

University of Alberta

Forest Floor Development in Reclaimed Boreal Forest Soils of Northern Alberta

by

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fulfillment of the requirements for the degree of

Master of Science
in
Soil Science

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ABSTRACT

Following oil extraction by surface mining, sites are reclaimed by reconstructing soils using salvaged organic and mineral materials, and planted to native tree species. This study assessed the influence of stand type (*Populus tremuloides* Michx., *Pinus banksiana* Lamb, and *Picea glauca* (Moench) Voss) on forest floor development, soil organic matter composition, and microbial community composition in 32 sites reclaimed 16 to 33 years ago. In three sites, spatial variability in soil nutrients was examined. *P. tremuloides* stands fostered more rapid forest floor development than coniferous stands, showed changes in soil organic matter composition with time that reflected inputs from the canopy, and was the only stand type with relationships between the canopy, forest floor, and all macronutrients. Furthermore, microbial community composition in reconstructed soils differed among stand types when canopy cover was above 30%. Canopy cover and stand type are important for reestablishment of plant-soil relationships at these reclaimed sites.

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Table of Contents

1. Introduction	1
1.1 Athabasca oil sands region and surface mining	1
1.2 Pre-disturbance vegetation	2
1.3 Forest floor characteristics	4
1.4 Government of Alberta recommendations	6
1.5 Characteristics of reclaimed soils.....	7
1.6 Spatial ecology	9
1.7 Objectives.....	10
Tables and Figures.....	12
Literature Cited	13
2. Canopy cover and stand type influence on forest floor development and characteristics in reclaimed boreal forest soils	18
2.1. Introduction	18
2.2. Materials and Methods.....	21
2.2.1. Study Area and Site Selection	21
2.2.2. Vegetation Survey and Soil Collection.....	23
2.2.3. Laboratory Analyses.....	25
2.2.4. Data Analyses.....	26
2.3. Results	29
2.3.1. Vegetation and Forest Floor Characteristics.....	29
2.3.2 Soil Organic Matter Composition	31
2.3.3 Soil Microbial Communities	33
2.4. Discussion	34
2.5. Conclusions	39
Tables and Figures.....	40
Literature Cited	47
3. Inferring processes from stand characteristics and soil nutrient patterns in reclaimed boreal forests of northern Alberta	56

3.1 Introduction	56
3.2 Materials and Methods	59
3.2.1 Study Sites	59
3.2.2 Spatially Explicit Sampling	60
3.2.3 Ion exchange resin analysis	62
3.2.4 Statistical analysis	63
3.3 Results	66
3.3.1 Spatial dependence of stand characteristics and soil nutrient supply rates	66
3.3.2 Relationships between variables after accounting for SPACE	69
3.4 Discussion	71
3.4.1 Implications of spatial structure	71
3.4.2 Comparing spatial structure among stand types	72
3.4.3 Modeling relationships between variables after accounting for SPACE on reclaimed sites	75
3.5 Conclusions	78
Tables and Figures	80
Literature Cited	89
4. Synthesis	98
4.1 Conclusions from temporal and spatial studies	98
4.1.1 Characteristics of the forest floor	98
4.1.2 Effect of canopy cover	99
4.1.3 Spatial patterns	100
4.1.4 Stand type differences	101
4.2 Project limitations and future research	102
4.3 Recommendations	103
4.3.1 Effect of canopy cover and stand type	103
4.3.2 Implications of management practices	105
4.3.3 Implications of spatial variability	106
Appendix A. LFH Survey Site Data	107
Appendix B. NMS Ordination of PLFA Profiles for Subsites	109

Appendix C. Full Models for Spatial ANOVA Analyses	110
Appendix D. Forest floor, Stand Characteristic and Soil Nutrient Supply Rate Semivariograms	112
Appendix E. Forest floor and Soil Nutrient Supply Rate Maps	116

List of Tables

Table 2-1 Distribution of time since reclamation (years) for the trembling aspen, jack pine, and white spruce reclaimed sites included in the study. Numbers in the table (1 to 45) correspond to site identification numbers, and reclamation prescriptions are shown in parentheses. Descriptions of reclamation prescriptions and materials are from Turcotte et al. (2009).	40
Table 2-2 Vegetation and forest floor characteristics of trembling aspen, jack pine, and white spruce reclaimed sites previously disturbed by oil sands mining. Data for the L, F, and H horizons represent the percent of the total forest floor thickness (cm) for each horizon. Mean values are presented with \pm one standard deviation (n=10-11).	41
Table 2-3 Regression parameters for relationships between dependent variables (forest floor thickness, soil carbon and nitrogen concentrations, and alkyl:o-alkyl ratio) and independent variables (time since reclamation, canopy cover, shrub cover, and stem density) in trembling aspen, jack pine, and white spruce reclaimed sites previously disturbed by oil sands mining. Regression parameters for canopy cover and time in each stand type were also determined. Parameters include R^2 (coefficient of determination of the regression model), and p (p-value with significance determined at $\alpha=0.05$).	42
Table 3-1 Forest floor mass (Mg/ha), soil pH, and canopy cover (# of layers) values and semivariogram results in reclaimed aspen, pine, and spruce reclaimed sites north of Ft. McMurray, AB. The range term is the distance where spatial dependence occurs, and spatial predictability indicates ability of the model to account for spatial structure in the data and predict unmeasured points on the landscape.	80
Table 3-2 Fall, spring, and summer N ($\mu\text{g}/\text{probe}/3$ months), P ($\mu\text{g}/\text{probe}/3$ months), and K ($\mu\text{g}/\text{probe}/3$ months) supply rates in aspen, pine, and spruce reclaimed sites north of Ft. McMurray, AB. Soil available nutrients were collected with plant root simulator process installed from 0-5cm in the mineral soil. The range term is the distance where spatial dependence occurs, and spatial predictability indicates ability of the model to account for spatial structure in the data and predict unmeasured points on the landscape.	81

Table 3-3 Fall, spring, and summer S ($\mu\text{g}/\text{probe}/3$ months), Ca ($\mu\text{g}/\text{probe}/3$ months), and Mg ($\mu\text{g}/\text{probe}/3$ months) supply rates in aspen, pine, and spruce reclaimed sites north of Ft. McMurray, AB. Soil available nutrients were collected with plant root simulator process installed from 0-5cm in the mineral soil. The range term is the distance where spatial dependence occurs, and spatial predictability indicates ability of the model to account for spatial structure in the data and predict unmeasured points on the landscape..... 82

Table 3-4 Aspen stand F-values and parameter estimates from spatial ANOVA models for nutrient supply rates ($\mu\text{g}/\text{probe}/3$ months) season, forest floor mass (FFM), distance to the nearest tree, canopy cover, and soil pH in a reclaimed stand north of Fort McMurray, AB. Since covariates were standardized, parameter estimates are relative to the scale of the covariate. 83

Table 3-5 Pine stand F-values and parameter estimates from spatial ANOVA models for nutrient supply rates ($\mu\text{g}/\text{probe}/3$ months), season, forest floor mass (FFM), distance to the nearest tree, canopy cover, soil pH in a reclaimed stand north of Fort McMurray, AB. Since covariates were standardized, parameter estimates are relative to the scale of the covariate. 84

Table 3-6 Spruce F-values and parameter estimates from spatial ANOVA models for nutrient supply rates ($\mu\text{g}/\text{probe}/3$ months), season, forest floor mass (FFM), distance to the nearest tree, canopy cover, soil pH in a reclaimed stand north of Fort McMurray, AB. Since covariates were standardized, parameter estimates are relative to the scale of the covariate. 85

List of Figures

- Figure 1-1** Schematic representation of soil reclamation treatments (Adapted from Turcotte et al. 2009)..... 12
- Figure 2-1** Representative NMR spectra of the light fractions isolated from a) three reclaimed trembling aspen and three b) white spruce sites. Aspen site spectra (sites 7, 34, 25) depict an age gradient of 17, 24, and 33 years, respectively and the spruce sites (sites 29, 26, 23) depict a canopy cover gradient from 6%, 36%, and 95%, respectively. 43
- Figure 2-2** NMR spectrum from a peatland soil (0-5 cm) from eastern Alberta (54.95°N, 112.47°W). The mean annual temperature at the site is 2.1°C and mean annual precipitation is 507 mm. Sampling and analysis was undertaken in 2005 (Quideau, unpublished data). 44
- Figure 2-3** NMDS ordination of mol(%) PLFA data from trembling aspen, jack pine, and white spruce reclaimed sites previously disturbed by oil sands mining. Each point on the ordination corresponds to one site, and numbers correspond to site identification numbers from Table 1. The cut-off for vectors is R^2 of 0.5. ... 45
- Figure 2-4** MRT analysis of mol(%) PLFA data from trembling aspen, jack pine, and white spruce reclaimed sites previously disturbed by oil sands mining. Groups are based on canopy cover (%), reclamation prescription (see Table 1 for description), and stand type (aspen, jack pine, and white spruce). 46
- Figure 3-1** Sampling locations in the aspen, pine and spruce reclaimed sites. Seventy-five sampling points were randomly generated with 15 initial points 2-10m from the preceding point and 60 additional points established 0-2m from the 15 initial points. One sampling point was established in each cardinal direction from one of the 15 initial points. 86
- Figure 3-2** Tree locations in the aspen, pine and spruce reclaimed sites..... 87

Figure 3-3 Spatial distributions of trees in aspen, pine and spruce reclaimed stands. G-values, which measure the distribution of event to nearest event distances, were calculated for each stand. An observed G-value within the Monte Carlo envelopes indicates a random and uniform distribution of trees. G-values above the envelope indicate aggregated distributions of trees, and G-values below the envelope indicate a regular distribution of trees. 88

List of Symbols and Abbreviations

AIC	Akaike's information criterion
ANOVA	Analysis of variance
AOSR	Athabasca oil sands region
C	Sill
C ₀	Sill-nugget
DBH	Diameter at breast height
DP	Direct placement
EPEA	Alberta Environmental Protection and Enhancement Act
F	Fibric forest floor horizon
F _m	F horizon characterized by fungal hyphae
F _r	F horizon characterized by root residues
F _s	F horizon characterized by bryophytes
F _z	F horizon characterized by faunal activity
G(r)	Event to event nearest neighbor distribution
H	Humic forest floor horizon
L	Litter forest floor horizon
MRPP	Multi-response permutation procedure
MRT	Multivariate regression tree
NMDS	Non metric multidimensional scaling
OM	Organic matter
PLFA	Phospholipid fatty acid
PMM	Peat mineral mix
PRS	Plant root simulator
RAMP-CP ¹³ C NMR	Ramped-cross-polarization ¹³ C nuclear magnetic analysis
REML	Restricted maximum likelihood model fitting
SOM	Soil organic matter
USDA	United States Department of Agriculture

1. Introduction

1.1 Athabasca oil sands region and surface mining

The Athabasca oil sands region (AOSR) in northern Alberta represent one of the largest deposits of oil in the world with approximately 1.7 trillion barrels of bitumen, of which 170 billion barrels are estimated to be recoverable by current technologies. Currently, over 600 km² of boreal forest has been disturbed by surfacing mining (Alberta Environment, 2009). Bitumen, a viscous hydrocarbon, cannot be refined in normal petroleum processing facilities, but it can be turned into a synthetic crude oil product through upgrading and further processing (Fung & Macyk, 2000). Oil sands deposits are not just a mixture of sand and bitumen, but also contain some silts and clays, with water and bitumen filling the pore space between the sand, silt and clay particles (McRory, 1982). In parts of the Athabasca oil sands region (AOSR) oil sands deposits are close enough to the surface for mining with truck and shovel operations. Mining first involves selectively salvaging surface soils (~1 m) and geological materials (1-3 m) for use as substrates during reclamation. Surface mining represents a substantial disturbance more severe than any other anthropogenic disturbance, (e.g.; harvesting), because of the need to reconstruct soils in addition to re-establishing vegetation in reclaimed landscapes. Indeed, the reconstruction of a soil functionally similar to a natural soil is a necessary prerequisite to the foundation of sustainable ecosystems.

1.2 Pre-disturbance vegetation

Prior to disturbance, the most common trees in upland forests in the region include trembling aspen and white spruce on finer textured soils and jack pine on coarser textured soils (Thompson *et al.*, 1978). Trembling aspen is the most common species by areal extent, and white spruce is an important late successional species (Thompson *et al.*, 1978). All three species (aspen, spruce, pine) are fire adapted species, and have different growth characteristics and reproductive strategies. Aspen commonly establishes earlier than conifers in sites following disturbance (Graham *et al.*, 1963). Furthermore, aspen primarily reproduces vegetatively (Barnes 1966), which should enable earlier natural reproduction in reclaimed sites compared to jack pine and white spruce. White spruce successfully establishes in the understory of aspen stands as a shade tolerant species and may eventually replace aspen as the dominant species in the canopy given enough time after disturbance (Lieffers *et al.*, 1996). The successful establishment of white spruce, and the successional pathway that takes place, depend in particular on the availability of a seed source and disturbance severity (Peters *et al.*, 2006). If there is a sufficient seed source, even-aged mixedwood stands tend to establish after severe disturbance, whereas less severe disturbances tend to lead to uneven-aged mixedwood stands (Peters *et al.*, 2006). Jack pine trees are typically found on nutrient and moisture poor sites and are much more prevalent than aspen and white spruce in these conditions (Beckingham & Archibald, 1996). Aspen and spruce are most commonly associated with gray luvisols in the Athabasca oil sands region, whereas jack pine tends to grow on

dystric brunisols. Luvisols form on well to imperfectly drained sites in sandy loam to clay textured parent materials with relatively high base saturation, while dystric brunisols are coarser textured soils with low base saturation (Turchenek & Lindsay, 1982).

Natural forests in northern Alberta are classified into ecosites, which are a function of soil and vegetation characteristics (Beckingham & Archibald, 1996). Reclamation seeks to emulate, if not necessarily recreate, the conditions that existed prior to mining and this requires taking into account both vegetation and soil characteristics. Trembling aspen and white spruce are most commonly found in d (low-bush cranberry) ecosites (Beckingham & Archibald, 1996). These d (low-bush cranberry) ecosites have mesic moisture conditions, medium nutrient regimes and are found where parent materials are moderately fine to fine textured tills or glaciolacustrine materials; they are considered to be a reference ecosite for the boreal highlands of Alberta (Beckingham & Archibald, 1996). Aspen is also found in b (blueberry) ecosites, where jack pine may also be present. As an intermediate ecosite between a (lichen) and d (low-bush cranberry) ecosites, b (blueberry) ecosites range from subxeric to submesic moisture regimes and nutrient regimes that are intermediate between poor (a ecosites) and medium (d ecosites). Parent materials in b (blueberry) ecosites are relatively coarse textured glaciofluvial materials. While occurring in b ecosites, jack pine is more prevalent in a ecosites that are characterized by open canopied jack pine stands with a carpet of lichen in the understory; a ecosites are rapidly draining and acidic, have

a poor nutrient status, and are found on coarse-textured eolian, glaciofluvial or fluvial eolian parent materials.

1.3 Forest floor characteristics

The presence of a forest floor overlying mineral soil is a key characteristic of forest ecosystems. Forest floor develops from the accumulation of leaves, twigs and woody materials and may also have inputs from mosses (Soil Classification Working Group, 1998). Forest floors are typically found in upland forest soils that are relatively well drained (Soil Classification Working Group, 1998), with horizons classified as Litter (L), Fibric (F), and Humus (H) layers, each with distinct characteristics. Not all forest floors have all three horizons, and these can vary greatly in terms of their relative proportions. The L layer retains the original structure of the plant materials, which are easily identifiable. The F layer consists of partially decomposed organic materials, and some of their original structures may be difficult to discern. The H layer is decomposed organic matter where the original structures are no longer identifiable. The H layer has undergone more decomposition relative to the F layer, and may be intermixed with the mineral soil at the interface between organic and mineral horizons (Soil Classification Working Group, 1998).

The forest floor plays an important role in forest ecosystems. In Canadian forests, the amount of carbon stored in the forest floor alone is comparable to that in vegetation (Goodale *et al.*, 2002), and is especially valuable for soil moisture

retention (Prescott *et al.*, 2000). Moreover, the forest floor is important as a major store of nutrients (Gosz *et al.*, 1976), and as a site of microbial activity (Bauhus *et al.*, 1998). Aspen, pine and spruce stands differ both in the amount of forest floor and the rate of forest floor development. Forest floor thicknesses in natural mature stands from the boreal forest of Northwestern Alberta typically range from 6.3 to 9.0 cm in aspen, and 8.9 to 13.0 cm in spruce stands (Lindo & Visser, 2003; Kischuk, 2004). Jack pine stands have thinner forest floors, with depths ranging from 1 to 4 cm observed in mature stands (Norris *et al.*, 2009). In terms of litter production, aspen stands produce approximately double the litter in a given period as compared to spruce and pine stands (Gower *et al.*, 1997). Rates of forest floor development are a function of both litterfall production and decomposition rates, which in turn are affected by organic matter composition and the structure and activity of the associated soil microbial community.

In addition to differences in the rate of forest floor development, there are differences in key forest floor characteristics depending on stand type. Specifically, differences in soil organic matter and microbial community composition have been observed between deciduous and coniferous stands in northern Alberta (Hannam *et al.* 2004; Hannam *et al.* 2006). Differences in soil nutrient availability have also been observed. Pine stands tend to have lower nutrient concentrations and nutrient availability as compared to spruce stands (Prescott *et al.* 1992; Johansson 1995). Furthermore, aspen forest floors have relatively high rates of nitrogen mineralization, and as nitrogen is the most limiting nutrient in the boreal forest, this can have a substantial impact on overall

soil fertility (Flanagan & Van Cleve 1983).

1.4 Government of Alberta recommendations

The Alberta Environmental Protection and Enhancement Act (EPEA) requires that disturbed land be returned to an “equivalent land capability”, defined as the ability to support similar land use values for commercial forest and wildlife habitat as existed before mining (Alberta Land Conservation and Reclamation Council 1999). Specifically, reclamation involves a number of steps that begin before mining, and ends with revegetation. Reclamation begins with a survey of the undisturbed landscape in order to assess the volume and suitability of materials available for use in reclamation (Fung and Macyk, 2000). Subsurface geological materials overlying the oil sands deposits are then stripped (~40 m), and stored in heaps outside the active mining areas (Turcotte *et al.*, 2009; Fung and Macyk, 2000). As peat is available in large quantities in the AOSR it is used as a soil conditioner to increase the soil organic carbon and nutrient content, and enhance its water holding capacity. Peat is mixed with mineral soil (PMM) (25-50 % vol_{peat}/ 75-50 % vol_{mineral}) and applied in a 20 to 50 cm layer on top of the reclamation profile (Fung and Macyk, 2000). If the underlying material in a reclaimed soil profile consists of lean oil sands or saline overburden, an intermediate 80 cm layer of nonsaline material is placed in between the lean oil sand or saline overburden and the PMM (Oil Sands Vegetation Reclamation Committee, 1998). Reclamation treatments are classified depending on the cover

material and underlying soil materials (Figure 1-1). After soil reconstruction, all sites are seeded with barley for slope stabilization purposes and fertilizer is applied ranging from 35-80 kg N/ ha, 18-46 kg P/ ha, and 10-44 kg K/ ha (Lanoue, 2003). In the following year, one-year-old aspen, jack pine, or white spruce are planted.

Monitoring programs for reclamation success evaluate land capability with end land use needs in mind (Oil Sands Vegetation Reclamation Committee 1998). Soil capability is considered a key criterion for long-term forest productivity, and the development of the forest floor is necessary to ensure reclaimed sites meet soil capability targets. As part of soil capability, soil nutrient availability is critical for plant growth, and while plants rely initially on the peat mineral mix they will eventually need to rely on nutrients cycled from the canopy through the forest floor. Furthermore, the development of a forest floor increases nutrient availability as diffuse nutrients are scavenged from the soil by plants and concentrated in the forest floor (Knabe 1973; Bradshaw 2000). Evaluating reclamation success hence requires a fundamental understanding of soil processes at both natural and reclaimed sites, and whether processes present in natural forests are reestablishing at reclaimed sites.

1.5 Characteristics of reclaimed soils

Previous research in soil biogeochemical processes of reclaimed sites from northern Alberta focused on investigating the success of different reclamation

prescriptions in creating conditions similar to natural mature sites. McMillan *et al.* (2007) found that the use of forest floor mineral mixes (FFM), a mixture of mineral soil and salvaged upland forest floor, led to higher microbial activity compared to the use of peat alone. However, soils reconstructed with both forest floor and peat, still differed significantly from natural forest floors. Reclaimed soils were also found to have relatively high rates of nitrification as compared to natural sites (McMillan *et al.*, 2007). Sites reclaimed with PMM have also been observed to differ from natural sites in terms of their nutrient status, with higher nitrate, but lower ammonium, phosphorus, and potassium than in upland forest soils typically found in the AOSR (Rowland *et al.*, 2009). Furthermore, reclaimed sites differ from natural forests in terms of soil organic matter composition, with reclamation prescriptions A and B (Fig 1-1) particularly different from natural analogues (Turcotte *et al.* 2009). MacKenzie and Quideau (2010) suggested that reclaimed sites may be behaving like recently disturbed natural sites in terms of soil microbial community structure and nutrient availability. Furthermore, they underlined the importance of the presence or absence of vegetation on these properties. While we know that differences exist between natural and reclaimed sites, the role of different canopy types on these properties has not been investigated in reclaimed sites. Forest floor development, soil organic matter composition, soil microbial community composition, and soil nutrient availability are all strongly affected by stand type in natural stands (Gower *et al.*, 1997; Hannam *et al.*, 2004; Hannam *et al.*, 2006; Prescott 2002). Differences among reconstructed soils as a result of the type of trees planted on site should become

apparent with time if natural processes are reestablishing at these reclaimed sites.

1.6 Spatial ecology

Thus far the role of spatial variability has not been considered in reclamation research in northern Alberta. Everything in nature is connected via spatial relationships as a result of biological, chemical, and physical processes (Levin 1992; Legendre 1993). Spatial variability is often regarded as random noise, but understanding the patterns produced by different factors can provide valuable insight into ecological processes at different scales (Ettema & Wardle 2002).

Forests are characterized by spatial patterns in soil nutrients at small scales, whereas agricultural or disturbed landscapes tend to be relatively more homogenous and exhibit spatial patterns at larger scales (Fraterrigo *et al.* 2005). Changes of spatial patterns in soil nutrients from large scale to small scale can be expected as forest ecosystems recover from disturbance. Initially, spatial scales should be larger and defined by the scale at which reclamation substrates are applied by heavy machinery. As the canopy develops individual trees will begin to have an influence on soil properties at small scales because of the uptake of nutrients by roots and return of nutrients from litterfall and throughfall. Individual trees can therefore be expected to increase resource heterogeneity and create spatial patterns at smaller scales than exist initially. Understanding the spatial pattern in soil properties at reclaimed sites is critical, as the re-establishment of

small scale spatial variability may have long term consequences for future site biodiversity (Ricklefs 1977).

1.7 Objectives

This study investigated forest floor development in reclaimed sites that were disturbed as a result of oil sands mining. The study has two components, the first looks at forest floor development with time and the second looks at spatial patterns in forest floor development, stand characteristics, and soil nutrients. As vegetation plays an important role in influencing soil characteristics, the trends in forest floor development and changes in soil properties were expected to differ as a function of the type of trees that were planted on the reclaimed sites. The following specific objectives were included:

- To investigate the trajectory of forest floor development over time in reconstructed soils planted to trembling aspen, jack pine, and white spruce.
- To determine if soil organic matter composition and soil microbial communities were changing as sites get older, and whether differences in these characteristics were observable among stand types.
- To characterize the spatial pattern of forest floor development and soil nutrient availability in different stand types.

