamation process. Overall this work is an important step for the reestablishment of self-sustaining, functional boreal forest ecosystems with close-to-nature characteristics in the post-mining landscape.

This PhD thesis includes two primary long-term field experiments based on a cyclic sampling design, which allows the characterization of spatial heterogeneity, greenhouse experiments, which aim to characterize and improve capping material for reclamation, and a conclusions chapter, which aims to apply our findings in the reclamation landscape (Figure 1-1). The target of all studies is an improvement of upland Oil Sands Mine reclamation in the AOSR. Each of the projects was analyzed in a separate chapter and consists out of an introduction, materials and methods, results, discussion, and conclusions part. Separate data chapters are framed by a general introduction and a tech transfer chapter, which describes potential application of findings for land reclamation practice.

In our studies, we use measures that allow determination of ecosystem function. We measured bioavailable nutrients using Plant Root Simulator (PRS™) probes, as they allow a more ecologically meaningful evaluation of nutrient profiles over time. PRS™ probes employ membrane-bound ionic exchange resins and are designed to capture nutrient anions and cations available in the soil solution. The use of ion exchange resin membranes has been demonstrated to be more efficient and biologically relevant than traditional extraction methods in the context of nutrient bioavailability (Qian and Schoenau 2002). Soil biological activity was evaluated measuring bulk soil respiration, which is mainly composed out of autotrophic and heterotrophic respiration. Soil microbial biomass (SMB) was used to evaluate the amount of living biomass of soil organic matter (SOM). Nutrients bioavailability and soil respiration are affected by SMB.

Besides the biological indicator SMB, we evaluated thermal SOM stability, as the stability of SOM might affect rates decomposition and following soil respiration. Vegetation performance was assessed based on biometrics, foliar nutrient thresholds for deficiency and toxicity, as well as for nutrient stoichiometry. Root exudation was evaluated based on changes in rhizosphere polysaccharide concentration.

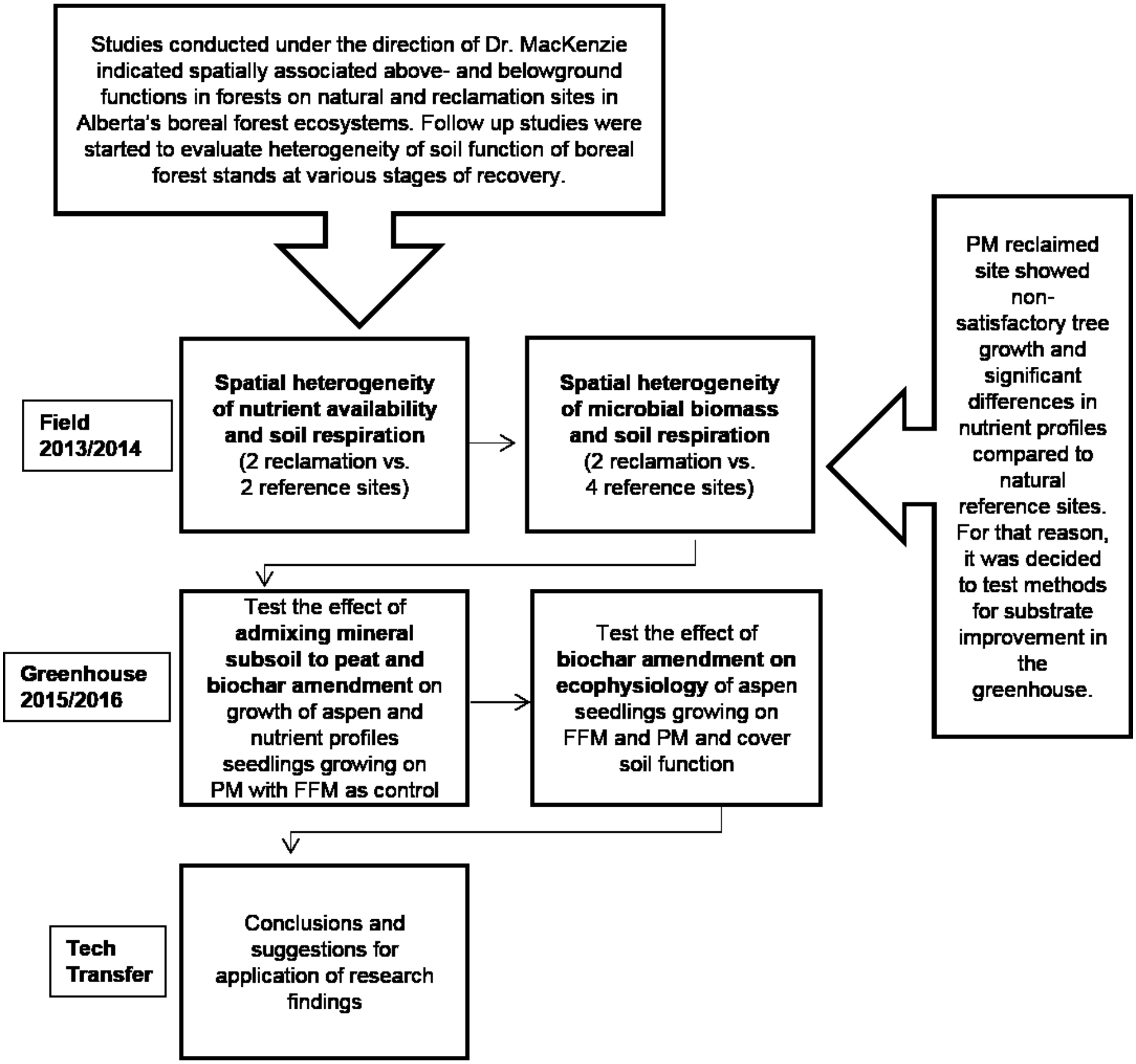


Figure 1-1: Flowchart of studies conducted

Chapter 2 Comparing spatial heterogeneity of bioavailable nutrients and soil respiration in boreal sites recovering from natural and anthropogenic disturbance

2.1 INTRODUCTION

Surface mining of oil sands in the Athabasca Oil Sands Region (AOSR), Alberta, Canada creates a large-scale ecosystem disturbance requiring land reclamation (Alberta 2014a). At the end of 2015, 940.95 km^2 have been disturbed by oil sands surface mining, 6,163 ha have been permanently reclaimed, and 104 ha of the total disturbed area has been certified reclaimed (Environment&Parks 2015). Operators are required to reclaim disturbed land to equivalent land capability (Alberta 2014a). The presence and growth of local plant species are used to assess recovery of boreal forests on these disturbed areas (Alberta 2013; Environment&Parks 2015). Reclamation targeting upland forests is realized by placing cover soil over suitable subsoil or overburden. Cover soil materials used for reclamation are forest floor mineral-mix (FFM) when salvaged from upland forest ecosystems (Alberta Environment and Water 2012) or peat or peat mineral-mix (PM), when salvaged from bogs and fens, which are characterized by higher contents of organic materials (Alberta Environment and Water 2012). Early vegetation recovery using FFM as cover soil has been demonstrated to be more successful (Mackenzie and Naeth 2010). However, the material is less abundant than PM within the mine footprint, and thus PM will be used in most reclamation sites targeting upland forest (Alberta Environment and Water 2012; Pinno and Hawkes 2015).

Boreal ecosystems are naturally disturbed by fire, insect or disease outbreaks, windthrow, landslides, and erosion, which depending on intensity, type and prevalence affect vegetation and biogeochemical cycling (Maynard et al. 2014). However, wildfire is the most significant non-anthropogenic disturbance in the boreal forest (Armstrong 1999) and is the major stand-renewing event in Canada's boreal zone (Brandt et al. 2013). Human based disturbances of boreal forest are related to resource extraction (e.g., harvesting and mining), but also to acid deposition and flooding (Maynard et al. 2014). For boreal mixedwood forests of Alberta, it has been demonstrated that ten years after clearcut harvesting soil properties are not adversely affected (Kishchuk et al. 2015). Given the successful regeneration of forest stands following harvesting or fire we used a burned and harvested site as a benchmark to evaluate the performance of upland mine reclamation sites.

Species diversity in forest ecosystems has been suggested to be driven by resource heterogeneity, especially variability in light and soil resources (resource heterogeneity hypothesis) or supply rates of limiting resources (resource quantity hypothesis) (Bartels and Chen 2010). For forest ecosystems, it has been argued that both hypotheses play an important role on a temporal scale (Bartels and Chen 2010). For surface patterns that are spatially continuous, like soil, spatial heterogeneity refers to the variability of values among subregions or to the distribution of those parameters in space (Dutilleul 1993). Spatial heterogeneity of soil nutrients has been demonstrated to be an essential factor in recovering forests, as low resource heterogeneity might support the establishment of species that can cope with high levels of competition; or weaken species that need unique regeneration niches for successful reestablishment (Fraterrigo et al. 2005). Nutrient heterogeneity is also influencing spatial heterogeneity of other organisms (e.g., ants and soil biota) indirectly by influencing plant community composition (Fraterrigo et al. 2005). The importance of spatial heterogeneity for plants is associated to the spatial scale of the individual root system, which it must either match or be less than to respond to heterogeneity (Hutchings et al. 2003).

Soil respiration is an important component of belowground function. It is driven by heterotrophic respiration, which is caused by microbes and soil fauna and autotrophic respiration, which is caused by roots (Hanson et al. 2000). All metabolic functions in soil that produce carbon dioxide (CO_2) are taken together in summation soil respiration (Lundegårdh 1927). The dissolution of carbonates can affect the C flux from soil and may lead to an over estimate of biologic activity (Tamir et al. 2011). Soil respiration is known to be one of the largest fluxes of the global carbon cycle and the primary path for returning CO_2 fixed by plants to the atmosphere (Schlesinger and Andrews 2000). Small changes in soil respiration could potentially have large influences on the atmospheric CO_2 concentration (Schlesinger and Andrews 2000). The flux of CO_2 from soils is strongly related to plant productivity, which is also driving the availability of organic residues for decomposers (Raich and Schlesinger 1992; Schlesinger and Andrews 2000). Adding organic C to soils results in increased rates of soil respiration (Högberg and Ekblad 1996). This indicates that using PM for land reclamation might increase the CO_2 flux on a landscape scale in the Athabasca Oil Sands Region (AOSR) and result in a different function from established forest ecosystems (source vs. sink). However, this is not known to date. A recent study conducted in the AOSR detected that above- and belowground functions of boreal aspen stands are linked by soil respiration and it has been suggested that reclamation success might be evaluated by spatial heterogeneity of soil respiration (Das Gupta and Mackenzie 2016).

Spatial patterns of nutrient availability might be of great importance for the successful reestablishment of native plant species but have not been observed in great detail on reclamation sites in the AOSR. As both harvested and post-fire sites are known to reestablish native vegetation in the boreal forest, we are comparing both nutrient heterogeneity and quantity in line with this study. We hypothesized that harvested and post-fire sites show a more heterogeneous pattern with differences in soil nutrient availability (resource quantity), as both cover soil salvage and placement will lead to homogenization and reduced heterogeneity of soil nutrient availability (Figure 2-1). Secondly, we hypothesize that soil respiration will follow a seasonal pattern on benchmark sites and FFM reclaimed sites as a belowground link in the form of root propagules, or a seedbed is more likely readily established compared to PM.

The goal of this study is to identify if reclaimed sites can provide similar niches for organisms (e.g., bioavailable nutrients) that can be found on the natural ecosites present in the Athabasca Oil Sands Region (AOSR), and those ecosites are targeted as reclamation end goals in closure plans for Oil Sands mining operations. The successful establishment of native plant species is becoming a more important parameter in the context of certification of reclaimed sites, and there is an interest to understand what drives this in an economical but also ecological context in boreal Alberta. Spatial patterns of soil respiration could potentially be used as an indicator of an established linkage of aboveground and belowground function and potentially indicate early successful ecosystem recovery.

2.2 METHODS

2.2.1 Study sites

Data was collected during the 2013 growing season in the AOSR north of Fort McMurray (57°20'12.25"N 111°32'22.92"W), Alberta, Canada. Two reclamation sites at Syncrude’s Aurora Soil Capping study (ASCS) were used, one reclaimed with FFM in 2012 (Figure 2-2a) and one reclaimed with PM in 2012 (Figure 2-2b). These were compared to a reference site naturally recovering from harvesting in 2006 (Figure 2-2c), classified as type **ab** ecosite (Beckingham and

Archibald 1996), and a site regenerating from wildfire in 2012 (Figure 2-2d), classified as type **b** ecosite (Beckingham and Archibald 1996). Natural upland forest ecosystems observed in this study lie within Alberta's boreal forest natural region, in the Central Mixedwood Natural Subregion (Natural Regions Committee 2006). The Central Mixedwood Natural Subregion is characterized by upland forests of aspen mixedwood and white spruce, but also with areas of treed fens or jack pine stands on coarser materials in the east (Natural Regions Committee 2006). Summers in the Central Mixedwood Natural Subregion are short and warm while winters are long and cold (Natural Regions Committee 2006). The capping on the FFM reclaimed site consisted of 20 cm FFM over 130 cm of subsoil salvaged from below 100 cm, while capping on the PM reclaimed site consisted of 30 cm of peat over 120 cm subsoil. FFM was salvaged from the LFH and the top 10-15 cm of soil from a coarse-textured upland type **a** or **b** **ecosite**s. Type **a** ecosites are characterized by xeric to subxeric moisture regimes and poor to very poor nutrient regimes, type **b** ecosites are characterized by submesic to subxeric moisture regimes and medium to poor nutrient regimes (Beckingham and Archibald, 1996). PM was salvaged from a **j** ecosite (poor fen), which was characterized by an intermediate nutrient regime and a subhydric moisture regime (Beckingham and Archibald 1996), as well as from a **k** *ecosite* (rich fen), which is characterized by a rich nutrient regime and a subhydric moisture regime (Beckingham and Archibald 1996). Soils of both fen ecosite types were composed of organic matter (OM) derived from sedges, golden, tufted and brown moss, and peat moss (Beckingham and Archibald, 1996). Soils found on the reference sites were characterized as Orthic Dystric Brunisol at the harvested site and Orthic Gray Luvisol at the type **b** ecosite. Basic soil characteristics of top soils collected at all sites are reported in Table 1-1. All plots were set up in plane areas to minimize effects of slope and resulting effects on the moisture regime. Due to the high sampling intensity per treatment replicates of treatments were not established.

2.2.2 Spatial Sampling

A spatial sampling protocol was used to collect data on bioavailable nutrients (81 points) (Figure 2-3a) and soil respiration (42 points) (Figure 2-3b) in a 20 x 50 m plot. We followed the approach used by Smithwick et al. (2005) who suggested cyclic sampling design, which is reversed in the middle three rows to account for anisotropy. Using a cyclic sampling design allowed us the study of spatial patterns due to the establishment of comparable power at diverse lag distances and maximized the effectiveness of sampling (Clayton and Hudelson 1995). The minimum detectable spatial lag was 0.5 m for bioavailable nutrients and 2 m for soil respiration.

Bioavailable nutrients

Bioavailable nutrients were measured by installing Plant-Root-Simulator™ probes (PRS) in situ (n=81) during the growing season in the topsoil of research sites according to the manufacturer instructions (Western AG Innovations, Saskatoon, SK). Probes were installed over a 57 day period from June – August 2013 in the topsoil of all sites. PRS™ probes employ membrane-bound ionic exchange resins and are designed to capture nutrient anions and cations available in the soil solution. Probes were incubated in situ during the growing season of 2013. The use of PRS technology allows a more realistic approach than conventional soil tests for evaluating rhizosphere conditions as labile inorganic nutrients are measured in-situ with little disturbance and avoid the use of extractants on soil (Qian and Schoenau 2002). Upon removal from sites at the end of August, probes were immediately cooled, transported to the lab and rinsed with de-ionized water and sent to Western Ag Innovations. Probes were extracted with 0.5

M HCl and analyzed for NH_4^+ and NO_3^- by colorimetry (FIAlab 2600), and by ICP-OES (Perkin Elmer ICP-OES 8300) for Al, B, Cu, P, K, Mn, Zn, Fe, Mg, Ca, and S.

Soil Respiration

Soil respiration was measured monthly during the growing season in May, June, July, and August using a LiCor LI-8100 Soil Gas Flux System (LI-COR, Lincoln, Nebraska, USA) on preinstalled custom made 8" diameter respiration collars. Collars were installed to a depth of 2," and headspace was measured to ensure the accuracy of measurements. Measuring soil respiration allows to evaluate in-situ soil metabolic activity producing CO_2, resulting out of autotrophic and heterotrophic respiration, and eventually the chemical oxidation of carbonaceous compounds (Bunt and Rovira 1954; Lundegårdh 1927).

2.2.3 Statistical Analysis

R 2.1.5 (R Core Team 2012) was used for statistical analyses with agricolae package (de Mendiburu 2014) for post-hoc testing with Tukey HSD test, following permutational ANOVA in the lmPerm package (Wheeler 2010) for significance testing to compare means of both nutrient bioavailability and soil respiration. Permutational ANOVA was chosen for analysis to account for violation of ANOVA assumptions and sample size.

Multivariate statistics were used to examine the overall nutrient profile. Principal component analysis (PCA) was carried out with the ggbiplot package (Vu 2011) in R3.3.1(R Core Team 2016). Data were standardized and normalized before PCA by log transformation and adding a constant (log10(x+100)).

Isotropic variograms were fitted with GS+ 10.0 (Gamma Design Software). Four Varigrom models were tested (Linear, Exponential, Spherical, and Gaussian) to identify the model that best fitted the data. The residual sum of squares (RSS) was used in combination with the combination highest coefficient of determination (R^2) to identify best model fit. Following ordinary and standardized kriging maps were plotted to visualize spatial characteristics using GS+ 10.0 (Gamma Design Software). Cross-validation was used to evaluate kriging interpolations, which is based on a comparison of estimated versus actual measured values.

The nugget coefficient, nc, which is calculated from total variance (c0 + c1) and the associated nugget variance (c0) was used to calculate spatial dependence. If the nugget coefficient is >75 strong spatial dependence is indicated, 25-75 indicates moderate spatial dependence, and a value <25 indicates poor or no spatial dependence (Isaaks and Srivastava 1989). Also the coefficient of variation (CV) was calculated to provide a measure of global variation. The CV is calculated by dividing the standard deviation by the mean and was reported as a percentage.

2.3 RESULTS

2.3.1 Ordination of bioavailable nutrients

Macronutrients

To evaluate the overall differences of the macronutrient profiles of reference and reclamation sites PCA was conducted on the data (Figure 2-4). PC1 and PC2 explained 78.4 % of the variation within the four treatments. Differences between reference and reclamation treatments are mainly explained on the axis of PC1, which explained 59.9 % variation (Figure 2-4). Increased bioavailable P and K was positively associated with the Harvest **ab** and the Fire **b** site and shifted those treatments to the right of PC 1 (Figure 2-4). Reclamation treatments PM and FFM were positively associated with increased N, S, and Ca, which shifted those treatments to the left of PC 1(Figure 2-4).

The axis of PC 2, which explained 18.5 % variation was positively associated to increased P and Mg availability and shifted FFM and the Fire **b** treatment upwards compared to the PM and Harvest **ab** treatments (Figure 2-4).

Ellipses are indicating the 68% confidence interval for treatments overlapped for the Fire **b** and Harvest **ab** site indicating great similarity. The 68% confidence interval ellipse for FFM was closer to the reference treatments than the ellipse for PM, indicating greater similarity of FFM to reference sites. The ellipse for PM was smaller compared to other treatments indicating a reduced overall variability.

Micronutrients

PCA of bioavailable micronutrients explained a total of 64.3% on the axes of PC1 and PC2. All treatments overlapped within their 68% confidence interval (Figure 2-5). The reclamation treatments PM and FFM showed a shift to the left on the axis of PC1, which was associated with increased B, Fe, Al, and Cu availability. A downward shift of FFM, Harvest **ab**, and the Fire **b** site on the axis of PC2 was associated with increased Mn and Zn availability (Figure 2-5).

2.3.2 Spatial patterns of bioavailable nutrients

Nitrogen

Mean bioavailable N was highest on the PM treatment, which was significantly greater than the mean N availability on FFM, Harvest **ab**, and Fire **b** (Table 2-2). Mean N availability on

PM was about 100% greater than on FFM and more than 1000% greater than on the reference sites Harvest **ab** and Fire **b** (Table 2-2). Minimum values for N availability on PM was greatest, followed by Fire **b**, FFM, and Harvest **ab** (Table 2-2). The N availability maximum was highest on FFM, followed by PM, Fire **b**, and Harvest **ab**. Global variation (CV) was greatest on FFM, followed by Fire **b**, Harvest **ab**, and lowest on PM.

Nitrogen had the finest spatial range on PM, followed by Fire **b**, FFM, and Harvest **ab** showed larger scale spatial autocorrelation than on other sites (Table 2-2). Kriging maps supported and visualized those findings showing fine points with lower N availability, but also higher N availability than the mean N availability in PM. The left side of the kriging map showed a vast area with higher variation in N bioavailability, while to the right N bioavailability ranged from100 to 200 μg $10cm^{-2}$ N showing low variability (Figure 2-6). The Fire **b** site showed predicted variation at much lower N bioavailability, which was slightly higher than on FFM (Figure 2-6). On FFM kriging maps (right) areas were predicted that were similar in N bioavailability as on the reference sites Fire **b** and Harvest **ab**, however also areas with high N bioavailability (left, Figure 2-6). The Harvest **ab** site showed predicted vast areas with low N bioavailability (Figure 2-6). Strong spatial dependency of N bioavailability was only observed for the PM reclaimed sites, while all other sites showed poor or no spatial dependency.

Phosphorus

Mean bioavailable P was highest on the Fire **b** site, followed by Harvest **ab** and FFM, which both showed no significant difference in mean P bioavailability (Table 2-2). PM had a significantly lower mean P bioavailability compared to all other treatments (Table 2-2). The minimum availability was greatest on Fire **b**, all other tree sites tested had areas with no measurable P availability. The maximum was greatest on Fire **b** followed by Harvest **ab**, FFM, and PM. The maximum availability on peat was more than 40 times smaller than on Fire **b** and about 40 times smaller than on the Harvest **ab** site (Table 2-2). Global variation of P was greatest on Harvest **ab** followed by Fire **b**, PM and was smallest on FFM (Table 2-2).

The spatial range of P was coarse on PM with an autocorrelation >22 m while being fine on all other sites were spatial autocorrelation was detected at a range of about 1.5 m (Table 2-2). Kriging maps predicted a very low to not existing P availability with little to no variation on PM (Figure 2-6). Kriging maps of FFM indicated greater variability and increased availability of P compared to PM, but visually lower than on the Harvest **ab** site, which showed areas with lower availability, but also hot spots. The Fire **b** sit was predicted to have bigger areas with high P availability and some spots with moderate P availability compared to other sites but also some smaller hotspots (Figure 2-6). Spatial dependency was poor or not existent for all sites except Harvest **ab** where a moderate dependency was detected.

Potassium

Mean K bioavailability was highest on Fire **b**, followed by Harvest **ab**, FFM and PM (Table 2-2). The mean K bioavailability was more than 2000% greater on Fire **b** compared to PM (Table 2-2) and K availability on Harvest **ab** was about 1300% times greater compared to PM (Table 2-2). Minimum values were greatest on H**2.3.3 Spatial pattern of soil respiration**

Seasonal Pattern of soil respiration

Peat mineral-mix showed no consistent pattern when visually comparing soil respiration rates from May to August (Figure 2-7). FFM showed a recurring pattern with higher respiration rates in lower parts of kriged maps and reduced respiration rates in upper parts of kriged maps, which were especially observable for months May, June, and July (Figure 2-7). On the Harvest **ab** site reoccurring patterns with higher and lower activity could be observed as well, when comparing kriged maps for June, July, and August (Figure 2-7). For example, the top left corner, as well as the bottom left corner, showed higher rates of soil respiration throughout the months of measurements (Figure 2-7). Similarly, this observation could be made on Fire **b** where areas with returning rates of increased soil respiration could be identified for example in the bottom left corner throughout May, June, July, and August (Figure 2-7).

May

Mean soil respiration in May was greatest on PM and significantly higher than on FFM and Fire **b**, no measurements could be reported for Harvest **ab** due to limitations in site access (Table 2-3). Soil respiration on PM exceeded FFM by 50% and Fire **b** by about 100%. Greatest minimum values for soil respiration in May were observed on PM followed by Fire **b** and FFM (Table 2-3). Maximum values were highest on PM, followed by FFM and Fire **b** (Table 2-3). Global variation was about 300% greater on Fire **b** compared to FFM and about 100% greater compared to PM (Table 2-3).

Spatial autocorrelation was fine on Fire **b**, followed by PM and FFM (Table 2-3). Reclamation sites showed a coarser range, which was greater >22m on FFM (Table 2-3). Kriging maps for soil respiration in May predicted higher respiration rates on PM comparted to FFM and Fire **b** (Figure 2-7). Kriging maps also showed the smaller scale variability on Fire **b** compared to PM and FFM (Figure 2-7). Spatial dependency was poor or no existent for PM and FFM but strong on Fire **b** (Table 2-3).

June

Mean soil respiration in June was highest on FFM and significantly exceeded respiration rates of all other sites (Table 2-3). Respiration rates on PM were second highest but not significantly higher than on Fire **b** (Table 2-3). Mean respiration was lowest on Harvest **ab** (Table 2-3). Minimum values were greatest on FFM followed by PM, Fire **b** and Harvest **ab** (Table 2-3). Maximum respiration value was highest on FFM, followed by PM, Fire **b** and Harvest **ab** (Table 2-3). Global variation in June was greatest on Harvest **ab** followed by PM, FFM and Fire **b** (Table 2-3).

Spatial autocorrelation was detected at a fine scale in PM, FFM, and Harvest **ab** and at a very coarse scale at Fire **b** (Table 2-3). Kriging maps of soil respiration confirmed those findings showing smaller patches on PM, FFM, and Harvest **ab** compared to Fire b (Figure 2-7). Spatial dependency of soil respiration was strong on PM but poor or not existent at FFM, Harvest **ab**, and Fire **b** (Table 2-3).

July

Mean soil respiration was highest on FFM followed by Fire **b**, PM and Harvest **ab** (Table 2-3). Highest minimum soil respiration values in July were found on Fire **b** followed by PM, FFM, and Harvest **ab** (Table 2-3). The respiration maximum was highest on FFM and exceeded values of all other sites by 100 % (Table 2-3). Global variation was highest on Harvest **ab** followed by FFM and on both sides about 75% greater compared to PM and Fire **b** (Table 2-3).

Spatial autocorrelation was very coarse (>22 m on FFM), and coarse on PM followed by Fire **b** (Table 2-3). Harvest **ab** showed a finer autocorrelation with a range of 6.63 m (Table 2-3). Kriging maps predicted lower variability on PM and Fire **b** compared to FFM and Harvest **ab** (Figure 2-7). Spatial dependency was poor or not existent during July on PM, FFM, and Fire **b**, while strong dependency was detected on Harvest **ab** (Table 2-3).

August

Mean respiration rates in August highest on FFM, were respiration rates exceeded respiration of the second highest site Fire **b** by close to 100% (Table 2-3). Respiration rates on Fire were slightly higher compared to Harvest **ab** and significantly higher compared to PM (Table 2-3). Respiration on Harvest **ab** was not significantly higher than on PM (Table 2-3). Highest minimum values were detected on Fire **b**, followed by FFM and Harvest **ab** and were lowest on PM (Table 2-3). Maximum values were highest on FFM; the maximum was more than 100% greater than maxima on other sites (Table 2-3). Second highest was Harvest **ab** followed by Fire **b**, the maximum values here were close to 100% higher than on PM (Table 2-3). Global variation in August was highest on Harvest **ab** followed by FFM, Fire **b** and PM (Table 2-3).

Spatial autocorrelation was very coarse (>22 m) on Fire **b**, on Harvest **ab** compared to PM also coarse autocorrelation was detected (Table 2-3). FFM was characterized by a fine scale autocorrelation (Table 2-3). Kriging maps predicted low respiration rates with little variation for PM (Figure 2-7). FFM and Harvest **ab**, but also Fire b showed greater variation (Figure 2-7). Especially FFM and Harvest **ab** showed patches with higher but also lower soil respiration (Figure 2-7). Spatial dependency was poor or not existent at PM, Harvest **ab**, and Fire **b** but strong at FFM (Table 2-3).

2.4 DISCUSSION

2.4.1 Ordination of bioavailable nutrients

Macronutrients

Biomass accumulation in forest stands following severe disturbance appears to be linked to nutrient availability (Johnson et al. 2000). Reference sites recovering from disturbance showed measurable differences in bioavailable macronutrients compared to the tested reclamation treatments PM and FFM with FFM being more similar to natural references than PM. Research has shown that plants species adapted to infertile conditions differ in ecophysiological traits compared to plants that strive in fertile systems (Wardle 2002). It is understood that under conditions of fluctuating resource availability, sites are more susceptible to the invasion of non-native or unwanted species (theory of fluctuating resource availability) (Davis et al. 2000). This implies that especially young reclamation sites with differing nutrient profiles (e.g., N, P) and no stable target vegetation community might be prone to invasion of undesired species. Additionally, the availability of essential soil minerals is playing a key factor in mutual benefits provided by soil microorganisms (e.g., mycorrhizal fungi) to plants (Reynolds et al. 2003). For forest ecosystems, it is suggested that resource quantity is a key driver for understory diversity during stages of stem exclusion and mature stages when resource quantity is low, while resource heterogeneity might be of greater importance for understory diversity in old growth forests (Bartels and Chen 2010). Hence, it might be beneficial to make profiles of bioavailable nutrients in both resource quantity and heterogeneity more similar to natural benchmarks when targeting type **a** or **b** ecosites as an end goal for oil sands mine reclamation.

Micronutrients

Boron deficiency has been reported to have the potential to cause stunting growth, poor tree survival, or to cause stem defects in natural forest stands following macronutrient fertilization, fire, or erosion (Stone 1990). For forests stands of Pacific Northwest America, it has also been shown that Mo can limit asymbiotic N fixation (Silvester 1989). Other studies conducted with slash pine and loblolly pine for which micronutrients deficiencies are uncommon showed that Mn additions can be influencing growth (Jokela et al. 1991). Data for bioavailable B, Fe, Al, Cu, Zn, and Mn suggested similarity of micronutrient availability but also showed that there is some separation (Figure 2-5). However, it is important to understand that micronutrient deficiencies can significantly affect vegetation performance on reclamation sites. Especially on This is especially relevant on PM where bioavailability of Cu or Mn might be reduced due to complexation in substrates with high organics and high pH (Broadley et al. 2012a). We suggest that micronutrient bioavailability in cover soils used for land reclamation is monitored and eventually controlled on reclamation sites if needed.

2.4.2 Spatial pattern of bioavailable nutrients

Nitrogen

In the boreal biome, N bioavailability is considered to be of limiting nature in forest growth and is also determining terrestrial biodiversity or a factor in eutrophication of aquatic ecosystems (Sponseller et al. 2016). The forest floor patchiness can influence spatial patterns of revegetation and regenerated vegetation itself interacts with nutrient availability (Guo et al. 2004). Data from our study showed that global variation in nutrient bioavailability was higher in FFM and Fire **b** compared to Harvest **ab** and PM. As vegetation is already established on Harvest **ab** and is older compared to PM, FFM, and Fire **b** comparing site nutrient availability is challenging, as vegetation might contain significant amounts nutrients. Higher nutrient retention in the biomass of the Harvest **ab** treatment is likely, as in early stages following disturbance (harvest) biomass accumulates rapidly binding essential or potentially limiting nutrients (Vitousek and Reiners 1975). As it has been shown that both above and belowground productivity of young regenerating post-logged and post fire stands does not differ (Seedre et al. 2014; Yuan and Chen 2013); it can be expected that the post fire site will develop on a similar trajectory as the logged site over time, showing an overall reduced availability of N. Overall it can be observed that N bioavailability on FFM and PM is exceeding availability compared to natural sites following disturbance, potentially opening a window for invasion of unwanted species following the hypothesis of Davis et al. (2000). To reduce overall N bioavailability on reclamation sites PM or FFM could be placed shallower over suitable overburden or when salvaging materials higher amounts of suitable underlying materials could be stripped to reduce overall N availability in reclamation cover soils. Kriging maps demonstrated that bioavailability of N on both reclamation sites varies, similar to that on Fire **b** post natural disturbance. Future studies should investigate if patterns of variable N availability determine species-specific successful plant reestablishment.

Phosphorus

Phosphorus soil chemistry has been shown to be impacted by plant-soil feedbacks in forest stands (Gómez-Aparicio et al. 2017). Plant-soil feedbacks influence species abundance, coexistence, and succession in plant communities (Gómez-Aparicio et al. 2017). In our study reference sites recovering from harvesting and fire showed an overall higher bioavailability of P compared to FFM and PM, with Harvest **ab** not being significantly different to FFM. It has been suggested that during stages of secondary boreal forest succession soil P resources maybe outpaced by their demand (Hume et al. 2016), and for that reason, an overall reduced availability on reclamation sites as observed in our study compared to natural sites has to be evaluated critically. Phosphorus availability, as well as N availability, can affect future productivity and C storage of terrestrial ecosystems (Wieder et al. 2015). Kriging maps in our study demonstrated no variation of P on the PM treatment and variability on the FFM treatment that was low compared to Harvest **ab** and Fire **b** treatment. Soil P availability has been shown to be a driving factor regulating plant intra-specific competition (Facelli and Facelli 2002). Plants growing with high available P develop higher mean shoot and root biomass (Facelli and Facelli 2002). Hence it can be anticipated that in patches with increased P availability vegetation will establish with greater success. Phosphorus can be volatilized at higher fire temperatures in excess of 774 ºC and losses can range from 10 – 50% depending on burning intensity, but studies conducted in the United States also reported increases of P in the litter layer following fire (Neary et al. 1999). We suggest that P bioavailability on reclamation sites should be increased to a level that is similar to Fire **b**, as it can be assumed that availability on Fire **b** belowground included parts of aboveground nutrients. Large-scale autocorrelation of P at low levels is concerning as it might allow the establishment of undesired species

Potassium

Potassium has been shown to be more heterogeneous in soils following fire than other cations as for example Ca and Mg (Outeiro et al. 2008). K, as well as P, is a readily by plants cycled nutrient and is more concentrated in the topsoil (Jobbágy and Jackson 2001). Potassium plays an important role in enzyme activation and osmoregulation; deficiency predisposes plants to abiotic and biotic stresses (Hawkesford et al. 2012). Areas with higher availability of K to seedlings might increase chances of successful plant establishment. Our study demonstrated that variability following fire was greater as on the harvested site. Reclamation sites showed some variability but at lower rates of K availability. A study conducted on reclamation sites in Alberta identified only coarse scale patterns for K and associated those to the application of PM reclamation cover soil (Sorenson et al. 2017). However, we found autocorrelation at a fine scale comparable to reference sites. Those findings underline that generalizations in the reclamation environment for cover soil quality cannot be made and that specialized assessments are necessary to optimize reclamation site quality.