- To determine if differences exist between stand types with respect to the reestablishment of relationships between the forest floor, canopy and soil nutrients.

Tables and Figures

Figure 1-1 Schematic representation of soil reclamation treatments
(Adapted from Turcotte *et al.* 2009)

Depth (cm)	Syncrude			Suncor	
	A	B	E	H	I
10	PMM	DP	PMM	PMM	PMM
20	Subsoil	DP	Subsoil	Tailings sand	Overburden
30					
40	Tailings sand	Tailings sand	Overburden	Tailings sand	Overburden
50					
60					
70					
80					
90					
100	Tailings sand		Overburden		
PMM: Peat 25-50% (vol/vol) + mineral soil mixture that was stockpiled prior to application DP: Salvaged peat and mineral soil directly placed from undisturbed to disturbed area Subsoil: Mineral soil salvaged down to 3m Tailings sand: Residual sand following bitumen extraction Overburden: Geological substrate removed to access the oil sands					

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2. Canopy cover and stand type influence on forest floor development and characteristics in reclaimed boreal forest soils

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2.1. Introduction

Northern boreal forests store over 80% of their total carbon belowground, either in the mineral soil or as a surface accumulation of organic matter, known collectively as the forest floor (Dixon *et al.*, 1994). In Canadian forests, the amount of carbon stored in the forest floor alone is comparable to that in vegetation (Goodale *et al.*, 2002). The forest floor plays a central role in boreal forests and is especially valuable for soil moisture retention (Prescott *et al.*, 2000), as a major storage of nutrients (Gosz *et al.*, 1976), and as an important site of microbial activity (Bauhus *et al.*, 1998).

Forest floor development is the product of two opposing influences: litter inputs from vegetation and decomposition outputs. In the western boreal forest of Canada, forest stands can be dominated by deciduous broadleaf trees such as trembling aspen (*Populus tremuloides* Michx.), or evergreen coniferous trees such as jack pine (*Pinus banksiana* Lamb.) and white spruce (*Picea glauca* (Moench) Voss; Johnson *et al.*, 1995). Litter fall under trembling aspen has been reported to be approximately twice that under black spruce, and ranges from being two to

almost four times greater than under jack pine (Gower *et al.*, 1997; Bernier *et al.*, 2007). Species composition of the canopy (stand type) also exerts an influence on decomposition rates as a result of its litter type, at least during the early stages of decay (Prescott, 2010). A variety of broadleaf litters, including aspen, decompose faster than coniferous litters under similar environmental conditions, although decomposition rates become similar after 1-3 years of decay (Prescott *et al.*, 2004). As a result of slower decomposition, forest floors under white spruce tend to be thicker than under aspen. For instance, an average forest floor thickness of 13 cm was recorded in mature white spruce stands from Northwestern Alberta, while a thinner forest floor of 9 cm was observed in aspen stands (Kishchuk, 2004). In comparison, forest floors in jack pine stands tend to be thinner with thicknesses of 1-4 cm (Norris *et al.*, 2009).

Previous work in the boreal forest of northern Alberta has established the link between stand type, forest floor composition, and its microbial community (Hannam *et al.*, 2004 & 2006; Swallow *et al.*, 2009). As shown by solid-state nuclear magnetic resonance, aspen forest floors were characterized by more labile (less aromatic) carbon and more oxidized (carbonyl) carbon than spruce stands (Hannam *et al.*, 2004). Phospholipid fatty acid and substrate-induced respiration analyses were used to describe the structure and function of forest floor microbial communities and to investigate their potential response to both stand type and disturbance (Hannam *et al.*, 2006; Swallow *et al.*, 2009). Distinct differences were found among stand types using both techniques, where mixed and white spruce forest floors were statistically different from aspen (Hannam *et al.*, 2006). These

vegetation-induced differences appeared fairly resilient to disturbance, as no changes were observed after harvesting or low to medium intensity fire (Swallow *et al.*, 2009).

Surface mining of oil sands deposits has currently disturbed 602 km² of boreal forest in northeastern Alberta (Alberta Government, 2010; The Oil Sands Developers Group, 2009). Soils in reclamation areas are reconstructed using a mixture of peat and mineral soil materials that have been salvaged prior to mining. These areas are then planted with trembling aspen, jack pine and white spruce. Prior research demonstrated that organic matter composition and nutrient availability in these reclaimed soils differed, at least originally, from mature upland forest soils (Turcotte *et al.*, 2009; Rowland *et al.*, 2009). In addition, reclamation practices have been observed to lead to functional and structural differences among reclaimed sites. For instance, composition of the organic amendment used to cap these reconstructed soils affected nitrogen availability and microbial activity (McMillan *et al.*, 2007), while soil microbial communities were influenced by the presence or absence of vegetation in reclaimed soils younger than 6 years (MacKenzie & Quideau, 2010). However, the specific influence of canopy type and canopy cover on soil organic matter and soil microbial community composition in older reclaimed sites has yet to be examined.

This study aimed to assess forest floor development in trembling aspen, jack pine, and white spruce reclaimed sites previously disturbed by oil sands mining. Older sites (16-31 years) were targeted for this study, as to ensure the presence of a forest floor. The overall objective was to compare the development

of the forest floor over time in relation to stand type and canopy cover.

Specifically, we were interested in examining the potential effect of vegetation on the rate of carbon and nitrogen accumulation at the surface of these soils.

Secondly, we wanted to test if vegetation-induced differences in soil organic matter composition and associated microbial communities developed over time in a fashion similar to what is found in natural boreal forest soils.

2.2. Materials and Methods

2.2.1. Study Area and Site Selection

The reclamation sites are located north of Fort McMurray, Alberta (56°43'N 111°21'W). The climate is characterized by relatively short cool summers and long cold winters. The mean monthly air temperature ranges from -19°C in January to 17°C in July, with a mean annual temperature of 0.7°C (Environment Canada, 2009). The mean annual precipitation is 456 mm, with 342 mm occurring as rainfall during the growing season. The dominant tree species in upland forests in the boreal mixedwood region include trembling aspen, jack pine, and white spruce. Trembling aspen is the most common species by areal extent (Thompson, 1978). Trembling aspen and white spruce are typically found on soils classified as gray luvisols in the Canadian soil classification system (Soil Classification Working Group, 1998), and as haplocryalfs in the USDA system (Soil Survey Staff, 1999). These soils tend to form on well to imperfectly drained sites in sandy loam to clay textured parent materials with relatively high base saturation (Turchenek & Lindsay, 1982). Jack pine in the region is found on

dystric brunisols (Soil Classification Working Group, 1998), equivalent to dystrocryepts in the USDA system (Soil Survey Staff, 1999). These soils usually form in coarser textured parent materials with low base status (Turchenek & Lindsay, 1982).

Thirty-two study sites were established on the Syncrude and Suncor mining leases located 25 to 45 km north of Ft. McMurray. Sites were within 20 km of each other, and ranged in age from 16 to 33 years since reclamation (Table 2-1). Due to the still young age of the oil sands industry, 33 years since reclamation corresponded to the oldest sites available for this study. Sites were selected in order to include a balanced range of ages since reclamation in each of the three stand types under study, resulting in a total of eleven aspen, eleven spruce, and ten pine sites. As much as possible, these sites were chosen to be on a gentle slope to level position. Additional characteristics such as reclamation prescription and fertilization rate were considered so that they could be included in the data analysis. Generally, the mining areas were refilled with overburden material, and at least 80 cm of tailings sand or salvaged subsoil material was placed on the overburden (Table 2-1). Most of the reconstructed soils were then capped with a peat mineral mix ($25-50\% \text{ vol}_{\text{peat}}/75-50\% \text{ vol}_{\text{mineral}}$) ranging in thickness from 6 to more than 20 cm. All sites were seeded with barley for slope stabilization purposes and fertilizer was applied. Some sites received a single fertilizer application while the others received five consecutive annual applications; total fertilization of sites ranged from 35-80 kg N/ ha, 18-46 kg P/ ha, and 10-44 kg K/ ha (Lanoue, 2003). In the following year one-year-old aspen,

jack pine, or white spruce container planting stock, grown from local seed sources at a commercial nursery, were planted. Planting densities averaged 2161 stems/ ha in the aspen stands, 1524 stems/ ha in the pine stands, and 2855 stems/ ha in the spruce stands. No further stand tending treatments were applied after the initial planting of the seedlings.

2.2.2. *Vegetation Survey and Soil Collection*

One hundred meter transects (100 m) were established on 32 study sites in June 2008. Vegetation and forest floor descriptions were undertaken at 10 m intervals along each transect. A circular plot with a 1.79 m radius (10 m²) was established at each interval where tree and dominant shrubs, forbs, or grass species were identified. The most common shrubs on the study sites were red osier dogwood (*Cornus stolonifera Michx.*), red raspberry (*Rubus idaeus L.*), saskatoon (*Amelanchier alnifolia Nutt.*), and various willow species (*Salix* spp.), while dominant grass species were smooth brome (*Bromus inermis Leyss.*), wild oats (*Avena fatua L.*) and several fescue species (*Festuca* spp.). Clover species (*Trifolium* spp.) and dandelion (*Taraxacum officinale Weber*) were the most common forb species. Tree measurements on trees taller than 1.3 m included: number of trees, species, diameter at breast height (DBH), height, and height to live branches. Canopy cover was estimated at the centre of each plot at breast height using a convex densiometer (Robert E. Lemmon Forest Densiometer – Model A).

In each plot the humus form was characterized using the classification system from Green *et al.* (1993) by identifying the L, F, H horizons in terms of their varying degree of decay and the relative importance of faunal and microbial activity; the L layer consists of relatively fresh plant residues that retain their original structure and are easily identifiable, the F layer contains partially decomposed materials, where partial plant structures are still macroscopically discernible, and the H layer, which is composed of well decomposed plant residues, where original structures are not discernible. Subordinate horizons for the F horizons are further distinguished based on the relative importance of fungal hyphae (Fm), root residues (Fr), bryophytes (Fs), and faunal activity (Fz).

The study sites and plots were revisited in late June of 2009, and soil samples were collected 1 m away from the original plots to avoid the previous disturbance to the vegetation and forest floor. Samples were analyzed for total soil carbon and nitrogen concentrations, and used for phospholipid fatty acid (PLFA) and ramped-cross-polarization (RAMP-CP) ^{13}C nuclear magnetic resonance (NMR) analyses. For all analyses, the forest floor and the top 5 cm of mineral soil were sampled together. Samples for soil carbon, soil nitrogen, and NMR analyses were taken at each of the 10 plots along the transect and combined into one representative sample per site. Samples for PLFA characterization were collected at position 1, 4 and 7 on each transect, kept refrigerated on ice, and then stored at -20°C within 24 hours. Upon return to the laboratory, these were frozen at -86°C and then freeze-dried before undertaking analysis.

2.2.3. Laboratory Analyses

For total soil carbon and nitrogen analyses, as well as NMR analysis, samples were sieved with a 4 mm screen in order to exclude the fresh litter materials corresponding to the L horizon. Soil carbon and nitrogen samples were air dried and ground with a ball mill until homogeneous. Samples were analyzed for soil carbon and nitrogen concentrations by dry combustion using a Costech ECS 4010 CHNS-O Elemental Combustion System (Costech Analytical Technologies Inc., Valencia, CA). Samples for NMR analysis were physically fractionated in order to isolate the low density OM fraction (light fraction) according to the method outlined in Turcotte *et al.* (2009). Soils were immersed in distilled water at a 1:5 ratio and shaken on a reciprocal shaker for 1 hour to ensure dispersion. Samples were then wet sieved at $>53 \mu\text{m}$ to collect the sand-sized and organic particulates, which were transferred to distilled water for density separation by flotation to isolate the light fraction from its mineral associated fraction. The light fractions were dried at 65°C , and ground. Samples were characterized by RAMP-CP ^{13}C NMR spectroscopy on a Bruker Avance 400 ($B_0 = 9.4 \text{ T}$, $\nu_L(^{13}\text{C}) = 100.6 \text{ MHz}$) NMR spectrometer. Spectra were acquired from samples spinning at 13 kHz and exposed to a ^1H 90° pulse width of $4.0 \mu\text{s}$, a 1 ms contact time and a 5 s pulse delay. Glycine was used to determine the Hartmann-Hahn matching condition and four to eight thousand scans were collected for each sample with a line broadening of 200 Hz. The ^{13}C chemical shifts were referenced relative to TMS ($\delta_{\text{iso}} = 0.0 \text{ ppm}$) using adamantane as a secondary reference. Bruker's WIN-NMR package was used to estimate the

relative areas of five regions between 0 and 192 ppm including the alkyl C attributed region (0-45 ppm), *O*-alkyl C (45-112 ppm), aromatic C (112-140 ppm), phenolic C (140-165 ppm), and carbonyl C (165-192 ppm) (Preston *et al.* 2000). Corrections for spinning side bands were applied under the assumption that the sidebands of each signal had the same intensity, with spectral divisions assigned based on local minima.

To characterize the soil microbial community, phospholipid fatty acids were extracted with a modified Bligh and Dyer technique to isolate polar lipids from 1.5 g of freeze dried material (Bligh & Dyer, 1959; White & Ringelberg, 1998). As described in Hamman *et al.* (2006), polar lipids were extracted and purified on pre-packed silicic acid columns (Agilent Technologies, Wilmington, DE, USA) to separate them from neutral lipid and glycolipids. Polar lipid extracts then underwent a mild alkaline methanolysis in order to form fatty acid methyl esters (FAMES). The FAMES were quantified using an Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE, USA). The fatty acids were designated with the X:Y ω Z nomenclature where X is the number of carbon atoms, Y is the number of double bonds, and Z the first double bond from the aliphatic (ω) end of the molecule.

2.2.4. Data Analyses

For the statistical analyses, all samples collected at a given site were combined into one and measurements were summarized into site averages. These

site values were used in all subsequent data analyses. Relationships between forest floor thickness, soil carbon and nitrogen concentration, alkyl C :O-alkyl C, time since reclamation, and site vegetation factors were analyzed with linear and multiple regression techniques and sites as replicates in SAS (SAS Institute Inc., 2002, Cary, NC). Forest floor thickness, soil carbon and nitrogen concentrations, and the alkyl C:O-alkyl C ratio were analyzed with linear regressions where time was included as the independent factor. Multiple regression analyses were run with forest floor thickness, soil carbon and nitrogen concentrations and the alkyl C :O-alkyl C ratio as dependent factors, and time since reclamation, canopy cover, understory shrub cover, and stem density as independent factors. Residuals conformed to the assumptions of normality and homogeneity of variance, and significance was determined at α of 0.05. Models were selected based upon the adjusted R^2 values and the Akaike's Information Criterion (AIC) value.

PLFA data were analyzed using non-metric multi-dimensional scaling (NMDS) followed by the multi-response permutation procedures (MRPP) with the PC Ord software package (version 5.10, MjM Software Design, Gleneden Beach, OR). The NMDS analysis organizes data in two or more dimensions based on distances between data points, and presents the advantage that data do not need to conform to the requirement of a normal distribution (McCune & Grace, 2002). Analysis of the PLFA data was conducted on the following twenty five biomarkers: i15:0, a15:0, 15:0, i16:0, 16:1 ω 9c, i17:0, a17:0, cyclo17:0 17:0, 18:1 ω 7c, cyclo19:0 for bacteria (Frostegård & Bååth, 1996); 15:1 ω 6c, 16:1 ω 9c, and 17:1 ω 8c for gram negative bacteria; i14:0 and i18:0 for gram positive bacteria

(Myers *et al.* 2001); 18:2 ω 6c (Frostegård & Bååth, 1996), 18:1 ω 9c, 20:1 ω 9c (Myers *et al.*, 2001), 16:1 ω 5c, and 18:3 ω 6c (Hamman *et al.*, 2007) for fungi; 10Me19:0 (O'Donnell *et al.*, 1982), 10Me16:0, and 10Me18:0 for actinomycetes; and 20:4 ω 6c for protists (Myers *et al.*, 2001). All data were expressed on a mol% basis and arc sine square root transformed. The Sorensen (Bray-Curtis) distance measure was used for the analysis (McCune & Grace, 2002). The secondary matrix included canopy type, reclamation prescription, and fertilizer rate as grouping factors. Canopy cover, dominant shrub cover, forest floor thickness, soil carbon concentration, and the summed PLFA values for bacteria, gram negative bacteria, gram positive bacteria, fungi, actinomycetes, and protists were included as potential vectors. The significance of the grouping factors (canopy type, reclamation prescription fertilizer rate) was tested using a MRPP with a minimum α of 0.05. The MRPP is a non-parametric test that generates three values to compare against random expectations: a *p* value, which indicates overall significance of the comparisons; a *T* value that indicates separation among groups, and an *A* value that tests for homogeneity within groups (McCune & Grace, 2002). Pair-wise comparisons between groups were also conducted using MRPP.

Multivariate regression tree (MRT) analysis was performed to compare microbial community structure (PLFA) based on a number of environmental variables (De'ath, 2002). Briefly, MRT creates dichotomies where sites are clustered hierarchically based on similarity. Sites are clustered in order to minimize the differences within each cluster by repeatedly splitting the data based on the environmental variables included in the analysis. A Bray-Curtis distance

measure was used, and the transformed mol% PLFA data from the ordination was used for the MRT analysis as well. Environmental variables included canopy type, reclamation prescription, time since reclamation, canopy cover, and shrub cover. The MRT was computed with the R software package (version 2.10.1, R development Core Team) and the mvpart library (Therneau & Atkinson, 2005).

2.3. Results

2.3.1. Vegetation and Forest Floor Characteristics

Canopy cover ranged from 23-85% in the aspen stands, 6-81 % in the pine stands, and 6-94% in the spruce stands (Table 2-2). A similarly wide range of shrub cover was observed, with 2-68% under aspen, 0-25% under pine, and 0-38% under spruce. While differences in canopy and shrub cover among stand types were not statistically significant across all age classes, the aspen stands at similar age classes had on average higher cover than the pine and spruce stands. The spruce stands in particular had eight sites with canopy cover less than 40%, including three sites that were reclaimed more than 24 years ago. Canopy cover in the aspen stands was distinct from the coniferous stands in that canopy cover was always above 65% in sites that had been reclaimed for more than 25 years. There was a significant non-linear relationship between time and canopy cover in the aspen stands as modeled with a Gompertz curve ($p < 0.01$), a sigmoidal relationship typically used to model growth. On the other hand, there was no

relationship between canopy cover and time in either the pine or the spruce stands (Table 2-3).

Forest floor development did not vary greatly among stand types in terms of the relative proportions of the L, F and H layers (Table 2-2). On a thickness basis, approximately half of the developing forest floor was composed of the L layer, and the other half was composed of the F layer. While there had been sufficient time for an F layer to develop on all of the study sites, there was very little to no H development on any of these sites. The F layers had an absence of faunal droppings, little evidence of mesofaunal activity, and rare fine roots. Despite also being relatively rare, some fungal mycelia were present and were the dominant morphological characteristic. Consequently, the F horizons were classified as Fm and the humus forms as hemimors (Green *et al.*, 1993).

Forest floor thickness was significantly greater under aspen than under either spruce ($p < 0.01$) or pine ($p = 0.04$; Table 2-2). Forest floor in the trembling aspen stands increased in thickness with time since reclamation, although this trend was not significant at an alpha of 0.05, whereas jack pine and white spruce stands showed no such trend (Table 2-3). Soil carbon concentrations (%) increased significantly with time since reclamation in the aspen and pine stands, but not in the spruce stands. Soil nitrogen concentration (%), which was highly correlated ($r = 0.97$, $p < 0.01$) with soil carbon concentration (data not shown), also increased with time in the aspen and jack pine stands, but not in the white spruce stands (Table 2-3).

In addition to time since reclamation, canopy cover and shrub cover played an important role in forest floor development at the study sites, although differences existed among stands in terms of which factor was more important (Table 2-3). In the aspen stands, canopy cover best explained differences in forest floor thickness, while forest floor thickness was correlated to shrub cover in the pine stands. In the white spruce stands, canopy cover and shrub cover were both significantly related to forest floor thickness. Similarly, canopy cover was significantly related to soil carbon and nitrogen concentrations in the aspen stands, while both canopy cover and shrub cover were the most important factors influencing soil carbon concentration in the spruce stands (Table 2-3).

2.3.2 Soil Organic Matter Composition

As indicated by the alkyl C:*O*-alkyl C ratios of the isolated light fractions, soil organic matter composition significantly changed with time since reclamation in the aspen stands, but not in the spruce stand; a non-significant trend was observed in the jack pine stands (Table 2-3). Many of the younger sites (17-20 years) had comparable alkyl C:*O*-alkyl C ratios of approximately 0.20-0.25 regardless of stand type, whereas the alkyl C:*O*-alkyl C ratios of the older sites (>25 years) were quite different and depended on stand type (data not shown). The older aspen and pine stands showed an alkyl C:*O*-alkyl C ratio between 0.30-0.40, whereas the spruce stands showed little increase with time since reclamation. While there was no trend with time in the spruce stands, a significant

relationship existed between the alkyl C:O-alkyl C ratio and canopy cover for these stands (Table 2-3).

As illustrated on representative NMR spectra from chosen sites, all light fractions showed a dominant peak at 72-74 ppm, indicative of the C-2, C-3, and C-5 carbons from cellulose and hemicelluloses (Figs. 2-1 & 2-2). Additional peaks at 63 ppm and 105 ppm can be assigned to the C-6 and anomeric carbons from these carbohydrates (Hannam *et al.*, 2004). In the alkyl region of the spectra, samples from the younger aspen and spruce stands had distinctive peaks at 18-21 ppm and 30-31 ppm, which were characteristic of a peat signature (Fig. 2-2). The peak around 20 ppm corresponds to terminal methyl groups, while the one at 30 ppm represents polymethylene type carbons (Skjemstad *et al.*, 1983). As time since reclamation increased, the signal at 30 ppm clearly increased for the aspen samples (Fig. 2-1). An increase in the signal at 174 ppm was also observed, which may include carboxyl groups from organic acids or amide groups. On the other hand, the NMR spectra for the spruce stands did not show any readily apparent changes with time, but instead, presented an increased intensity of the 30 ppm peak as cover increased from 6 to 95 % (Fig. 2-1). Finally, in the aromatic and phenolic areas of the spectra, a peak at 132 ppm along with a shoulder at 116-117 ppm indicated C-substituted aromatic carbons, while the peaks in the 150-160 ppm corresponded to carbons from phenolic structures; these peaks did not show any consistent changes with either time or canopy cover.

2.3.3 Soil Microbial Communities

Non-metric multidimensional scaling (NMDS) analysis of mol (%) PLFA data produced a 3-dimensional solution with a final stress of 10.6 after 113 iterations (Fig. 2-3). Axes 1 and 2 were presented because these best explained the variation in the data, and there was little additional variance (13 %) explained by the third axis. There were no significant differences among sites based on reclamation treatment ($p=0.25$) or fertilization regime ($p=0.18$). On the other hand, stand-related grouping patterns were significantly different ($T=-4.53$, $A=0.064$, $p<0.01$), and all canopy types grouped separately. Aspen stands grouped differently from the pine ($T=-1.92$, $A=0.03$, $p=0.048$) and spruce stands ($T=-3.47$, $A=0.06$, $p<0.01$). Pine stands also grouped differently from spruce stands ($T=-4.52$, $A=0.06$, $p<0.01$). Correlation vectors of stand structural features (r^2 cutoff of 0.50) indicated that fungal PLFAs were relatively more prevalent in pine and spruce stands, while bacterial PLFAs were relatively more prevalent in aspen stands.