Sulphur

Sulphur is a macronutrient that can limit tree seedling growth (Ericsson 1995). However, it has also been reported that excess S can negatively impact plant metabolism and can affect dry weight accumulation, yield, and delay of flowering (Rennenberg 1984). Increased levels of S have been shown to negatively affect some macroinvertebrates found in Canadian pine forests (Carcamo et al. 1998). Our study demonstrated significantly increased bioavailability of S on both reclamation sites. Sulphur availability was by magnitudes greater on PM compared to reference sites. S availability on PM had coarse autocorrelation indicating that high values existed throughout the site. Sorenson et al. (2017) reported varying spatial ranges of S bioavailability, which also changed during seasons but found similarly high values in the spruce stand as in our study on PM. It has been suggested that the intercept in between canopy and S bioavailability is related to high deposition on oil sands reclamation sites. Further field studies should investigate if plant phenology and invertebrates are affected by higher S bioavailability on reclamation sites. Another option would be to reduce topsoil S concentration by blending PM or FFM with materials that contain lower amounts of S to reduce the bioavailability.

2.4.3 Spatial pattern of soil respiration

Our study identified reoccurring patterns of specific respiration activity using kriging maps, especially in Harvest **ab**, Fire **b** and to some degree in FFM. PM showed no reoccurring pattern suggesting that no biologic legacy governing biologic activity is established at this early stage. In a chronosequence of boreal aspen stands spatial patterns of soil respiration have been reported to link above and below ground function at the canopy closure and mature stage (Das Gupta and Mackenzie 2016). Our study demonstrates that a similar pattern can be identified visually on a forest sites following harvesting, indicating a link of existing above and belowground function but contrary to Das Gupta & MacKenzie (2016) also following fire and to some degree on FFM reclaimed sites. It is likely that the identified link is related to density of plant propagules contained in topsoil of the Fire **b** and FFM site, which are quickly recovering following disturbance. It has been shown that boreal forest plants successfully recover due to resprouting from an intact rhizome, germination from buried seed propagule banks, or dispersal of propagules (Rydgren et al. 1998). Hence, dispersal of seeds might not have played an important role in measured patterns of respiration at this early stage, as roots of dispersed seedlings would not have been capable of forming detectable interactions. Future studies should investigate if reclamation sites develop similar detectable patterns as these potentially could be used as an indicator for a successfully established link of above and belowground ecosystem function.

May

In-situ soil respiration has been suggested to be an important measure for evaluating forest recovery following disturbance (Weber 1990). It has been shown that soil respiration is directly linked to photosynthetic activity forest stands (Hogberg et al. 2001; Moyano et al. 2008). During May we observed a higher respiration rates on PM suggesting higher heterotrophic activity compared to FFM and Fire **b**, and we suggest that autotrophic respiration was not playing a major role during the early growing season in May. Bud break of aspen for Northern Alberta has been observed on day 135 of the year (Schreiber et al. 2013), what would be equivalent to May 15 of 2013 and measurements were taken on May 27. For *Pinus sylvestris* it has been shown that high photosynthate sink activity occurs in spring and during late wood formation in summer (Oberhuber et al. 2011), we assume that similar processes affected rhizosphere metabolic activity in our study.

June

In June respiration rates on FFM picked up and were highest. A study conducted with FFM similarly found increased respiration rates of a similar FFM substrate and linked those findings to higher microbial biomass compared to a PM treatment (McMillan et al. 2007). It is likely that increased temperature in June supported an increase of soil respiration at the measured sites (Lloyd and Taylor 1994) in combination with increased vegetation cover commonly found on FFM reclaimed sites (MacKenzie and Naeth 2007; Mapfumo 2003), which likely contributed to increased autotrophic soil respiration.

July

In July, we observed a decrease of respiration on all sites, with highest rates observed on FFM indicating high soil biological activity, which might be related to reduced soil moisture in July. Fire **b** showed no changes in respiration rates which likely is related to more consistent conditions on the fine-textured luvisolic soil and potentially different patterns of precipitation. Water potential of soils has been shown to be closely related to soil respiration (Orchard and Cook 1983). Tang & Baldochi (2005) have observed a reduction in soil respiration during summer with a decrease in available water.

August

In August further reduction of respiration rates was observed on all sites, which likely was related to the by Tang & Baldochi (2005) observed phenomenon of reduced respiration rates with reduced water availability. A decrease in available water during the growing season has been observed for Canadian boreal jack pine (*Pinus banksiana*) stands (Baldocchi et al. 1997).

2.2.4 Possible methodological limitations

The spatially explicit sampling design with a high sample size per site, but no site replication, allowed capturing site individual spatial heterogeneity but disallowed significance testing for variogram parameters and global measures of spread. This is a common problem for studies capturing spatial heterogeneity (Bengtson et al. 2007; Lavoie and Mack 2012; Smithwick et al. 2005). As cover soil materials, but also cover soil placement varies within the AOSR detected spatial soil resource heterogeneity and quantity can only be extrapolated to sites reclaimed under comparable circumstances. The sampling design for nutrient bioavailability differed from the sampling design for soil respiration and did not allow me to link theseparameters, however, allowed to evaluate the by DasGupta & MacKenzie (2016) described patterning of respiration. Additionally, heterogeneity of bioavailable nutrients could be evaluated in greater detail. Given the different characteristics of cover soils there is potential for an error related to dissolution of carbonates as this may affect the C flux from soil and can lead to an over estimate of biologic activity (Tamir et al. 2011). As reclamation sites were characterized by increased Ca^{2+} bioavailability (Figure 2-4) a potential release of CO_2 resulting from carbonate dissolution may be possible.

2.5 CONCLUSIONS

Bioavailable nutrient profiles of a PM and FFM sites differed significantly in nutrient profiles from natural sites disturbed by harvesting and fire, with the FFM site showing greater similarity, especially in bioavailability of macro nutrients. Contrary to our initial hypothesis we could identify spatial variability of bioavailable macro nutrients in both reclamation treatments. FFM showed spatial heterogeneity for N, P, K, and S. PM also showed some variation for N and

K, but very low variability for P and S, which is related to very low and very high values for PM. Critical is a significant difference soil nutrient quantity, especially on the tested PM. Future studies need to evaluate if a similar pattern or heterogeneity can be identified on other reclamation sites and how the substrate can be improved, eventually by increasing mineral component of PM or use of soil amendments. Our study demonstrates that reclamation procedures can reestablish heterogeneity in bioavailable nutrients. Targeted P and K fertilization might be used to increase bioavailability on PM reclaimed sites to increase similarity to type **a** and **b** ecosites targeted in closure plans. Increasing the mineral soil content of PM either during salvage, stockpile or placement, which is done in oil sands reclamation when the opportunity arises, might be an easy remedy to reduce N and S bioavailability in PM. Spatial heterogeneity of nutrient bioavailability on FFM reclaimed sites likely is a factor that positively affects reestablishment of vegetation. It needs to be tested if by increasing heterogeneity of bioavailable nutrients on PM reclaimed sites to a level that is similar as on benchmark sites vegetation recovery can be improved. Admixing of mineral subsoil with peat and use of soil amendments are promising procedures to increase similarity of PM to natural soils or FFM.

Our study demonstrated that spatial heterogeneity of soil respiration varied on all sites during the growing season. However, only the Fire **b**, Harvest **ab**, and FFM sites showed reoccurring patterns during the season indicating that a link in between above and below ground function is established. We conclude that FFM reclaimed sites show ecological function at an early stage and sites may have the potential to be certified reclaimed at an early stage. Future work should verify our findings in a blocked and replicated design to confirm our findings. Our study did not find higher respiration rates on PM compared to FFM. However our experimental design did not allow us to distinguish in between autotrophic and heterotrophic respiration rates, and biomass accumulation was not considered as an offset. Future studies need to evaluate soil respiration rates from the cover soil in a morecontrolled environment.

Table 2-1: Topsoil characterization of research sites (n=3). Mean and standard error. PM peat mineral-mix, FFM forest floor mineral-mix, Harvest **ab** by timber harvesting disturbed **a/b** ecosite, Fire **b** a by forest fire disturbed type **b** ecosite.

Treatment	**pH**	**EC (µS/cm)**	**TC (%)**
PM	7.47 (0.19)	1423.33 (80.07)	15.30 (0.34)
FFM	5.70 (0.11)	213.33 (17.64)	1.57 (0.14)
Harvest ab	5.67 (0.19)	73.33 (18.56)	2.72 (0.21)
Fire b	6.56 (0.08)	212.70 (5.63)	5.48 (0.28)

Table 2-2: Selected macro nutrient characteristics for research sites. Different letters indicate significant differences of the mean based on permutational ANOVA and Tukey HSD ($p<0.05$) for same nutrients in between treatments only. PM peat mineral-mix, FFM forest floor mineral-mix, Harvest **ab** by timber harvesting disturbed **a/b** ecosite, Fire **b** a by forest fire disturbed type **b** ecosite. TIN reported as sum of NO_3^- and NH_4^+; P as equivalent of $H_2PO_4^-$, HPO_4^{2-}; K as equivalent of K^+; S as equivalent of SO_4^{2-}.

Treatment	Element	Mean (±SE) µg $10cm^{-2}$	Min	Max	CV (%)	Range (m)	Spatial dependence	Dependence Class	R^2	Model
PM	TIN	79.32a (5.30)	7.98	225.44	60.12	1.71	1962.00	strong	0.44	Spherical
	P	0.30c (0.04)	0.00	1.43	119.20	22.51	1.00	poor or no	0.31	Linear
	K	10.59d (0.67)	3.23	49.49	57.09	1.1	7.30	poor or no	0.57	Exponential
	S	1394.87a (27.64)	777.41	1998.93	17.84	22.54	1.13	poor or no	0.14	Linear
FFM	TIN	36.73b (10.07)	0.68	565.98	246.76	6.01	2.25	poor or no	0.37	Gaussian
	P	3.39b (0.23)	0.00	12.52	62.23	1.65	2.13	poor or no	0.16	Exponential
	K	50.73c (6.87)	2.22	386.3	121.94	22.52	1.0	poor or no	0.04	Linear
	S	382.41b (30.11)	29.83	1315.07	70.86	0.34	41.65	moderate	0.18	Exponential
Harvest ab	TIN	6.24c (0.47)	0.36	29.48	68.29	22.52	1.96	poor or no	0.35	Linear
	P	5.94b (1.04)	0.00	55.94	157.64	1.29	30.07	moderate	0.10	Spherical
	K	136.54b (10.15)	30.47	501.26	66.89	0.36	8.21	poor or no	0.12	Exponential
	S	23.07c (1.90)	2.22	115.17	74.24	1.19	8.06	poor or no	0.18	Exponential
Fire b	TIN	7.13c (0.79)	1.92	56.22	100.02	3.3	2.00	poor or no	0.11	Gaussian
	P	10.51a (1.45)	0.34	63.96	124.48	1.29	5.65	poor or no	0.12	Exponential
	K	225.60a (21.83)	16.96	1068.29	87.07	1.09	363.10	strong	0.23	Spherical
	S	32.57c (4.69)	1.62	226.02	129.45	1.19	8.06	poor or no	0.11	Spherical

Table 2-3: Soil respiration of research sites. Different letters indicate significant differences of the mean based on permutational ANOVA and Tukey HSD ($p<0.05$) for same nutrients in between treatments only. PM peat mineral-mix, FFM forest floor mineral-mix, Harvest **ab** by timber harvesting disturbed **a/b** ecosite, Fire **b** a by forest fire disturbed type **b** ecosite.

Treatment	**Month**	**Mean (±SE) µmol CO_2 m^{-2} s^{-1}**	**Min**	**Max**	**CV (%)**	**Range (m)**	**Spatial dependence**	**Dependence Class**	**R^2**	**Model**
PM	May	3.47a (0.09)	2.69	4.88	16.12	19.22	2.30	poor or no	0.77	Gaussian
	June	4.61b (0.22)	2.68	8.94	29.27	2.74	775.0	strong	0.49	Gaussian
	July	2.88c (0.13)	2.08	5.04	28.47	21.03	1.33	poor or no	0.14	Linear
	August	1.43c (0.07)	0.51	2.32	28.66	17.55	2.01	poor or no	0.46	Gaussian
FFM	May	2.11b (0.15)	0.46	4.70	46.02	35.43	2.65	poor or no	0.44	Spherical
	June	7.58a (0.33)	3.46	11.95	28.29	7.63	5.39	poor or no	0.41	Exponential
	July	5.50a (0.39)	1.88	13.92	46.92	29.18	4.57	poor or no	0.65	Spherical
	August	4.57a (0.37)	1.42	13.65	52.82	3.4	321.0	strong	0.57	Gaussian
Harvest ab	June	2.65c (0.17)	0.98	5.59	39.45	6.73	15.99	poor or no	0.14	Spherical
	July	1.90d (0.14)	0.82	4.94	50.07	6.63	456.0	strong	0.28	Spherical
	August	1.75bc (0.15)	0.72	5.31	56.13	43.46	3.17	poor or no	0.36	Exponential
Fire b	May	1.77b (0.41)	1.28	3.44	141.47	5.45	601	strong	0.19	Spherical
	June	3.82b (0.13)	2.45	6.11	22.96	51	3.58	poor or no	0.47	Exponential
	July	3.83b (0.12)	2.26	6.18	21.48	19.22	2.0	poor or no	0.51	Spherical
	August	2.35b (0.10)	1.57	4.21	28.73	44.45	5.8	poor or no	0.57	Gaussian

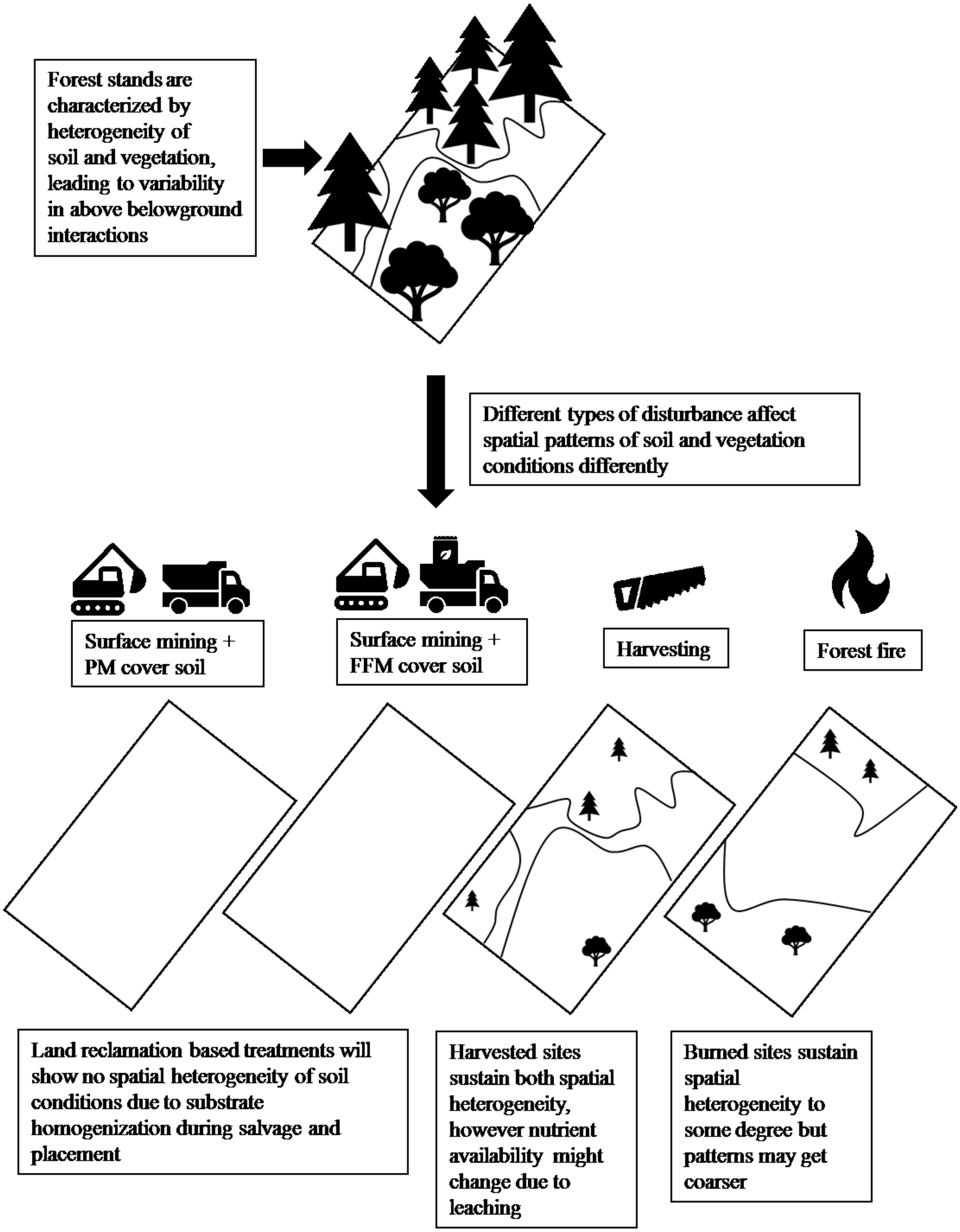


Figure 2-1: Spatial pattern of soil function following different types of disturbance.

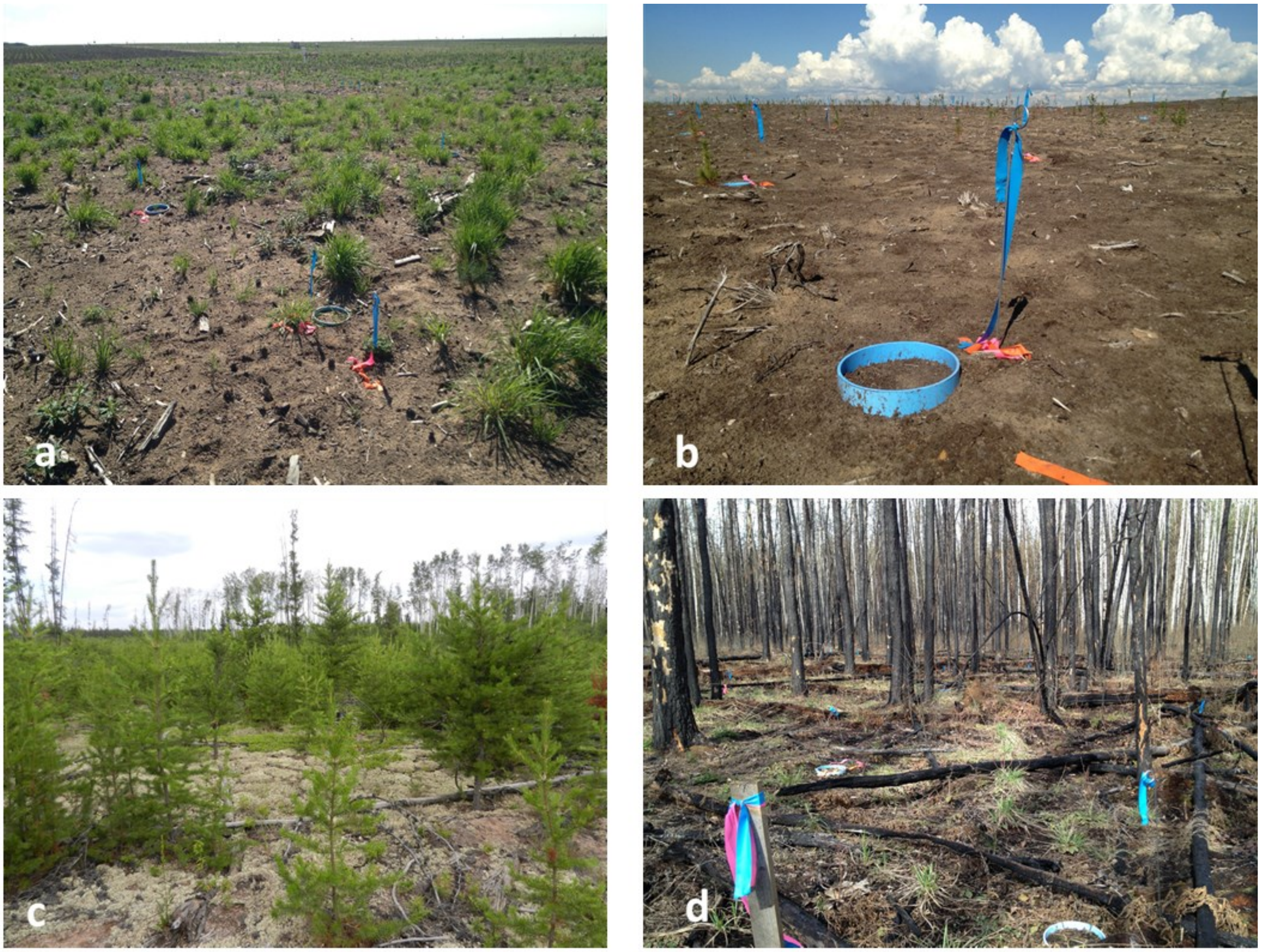


Figure 2-2: Pictures of sites compared in this study. a) Forest Floor Mineral Mix (FFM); b) Peat Mineral Mix reclaimed site (PM); c) Harvested **ab** ecosite; d) Fire **b** ecosite

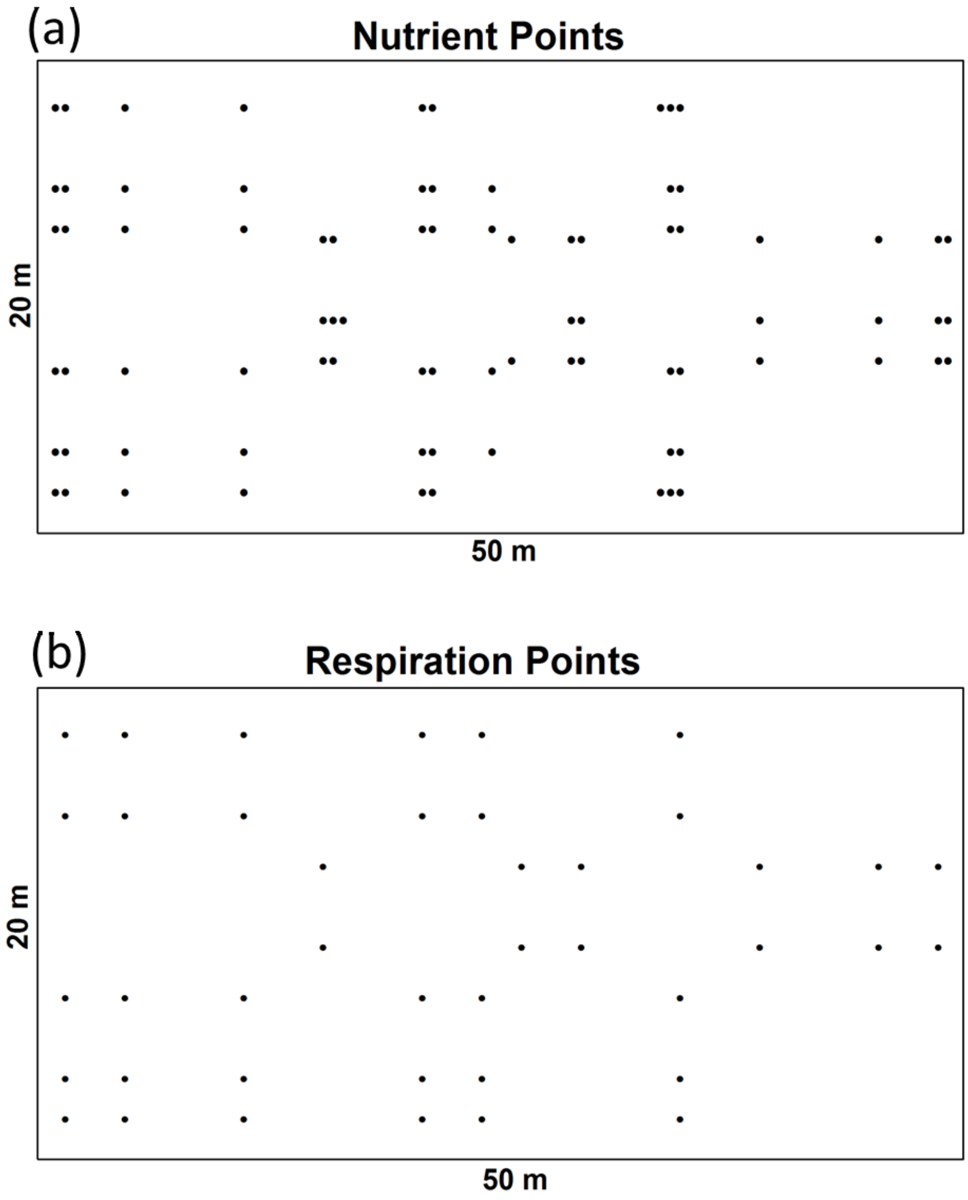


Figure 2-3: Spatial sampling protocol for data collection during the growing season of 2013 in sites in the Athabasca oils sands region. a) Design for measurement of bioavailable nutrients with 81 sampling points. b) Design for measurement of soil respiration with 42 sampling points.

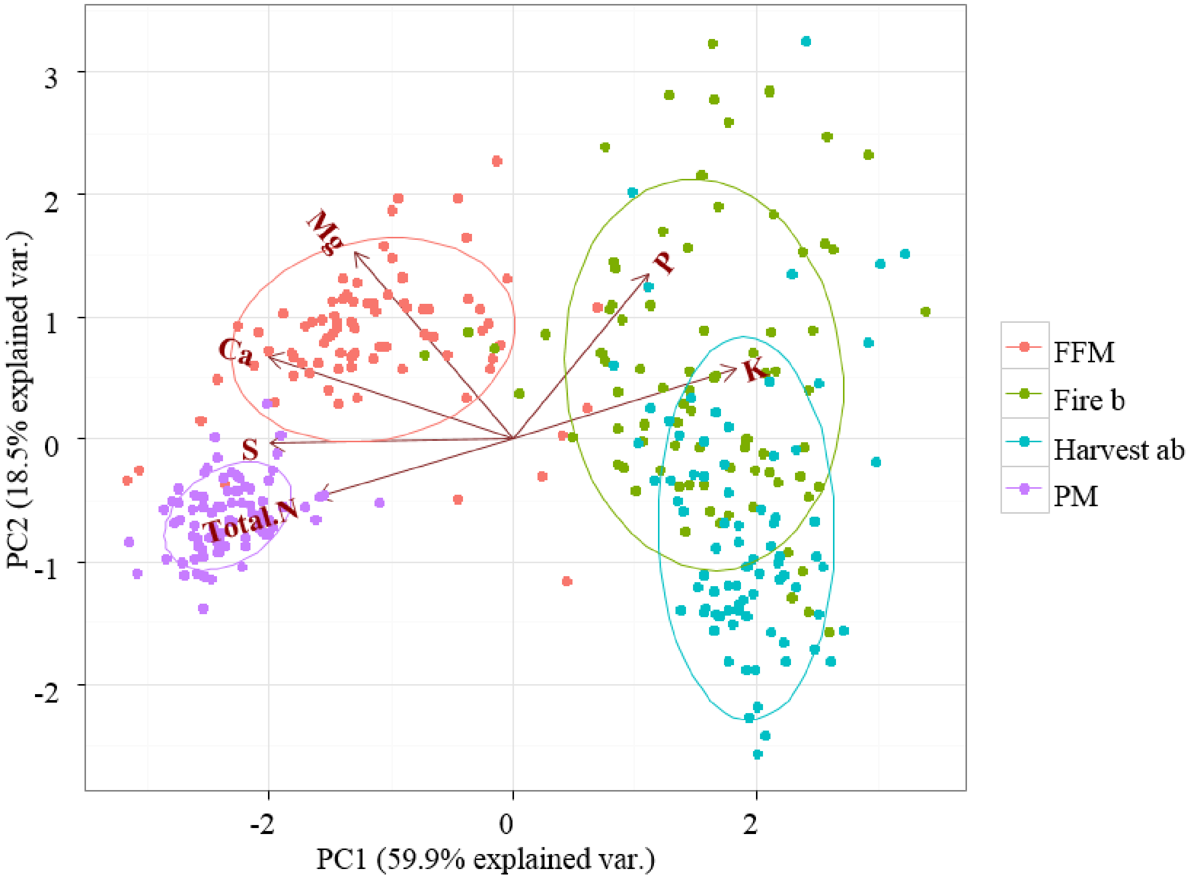


Figure 2-4: Principal component analysis (PCA) of bioavailable macronutrients. Nutrient bioavailability was measured with PRS™ probes. PM peat mineral-mix, FFM forest floor mineral-mix, Harvest **ab** by timber harvesting disturbed **a/b** ecosite, Fire **b** a by forest fire disturbed type **b** ecosite.

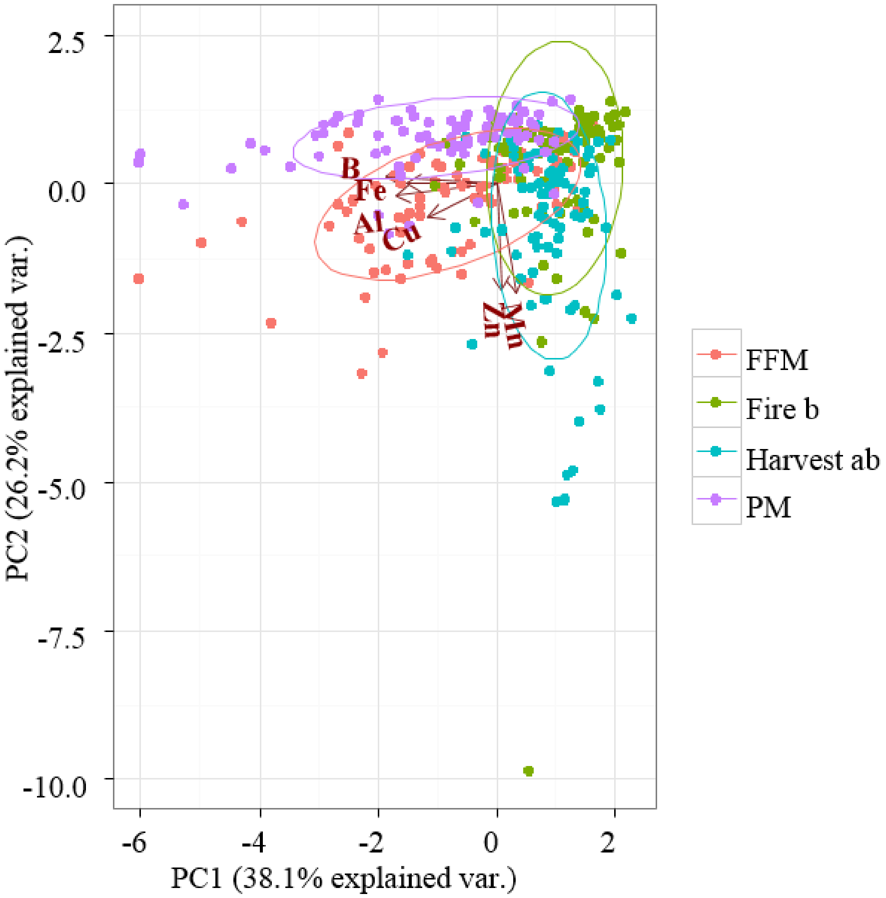


Figure 2-5: Principal component analysis (PCA) of measured bioavailable micronutrients. Nutrient bioavailability was measured with PRS™ probes. PM peat mineral-mix, FFM forest floor mineral-mix, Harvest **ab** by timber harvesting disturbed **a/b** ecosite, Fire **b** a by forest fire disturbed type **b** ecosite.

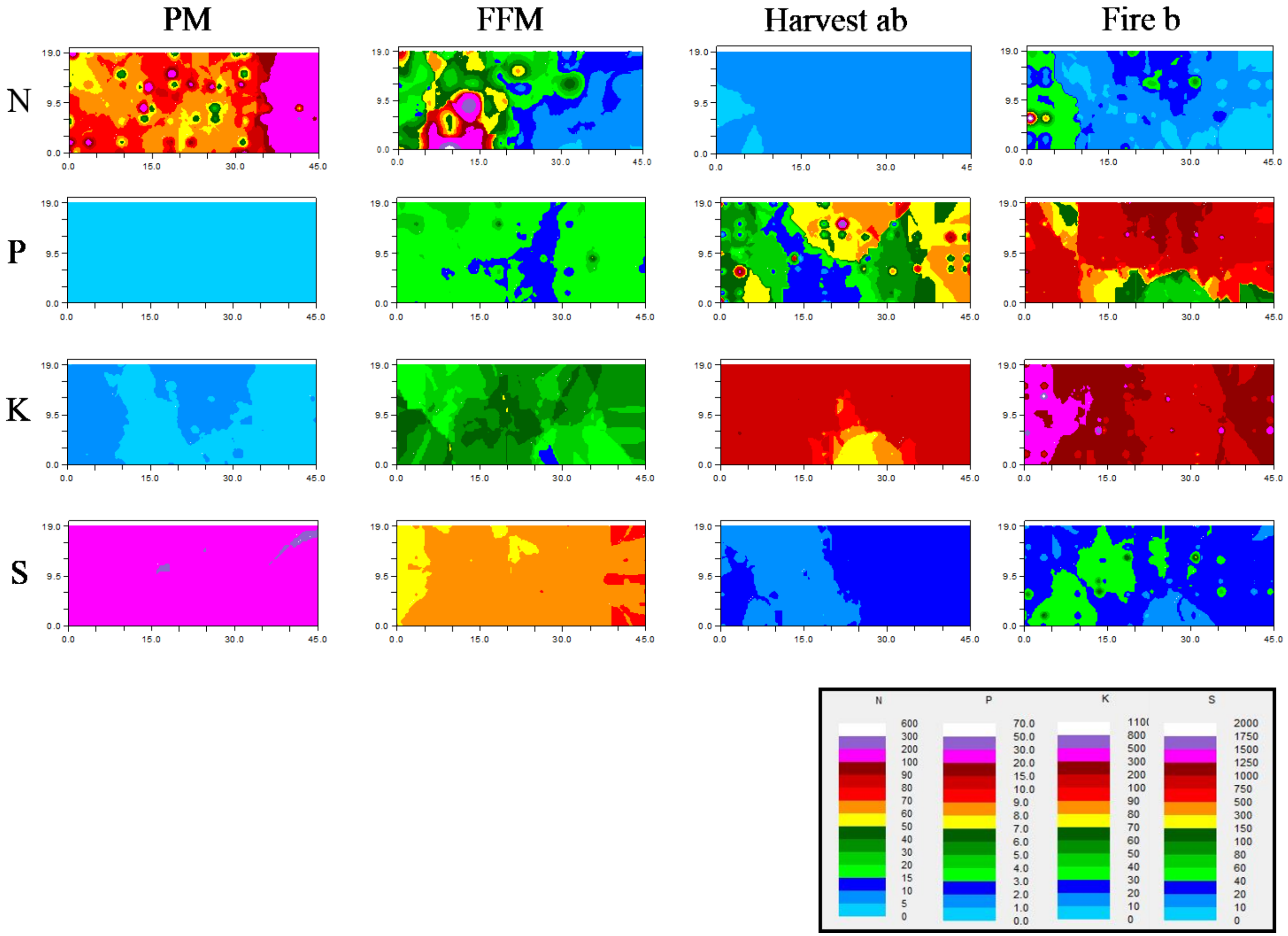


Figure 2-6: Kriging maps of bioavailable N, P, K, and S based on fitted variogram models. Modelled spatial nutrient bioavailability is expressed in µg 10cm^{-2}. PM peat mineral-mix, FFM forest floor mineral-mix, Harvest **ab** by timber harvesting disturbed **a/b** ecosite, Fire **b** a by forest fire disturbed type **b** ecosite. TIN reported as sum of NO_3^- and NH_4^+; P as equivalent of $H_2PO_4^-$, HPO_4^{2-}; K as equivalent of K^+; S as equivalent of SO_4^{2-}.

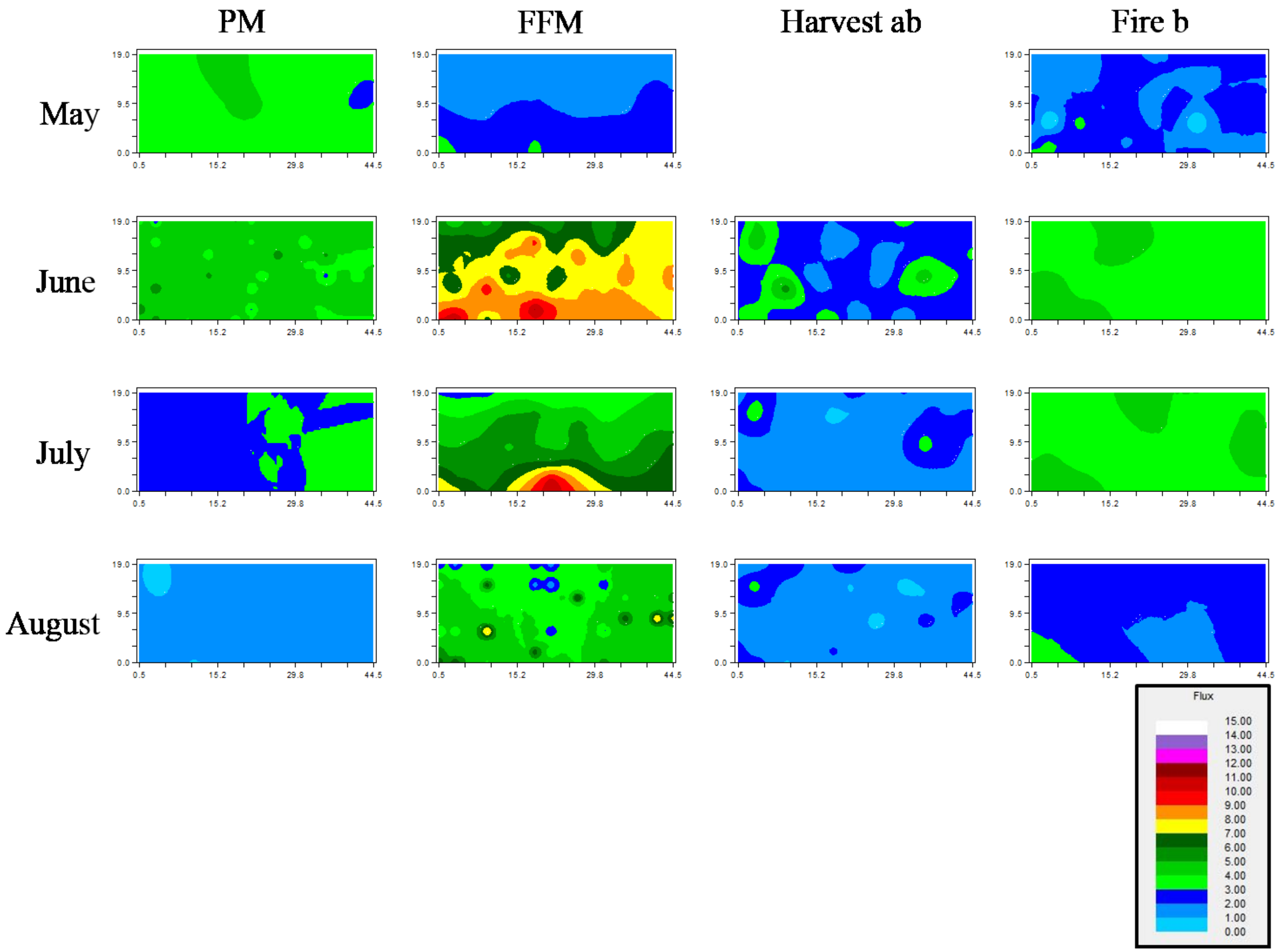


Figure 2-7: Kriging maps of soil respiration based on fitted variogram models. Modelled spatial soil respiration is expressed in µmol CO_2 m^{-2} s^{-1}. PM peat mineral-mix, FFM forest floor mineral-mix, Harvest **ab** by timber harvesting disturbed **a/b** ecosite, Fire **b** a by forest fire disturbed type **b** ecosite.

Chapter 3 Spatial pattern of soil microbial biomass and soil respiration of boreal forest stands at different stages of recovery in comparison to young reclaimed sites

3.1 INTRODUCTION

The Athabasca Oil Sands deposit contains an estimated 869 billion barrels of bitumen (Mossop 1980) and is one of the largest to date known oil reserves on earth. Oil sands in Alberta were originally deposited in the Cretaceous age about 110 million years ago (Mossop 1980). Surface mining activities occurring today result in large-scale anthropogenic disturbance, requiring land reclamation to return the closure landscape to equivalent land capability. Fire is the primary natural disturbance in the boreal forest, and it has been suggested that mimicking fire might allow maintaining ecological integrity following anthropogenic disturbance (Armstrong 1999). However, fire characteristics (e.g., intensity, scale, time of occurrence) can have varying effects on sites and scales that range from a tree stand to global levels (Armstrong 1999). On the stand level, species patch sizes, tree size and spacing have been shown to affect the spatial pattern of microbial biota in soils (Ettema and Wardle 2002). Patch sizes or spatial heterogeneity of soil organisms has long been interpreted to be random. However, it is now recognized that using a spatial statistics approach can support a better understanding of factors driving soil ecological relationships (Ettema and Wardle 2002). The spatial structure of soil biota and the factors influencing them can influence the spatial characteristics of plant communities (Ettema and Wardle 2002).

An important fraction of living organisms in soil is soil microbial biomass (SMB). Soil microbial biomass is the total mass of living component of soil organic matter (Singh et al. 1989) and contributes to important functions. The soil microbiota influence aggregation and affect plant nutrient uptake and following growth and productivity (Jia et al. 2017). Important functions in soil as providing labile C, N, P, and S while also being a sink for those elements are linked to SMB (Dalal 1998). In addition to soil biota being an agent for degradation and transformation of organic material in soil (Dalal 1998), SMB moderates biogeochemical processes of terrestrial ecosystems (Jia et al. 2017). Even though SMB plays a role in ecosystem function, little is known about the effects of oil sands mining and reclamation on SMB in boreal forest ecosystems. A study conducted in the UK discussed soil microbial biomass as an indicator for environmental change and pointed towards a need for high sampling intensity and problems with variable environmental conditions at sampling locations (Hargreaves et al. 2003). A recent study suggests that SMB can be used as a sensitive indicator for biological processes (Jia et al. 2017). In this study, we measure the spatial heterogeneity of SMB following disturbance caused by fire and logging, as well as oil sands reclamation sites, and compare that heterogeneity to that in a mature tree stand. We then evaluate SMB as a spatial indicator for the reestablishment of belowground biological processes.

In addition to microbial biomass, we measured in situ soil respiration monthly during the growing season. Soil respiration is driven by heterotrophic and autotrophic respiration (Hanson et al. 2000) and sums up all metabolic processes that produce carbon dioxide (CO_2) (Lundegårdh 1927). Measurements of soil respiration can also be affected by abiotic processes as dissolution of carbonates (Tamir et al. 2011). In the context of the global C cycle, soil respiration is one of the biggest soil gaseous fluxes and small changes soil gaseous fluxes can have major effects on the atmospheric CO_2 concentration. For a chronosequence of boreal aspen stands in the AOSR it has been demonstrated that soil respiration links aboveground and belowground function, and that fire alters the spatial structure with heterogeneity returning at the canopy closure stage (after ten years) (Das Gupta and Mackenzie 2016). Unpublished work (Chapter 2) from this study evaluated the spatial pattern of soil respiration on four sites, of which two were disturbed by harvesting and fire, and two were reclaimed one with peat mineral mix (PM) and one with forest floor mineral mix (FFM). Kriging detected seasonally reoccurring patterns, indicating an established above below ground linkage on all tested sites but PM (Dietrich and MacKenzie 2017). In this study, we re-measured those sites in the following growing season to evaluate spatial patterns of soil respiration as a potential indicator for established aboveground and belowground linkage. For further reference, we added a burned type **a** ecosite and a mature type **a** ecosite.

We hypothesize that undisturbed and older stands will be characterized by smaller-scale spatial pattern with overall higher SMB and that stands recovering fire and logging from disturbances, as well as oil sand reclaimed sites, will show a larger scale pattern with less heterogeneity (Figure 3-1). Older sites will be characterized by seasonally reoccurring patterns of soil respiration and sites affected by disturbance will show larger scale spatial patterns of soil respiration due to a more homogenous distribution of soil resources.

3.2 METHODS

3.2.1 Study sites

Data was collected during the 2014 growing season in the Athabasca Oil Sands Region north of Fort McMurray (57°20'12.25"N 111°32'22.92"W), Alberta, Canada. Two oil sand reclamation treatments at Syncrude’s Aurora Capping Study (ASCS) were chosen for study, one reclaimed with forest floor mix (FFM) over suitable subsoil in 2012 (Figure 3-2), and the other reclaimed with Peat (PM) over suitable subsoil in 2012 (Figure 3-2).

The two soil reclamation treatments were noted in this study as FFM and PM. The FFM treatment was on a soil cover design consisting of 20 cm FFM over 130 cm of subsoil, which is mineral soil material salvaged from upland boreal forests from a depth of approximately 15 to 250 cm, and the PM treatment was a soil cover consisting of 30 cm of peat over 120 cm subsoil. FFM was salvaged from coarse-textured Brunisol upland soils that are **a** and **b** ecosites. Type **a** ecosites are characterized by xeric to subxeric moisture regimes and poor to very poor nutrient regimes, while type **b** ecosites are characterized by submesic to subxeric moisture regimes and medium to poor nutrient regimes (Beckingham and Archibald, 1996). Peat for the PM treatment of the ASCS was salvaged from an area with predominantly **j** ecosites (poor fen), which is characterized by an intermediate nutrient regime and a subhydric moisture regime, as well as from a **k** ecosite (rich fen). Soils of both ecosite types are composed of organic matter (OM) derived from sedges, golden, tufted and brown moss, and peat moss (Beckingham and Archibald, 1996).

Reclamation sites were compared to the following sites: a) a reference site (Harvested **ab**) naturally recovering from harvesting since approximately 2006, which is a type **a** and **b** ecosite (transitional) (Figure 3-2), b) two sites recovering from wildfire that occurred in 2011; Fire **a** being an **a** ecosite and Fire **b** being a **b** ecosite, and c) a mature, undisturbed **a** ecosite (Mature **a**). Soils found on the reference sites were characterized as an Orthic Dystric Brunisol at the Harvested **ab** site, Mature **a** site and Fire **a** site, while the soil was classified as an Orthic Gray Luvisol at the Fire **b** site. All plots were located on level slopes (<2%) to minimize effects of slope and effects on the moisture regime. Due to the high sampling intensity per treatment performed for this study, replication of treatments was not established.

3.2.2 Data Collection

A spatial sampling protocol was established for microbial biomass carbon (MBC), which functions as a measure for SMB (81 points) (Figure 3-3a) and for soil respiration (42 points) (Figure 3-3b) on a plot of 20 m x 50 m based on a cyclic sampling design. The minimum detectable spatial lag was 0.5 m for the design with 81 points and 2 m for the design with 42 points. For microbial biomass points, soil samples were taken from 0 to 7.3 cm depth with bulk density sampling cores. Samples included LFH and mineral topsoil or equivalent amounts of cover soil. We followed the suggested sampling design of Smithwick et al. (2005), which is reversed in the middle three rows to account for anisotropy. Using a cyclic sampling design allowed us the study of spatial patterns due to the establishment of comparable power at diverse lag distances and maximized the effectiveness of sampling (Clayton and Hudelson 1995).

Soil microbial biomass carbon (MBC) was measured on a subset (n=18) of the entire spatial dataset by the simultaneous chloroform fumigation extraction (sCFE) “slurry” method (Fierer 2003). Extracts were analyzed with a Shimadzu TOC-V CHS/CSN Model (Shimadzu Corporation Kyoto, Japan). Before the extractions, soil samples were incubated under controlled conditions in an incubator (30°C, 10% Vol. H_2O content) for a period of two weeks. To minimize the effect of environmental conditions affecting soil microbial biomass, soils can be incubated in the laboratory under controlled temperature and moisture conditions. After an incubation period of 5-10 days, the effects of soil sampling, sample handling, and sample preparation can be eliminated (Shan-Min et al. 1987).

To estimate the MBC of the whole spatial data set (486 samples), Diffuse Reflectance Infrared Fourier Transform (DRIFT) Spectroscopy was used. Before calibration, for prediction of soil properties principal component analysis (PCA) was conducted to detect outliers in the raw data (Unscrambler 10.3 CAMO Software AS Oslo, Norway). Optimum frequency ranges and various pre-processing algorithms (e.g., multiplicative scatter correction, standard normal variate, and quantile normalization) were tested to reduce or eliminate noise and bias in raw data. (Unscrambler 10.3 CAMO Software AS Oslo, Norway). Samples were divided randomly into training sets and test sets. The partial least squared regression (PLSR) calibrations were carried out using 'leave-one-out' cross-validation to estimate the optimal numbers of factors to avoid over-fitting. Calibrations were validated using the test groups. (Unscrambler 10.3 CAMO Software AS Oslo, Norway). Accuracy and performance of Models were evaluated using the coefficient of determination (R^2), root mean square error (RMSE) and residual prediction deviation (RPD) (Unscrambler 10.3 CAMO Software AS Oslo, Norway). Data were then used to extrapolate microbial biomass carbon data to the whole data set (n=81) using FT-IR. Infrared reflectance characteristics have been shown to be correlated with microbial biomass (Coûteaux et al. 2003; Fritze et al. 1994). The final relationship modeled and used for extrapolation had a coefficient of correlation (R^2) of 0.86, when comparing predicted vs. measured values (Figure 3-4). Extrapolated data was then used in combination with individual site bulk density (Table 3-1) to calculate microbial biomass per volume topsoil to a depth of 7.3 cm on a square meter base, as the soil treatments had differing bulk densities. Working with MBC data based on concentration only would have communicated a not comparable picture, as bulk densities were different and this affects the spatial and volumetric topsoil MBC content (Table 3-1). Bulk density was calculated based on randomly selected bulk density samples, which were sampled from the topsoil at random locations on each research site (n=3).

Soil respiration was measured monthly during the growing season in May, June, July, and August using a LiCor LI-8100 Soil Gas Flux System (LI-COR, Lincoln, Nebraska, USA) on preinstalled custom made 8-inch diameter respiration collars. Collars were installed to a depth of 2 inches, and head space was measured to ensure the accuracy of measurements. Measuring soil respiration allows to evaluate in-situ soil metabolic activity producing CO_2, resulting out of autotrophic and heterotrophic respiration, and eventually chemical oxidation of carbonaceous compounds (Bunt and Rovira 1954; Lundegårdh 1927).

3.2.3 Statistical Analysis

R 2.1.5 (R Core Team 2012) was used for statistical analyses with agricolae package (de Mendiburu 2014) for post-hoc testing with Tukey HSD test, following permutational ANOVA in the lmPerm package (Wheeler 2010) for significance testing to compare means of both microbial biomass and soil respiration. Permutational ANOVA was chosen for analysis to account for potential violation of ANOVA assumptions and sample size.

Isotropic variograms were fitted with GS+ 10.0 (Gamma Design Software) (see appendix). Four variogram models were tested (Linear, Exponential, Spherical, and Gaussian) to identify the model that best fitted the data. The residual sum of squares (RSS) was used in combination with the combination highest coefficient of determination (R^2) to identify best model fit. Following ordinary, standardized kriging maps were plotted to visualize spatial characteristics using GS+ 10.0 (Gamma Design Software). Cross-validation was used to evaluate kriging interpolations, which is based on a comparison of estimated versus actual measured values.

The nugget coefficient (nc), which is calculated from total variance (c0 + c1) and the associated nugget variance (c0) was used to calculate spatial dependence. If the nugget coefficient is >75, strong spatial dependence is indicated, 25-75 indicates moderate spatial dependence, and a value <25 indicates poor or no spatial dependence (Isaaks and Srivastava 1989). The coefficient of variation (CV) was calculated to provide a measure of global variation. The CV is calculated by dividing the standard deviation by the mean and was reported as a percentage.

3.3 RESULTS

3.3.1 Spatial heterogeneity of MBC

The lowest top soil MBC mean values were detected on the Fire **a**, FFM, and PM treatment values with mean with mean values being significantly lower than on all other sites (Table 3-2). A significantly higher mean value for MBC was detected on the Harvest **ab** site, which in comparison to the Fire **b** site and the Mature **a** site was significantly lower (Table 3-2). The Mature **a** site was characterized by the highest mean value for MBC, which was significantly higher than the mean for MBC on the Fire **b** site (Table 3-2). Global variation (CV) was highest on the Harvest **ab** site, followed by the Fire **a**, FFM, PM, Fire **b**, and Mature **a** site (Table 3-2).

Variogram fitting for all sites revealed that the Harvest **ab**, Fire **a**, and PM site showed a coarse spatial range indicating autocorrelation of MBC to 22.5 m distance (Table 3-2). Fire **b** had a range of 6.74 m indicating autocorrelation at a more moderate distance, while both FFM and

Mature **a** showed fine-scale variation with autocorrelation of MBC below 1 m (Table 3-2). For all sites, poor or no spatial dependency was detected for MBC (Table 3-2).

Standardized kriging maps predicted larger areas with lower heterogeneity on PM and Fire **a**, while FFM showed more heterogeneity (Figure 3-5). MBC was predicted to be lowest at the Fire **a**, PM, and FFM sites, with some small-scale hotspots with higher MBC values on FFM (Figure 3-5). Harvested **ab** showed higher predicted MBC values with higher overall heterogeneity (Figure 3-5). Fire **b** showed higher overall MBC values on kriging maps with medium sized patches (Figure 3-5). Highest MBC values were predicted on the Mature **a** site with high fine-scale variability (Figure 3-5).

3.3.2 Spatial heterogeneity of soil respiration

May

Soil respiration in May was lowest on the Harvest **ab** site (Table 3-3). Fire **b** and PM showed a significantly greater mean respiration rate compared to Harvest **ab** (Table 3-3) and were non-significantly different from each other. Highest respiration rate was identified on FFM and was significantly greater than on all other sites (Table 3-3). For the Mature **a** and Fire **a** site no soil respiration data were available in May (Table 3-3). Global variation (CV) was highest on the PM site followed by the Fire **b**, FFM, and Harvest **ab** site (Table 3-3).

June

In June soil respiration again was lowest on the Harvest **ab** site but was non-significantly different to PM and Fire **b** where respiration rates were slightly higher (Table 3-3). FFM showed significantly higher mean respiration in comparison to other treatments (Table 3-3). Global variation was highest on Harvest **ab** site followed by the FFM, PM, and Fire **b** site (Table 3-3).

July

In July mean soil respiration was lowest on the Fire **a** site, which was significantly lower than on all other sites (Table 3-3). Soil respiration on Harvest **ab** in July was significantly higher than on Fire **a** but lower in comparison to all other sites (Table 3-3). On the Fire **b** site soil respiration in July was non-significantly different to the PM site, but higher than on the Fire **a** and Harvest **ab** site, on FFM and Mature **a** soil respiration was significantly higher than on Fire **b** (Table 3-3). Soil respiration on PM was non-significantly different in comparison to FFM and significantly higher compared to all other sites except the Mature **a** site where respiration was significantly higher (Table 3-3). On the FFM site, soil respiration was significantly higher in comparison to all other sites except to PM and Mature **a** where differences were non-significant (Table 3-3). The Mature **a** site showed the overall highest rate of soil respiration in July, which was non-significantly different to FFM but significantly higher in comparison to all other sites (Table 3-3). Global variation of soil respiration in July was highest on the Harvest **ab** site, followed by the Fire **a**, PM, Mature **a**, FFM, and the Fire **b** site (Table 3-3).

August

In August mean soil respiration was lowest on the Harvest **ab** site followed by Fire **a**, FFM, and Fire **b** where soil respiration rates were non-significantly different (Table 3-3). Significantly higher mean soil respiration was measured on the Mature **a** site where respiration rates were highest (Table 3-3). The PM site showed the second highest mean respiration rate and was non-significantly different to the Mature **a** site (Table 3-3). Global variation of soil respiration in August was highest on FFM followed by the Fire **a**, Harvest **ab**, Mature **a**, PM, and the Fire **b** site (Table 3-3).

Variogram fitting for soil respiration on all sites was conducted. No fit could be detected for the PM site in June and August, the FFM site in May and June, the Harvest **ab** site in May, the Fire **b** site in June, July, and August, as well as for the Mature **a** and Fire **a** site in July and August (Table 3-3). On PM a change from a coarse scale (> 25m) to a finer scale pattern (2.75 m) from May to July could be detected with a change in spatial dependence from poor or non-existent to very strong in July (Table 3-3). Coarser scale spatial patterns in soil respiration with poor or no spatial dependence were detected on FFM in July and August with a range of 21.28 m and 21.01 m, respectively (Table 3-3). On the Harvest **ab** site, a very coarse scale spatial pattern (>25 m) with poor or no spatial dependence was detected in June and July, which was reduced to a more a range 21.02 m in August also showing poor or no spatial dependence (Table 3-3). On the Fire **b** site, coarse-scale spatial pattern (> 25 m) was detected in May with poor or no spatial dependence (Table 3-3).

Standardized kriging maps showed a smaller scale spatial range in PM with increased variability later in the growing season when comparing May and July (Figure 3-6). Visually no seasonal repeating pattern could be detected in PM (Figure 3-6). Kriging maps for FFM predicted overall higher respiration rates in July with smaller scale variation in comparison to respiration in August (Figure 3-6). Visually no seasonal repeating pattern could be detected in FFM (Figure 3-6). On the Harvest **ab** site, higher respiration rates were predicted for June and July in comparison to August (Figure 3-6). Areas with lower respiration rates were predicted for the center of the mapped area and following visual inspection showed similar patterns in June and July (Figure 3-6). The Fire **b** site showed a larger scale pattern in May (Figure 3-6).

3.4 DISCUSSION

3.4.1 Spatial heterogeneity of MBC

Our study showed increased topsoil microbial biomass on the Mature **a** site, which likely was related to increased root biomass and root exudate content found in older forest stands. Plant and especially root biomass are generally correlated with soil microbial biomass (Wardle 1992), and in the case of boreal forests, it has been demonstrated that with stand age the fine root biomass increases (Yuan and Chen 2010). A fine-scale spatial pattern with a range of 0.45 m of soil microbial biomass was identified on the Mature **a** site potentially indicating fine scale effects of tree roots and root exudation. Soil microbial biomass in forest soils often is reflecting the influence zone and positioning of trees and has been observed to occur in patch sizes ranging from one to several meters (Ettema and Wardle 2002). The Harvest **ab** site, as well as the Fire **b** site, showed in comparison to the Mature **a** site lower mean soil microbial biomass. Our findings are contrary to a study conducted by Hannam et al. (2006) who reported that harvesting does not affect microbial biomass. Differences identified might be related to the fact that Hannam et al. (2006) only sampled the FH layer and did not directly freeze samples in the field, what might have affected the availability of labile organic components for microorganisms. Another factor might be the relatively low sample size (n=3) in the study conducted by Hannam et al., as variability within sites can be high (Table 3-2, Figure 3-5) and random sampling might have been conducted for samples that are not representative for harvested sites and mature stands. When considering the soil microbial biomass on Fire **a**, Harvest **ab**, and Mature, which are characterized by similar soil types and vegetation a trend for increasing MBC and following SMB can be observed (Table 3-3). Contrasting the Fire **b** site showed increased microbial biomass in comparison to other disturbed sites even though soil texture on the type **b** ecosite was finer and finer soil texture has been reported to be associated with lower microbial biomass (Merckx et al. 1985). However, fire severity on the Fire **b** site was lower and more carbon as substrate was available following fire in comparison to other sites (Table 3-1). Fire severity has been shown to significantly affect soil microbial biomass in boreal forests (Holden et al. 2016), as well as seedling recruitment (Keeley et al. 2008). The Fire **b** site was in comparison to the Fire **a** site characterized by a more rapid recovery of vegetation following fire (Figure 3-2). Likely aspen (*Populus tremuloides* Michx.), which is commonly found on type **b** ecosites, recovered faster compared to jack pine (*Pinus banksiana* Lamb.). Aspen is a clonal tree species and regenerates from root suckers and in a silvicultural context is known to outcompete other species due to its vigorous recovery potential (Frey et al. 2003). Faster recovery on the Fire **b** site likely increased the availability of root exudates in the topsoil and following affected soil microbial biomass. Both reclaimed sites PM and FFM, as well as the Fire **a** site showed overall lower microbial biomass indicating that less labile substrate supporting microbial biomass was available. For the PM, Harvest **ab**, and Fire **a** site we could confirm our initial hypothesis that disturbance creates larger scale spatial patterns. The FFM site, however, showed a small scale spatial pattern while PM and Fire **a** showed a larger scale spatial pattern, more similar to the Harvest **ab** site (Table 3-2, Figure 2-5). As pre-salvage conditions for FFM are likely similar to conditions found on the Harvest **ab** site, a finer scale spatial pattern on FFM might be resulting out of mechanical turbation of topsoil. Soil mixing might occur in line with the salvage and transport processes and might lead to increased heterogeneity in FFM cover soil. Increased heterogeneity in soil resources could be an additional factor for the by MacKenzie and Naeth (2007) described more successful reestablishment of native species on FFM cover soils, which also could be observed on our research sites (Figure 2-2). However this needs to be further tested. Increased spatial heterogeneity in soil resources leads to a higher amount of niches with different characteristics that allow the reestablishment of species with different niche requirements (resource-ratio hypothesis of plant succession (Tilman 1985)). The coexistence of species can be positively affected by increasing spatial heterogeneity in soil resources due to the greater partitioning of resources (Ettema and Wardle 2002). Hence, increasing spatial heterogeneity of soil resources on PM reclaimed sites or following harvesting could positively affect reclamation and reforestation targets, especially when the reestablishment of forests with close to undisturbed conditions is desired.

3.4.2 Spatial heterogeneity of soil respiration

May

Differences in site-specific soil respiration detected in May could be related to differences in site-specific photosynthetic activity, which has been reported to affect soil respiration rates directly (Hogberg et al. 2001; Moyano et al. 2008). As in the 2014 growing season vegetation on FFM already was well developed in comparison to PM, where no understory vegetation beside trees was established, it is likely that vegetation establishment drove increased respiration rates (Figure 3-2). When comparing soil respiration rates of May 2014 to May 2013 respiration data acquired from the same sites, it can be observed that mean respiration rates in 2013 were highest on the PM site; rates were significantly lower on FFM in 2013, while in 2014 respiration on the FFM were highest and significantly lower on PM. Besides changes in vegetation differences in soil temperature and precipitation could have caused differences in between the years, as those are also important factors affecting soil respiration (Raich and Schlesinger 1992). Weather data collected at the Aurora capping study showed a difference of 5 ºC in mean temperature in May and great differences in precipitation for May comparing 2013 and 2014 (see appendix for diagrams on weather during the growing season). In our study, no model for soil respiration in May could be fitted for the Harvest **ab** site as well as the FFM site. Our observations suggest that below ground functions are changing over time and that patterns change from year to year, as for 2013 data models could be fitted. To quantify potential effects in future studies either a different sampling design needs to be used, which can capture both smaller scale and larger scale autocorrelation, or different variogram models need to be tested, as for example semi-variogram models for random-effects.

June

Respirations rates detected in June were highest on FFM. While it has been suggested by McMillan et al. (2007) that increased microbial biomass can be a driver for increased rates of soil respiration, our study demonstrated that in the field also other parameters need to be considered as microbial biomass on PM and FFM was not significantly different (Table 3-2). Likely increased rates of soil respiration correlate to increased plant growth and vegetation establishment on FFM as this is a described for the cover soil type insert (Mackenzie and Naeth 2007; Mapfumo 2003). When comparing the soil respiration data of the 2014 growing season in June to data reported for the same site in 2013 by Dietrich & MacKenzie (2017), similar trends for mean respiration could be identified. However, no variogram models could be fitted for the June 2014 respiration data, except for the Harvest **ab** site where a coarse scale spatial range could be identified. The spatial Range on Harvest **ab** changed from a fine to a coarse scale spatial range in June, which again indicates that spatial patterns change over time. Changes in respiration could potentially be related to lower precipitation in June 2014 in comparison to 2013 (see appendix for diagrams on weather during the growing season), as a reduction of available water in soils affects rates of soil respiration (Tang and Baldocchi 2005). Future work should quantify in greater detail, which parameters drive changes in soil respiration and include carbon fixation, which would allow quantifying site function in greater detail.

July

In July highest respiration rates were detected on the Mature **a** site. Respiration on all sites was lower than in the previous month (June), where data for June was available, except for the PM site. Reductions in soil respiration during summer have been observed by Tang and Baldochi (2005). Reduced water availability likely drove reduced rates of soil respiration (see appendix for diagrams on weather during the growing season). Water availability has been shown to affect photochemistry and carbon metabolism in forests (Chaves et al. 2002), and rate of photosynthesis has been shown to drive soil respiration in forests (Hogberg et al. 2001). On well-drained soils, as found on the mature **a** site, roots can penetrate below a depth of 2.7 m. On the PM site, an increase in mean soil respiration in comparison to the previous month could be observed in July. Increased rates of respiration on the PM site could point towards an increased availability of water leading to increased soil respiration or potentially increased chemical decomposition of organic compounds as described by Bunt and Rovira (1994). While only on the PM site small-scale variation could be identified in July larger scale patterns were detected on the FFM site and the Harvest **ab** site, with kriging predictions being similar to June observations on the Harvest **ab** site only. Our findings indicate established linkage between above- and belowground functions on the Harvest **ab** site, that however only was detectable in June and July.

August

Respiration rates in August decreased on all sites except on Fire **a** in comparison to measurements from previous months, following the by Tang and Baldochi (2005) described decrease during the growing season. As on the Fire **a** site this phenomenon could not be observed it can be anticipated that seasonal changes in respiration might reestablish at later stages of recovery following fires with increased severity on type **a** ecosites. However, this needs to be tested in future studies. Variogram models could only be fitted for the FFM site and the Harvest **ab** site on other sites no variograms could be fitted indicating that with the used sampling design and tested models either no spatial patterns could be detected or where existent.

Seasonal Pattern of soil respiration

In comparison to the in Chapter 2 of this thesis documented reoccurring patterns for soil respiration in 2013, no significantly evident pattern could be detected in 2014. However, kriging identified visually reoccurring patterns on the Harvest **ab** site. This finding underlines that care must be used when interpreting data based on one-year measurements. Seasonal changes in weather can affect respiration measurements in the field, and evaluation of soil characteristics might be more appropriate under controlled conditions, especially when like in this study same day measurements due to high sample sizes are not possible. Analog to soil respiration data observed in 2013 seasonal changes with highest respiration values in June 2013 could be observed in June during the 2014 growing season. High rates of soil respiration indicate high rates of plant productivity (Rustad et al. 2001) and following it can be anticipated that net ecosystem productivity is highest in June on natural benchmarks sites and FFM reclaimed sites.

For the PM site, this observation could not be made indicating that potentially other factors affected rates of soil respiration, as chemical oxidation.

3.4.3 Possible methodological limitations

The spatially explicit sampling design with a high sample size per site, but no site replication, allowed capturing site individual spatial heterogeneity but disallowed significance testing for variogram parameters and global measures of spread. This is a common problem for studies capturing spatial heterogeneity (Bengtson et al. 2007; Lavoie and Mack 2012; Smithwick et al. 2005). As cover soil materials, but also cover soil placement varies within the AOSR detected spatial soil resource heterogeneity and quantity can only be extrapolated to sites reclaimed under comparable circumstances. The sampling design for SMB differed from the sampling design for soil respiration and did not allow to link both parameters, however, allowed to evaluate the by DasGupta & MacKenzie (2016) described patterning of respiration. Additionally, heterogeneity of SMB could be evaluated in greater detail. Soil microbial biomass measurements may also have been affected by freezing following sampling in the field, as freezing can affect soil microbial biomass and activity (Sorensen et al. 2018). Given the different characteristics of cover soils there is potential for an error related to dissolution of CO_2 as this may affect the C flux from soil and can lead to an over estimate of biologic activity (Tamir et al. 2011). As reclamation sites were characterized by increased Ca^{2+} bioavailability in 2013 (Figure 2-4) a potential release of CO_2 resulting from carbonate dissolution may be possible.

3.5 CONCLUSIONS

Spatial patterns of topsoil microbial biomass are similar on PM reclaimed sites and sites severely disturbed by fire, with more fine scale pattern developing with increased stand age on reference sites. Reestablishment of spatial patterns of soil microbial biomass could potentially be used as an indicator for successfully reestablished root systems and microbial organisms that metabolize to vegetation associated roots exudates. With time since disturbance spatial patterns of microbial biomass tend towards finer scale variations on type **a** and **ab** ecosites. FFM shows initially smaller scale variation in microbial biomass from early stages on what potentially explains greater success rates of reestablishment of native vegetation. Lower intensity fires on type **b** ecosites reestablish spatial patterns potentially more rapidly. However, comparison to mature sites and severely burned sites is necessary.

Reoccurring spatial patterns of soil respiration potentially can be used as indicators of successfully established linkage in between above and belowground parameters. Seasonal changes in weather can affect patterns of soil respiration and can affect the repeatability of findings. Measurements under better-controlled conditions might be better suited to evaluate site characteristics.

Table 3-1: Mean top soil characteristics with standard error (±SE) measured on randomly selected subsamples.