Stand type differences were detected in the MRT analysis, but not as strongly as differences in canopy cover (Fig. 2-4). Canopy cover greater than 30% was the most important grouping factor. When canopy cover was less than 30%, reclamation prescriptions best explained differences among sites. Stand type was important as a factor only when canopy cover exceeded the threshold value of 30%. The importance of canopy cover in influencing soil microbial community composition appears to be highlighted further in the ordination results (Fig. 2-3). Those aspen sites that did not group with the majority of the other aspen sites all

had relatively low canopy cover. Sites 15, 16, and 21 were below the 30% threshold and site 41 was below the 64% threshold. Additionally, the pine sites (32 and 44) that did not group with the other pine sites also were below the 30% threshold. Finally, while time since reclamation and shrub cover were included as environmental variables for the MRT analysis, neither one appeared in the results as a significant grouping factor.

2.4. Discussion

Forest floors in the reclaimed aspen and spruce stands had not yet reached thicknesses similar to those found in mature boreal forests. They showed average thicknesses of 3.6 cm under aspen and 2.0 cm under spruce (Table 2-2). In comparison, forest floor thicknesses in natural mature stands from the boreal forest of Northwestern Alberta typically range from 6.3 to 9.0 cm in aspen and 8.9 to 13.0 cm in spruce stands (Lindo & Visser, 2003; Kishchuk, 2004). Depending on environmental conditions and the extent of disturbance, recovery of the forest floor following clear cutting can take anywhere from 5 to 80 years (Preston *et al.*, 2000), hence it is likely that forest floors at the reclaimed sites had not had sufficient time (33 years or less) to reach maximum thicknesses. The average thickness of the forest floor in the reclaimed aspen stands was comparable to those observed (1.8-2.7 cm) in a 40-year-old aspen plantation (Alban, 1982). On the other hand, the average spruce forest floor thickness was thinner than in similarly aged natural stands, ranging from 2.2-3.3 cm in a 40 year old Minnesota

stand (Alban, 1982) to 3.5 cm in a 29 year old Swedish stand (Cerli *et al.*, 2006). Forest floor development in pine stands tend to level off faster than in spruce stands (Krause, 1998), which appears to be the case in this study. In contrast to the aspen and spruce stands, forest floors in the reclaimed pine stands, with a thickness averaging 2.5 cm and reaching a maximum of 4.0 cm (Table 2-2), were comparable to forest floors in natural jack pine stands (Norris *et al.*, 2009). Based on forest floor bulk densities (0.075-0.104 Mg m⁻³) and total carbon concentrations (422.8-475.5 g kg⁻¹) as reported in Redding *et al.* (2005), the average amount of carbon currently stored in the forest floor at the aspen stands can be estimated at 13 Mg C/ha, and 9 Mg C/ha for the pine and spruce stands. Compared to natural stands, the aspen reclaimed sites have only accumulated approximately 25% of their expected steady state carbon, and the spruce stands approximately 15% of expected steady state carbon (Wang *et al.*, 1995; Cerli *et al.*, 2006).

Morphologically, while the forest floors most closely resembled hemimors, they did not quite fit this classification because of little visible fungal mycelia and fine roots, indicating low biological activity. The H layer was completely absent or very thin in most cases. In total, the forest floor from only four sites presented the development of an H layer, and in all cases, these H layers comprised less than 10% of the total forest floor thicknesses. In contrast, natural aspen stands aged 35-70 years in northeastern British Columbia were reported to commonly have H horizons and both Fz and Fm horizons, which indicated the presence of both fungal and faunal activity (Fons *et al.*, 1998). Thirty-three years,

the age of our oldest site, was clearly not sufficient time for an H horizon to develop on these reclaimed soils, where biological activity appeared to be low. Low biological activity may be the result of decreased mesofauna. Mesofauna may have decreased in number relative to undisturbed natural soils, as they have been observed to decrease in density by as much as 93% following disturbance such as forest floor removal, soil compaction, and whole tree harvesting (Battigelli *et al.*, 2004). Mesofauna, including litter shredders, play an important role in organic matter decomposition (Seastedt, 1984), but their role in soil development at the reclaimed sites has not been well reported in the literature.

Canopy cover stands out as having an important role in forest floor development. Forest floor thickness and soil carbon concentration were influenced by canopy cover in the aspen and spruce stands (Table 2-3). Time was less important than canopy cover in the spruce stands, which may be a result of the much slower establishment of canopy cover in time. Time was important in the aspen stands, but aspen was the only stand type that presented a significant relationship between canopy cover and time with consistently higher canopy cover in older sites. In addition to canopy cover, shrub cover appeared to play an important role in coniferous stands (Table 2-3), and previous work has suggested that shrub cover may play an important role in forest floor development in these reclaimed ecosystems (Rowland *et al.*, 2009). Results from this study further suggest that the benefit of shrub cover on forest floor development may be more important for reclaimed conifer sites than aspen sites because of the higher canopy cover and litterfall rates under aspen (Gower *et al.*, 1997).

The increase in soil carbon and nitrogen concentrations that was observed in the aspen and pine stands indicated that the incipient forest floor was altering the surface soil conditions on these sites (Table 2-2). However, a change in the light fraction composition with time was only observed for the aspen stands (Table 2-3 & Fig. 2-2). The light fraction is a useful soil C pool for observing changes in soil organic matter following disturbance as it is the pool most sensitive to environmental changes (Six *et al.*, 1998; Dygryze *et al.*, 2004), and changes in light fraction composition have been previously observed in oil sands reclaimed sites (Turcotte *et al.*, 2009). In the oil sands region of northeastern Alberta, soils are reconstructed using a mixture of peat and mineral materials, as peat is available in large areas of the mining footprint and can be used as an organic amendment and soil conditioner (Fung & Macyk, 2000). While the original peat material is characterized by a low alkyl C signal at 30 ppm (Fig. 2-2), the increase in this signal with time since reclamation in the aspen soils, or with increasing canopy cover in the spruce soils (Fig. 2-1), as well as the corresponding increases in the alkyl C:*O*-alkyl C ratios (Table 2-3) can be attributed to an increase in litter input from the regrowing canopy. Specifically, the signal at 30 ppm corresponds to carbon from long chain aliphatic structures, such as those as found in lipids and cutin of woody plants (Kögel-Knabner, 2002). Additionally, for the aspen stands, the observed increase in the peak at 174 ppm, corresponding to carbonyl, the most oxidized form of carbon (Fig. 2-1), can be related to conditions found in natural boreal forests, where aspen forest floors

have been reported to have significantly higher carbonyl concentrations than white spruce forest floors (Hannam *et al.*, 2004).

The soil microbial community was significantly influenced by stand type in our study (Fig. 2-3). Differences in microbial community structure and function linked to tree species have been reported for podzolic soils from southeast Finland (Priha *et al.*, 2001) as well as for mixedwood forest floors from western Alberta (Hannam *et al.*, 2006). These differences have been related to the interactive effect of microclimate (e.g. soil moisture), pH, and the organic substrate composition (Waldrop & Firestone, 2006; Högberg *et al.*, 2007). The presence of spruce, either in pure or in mixed stands, appears to play a key role in affecting not only the soil microbial community composition but also the composition of the understory vascular plant and epigeic arthropod communities (Hannam *et al.*, 2006; Fenniak, 2001; Work *et al.*, 2004). In our study, provided that canopy cover exceeded 30%, microbial community in the spruce stands significantly differed from that in the pine stands (Fig. 2-4). Additionally, the coniferous stands were positively correlated to the presence of fungi, while higher bacterial concentrations were correlated to the aspen stands (Fig. 2-3); these relationships are consistent with stand influences on microbial communities in natural stands. Actinomycetes are relatively more prevalent in aspen stands as compared to pine stands, and actinomycetes are actively antagonistic to fungal communities (Jayasinghe & Parkinson, 2008). When canopy cover was less than 30%, differences in soil microbial community structure were related to reclamation prescription rather than to stand type (Fig. 2-4). Differences in soil microbial

communities linked to reclamation treatments were previously observed in a series of oil sands reclaimed soils, which ranged in age from 1 to 33 yrs since reclamation (Dimitriu, 2009 & 2010).

2.5. Conclusions

By targeting older sites (> 15 yrs), our study allowed us to detect differences in forest floor development linked to canopy cover and stand type (Fig. 2-4), which suggests that soil-vegetation relations are reestablishing at these sites. The establishment of an aspen canopy was particularly effective for the rapid development of a forest floor and associated changes in soil organic matter composition. When canopy cover surpassed a threshold value of 30%, significant differences in the soil microbial communities emerged that were driven by stand type rather than reclamation prescription. The earlier establishment of a tree canopy, and rapid development of the forest floor in the aspen stands may have long term consequences for the productivity of these reclaimed sites. Future research is necessary to identify the contribution of mesofauna to forest floor development in reclaimed sites, and to examine how the development of the forest floor compares to similarly aged sites disturbed by fire in the region.

Tables and Figures

Table 2-1 Distribution of time since reclamation (years) for the trembling aspen, jack pine, and white spruce reclaimed sites included in the study. Numbers in the table (1 to 45) correspond to site identification numbers, and reclamation prescriptions are shown in parentheses. Descriptions of reclamation prescriptions and materials are from Turcotte *et al.* (2009).

Time since reclamation (yrs)	Aspen (n=11)	Pine (n=10)	Spruce (n=11)
16		31(H), 32(H)	
17	7(I), 15(E), 16(E), 22(E)	1(B), 42(H)	43(I)
18		3(B), 44(H), 45(H)	21 (A), 29(I), 37(I)
19	41(H)	11(B)	18 (I), 40(I)
21	13(A), 14(E)		
24	34(I)		17(E)
25	12(E), 38(I)		33(I)
26		4(H), 19(I)	28(I)
27			26(I)
28			27(I)
33	25(Peat)		

A: Peat mineral mix/subsoil/tailings sand
B: Direct placement/tailings sand
E: Peat mineral mix/subsoil/overburden
H: Peat mineral mix/tailings sand
I: Peat mineral mix/overburden
Peat: Former peat stockpile

Peat mineral mix: 25-50% (vol/vol) peat + mineral soil mixture that was stockpiled prior to application
Direct placement: Salvaged peat and mineral soil directly place from undisturbed to disturbed area
Subsoil: Mineral soil salvaged down to 3m
Tailings sand: Residual sand following bitumen extraction
Overburden: Geological substrate removed to access oil sands deposits

Table 2-2 Vegetation and forest floor characteristics of trembling aspen, jack pine, and white spruce reclaimed sites previously disturbed by oil sands mining. Data for the L, F, and H horizons represent the percent of the total forest floor thickness (cm) for each horizon. Mean values are presented with \pm one standard deviation (n=10-11).

Variable	Canopy Type	Mean
Canopy Cover (%)	Aspen	57 \pm 25
	Pine	48 \pm 28
	Spruce	39 \pm 28
Shrub Cover (%)	Aspen	24 \pm 21
	Pine	7 \pm 9
	Spruce	9 \pm 12
Forest Floor Thickness (cm)	Aspen	3.6 \pm 1.3*
	Pine	2.5 \pm 0.9
	Spruce	2.0 \pm 0.8
L horizon (%)	Aspen	57 \pm 11
	Pine	53 \pm 9
	Spruce	50 \pm 17
F horizon (%)	Aspen	43 \pm 11
	Pine	47 \pm 9
	Spruce	50 \pm 17
H horizon (%)	Aspen	0 \pm 0
	Pine	0 \pm 0
	Spruce	0 \pm 0

*Denotes significance at $\alpha=0.05$

Table 2-3 Regression parameters for relationships between dependent variables (forest floor thickness, soil carbon and nitrogen concentrations, and alkyl:o-alkyl ratio) and independent variables (time since reclamation, canopy cover, shrub cover, and stem density) in trembling aspen, jack pine, and white spruce reclaimed sites previously disturbed by oil sands mining. Regression parameters for canopy cover and time in each stand type were also determined. Parameters include R^2 (coefficient of determination of the regression model), and p (p-value with significance determined at $\alpha=0.05$).

Dependent Variable	Treatment	Independent Variable (s)	R^2	p
Forest Floor Thickness (cm)	Aspen	Time	0.31	0.07
		Canopy Cover	0.33	0.04
	Pine	Time	0.07	0.44
		Shrub Cover, Stem Density	0.49	0.1
		Time, Canopy Cover, Shrub Cover	0.85	<0.01
Soil Carbon Concentration (%)	Aspen	Time	0.77	<0.01
		Time, Canopy Cover	0.83	<0.01
	Pine	Time	0.63	<0.01
	Spruce	Time	0.15	0.21
		Canopy Cover, Shrub Cover	0.45	0.09
Soil Nitrogen Concentration (%)	Aspen	Time	0.77	<0.01
		Time, Canopy Cover	0.83	<0.01
	Pine	Time	0.63	<0.01
	Spruce	Time	0.17	0.21
		Canopy Cover, Shrub Cover	0.20	0.41
Alkyl C: O-alkyl C	Aspen	Time	0.46	0.02
	Pine	Time	0.29	0.11
	Spruce	Time	0.06	0.46
		Canopy Cover	0.45	0.02
Canopy cover	Aspen	Time (non-linear)	0.41	<0.01
	Pine	Time	0.10	0.36
	Spruce	Time	0.16	0.21

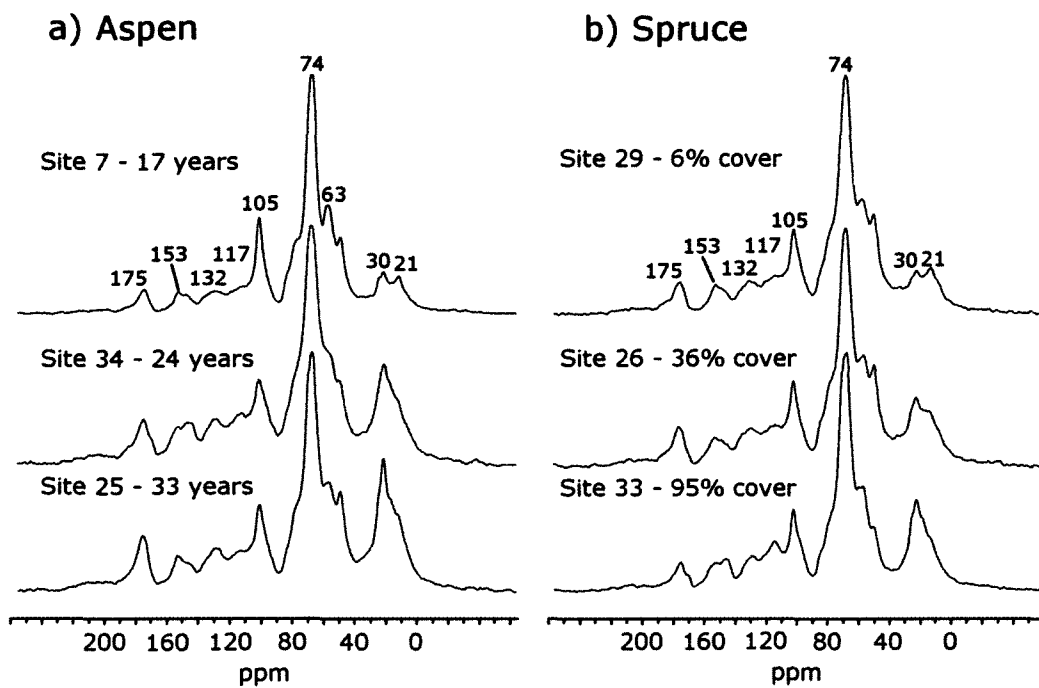


Figure 2-1 Representative NMR spectra of the light fractions isolated from a) three reclaimed trembling aspen and three b) white spruce sites. Aspen site spectra (sites 7, 34, 25) depict an age gradient of 17, 24, and 33 years, respectively and the spruce sites (sites 29, 26, 23) depict a canopy cover gradient from 6%, 36%, and 95%, respectively.

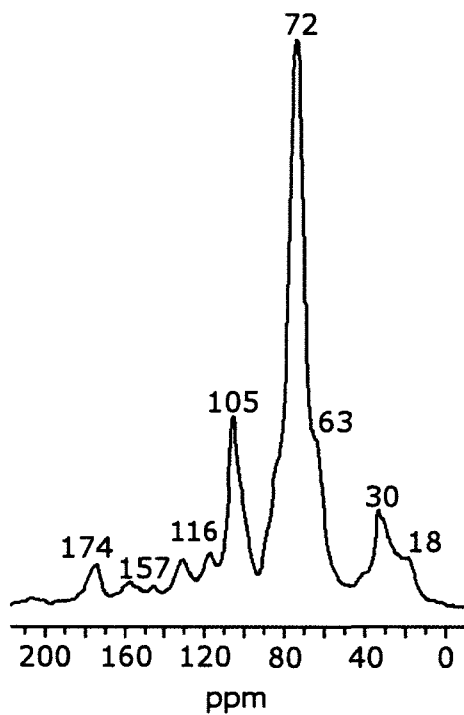


Figure 2-2 NMR spectrum from a peatland soil (0-5 cm) from eastern Alberta (54.95°N, 112.47°W). The mean annual temperature at the site is 2.1°C and mean annual precipitation is 507 mm. Sampling and analysis was undertaken in 2005 (Quideau, unpublished data).

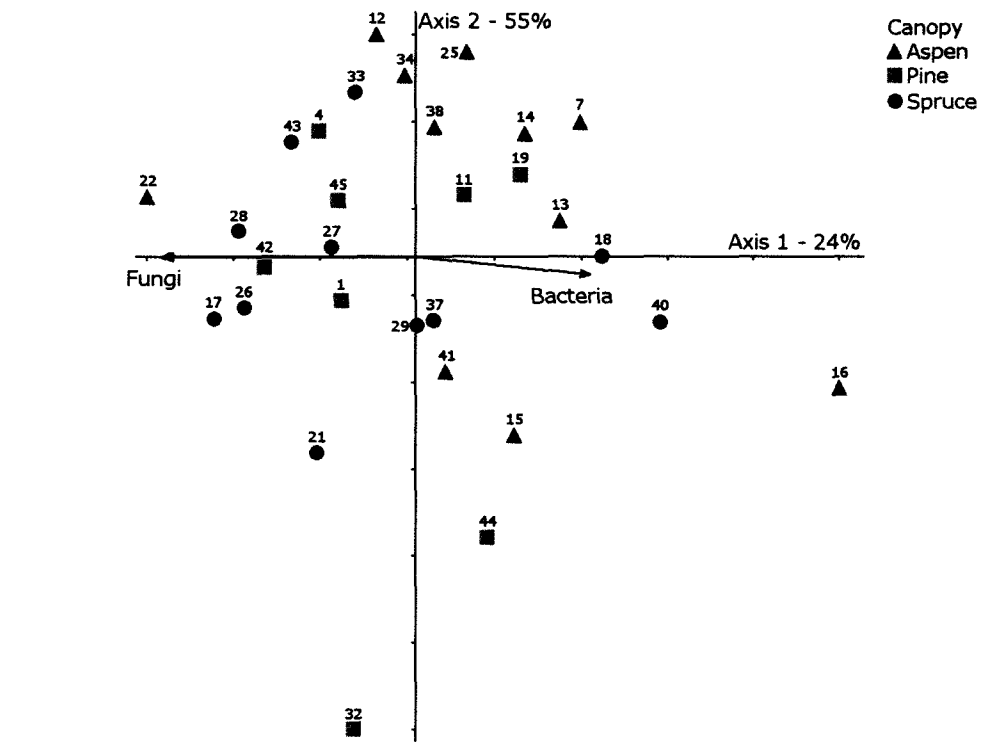


Figure 2-3 NMDS ordination of mol(%) PLFA data from trembling aspen, jack pine, and white spruce reclaimed sites previously disturbed by oil sands mining. Each point on the ordination corresponds to one site, and numbers correspond to site identification numbers from Table 1. The cut-off for vectors is R^2 of 0.5.

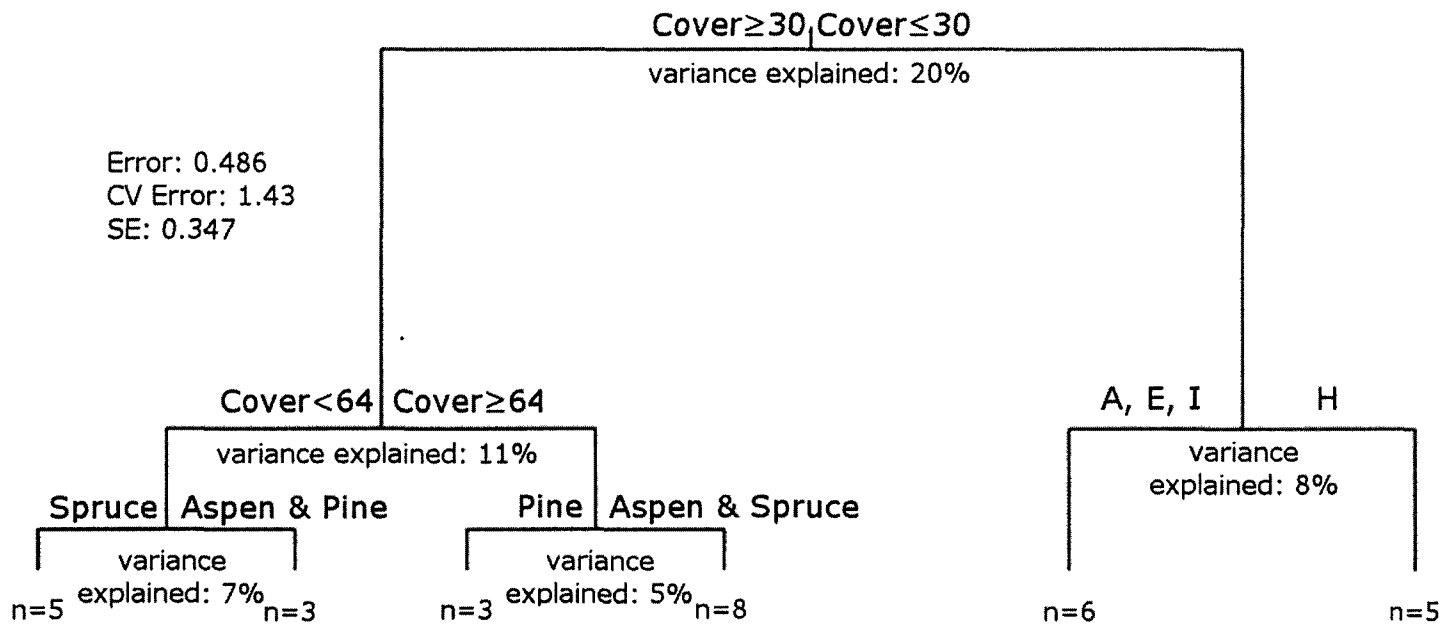


Figure 2-4 MRT analysis of mol(%) PLFA data from trembling aspen, jack pine, and white spruce reclaimed sites previously disturbed by oil sands mining. Groups are based on canopy cover (%), reclamation prescription (see Table 1 for description), and stand type (aspen, jack pine, and white spruce).

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3. Inferring processes from stand characteristics and soil nutrient patterns in reclaimed boreal forests of northern Alberta

3.1 Introduction

Four main factors contribute to nutrient cycling in forest ecosystems including: stand type and litterfall rates; forest floor decomposition rates; structure and function of the microbial community; and seasonal variability in soil temperature and moisture (Binkley and Giardina 1998, Augusto *et al.* 2002, Waldrop and Firestone 2006). Stand type plays an important role in nutrient cycling as litterfall rates and precipitation throughfall, which vary among stand types, are responsible for the majority of nutrients returned to soil in mature forests (Prescott 2002). Stand type has a substantial impact on soil biogeochemical cycling by influencing litter quality and quantities and their decomposition rates (Binkley and Valentine 1991, Prescott 2002), throughfall nutrient concentrations (Kristensen *et al.* 2004), filtering of particulates, and rates of nutrient uptake (Miles 1985). In addition to the effects of stand type, decomposition rates, microbial dynamics, and seasonal variability in temperature and moisture have been shown to have important consequences on nutrient cycling, the effects of which are hard to separate from each other. Litter nutrient concentrations and turnover have an important effect on site fertility and soil nitrogen (N) availability is closely related to litter N content in boreal forests of northern Alberta (Jerabkova *et al.* 2006). Seasonal variability in moisture and temperature is primarily responsible for changes in microbial activity (Waldrop and Firestone 2006), which has been closely linked to the availability of nutrients

in forest ecosystems due to their important role in decomposition (Leckie *et al.* 2004). Finally, evidence suggests that based on the dominance of these factors, nutrient availability in different ecosystems is controlled by either top-down (plant related), or bottom-up (microbe related) processes (Bever, 1994).

Accumulation of soil organic matter (SOM) in forest ecosystems is considered one of the key elements required to evaluate the success of reclamation of disturbed land (Bradshaw 2000). Organic matter in reclaimed oil sands sites in northern Alberta are initially amended with a peat-mineral mix (PMM) that is applied as a salvaged soil cover material (Fung and Macyk 2000). However, the re-establishment of natural upland forest soil processes require organic matter litter input from vegetation on reclaimed sites (Vetterlein and Hüttel 1999). Organic matter accumulation improves the fertility of surface soils over time as plants scavenge diffuse nutrients, accumulate them in leaf tissues, some portion of which is returned to the soil in the form of organic matter (Knabe 1973; Bradshaw 2000). Rowland *et al.* (2009) found that PMM differed from SOM of natural boreal forest ecosystems in terms of available nutrients, with high NO_3^- , Ca, Mg and S, but low NH_4^+ , P and K. Percent canopy cover and stand type have an influence on the development of the forest floor, soil organic matter composition, and soil microbial community composition of reclaimed sites in the region (Sorenson *et al.*, *in review*) but the influences of stand type, forest floor, and season on the spatial variability of available nutrients have not yet been investigated.

Everything in nature is connected via spatial relationships as a result of

biological, chemical, and physical processes (Levin 1992; Legendre 1993). Spatial variability is often regarded as random noise, but understanding the patterns produced by different factors can provide valuable insight into ecological processes at different scales (Ettema & Wardle 2002). Ecological processes often operate at multiple scales, potentially influencing a variety of variables simultaneously (McIntire and Fajardo 2009) and the influence of multiple factors can be difficult to separate with conventional statistical analyses. Rather than treating SPACE as a confounding factor, it can be explicitly incorporated into statistical models as a variable to account for unmeasured or unknown variables, and information about ecological processes can be inferred from spatial patterns and relationships (McIntire and Fajardo 2009). In particular, the success of land reclamation can be evaluated with spatial statistics because forests are characterized by small scale spatial patterns, whereas agricultural or disturbed landscapes tend to be relatively more homogenous and exhibit spatial patterns at larger scales (Fraterrigo *et al.* 2005). Changes of spatial patterns in soil nutrient availability, from large scale to small scale, can be expected as forest ecosystems recover from disturbance. Additionally, the analysis of soil-plant relations via spatially explicit techniques can be very informative, as soil is spatially connected to vegetation by a number of ecological processes, for example N transformations (Bengston *et al.* 2006; Saetre 1999).

This study focused on the restoration of soil-plant relations in reclaimed oil sands sites from northern Alberta. These reclaimed sites provide a unique opportunity to investigate the spatial pattern and re-establishment of soil-plant

relations in developing ecosystems. The objectives of this study were to quantify the spatial dependence of stand characteristics and soil nutrient availability in planted trembling aspen, jack pine, and white spruce stands on reclamation sites. Aspen improve site fertility relative to coniferous stands because of higher N mineralization (Flanagan and Van Cleve 1983), faster NO_3^- turnover and higher accumulation of NO_3^- (Ste-Marie and Paré 1999). Of particular interest was whether or not patchiness in soil nutrient supply rates changes with season, and if differences between aspen, pine and spruce reclaimed sites exist. Furthermore, relationships between canopy cover, stem location and soil nutrient supply rates after accounting for SPACE were investigated to determine if differences between top-down (plant) and bottom-up (microbe) controls exist in different stand types.

3.2 Materials and Methods

3.2.1 Study Sites

The study sites were located in the Athabasca oil sands region north of Ft. McMurray, Alberta (56°43'N 111°21'W). The mean monthly air temperature in the study area ranges from -19°C in January to 17°C in July, with a mean annual temperature of 0.7°C (Environment Canada 2009). The mean annual precipitation is 455.7mm, with 342.2mm occurring as rainfall during the growing season (Environment Canada 2009). On these sites subsurface soil and near surface geological deposits (to a depth of 3m) were salvaged before mining and used as subsoil in reclaimed areas (Fung and Macyk 2000). Reclaimed sites were capped with peat-mineral mix (25-50 % v/v) (PMM) to provide an organic matter rich

cover soil. All three sites were capped with PMM that exceeded 20 cm in depth, but the subsoil materials varied. Subsoil in the aspen and pine sites consisted of salvaged subsoil (2-3 m salvaging depth) over tailings sand, whereas at the spruce site the subsoil consisted of geologic (overburden) material (3-45 m salvaging depth). Reclamation practices are described in more detail elsewhere (see Turcotte et al. 2009 or Rowland et al. 2009). Following capping, one-year old aspen, jack pine, or white spruce planting stock, container grown from local seed sources were planted. Planting densities averaged 2161 stems/ ha in the aspen stand, 1524 stems/ ha in the pine stand and 2855 stems/ ha in the spruce stand, with an average distance of 2-3 m between trees. No further stand tending treatments were applied after the initial establishment of the seedlings.

3.2.2 Spatially Explicit Sampling

Study plots were established to investigate the spatial relationships between forest floor development, canopy cover, and nutrient availability in reclaimed ecosystems. The spatially explicit sampling method used followed a random walk design (Underwood 1997) consisting of 75 randomly generated sampling points at each site (Figure 3-1). The sampling pattern was designed in order to ensure that there were at least 45 pair-wise comparisons at one meter spatial lags between 0 and 30 m. Bearings and distance were used to locate 15 initial points, randomly generated 2-10 m from the preceding point. Sixty additional points were randomly generated, with one sampling point was

established in each cardinal direction, randomly generated between 0 and 2 m away from each of the 15 initial points.

In August 2008, forest floor and peat mineral mix samples were collected at each of the 75 sampling points in all three study plots. Forest floor samples were collected with a 15.2cm diameter cylinder, the depth of the forest floor was measured, and samples were air dried and weighed. Peat mineral mix samples were collected with a cylindrical sampler from a depth of 0-5 cm. The bulk density of the forest floor sample was calculated, and forest floor mass (FF mass) was determined on an areal basis (Mg/ha). Soil pH was measured using the method outlined in Kalra and Maynard (1991). Soil samples were first sieved with a 2mm screen and ten grams of sample were mixed with 20ml of 0.01M CaCl₂. The suspension settled for thirty minutes and the pH of the supernatant solution was measured and recorded.

In each site the location of all trees were surveyed in August 2009 using a Vertex III and Transponder T3 (Haglöf Sweden AB) and an automatic level (FaberCastell Automatic Level B-4). Trees up to 10 m from a sampling location were included, and tree locations are presented in Figure 3-2. For each tree, height to live crown, crown width, and crown length were measured. To measure crown width, the distance from stem to crown edge was measured in four cardinal directions and an average was taken in order estimate crown radius. The number of crowns extending over each spatially explicit soil sampling position (canopy cover) and the distance to the nearest tree from each sampling point were

estimated using the *spatstat* library (Baddeley and Turner 2005) in R (R Development Core Team, 2010).

3.2.3 Ion exchange resin analysis

Plant root simulator (PRS) probes (Western Ag Innovations Inc., Saskatoon, SK, Canada) were used to measure available nutrients in the soil. Probes consisted of cation or anion resin membranes (10 cm² area), with approximately 215 meq of exchange capacity, enclosed in a plastic frame (for details see Qian and Schoenau 2002). The probes were installed vertically in the soil with the top of the probe membrane at the interface of the forest floor and PMM and the bottom located 5cm down. Probes were installed at each sampling point in all three sites for three periods, fall (August 15, 2008 to November 2, 2008), spring (November 2, 2008 to May 9, 2009), and summer (May 9, 2009 to July 17, 2009). Since all probes were installed for approximately 3 months of unfrozen soil conditions, the data are comparable and reported as nutrient supply rate for a three month period. After retrieval, probes were washed with deionized water and each probe pair (cation and anion) were bagged separately and sent for analysis. Each probe pair was eluted with 0.5N HCl to extract nutrients for analysis (Qian and Schoenau 2002). Ammonium (NH⁴⁺), nitrate (NO³⁻), and phosphate (PO₄³⁻) were analyzed colourimetrically using a segmented flow Autoanalyzer III (Brand and Lubbe, Inc., Buffalo, NY.). Potassium (K⁺), sulfate (SO₄²⁻), calcium (Ca²⁺), and magnesium (Mg²⁺) were quantified by inductively-

coupled plasma (ICP) spectrophotometry (PerkinElmer Optima 3000-DV, PerkinElmer Inc., Shelton, CT). As NH_4^+ supply rates were very small compared to nitrate, and in many cases below detection, only total inorganic N supply rates were reported.

3.2.4 Statistical analysis

For each stand the nearest-neighbor distribution $G(r)$ was calculated using the spatstat library (Baddeley and Turner 2005) in R (R Development Core Team, 2010). The G function measures the distribution of distances from an arbitrary event to its nearest event (Bivand *et al.* 2008). Event in this study refers to a stem location, therefore event to nearest event refers to distance to nearest tree stem in this context. $G(r)$ is formally defined as a probability that the distance from a random chosen event to its nearest neighbor is less than or equal to a given nearest neighbor distance (r). Point patterns were determined for tree locations in each stand type, and $G(r)$ was then calculated from these point patterns. Monte carlo envelopes were computed based on 99 simulations and the spatial distribution of trees were determined based on these envelopes which indicate the maximum and minimum simulation values. $G(r)$ values above the upper envelope indicate tree clustering, while functions within the envelope indicate a randomly distributed trees, and functions below the lower envelope indicate regularly distributed trees.

Semivariograms were calculated to examine the scale of spatial dependence in the forest and soil properties of each stand type. All semivariograms were generated using the `gstat` library (Pebesma 2004) in R. Soil properties included FF mass (Mg/ha), soil pH, and nitrogen (NH_4^+ and NO_3^-), phosphorus (PO_4^{3-}), potassium (K^+), sulfur (SO_4^{2-}), calcium (Ca^{2+}) and magnesium (Mg^{2+}) supply rates. In each semivariogram there were 75 independent samples and 2775 pairwise comparisons between all points (75 x 75 distance matrix, minus 75 for the diagonal, divided by 2 for half of the matrix). Data was log transformed before calculating the semivariogram in order to reduce the influence from extreme values, and large scale trends were removed if present. Summer N values were not log transformed because there were too many zeros in the data. Experimental semivariograms were generated to a maximum of 30 m as this is half of the maximum distance between sampling points, and the influence of outlying variables was accounted for by using a robust measure (Cressie 1993) of the sample semivariogram (`cressie=TRUE` was added to the variogram call function). Once experimental semivariograms had been calculated, exponential, matern, spherical and gaussian theoretical models were fit to the data. Semivariogram sills were fit to the data using restricted maximum likelihood (REML) fitting (`fit.variogram.reml` function), and ranges were fit by non-linear regression (`fit.variogram` function) as REML fitting of ranges is not available in the `gstat` package (Bivand *et al.* 2008). The theoretical semivariogram with the lowest sum of squares was then used for the description of spatial dependence. A semivariogram consists of three primary components termed the range, nugget,

and sill (see Ettema and Wardle 2002). Range indicates the distance at which spatial independence is reached and therefore represents the patch size. The sill is the semivariance at that range, and the nugget is the semivariance at a distance of zero which specifies the measurement error. The relative difference between C_0 (sill-nugget) and C (sill) corresponds to noise in the data and the strength of spatial patterns detected up to the range (Ettema and Wardle 2002). In this study, $(C_0-C)/C$ is referred to as spatial predictability, with 1 being perfect predictability. Maps were generated via kriging for forest floor mass, soil pH, and soil nutrient supply rates in each stand. Graphs of semivariograms and maps are presented in Appendix D and Appendix E, respectively.

Relationships between soil nutrient supply rates, FF mass, and canopy characteristics were investigated for each stand type using a spatial analysis of variance. One model was run for each nutrient in each stand type. Season, FF mass, soil pH, distance to tree, and canopy cover (number of crowns over a sampling point) were included as fixed effects in the model. All the nutrients were included as either predictor or response variables within each spatial ANOVA. Swapping response and predictor variables acts to explicitly model as much of the intercorrelations as possible and generates a more conservative assessment of the influence of FF mass, distance to a stem, and canopy cover on soil nutrient supply rates (Mackenzie *et al.* 2008). Using the generalized least squares approach in R (Pinheiro *et al.* 2009), we analyzed the marginal effects of each term (anova

function with type="marg" option). Therefore the effect reported is the effect after all other interactions have been removed.

The three spatial ANOVAs are represented by:

$$y_k = \alpha_k + \boldsymbol{\beta}_k \mathbf{X}_k + \varepsilon_k$$

$$\varepsilon \sim \text{Normal}(0, \sigma^2_k, \Lambda_k)$$

where the subscript k is one of the 18 ANOVA models used to predict the set of six response variables (y): N supply rate, PO_4^{3-} supply rate, K^+ supply rate, SO_4^{2-} supply rate, Ca^{2+} supply rate, Mg^{2+} supply rate. \mathbf{X} is the matrix of data and interactions, $\boldsymbol{\beta}$ is the vector of parameters for the fixed covariates, ε is the residual error, and σ^2 is the variance for the error terms. We used the exponential covariance model (Pinheiro *et al.* 2009) to account for within-group spatial dependence, as reflected by the covariance matrix, Λ . The full explicit models shown are in Appendix C.

3.3 Results

3.3.1 Spatial dependence of stand characteristics and soil nutrient supply rates

Nearest neighbor analysis indicated that the spatial patterns of tree distribution differed across stand types (Figure 3-3), where the spatial distribution of pine and spruce were random up to 2 m, as would be expected in a planted stand, but aspen exhibited a clustered pattern. The maps of stem location suggest that the conifer stands were regularly distributed, although with some clumping,

but not as much as in the aspen stand (Figure 3-2). The aspen stand had a lower mean nearest neighbor distance (1.0 ± 0.5 m), as compared to pine (1.5 ± 0.6 m) and spruce stands (1.3 ± 0.6 m). Given the aggregation of trees observed in the aspen stand, tree scale influence may be associated with clusters of trees rather than individual stems in this stand. Canopy cover exhibited spatial dependence at different scales in different stand types (Table 3-1), with ranges corresponding to tree scale in the aspen and pine stands (3 and 5 m, respectively) suggesting tree to tree variability. However, there was a larger range in the spruce stand (19 m) implying uniformity in canopy cover within larger patches (Table 3-1), and indicating there was likely less mortality than in the spruce stand.

Spatial dependence in FF mass and soil pH also varied among stand types (Table 3-1). Aspen FF mass and soil pH exhibited ranges that were larger than tree scale and showed little spatial predictability implying relative uniformity (Table 3-1). The pine stand FF mass had a range at tree scale, but low spatial predictability, whereas soil pH had a range larger than the study area and strong spatial predictability indicating a large scale gradient. Spruce stand FF mass and soil pH exhibited ranges larger than tree scale, with low spatial predictability in FF mass and high predictability in pH (Table 3-1).

Spatial dependence was detected in soil nutrient supply rates in the aspen stand and most nutrients showed evidence of seasonality (Tables 3-2 and 3-3). Nitrogen supply rates were at a tree scale in the fall and spring, but varied at a larger scale in the summer (Table 3-2). The summer increase in range was accompanied by a decrease in N supply rate. Phosphorus varied at a tree scale in

the fall and at a scale larger than the study area in the spring, however low spatial predictability indicated noise in the data and a weak spatial pattern (Table 3-2). A pure nugget was detected for PO_4^{3-} in the summer indicating a smaller scale of variation than the minimum scale of the study, or large measurement error.

Potassium exhibited low spatial dependence in the fall and spring, and a large scale gradient with the highest supply rate in the summer (Table 3-2). Although SO_4^{2-} supply rates were much higher in the fall and spring there was low spatial dependence until summer, where it exhibited a tree scale patchy distribution with lower concentration (Table 3-3). Calcium did not vary greatly between seasons with low spatial predictability. Magnesium was patchy at tree scale in summer but was patchy at larger than tree scale in spring.

The pine stand exhibited spatial dependence and seasonal variability for all nutrients except Mg^{2+} (Tables 3-2 and 3-3). Nitrogen was patchy at the tree scale in spring and at larger than tree scale in the summer (Table 3-2). In addition to a larger range, the summer also had the highest N supply rates of all stand types. Phosphorus was patchy at the tree scale in fall, which increased to larger than tree scale in spring, and a gradient larger than the study area in the summer (Table 3-2). Potassium exhibited a large scale gradient in the fall, but spatial dependence was limited in the spring and summer. Spatial dependence in SO_4^{2-} was limited in all seasons, and supply rates decreased from fall to summer, as in the aspen stand (Table 3-3). Calcium exhibited a gradient larger than the study area in fall and summer, but tree scale patchiness in the spring. Magnesium spatial dependence was low in the fall and summer, and was barely larger than tree scale

in the spring.

Spatial dependence was also observed in the spruce stand, with some seasonality (Tables 3-2 and 3-3). Nitrogen supply rates were highest in the fall and lowest in the summer, but little spatial dependence was evident in any season (Table 3-2). Phosphorus had a similar supply rate in all seasons with slightly larger than tree scale patchiness in the spring and tree scale patchiness in the summer. Potassium supply rates were patchy at tree scale in fall and spring, but spatial dependence was limited in the summer (Table 3-2). Sulfur supply rates were much higher in the spruce stand than in the aspen and pine stands, but exhibited very little spatial dependence (Table 3-3). Calcium exhibited little spatial dependence in the fall and summer, but was patchy at the tree scale in spring. However, a spatial predictability value of 1.0 should be treated with caution, as a zero nugget implies no error which is unlikely (Table 3-3). Spatial dependence in Mg^{2+} was weak in the fall, patchy at the tree scale in spring and slightly larger than tree scale in the summer (Table 3-3).

3.3.2 Relationships between variables after accounting for SPACE

After accounting for the effect of SPACE, changes in soil nutrient availability were related to FF mass, canopy cover, and distance to nearest tree, but these relationships varied among the stands investigated (Tables 3-4, 3-5, 3-6). Results from the spatial ANOVA include an F-value, which determines whether a relationship is significant, and a parameter estimate that provides insight into both

the direction and magnitude of a relationship. Since covariates were standardized, the influence of independent factors can be directly compared. For every increase of one in the independent factor, the dependent factor increases by the value of the parameter estimate.

In the aspen stand, N was significantly affected by distance to nearest tree, canopy cover, and soil pH (Table 3-4). Canopy cover had the strongest positive effect on soil N supply rates, while pH had a strong negative effect as indicated by the parameter estimates. Phosphorus had a significant intercept value, indicating that SPACE was a significant factor. Phosphorus supply rates were also significantly affected by season, with a lower phosphorus supply rates in the spring and summer, and by FF mass (Table 3-4). Potassium and Ca^{2+} were both significantly affected by FF mass, which had approximately twice the effect on K^+ as compared to Ca^{2+} . Calcium was also affected by canopy cover, and both canopy cover and FF mass had significant positive parameter estimates. Although overall season was not a significant factor on Ca^{2+} , spring and summer parameter estimates were significant and negative (Table 3-4). Sulfur was positively affected by canopy cover, varied between seasons, and SPACE was significant, while Mg^{2+} was only significantly related to soil pH.

In the pine stand, N varied significantly between seasons, with a positive estimate in the spring, and SPACE was significant, but there were no relationships between N and stand characteristics like in the aspen stand (Table 3-5). Phosphorus was significantly related to distance to the nearest tree with a positive parameter estimate. Potassium showed no significant relationships in the spatial

ANOVA, but there was a positive parameter estimate for summer K^+ which is difficult to interpret. Sulfur varied seasonally, with negative parameter estimates in the spring and summer. Sulfur was also positively affected by FF mass, and a significant SPACE effect in the data (Table 3-5). Contrary to SO_4^{2-} , Ca^{2+} was negatively affected by FF mass, and had a negative spring parameter estimate although season was not significant.

The spruce stand had distinctly fewer relationships than the aspen and pine stands (Table 6). In the spruce stand, both N and SO_4^{2-} were affected by unmeasured variables, as indicated by a significant intercept term. Nitrogen and SO_4^{2-} also varied seasonally, with negative parameter estimates in the spring and summer for both nutrients (Table 3-6). Potassium was the only nutrient that was significantly related to a stand characteristic which was a positive canopy cover effect.

3.4 Discussion

3.4.1 Implications of spatial structure

Spatial dependence is a common feature in forest ecosystems, where many stand characteristics have a patchy distribution (Carey 2003), including soil resources such as nutrient availability (Frateriggo *et al.* 2005). Resource heterogeneity has been hypothesized as an important driver of species diversity (Ricklefs 1977) and disturbance has been shown to decrease resource heterogeneity (Frateriggo *et al.* 2005). However, this does not seem to be the case on the 21-26 year old reclaimed sites examined here, where spatial dependence

was observed in a number of site characteristics and nutrient supply rates, which varied from the tree scale, to larger than tree scale, to larger than the study site.

Spatial dependence occurs as a result of relationships among environmental factors that were further investigated with a spatial analysis of variance model. This model accounts for the effect of SPACE and when significant indicates that a mechanistic relationship exists among variables. Because SPACE is a surrogate for unmeasured variables (McIntire and Fajardo, 2009), a significant intercept indicates that factors unaccounted for were having an effect on the spatial pattern of the dependent variables and a non-significant intercept indicates that all the relationships influencing a variable were accounted for in the study. We believe that in this study the absence of a SPACE effect indicates that stand type is exerting a top-down control on nutrient supply, whereas the presence of a SPACE effect likely indicates that microbial community structure and function are exerting bottom-up controls.

3.4.2 Comparing spatial structure among stand types

Canopy cover was expected to be patchy at scales similar to forest floor mass, but this was not the case as forest floor mass showed little spatial dependence in all three stands. Spatial dependence in soil pH was generally at a larger scale than what has previously been observed in coniferous boreal forest stands, where a range of 3.7 m has been observed (Saetre 1999), and did not appear to be related to the forest floor or canopy in the aspen and pine stands (Table 3-1). Soil pH did vary at scale similar to canopy cover in the spruce stand.

Non-corresponding spatial dependence suggests that historic canopy cover and/or decomposition rates were more important for FF mass, and that mineral soil characteristics had more of an effect on soil pH except in the spruce stand where needle litter might have contributed to lower soil pH (Binkley and Valentine 1991) given the similarity in range with canopy cover.