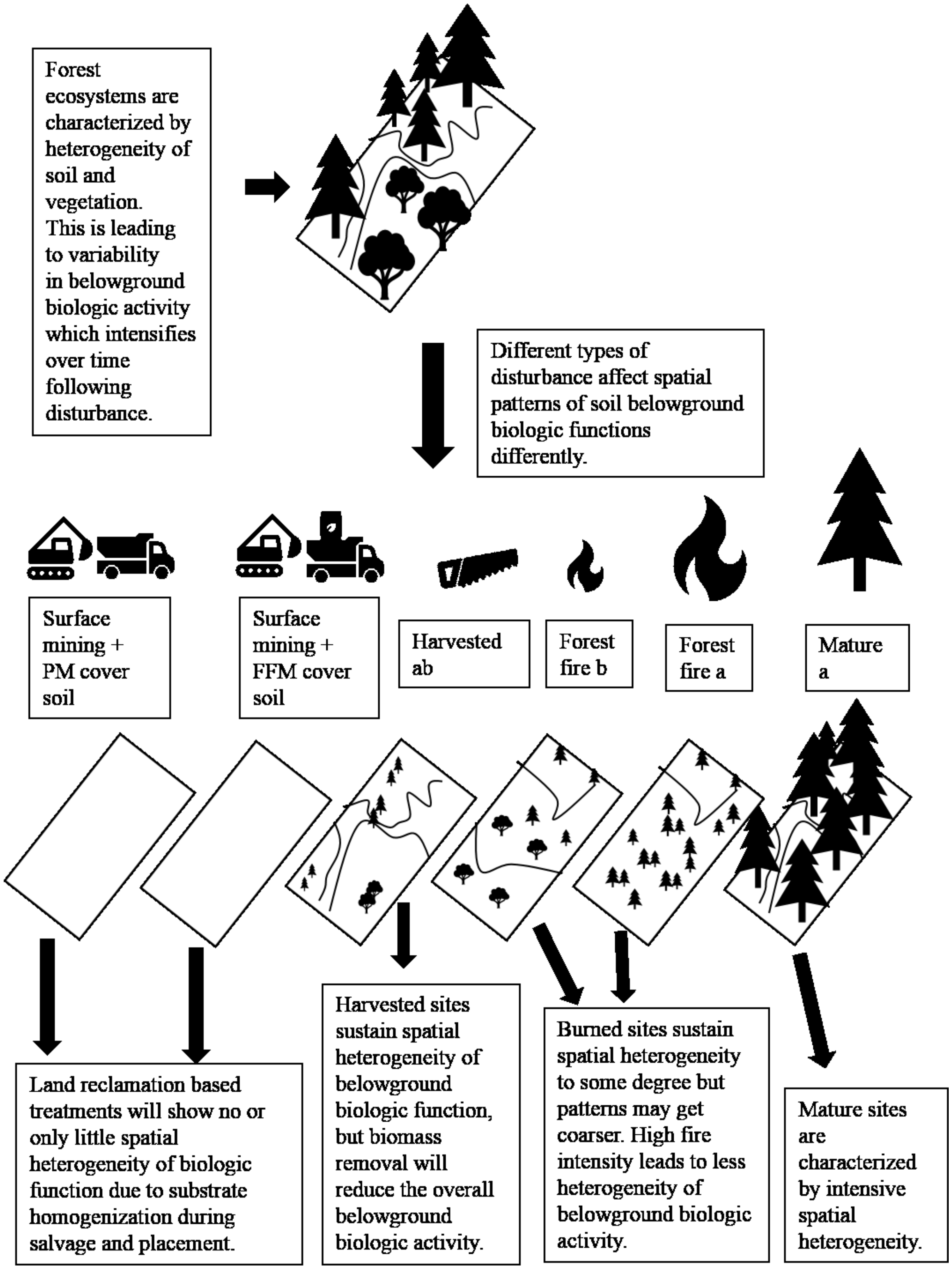
Treatment	**Bulk density g cm^{-3} (n=3)**	**MBC mg g^{-1} dw (n=18)**	**TN (w%w) (n=15)**	**TC (w%w) (n=15)**	**pH (n=3)**	**EC µS/cm (n=3)**
PM	0.40 (0.01)	0.16 (0.02)	0.88 (0.10)	16.11 (1.49)	7.07 (0.14)	190.3 (27.0)
FFM	1.27 (0.02)	0.04 (0.00)	0.10 (0.02)	2.12 (0.23)	6.73 (0.03)	29.2 (6.39)
Harvest ab	1.16 (0.02)	0.09 (0.02)	0.15 (0.01)	1.97 (0.29)	5.47 (0.07)	57.3 (11.95)
Fire b	1.45 (0.01)	0.10 (0.01)	0.18 (0.02)	5.85 (0.73)	5.57 (0.03)	31.2 (1.30)
Mature a	1.64 (0.01)	0.09 (0.01)	0.23 (0.01)	4.90 (0.76)	5.89 (0.07)	39.7 (8.84)
Fire a	1.41 (0.01)	0.03 (0.00)	0.18 (0.02)	2.42 (0.30)	5.95 (0.18)	36.5 (5.88)

Table 3-2: Spatial characteristics of soil microbial biomass carbon (MBC) as extrapolated to all sampling points with FT-IR (n=81) in site-specific topsoil horizons.

Treatment	**Mean MBC (±SE) (g m^2 7.3cm^{-1})**	**Min**	**Max**	**CV (%)**	**Range (m)**	**Spatial dependence**	**Dependence Class**	**R^2**	**Model**
PM	5.03d (0.20)	1.11	13.81	34.38	22.51	1	poor or no	0.258	Linear
FFM	4.96d (0.24)	0.81	11.81	43.24	0.72	10.97	poor or no	0.37	Exponential
Harvest ab	7.44c (0.46)	0.24	26.20	55.71	22.53	1	poor or no	0.102	Linear
Fire b	9.28b (0.33)	2.75	19.97	32.20	6.74	2	poor or no	0.51	Exponential
Mature a	11.51a (0.39)	4.32	23.77	30.10	0.45	8.78	poor or no	0.152	Exponential
Fire a	3.81d (0.23)	0.04	9.03	52.92	22.52	1	poor or no	0.232	Linear

Table 3-3: Soil respiration as measured in-situ. Variograms were modeled testing and fitting linear, exponential, gaussian, and spherical variogram models. Significance testing in between same months of different treatments only.

Treatment	Month	Mean (±SE) μmol CO_2 m^{-2} s^{-1}	Min	Max	CV (%)	Range (m)	Spatial dependence	Dependence Class	R^2	Model
PM	May	1.80b (0.12)	0.29	3.48	44.22	44.84	8.09	poor or no	0.734	gaussian
	June	4.31b (0.24)	2.22	11.63	35.70	-	-	-	-	-
	July	4.76bc (0.25)	1.98	9.98	34.11	2.75	2899.00	strong	0.393	gaussian
	August	4.09a (0.19)	2.18	8.06	30.17	-	-	-	-	-
FFM	May	4.16a (0.23)	1.16	7.35	35.52	-	-	-	-	-
	June	10.86a (0.61)	4.20	24.61	36.35	-	-	-	-	-
	July	5.10ab (0.23)	2.57	8.75	29.38	21.28	2.75	poor or no	0.599	gaussian
	August	2.00b (0.26)	0.30	8.65	84.05	21.01	1.00	poor or no	0.131	linear
Harvest ab	May	0.97c (0.05)	0.16	1.74	32.74	-	-	-	-	-
	June	3.62b (0.40)	1.23	17.09	72.48	51	4.28	poor or no	0.495	spherical
	July	3.06d (0.26)	1.44	11.47	53.80	51	4.86	poor or no	0.493	exponential
	August	1.86b (0.12)	0.41	4.10	40.89	21.02	1.66	poor or no	0.174	linear
Fire b	May	1.68b (0.10)	0.25	3.99	38.62	51	3.03	poor or no	0.132	exponential
	June	4.49b (0.15)	3.10	7.99	22.06	-	-	-	-	-
	July	3.99c (0.13)	2.43	5.78	21.29	-	-	-	-	-
	August	2.61b (0.08)	1.69	3.98	19.23	-	-	-	-	-
Mature a	July	5.66a (0.27)	2.71	12.42	31.20	-	-	-	-	-
	August	4.37a (0.23)	1.41	10.39	34.19	-	-	-	-	-
Fire a	July	1.73e (0.13)	0.41	6.46	50.18	-	-	-	-	-
	August	1.91b (0.15)	0.46	5.59	52.32	-	-	-	-	-



Forest ecosystems are characterized by heterogeneity of soil and vegetation. This is leading to variability in belowground biologic activity which intensifies over time following disturbance.

Different types of disturbance affect spatial patterns of soil belowground biologic functions differently.

Land reclamation based treatments will show no or only little spatial heterogeneity of biologic function due to substrate homogenization during salvage and placement.

Harvested sites sustain spatial heterogeneity of belowground biologic function, but biomass removal will reduce the overall belowground biologic activity.

Burned sites sustain spatial heterogeneity to some degree but patterns may get coarser. High fire intensity leads to less heterogeneity of belowground biologic activity.

Mature sites are characterized by intensive spatial heterogeneity.

Figure 3-1: Spatial heterogeneity of key soil biological characteristics at mature stage and early recovery following fire, harvesting, land reclamation in a boreal forest ecosystem.

Peat Mineral Mix (PM),
Reclaimed 2012

Forest Floor Mineral Mix (FFM),
Reclaimed 2012

Harvested ab,
Natural recovery 2006

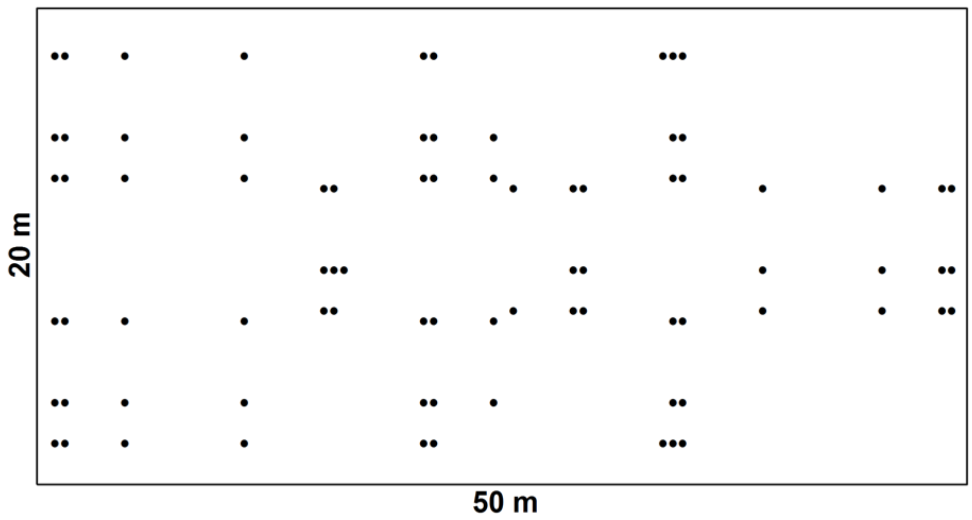
Fire b,
Natural recovery 2011

Mature a

Fire a,
Natural recovery 2011

Figure 3-2: Pictures of research sites taken during the growing season of 2014.

a) MBC Points



b) Respiration Points

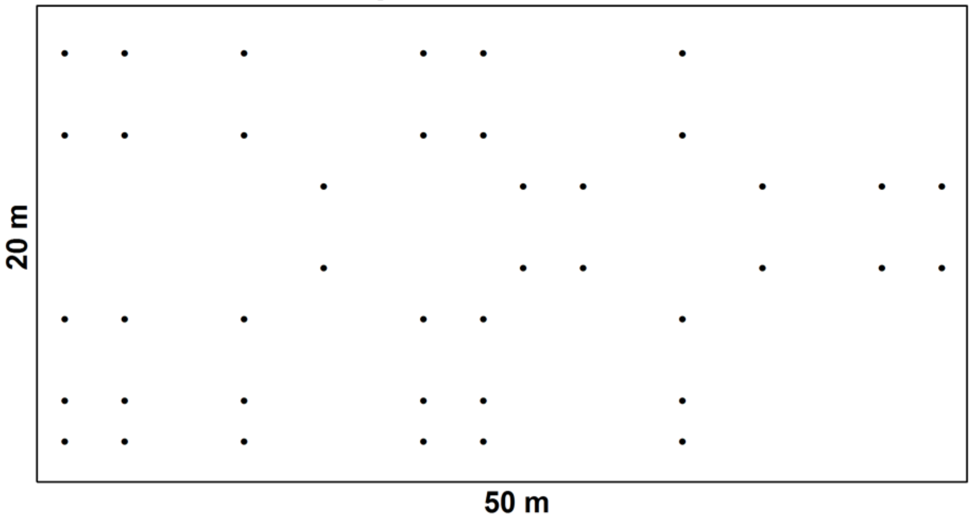


Figure 3-3: Spatial sampling design for microbial biomass carbon (a) and soil respiration (b).

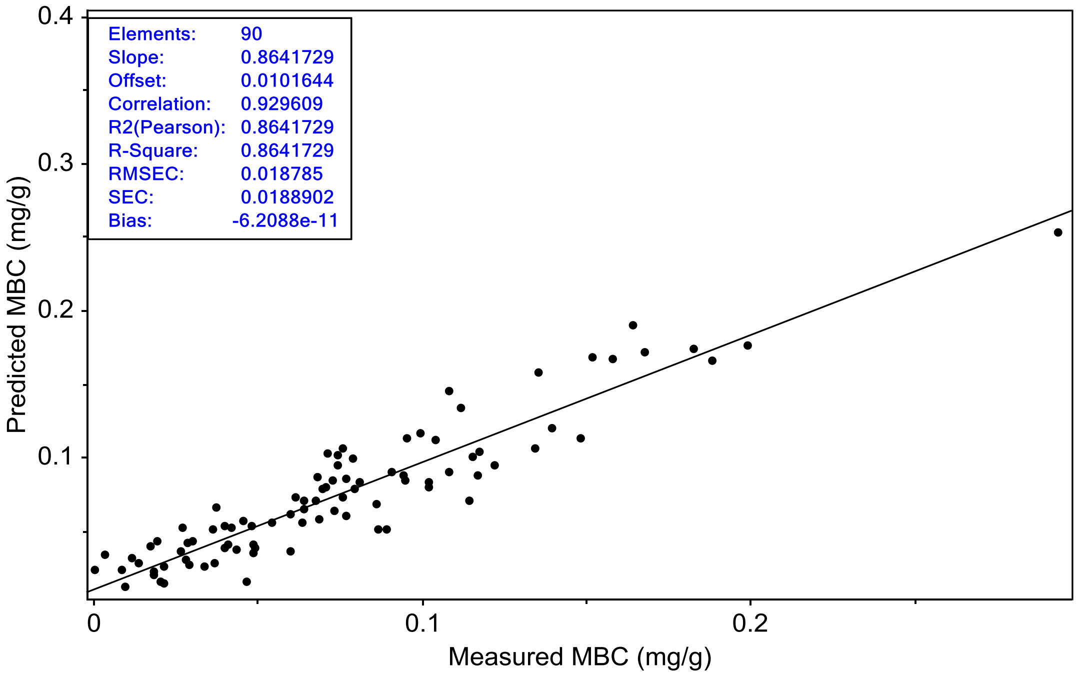


Figure 3-4: Extrapolation model used for microbial biomass carbon estimates.

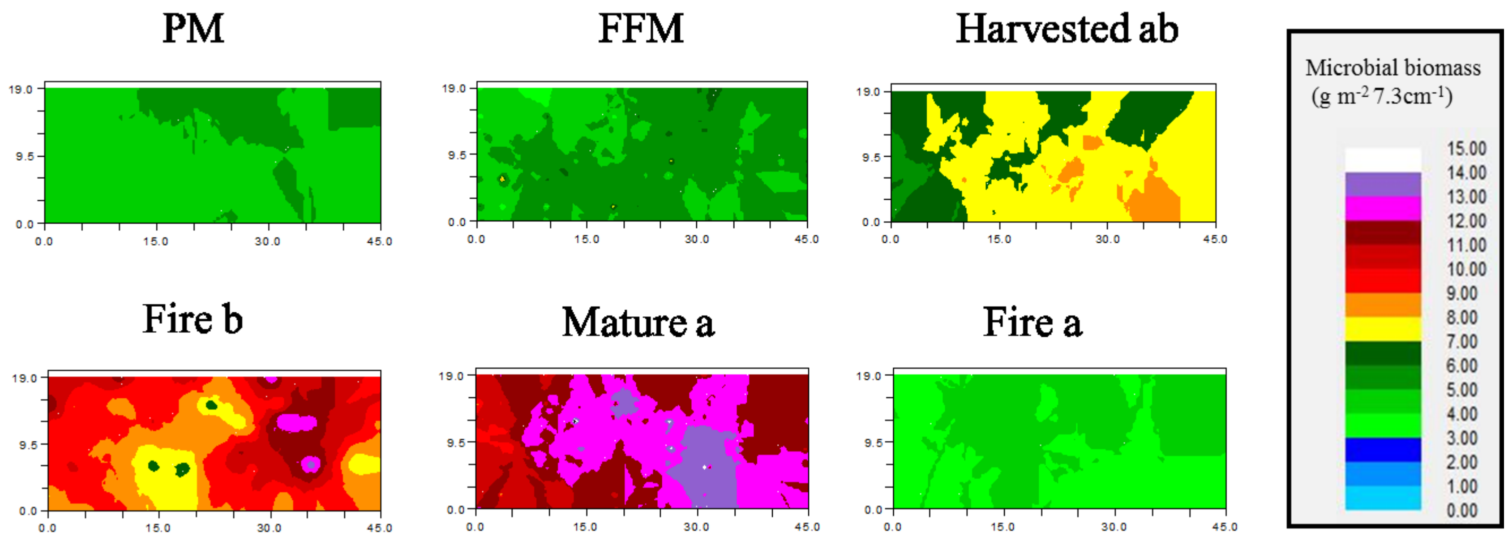


Figure 3-5: Ordinary kriging maps of topsoil microbial biomass carbon.

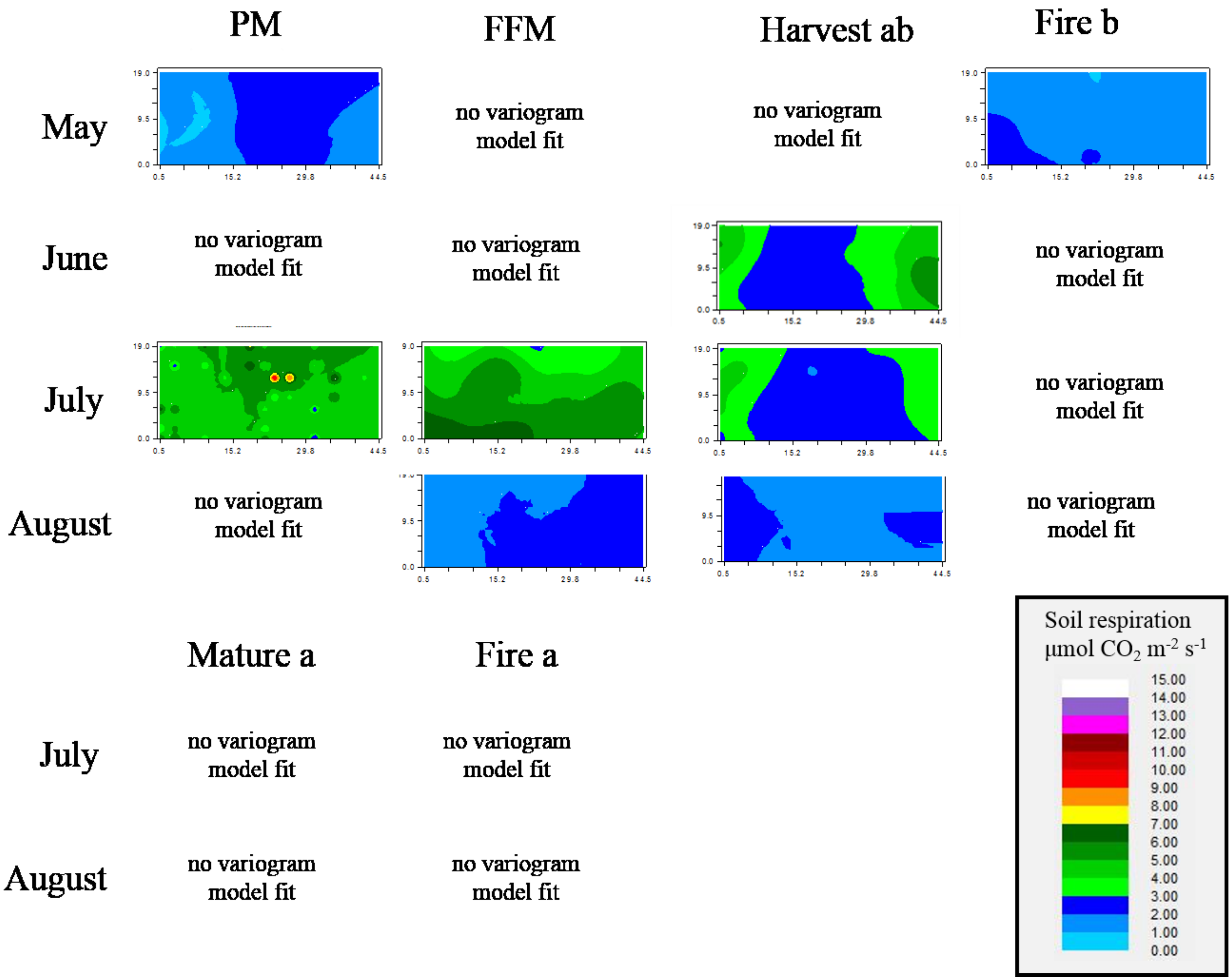


Figure 3-6: Ordinary kriging maps of soil respiration during the growing season of 2014.

Chapter 4 Building a Better Soil for Upland Surface Mine Reclamation in Northern Alberta: Admixing Peat, Subsoil and Peat Biochar in a Greenhouse Study with Aspen

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4.1 INTRODUCTION

Land reclamation following surface mining frequently results in poor ecosystem function due to a lack of organic matter (OM) in cover soils. This applies to oil and gas operations in Wyoming (Mason et al. 2011), coal extraction in Germany (Šourková et al. 2005), or Bauxite mining in Australia (Jasper 2007; Lewis et al. 2009). However, surface mining of oil sands in boreal mixed-wood forests of northern Alberta suffers from the opposite problem. Selectively salvaged peat from lowland ecosystems during pre-mining (Fung and Macyk 2000) produces an excess of OM. This salvaged peat is used to reclaim upland ecosystems as a means of stimulating soil fertility, but the functional parameters created by this practice are not similar to those found in natural upland ecosystems (MacKenzie and Quideau 2010, 2012; Quideau et al. 2013; Sorenson et al. 2011). In fact, reclaimed ecosystems using peat are potentially put on recovery trajectories that create novel ecosystems (Audet et al. 2014).

In Alberta, surface mining of oil sands creates a large-scale ecosystem disturbance requiring *"reclamation .... to its equivalent capability"* (Alberta 2014a: 99). Conservation and reclamation regulations for the province of Alberta currently define equivalent capability as the ability to support various land uses, similar to what existed prior to surface mining, but not necessarily identical (Alberta 2014b). Legislation also requires evaluation of physical, chemical and biological characteristics in reclaimed ecosystems, including soils and vegetation following reclamation (Alberta 2014b). Together these two points imply re-establishment of ecosystem function on both local and regional scales. Of the 904 km^2 disturbed to date, only 104 ha of the total disturbed area has been certified reclaimed (Environment & Parks 2015). Another 5,901 ha have been permanently reclaimed, but must be monitored for more than 15 years before being certified (Alberta 2013). Currently, the presence and growth of local plant species are used to assess recovery of boreal forests on these disturbed areas (Alberta 2013; Environment&Parks 2015) along with concepts of fertility that may not be realistic for wildland ecosystems. Upland reclamation of surface mines in the AOSR is carried out by placing coversoil, mostly peat, over suitable subsoil or overburden. Operators are legally required to salvage all available topsoil (Alberta Environment 2004), which includes limited salvaged material from uplands that contain native seed and propagules. This material, when using it for reclamation, is called forest floor mineral mix (FFM). While vegetation recovery using FFM or LFH donor material as cover soil is better for reclamation (Mackenzie and Naeth 2010), the material is scarce and current reclamation practices use peat instead on sites targeting boreal upland forest recovery.

To date, reclamation practices in the oil sands mining-affected areas of northeastern Alberta have not considered the influence of wildfire as part of a possible recovery strategy. The intensity, type, and prevalence of fire in these boreal ecosystems influences vegetation and biogeochemical cycling (Maynard et al. 2014), and structures Western Canada’s boreal mixed woods (Cumming 2001), as fire is the main natural disturbance of these ecosystems (Cumming 2001; Lecomte et al. 2006). It thermally alters organic matter, turning vegetation into charcoal, a stable carbon compound and a long-term carbon sink (Ohlson et al. 2009). Charcoal is a residue of wildfire (Preston 2009), and native vegetation might be as it is adapted to fire as a natural disturbance, be adapted to the influence of charcoal as a soil component, but this is not known to date. Biochar is a man-made surrogate for charcoal and can have the same influence on soil biogeochemical properties. Biochar has been used as a soil amendment for forest restoration since the early 1820's and is known to increase tree biomass (Thomas and Gale 2015). The addition of biochar to peat in greenhouse trials increased plant biomass production and reduced decomposition rates (Tian et al. 2012). Thomas and Gale (2015) suggested that biochar could potentially replace other forms of organic matter or liming agents in the field of forest restoration. Biochar might also stabilize carbon, change biogeochemical characteristics, influence nutrient mineralization, enhance plant performance, reduce greenhouse emissions, and increase soil microbial biomass in reclaimed soils (Anderson et al. 2011).

A possible solution to the issue of adding too much organic matter to reclamation soils in the AOSR is admixing peat with non-saline/sodic sub-soil material to create a soil prescription similar to natural, undisturbed forest soils in the region. These natural soils have low surface organic matter content and can be coarse textured with rapid drainage (Fung and Macyk 2000). Reducing surface OM content through admixing might better simulate these properties. Another solution for reclamation could creating biochar using available peat sources and incorporating it into peat-subsoil admixtures. Biochar can be chemically similar to pyrogenic carbon produced after wildfire and has been shown to influence soil biogeochemical processes (DeLuca et al. 2009).

Our study included two parts. In part one, we assessed the effect of admixing peat with non-saline/sodic sub-soil material on aspen biomass production and nutrient availability to create a reclaimed soil more similar to FFM. In part two, we examined the effect of including pyrolysed peat (biochar) as a Pyrogenic Carbon (PyC) amendment to admixed soils on aspen biomass production and nutrient availability, to determine if this produced soil more similar to FFM.

4.2 METHODS

4.2.1 Greenhouse Set-up

A greenhouse experiment was set up in March 2015 at the plant growth facility located at the University of Alberta, Edmonton, Alberta, Canada. Aspen *(Populus tremuloides* Michx.*)* seeds were germinated and grown for six weeks in styroblock containers (Beaver Plastics ID Code 540/8) and filled with Sunshine® Peat Moss. An 8ml container size was chosen to reduce the transfer of nutrients into reclamation soil types. All seedlings were fertilized two weeks after germination with 11-41-8 (NPK) tree seedling fertilizer (Agrium Advanced Solutions) according to manufacturer instructions. In mid-April 2015, seedlings were transferred into pots containing different admixed soil treatments (see below). Only seedlings showing equivalent growth were selected for transfer into treatment pots. Seedlings were watered daily to field capacity. Additional lighting was set to 16 h per day (6:00-22:00 hours).

Pots used for the trial were 20 cm in diameter and 10 cm in height for a total of volume 2.42 l. Pots were filled with one of six different admixtures of peat and sub-soil salvaged from the pre-mining environment. Peat was salvaged from lowland ecosystems and had a woody nature, consistent with paluaudification, while sub-soil was salvaged from a coarse-textured jack pine (*Pinus banksiana* Lamb.) ecosystem. Admixtures were produced by mixing large quantities (40 l total per run) of peat and sub-soil in a clean cement mixer in different volumetric proportions for 10 minutes. FFM was mixed following the same procedure and consisted of the

LFH and the top 10-15 cm of A/B horizon from the same coarse textured **a** or **b** ecosites. Type **a** ecosites are characterized by xeric to subxeric moisture regimes with poor to very poor nutrient regimes, while type **b** ecosites are characterized by submesic to subxeric moisture regimes with medium to poor nutrient regimes (Beckingham and Archibald 1996). Each experiment (admixing and admixing with biochar) was replicated six times with trees and three times without trees (Table 4-1). The experimental set up was a completely randomized design and pots were rotated in the greenhouse weekly.

Biochar was produced by gradual carbonization of peat in a muffle furnace in a reduced oxygen environment (tinfoil and sand bed). Peat was charred by fast-ramping (30 minutes) to a temperature of 500°C where it was held for one hour. Biochar was of fine structure with a BET (Brunauer–Emmett–Teller) surface area of 23 m^2 g^{-1}, total carbon content of 23.58% (C), hydrogen content of 0.78% (H), nitrogen content of 0.51 % (N), and oxygen content of 5.78 % (O). Each pot received 32.2 g of biochar, equivalent to 10 MT biochar/ha, mixed into the treatment pots. Treatment pots were incubated in the greenhouse for two weeks before trees were transferred into individual pots. Pots were watered with deionized water during the incubation period, to maintain 80 % Field Capacity.

4.2.2 Biomass and Laboratory Analysis

We measured bioavailable nutrients by installing Plant-Root-Simulator™ probes (PRS) in pots without trees according to the manufacturer instructions (Western AG Innovations, Saskatoon, SK). PRS™ probes employ membrane bound ionic exchange resins to capture nutrient anions and cations available in soil solution. Probes were incubated in-situ for one week prior to the end of the experiment. Upon removal from the pots, probes were rinsed with de-ionized water, sent to Western Ag Innovations, extracted with 0.5 M HCl and analyzed by colorimetry (FIAlab 2600) for NH_4^+ and NO_3^-, and by ICP-OES (Perkin Elmer ICP-OES 8300) for Al, B, Cu, P, K, Mn, N, Zn, Fe, Mg, Ca, and S.

The experiment ended in mid-July 2015 at which point the trees had been growing in the experimental treatments for 12 weeks. Roots were washed, and soil samples were collected. Plants were dried to a consistent weight at 65°C and total biomass of each tree was recorded. Leaves were ground up using a Ball Mill MM200 (Brinkmann Retsch). Elemental analysis of foliar material was conducted after its digestion with 65% HNO_3 in a MARS 5 microwave digester (CEM corporation). Foliar C and N were measured by dry combustion (COSTECH 4010 Elemental Analyser) and Al, B, Cu, P, K, Mn, Zn, Fe, Mg, Ca, Mo, Ni, Co, Na, Cr, and S were measured using ICP-OES (ThermoFischer iCap 6300 Duo). Elemental concentrations were controlled and verified with standard reference materials.

Soil samples were dried at 60°C to a consistent weight. All samples were sieved to remove plant debris, rocks and other soil components greater than 2.0 mm and then ground using a Ball Mill MM200 (Brinkmann Retsch). Samples were transferred to 20-mL scintillation vials and stored before analysis.

Soil thermal stability was measured on twenty percent of the samples using Differential Scanning Calorimetry (DSC; STA 6000, Perkin Elmer). Approximately 20 mg of each sample were loaded in an open ceramic pan under an oxidative atmosphere (flow rate: 20 mL min^{-1} Oxygen gas and 80 mL min^{-1} Nitrogen gas) at a scanning rate of 20°C min^{-1}. The heat of combustion (Q in J g^{-1}) was determined by integrating the DSC curve over the exothermic region (150°-550°C). The area on the DSC curve was divided into three groups based on resistance to oxidation: (1) labile organic matter (150°C-375°C), (2) recalcitrant organic matter (375°C-

475°C) and (3) highly-recalcitrant organic matter (475°C-550°C) modified from Merino et al. (2014), Merino et al. (2015), and Merino et al. (2016). These partial heats of combustions were designated as *Q1*, *Q2* and *Q3*, respectively following Merino et al. (2014), for convenience. Soil thermal stability was calculated as a ratio of recalcitrant (*Q2*) over the labile organic matter (*Q1*).

Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) was performed on all samples to predict soil thermal stability measured by DSC (Merino et al. 2015). Samples were placed in stainless steel sample cups and loaded into a Cary 600 Series Fourier Transform Infrared Spectrophotometer (FTIR, Agilent Technologies) equipped with diffuse reflectance accessory (AutoDiffusIR^{TM}, PIKE Technologies). IR spectra of each soil sample were generated based on 16 sample scans per run at a spectral resolution of 4.0 cm^{-1}. All samples were analyzed in triplicate. IR spectra were baseline corrected and averaged before partial least square regression analysis (PLSR).

PLSR analysis of the prediction of heats of combustion from FTIR spectral data was performed using Unscrambler X (CAMO Software AS ver.10.3). The heat of combustion from labile organic matter (*Q1*) was predicted from IR spectral ranges of 1000 cm^{-1} - 1300 cm^{-1} (-C-H bending and C-O stretching), 2850 cm^{-1} - 2950 cm^{-1} (alkyl C-H stretching) and 3380 cm^{-1} - 3410 cm^{-1} (O-H stretching). The heat of combustion from recalcitrant organic matter (*Q2*) was predicted on the regions for unsaturated bond vibrations: 750 cm^{-1} - 850 cm^{-1} (=C-H bending) and 1500 cm^{-1} - 1850 cm^{-1} (C=C stretching and C=O stretching).

4.2.3 Statistical Analysis

R 2.1.5 (R Core Team 2012) was used for statistical analyses with *agricolae* package (de Mendiburu 2014) for post-hoc testing with *Tukey HSD* test and with permutational ANOVA in the *lmPerm* package (Wheeler 2010) for significance testing. Biochar amended treatments were tested separately within another set of the admixed soil treatments. Following separate analysis, all treatments were compared by Principal component analysis (PCA) using the *ggbiplot* package (Vu 2011) in R3.3.1 (R Core Team 2016). Data were standardized before PCA by log transformation and adding a constant (log10(x+100)).

4.3 RESULTS

4.3.1 Substrate Characterisation

Values for pH ranged from 6.8 in FFM to a high of 8.0 in S100 and P30S70 soils with biochar added in both. Treatments with peat concentrations >50% had pH values ranging from 7.6 to 7.9. Values for pH were higher in soils with biochar than in soils without biochar (Table 4-1) while pH of FFM soils was lower than all other treatment soils. EC values in all soil mixes with biochar ranged from 292 to 547µs/cm and were higher than the EC of FFM (161 µs/cm). EC values of all soils without biochar were also higher than FFM except for S100 (87µs/cm) (Table 4-1). Organic matter (OM) content values were higher in soil mixes with biochar than without biochar except for P80S20 (Table 1). OM content for FFM soils was 4.1%, slightly higher than S100 and P30S70 values regardless of biochar being present but substantially lower than those soil mixtures with higher percentages of peat (Table 4-1). Total Carbon (TC) and

Total Nitrogen (TN) were higher in treatments with more peat. Biochar amendment increased TC and TN compared to unamended treatments (Table 4-1).