The spatial dependence of N in the aspen and pine stands appeared to be similar to what has been observed in natural ecosystems (Cain *et al.* 1999; Bengston *et al.* 2006; Bengston *et al.* 2007). As far as we know, seasonal changes in spatial dependence have rarely been investigated, but Cain *et al.* (1999) observed spatial dependence in N availability during some sampling periods but not others. Tree scale spatial dependence in N supply rates observed in at least one season in both the aspen and pine stands of this study appeared to correspond with the spatial scale of N mineralization and immobilization (ranges of 3.5 m and 2.7 m, respectively) that was observed in boreal forests in Sweden (Bengston *et al.* 2006). Tree scale variation may be the result of a number of additional and related influences such as nutrient uptake or variation in microbial communities. While microorganisms can be patchy at a variety of distances, patch sizes in microbial community composition in mixed spruce-birch stands of 4-5 m have been observed (Saetre and Bääth 2000). Larger spatial patterns have also been observed with N mineralization in coastal British Columbian forests (range 18.7 to 115.5 m) and are believed to be related to shifts in canopy cover or changes in topographic features (Bengston *et al.* 2007). The spatial dependence of N in aspen and pine stands appears to shift seasonally from tree scale to larger scales perhaps

indicating a change in process from top-down to bottom-up controls. The larger scales may be related to total C and microbial activity, which can be patchy from tens to hundreds of meters in agroecosystems (Fromm *et al.* 1993). Cover soils are laid down and spread by large heavy equipment which may lead to homogeneity similar to annual plowing, however 21-26 years after reclamation, seasonality appears to drive changes in soil processes spatially.

While there was little spatial dependence of PO_4^{3-} in the aspen stand, the pine stand showed changes in spatial dependence among seasons. Spatial dependence in the fall P supply rate was at tree scale suggesting a tree driven process and leaf and soil P concentration have been observed to occur at similar scales in natural systems (Gallardo and Covelo 2005). Larger spatial dependence has also been observed in soil P availability in a coastal British Columbian forest (Bengston *et al.* 2007) with a range similar to those measured here in the spring and summer (23 and >30m) . Bengston *et al.* (2007) suggested that a large scale topographic feature may be responsible for the pattern of PO_4^{3-} and the greater than plot scale patchiness in the summer may be the result of differences in soil characteristics related to topography. Large scale spatial dependence also existed for potassium in the aspen and pine stands, which was similar to the patchiness observed in the coastal BC forest (range of 41 m). Given the relatively small area occupied by the plots in this study, larger than the study area gradients may be the result of differences in the application of cover soil. The relative proportions of peat and mineral soil in the PMM may have varied between applications, leading to large gradients in supply rates of nutrients heavily influenced by processes in

the mineral soil. A lack of spatial dependence was largely observed in Ca^{2+} in the aspen and spruce stands, and spatial dependence smaller than the minimum distance in this study has been observed in natural stands (Søe and Buchmann 2005). Others have found Ca^{2+} to be patchy at scales larger than what occurred in the pine stand in this study (Bell *et al.* 1993).

While previous studies have often focused on implied relationships due to similarity in spatial dependence (e.g. Gallardo and Covelo 2005; Cain *et al.* 1999; Saetre and Bääth 2000), the challenge with this type of analysis is that both the nature of the cause and effect relationship, as well as the role of other factors, are not clear. We explicitly tested these relationships by accounting for the effect of SPACE while modeling relationships between variables. This enabled a much more conclusive evaluation of canopy influences on soil nutrient supply rates than semivariograms alone.

3.4.3 Modeling relationships between variables after accounting for SPACE on reclaimed sites

Distance to tree was positively related to soil N supply rates in the aspen stand and indicated that N concentrations decreased by 230% per m as we approached the stem (Table 3-4). This suggests that uptake is an important factor for tree scale patchiness in N supply rates. In contrast to the deciduous stand, N had no relationship with stand characteristics in the coniferous sites, but was dependent on SPACE and season (Table 3-5 and 3-6), which is reflected in a change in seasonal N supply rates, but not patchiness (Table 3-2). The significant

SPACE effect in N indicates that some of the factors related to N supply rates in coniferous stands were missed in this study and may be the result of differences in microbial activity across the sites as partially reflected in a significant seasonal effect. Furthermore, spruce has been found to have more of an effect on soil microbial community composition than aspen (Hannam *et al.* 2006), as well as more influence on the spatial dependence of soil microbial communities than birch (Saetre and Bääth 2000). Soil microorganisms have been linked to N concentrations in reclaimed sites and tend to have smaller patches (~14 cm) than those investigated in this study (Mummey *et al.* 2002). Nitrogen and microorganisms in natural forests frequently exhibit spatial dependence at similar scales (Bengston *et al.* 2006; Gross *et al.* 1995; Saetre 1999), which suggests that spatial patterns in N cycling are biologically controlled (Bengston *et al.* 2007) and here we suggest that different stand types exhibit different types of biological control.

Opposite to N, PO_4^{3-} was significantly affected by SPACE and season in the aspen stand, but only by distance to tree in the pine stand. The effect of SPACE on PO_4^{3-} in aspen may be related to interaction with other ions. In the aspen stand PO_4^{3-} supply rates were significantly related to Ca^{2+} supply rates ($F=6.33$, $p=0.01$). Immobilization of PO_4^{3-} by Ca^{2+} may be contributing to patterns of P availability in this stand. In the pine stand, for every meter away from the base of a tree a 310 % increase in PO_4^{3-} was observed, again most likely as a function of reduced uptake. These drastic differences in the use of N and P by conifer and deciduous trees have been reflected in the literature (see Son and

Gower 1991).

Sulfur showed a similar pattern in all three stands. The most important factor in each stand was SPACE and increased S in the fall. The strong SPACE effect in SO_4^{2-} may be the result of atmospheric SO_2 deposition due to oil sands activity. Deposition of SO_2 in the region has been estimated to be between 2.4-10.6 $\text{kg ha}^{-1} \text{yr}^{-1}$ (EPCM Associated Ltd. 2002). Sulfate in wet deposition is often in excess of vegetation requirements and therefore no uptake by the canopy occurs (Richter *et al.* 1983; Lindberg *et al.* 1986). Atmospherically deposited SO_4^{2-} can therefore be expected to have moved through the canopy to the mineral soil and contributed to the SPACE effect in the SO_4^{2-} data.

Literature suggests that aspen acts as an effective cation pump by reallocating cations from the mineral soil to the humus layer through litter fall (Alban 1982; Corns 1989; Paré and Bergeron 1996). The aspen stand appears to be reestablishing cation pump activity in this study, as FF mass was associated with higher soil K^+ and Ca^{2+} supply rates in the spatial ANOVA. This relationship was negative in the pine stand, suggesting that the buildup of the forest floor was associated with lower available Ca^{2+} in this stand, perhaps as a result of immobilization in the tissues of the trees. The differences between aspen and pine stands may be the result of greater exchangeable nutrients in the forest floor of aspen stands as compared to pine stands (Ste Marié *et al.* 2007), and aspen have also been observed to enrich forest floor and mineral soil exchangeable K (Bélanger *et al.* 2004; Legare *et al.* 2005). Aspen have been found to have relatively high concentrations of nutrients in their tissues (Pastor and Bockheim

1984), which may be an important consequence of the role of aspen as a cation pump.

Canopy cover was associated with higher nutrient supply rates in the aspen and spruce stands. More canopy layers over a sampling point were associated with increases in N, SO_4^{2-} and Ca^{2+} supply rates in the aspen stand, and higher K^+ supply rates in the spruce stand. These relationships indicate that development of the canopy is associated with a return of the nutrients to the soil, particularly in the aspen stand. One possible explanation for this relationship is the return of nutrients via throughfall. Precipitation in forests tends to be enriched in Ca^{2+} , Mg^{2+} , K^+ (Parker 1983) and SO_4^{2-} (Price and Watters, 1989) as a result of contact with the canopy. In natural stands, Ca^{2+} tends to be the dominant base-cation contributed by bulk precipitation followed by Mg^{2+} , Na^+ , and K^+ (Fujinuma *et al.* 2005). That N was only related to canopy cover in the aspen stand is consistent with what has been observed in natural stands, as throughfall N has also been found to be higher in deciduous stands, leading to higher soil solution NO_3^- concentrations (Kristensen *et al.* 2004). Given the relationship between canopy cover and some soil nutrient supply rates, throughfall may be important for the return of nutrients from the canopy to the soil in reclaimed stands, and this relationship warrants future investigation.

3.5 Conclusions

The reestablishment of soil-plant relations was apparent in reclaimed sites

in northern Alberta. The establishment of these links was more apparent in the aspen stand, as it was the only stand where the canopy or the forest floor had an effect on all the macronutrients. Vegetation or forest floor links were present with phosphorus, sulfur and calcium in the pine stand, and in the spruce stand only potassium was associated with any of these factors. These relationships are of note as canopy species composition can intensify differences in soil fertility (Prescott 2002). Furthermore, the role of proximity to a tree and canopy cover having contrasting effects on soil nutrients may increase spatial heterogeneity in soil nutrients with time in the aspen stand. As spatial heterogeneity in soil nutrients is an important contributing factor to biodiversity (Carey 2003), differences in the influence of the canopy on soil nutrient availability may have long term consequences for these reclaimed sites.

Tables and Figures

Table 3-1 Forest floor mass (Mg/ha), soil pH, and canopy cover (# of layers) values and semivariogram results in reclaimed aspen, pine, and spruce reclaimed sites north of Ft. McMurray, AB. The range term is the distance where spatial dependence occurs, and spatial predictability indicates ability of the model to account for spatial structure in the data and predict unmeasured points on the landscape.

Stand	Variable	Value	Range	Spatial Predictability
Aspen	Forest Floor Mass	40 ± 10	18	0.01
	Soil pH	6.7 ± 0.1	19	0.03
	Canopy Cover	1.3 ± 1.1	3	0.99
Pine	Forest Floor Mass	70 ± 20	7	0.40
	Soil pH	6.7 + 0.3	>30	0.95
	Canopy Cover	2.1 ± 1.3	5	0.58
Spruce	Forest Floor Mass	40 ± 20	10	0.41
	Soil pH	7.2 ± 0.2	19	0.73
	Canopy Cover	0.9 ± 0.7	19	0.82

Table 3-2 Fall, spring, and summer N ($\mu\text{g}/\text{probe}/3$ months), P ($\mu\text{g}/\text{probe}/3$ months), and K ($\mu\text{g}/\text{probe}/3$ months) supply rates in aspen, pine, and spruce reclaimed sites north of Ft. McMurray, AB. Soil available nutrients were collected with plant root simulator process installed from 0-5cm in the mineral soil. The range term is the distance where spatial dependence occurs, and spatial predictability indicates ability of the model to account for spatial structure in the data and predict unmeasured points on the landscape.

Stand	Season	Nitrogen			Phosphorus			Potassium		
		Supply Rate	Range	Spatial Predictability	Supply Rate	Range	Spatial Predictability	Supply Rate	Range	Spatial Predictability
Aspen	Fall	10.4 ± 14.4	3	0.66	4.8 ± 3.0	3	0.40	103.2±61.7	7	0.10
	Spring	15.6 ± 25.0	5	0.96	3.3 ± 3.8	>30	0.34	123.4±93.6	0	0.00
	Summer	8.5 ± 19.4	16	0.85	4.2 ± 3.0	0	0.00	144.8±96.2	>30	0.99
Pine	Fall	17.1 ± 24.2	19	0.4	1.6 ± 2.8	7	0.57	36.8±23.9	>30	0.64
	Spring	36.5 ± 54.5	4	0.65	1.4 ± 3.7	23	0.79	63.4±44.1	0	0.00
	Summer	50.7 ± 81.2	10	0.91	1.1 ± 0.8	>30	0.55	75.0±43.3	6	0.30
Spruce	Fall	8.3 ± 5.4	0	0	0.9 ± 0.5	3	0.25	47.8±16.7	4	0.81
	Spring	4.6 ± 2.9	>30	0.29	0.6 ± 0.4	12	0.51	29.5±10.9	7	0.74
	Summer	2.6 ± 5.5	0	0	0.8 ± 0.3	7	0.59	42.9±21.5	7	0.37

Table 3-3 Fall, spring, and summer S ($\mu\text{g/probe/3 months}$), Ca ($\mu\text{g/probe/3 months}$), and Mg ($\mu\text{g/probe/3 months}$) supply rates in aspen, pine, and spruce reclaimed sites north of Ft. McMurray, AB. Soil available nutrients were collected with plant root simulator process installed from 0-5cm in the mineral soil. The range term is the distance where spatial dependence occurs, and spatial predictability indicates ability of the model to account for spatial structure in the data and predict unmeasured points on the landscape.

Stand	Season	Sulfur			Calcium			Magnesium		
		Supply Rate	Range	Spatial Predictability	Supply Rate	Range	Spatial Predictability	Supply Rate	Range	Spatial Predictability
Aspen	Fall	631.8±194.1	4	0.35	2989.9±316.4	>30	0.18	390.7±54.0	0	0.00
	Spring	370.8±292.8	24	0.38	2628.4±447.1	21	0.45	366.2±89.3	21	0.71
	Summer	134.6±125.4	3	0.98	2527.0±431.3	>30	0.30	364.3±81.2	3	0.62
Pine	Fall	629.8±249.6	6	0.22	2813.2±409.2	>30	0.88	400.9±82.9	11	0.48
	Spring	484.7±318.7	3	0.20	2567.7±371.5	3	0.62	404.7±77.0	9	0.61
	Summer	266.4±226.2	>30	0.25	1900.2±444.7	>30	0.99	313.8±91.9	10	0.36
Spruce	Fall	1582.5±177.4	0	0.00	2222.3±297.1	10	0.49	541.3±75.3	9	0.21
	Spring	1525.6±163.2	0	0.00	2019.6±184.1	2	1.00	428.0±62.7	7	0.73
	Summer	1459.8±228.5	3	0.13	2124.7±281.4	9	0.09	488.4±76.1	10	0.53

Table 3-4 Aspen stand F-values and parameter estimates from spatial ANOVA models for nutrient supply rates ($\mu\text{g}/\text{probe}/3$ months) season, forest floor mass (FFM), distance to the nearest tree, canopy cover, and soil pH in a reclaimed stand north of Fort McMurray, AB. Since covariates were standardized, parameter estimates are relative to the scale of the covariate.

Factor	N	P	K	S	Ca	Mg
F-values						
Intercept (Space +Error)	0.62	5.03*	0.14	21.38***	3.37	0.55
Season	2.58	4.62*	0.5	20.90***	2.72	0.39
Forest floor mass	2.31	4.75*	17.61***	0.67	4.38*	0.40
Distance to nearest tree	5.24*	<0.01	0.01	<0.01	0.66	3.82
Canopy Cover	11.02**	0.06	3.62	17.06***	5.83*	2.40
pH	8.15**	<0.01	1.95	3.26	1.90	5.84*
Parameter Estimates						
Intercept (Fall + Error)	-0.78	2.24*	-0.37	4.62***	1.83	-0.74
Season - Spring	-0.16	-1.76	0.68	-6.46***	-2.10*	0.80
Season - Summer	1.55	-3.02**	-0.08	-3.64***	-1.98*	0.74
Forest floor mass	1.52	-2.18*	4.20***	-0.81	2.09*	0.63
Distance to nearest tree	2.29*	-0.04	0.12	0.08	-0.81	1.95
Canopy Cover	3.32**	0.25	1.90	4.13***	2.41*	-1.55
pH	-2.86**	0.05	1.40	1.80	1.38	-2.42*

*Significant at $P < 0.05$

**Significant at $P < 0.01$

***Significant at $P < 0.001$

Table 3-5 Pine stand F-values and parameter estimates from spatial ANOVA models for nutrient supply rates ($\mu\text{g/probe}/3$ months), season, forest floor mass (FFM), distance to the nearest tree, canopy cover, soil pH in a reclaimed stand north of Fort McMurray, AB. Since covariates were standardized, parameter estimates are relative to the scale of the covariate.

Factor	N	P	K	S	Ca	Mg
F-values						
Intercept	7.13**	0.7	1.88	24.75***	1.40	0.23
Season	6.19**	0.37	2.67	15.75***	2.60	0.51
Forest floor mass	0.04	1.27	1.67	6.26*	5.27*	2.14
Distance to nearest tree	0.88	9.43**	2.56	0.93	0.02	1.11
Canopy Cover	0.28	3.58	1.35	0.21	2.32	1.86
pH	3.66	0.01	0.92	0.13	3.24	0.36
Parameter Estimates						
Intercept	-2.67**	0.84	-1.37	4.97***	1.18	0.48
Season - Spring	3.52***	-0.83	0.89	-5.58***	-2.07*	-0.91
Season - Summer	1.79	0.63	2.28*	-3.38***	-0.22	-0.07
Forest floor mass	0.21	1.13	-1.29	2.50*	-2.30*	1.46
Distance to nearest tree	0.94	3.07**	-1.6	0.96	0.15	1.05
Canopy Cover	0.53	1.89	-1.16	-0.46	-1.52	1.36
pH	-1.91	-0.07	0.96	0.36	1.80	-0.6

*Significant at $P < 0.05$

**Significant at $P < 0.01$

***Significant at $P < 0.001$

Table 3-6 Spruce F-values and parameter estimates from spatial ANOVA models for nutrient supply rates ($\mu\text{g}/\text{probe}/3$ months), season, forest floor mass (FFM), distance to the nearest tree, canopy cover, soil pH in a reclaimed stand north of Fort McMurray, AB. Since covariates were standardized, parameter estimates are relative to the scale of the covariate.

Factor	N	P	K	S	Ca	Mg
F-values						
Intercept	20.86***	1.21	2.51	10.22**	0.38	<0.01
Season	21.04***	2.22	4.70	7.36***	0.06	0.22
Forest floor mass	2.87	0.01	0.03	2.57	0.89	0.08
Distance to nearest tree	2.82	1.74	0.17	2.75	1.57	0.46
Canopy Cover	2.03	0.87	6.53*	0.85	0.04	0.31
pH	0.49	0.04	0.17	0.37	0.02	<0.01
Parameter Estimates						
Intercept	4.57***	1.10	1.58	3.20**	0.62	0.02
Season - Spring	-6.44***	-0.26	-1.02	-3.77***	-0.17	-0.35
Season - Summer	-3.20**	-1.82	-2.98**	-2.84*	-0.34	-0.67
Forest floor mass	1.69	0.12	-0.16	1.60	0.94	-0.28
Distance to nearest tree	1.68	1.32	0.41	1.66	1.25	-0.68
Canopy Cover	-1.42	-0.93	2.56*	-0.92	0.20	-0.56
pH	0.70	0.21	-0.41	0.61	-0.15	-0.02

*Significant at $P < 0.05$

**Significant at $P < 0.01$

***Significant at $P < 0.001$

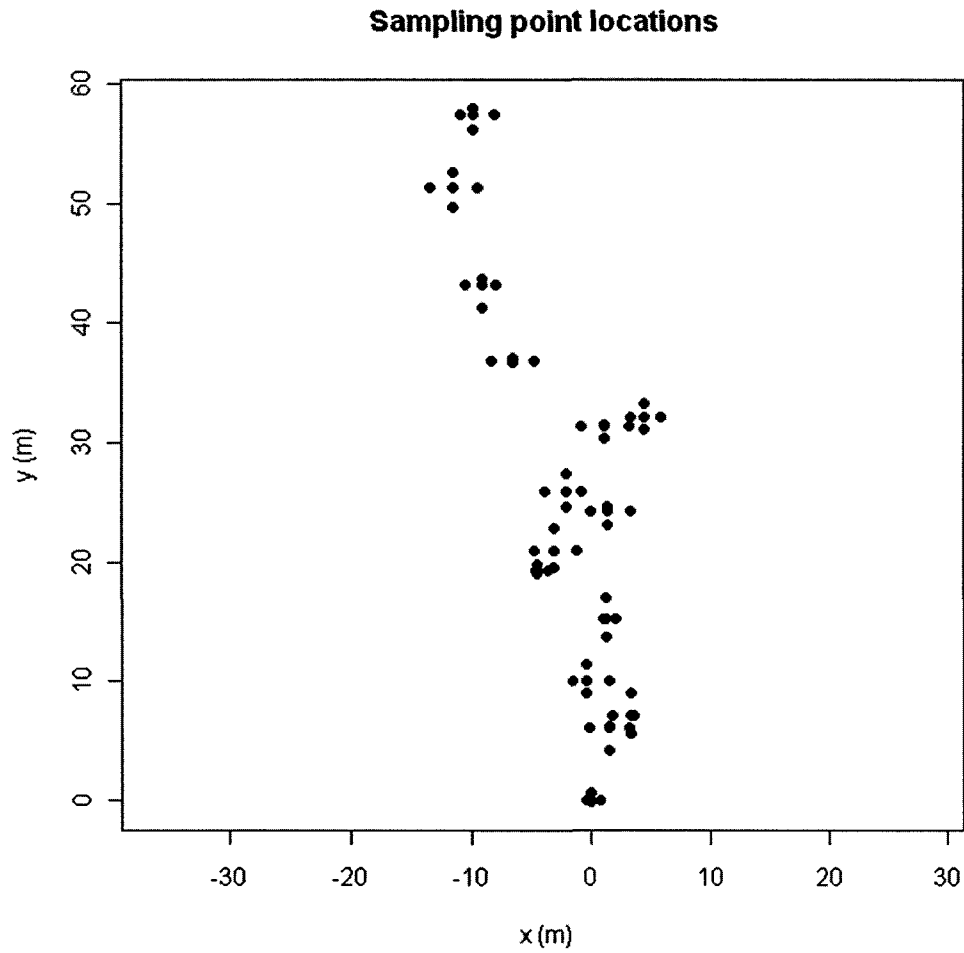


Figure 3-1 Sampling locations in the aspen, pine and spruce reclaimed sites. Seventy-five sampling points were randomly generated with 15 initial points 2-10m from the preceding point and 60 additional points established 0-2m from the 15 initial points. One sampling point was established in each cardinal direction from one of the 15 initial points.

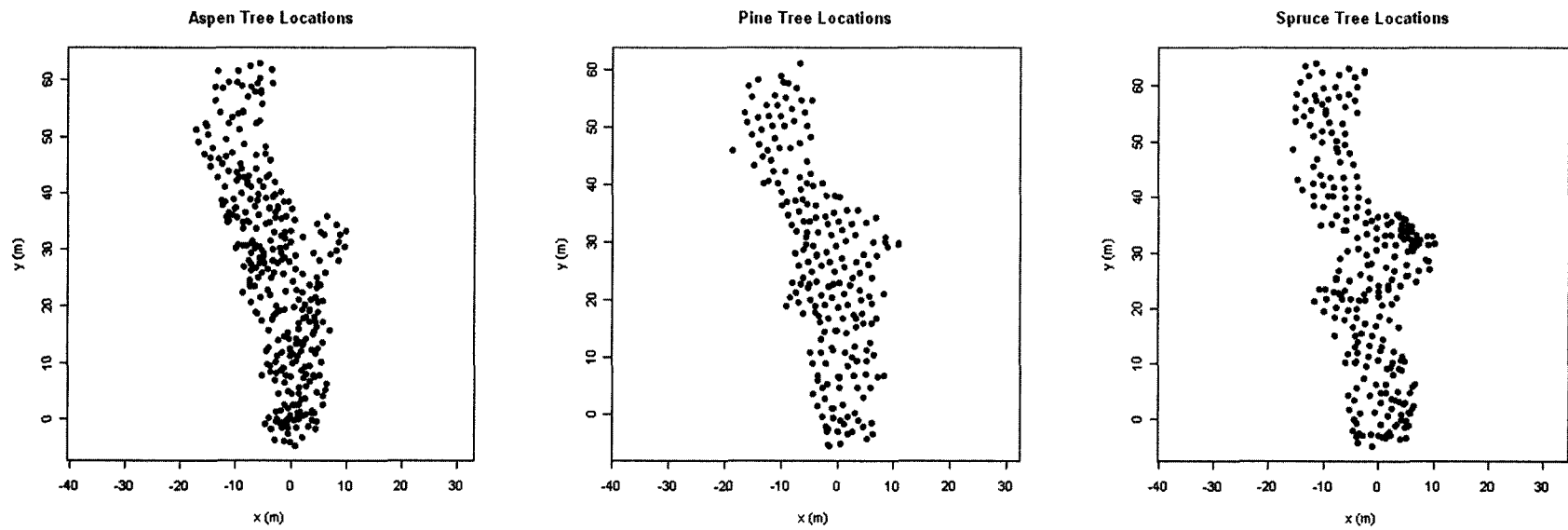


Figure 3-2 Tree locations in the aspen, pine and spruce reclaimed sites.