4.3.2 Effect of Admixing Peat

Biometrics data

Biomass of seedlings grown in FFM was significantly greater than seedling biomass of all other treatments except for P50S50. Biomass of seedlings grown in P80S20, P90S10 and P100 were significantly lower than FFM and P50S50 (Table 4-2). Seedlings grown in FFM were significantly taller than all other treatments and almost 90% taller than the best performing admixture P50S50 (Table 4-2).

Foliar data

Mean foliar concentrations of N were significantly higher in P80S20, P90S10 and P100 than all other treatments including FFM (Table 4-2). Concentrations of P were significantly greater in FFM and S100 than all other treatments and up to 300% greater than in treatments with >80% peat admix ratio. Mean values of P in P30S70 were also significantly greater than P80S20, P90S10 and P100. Foliar concentrations of K were significantly higher in P90S10 than P30S70 and S100 (Table 4-2). Mean K concentration from FFM was only significantly greater than K concentrations in S100 (Table 4-2). Average foliar S concentration was significantly higher (100% greater) in P80S20 than P50S50, P30S70, S100 and FFM (Table 4-2). Foliar Mg concentrations were significantly higher in S100 than all other treatments including FFM (Table 4-2). Foliar Ca concentrations were significantly higher in P30S70 than P90S10 and P100 (Table 4-2). Foliar Na concentrations were significantly higher in P90S10, and P80S20 than P50S50, P30S70 and FFM, the foliar concentration of Na was up to 600% greater in treatments with high peat admix ratios (Table 4-2). Foliar Mn concentrations were significantly higher in S100 than in FFM and P30S70 and all three treatments were significantly higher than all admix treatments with peat (Table 4-2). Foliar Cu concentrations were highest in FFM but not significantly higher than in S100, P80S20, P90S10, and P100 (Table 4-2). Cu concentrations in the foliage from P50S50 and P30S70 treatments were less than 50% of concentrations in FFM seedlings and significantly different to FFM with means below 5 µg g^{-1}.

The axes of principal component (PC) 1 and PC 2 of standardized foliar nutrient concentrations from aspen seedlings explained 50% of the variation in data (Figure 4-1). The axis of PC1 showed the influence of peat and subsoil proportions. FFM samples grouped midway between all peat (P100) samples on the left and all subsoil (S100) samples on the right. As peat concentration decreased, groups moved to the right with samples containing P50S50 and P30S70 overlapping with FFM samples. Higher foliar concentrations of P, Mn, Co and Mg were found in S100 samples. Foliar K and N concentrations were positively related to P100 samples while P was negatively related to samples containing more peat. The axis of PC 2 showed higher foliar Na and S concentrations that were negatively related to FFM samples. Analyses of PC 3 and PC 4 (data not shown) increased total explained variation to 73.3 percent but could not identify significant differences among peat treatments. However, this analysis highlighted differences in foliar Zn, Mn, P, and Cu concentrations in FFM from other treatments. Foliar concentrations of Zn were not significantly different among treatments when tested separately.

PRS and SOM stability data

Bioavailability of N, S, Mg and Ca were significantly higher in P80S20, P90S10 and P100 treatments than P50S50, P30S70, S100 and FFM treatments. Bioavailability of P was significantly higher in FFM and exceeded levels of other treatments by 400%. Bioavailability of K was also significantly higher in FFM than in all other treatments except for P90S10. SOM stability was significantly greater in P80S20 than in P50S50, P30S70, S100 and FFM.

PCA axes PC1 and PC2 of standardized bioavailable nutrients and soil organic matter stability explained 70.1% of the variance in data (Figure 4-2). As in Figure 4-1, PC1 axis in Figure 4-2 showed the influence of peat and subsoil proportions with FFM samples grouped midway between all peat (P100) samples on the left and all subsoil (S100) samples on the right. As peat content decreased, and subsoil content increased, groups moved to the right with samples containing P50S50 and P30S70 in line with FFM samples. Axis PC2 in Figure 4-2 showed a separation between FFM samples and all admixed soil samples, which suggests differences between natural soils (FFM) and the admixed soils created for this study. Bioavailability of P was positively related to FFM while most other nutrients were positively related to peat content and negatively related to subsoil content. Figure 4-2 clearly showed treatment P50S50 had a nutrient profile similar to FFM with regards to the variation explained by the axis of PC1. Differences between P50S50 and FFM related to the differences of bioavailable P, Mn, K, and Cu on Axis PC2.

4.3.3 Effect of Admixing Biochar to reclamation soil mixes

Biometrics data

Biomass of seedlings grown in FFM was significantly greater than the biomass of seedlings grown in all other soils mixtures with biochar except P50S50+ which, on average, produced only 55% as much biomass as seedlings grown on FFM. Biomass of seedlings in P50S50+ did not differ significantly from all other admixture treatments with biochar (Table 4-3). Seedling height followed the same pattern as seedling biomass. Seedlings in FFM grew significantly taller than all other admixtures with biochar during the study except seedlings in P80S20+ with biochar; however, seedlings grown on FFM were more than 70% taller than the best performing admixture. Seedling height in P80S20+ did not differ significantly from any of the seedlings grown in other soil admixtures with biochar (Table 4-3).

Foliar data

Foliar N concentration in FFM seedlings did not differ significantly from any other soil treatments with biochar except S100+. Foliar N concentration was significantly greater in P100+ than P50S50+, P30S70+, and S100+ (Table 4-3). Foliar P concentration was significantly greater in FFM seedlings than all other soil admixing treatments with biochar except for P50S50+. P concentration in P50S50+ was significantly greater than P80S20+, P90S10+ and P100+ (Table 4-3). Foliar K concentration was significantly higher in P80S20+ and P90S10+ than in S100+, while foliar concentrations of K from FFM did not differ significantly from any seedlings of the treatments with biochar. Foliar concentrations of S and Mg did not differ significantly among admixtures with biochar or from FFM (Table 4-3). Foliar concentrations of Ca were significantly greater in P50S50+ than all other soil admixing treatments with biochar including FFM. Foliar concentrations of Na in FFM and P50S50+ were significantly lower than S100+, but there were no significant differences in Na concentrations among any of the other soil admixing treatments. However, foliar Na concentrations were 100% to 600% greater than in FFM (Table 4-3). Foliar Mn concentrations were significantly smaller in all peat subsoil admix treatments compared to FFM. Foliar Mn concentrations from FFM were more than 100% greater (Table 4-3). Foliar Cu concentrations were higher on FFM compared to all treatments. However the difference was significant in all cases (Table 4-3). Treatments P80S20+, P50S50+, and P30S70+ were significantly different to FFM in foliar Cu concentration with mean Cu concentrations below 5µg g^{-1} (Table 4-3).

PRS available nutrients and SOM stability data

N bioavailability, determined using PRS™ probes, was significantly higher in P100+ than in P50S50+, P30S70+, S100+, and FFM (Table 4-3). Bioavailability of P and K were significantly higher in FFM than all other soil treatment samples with biochar. Bioavailability of S did not differ significantly among any of the samples. Mg bioavailability was significantly higher in P80S20+ than FFM and S100+. Ca bioavailability was significantly greater in P80S20+ and P90S10+ than in P30S70+, FFM and S100+ (Table 4-3). SOM stability in treatments P100+, P90S10+, and P80S20+ was significantly greater than in P50S50+ and FFM. SOM stability was significantly lower in P30S70+ and S100+ (Table 4-3).

4.3.4 Biochar effect on admixing treatments

Permutational two-way ANOVA could not identify significant differences when comparing peat admix treatments with and without peat biochar amendment. For that reason the entire study was analysed using multivariate statistics (PCA) in order to capture the overall effect of a biochar amendment.

Figure 4-3 shows PCA results for all data together, including available nutrients, SOM stability, foliar nutrient concentrations, and biometrics (height, biomass). Axes PC1 and PC2 explained 53 % variation. The ordination showed a trend that with biochar amendment the treatments P100, P90S10, P80S20, P30S70, and S100 shifted closer towards FFM, suggesting overall characteristics of those treatments were similar to the reference substrate FFM (see arrows in figure 4-3). Treatment P50S50+ was less similar to FFM. Analysis of PC3 and PC4 for this PCA (data not shown) increased the total explained variation to 71.6 % but did not identify trends or significant differences with biochar amendment.

4.4 DISCUSSION

4.4.1 Admixing

Improving growth

Aspen seedling biomass was greatest in P50S50 and did not differ significantly from aspen growth in FFM. Compared to the classical placement of reclamation materials in layers in upland oil sands mine reclamation (e.g. 30 cm of peat over 120 cm subsoil (Barber et al. 2015)), reclamation might be more successful with those materials by mixing materials to create conditions similar to natural soils. However, height growth in our admixture treatments was still significantly less than FFM soil. Height growth has a strong influence on competitive success, especially when competing for light in a weedy environment (Weinig 2000). Davis et al. (1999) reported that seedling survival was significantly affected by interactions of both water and light availability. Considering the increased height and biomass in P50S50, relative to the other admix treatments, survival rates of seedlings could be greatest on this cover soil prescription.

Assimilation of foliar nutrient concentrations to FFM grown seedlings

The foliar nutrient profile was influenced by admixing mineral subsoil and peat. Treatments P50S50 and P30S70 were most similar to FFM, with differences mainly explained by foliar S and Na concentrations (Figure 4-1). When all other treatments are compared to FFM, differences in foliar nutrient profiles can be interpreted as factors that reduced growth, even though in some cases, differences are not significant (Table 4-2). The shift in foliar nutrient profiles along axis PC1 (Figure 4-1) suggests that those treatments were significantly different. The driving elements on axis PC1 are P, Mn ,Co, Mg, Fe, Al, Cr, Cu, and Ca concentrations (Figure 4-1), which point towards treatment S100 and are negatively correlated with the foliar N and K concentrations related to treatments with a higher peat content as P100, P90S10, and P80S20. A more detailed analysis with permutational ANOVA and Tukey HSD revealed that concentrations of N, P, K, Mn, Cu, S, and Na were of greater importance (Table 4-2).

Kirkby (2012) stated that shoot dry matter concentration of N greater than 15 mg g^{-1} is required for adequate growth. All treatments, except S100, had foliar N concentrations greater than this critical value. With Foliar N concentration in peat ad-mix treatments higher than concentrations in FFM, which showed greater growth than the other treatments, N availability was not a limiting factor for aspen seedling growth in this experiment. Therefore, application of N fertilizer on peat cover soils for reclamation targeting type **a** or **b** ecosites is not needed, as supply rates of N are higher than on FFM, which is sourced from the targeted upland forest ecosystems.

Attiwill and Adams (1993) suggested that growth in forests is probably limited by P or some other elements, rather than N. The shoot concentration of P required for adequate growth is 2 mg g^{-1} (Kirkby 2012). None of the treatments reached that foliar P concentration, however, FFM and S100 had significantly higher foliar P concentrations than treatments with higher admix ratios of peat. For example, P100 had only 50 % as much foliar P concentration as P50S50 and significantly less accumulated biomass. P limitation can reduce shoot growth rate, inhibit formation of reproductive organs, or lead to restricted seed formation (Hawkesford et al. 2012). Those factors are critical for establishing a self-sustaining forest ecosystem. Our study showed that admixing increased foliar concentrations of P, however the increase was insufficient when foliar concentrations and growth of admix treatments were compared to FFM.

Foliar concentrations of K decreased with admixing subsoil to peat but was similar to FFM up to a ratio of 50% subsoil. Foliar concentrations of K were greater than 10 mg g^{-1}, the value reported by Kirkby (2012) for adequate function. Only treatments P30S70 and S100 had K concentrations below 10 mg g^{-1}. K plays an important role in enzyme activation and osmoregulation (stomatal control), and K deficiency predisposes plants to abiotic and biotic stresses (Hawkesford et al. 2012). In the boreal forest ecosystems, the increased probability for plant damage under conditions of drought, or low temperature are important (Hawkesford et al. 2012). Massive drought-induced aspen mortality has been observed (Hogg et al. 2008; Michaelian et al. 2011), as well as thaw-freeze events inducing mortality, dieback, or reduced growth (Hogg et al. 2008).

Differences in foliar Mn concentrations could be importance since Mn deficiency is common in soils with high OM and pH (Broadley et al. 2012a). The tested ad-mixes were all high in OM (peat) and are characterized by basic pH values ranging from 7.8 to 7.6 (Table 4-1).

Only mineral subsoil (S100) and FFM had pH values slightly below pH 7. Admixing of subsoil lowered pH slightly, which could potentially improve Mn availability of reclamation cover soils. The critical Mn value lies in between 10 and 20 µg g^{-1} (Broadley et al. 2012a), therefore a deficiency of Mn in our study was unlikely, but our data showed Mn concentrations lower in peat admix treatments than in FFM. However, our study was conducted over a short period (90 days). Future work needs to test if admixed cover soils can supply adequate Mn to support native vegetation over longer periods of time. Again, this is important for boreal reclamation, as Mn deficient plants are more susceptible to damage by freezing or soil born fungal root-rot diseases (Broadley et al. 2012a).

Similar to Mn, Cu deficiency has been described for soils that are high in OM (Broadley et al. 2012a) and is related to the complexation of Cu with organic substances. Another factor that drives Cu deficiency is high N availability (Broadley et al. 2012a). Values for critical Cu deficiencies range from 1-5 µg g^{-1} depending on plant organ and species (Broadley et al. 2012a). Typical symptoms of Cu deficiency include stunted growth, chlorosis/necrosis starting at the apical meristem, or bleaching of young leaves (‘white tip’ or ‘reclamation disease’) (Broadley et al. 2012a), and similar symptoms were observed on seedlings in our study. Foliar concentrations were in the range of 5 µg g^{-1} for the majority of admix treatments, but in P50S50 and P30S70 lower, so deficiencies potentially possible. Given the nature of reclamation soils being used, future work should evaluate potential Cu deficiencies in long-term field trials as N metabolism, lignification, and pollen formation are affected by Cu deficiency (Broadley et al. 2012a).

Differences between P50S50 and FFM, and other treatments were also explained by axis PC2 (Figure 4-1). Foliar S and Na concentrations drive the variation between treatments along this axis. While S is a macro nutrient that can limit tree seedling growth (Ericsson 1995), Rennenberg (1984) suggested that excess S can negativly impact plant metabolism and can affect dry weight accumulation, yield, and delay flowering. With the short growing season in boreal climates and limited knowledge on the response of native vegetation to increased S concentrations, future studies should evaluate potential effects on flowering delay as this might be critical for reproduction of plants. Na is known to have potentially toxic effects on plants, even at low levels, and can negatively affect plant growth and development (Luan et al. 2009). Higher foliar Na concentrations were observed in all treatments relative to FFM. However, P50S50 had the lowest foliar Na concentration, which was not significant different from seedlings grown on FFM.

Assimilation of biogeochemical characteristics to FFM by admixing

Our study showed that admixing peat with mineral subsoil resulted in more similar biogeochemical characteristics with regard to nutrient availability and SOM stability, when compared to FFM. This is important when reclamation practices target plant communities that are adapted to specific nutrient regimes. When targeting type **a/b** ecosites, using a cover soil with high nutrient availability is inappropriate, as distinct plant communities occupy sites with low nutrient availability as a competitive strategy (Chapin et al. 1986). Results from our study suggest that nutrient availability in the admix treatment P50S50 is most similar to FFM based on the variation explained on axis PC1 (Figure 4-2), which is driven by decreased availability of P, Mn, K, and Cu. By fertilizing P50S50 with an adequate rate of those nutrients, very similar nutrient availability compared with FFM could be established. This would be of great importance for re-establishing native plant communities, as soil fertility is in many cases an excellent predictor for the composition of plant communities (Chapin et al. 1986; Christensen and Peet 1981). Future field studies should evaluate the effect of targeted fertilization on the establishment and resilience of native plant communities on P50S50 cover soil compared to equivalent ecosites following disturbance, and sites reclaimed using current standard procedures.

4.4.2 Amending biochar to admix treatments

Improving growth

Treatment P50S50+ showed the greatest biomass accumulation and did not differ significantly from FFM grown aspen seedlings, however, heights of seedlings in P50S50+ were significantly lower compared to FFM, which is critical when competing with other plants. Biedermann and Harpole (2013) showed that a biochar amendment can increase plant productivity by various mechanisms, however growth equivalent to FFM was not achieved. This might be the result of producing the biochar from peat (P100), which might be considered low quality feedstock given the low BET surface area of $23m^2$ and a low C content. Properties of biochar are affected by feedstock quality and pyrolysis temperature (Kloss et al. 2012).

Assimilation of foliar nutrient concentrations to FFM

Foliar nutrient concentrations of N, K, S, Mg, and Na in biochar amended treatments P100+, P90S10+, P80S20+, P50S50+, P30S70+ were similar to foliar nutrient concentrations in FFM than in treatments without biochar. Foliar P concentrations, however were significantly different compared to FFM, indicating that the amendment of biochar did not improve P availability. Foliar P concentrations in biochar-amended treatments were still below critical values, indicating that the biochar used in this study did not stimulate uptake. Steiner et al. (2007) described a positive biochar effect on P uptake using secondary forest wood as a feedstock, however in our study probably not existing for P as the feedstock and other OM was very low in P. Similar observations were made for foliar concentration of Mn and Cu. Biochar amendment increased pH and SOM content (Table 4-1) and subsequently reduced bioavailability of Mn and Cu. Biochar amendments reduced the fraction of exchangeable Cu and might have a negative effect on Cu availability to plants, as peat admix treatments had low Cu availability. Foliar symptoms indicating Cu deficiency were observed in biochar amended admix treatments. Foliar concentrations of N, P, K, S, Mg, and Na in P50S50+ did not differ significantly from FFM foliar concentrations, but Mn and Cu were lower. Significantly higher foliar concentrations of Ca in P50S50+ were within the range of 1,000 and 50,000 mg g^{-1}, which was reported for plants depending on organs and growing conditions (Hawkesford et al. 2012) and likely had no adverse effects.

Assimilation of biogeochemical characteristics

Adding biochar produced similar N bioavailability among all treatments except in P100+. Biochar has increased bioavailability of NH_4-N shortly following application, but decreased NO_3-N concentrations consistently (Nelson et al. 2011). In our study, biochar amended treatments had a higher N availability than unamended treatments. When targeting a long-term status with lower N bioavailability that is comparable to FFM a biochar amendment might be useful, but that needs to be tested in the reclamation environment. None of the admix treatments amended with biochar reproduced similar P bioavailability. Nelson et al. (2011) stated that biochar can reduce the amount of exchangeable P when no P fertilization is applied. Since the bioavailability of P from the peat and subsoil used in our study was low, future studies should evaluate the effects of P fertilization with biochar amendment, or test biochars that are produced from feedstock that has a higher P content. Oram et al. (2014) showed that biochar can improve the availability of K to plants, but biochar did not elevate the availability of K to a level comparable with FFM in our study. For S bioavailability no significant differences with biochar amended treatments were identified. However the average bioavailability of S on treatments with higher peat contents (P100+ to P50S50+) was five times higher than FFM. Biochar releases essential elements like S (Uchimiya et al. 2010), however, increased S availability needs to be evaluated carefully as excess S in plants can potentially have negative effects (Rennenberg 1984). Bioavailability of Ca and Mg in treatments P100+ to P30S70+ showed that admixing mineral subsoil and peat in combination with biochar amendment resulted in sufficient availability of those elements relative to FFM. SOM stability of P50S50+ was not significantly different from FFM. Stability of SOM can be used as a proxy to indicate how easily C and N can be mineralized (Plante et al. 2011). For this reason P50S50+ will provide a similar nutrient mineralization rate and availability as FFM. As the effects of biochar on the stabilisation of pre-existing SOM are not currently understood (Kimetu and Lehmann 2010), future work should examine the effect of biochar on peat, which is the surrogate of SOM in upland reclamation.

4.4.3 Evaluating the effect of peat biochar on peat subsoil admixtures using multivariate statistics

An analysis of all factors together, including available nutrients, foliar concentrations, biometric parameters, and biochar amendment (Figure 4-3) revealed that treatment P50S50 without biochar was most similar to FFM. However, biochar amendment shifted all other treatments towards FFM. This might be of great importance when there is a material shortage of peat or subsoil to recreate similar characteristics as found on FFM. The addition of biochar to peat cover soils seems promising, as it increases the similarity of overall biogeochemical characteristics and seedling performance to FFM. Research has shown that a biochar amendment can improve productivity of soils by affecting soil physical and chemical parameters (Ameloot et al. 2013). However, negative effects on productivity have been also reported (Ameloot et al.

2013). Future work should focus on evaluating the potential use of biochar in upland oil sands mine reclamation over longer periods of time in field settings and assess the impact of biochar derived from different feedstocks.

4.5 CONCLUSIONS

Admixing mineral subsoil with peat created cover soil characteristics similar to FFM. The best ratio in terms of tree growth, foliar nutrient, and available nutrient profiles was 1:1. However, foliar data indicated that P and Mn availability was insufficient, and Cu was potentially deficient. Availabilities of those nutrients were not improved by admixing. PRS™ nutrient data also suggested that K availability was significantly lower on peat cover soils and subsoil compared to FFM. In regard to all other tested nutrients, a great similarity in foliar concentrations and bioavailability could be achieved with a 1:1 admix ratio. Concentrations P and Mn in biochar amended admix treatments were still too low, and Cu was also potentially deficient in various admix treatments with biochar amendment. In all admix treatments with and without biochar, PRS™ data suggested that K availability needs to be evaluated further. Biochar amendment increased the similarity of most peat admix treatments to FFM. This might be of importance when targeting plant communities adapted to certain soil characteristics or when an ideal admix ratio cannot be achieved due to availability of salvaged materials. Further studies should investigate the use of soil amendments that can additionally improve the availability of P, K, Mn, and Cu. We are suggesting to test local waste products (e.g. biosolids - as those are known to contain high amounts of e.g. Cu) or biochars produced out of materials rich in deficient elements to address differences in nutrient profiles preferably in line with a field study. Further research should also evaluate the influence of the addressed parameters on establishment of native boreal forest vegetation, ideally over several growing seasons. These studies should also assess recovery from natural disturbances, for example fire or experimental drought, to evaluate the long-term suitability of cover soil prescriptions in context of resistance and resiliency of targeted plant communities. Careful attention must be paid when applying greenhouse study results in the field. However, we are confident that considering our findings in land reclamation practice will be useful when aiming to re-establish **a** or **b** ecosites.

Table 4-1: Experimental design and substrate characteristics.

Treatment	Peat/Subsoil ratio	Biochar	Material type	pH (aq)	EC (µs/cm)	%SOM (w/w)	%TC (w/w)	%TN (w/w)
P100	100/0	w/o	Peat	7.75 (0.04)	470 (64)	36.11 (0.03)	17.93 (0.14)	0.922 (0.119)
		w(+)		7.79 (0.16)	547 (146)	40.56 (0.05)	20.99 (0.02)	1.07 (0.13)
P90S10	88.9/11.1	w/o	Peat-Mineral-Mix	7.61 (0.04)	320 (27)	23.82 (0.04)	15.97	0.820
		w(+)		7.81 (0.03)	404 (56)	24.66 (0.64)	16.06	0.976
P80S20	80/20	w/o	Peat-Mineral-Mix	7.61 (0.02)	252 (323)	19.16 (0.07)	14.41	0.739
		w(+)		7.74 (0.04)	292 (21)	18.54 (0.05)	14.44	0.897
P50S50	50/50	w/o	Peat-Mineral-Mix	7.63 (0.03)	282 (32)	6.25 (0.11)	9.12	0.464
		w(+)		7.94 (0.02)	302 (33)	6.70 (0.06)	9.22	0.630
P30S70	33.3/66.7	w/o	Peat-Mineral-Mix	7.69 (0.05)	172 (13)	3.23 (0.06)	5.60	0.311
		w(+)		7.96 (0.03)	256 (29)	3.87 (0.05)	5.73	0.482
S100	0/100	w/o	Mineral Subsoil	6.89 (0.08)	87 (10)	0.70 (0.06)	0.31 (0.06)	0.006 (0.003)
		w(+)		8.03 (0.10)	371 (33)	0.99 (0.02)	0.50 (0.09)	0.186 (0.004)
FFM	NA	w/o	Forest-Floor-Mineral-Mix	6.77 (0.03)	161 (10)	4.09 (0.06)	2.09 (0.02)	0.074 (0.001)

Note: Treatment names are based on volumetric Peat to Subsoil ratios. Biochar was amended to Peat / Mineral Subsoil mix treatments at a rate of 10MT/ha (32.2g per pot) (w/o =no biochar added; w(+) = biochar added), pH (aq) and EC (µs/cm) reported as mean (standard error) n=9. %SOM based on loss on ignition n=3, expressed as percent based on weight loss. Total Carbon (TC) and Total Nitrogen (TN) n=3 based on combustion analysis for P100, S100, and FFM, weighted for admixing treatments based on S100 and P100 values. Treatment names indicate approximate admix ratios (e.g. 88.9 parts peat and 11.1 parts subsoil = P90S10

Table 4-2: Comparison of mean value (±SE) by treatment effects on biometrics, selected foliar element conc., selected bioavailable nutrients (PRS™), and SOM Stability.

	Biometrics		**Foliar**									**PRS™**						**SOM**
Treatment	Biomass	Height	N	P	K	S	Mg	Ca	Na	Mn	Cu	N	P	K	S	Mg	Ca	Stability
	g	cm	mg g^{-1}							µg g^{-1}		µg 10cm^2 7 days						ratio
P100	0.94*c*	11.8*b*	25.15*a*	0.42*c*	13.44*ab*	2.85*ab*	3.47*b*	15.52*b*	0.45*ab*	44.5*c*	5.9*ab*	136.53*a*	0.36*b*	28.02*b*	312.91*a*	152.11*a*	2588.22*a*	0.40*ab*
	(0.16)	(1.6)	(0.89)	(0.02)	(0.71)	(0.22)	(0.22)	(1.35)	(0.01)	(2.0)	(1.4)	(32.32)	(0.15)	(8.05)	(32.32)	(18.35)	(262.83)	(0.01)
P90S10	0.77*c*	10.3*b*	27.51*a*	0.47*c*	16.25*a*	2.96*ab*	3.37*b*	15.25*b*	0.56*a*	55.2*c*	6.5*ab*	188.35*a*	0.06*b*	35.09*ab*	351.39*a*	133.53*a*	2337.17*a*	0.40*ab*
	(0.09)	(1.0)	(1.36)	(0.01)	(1.73)	(0.37)	(0.22)	(0.79)	(0.09)	(4.6)	(0.7)	(7.95)	(0.02)	(6.2)	(7.95)	(17.71)	(310.83)	(0.01)
P80S20	0.73*c*	7.8*b*	25.44*a*	0.55*c*	11.91*ab*	4.21*a*	4.38*b*	21.46*ab*	0.58*a*	85.2*c*	9.0*ab*	123.37*a*	0.21*b*	17.65*b*	324.57*a*	142.90*a*	2671.21*a*	0.43*a*
	(0.13)	(0.8)	(1.96)	(0.03)	(1.44)	(0.99)	(0.53)	(2.89)	(0.11)	(17.6)	(1.8)	(30.37)	(0.09)	(3.45)	(30.37)	(4.33)	(156.27)	(0.01)
P50S50	2.10*ab*	12.6*b*	16.46*b*	0.76*bc*	13.27*ab*	1.92*b*	3.77*b*	20.88*ab*	0.19*bc*	83.9*c*	4.8*b*	22.92*b*	0.15*b*	20.25*b*	137.84*b*	54.30*b*	899.38*b*	0.29*c*
	(0.52)	(2.4)	(1.76)	(0.05)	(1.16)	(0.16)	(0.29)	(2.81)	(0.04)	(8.1)	(1.4)	(6.06)	(0.05)	(0.73)	(6.06)	(3.00)	(41.63)	(0.04)
P30S70	0.97*bc*	7.7*b*	15.93*b*	1.03*b*	9.90*bc*	2.04*b*	5.01*b*	24.78*a*	0.27*bc*	140.4*b*	4.6*b*	13.17*b*	0.42*b*	25.60*b*	79.34*b*	68.71*b*	745.65*b*	0.27*c*
	(0.11)	(1.1)	(0.88)	(0.09)	(0.97)	(0.11)	(0.58)	(2.69)	(0.03)	(18.8)	(1.1)	(3.50)	(0.04)	(1.71)	(3.50)	(17.28)	(157.90)	(0.02)
S100	1.10*bc*	7.9*b*	14.41*b*	1.63*a*	6.21*c*	1.94*b*	7.27*a*	21.93*ab*	0.36*abc*	259.6*a*	7.7*ab*	13.05*b*	0.46*b*	15.87*b*	13.11*b*	23.98*b*	129.75*b*	0.15*d*
	(0.12)	(0.5)	(0.81)	(0.17)	(0.6)	(0.21)	(0.42)	(0.83)	(0.07)	(9.0)	(0.8)	(5.28)	(0.25)	(6.51)	(5.28)	(6.57)	(38.98)	(0.02)
FFM	2.65*a*	23.6*a*	18.88*b*	1.72*a*	12.27*ab*	1.84*b*	4.40*b*	18.80*ab*	0.10*c*	175.6*b*	11.3*a*	35.31*b*	2.50*a*	59.92*a*	64.95*b*	69.71*b*	607.58*b*	0.33*bc*
	(0.35)	(3.0)	(1.38)	(0.10)	(0.93)	(0.10)	(0.39)	(1.50)	(0.02)	(11.4)	(1.6)	(4.48)	(0.22)	(8.68)	(4.48)	(9.23)	(52.87)	(0.02)

Note: Foliar element conc. (n=6), selected bioavailable nutrients (PRS™) (n=3), and SOM Stability (n=9). tested with permutational ANOVA and TUKEY HSD as a posthoc test with alpha=0.05. Different letters indicate significant differences within columns only.

Table 4-3: Comparison of mean value (±SE) by treatment effects on biometrics, selected foliar element conc., selected bioavailable nutrients (PRS™), and SOM Stability.

	Biometrics		**Foliar**									**PRS™**						**SOM**
Treatment	Biomass	Height	N	P	K	S	Mg	Ca	Na	Mn	Cu	N	P	K	S	Mg	Ca	Stability
	g	cm	mg g^{-1}							µg g^{-1}		µg 10cm^2 7 days						ratio
P100+	1.19*b*	11.4*b*	22.65*a*	0.53*c*	11.77*ab*	2.33*a*	3.32*a*	16.87*b*	0.34*ab*	55.9*b*	5.7*ab*	193.48*a*	0.36*b*	28.96*b*	383.71*a*	128.25*ab*	2110.43*ab*	0.41*a*
	(0.29)	(2.1)	(1.19)	(0.05)	(1.41)	(0.18)	(0.44)	(1.91)	(0.05)	(9.37)	(0.81)	(78.76)	(0.16)	(3.69)	(199.18)	(7.95)	(87.97)	(0.01)
P90S10+	1.39*b*	13.6*b*	20.99*ab*	0.47*c*	12.62*a*	2.54*a*	3.51*a*	18.38*b*	0.41*ab*	55.1*b*	5.6*ab*	98.05*ab*	0.26*b*	29.11*b*	378.63*a*	135.79*ab*	2328.16*a*	0.41*a*
	(0.31)	(2.8)	(1.86)	(0.04)	(0.51)	(0.49)	(0.60)	(2.29)	(0.10)	(11.32)	(2.09)	(14.94)	(0.09)	(2.38)	(113.47)	(12.35)	(209.33)	(0.01)
P80S20+	1.34*b*	13.7*ab*	20.05*ab*	0.64*c*	13.36*a*	2.22*a*	3.41*a*	17.03*b*	0.28*ab*	50.9*b*	4.9*b*	64.17*ab*	0.17*b*	28.16*b*	351.36*a*	144.58*a*	2448.18*a*	0.43*a*
	(0.35)	(2.6)	(1.65)	(0.16)	(1.01)	(0.06)	(0.56)	(2.47)	(0.09)	(13.42)	(0.65)	(7.47)	(0.15)	(2.07)	(70.90)	(22.99)	(439.05)	(0.01)
P50S50+	1.52*ab*	13.2*b*	16.01*bcd*	1.31*ab*	11.38*ab*	2.12*a*	4.62*a*	29.70*a*	0.20*b*	74.5*b*	3.9*b*	43.13*b*	0.47*b*	32.46*b*	385.34*a*	111.58*ab*	1892.16*ab*	0.33*b*
	(0.2)	(0.8)	(0.64)	(0.18)	(0.96)	(0.10)	(0.42)	(2.05)	(0.02)	(7.13)	(0.60)	(7.14)	(0.21)	(2.47)	(66.82)	(11.52)	(61.06)	(0.02)
P30S70+	1.37*b*	12.3*b*	13.47*cd*	0.81*bc*	10.61*ab*	2.13*a*	3.39*a*	16.93*b*	0.27*ab*	73.5*b*	4.5*b*	12.93*b*	0.73*b*	33.06*b*	182.71*a*	86.29*abc*	1203.73*bc*	0.22*c*
	(0.21)	(2.3)	(1.41)	(0.16)	(0.47)	(0.21)	(0.31)	(1.94)	(0.05)	(14.90)	(1.75)	(2.43)	(0.40)	(1.48)	(35.48)	(19.74)	(201.99)	(0.01)
S100+	0.79*b*	8.0*b*	12.55*d*	1.02*bc*	8.37*b*	2.96*a*	4.30*a*	18.68*b*	0.62*a*	86.5*b*	6.1*ab*	7.51*b*	0.89*b*	29.31*b*	86.26*a*	34.65*c*	448.39*c*	0.17*c*
	(0.2)	(1.3)	(0.94)	(0.13)	(1.03)	(1.21)	(0.35)	(2.48)	(0.19)	(15.05)	(1.18)	(1.39)	(0.14)	(2.36)	(18.02)	(6.29)	(67.84)	(0.02)
FFM	2.65*a*	23.6*a*	18.88*abc*	1.72*a*	12.27*ab*	1.84*a*	4.40*a*	18.80*b*	0.10*b*	175.6*a*	11.3*a*	35.31*b*	2.5*a*	59.92*a*	64.95*a*	69.71*bc*	607.58*c*	0.33*b*
	(0.35)	(3.0)	(1.38)	(0.10)	(0.93)	(0.10)	(0.39)	(1.50)	(0.02)	(11.43)	(1.64)	(4.48)	(0.22)	(8.68)	(4.48)	(9.23)	(52.87)	(0.02)

Note: Foliar element conc. (n=6), selected bioavailable nutrients (PRS™) (n=3), and SOM Stability (n=9). tested with permutational ANOVA and TUKEY HSD as a posthoc test with alpha=0.05. Different letters indicate significant differences within columns only. Treatments with a plus symbol (+) indicate biochar amendment with a rate of 10 MT/ha.