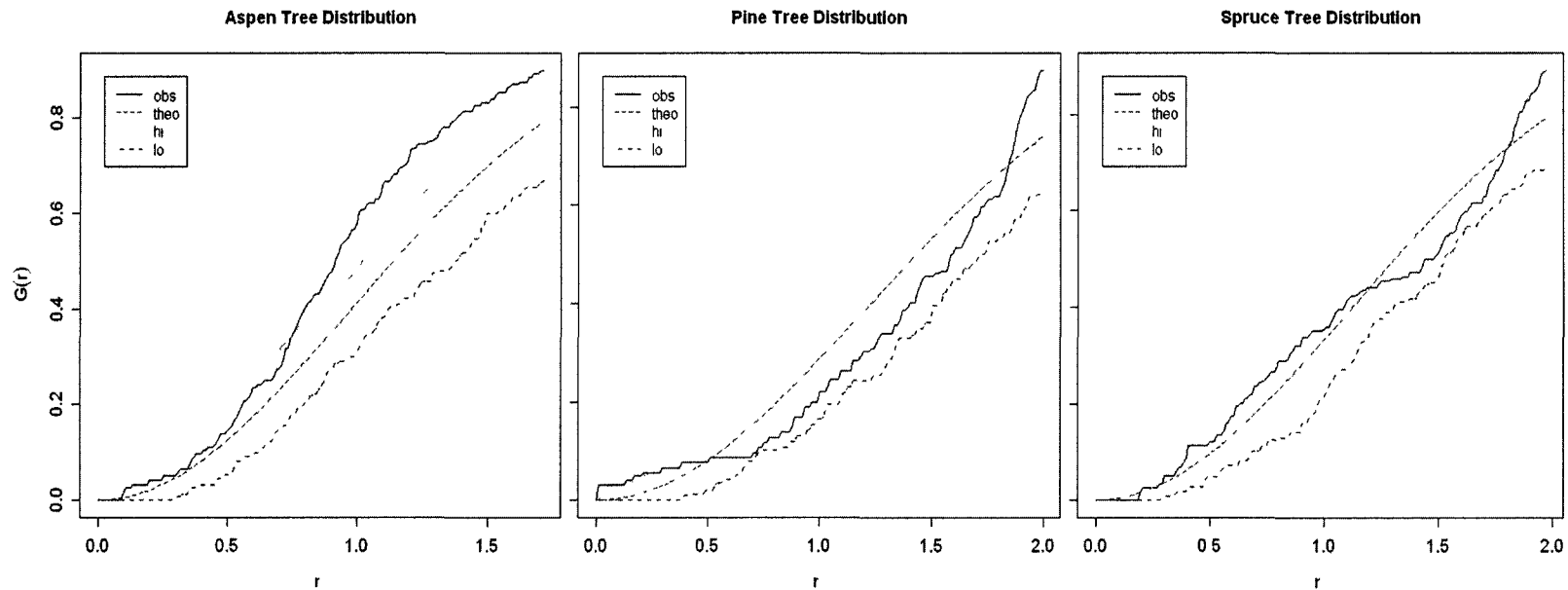


Figure 3-3 Spatial distributions of trees in aspen, pine and spruce reclaimed stands. G-values, which measure the distribution of event to nearest event distances, were calculated for each stand. An observed G-value within the Monte Carlo envelopes indicates a random and uniform distribution of trees. G-values above the envelope indicate aggregated distributions of trees, and G-values below the envelope indicate a regular distribution of trees.

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4. Synthesis

4.1 Conclusions from temporal and spatial studies

4.1.1 Characteristics of the forest floor

Forest floor thicknesses in the aspen and spruce stands of this study have not reached the depths found in natural stands. Aspen forest floor depths on reclaimed sites were comparable to similarly aged planted stands in other locations, whereas spruce forest floors were thinner by comparison. Relatively low canopy cover in the spruce stands may be responsible for the lack of forest floor development. While the pine stands were within the ranges found in natural stands, pine stands have particularly thin forest floors (1 – 4 cm).

Morphologically, while the forest floors most closely resembled hemimors, they did not quite fit this classification because of little visible fungal mycelia and fine roots, indicating low biological activity. The low biological activity may be the result of a lack of mesofauna, which are heavily affected by disturbance

Additionally, H horizons were present on only four sites, and in all cases, these H layers comprised less than 10% of the total forest floor thicknesses. Natural aspen stands aged 35-70 years in northeastern British Columbia have been reported to commonly have H horizons and both Fz and Fm horizons (indicating the presence of both fungal and faunal activity), which were completely missing from these reclaimed sites. The aspen stands had thicker forest floors relative to the coniferous stands, a trend of increasing forest floor thickness, and observable changes in SOM composition with time since reclamation. The increase in forest

floor thickness with time indicates that the aspen stands exhibit a consistent organic matter input to the soil, and may be expected to reliably develop a forest floor. Additionally, the change in SOM composition with time indicates that litterfall and organic matter accumulation processes have been substantial enough to alter the surface SOM composition and create conditions more similar to a natural forest, as indicated by the observed increase in the alkyl C region on the NMR spectra.

Morphologically the forest floor in these reclaimed stands was distinct from what has been observed in natural stands. While there are many possible explanations for the morphological dissimilarity, a lack of mesofauna may be a reason why biological activity appeared to be low. Mesofauna may have decreased in number relative to undisturbed natural soils, as they have been observed to decrease in density by as much as 93% following disturbance such as forest floor removal, soil compaction, and whole tree harvesting. Furthermore, preliminary investigations suggest that densities and community structure differ between reclaimed and natural soils for most soil mesofauna. Future research is necessary to identify the prevalence of mesofauna and investigate their contribution to forest floor development at reclaimed sites.

4.1.2 Effect of canopy cover

Canopy cover stood out as a particularly important factor as it had substantial impacts on soil microbial community structure. When canopy cover

exceeded a threshold of 30%, differences in soil microbial communities became driven by stand type. On the other hand, when canopy cover was below 30%, reclamation prescription better explained differences among sites in terms of their soil microbial community composition. Reclamation prescription H was different from prescriptions A, E, and I on sites with less canopy cover, likely as a result of subsoil differences. Prescriptions A, E and I include a PMM cover over salvaged subsoil or overburden whereas prescription H consists of PMM over tailings sand. These findings are somewhat in contrast with those from Rowland *et al.* (2009), who reported that reclamation prescriptions E, H, and I were different from prescriptions A and B in terms of soil bioavailable nutrients. Rowland *et al.* (2009) included a number of sites that were younger than the age range included in my study, and they did not investigate the specific influence of canopy cover on reclaimed site characteristics, which may explain the differences observed. In all cases, for the range of reclaimed sites that I investigated for my study, the main influence on soil microbial community composition shifted from reclamation treatment to stand type when canopy cover exceeded 30%.

4.1.3 Spatial patterns

Spatially explicit investigation into forest floor development revealed evidence of spatial patterns at scales between 0 and 30 m, as well as links between soil nutrients and the canopy. As spatial heterogeneity plays an important role in forests and contributes to biodiversity, the presence of spatial heterogeneity in soil

nutrients may have important long-term consequences for biodiversity at these reclaimed sites. The aspen stand in this study stood apart from the pine and spruce stands in terms of connections between the forest floor, canopy and soil nutrients. Nitrogen, phosphorus, potassium, sulfur and calcium were linked to either forest floor mass or canopy cover in the aspen stand, whereas the pine stand only exhibited relationships with phosphorus, sulfur and calcium. In the spruce stand, the only spatial relationship that appeared to exist was that canopy cover had a positive effect on soil potassium. The presence of these numerous spatial relationships in the aspen stand indicates that soil-plant relationships established earlier as compared to the pine and spruce stands, as the aspen stand was 5 to 6 years younger than the coniferous sites.

4.1.4 Stand type differences

Both the temporal and spatial components of this study told similar stories in terms of the reestablishment of ecosystem function and differences among stand types. In both components of the study the establishment of canopy stood out as a critical factor. A higher canopy cover was associated with a thicker forest floor, greater changes in soil organic matter and microbial community composition, as well as with higher soil nutrient availability. Additionally, it appears that links between soil and vegetation establish sooner in the aspen stand, as can be concluded from observed changes with time in forest floor depth and soil organic matter composition (Chapter 2), and additional links between the

canopy, forest floor and soil nutrients than were present in the coniferous stands (Chapter 3). The restoration of ecosystem processes is at the basis of successful reclamation and earlier establishment of soil-vegetation links is critical for the long term success of reclaimed sites.

4.2 Project limitations and future research

Research into forest floor development with time and the spatial variability of forest floor development in reclaimed sites was constrained by a number of limitations as a result of the industrial environment where the research took place. I would like to point out some limitations that may warrant future investigation, as well as research that may build on this thesis.

This study did not directly address how forest floor development in reclaimed sites compares to forest floor development in similarly aged natural stands in the region. Previous studies have found that reclaimed soils are distinct from natural boreal forest soils with respect to a number of characteristics. However, these studies did not directly compare reclaimed sites to natural stands that had been disturbed by fire a comparable number of years ago, which may be very informative for future investigations. While comparisons with literature from other parts of the country were available, how the forest floor in different reclaimed stand types compares to the forest floor in similarly aged naturally disturbed sites in the region remains unanswered.

A key limitation of the investigation into spatial patterns of forest floor development was a lack of replication of study sites. While observations about

spatial dependence of variables and the relationship between soil nutrient availability and site characteristics within a site were possible with the current study, direct comparisons of the magnitude of relationships among stand types was not. Replication is also necessary in order to conclusively attribute the presence of different relationships among sites to stand type and not to an unmeasured factor such as soil differences. A replicated spatially explicit study of the soil-vegetation links investigated in this study will enable direct comparisons among stand types with respect to the strength of relationships and will be able to conclusively attribute the presence or absence of relationships to stand type.

4.3 Recommendations

4.3.1 Effect of canopy cover and stand type

Soil capability is considered to be a key criterion for forest productivity monitoring in oil sands reclaimed sites, and refers to the ability of a soil to support the requirement of vegetation. The amount of organic matter is considered to be a key variable in soil capability, but the quality of organic matter, not just the quantity, is important. Organic matter quality strongly influences microbial community activity and nutrient turnover. While the PMM provides a source of carbon for microorganisms and nutrients, organic matter most similar to that of a natural forest will come from the development of the forest floor, and a cycling of nutrients through the forest floor is needed to meet future plant nutrient requirements. Two main management implications come out of this research with respect to forest floor development, and therefore future soil capability. First, it is

important to ensure early canopy closure as this closely relates to forest floor development and subsequent changes in key soil characteristics. Secondly, the use of aspen should be considered to jump start forest floor development, as aspen sites have more consistent canopy establishment and forest floor development than the coniferous sites.

High canopy cover stands out as being an important prerequisite for the development of the forest floor and associated ecosystem processes. Canopy cover was associated with forest floor development, soil organic matter composition and the divergence of soil microbial communities among stand types. The investigation into spatial patterns in forest floor development also revealed that the canopy cover was associated with increases in soil nutrient availability, potentially due to throughfall and litterfall. The canopy appears to be an important driver for many processes in reclaimed sites, and is a defining feature of forests. Quick establishment of the canopy appears to be critical for the early reestablishment of soil organic matter and nutrient fluxes and processes. Based on the results of this study it appears that the establishment of the canopy differs among stand types. While differences were not statistically significant, the aspen stands had more consistent canopy establishment than coniferous stands.

Clear differences between aspen and coniferous reclaimed stands exist with respect to the development of a forest floor. Aspen stands had thicker forest floors and increasing forest floor depths with time. Changes in soil organic matter composition with time were also apparent in the aspen stands, and were not apparent in either the pine or spruce stand. In the spatial study, there were more

soil-vegetation links, such as increased nitrogen supply rates with increased canopy cover, in the aspen sites than in the pine or spruce sites. Both studies suggest that connections between the canopy and the soil establish earlier in aspen stands, with earlier canopy closure likely an important reason.

4.3.2 Implications of management practices

Reclamation management practices should take into account the role of canopy cover in the development of a forest floor. Higher planting densities and management of grasses to reduce competition with trees may be needed to ensure early canopy closure in newly reclaimed sites. In sites where coniferous stands are to be established, a deciduous shrub community in the understory appears to be important for the development of a forest floor. Furthermore, jack pine trees are naturally found in nutrient and moisture poor conditions with thin forest floors and relatively little soil organic matter. Attempting to replicate these conditions may have advantages for the establishment of jack pine stands. Not only would these sites be more characteristic of a ecosites, but there would be less competition with fast growing nutrient and water demanding species.

Additionally, aspen could be used to quickly establish canopy closure and the development of a forest floor, with white spruce subsequently planted in the understory. This approach not only has the advantage of increased likelihood of canopy closure, but it also mimics ecological succession with a pioneer species quickly colonizing a disturbed site and a shade-tolerant species establishing later

in the understory and eventually replacing the pioneer species as the dominant tree in the stand. While many priorities exist when selecting tree species to establish on reclaimed sites, aspen may have advantages over pine and spruce with respect to earlier establishment of organic matter accumulation and nutrient cycling processes. Stand type should be considered in reclamation not only for the end goal of stand composition but also for the differences in the reestablishment of soil organic matter accumulation and nutrient fluxes and processes.

4.3.3 Implications of spatial variability

Finally, the importance of spatial variability has not been sufficiently considered with respect to reclamation of forested landscapes in northern Alberta. Measuring spatial variability in reclaimed sites could be a useful tool for assessing the reestablishment of characteristics similar to undisturbed landscapes. Forest ecosystems are characterized by spatial variability at small scales, and patches of low nutrient availability can be a refuge for certain plants from competition with higher nutrient demanding species, thereby increasing biodiversity. Given that nitrogen is a biologically driven nutrient and frequently the most limiting nutrient in the boreal forest, spatial variability in nitrogen at small scales could be used as an indicator of the reestablishment of characteristics similar to natural forests.

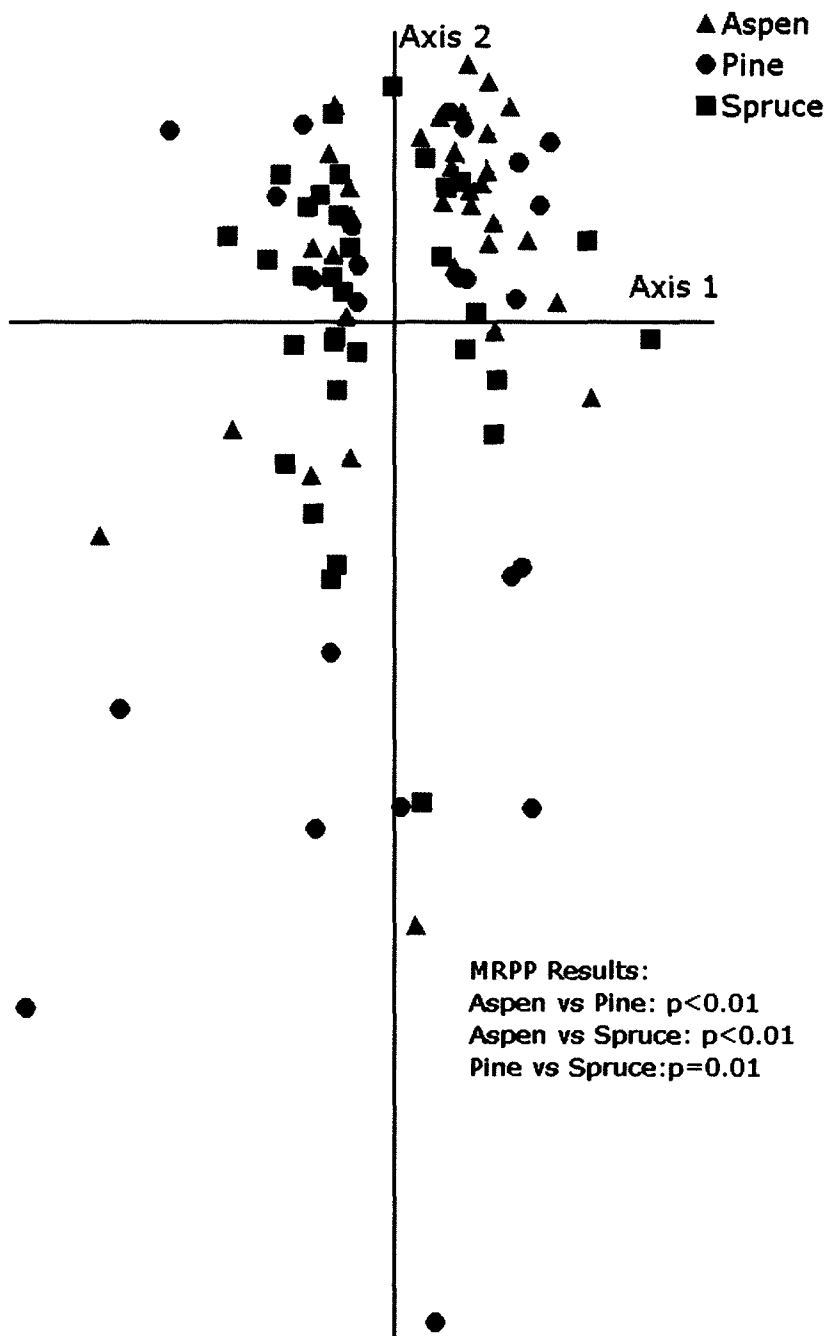
Appendix A. LFH Survey Site Data

Appendix A. Site characteristics of reclaimed sites in forest floor development study. Characteristics include transect number from LFH survey, reclamation polygon number, long term soil vegetation monitoring plot (S-V Plot), cover material, reclamation prescription, canopy type, year site was reclaimed, inclusion in biogeochemistry phase 1, mining lease, forest floor depth (cm), canopy cover (%), and UTM coordinates (NAD 83, 12U).

Transect	Polygon	S-V Plot	Cover Material	Rec. Prescr.	Canopy Type	Rec. Year	BGC1	Lease	Forest floor depth	Canopy Cover	Northing	Easting
1	658	3	DP/TSS	B	Pine	1992	Y	Syncrude	1.74	81.36	6324927	459744
3	691	-	DP/2°/TSS	B	Pine	1991	N	Syncrude	1.69	66.48	6324105	459453.9
4	2247	-	ptmix/TSS	H	Pine	1983	N	Syncrude	3.45	80.73	6322907	459859.1
7	1025	-	ptmix/OB	I	Aspen	1992	N	Syncrude	4.6	77.43	6317657	463045.9
11	1013	-	DP/OB	B	Pine	1990	N	Syncrude	4.03	36.33	6316660	462414.3
12	1958	43	ptmix/2°/OB	E	Aspen	1984	Y	Syncrude	2.58	75.20	6316545	465779.9
13	1924	41	ptmix/2°/TSS	A	Aspen	1988	N	Syncrude	3.33	23.46	6326858	462883
14	1924	-	ptmix/TSS	H	Aspen	1988	N	Syncrude	4.03	70.65	6324689	463814.6
15	1035	35	TSS/ptmix/TSS	A	Aspen	1992	N	Syncrude	1.29	24.86	6323415	462399.9
16	997	-	ptmix/TSS	H	Aspen	1992	N	Syncrude	2.79	28.55	6323042	460985
17	1954	-	ptmix/2°/OB	E	Spruce	1985	N	Syncrude	1.02	37.37	6316798	465310
18	1021	-	ptmix/LOS	I	Spruce	1990	N	Syncrude	3.34	77.15	6316701	465741
19	1966	-	ptmix/LOS	I	Pine	1983	N	Syncrude	1.92	47.04	6316620	465814
21	693	-	ptmix/2°/TSS	A	Spruce	1991	N	Syncrude	2.19	30.27	6324701	459580
22	665	-	ptmix/2°/OB	E	Aspen	1992	N	Syncrude	2.75	29.20	6323014	459594
25	3	-	deep ptmix	Deepptmix	Aspen	1976	N	Suncor	5.53	75.38	6315509	470442
26	27	5	ptmix/(oily)OB	I	Spruce	1982	N	Suncor	1.23	35.78	6315819	467650.8
27	21	31	ptmix/OB	I	Spruce	1981	N	Suncor	1.63	32.30	6316029	467211.2
28	31	-	ptmix/OB	I	Spruce	1983	N	Suncor	1.66	59.83	6316488	467199
29	50	28	ptmix/OB	I	Spruce	1991	Y	Suncor	1.79	5.59	6316512	467364.4

31		30	ptmix/TSS	H	Pine	1993	N	Suncor	2.14	59.86	6315478	468484
32		17	TSS/ptmix/TSS	H	Pine	1993	Y	Suncor	1.61	10.98	6315481	469638.4
33	35	32	ptmix/OB	I	Spruce	1984	Y	Suncor	3.36	94.98	6317394	466736.2
34	40	32	ptmix/OB	I	Aspen	1985	Y	Suncor	3.2	84.84	6317372	466711.5
37	52	-	ptmix/OB	I	Spruce	1991	N	Suncor	1.77	31.95	6317273	467569.3
38	34	25	ptmix/OB	I	Aspen	1984	Y	Suncor	5.53	78.97	6320133	469656.7
40	49	-	ptmix/OB	I	Spruce	1990	N	Suncor	2.36	12.74	6319308	469814.3
41	47	-	TSS/ptmix/TSS	H	Aspen	1990	N	Suncor	4.19	57.80	6316965	470529.7
42	42	33	ptmix/TSS	H	Pine	1992	N	Suncor	2.53	6.04	6317059	471380.3
43	57	-	ptmix/OB	I	Spruce	1992	N	Suncor	2.08	14.80	6316640	467629.1
44	66	1	ptmix/TSS	H	Pine	1991	Y	Suncor	1.82	25.25	6315004	471810.2
45	67	-	ptmix/TSS	H	Pine	1991	N	Suncor	3.54	67.24	6317315	471979.3

Appendix B. NMS Ordination of PLFA Profiles for Subsites



Ordination of subsites indicated that while within site agreement was higher than within stand type agreement, there was substantial variation between subsamples.

Appendix C. Full Models for Spatial ANOVA Analyses

For model $k=1$, mineral soil nitrogen supply rate was:

$$N_m = \alpha + \beta_1 \text{Seas}_{\text{fall}} + \beta_2 \text{Seas}_{\text{spring}} + \beta_3 \text{Seas}_{\text{summer}} + \beta_4 \text{FFM} + \beta_5 \text{Dist} + \beta_6 \text{Cover} + \beta_7 \text{pH} + \beta_8 P_m + \beta_9 K_m + \beta_{10} S_m + \beta_{11} \text{Ca}_m + \beta_{12} \text{Mg}_m + \varepsilon$$

where Season is season, N is mineral soil nitrogen supply rate, FFM is forest floor mass, Dist is distance to the nearest tree, Cover is canopy cover, pH is soil pH, P is mineral soil phosphorus supply rate, K is mineral soil potassium supply rate, S is sulfur mineral soil supply rate, Ca is mineral soil calcium supply rate, Mg is mineral soil magnesium supply rate. The error term was described with a spatial exponential covariance model. Each of these models was calculated separately for each stand type.

For model $k=2$, mineral soil phosphorus supply rate, this was:

$$P_m = \alpha + \beta_1 \text{Seas}_{\text{fall}} + \beta_2 \text{Seas}_{\text{spring}} + \beta_3 \text{Seas}_{\text{summer}} + \beta_4 \text{FFM} + \beta_5 \text{Dist} + \beta_6 \text{Cover} + \beta_7 \text{pH} + \beta_8 N_m + \beta_9 K_m + \beta_{10} S_m + \beta_{11} \text{Ca}_m + \beta_{12} \text{Mg}_m + \varepsilon$$

For model $k=3$, mineral soil potassium supply rate, this was:

$$K_m = \alpha + \beta_1 \text{Seas}_{\text{fall}} + \beta_2 \text{Seas}_{\text{spring}} + \beta_3 \text{Seas}_{\text{summer}} + \beta_4 \text{FFM} + \beta_5 \text{Dist} + \beta_6 \text{Cover} + \beta_7 \text{pH} + \beta_8 N_m + \beta_9 P_m + \beta_{10} S_m + \beta_{11} \text{Ca}_m + \beta_{12} \text{Mg}_m + \varepsilon$$

For model $k=4$, mineral soil sulfur supply rate, this was:

$$S_m = \alpha + \beta_1 \text{Seas}_{\text{fall}} + \beta_2 \text{Seas}_{\text{spring}} + \beta_3 \text{Seas}_{\text{summer}} + \beta_4 \text{FFM} + \beta_5 \text{Dist} + \beta_6 \text{Cover} + \beta_7 \text{pH} + \beta_8 N_m + \beta_9 P_m + \beta_{10} K_m + \beta_{11} \text{Ca}_m + \beta_{12} \text{Mg}_m + \varepsilon$$

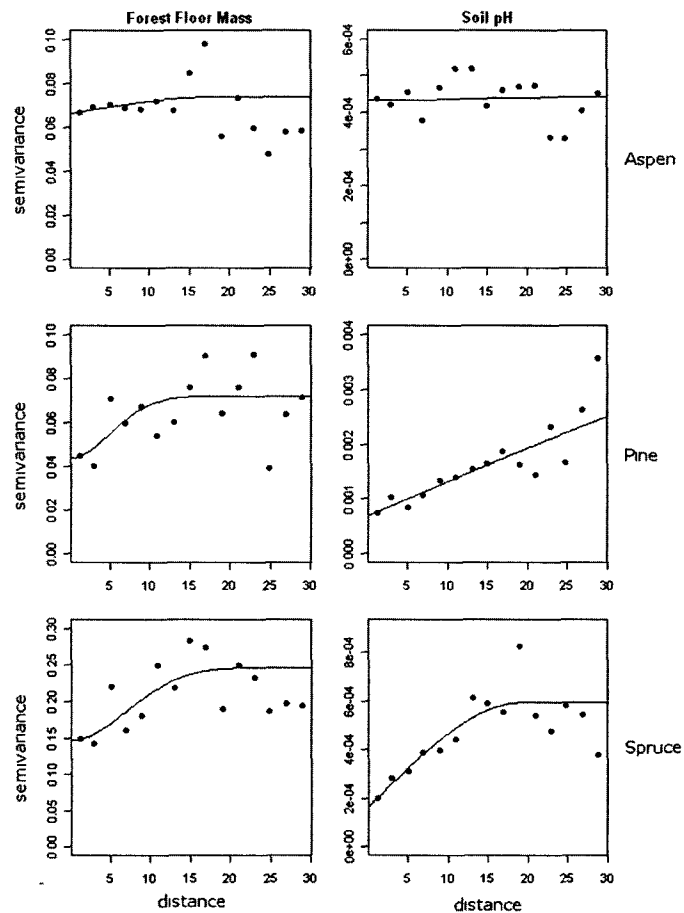
For model $k=5$, mineral soil calcium supply rate, this was:

$$\text{Ca}_m = \alpha + \beta_1 \text{Seas}_{\text{fall}} + \beta_2 \text{Seas}_{\text{spring}} + \beta_3 \text{Seas}_{\text{summer}} + \beta_4 \text{FFM} + \beta_5 \text{Dist} + \beta_6 \text{Cover} + \beta_7 \text{pH} + \beta_8 \text{N}_m + \beta_9 \text{P}_m + \beta_{10} \text{K}_m + \beta_{11} \text{S}_m + \beta_{12} \text{Mg}_m + \varepsilon$$

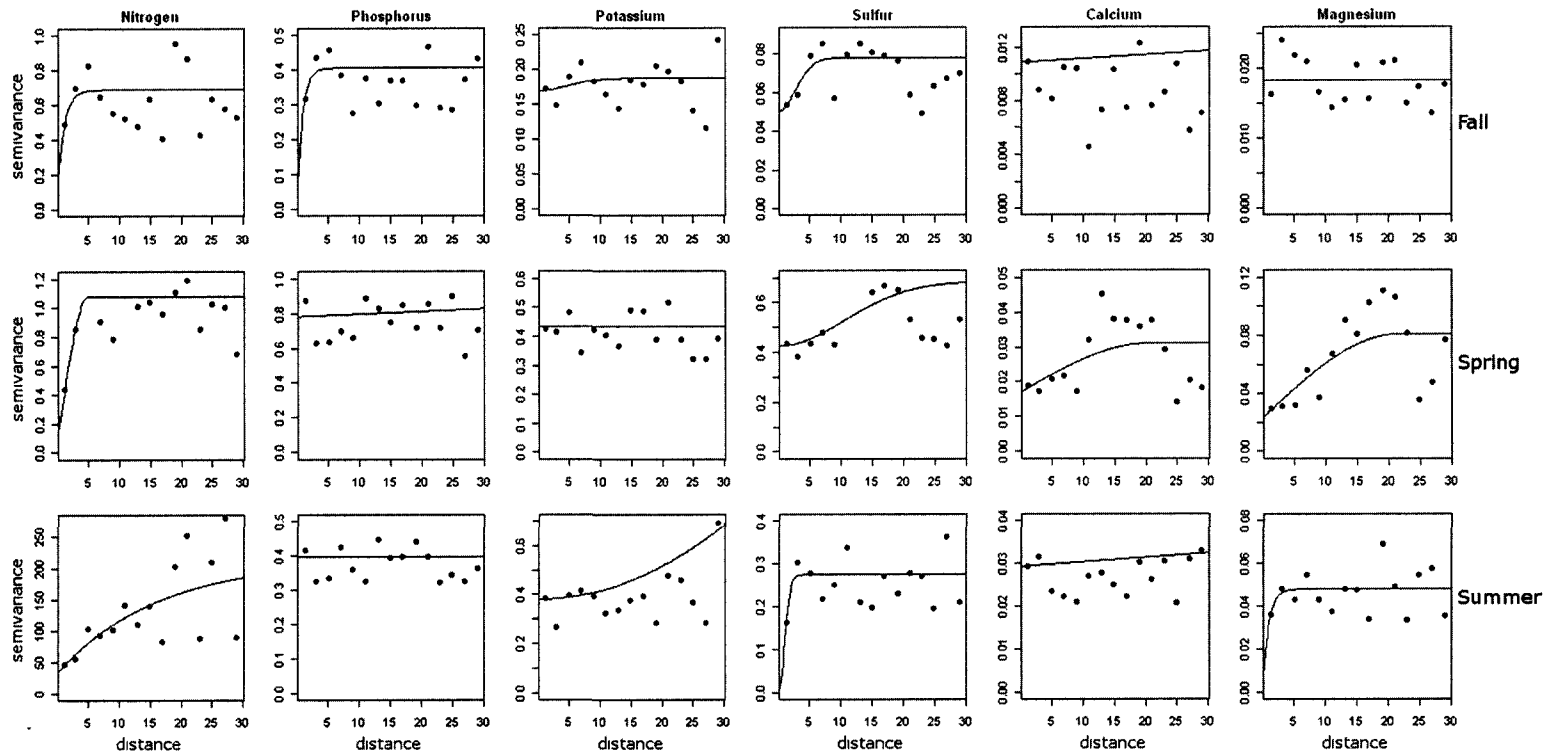
For model $k=6$, mineral soil magnesium supply rate, this was:

$$\text{Mg}_m = \alpha + \beta_1 \text{Seas}_{\text{fall}} + \beta_2 \text{Seas}_{\text{spring}} + \beta_3 \text{Seas}_{\text{summer}} + \beta_4 \text{FFM} + \beta_5 \text{Dist} + \beta_6 \text{Cover} + \beta_7 \text{pH} + \beta_8 \text{N}_m + \beta_9 \text{P}_m + \beta_{10} \text{K}_m + \beta_{11} \text{S}_m + \beta_{12} \text{Mg}_m + \varepsilon$$

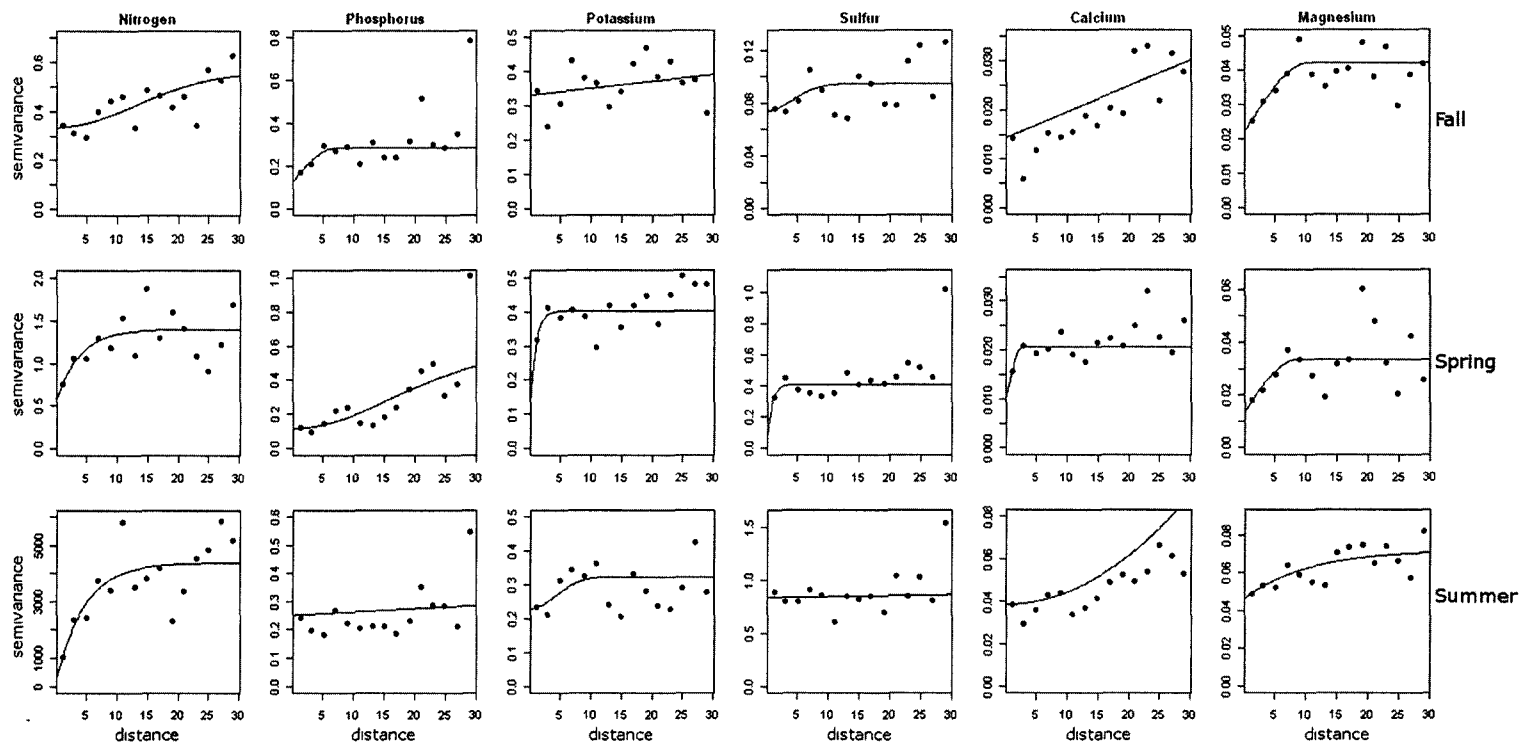
Appendix D. Forest floor, Stand Characteristic and Soil Nutrient Supply Rate Semivariograms



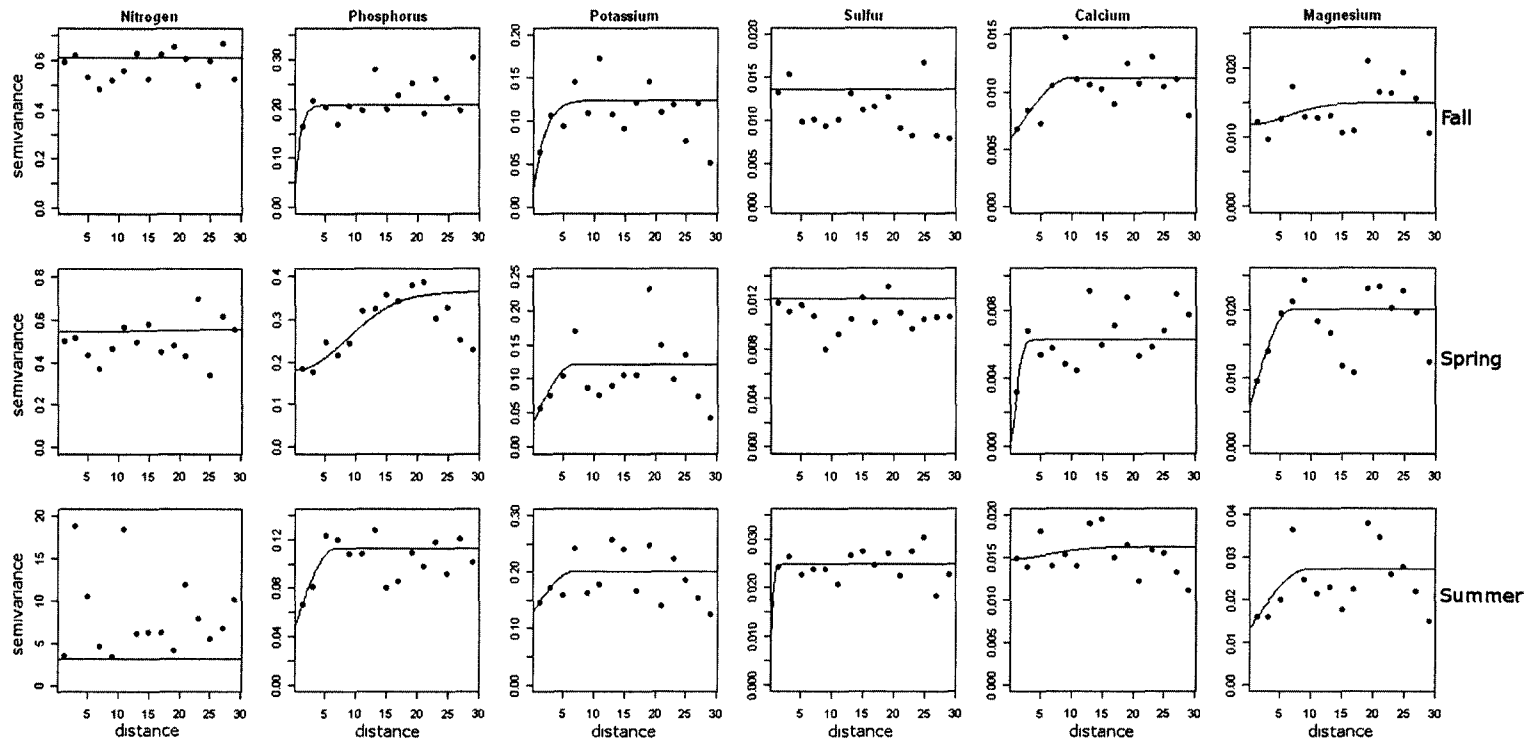
Semivariograms for log transformed forest floor (g/cm^2) and pH in aspen, pine, and spruce reclaimed sites north of Ft. McMurray, AB.



Aspen stand semivariograms for fall, spring, and summer log transformed N ($\mu\text{g/probe}$), P ($\mu\text{g/probe}$), K ($\mu\text{g/probe}$), S ($\mu\text{g/probe}$), Ca ($\mu\text{g/probe}$), and Mg ($\mu\text{g/probe}$) supply rates in a reclaimed site north of Ft. McMurray, AB. Soil available nutrients were collected with plant root simulator process installed from 0-5cm in the mineral soil. The summer soil N values were not log transformed because of too many zeros in the data.



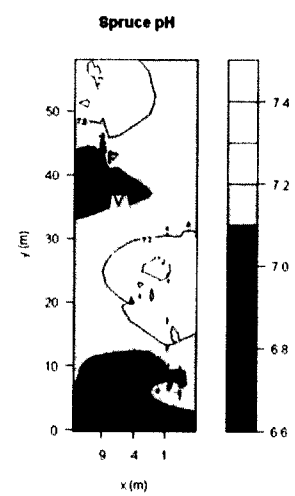
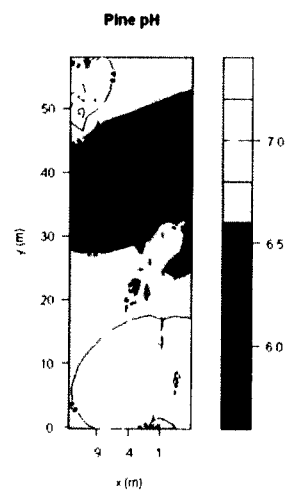
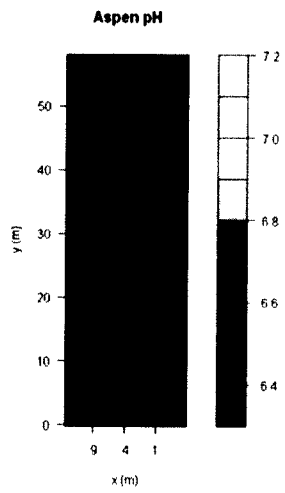
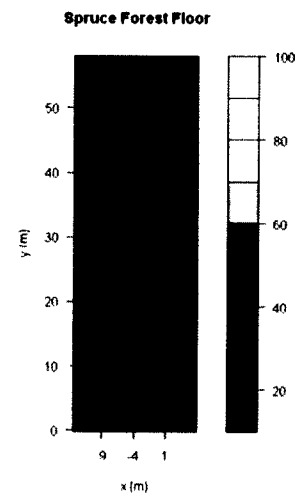
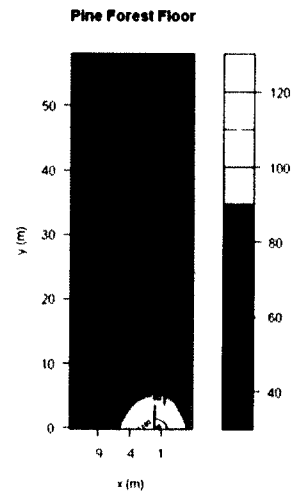
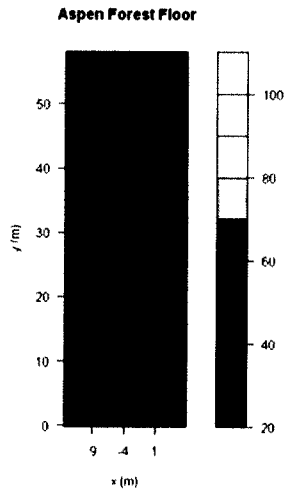
Pine stand semivariograms for fall, spring, and summer log transformed N ($\mu\text{g/probe}$), P ($\mu\text{g/probe}$), K ($\mu\text{g/probe}$), S ($\mu\text{g/probe}$), Ca ($\mu\text{g/probe}$), and Mg ($\mu\text{g/probe}$) supply rates in a reclaimed site north of Ft. McMurray, AB. Soil available nutrients were collected with plant root simulator process installed from 0-5cm in the mineral soil. The summer soil N values were not log transformed because of too many zeros in the data.

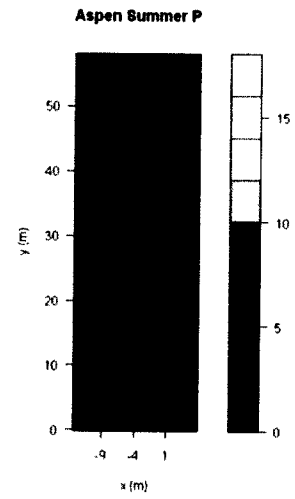
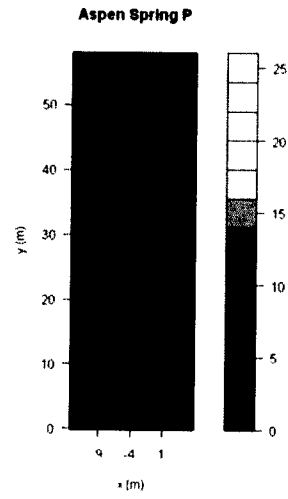
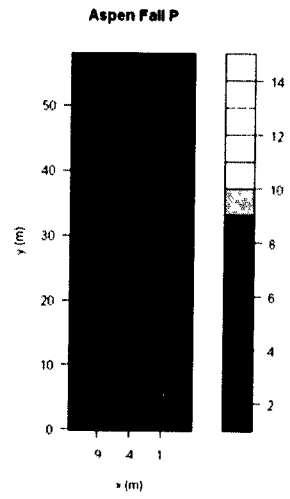
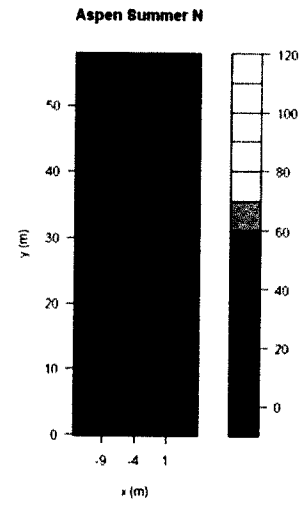
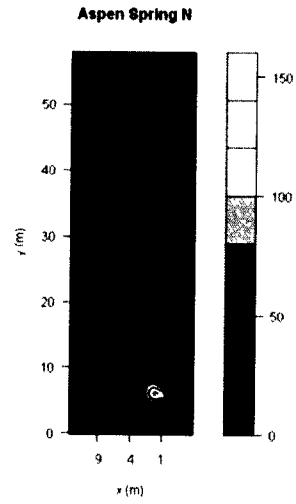
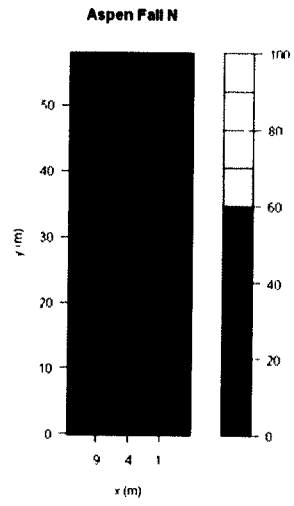


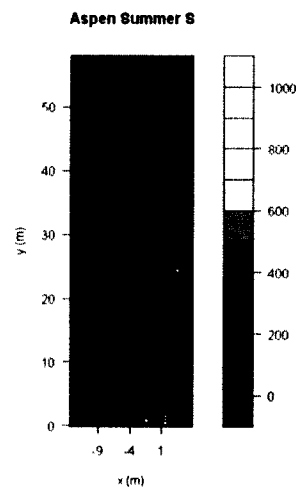
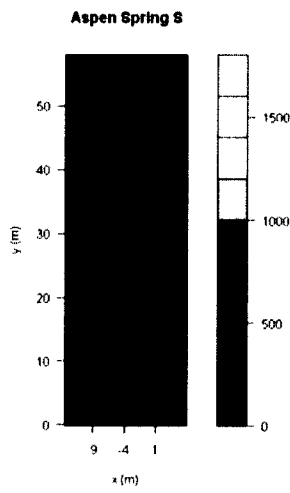
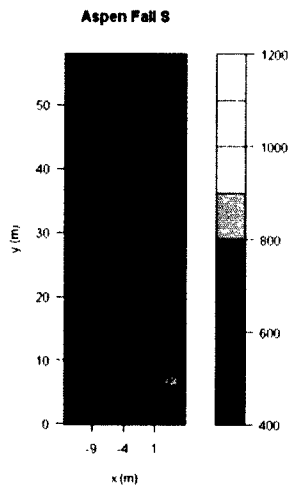
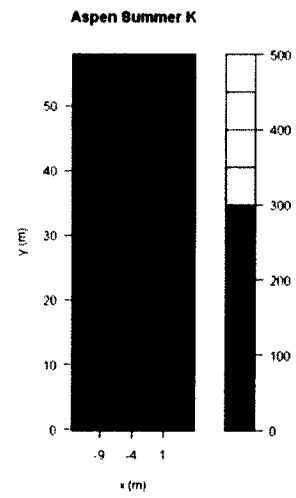
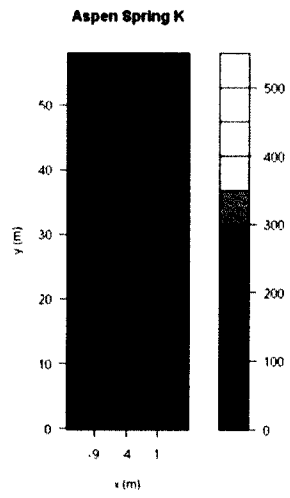
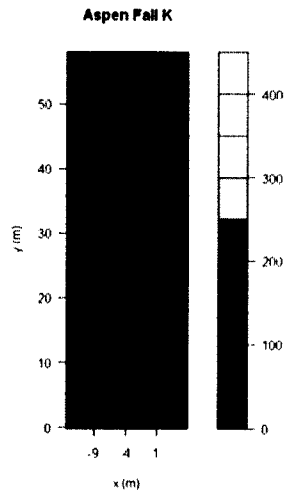
Spruce stand semivariograms for fall, spring, and summer log transformed N ($\mu\text{g/probe}$), P ($\mu\text{g/probe}$), K ($\mu\text{g/probe}$), S ($\mu\text{g/probe}$), Ca ($\mu\text{g/probe}$), and Mg ($\mu\text{g/probe}$) supply rates in a reclaimed site north of Ft. McMurray, AB. Soil available nutrients were collected with plant root simulator process installed from 0-5cm in the mineral soil. The summer soil N values were not log transformed because of too many zeros in the data.