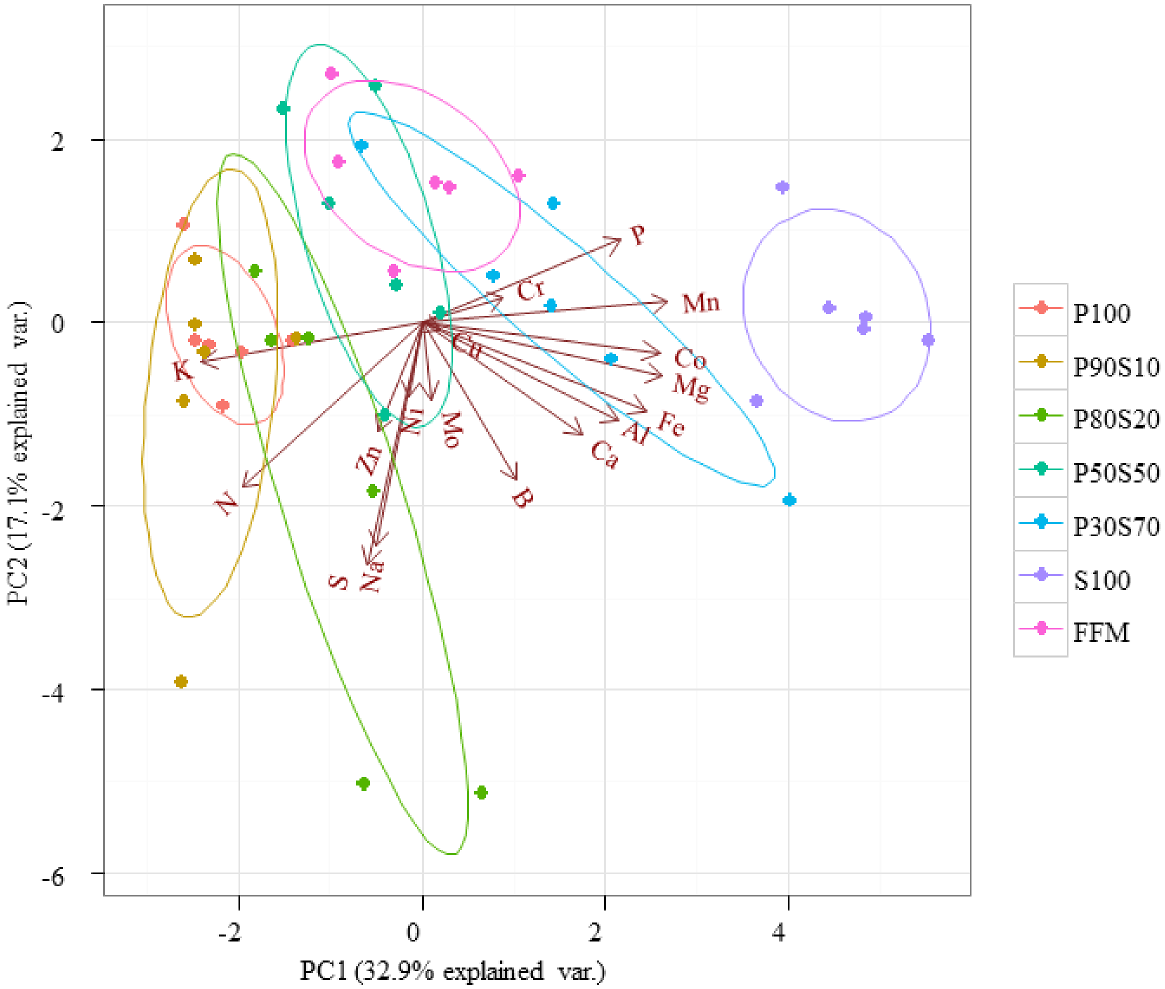

Figure 4-1: Principal Component Analysis (PCA) of standardized foliar nutrient concentration from aspen seedlings (n=6) with no biochar amendment. For treatment identification, see Table 1. Colored ellipses represent 68 percent confidence interval for each treatment.

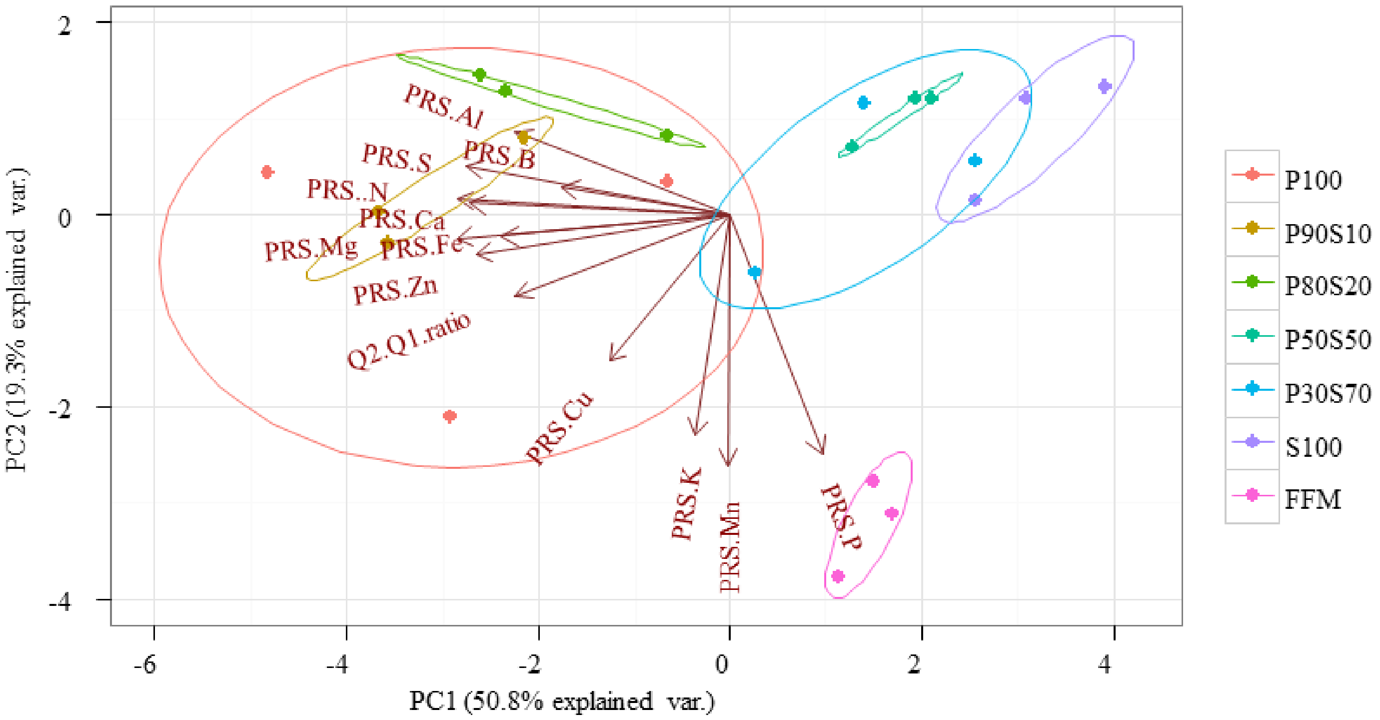

Figure 4-2: Principal Component Analysis (PCA) of standardized bioavailable nutrients (PRS™) (n=3) and soil organic matter stability (Q2/Q1 ratio) (n=3) in pots with no tree and no biochar amendment. For treatment, identification see Table 1. Colored ellipses represent the 68 percent confidence interval for each treatment.

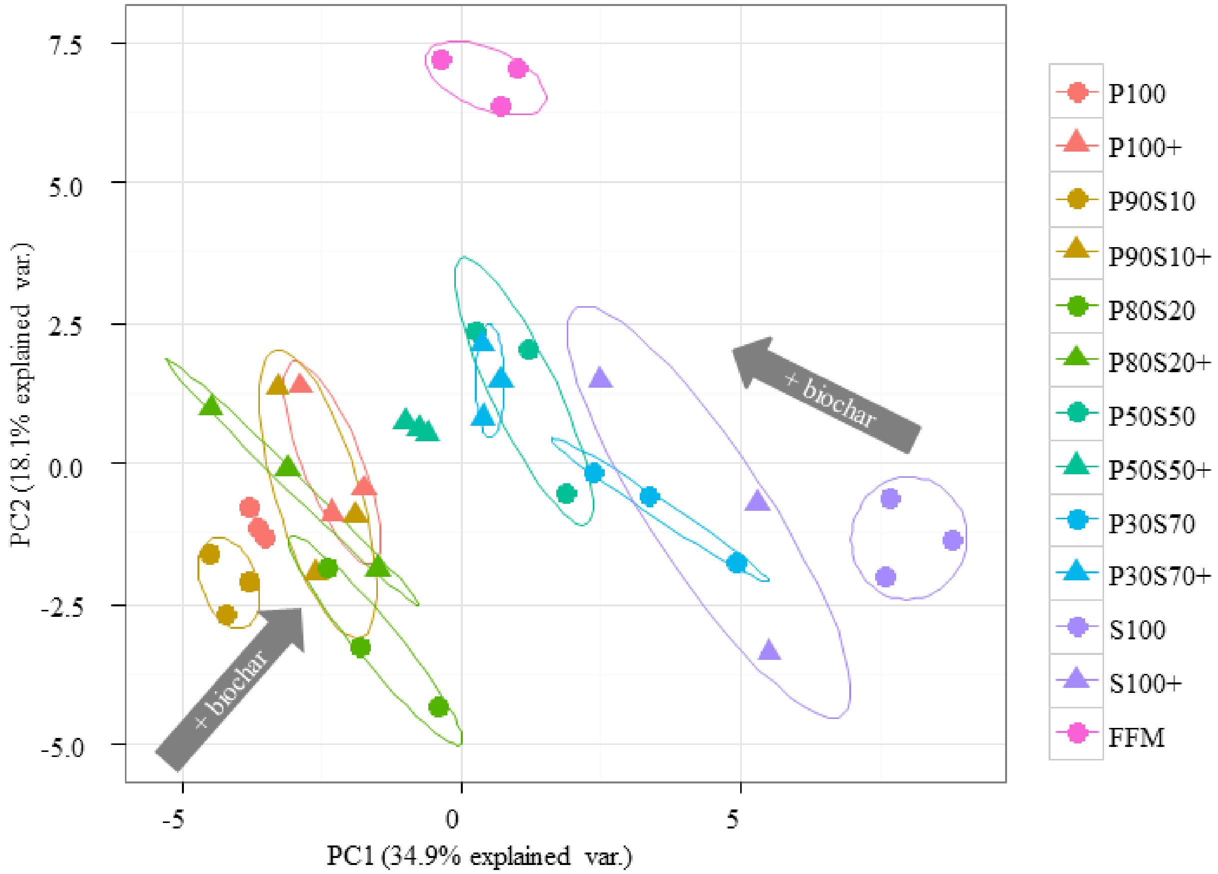

Figure 4-3: Principal Component Analysis (PCA) of standardized bioavailable nutrients (PRS™) (n=3), soil organic matter stability (Q2/Q1 ratio) (n=3) in pots with no tree and with and without biochar amendment, and equivalent averaged foliar element concentrations and biometric characteristics (biomass, height). Treatments marked with (+) indicate biochar amendment at a rate of 10MT/ha. Colored ellipses represent 68 percent confidence interval for each treatment. Arrows indicate a trend for a shift with biochar amendment for treatments P 100, P90S10, P80S20 (left arrow) and P30S70, and S100 (right arrow). Arrows are added for graphical purpose only.

Chapter 5 Biochar affects aspen seedling growth and function of reclaimed soils in the Athabasca oil sands region

A version of this chapter is under review: Sebastian T. Dietrich & M. Derek MacKenzie. 2017. Biochar affects aspen seedling growth and function of reclaimed soils in the Athabasca oil sands region. Canadian Journal of Soil Science, 2017, 2017-0113.R3

5.1 INTRODUCTION

Oil sands mining in the Athabasca Oil Sands Region (AOSR) has disturbed more than 900 km^2 land to date, and only 1.04 km^2 of the total disturbed area has been certified reclaimed (Environment and Parks 2015). Before mining, 64% of the landscape supported wetlands, while only 23% of the land supported uplands (Rooney et al. 2012). Mining operations and subsequent reclamation will convert wetlands to upland forests at the expense of peatlands (Rooney et al. 2012), and peat is being used as a major cover soil component for the establishment of upland forests. Heterotrophic peat decomposition in upland forests is known to be a component of soil respiration and is influenced by peat source and quality (e.g., feather or sphagnum moss peat, or state of decomposition) (Dioumaeva et al. 2002). Recent research shows that due to global warming, soil respiration will significantly increase (Pries et al. 2017), and peat placed in uplands will likely contribute to an increase in soil respiration rates in the post-mining landscape, leading to a destabilized C cycle.

Hernandez-Soriano et al. (2016) reported that biochar addition can enhance carbon storage in soils and one mechanism behind this phenomenon might be the adsorption of polysaccharides to aromatic C. Decomposition rates of biochars have been described as some of the slowest known for natural organic compounds (Kuzyakov et al. 2014). It is acknowledged that biochar also reduces the overall decomposition of soil organic matter (SOM), and it is suggested that biochars can remain stable in soils for centuries and improve soil carbon sequestration (Wang et al. 2016). For that reason, biochar may stabilize peat used as a surrogate for SOM in upland reclamation, but this needs to be evaluated before recommendations can be made about best practices.

Biochar addition to soil also significantly reduces leaching of plant nutrients, such as P and N (Laird et al. 2010). In some ecosystems, it has been clearly demonstrated that biochar amendments increase plant productivity due to increased aeration and water holding capacity, improvement of microbial activity, plant nutrient status of soils, and soil chemical properties (Laghari et al. 2016). Studies involving biochar application in forest restoration are few, but one meta-analysis showed a mean increase of 41 % in total woody plant biomass indicating a potentially large effect on tree growth (Thomas and Gale 2015).

Studies evaluating potential benefits of biochar amendments to cover soils used in the AOSR are rare, but some evidence suggests overall improvements of peat mineral mix (PM), (Dietrich et al. 2017), which is the material sourced from lowlands and used for upland mine reclamation in the AOSR (described below). The objective of this study was to further investigate the effect of peat biochar as an amendment to PM and forest floor-mineral mix (FFM) in regard to seedling growth, soil and plant nutrient status, soil respiration, rhizosphere polysaccharide concentration, and SOM stability. Forest floor-mineral mix is a cover soil sourced from upland forests and is known to support the reestablishment of native forest vegetation in the AOSR more successfully (Mackenzie and Naeth 2010). We hypothesized that a biochar amendment to PM would affect a variety of ecologically important parameters in the post-mining landscape (Figure 5-1).

5.2 MATERIALS & METHODS

5.2.1 Experimental design

A completely randomized greenhouse experiment was set up in March 2015, where aspen (*Populus tremuloides Michx.*) seeds were germinated and grown for six weeks in Styroblock Containers (Beaver Plastics, Acheson Alberta ID Code 540/8) filled with Sunshine® Peat Moss. An 8ml container size was chosen to reduce the transfer of nutrients into reclamation cover soil. All seedlings were fertilized two weeks after germination with 11-41-8 (NPK) tree seedling fertilizer (Agrium Advanced Solutions) according to manufacturer instructions. Pots used for the trial were 20 cm in diameter and 10 cm in height, for a total volume 2.42 liter. Pots were filled with either PM, PM (+), FFM, or FFM (+), where (+) symbol indicates treatments with biochar. FFM was chosen a control material for comparison due to the by Mackenzie and Naeth (2010) described characteristics in the reclamation environment. Before seedling transfer from styroblock containers to treatment pots, treatment pots were incubated in the greenhouse for two weeks. Pots were watered with deionized water during the incubation period and during the experiment to maintain 80 % field capacity. In mid-April, six weeks after seedling emergence, plants were transferred into pots containing different treatments. Additional lighting was set to 16 h per day (6:00-22:00 hours). Pots were rotated in the greenhouse weekly to avoid potential effects of microclimate variation. Each treatment was replicated six times with trees and three times without trees for analysis of soil bioavailable nutrients. The experiment ended in mid-July, after 12 weeks of growth in treatment pots and after 18 weeks of total seedling growth.

Forest floor-mineral mix was salvaged from the LFH horizon and the top 10-15 cm of A/B horizon from coarse-textured upland type **a** or **b** ecosites. Type **a** ecosites are characterized by xeric to subxeric moisture regimes and poor to very poor nutrient regimes; type **b** ecosites are characterized by submesic to subxeric moisture regimes and medium to poor nutrient regimes (Beckingham and Archibald, 1996). PM was salvaged from a **j** ecosite (poor fen), which is characterized by an intermediate nutrient regime and a subhydric moisture regime, as well as from a **k** ecosite (rich fen), which is characterized by a rich nutrient regime and a subhydric moisture regime within Aurora North mine. Soils of both ecosite types are composed of OM derived from sedges, golden, tufted and brown moss, and peat moss (Beckingham and Archibald 1996). Increased mineral components of PM are a result of including underlying mineral horizons when salvaging the material. All cover soils were homogenized by mixing in a clean cement mixer for 10 minutes. Basic soil characteristics are reported in Table 5-1.

Peat biochar was produced from peat salvaged in the Aurora North Mine with a total C content (TC) of 17.93 % and a total N content (TN) of 0.92 %. The material was pyrolized by gradual carbonization in a muffle furnace in a reduced oxygen environment (tinfoil wrapped and submerged in sand bed). The peat was charred by fast-ramping (30 minutes) to a temperature of 500°C where it was held for one hour. The biochar was of fine structure and had a BET (Brunauer–Emmett–Teller) surface area of 23 m^2 g^{-1}, a TC content of 23.58% (C), a hydrogen content of 0.78% (H), and oxygen content of 5.78% (O). Each pot received 32.2 g of biochar, equivalent to 10 MT biochar/ha mixed into the treatment soils. Biochar was added to pots individually to ensure consistent application rates and thoroughly mixed in.

5.2.2 Soil respiration

Soil respiration was measured weekly using a LiCor LI-8100 Soil Gas Flux System in combination with a LiCor LI-8150 Multiplexer (LI-COR, Lincoln, Nebraska, USA) and a custom made closed chamber system. Chambers were made out of PVC pipe, and caps with fitted fixtures were connected to the multiplexer, measurements of the headspace were controlled weekly. The chamber atmosphere was equilibrated with atmospheric conditions 45 seconds before and after measurements. Two measurements were taken per measurement cycle in between which the measurement chambers were equilibrated. Observations were averaged. This set-up allowed measurements within a comparable time frame to avoid influences of changes in daily temperature and moisture regimes. Respiration was measured in pots with no trees only (n=3) to exclude autotrophic root respiration, as variable growth rates of seedlings would have affected soil respiration measurements.

5.2.3 Bioavailable nutrients

Bioavailable nutrients were measured by installing Plant-Root-Simulator™ probes (PRS) in pots with no trees (n=3) according to the manufacturer instructions (Western AG Innovations, Saskatoon, SK). PRS™ probes employ membrane-bound ionic exchange resins and are designed to capture nutrient anions and cations available in the soil solution. Probes were incubated in situ for one week period prior to the end of the experiment. Upon removal from the pots, probes were rinsed with de-ionized water and sent to Western Ag Innovations. The probes were extracted with 0.5 M HCl and analyzed by colorimetry (FIAlab 2600) for NH_4^+ and NO_3^-, and by ICP-OES (Perkin Elmer ICP-OES 8300) for Al, B, Cu, P, K, Mn, N, Zn, Fe, Mg, Ca, and S.

5.2.4 Laboratory analysis

SOM content was estimated by loss on ignition (n=3) (Nelson and Sommers 1996), soil pH and EC were measured in aquatic solution (1:5 soil to water) (n=3) (Mclean 1982). TC was measured following combustion (Nelson and Sommers 1996) as well as TN was measured following combustion (n=3) (Bremner 1996).

At the end of the experiment, trees were harvested, roots were washed, and soil samples were collected. Plants were dried to a consistent weight at 65°C and biomass were recorded. Leaves were ground up using a Ball Mill MM200 (Brinkmann Retsch). Elemental analysis of foliar material was conducted after its digestion with 65% HNO_3 in a ThermoFischer MARS 5 microwave digester. Foliar, soil, and biochar C and N were measured by dry combustion (COSTECH 4010 Elemental Analyser) and Al, B, Cu, P, K, Mn, Zn, Fe, Mg, Ca, Mo, Ni, Co, Na, Cr, S, As, Cd, and Pb were measured using ICP-OES (ThermoFischer iCap 6300 Duo).

Soil samples were dried at 60°C to a consistent weight. All samples were sieved to remove plant debris, rocks and other soil components greater than 2.0 mm and then ground using a Ball Mill MM200 (Brinkmann Retsch). Samples were transferred to labeled 20-mL scintillation vials and stored in a cool, dry place before analysis.

Soil organic matter stability was measured on pots with trees (n=6) using Differential Scanning Calorimetry (DSC; STA 6000, Perkin Elmer) to test potential effects on plant growth. Approximately 20 mg of each sample were loaded in an open ceramic pan under an oxidative atmosphere (flow rate: 20 mL min-1 Oxygen gas and 80 mL min-1 Nitrogen gas) at a scanning rate of 20°C min-1. The heat of combustion [Q (J g^{-1})] was determined by integrating the DSC curve over the exothermic region (150° - 550°C). Within this region the area on the DSC curve was divided into three groups based on their degree of resistance to oxidation: (1) labile organic matter (150°C - 375°C), (2) recalcitrant organic matter (375°C - 475°C) and (3) highly-recalcitrant organic matter (475°C - 550°C). The ratios were modified following (Merino et al. 2014; 2015; 2016). Following Merino et al. (2014) the partial heats of combustions were designated as Q1, Q2, and Q3. Soil thermal stability was calculated and defined as a ratio of recalcitrant (Q2) over the labile organic matter (Q1), which is Q2/Q1, and in this manuscript is further referred to as SOM stability.

For rhizosphere polysaccharide analysis, rhizosphere soil was collected directly following harvest and stored at -80°C until analysis. Rhizosphere soil was sampled following a modified handshaking procedure following Barillot et al. (2013). Total polysaccharides were analyzed following hydrolysis with H_2SO_4 and reaction with phenol using a spectrophotometer at 490 nm (Lowe 1993). We used the rhizosphere polysaccharide concentration as an indicator for root exudation, as polysaccharides are known as high molecular weight (M_r) exudates (mucilage) (Walker et al. 2003).

5.2.5 Statistical analysis

Significance testing was conducted in R 2.1.5 (R Core Team 2012) using permutational ANOVA with the lmPerm package (Wheeler 2010) and post- hoc testing was conducted with Tukey HSD using the agricolae package (de Mendiburu 2014) with an alpha of 0.05. Permutational ANOVA was chosen for analysis to account for violation of ANOVA assumptions (normality) and sample size. Correlation with growth was tested with the Spearman’s rank based correlation test in R3.3.1 (R Core Team 2016), to account for outliers and data distribution. Additionally, cover soil treatments were tested separately for correlation of selected parameters with growth to identify biochar effects within treatments.

Multivariate statistics, such as ordination, allowed us to examine the interaction of variables related to ecosystem function. Principal component analysis (PCA) was carried out with the ggbiplot package (Vu 2011) in R3.3.1 (R Core Team 2016). Data were standardized and normalized before PCA by log transformation and adding a constant (log10(x+100)). Boxplots and bar graphs were created with ggplot2 (Wickham 2009). A Welch two-sample t-test was conducted in R3.3.1 with an adjusted alpha of 0.1 to identify biochar effects on soil respiration within treatments only. Alpha was adjusted to account for higher variation of respiration caused by changes in temperature and moisture content over the course of the experiment, which both affect soil respiration significantly.

5.3 RESULTS

5.3.1 Growth

Aspen seedlings showed significant better height growth and biomass production on both FFM treatments than on PM without biochar (Table 5-3, Figure 5-2). PM (+) could support height growth that is statistically not different to FFM (Table 5-2, Figure 5-2). Accumulation of dry plant biomass on PM was increased 200 % with biochar amendment. A positive trend on seedling biomass productivity could be observed with biochar amendment on FFM (Figure 5-2).

5.3.2 Total polysaccharides rhizosphere soil

The Polysaccharide concentration in rhizosphere soil was significantly higher in PM treatments compared to FFM, and more than 100 % greater (Table 5-2). Biochar amendment had no significant effect on rhizosphere polysaccharide concentration in PM and FFM. However, for both treatments, a weak trend for an increase of rhizosphere polysaccharide concentration with biochar amendment could be identified (Figure 5-2).

5.3.3 Bioavailable macro nutrients

The nitrate availability was significantly higher in both PM treatments than in FFM without biochar amendment (Table 5-2). Biochar amendment had no significant effect on the bioavailable NO_3^- in PM but had a significant effect in FFM (+) where the NO_3^- bioavailability was increased to levels comparable to PM. NH_4^+ showed no significant differences in PM and FFM but was increased with biochar amendment in FFM (+) (Table 5-2). P availability was significantly higher in FFM, biochar had no significant effects but increased P availability (Table 5-2). In PM bioavailability of P was slightly decreased with biochar amendment (Table 5-2). Differences in K availability were nonsignificant in all treatments, but biochar had a positive effect on K availability in PM and FFM (Table 5-2). S availability was significantly higher in PM with and without biochar amendment compared to FFM without biochar (Table 5-2). The amendment of biochar increased the S availability in FFM significantly, to a level comparable to PM (Table 5-2). Mg availability was not significantly different in PM and FFM (Table 5-2). Biochar increased the availability of Mg in PM and FFM slightly (Table 5-2). Ca availability was significantly higher in both PM treatments, no significant effect of biochar amendment on Ca availability could be identified in PM (Table 5-2). FFM without biochar had the lowest Calcium availability (Table 5-2). The amendment of biochar to FFM increased the Calcium availability significantly (Table 5-2).

5.3.4 Foliar nutrient concentration and stoichiometry

Nitrogen showed no significant differences in foliar leaf concentration (Table 5-2) but was significantly negatively correlated with growth when considering the entire dataset (rho -0.68) (Table 5-3).

Significant differences in foliar nutrient concentrations could be detected for P, which was lowest in PM (+), followed by PM, FFM, and FFM (+) where foliar phosphorus concentrations were highest (Table 5-2). A significant negative correlation between growth and foliar P concentration (rho -0.66) could be identified for PM treatments, trees that had greater biomass had a lower foliar P concentration. For this reason, it can be anticipated that phosphorus availability was not a limiting factor in PM. However, when considering the proportion of P to N (Table 5-4), it can be observed that in relation to N PM treatments showed significantly lower N/P ratios compared to FFM treatments. In regard to the N/P ratio, all treatments are considered to have an insufficient P supply. Biochar amended treatment FFM (+) reached almost sufficient P supply rates in regard to the N/P ratio (Table 5-4).

Potassium was significantly lower in PM without biochar application than in the PM (+) (Table 5-2). The concentration was for both PM treatments not significantly different to the FFM treatments, in which the biochar amendment did not affect K concentration significantly (Table 5-2). The concentration of K was significantly positive correlated with growth in PM treatments, where the treatment group with biochar amendment showed higher concentrations (rho = 0.66) (Table 5-3). In regard to the N/K ratio, both PM and FFM without biochar showed insufficient amounts of K (Table 5-4). The foliar N/K stoichiometry of trees grown PM was significantly different to biochar amended PM and FFM treatments (Table 5-4). The cation quotient of K/Ca was in PM without biochar significantly lower compared to PM (+) and indicated deficiency

(Table 5-5). K/Mg quotient was significantly higher in PM (+) compared to all other treatments (Table 5-5). The K/Mg quotient was not significantly different for PM treatments without biochar compared to FFM and FFM (+) (Table 5-5). The amendment of biochar to PM lead to a foliar K/Na ratio in PM (+) that was similar to FFM treatments (Table 5-5). In PM the ratio was lower as in PM (+), which showed a more than 100% greater value (Table 5-5). In FFM the amendment of biochar did not affect the K/Na ratio significantly (Table 5-5).

Foliar S concentration was lowest in FFM, which without biochar was significantly lower than PM (Table 5-2). The amendment of biochar to FFM increased the S concentration to a level that was not significantly different to PM treatments. S concentration was significantly negative correlated with growth in PM (rho = -0.58) (Table 5-3). Plants with increased growth had lower foliar S concentrations.

Foliar Mg concentration was significantly higher in FFM treatments than in PM (+) and was not significantly different with PM (Table 5-2). The reducing effect associated with biochar amendment on the foliar Mg concentration in PM (+) compared to PM was nonsignificant (Table 5-2). No significant correlations of Mg concentration and growth could be identified in FFM and PM treatments (Table 5-3). The N/Mg ratio indicated optimal Mg status for PM, FFM, and FFM (+) and sufficient status for PM (+) (Table 5-4). The N/Mg ratio was significantly lower in PM (+) compared to FFM treatments (Table 5-4).

Calcium showed significant differences for the concentration in leaves in FFM and PM without biochar (Table 5-2). A nonsignificant decrease in foliar Ca concentration in PM, and a nonsignificant increase in FFM could be observed with biochar amendment (Table 5-2). No correlation with growth could be identified for the foliar Ca concentrations (Table 5-3).

Foliar Na concentration was significantly lower in FFM treatments compared to PM without biochar, which was characterized by a foliar Na concentration, which was more than 100 % higher as both FFM treatments (Table 5-3). Amendment of biochar to PM resulted in lower foliar Na concentrations, which were nonsignificant different to the Na concentration in FFM with and without Biochar (Table 5-2). Foliar Na concentration showed a significant, strong negative correlation with growth in PM treatments (rho = -0.7762) (Table 5-3). The negative effect of Na was still significant when considering the entire dataset for correlation analysis (Table 3). Plants with decreased growth showed a higher foliar sodium concentration (Table 5-2).

Molybdenum was included in the permutational ANOVA analysis (Table 5-2) as PCA results showed a correlation with growth in PM (Figure 5-5). No significant differences could be identified for foliar Mo concentrations (Table 5-2). However, a significant positive correlation (rho=0.5245) could be identified for FFM treatments (Table 5-3).

All other micronutrients and potentially toxic trace elements (As, Cd, Pb) were measured and tested (data not shown), but no significant differences or significant correlations or effects could be identified.

5.3.5 SOM characteristics and stability

Labile SOM (Q1) absorbed significantly less energy in FFM compared to PM treatments (Figure 5-3). The amendment of biochar had no significant effect, however, showed a trend for reduced absorption of Energy in FFM (+) (Figure 5-3). In biochar amended PM treatments the upper quartile of samples and the median showed less energy absorption in biochar amended treatments (Figure 5-3). This indicates that biochar amendment decreases lability of SOM in both FFM and PM.

Recalcitrant SOM (Q2) was nonsignificant different in FFM, FFM (+), and PM. FFM treatments absorbed less heat than PM treatments what indicated a lower amount of Recalcitrant SOM (Figure 5-3). PM (+) absorbed significantly more heat compared to FFM treatments but was not significantly different to PM. PM (+) had compared to PM a lower median and lower quartile energy of integrated heat flow indicating a trend of higher amounts of recalcitrant SOM in the biochar amended treatment.

Highly recalcitrant SOM (Q3) absorbed significantly less heat in FFM treatments compared to PM treatments (Figure 5-3). Samples of FFM in the upper quartile and maximum emitted energy as the heat flow was greater zero (Figure 5-3). In FFM biochar amended samples showed a trend for higher amounts of highly recalcitrant SOM with the upper quartile, median, and lower quartile being lower in FFM (+) compared to FFM (Figure 5-3). A similar trend could be observed for Q3 in PM treatments (Figure 5-3). PM (+) had a lower median and lower quartile heat flow and absorbed more energy (Figure 5-3).

The SOM stability (Q2/Q1 ratio) was not significantly different in all treatments (Figure 5-3) but showed an increase with biochar amendment (Table 5-2, Figure 5-3). A significant positive correlation could be identified for SOM stability and growth in PM (rho = 0.59). This correlation of SOM stability and growth was of greater significance and stronger when testing all treatments of the study (rho = 0.64) (Table 5-3). In FFM and PM biochar amendment increased the SOM stability (Figure 5-3).

5.3.6 Soil respiration

Mean soil respiration was slightly higher in PM (1.28 µmol CO_2 m^{-2} sec^{-1}) without biochar then in FFM without biochar (1.27 µmol CO2 m^{-2} sec^{-1}) (Figure 5-4). The amendment of biochar showed an inverse effect in PM and FFM. In PM peat biochar amendment lead to a significant decrease of heterotrophic soil respiration to 0.95 µmol CO_2 m^{-2} sec^{-1} in PM (+), which is about 25 % reduced compared to unamended PM (Figure 5-4). Mean respiration in PM(+) was lower than in all other treatments (Figure 5-4). Soil respiration of FFM (+) was increased and about 25 % greater compared to FFM (1.01 µmol CO2 m^{-2} sec^{-1}) (Figure 5-4).

5.3.7 Ordination

To evaluate the effect of biochar amendment on growth in PM (+) compared to PM a PCA of foliar nutrient concentrations, SOM stability, biometrics, and rhizosphere polysaccharides concentration was conducted (Figure 5-5). PC1 and PC2 explained a total of 63.4% of the variation within the two treatments. Differences in between both treatments are mainly explained on the axis of PC1, which explains 41.9 % variation. Increased foliar K concentration is positively correlated with plant height and total biomass (Figure 5-5). Those parameters are leading to separation of PM and PM (+) and a shift to the right of PM (+) in the PCA (Figure 5-5), while increased concentrations of other elements (e.g., Na) were associated with PM without biochar (Figure 5-5). On the axis of PC 2, which explained 21.5 % variation, with a downward shift in the PCA for 2 data points can be observed (Figure 5-5). This shift is associated with increased rhizosphere polysaccharide concentration, total biomass, SOM stability and foliar Mo concentration. The correlation of SOM stability and growth expressed by total biomass is confirmed by the PCA as well (Figure 5-5).

5.4 DISCUSSION

5.4.1 Growth of aspen seedlings

Aspen seedlings are known to be intolerant to shade and can have negative metabolite assimilation rates under conditions with reduced light availability (Loach 1970). Following this, it can be assumed that an increased height growth of seedlings results in competitive benefits of aspen seedlings with other plants following emergence. Kneeshaw et al. (2006) have shown that aspen has a decreased growth dependent mortality with increased size classes and higher mortality rates within smaller size classes of saplings (Kneeshaw et al. 2006). Therefore, the data may indicate that trees grown on PM without biochar amendment will show increased growth related mortality in the field setting, where growth related mortality could occur in context of biological competition. In Alberta, Canada tree performance is currently being evaluated following reclamation to ensure that trees are healthy and vigorous and can survive to maturity (AESRD 2013). The PCA for PM treatments demonstrated that increased growth of biochar amended seedlings is strongly positive correlated with an increased bioavailability K and negatively with various other elements. Improving reclamation cover soil characteristics by amending biochar could be a promising approach to increase the probability that monitoring goals as described by AESRD (2013), which include acceptable trees and commercial forests will be achieved. Additionally, increased growth will increase C sequestration on reclamation sites, as woody biomass functions in northern forests as a carbon sink (Myneni et al. 2001). Future studies should evaluate the effects of a biochar amendment on growth, and overall C sequestration in field trials and evaluate the overall C balance.

5.4.2 Total polysaccharides rhizosphere soil

Amendment of biochar showed no significant effect on rhizosphere soil polysaccharide concentration. However, a weak trend for higher concentrations was seen in PM and FFM with biochar amendment. Plants exude up to 21 % of photosynthetically fixed carbon into the rhizosphere (Marschner 1995). Dicots, in particular, are known to exude phenolics under nutrient limited conditions into the rhizosphere (Marschner et al. 1987), and biochar is known to contain phenolics (Nartey and Zhao 2014). When considering the total polysaccharide concentration as a proxy for root exudation, exudation of phenolics might have followed a similar pattern. The amendment of biochar could have altered the rhizosphere biogeochemistry in a way that reduces the exudation of phenolics by plants and increased the availability of photosynthetically fixed carbon for growth. However, this could not be shown in line with this study. For polysaccharides it has also been shown that biochar amendments reduced co-localization of aromatic-C:polysaccharides-C, and this has been suggested as a mechanism for C stabilization (Hernandez-Soriano et al. 2016). This mechanism might affect the measurement of rhizosphere polysaccharides as an indicator for overall root exudation. The difference in rhizosphere polysaccharides in PM treatments with biochar amendment, which PCA proved to be correlated to total biomass, could also be related to increased exudation by plants with a greater biomass. However, non-significant differences underline the need for isotope labeling techniques in future studies evaluating the effect of biochar on root exudation.

5.4.3 Bioavailable nutrients

Adding biochar increased NO_3-N bioavailability in FFM significantly, but had no effect in PM. A higher N availability leads to increased herbaceous cover that adversely affects the establishment of woody seedlings (Davis et al. 1999), and for that reason might be counter-productive when reestablishing forest stands, especially on PM, which is characterized by a higher bioavailability of N compared to FFM. However, no significant effect on N availability with biochar amendment could be observed on PM. It has been demonstrated that biochar amendment increases the bioavailability of NH_4-N shortly following application but decrease NO_3-N concentrations consistently (Nelson et al. 2011). As our study was carried out over a short period, conclusions for long-term effects cannot be drawn, but as tree seedling mortality can be high within the first growing season (Castro et al. 2004), potential negative effects of increased N bioavailability have to be tested in a field setting.

Nelson et al. (2011) suggested that biochar can reduce the amount of exchangeable P when no P fertilization is applied. That is what we observed in the PM cover soils. However, in FFM biochar amendment lead to higher bioavailability of P. The increase of bioavailability of P could be related to increased decomposition of OM within FFM with biochar amendment. Substantial increases in forest humus decomposition have been observed when higher amounts of charcoal are present (Wardle et al. 2008b).

5.4.4 Foliar nutrient concentrations and stoichiometry

Our study showed that N supply was sufficient in all treatments and in fact was negatively correlated with growth. Foliar N concentration in all treatments was greater than the 15 mg g^{-1} dry matter concentration for adequate growth required by plants (Kirkby 2012). It can be anticipated that N availability was not a limiting factor for aspen seedling growth in this experiment, especially considering sufficient N concentration in foliage of FFM, which had the lowest N bioavailability. Davis et al. (1999) described negative effects of high N availability on tree establishment, therefore lowering N bioavailability in PM to levels similar to FFM could be an effective method to improve tree establishment in the field and should be tested. It has been suggested that growth in forests is unlikely limited by N, but rather by other elements such as P (Attiwill and Adams 1993).

Foliar P concentrations in all treatments were greater than the described deficiency value of < 1.0 mg g^{-1} for *Populus tremula* (European Aspen) (Kopinga and Van den Burg 1995), which occupies a similar niche as *Populus tremuloides*. Biochar elevated foliar P concentrations of seedlings grown in FFM to the by Kopinga and Van den Burg (1995) described optimal concentration in the foliage. The foliar N/P stoichiometry, however, suggested the insufficient availability of P for PM and FFM treatments (Table 5-4) and might indicate that aspen can cope with lower P availability, as trees with a greater growth in PM treatments showed a lower N/P ratio. In fact, the stoichiometry might be affected by an oversupply of N in PM. Biochar amendment to FFM optimized the N/P stoichiometry, which may be related to increased OM mineralization rates with biochar amendment, as described by Wardle et al. (2008a). This finding underlines the potential importance of biochar in the boreal environment, as it increases mineralization of nutrients from organic materialand improves bioavailability to plants.

A significant increase of foliar K concentration inb trees planted in PM with biochar amendment was observed. K affects important functions as enzyme activation and osmoregulation (stomatal control), and deficiencies are known to predispose plants to abiotic and biotic stresses (Hawkesford et al. 2012). Foliar cation-quotients can be used to evaluate relative deficiencies in deciduous trees (Kopinga and Van den Burg 1995), which is exactly what the molar ratio of K/Ca in PM without biochar indicated (below 0.5, Table 5-5). Adding biochar significantly increased this ratio to no deficiency. Similar observations were made for the K to N ratios in PM and FFM without biochar (Table 5-4), where ratios below 25 indicate deficiency for deciduous trees (Kopinga and Van den Burg 1995). Again, adding biochar significantly increased these ratios to non-deficient.

This corroborates the potential of biochar to optimize foliar nutrient stoichiometry as indicated for P. For plants; maintaining a wider cytosolic K/Na ratio is critical as this affects the plant's electrophysiology (Shabala and Cuin 2008). Our study shows that a biochar amendment to PM cover soil led to a wider K/Na ratio, and tree growth was highly significant positive correlated with a wider range. We demonstrated that seedlings of aspen showed decreased growth with higher foliar sodium concentrations and this would affect their ability to compete with other plants in the natural environment. Aspen as a species has low salt tolerance compared to forages (e.g., slender wheat grass) (Alberta Agriculture and Forestry 2001).

In boreal forest ecosystems, plant damage due to conditions of drought, or low temperature play an important role (Hawkesford et al. 2012), and drought-induced aspen mortality has been observed in Alberta (Hogg et al. 2008; Michaelian et al. 2011). Potassium affects plants capability to resist and cope with those factors (Hawkesford et al. 2012) and therefore should be considered in the reclamation environment for improving tree survival. The biochar amendment in this study was shown to increase foliar K concentrations and might be an effective measure to increase plant resilience and survival.

The effect of biochar on foliar K concentration might be related to changes in the rhizosphere, where it has been shown to reduce plant Na uptake and decreases osmotic stress by releasing K^+, Ca^{2+}, and Mg^{2+} (Akhtar et al. 2015). Concentrations of K^+ and Na^+ in the rhizosphere affect the activation of specific transporters in the root (Rubio et al. 1995), and the amendment of biochar might mediate this effect by changing K^+ and Na^+ ratios in the soil solution. Increased foliar Na concentrations were observed in PM, which was 100% higher compared to PM+, FFM, and FFM+. While Na can have positive effects on plant growth and health (Broadley et al. 2012b), we observed a significant negative correlation with growth. Biochar amendment reduced the foliar Na concentrations in PM treatments to a level that is similar to FFM treatments. Given the genesis of peat in lowlands and the high solubility of Na salts (e.g., NaCl) in water, increased salt concentration generated by runoff from upland sites has to be anticipated for most peat based reclamation materials. The biochar we used demonstrated an ability to mediate negative effects of Na and should be considered as an amendment to PM cover soils.

Foliar Mo concentrations increased with biochar amendment and were significantly positive correlated with growth in FFM treatments, where biochar increased the concentrations by close to 100%. Deficiencies of Mo in plants have been reported for concentrations ranging in between 0.1 and 1 $\mu g\ g^{-1}$ (Broadley et al. 2012a), and seedlings with decreased growth showed concentrations within this range. As the differences in growth were nonsignificant and due to the characteristics of the cover soil being sourced from a natural and functioning ecosystem with low fertility, this effect can be disregarded but should be monitored in long-term studies. In case of Mo deficiencies biochar produced from the in this study used peat could be suitable amendment.

5.4.5 SOM stability

While we could not identify significant differences within the different enthalpies of heat with biochar amendment (Q1, Q2, Q3) or the ratio of Q2/Q1, we identified a significant positive correlation of Q2/Q1, which we defined as SOM stability, with growth (Table 5-3). This effect could be related to the increased decomposition of SOM with biochar amendment (Wardle et al. 2008b), resulting in increased nutrient availability to plants and SOM stability. An increase in nutrient bioavailability was observed within our study, especially for bioavailable P in FFM treatments (Table 5-2), but not for PM. Findings suggest that SOM stability might be not only a proxy for C and N mineralization (Plante et al. 2011), but also an indicator for mineralization of P for soils with SOM characteristics similar to FFM. In PM, we identified an increase of available K, which could be related to increased decomposition of peat or a release from the biochar. It has been shown, that biochar amendments increase the bioavailability of K (Oram et al. 2014), but the source of P needs to be evaluated further. As there is an overlying effect with Na bioavailability in PM treatments future work should focus on SOM stability and nutrient mineralization under better-controlled conditions, using, for example, washed biochar as an amendment to PM. As the effects of biochar on the stabilization of pre-existing SOM are not currently understood (Kimetu and Lehmann 2010), future work should examine the effect of biochar on peat, which is the surrogate of SOM in upland reclamation and vast upland forest areas have to be reclaimed with this material.

5.4.6 Soil respiration

Significant differences in soil respiration were observed between PM and FFM. Soil respiration rates can be used as an indicator for decomposition rates of organic matter (Minkkinen et al. 2007) and data from our study suggests that additional factors are influencing the activity of heterotrophic organisms. Foliar nutrient data indicated increased, and potentially toxic bioavailability of Na salts in PM cover soils, which likely has affected soil osmotic potential. Microbes can adapt to low osmotic potential by synthesis of osmolytes, which is a high energy demanding process (Yan et al. 2015). This process likely explains increased respiration rates of PM without biochar amendment, while not increasing heterotrophic mineralization rates or being indicative of increased decomposition. For that reason, we are suggesting that SOM characteristics as measured with DSC might be of great importance for biologic SOM turnover when no other overlaying effects are overlapping with the activity of heterotrophs. FFM cover soil shows an increase in soil respiration rates with biochar amendment, while also showing increased mineralization of nutrients (e.g. P) resulting in increased seedling growth. Reducing soil respiration rates of PM by amending biochar might be of great importance when improving the overall carbon balance of reclamation sites, our study suggested a decrease in average soil respiration that was about 25% for PM cover soil with biochar amendment, while increasing seedlings biomass accumulation. Field studies should evaluate if heterotrophic respiration and SOM decomposition of PM reclaimed upland sites can be reduced by peat biochar amendment, as shown for agricultural sites (Wang et al. 2016).

5.5 CONCLUSIONS

Peat biochar amendments are promising for improving C sequestration in line with PM based upland forest reclamation in the AOSR. Biochar amendments can mediate negative impacts of for example increased Na bioavailability in PM cover soils. Benefits of amending biochar are likely not limited to increased C sequestration and could include important factors as increased groundwater quality and improvement of site function on a landscape scale. Positive effects demonstrated with this greenhouse study have to be verified with field trials before recommendations for reclamation practice can be made. Factors like Na bioavailability affect microbial turnover of SOM and need to be considered when evaluating thermal derived SOM stability data in the context of microbial decomposition. Our study punctuates that a holistic approach is necessary when evaluating heterotrophic turnover of SOM, meaning that all potentially the microbial activity influencing factors should be considered and underlines that effects of biochar can be soil specific. It has to be acknowledged that not all PM cover soils have comparable characteristics and generalizations in regard to the quality of PM as a growing medium for trees and native vegetation cannot be made. Individual analysis of PM cover soil and suitability tests for targeting certain plant species is recommended.

Table 5-1 Mean (±SE) of basic soil characteristics.

Treatment	Biochar	SOM (%)	TC (%)	TN (%)	pH (aq)	EC (dS m^{-1})
PM	**w/o**	9.81 (1.72)	9.00 (0.42)	0.57 (0.06)	7.8 (0.0)	0.38 (0.06)
	w/ (+)	10.88 (0.43)	9.72 (0.59)	0.57 (0.03)	7.9 (0.0)	0.39 .(0.02)
FFM	**w/o**	4.09 (0.03)	2.09 (0.02)	0.07 (0.00)	6.8 (0.1)	0.16 (0.02)
	w/ (+)	4.92 (0.01)	2.32 (0.03)	0.28 (0.02)	7.5 (0.1)	0.22 (0.01)

Note: Soil organic matter (SOM), total carbon (TC) and total nitrogen (TN) reported as a percentage of sample dry weight. pH and EC (electric conductivity); (n=3). Substrates were characterized after an incubation period of 2 weeks. PM (peat mineral-mix), FFM (forest floor mineral-mix), w/o (without biochar), w/(+) (with biochar amendment).

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Table 5-2: Comparison of mean value (±SE) of foliar nutrient concentration, bioavailable macro nutrients, SOM stability, biomass, and rhizosphere carbohydrates.

Treatment	Biochar	Selected Foliar Nutrients								Selected Bioavailable Nutrients							SOM Stability	Total Biomass	Rhizosphere Carbohydrates
		mg g^{-1}							µg g^{-1}	µg 10cm^{-2} wk^{-1}							ratio	(g dw)	mg g^{-1}
		N	P	K	S	Mg	Ca	Na	Mo	NO_3	NH_4	P	K	S	Mg	Ca	(Q2/Q1)	Mass	Carbohydrates
PM	w/o	22.29*a*	1.39*bc*	10.82*b*	2.52*a*	3.99*ab*	27.28*a*	0.24*a*	1.12*a*	89.26*a*	5.41*a*	1.13*b*	54.20*a*	139.87*a*	90.10*a*	2162.70*a*	0.29*a*	0.95*b*	22.47*a*
		(1.12)	(0.09)	(0.92)	(0.16)	(0.57)	(2.49)	(0.05)	(0.13)	(2.06)	(1.53)	(0.20)	(9.29)	(24.01)	(10.19)	(167.14)	(0.01)	(0.13)	(2.51)
	w/ (+)	21.58*a*	1.19*c*	16.35*a*	2.32*a*	2.58*b*	20.37*ab*	0.12*ab*	1.49*a*	90.57*a*	5.04*a*	0.72*b*	74.29*a*	152.79*a*	96.31*a*	2257.39*a*	0.36*a*	2.81*a*	22.60*a*
		(1.56)	(0.08)	(1.60)	(0.06)	(0.29)	(2.20)	(0.01)	(0.45)	(7.99)	(1.28)	(0.09)	(2.52)	(18.46)	(3.83)	(114.05)	(0.06)	(0.50)	(2.20)
FFM	w/o	18.88*a*	1.72*ab*	12.27*ab*	1.84*b*	4.40*a*	18.80*b*	0.10*b*	0.65*a*	30.87*b*	4.43*a*	2.50*a*	59.92*a*	64.95*b*	69.71*a*	607.58*c*	0.32*a*	2.65*a*	7.08*b*
		(1.38)	(0.10)	(0.93)	(0.10)	(0.39)	(1.50)	(0.02)	(0.10)	(3.09)	(1.48)	(0.22)	(8.68)	(6.33)	(9.23)	(52.87)	(0.02)	(0.35)	(0.56)
	w/ (+)	18.11*a*	1.97*a*	13.62*ab*	2.17*ab*	4.32*a*	21.29*ab*	0.10*b*	1.20*a*	79.69*a*	8.58*a*	3.67*a*	68.52*a*	157.03*a*	84.69*a*	1171.61*b*	0.38*a*	3.22*a*	5.79*b*
		(0.53)	(0.18)	(0.47)	(0.14)	(0.13)	(1.87)	(0.01)	(0.15)	(14.21)	(0.72)	(0.55)	(2.41)	(13.06)	(4.16)	(66.19)	(0.02)	(0.29)	(0.81)

Note: n=6 for foliar nutrients, biomass, SOM stability, and rhizosphere carbohydrates; n=3 for bioavailable nutrients, soil respiration. PRS probes were incubated in pots without trees, soil respiration was measured in pots without trees. Different letters in columns only indicate significant differences ($p<0.05$) following permutational ANOVA and Tukey HSD. Q1 labile SOM, Q2 recalcitrant SOM.

Table 5-3: Spearman's rho statistic for description of rank-based association of Growth (Total Biomass (g) DW) with for foliar nutrient concentration, molar K/Ca ratio, molar K/Mg ratio, molar K/Na ratio, SOM stability, Rhizosphere Polysaccharide concentration.

Treatment		N	P	K	S	Mg	Ca	Na	Mo	K / Ca ratio	K / Mg ratio	K / Na ratio	SOM Stability	Rhizosphere Polysaccharides
PM	**p-value**	0.2207	**0.0184**	**0.0184**	**0.0478**	0.08	0.4433	**0.0029**	0.457	0.2453	0.07536	**0.0061**	**0.0446**	0.3757
	rho	-0.3817	**-0.6643**	**0.6643**	**-0.5804**	-0.5244	-0.2447	**-0.7762**	0.03497	0.3636	0.5315	**0.7133**	**0.5874**	0.1152
FFM	**p-value**	0.3543	0.9484	0.0708	0.7292	0.9484	0.3423	0.4038	**0.04001**	0.9484	0.4168	0.5515	0.7621	0.5686
	rho	-0.2937	0.0209	0.5384	-0.1118	0.0209	0.3006	0.2657	**0.5245**	0.02097	0.2587	-0.0420	0.0979	-0.0559
Dataset	**p-value**	**0.0002**	0.2658	0.0551	**0.013**	0.6275	0.5381	**0.0068**	0.2141	0.2119	0.837	**0.0095**	**0.0007**	0.2379
	rho	**-0.6833**	0.2365	0.3965	**-0.4991**	0.1043	-0.1321	**-0.5365**	0.1695	0.26434	0.04434	**0.4748**	**0.6417**	-0.2625

Note: Separate test of PM and FFM to account for substrate-specific biochar effects. Significant correlations ($p<0.05$) are bolded; ($n=6$).

Table 5-4: Evaluation of average foliar nutrient stoichiometry (±SE) in context of nutrient supply.

Element	**Supply**			**Average proportion of foliar nutrients to N (= 100) (±SE)**			
	Optimal	**Sufficient**	**Insufficient**	**PM**	**PM (+)**	**FFM**	**FFM (+)**
N	100	100	100	100	100	100	100
P	10-14	5-10	< 5	**2.85b (0.19)**	**2.56*b* (0.22)**	**4.15*a* (0.11)**	**4.92*a* (0.41)**
K	50 - 100	25 - 50	< 25	**17.57*b* (1.68)**	27.76*a* (2.16)	**23.85*ab* (2.50)**	27.13*a* (1.49)
Mg	10	5 - 10	< 5	10.53*ab* (1.70)	7.12*b* (1.05)	13.63*a* (1.36)	13.84*a* (0.70)

Note: Grey shaded area (left): “Critical values of the proportion of nutrition elements to N (=100) in the leaves for the estimation of relative nutrient deficiency (with regard to N) for deciduous trees” (adapted from Kopinga & Van den Burg 1995); non shaded area (right) calculated average molar proportions of measured foliar P, K, Mg to N ratio, with N (=100) and standard error (±SE), values in the insufficient range bolded (n=6). PM = peat mineral mix, PM (+) = peat mineral mix with biochar (applied at a rate of 10 MT / ha), FFM = Forest Floor Mix, FFM (+) = Forest Floor Mix with biochar (applied at a rate of 10 MT / ha). Different letters in the same row indicate statistical significant differences based on permutational ANOVA and TUKEY HSD (p<0.05).

Table 5-5: Average foliar stoichiometric cation-quotients (±SE).

Treatment	Biochar	K/Ca	K/Mg	K/Na
PM	w/o	**0.44***b* (0.07)	1.95*b* (0.39)	34.69*b* (7.96)
	w/ (+)	0.91*a* (0.18)	4.51*a* (1.07)	83.30*a* (10.50)
FFM	w/o	0.69*ab* (0.08)	1.84*b* (0.26)	86.33*a* (11.81)
	w/ (+)	0.68*ab* (0.07)	1.96*b* (0.08)	78.92*a* (9.78)

Note: Values that indicate relative nutrient deficiency for K for deciduous trees following Kopinga & Van den Burg (1995) are bolded (Ca and Mg ratios only). Different letters within the same column indicate statistical significant difference based on permutational ANOVA and TUKEY HSD (n=6) ($p<0.05$).

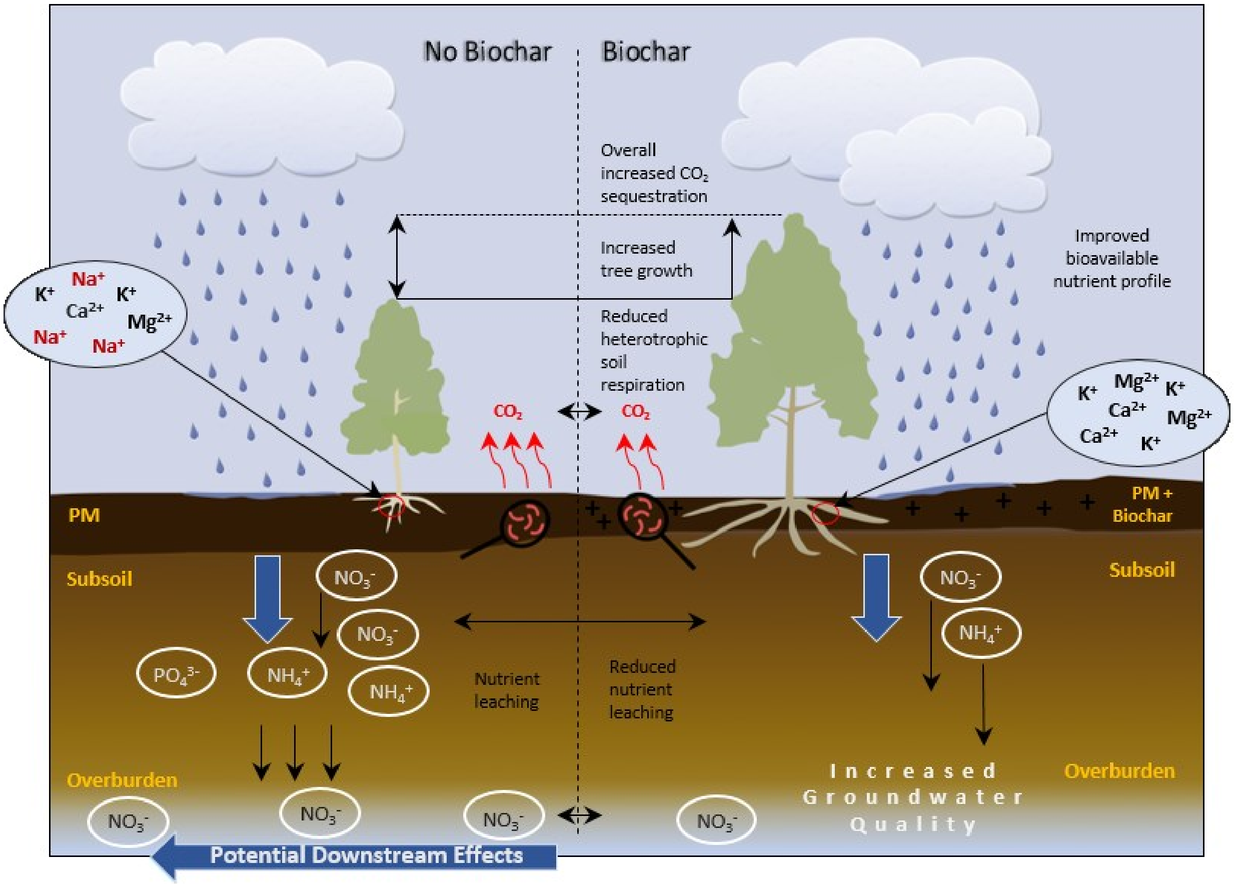

Figure 5-1: Diagram of potential hypothetical effects of a biochar amendment on peat mineral mix cover soils (PM) used commonly for upland boreal forest reclamation in the Athabasca Oil Sands Region.

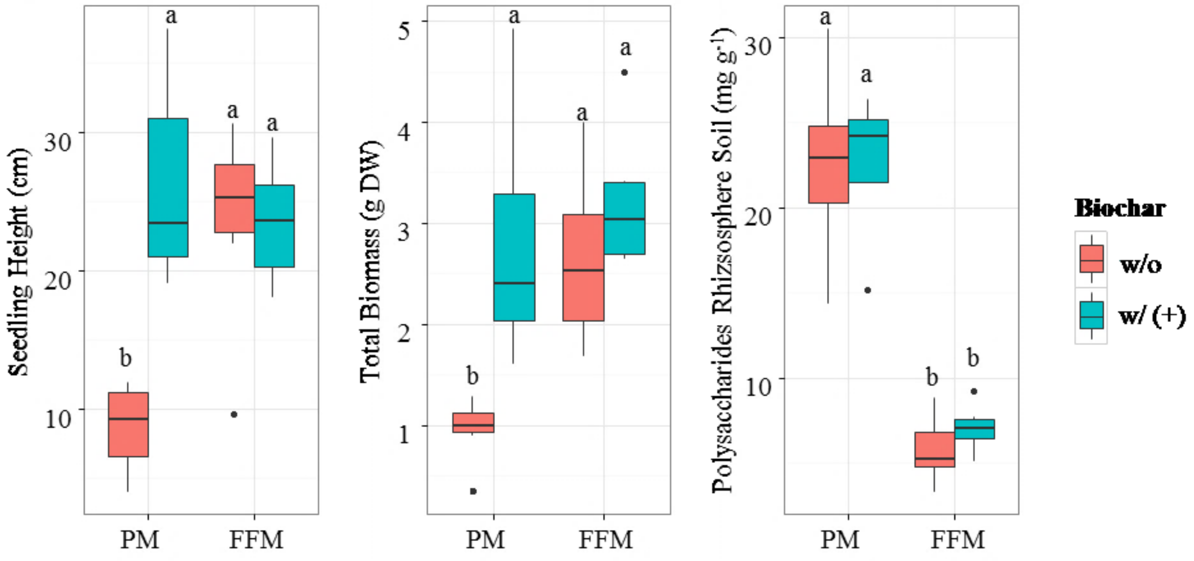

Figure 5-2: Boxplots of Seedling height (cm) (left), Seedling Biomass (g DW) (middle), Polysaccharides rhizosphere soil (mg g-1) (right), different letters indicate significant difference following permutational ANOVA and Tukey test ($p < 0.05$) (n=6). PM (peat mineral mix), FFM (forest floor mineral mix), w/o (without biochar), w/(+) (with biochar).

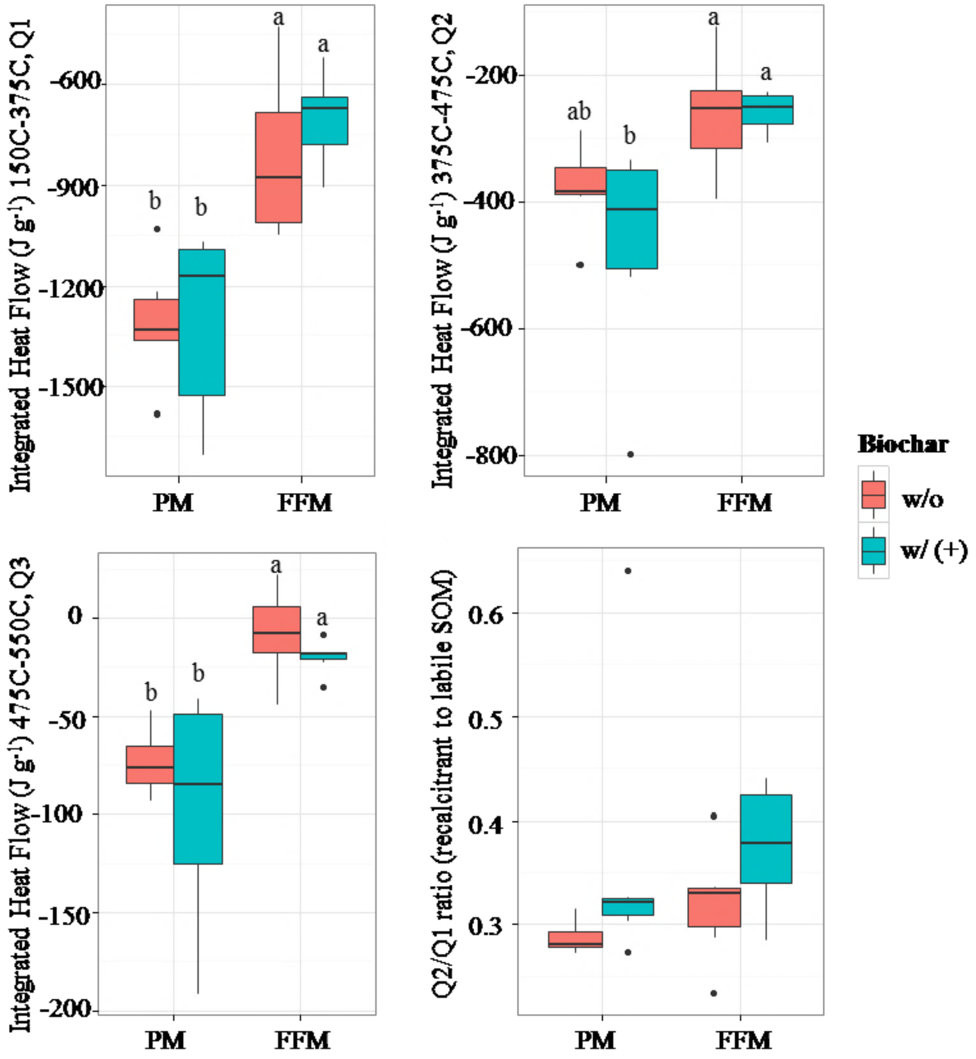

Figure 5-3: Boxplots of Heat Flow (J g^{-1}) 150 °C – 375 °C, Q1 (top left), 375 °C – 475 °C, Q2 (top right), 475 °C – 550 °C, Q2 (bottom left), and ratio of recalcitrant to labile SOM (Q2/Q1 ratio) (bottom right) (n=6), data generated with DSC, different letters indicate significant difference following permutational ANOVA and Tukey test ($p < 0.05$).

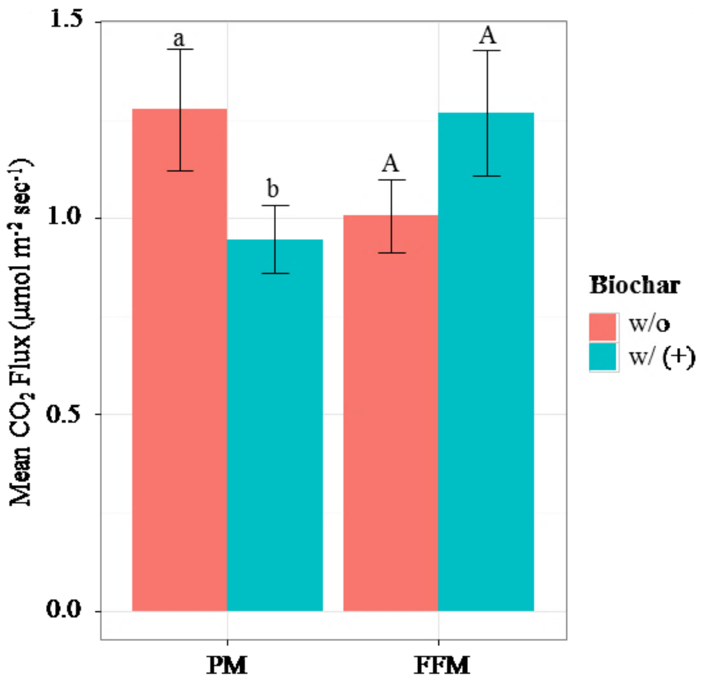

Figure 5-4: Mean CO_2 flux based on weekly measured heterotrophic soil respiration in pots with no trees (n=3), with standard error. Soil Respiration was tested with a Welch two sample t-test for a biochar effect within Treatments only ($p < 0.1$), as permutational ANOVA indicated significant differences within the treatments with alpha <0.1.

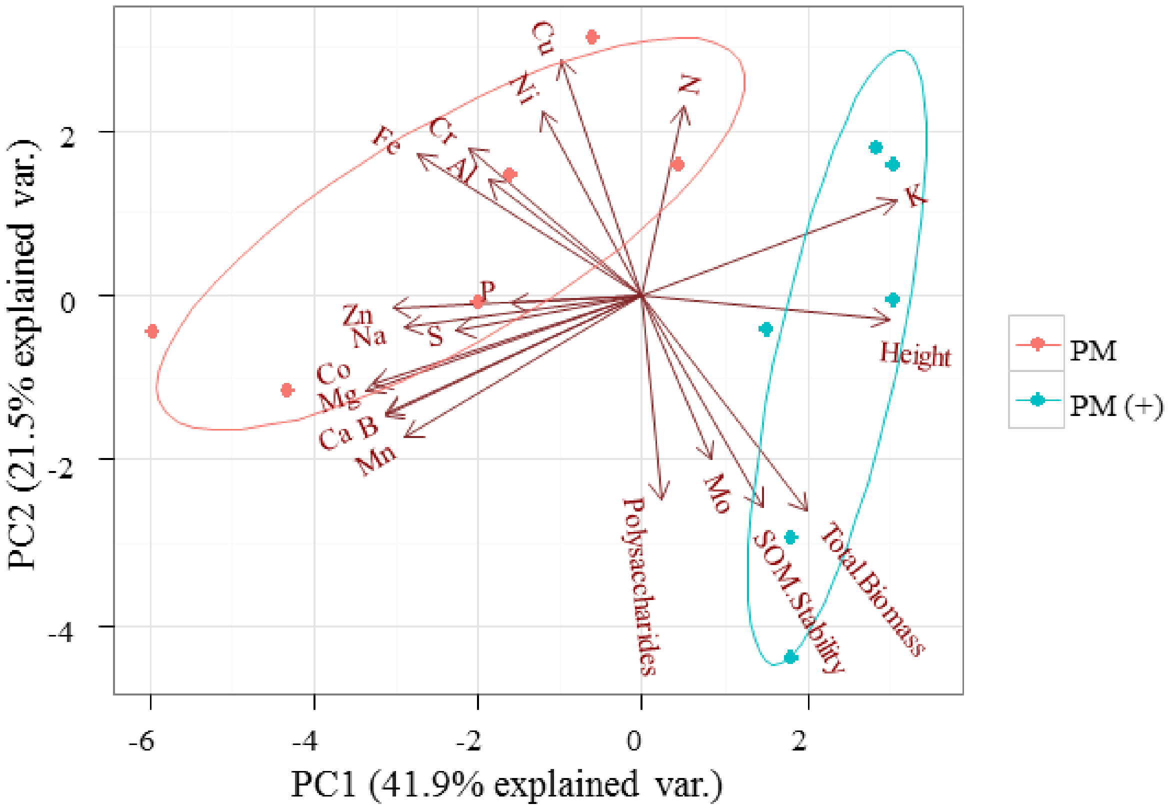

Figure 5-5: PCA of foliar element concentrations, rhizosphere soil polysaccharide concentration, SOM stability, aspen seedling (*Populus tremuloides* Michx.) total biomass and height for peat mineral mix treatments with (+) and without biochar (n=6).