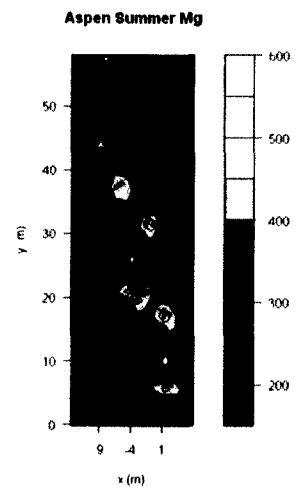
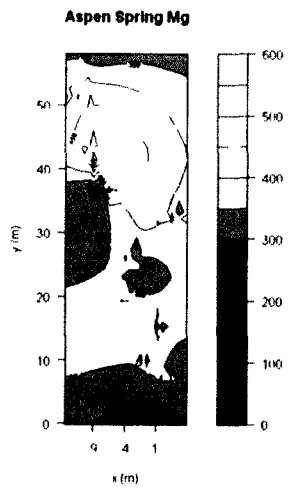
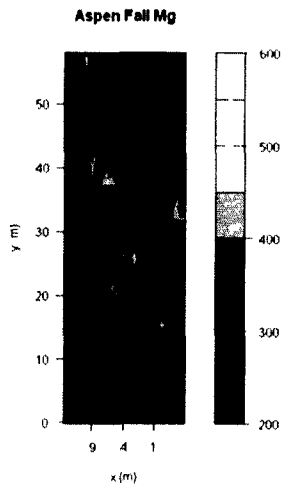
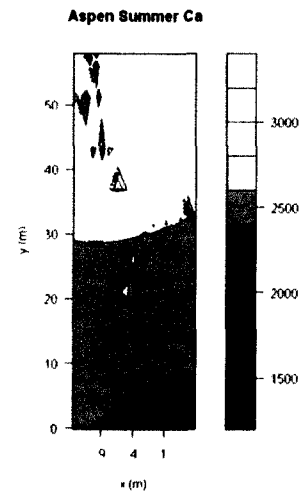
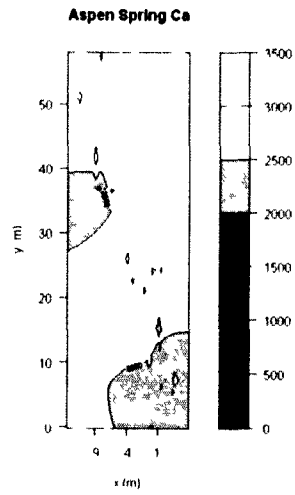
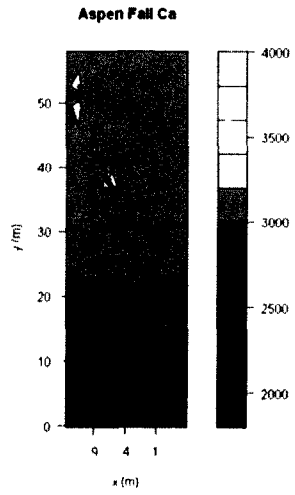
Appendix E. Forest floor and Soil Nutrient Supply Rate Maps

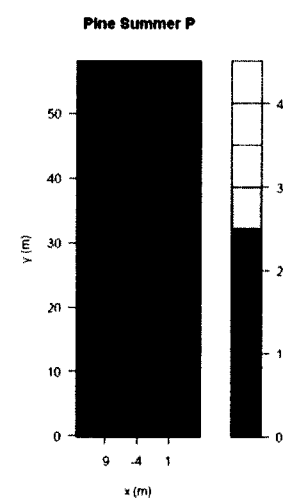
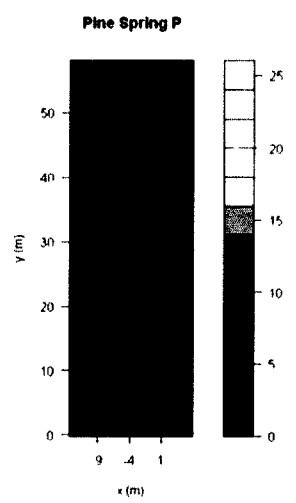
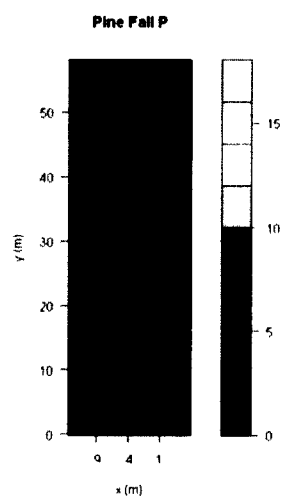
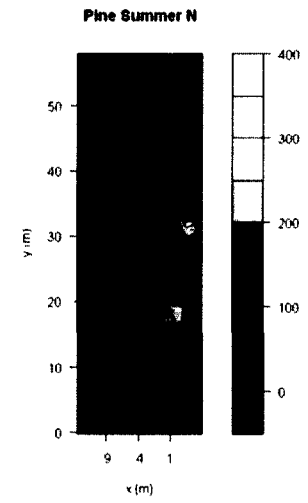
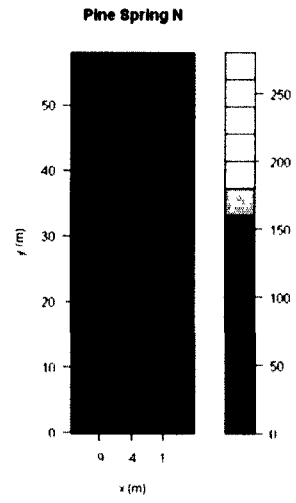
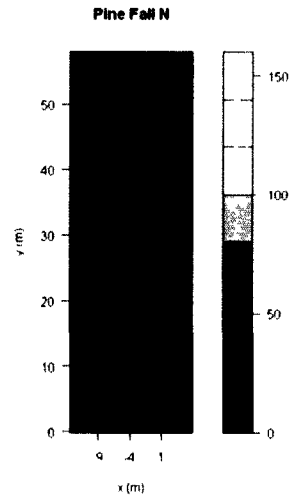
Maps were generated via kriging for forest floor mass (Mg/ha), soil pH, and soil nutrients ($\mu\text{g}/\text{probe}/\text{sampling period}$) for each season in each stand. Experimental semivariograms were generated to a maximum of 30 m as this is half of the maximum distance between sampling points, and the influence of outlying variables was accounted for by using a robust measure (Cressie 1993) of the sample semivariogram (`cressie=TRUE` was added to the variogram call function). Once experimental semivariograms had been calculated, exponential, matern, spherical and gaussian theoretical models were fit to the data. Semivariogram sills were fit to the data using restricted maximum likelihood (REML) fitting (`fit.variogram.reml` function), and ranges were fit by non-linear regression (`fit.variogram` function) as REML fitting of ranges is not available in the `gstat` package (Bivand *et al.* 2008). The theoretical semivariogram with the lowest sum of squares was then used for kriging, and maps of the predicted values were generated. Maps with only very small patches around sampling points are the result of little spatial structure in the data.

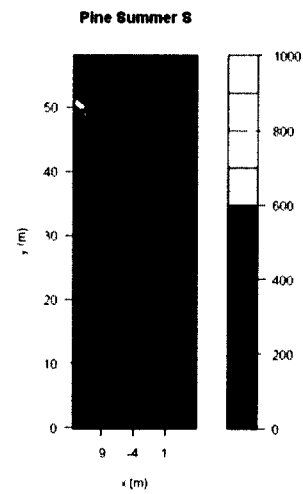
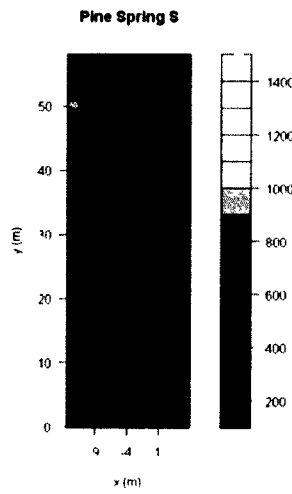
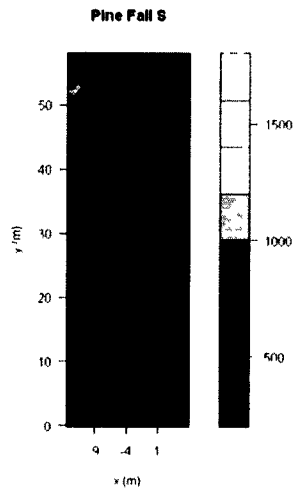
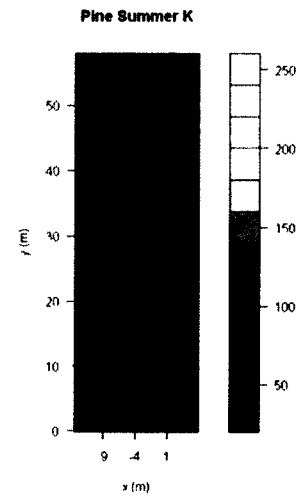
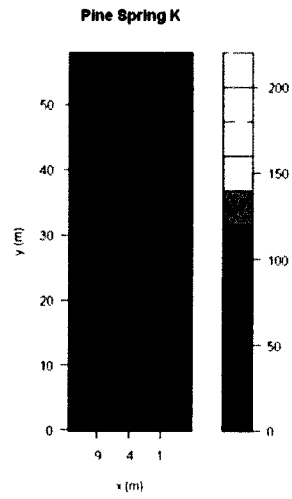
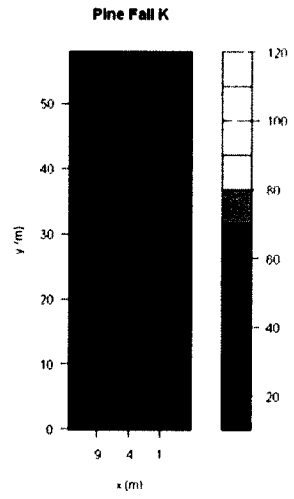


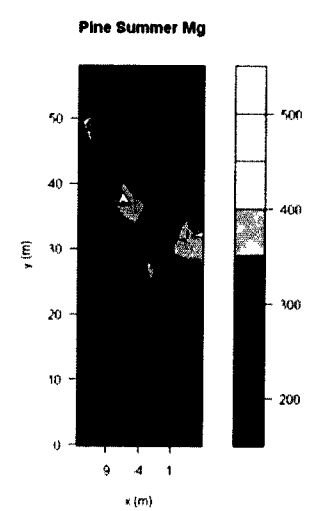
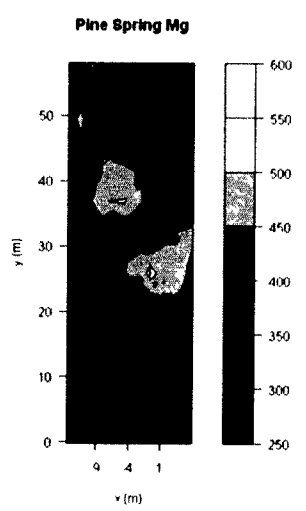
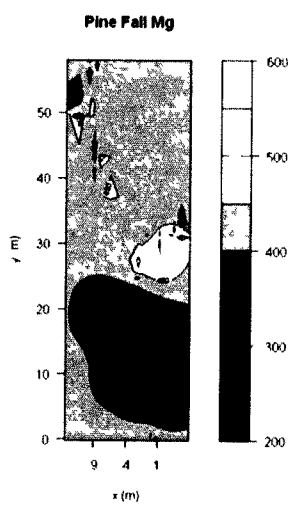
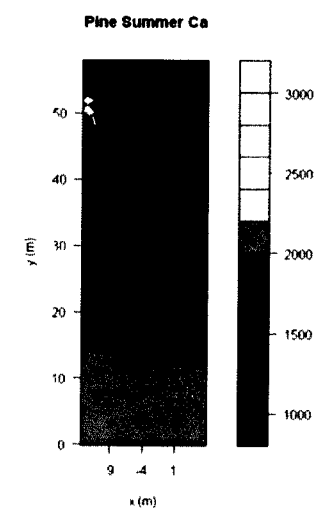
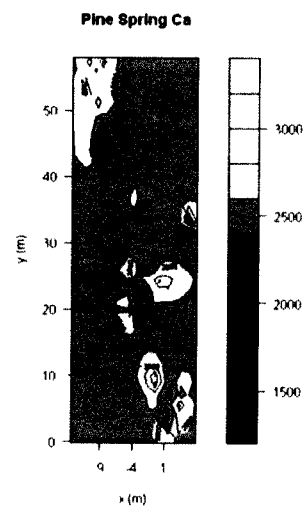
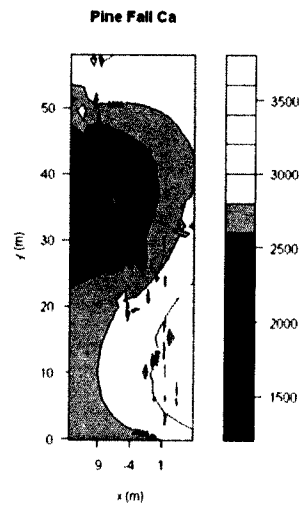


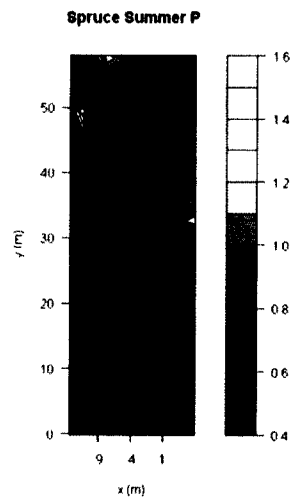
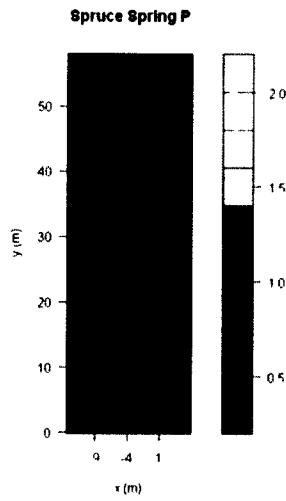
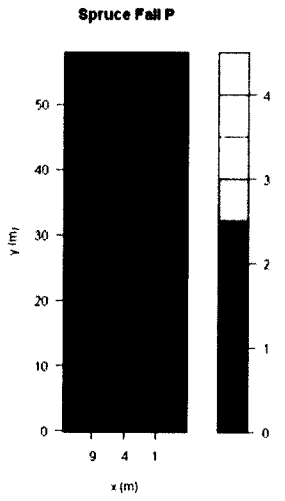
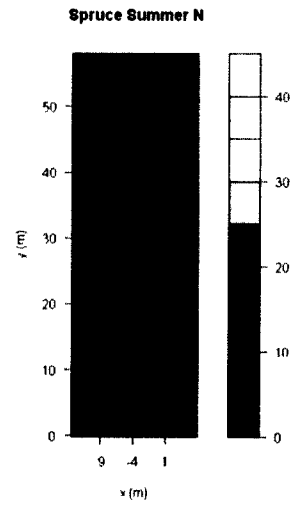
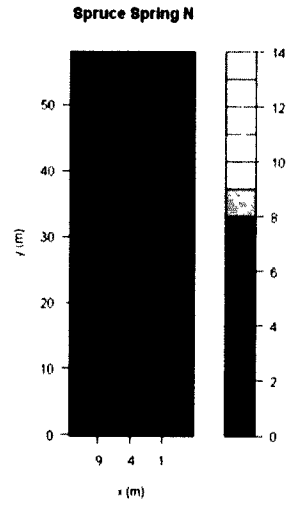
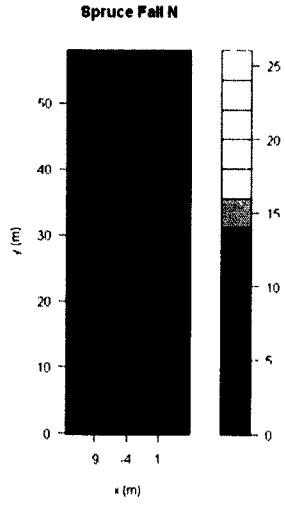


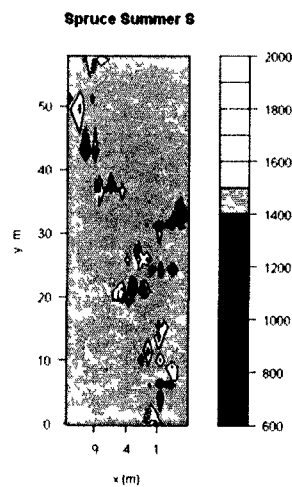
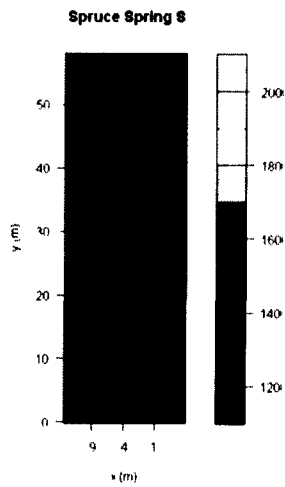
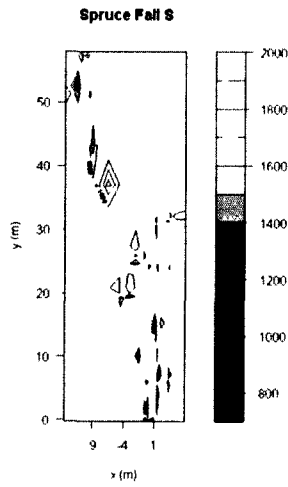
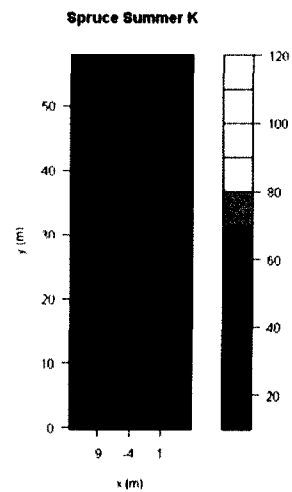
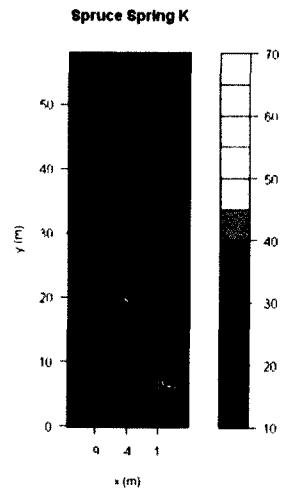
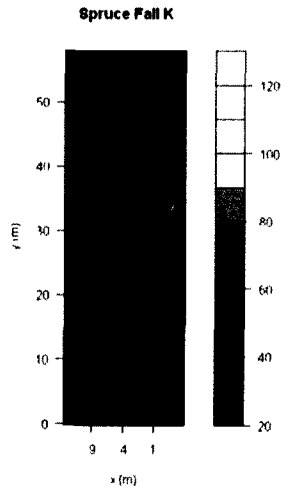




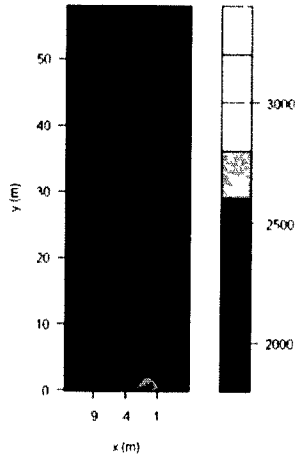




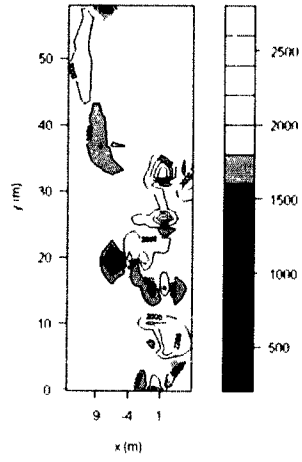




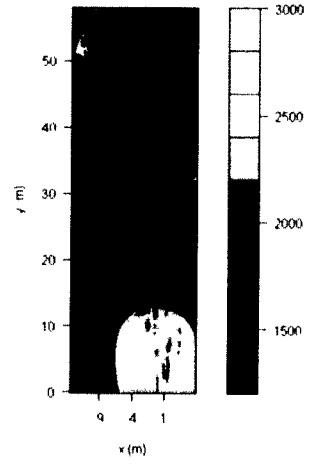
Spruce Fall Ca



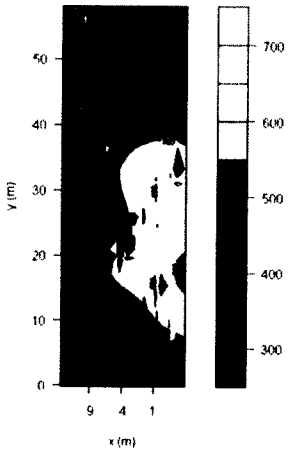
Spruce Spring Ca



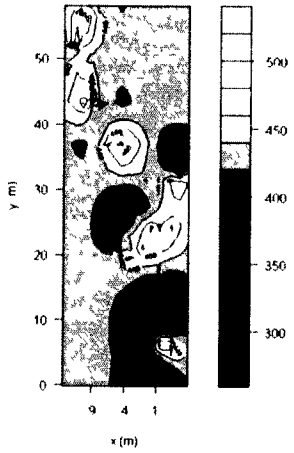
Spruce Summer Ca



Spruce Fall Mg



Spruce Spring Mg



Spruce Summer Mg