Chapter 6 Conclusions and technology transfer

6.1 Synthesis of research findings

Two data chapters were explicitly targeting spatial heterogeneity of soil function in field settings and allowed the comparison of young reclamation sites to sites at various stages of recovery in Alberta's boreal zone. Two greenhouse studies focused on the improvement of PM capping materials for upland oil sands mine reclamation by admixing of mineral subsoil with peat and by amendment of biochar. Key findings of the studies are summarized below. Note the key findings are based on the results of the reclamation materials (e.g., PM or FFM) of this specific study. In reality, there is a range of characteristics for each material type in oil sands reclamation and additional work may be required to confirm that the findings of this study are applicable across the full range of soil materials in reclamation.

Chapter 2: Comparing spatial heterogeneity of bioavailable nutrients and soil respiration in boreal sites recovering from natural and anthropogenic disturbance

i. Overall nutrient profiles of reclamation sites, as well as patterns and intensity of soil respiration on reclamation sites, are different compared to natural reference sites.
ii. Spatial heterogeneity exists for some bioavailable nutrients in FFM and PM cover soil, indicating that standard placement processes can recreate spatial heterogeneity. However, key bioavailable nutrients like P were significantly different in quantity (intensity) on PM and showed no spatial heterogeneity at all.
iii. Seasonal patterns of respiration showed variability on natural reference sites and potentially on FFM, indicating that disturbance had not removed belowground function completely.

iv. PM reclaimed sites showed no strong seasonal respiration patterns indicating homogeneous belowground function.

Chapter 3: Spatial pattern of soil microbial biomass and soil respiration of boreal forest stands at different stages of recovery in comparison to young reclaimed sites

i. The spatial pattern of soil respiration is different between sites and shows reoccurring patterns on the Harvested **a/b** site potentially indicating linkage of above- and belowground function.
ii. PM reclaimed site showed large-scale spatial patterns of soil microbial biomass like a site affected by severe fire, while FFM seemed to show smaller scale spatial patterns than sites disturbed by clear-cutting and severe forest fire, potentially indicating a faster recovery.
iii. Higher rates of soil respiration were observed on PM in July, following a different seasonal pattern as other tested sites and potentially indicate that chemical oxidation is affecting respiration or that differences in the water availability cause increased rates of soil respiration on PM longer during the growing season.

Chapter 4: Building a Better Soil for Upland Surface Mine Reclamation in Northern Alberta: Admixing Peat, Subsoil and Peat Biochar in a Greenhouse Study with Aspen

i. Seedling productivity increases with admixing subsoil to PM with and without biochar.
ii. There is an overall positive effect of biochar amendment when comparing biogeochemical, growth, and foliar nutrient stoichiometry parameters.
iii. Peat-subsoil mixes do not provide sufficient amounts of P and Cu to seedlings.

iv. Lower K and Mn availability in peat-subsoil mixes need to be evaluated in further studies.

Chapter 5: Biochar affects aspen seedling growth and function of reclaimed soils in the Athabasca oil sands region

i. Seedling growth increased significantly on PM cover soil with biochar amendment.
ii. Biochar improved K nutritional status and potentially interacted with Na bioavailability in PM, affecting growth.
iii. Soil respiration decreased significantly in PM with biochar amendment and increased in FFM.
iv. Soil organic matter stability was positively correlated with tree growth and increased with biochar amendment.

6.2 Applications and implications for land reclamation

Field studies demonstrated differences in mean soil respiration, nutrient bioavailability, and microbial biomass as well as differences in spatial heterogeneity of those parameters. The FFM reclaimed site showed greater similarities to natural benchmark sites, and for that reason, it is recommended to increase the similarity of PM cover soil to FFM and undisturbed upland surface soils of target ecosites. Reestablishing spatial heterogeneity of nutrient bioavailability and of factors that drive overall biologic activity in soils might lead to faster recovery with stands that have close-to-nature conditions and are more likely to provide long-term habitat for both native flora and fauna. Our greenhouse studies demonstrated that by admixing mineral subsoil with peat cover soil characteristics of peat-based soils can be improved. In this case, with peat and subsoil of this study, an admix ratio of 1:1 was the most ideal for seedling growth response and greatest similarity to FFM. Furthermore, we demonstrated that a biochar amendment could lead to an improvement of PM cover soils and depending on the substrate can improve tree growth significantly and reduce soil respiration what could lead to increased sequestration of carbon on reclamation sites. Biochar also reduced the bioavailability of Na to plants making it a potential amendment to soils with higher salt contents and could increase the availability of materials suitable for land reclamation. This study did not determine if a conventional nutrient amendment like fertilization would have had a similar positive effect. By admixing nutrient bioavailability of P, K, Cu, and Mn could not be adjusted to close to natural conditions and addition in the form of fertilizer or with suitable soil amendments might be necessary.

In figure 6-1 we demonstrate potential steps for improvement of cover soil placement that include an admixing and additional soil testing step. We suggest admixing of mineral subsoil with peat following placement by tillage to achieve optimal PM characteristics. This step would increase cover soil heterogeneity, as placed layers of peat and mineral subsoil would have variation in placement thickness. By tillage to a depth of 20 or 30 cm an admix ratio of 1:1 could be achieved when placing 10 or 15 cm of peat or PM over suitable subsoil. The practice would reduce substrate homogeneity, which currently can be assigned to PM reclaimed sites and likely would improve tree growth and the establishment of targeted vegetation.

Other methods for optimizing the peat mineral admix ratio could include over stripping, if the underlying mineral material has suitable characteristics. The normal practice for peat salvage is to over-strip or strip to mineral contact, so that PM is created (≤ 17 % TOC). However, if the underlying mineral is not salvaged with peat if it is not conducive to creating a homogenous mix (e.g., high clay content) or has chemical (e.g., oil, carbonates) or physical (e.g., high gravel content) limitations. Excluding the mineral component in these cases may be the correct reclamation practice, however, this study has shown that incorporating at least some mineral component with the peat to create a PM can improve vegetation growth. Therefore, post-placement admixing of peat and the underlying subsoil through mechanical means such as tilling or disking may improve the soil nutrient profile of the cover soil.

Additionally, cover soils should be tested and evaluated for biogeochemical characteristics including nutrient bioavailability. The step would allow a targeted fertilization or soil amendment with biochar or other amendments to increase the cover soil similarity to natural conditions. Reestablishing more similar to natural conditions would justify the practice of targeted fertilization. The reestablishment of realized niches for targeted plant communities in line with cover soil design is achievable and should be targeted.

Implementing those changes could lead to close-to-nature conditions with greater heterogeneity in vegetation and increased native species biodiversity and reestablishment. This may lead to a higher functional value of reclamation sites with potentially increased capacities in comparison to pre-disturbance conditions. A higher functional value could result out of increased carbon sequestration by locally higher forest growth rates on improved substrates with lower rates of soil respiration. The increased value following reclamation maybe seen in increased timber production compared to pre-disturbance conditions while fulfilling multiple functions of ecological importance.

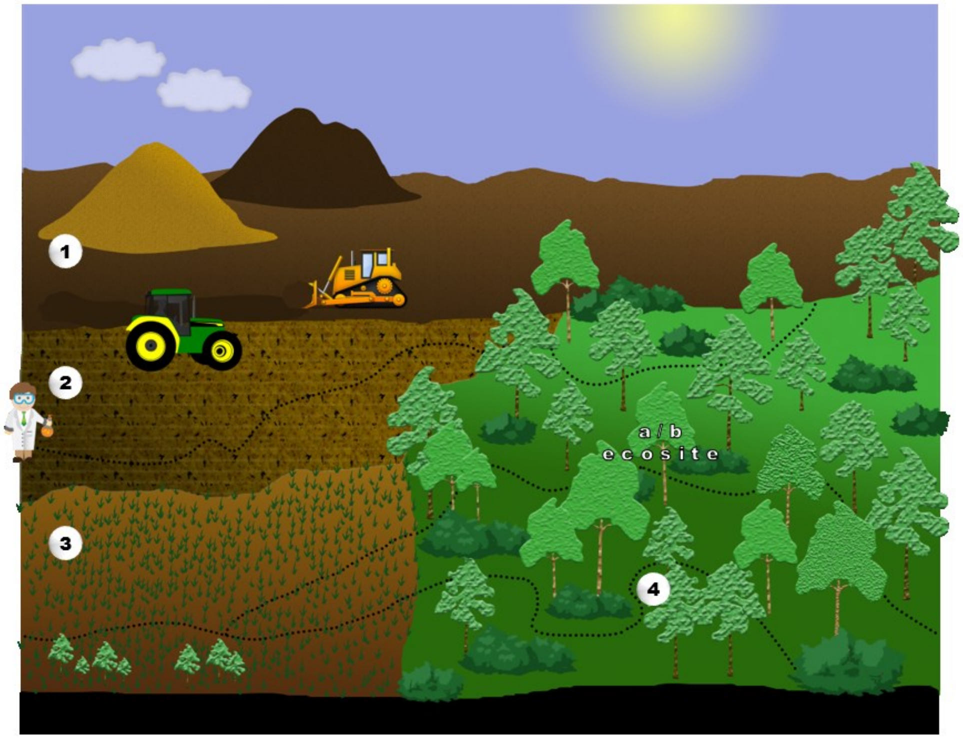

Figure 6-1: Application of study results in land reclamation following oil sands mining. 1) Cover soil placement of peat mineral mix over suitable subsoil. 2) Admixing of subsoil and peat by tillage and subsequent soil testing and evaluation. Increase of soil heterogeneity. 3) Establishment of vegetation with improved but also variable growth rates supporting heterogeneity in vegetation cover. 4) Reestablishment of forest stands with heterogenous characters and various realized niches for different types of vegetation.

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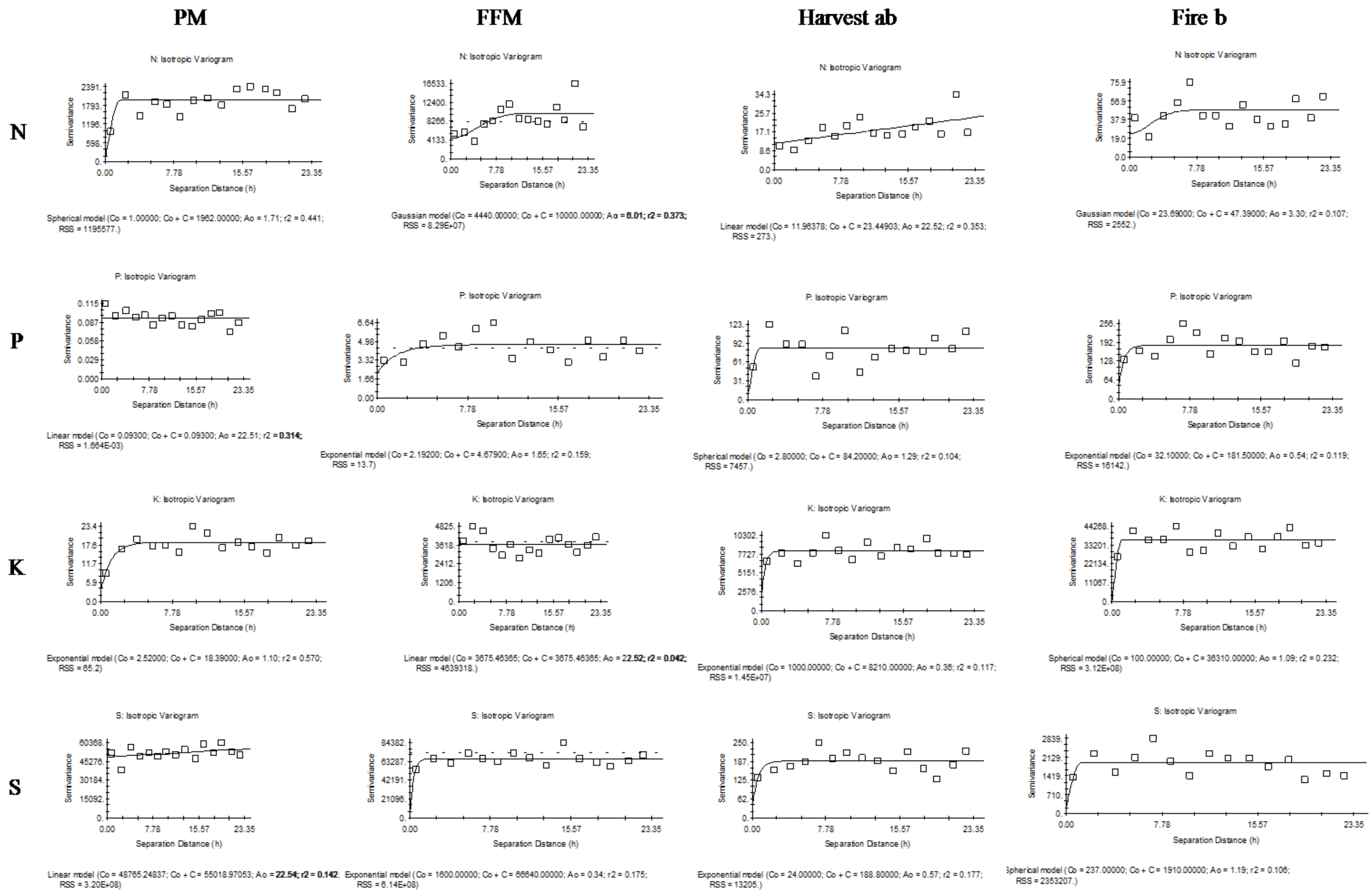
Appendix

Appendix Chapter 2



Spherical model (Co = 1.00000; Co + C = 1962.00000; Ao = 1.71; r2 = 0.441; RSS = 1195577.)

Gaussian model (Co = 4440.00000; Co + C = 10000.00000; Ao = **6.01; r2 = 0.373;** RSS = 8.29E+07)

Linear model (Co = 11.96378; Co + C = 23.44903; Ao = 22.52; r2 = 0.353; RSS = 273.)

Gaussian model (Co = 23.69000; Co + C = 47.39000; Ao = 3.30; r2 = 0.107; RSS = 2552.)

Linear model (Co = 0.09300; Co + C = 0.09300; Ao = 22.51; r2 = **0.314;** RSS = 1.664E-03)

Exponential model (Co = 2.19200; Co + C = 4.67900; Ao = 1.65; r2 = 0.159; RSS = 13.7)

Spherical model (Co = 2.80000; Co + C = 84.20000; Ao = 1.29; r2 = 0.104; RSS = 7457.)

Exponential model (Co = 32.10000; Co + C = 181.50000; Ao = 0.54; r2 = 0.119; RSS = 16142.)

Exponential model (Co = 2.52000; Co + C = 18.39000; Ao = 1.10; r2 = 0.570; RSS = 65.2)

Linear model (Co = 3675.46365; Co + C = 3675.46365; Ao = **22.52; r2 = 0.042;** RSS = 4639318.)

Exponential model (Co = 1000.00000; Co + C = 8210.00000; Ao = 0.36; r2 = 0.117; RSS = 1.45E+07)

Spherical model (Co = 100.00000; Co + C = 36310.00000; Ao = 1.09; r2 = 0.232; RSS = 3.12E+08)

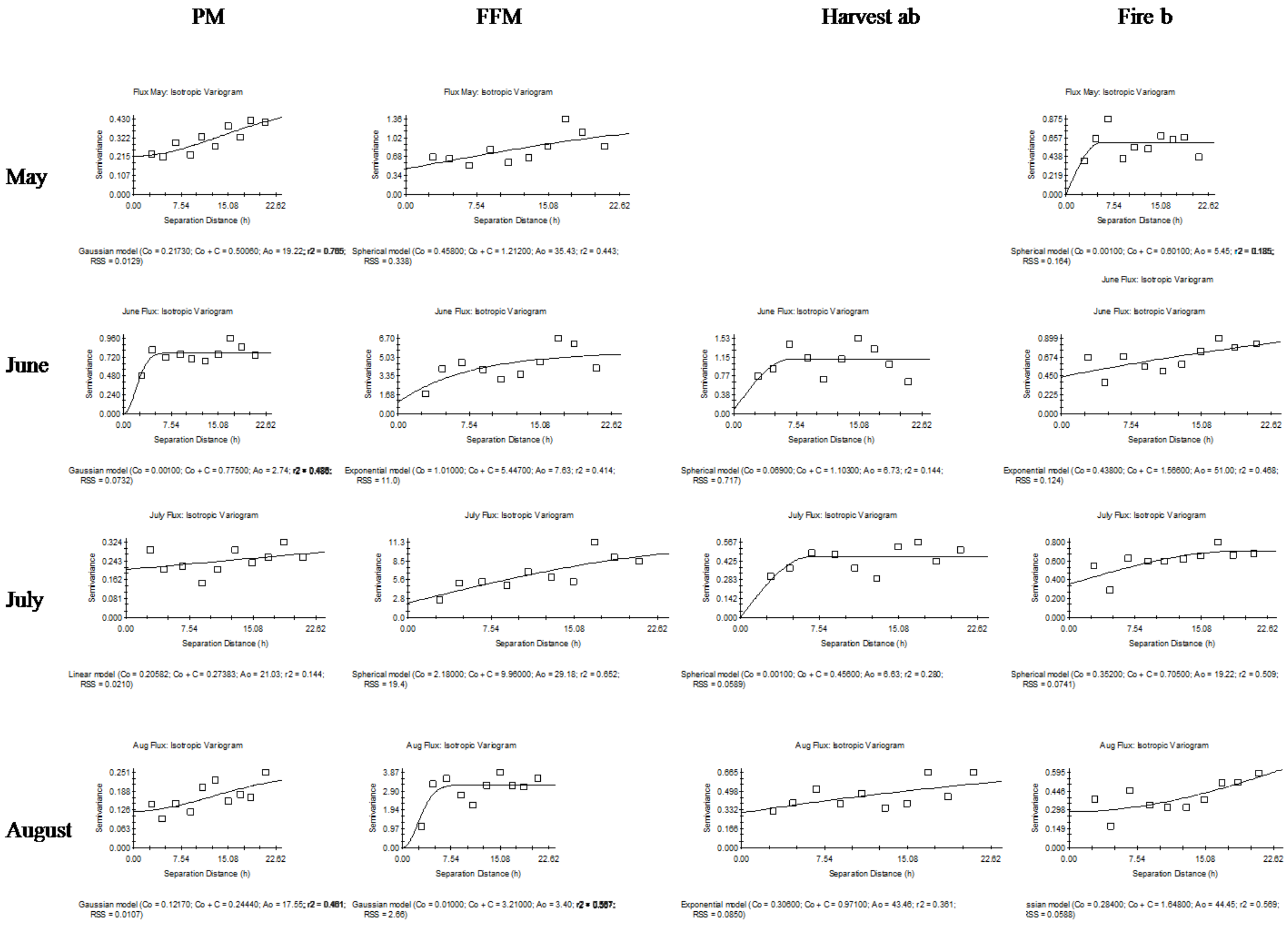
Linear model (Co = 48765.24837; Co + C = 55018.97053; Ao = **22.54; r2 = 0.142**; RSS = 3.20E+08)

Exponential model (Co = 1600.00000; Co + C = 66640.00000; Ao = 0.34; r2 = 0.175; RSS = 6.14E+08)

Exponential model (Co = 24.00000; Co + C = 188.80000; Ao = 0.57; r2 = 0.177; RSS = 13205.)

Spherical model (Co = 237.00000; Co + C = 1910.00000; Ao = 1.19; r2 = 0.106; RSS = 2353207.)

Variogram models for nutrient bioavailability during the growing season of 2013.



Gaussian model (Co = 0.21730; Co + C = 0.50060; Ao = 19.22; **r2 = 0.785**; RSS = 0.0129)

Spherical model (Co = 0.45800; Co + C = 1.21200; Ao = 35.43; r2 = 0.443; RSS = 0.338)

Spherical model (Co = 0.00100; Co + C = 0.60100; Ao = 5.45; **r2 = 0.185**; RSS = 0.164)

Gaussian model (Co = 0.00100; Co + C = 0.77500; Ao = 2.74; **r2 = 0.488**; RSS = 0.0732)

Exponential model (Co = 1.01000; Co + C = 5.44700; Ao = 7.63; r2 = 0.414; RSS = 11.0)

Spherical model (Co = 0.06900; Co + C = 1.10300; Ao = 6.73; r2 = 0.144; RSS = 0.717)

Exponential model (Co = 0.43800; Co + C = 1.56600; Ao = 51.00; r2 = 0.468; RSS = 0.124)

Linear model (Co = 0.20582; Co + C = 0.27383; Ao = 21.03; r2 = 0.144; RSS = 0.0210)

Spherical model (Co = 2.18000; Co + C = 9.96000; Ao = 29.18; r2 = 0.652; RSS = 19.4)

Spherical model (Co = 0.00100; Co + C = 0.45600; Ao = 6.63; r2 = 0.280; RSS = 0.0589)

Spherical model (Co = 0.35200; Co + C = 0.70500; Ao = 19.22; r2 = 0.509; RSS = 0.0741)

Gaussian model (Co = 0.12170; Co + C = 0.24440; Ao = 17.55; **r2 = 0.461**; RSS = 0.0107)

Gaussian model (Co = 0.01000; Co + C = 3.21000; Ao = 3.40; **r2 = 0.567**; RSS = 2.66)

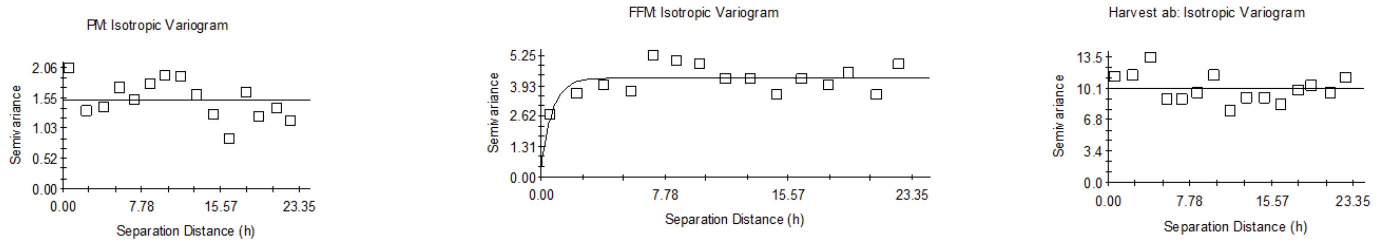
Exponential model (Co = 0.30600; Co + C = 0.97100; Ao = 43.46; r2 = 0.361; RSS = 0.0850)

ssian model (Co = 0.28400; Co + C = 1.64800; Ao = 44.45; r2 = 0.569; SS = 0.0588)

Variogram models for monthly measurements of soil respiration during the growing season of 2013.

Appendix Chapter 3

PM **FFM** **Harvested ab**

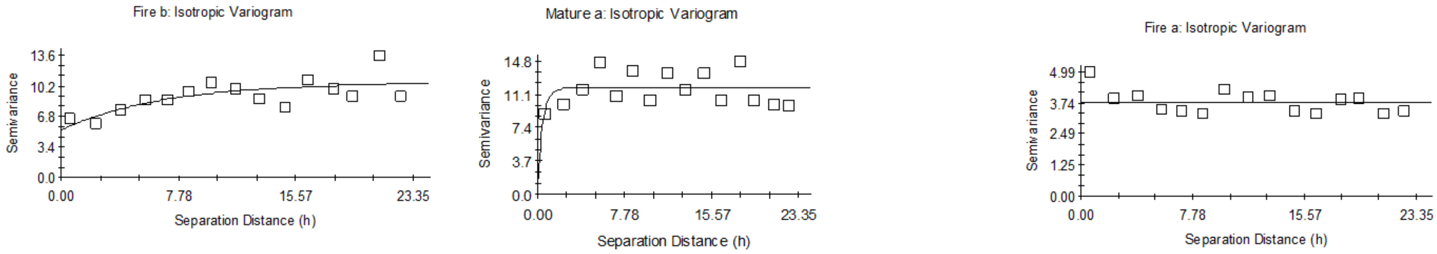


Linear model (Co = 1.52082; Co + C = 1.52082; Ao = 22.51; r2 = **0.258;** RSS = 1.55)

Exponential model (Co = 0.39000; Co + C = 4.27700; Ao = 0.72; r2 = 0.370; RSS = 4.10)

Linear model (Co = 10.13911; Co + C = 10.13911; Ao = 22.53; **r2 = 0.102**; RSS = 32.2)

Fire b **Mature a** **Fire a**

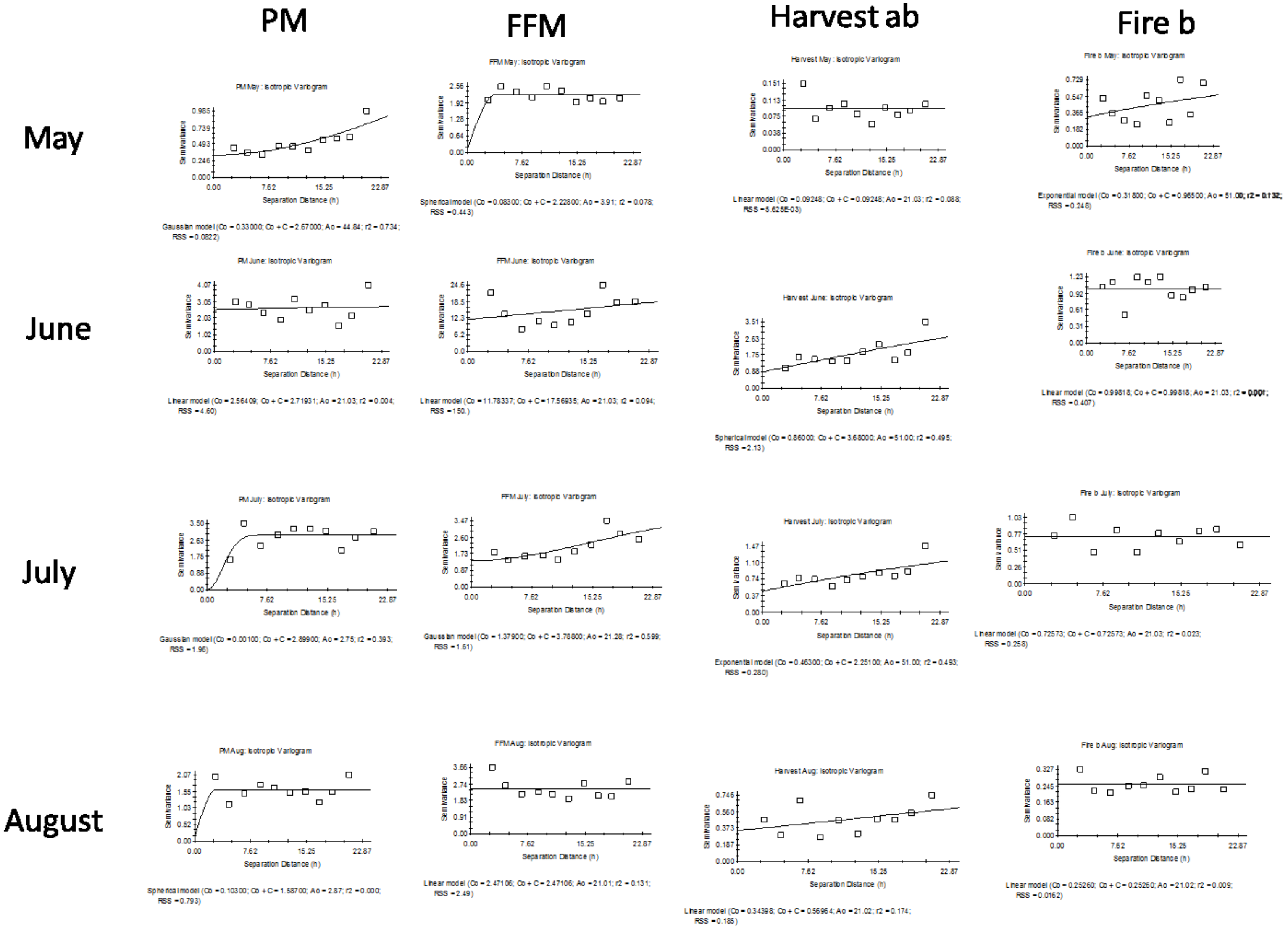


Exponential model (Co = 5.30000; Co + C = 10.61000; Ao = 6.74; r2 = 0.510; RSS = 23.5)

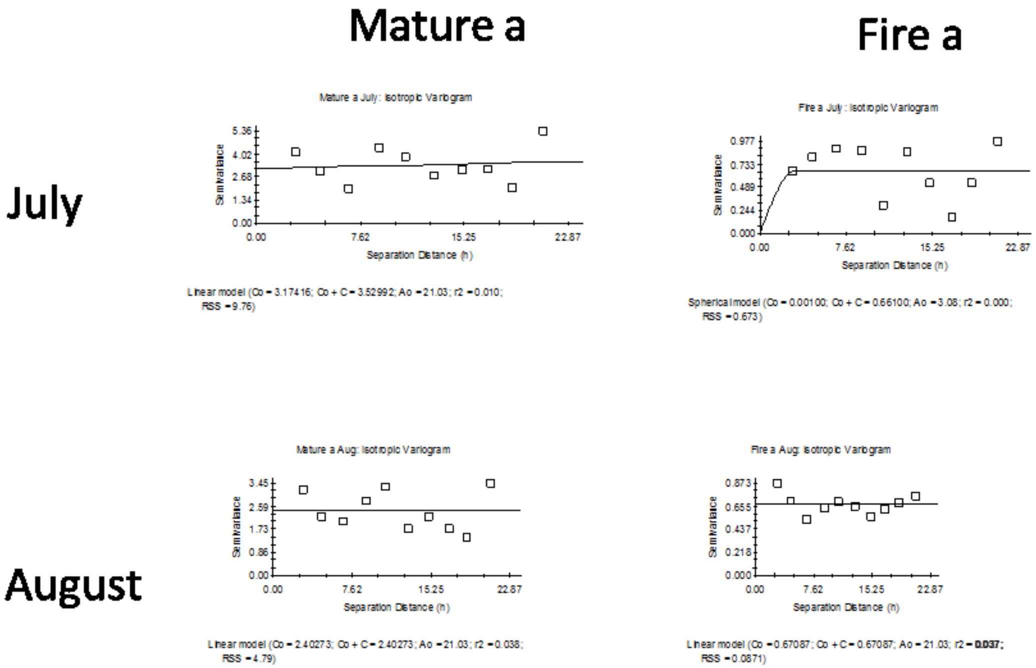
Exponential model (Co = 1.35000; Co + C = 11.85000; Ao = 0.**45; r2 = 0.152**; RSS = 42.2)

Linear model (Co = 3.78505; Co + C = 3.78505; Ao = 22.52; r2 = 0.232; RSS = 3.06)

Variogram models for microbial biomass carbon.



Variogram models for monthly measurements soil respiration during the growing season of 2014.



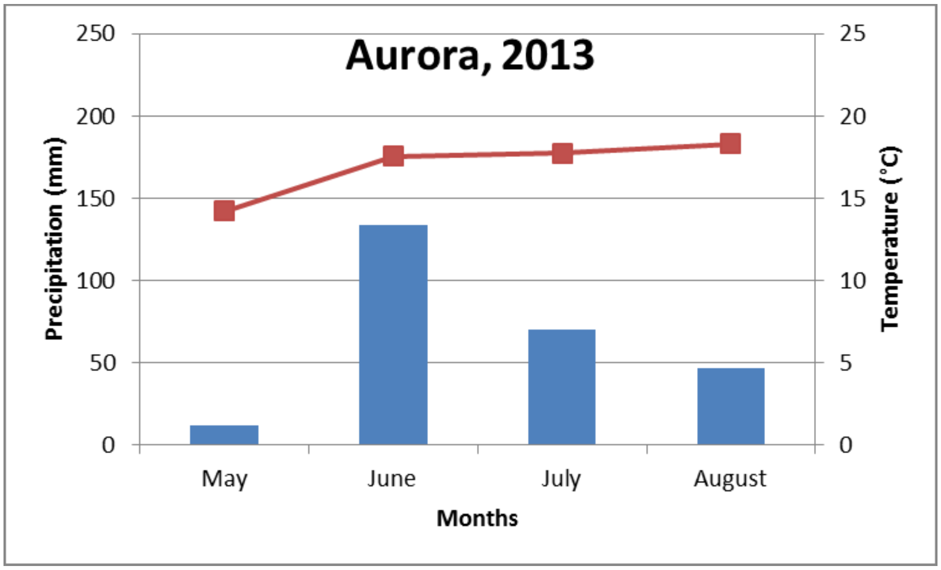
Linear model (Co = 3.17416; Co + C = 3.52992; Ao = 21.03; r2 = 0.010; RSS = 9.76)

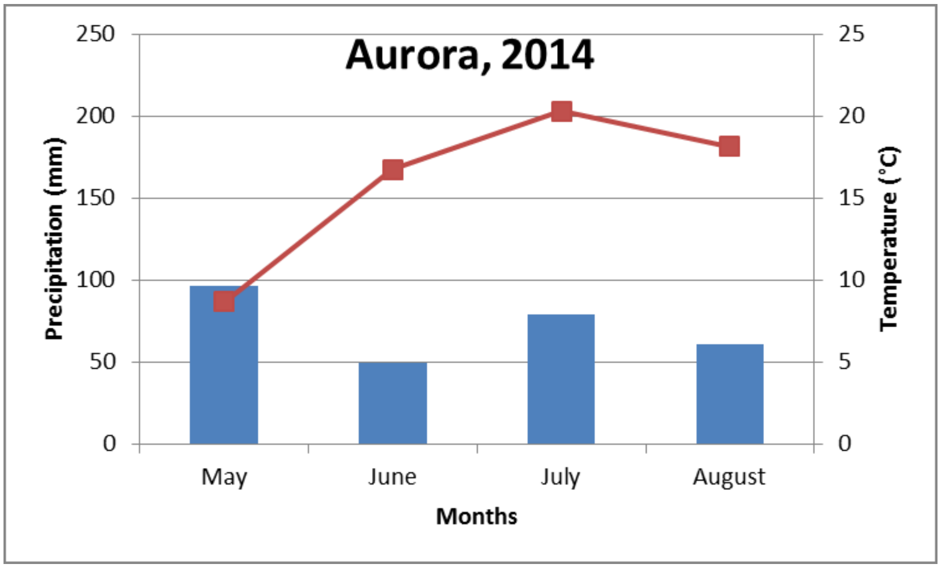
Spherical model (Co = 0.00100; Co + C = 0.66100; Ao = 3.08; r2 = 0.000; RSS = 0.673)

Linear model (Co = 2.40273; Co + C = 2.40273; Ao = 21.03; r2 = 0.038; RSS = 4.79)

Linear model (Co = 0.67087; Co + C = 0.67087; Ao = 21.03; r2 = 0.037; RSS = 0.0871)

Variogram models for soil respiration 2014.





Average precipitation (blue) and temperature (red) for the growing season of 2013 and 2014 as recorded at the Syncrude Aurora weather station.