SOIL CARBON STABILIZATION UNDER THREE RECLAIMED VEGETATION TYPES IN THE ALBERTA OIL SANDS

by

Meghan Laidlaw

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ABSTRACT

Prior to oil sands extraction, mineral soil from local boreal forest is salvaged and stored, together with additional peat obtained from local peatlands, for future reclamation use. The ability of reclaimed soils to stabilize carbon is an important indicator of soil functioning and successful reclamation. The objective of this research was to compare total soil carbon and the distribution of carbon in chemically and physically protected pools between three reclaimed vegetation treatments, and naturally fire-disturbed boreal forest sites. Twenty sites that were reclaimed or disturbed 20 – 40 years ago were chosen, and the top 10 cm of mineral soil was sampled. Density and size fractionation was used to separate soil organic carbon (SOC) into unprotected light fraction C, physically protected C, and chemically protected C. Aggregate size distribution, microbial biomass C, root biomass, and exchangeable cation concentrations were also measured. Reclaimed sites had three times more total SOC than natural sites and similar or greater SOC in physically and chemically protected pools. However, reclaimed sites were also estimated to have reached carbon saturation in chemically protected C pools, and half of the total SOC on average was unprotected, compared to 9 % unprotected C at natural sites. Accumulation of SOC in unprotected pools and C saturation in chemically protected pools at reclaimed sites suggest that further incorporation of SOC into chemically protected pools may be limited, possibly by soil texture (< 3% clay). Almost one third of reclaimed soil C was old, biochemically resistant C, compared to only 10 % at natural sites, and is probably peat that is persisting in reclaimed soils. Within reclaimed vegetation types, grassland sites had the greatest physically and chemically protected C, while deciduous sites had the greatest unprotected C, despite both vegetation types having similar total SOC content. Coniferous sites had the lowest total SOC content, but tended to have more physically and chemically protected C than deciduous sites, and may ultimately surpass grasslands if C accumulation rates continue to increase at these sites.

PREFACE

This thesis represents original, unpublished work by Meghan Laidlaw. It is based on work conducted at Syncrude and Suncor mines in the Alberta oil sands, and at naturally fire-disturbed sites in the surrounding area, by Ph. D. Candidate Jacynthe Masse, Eli Rechtschaffen, and myself. I was responsible for all major areas of research, including selecting research questions, collecting data, analyzing data, and writing the draft manuscript. I processed soil samples for SOC fractionation, total % C analysis, soil structure analysis, bulk density, and root biomass assessments. Jacynthe Masse and Eli Rechtschaffen assisted me in processing soil samples for microbial biomass carbon analysis and Keyano College provided the use of their lab facilities for immediate processing of these samples. Extractable biomass C and N were measured in the Analytical Services Laboratory at the University of Alberta. Jacynthe Masse also performed pH and conductivity analyses, and prepared samples for texture analysis. Soil texture analysis was performed at Trent University, and cation exchange capacity and exchangeable cation concentrations were measured at the University of Montreal. Dr. Jérôme Laganière provided advice and instructions on SOC fractionation methods. Dr. Les Lavkulich provided advice on young vs old carbon analysis and data interpretation. Dr. Gary Bradfield provided assistance with multivariate data interpretation. Drs. Cindy Prescott, Sue Grayston, Maja Krzic, and Sean Smuckler contributed to thesis edits. My supervisor, Dr. Cindy Prescott, provided assistance with research question formation, data interpretation and editing throughout the thesis.

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C	carbon
SOC	soil organic carbon
SOM	soil organic matter
MBC	microbial biomass carbon
MBC:SOC	microbial biomass carbon-to-soil organic carbon ratio
C:N	carbon-to-nitrogen ratio
MWD	mean weight diameter (aggregate stability)
ANOVA	one-way analysis of variance
CEC	cation exchange capacity
Ca	calcium
Mg	magnesium
K	potassium
Na	sodium
free LF	free light fraction (unprotected soil carbon)
POM	particulate organic matter (physically protected soil carbon)
S&C	silt and clay fraction (chemically protected soil carbon)
NaOCl	sodium hypo-chlorite (bleach)
ELC	equivalent land capability
PMM	peat-mineral mix
RDA	redundancy analysis

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1 INTRODUCTION

1.1 Project overview

Surface mining in the Athabasca oil sands region is a large-scale, disruptive process in which whole ecosystems and surface material up to 75 m deep are removed to access bitumen deposits. Following removal of bitumen, the mined area is reclaimed by filling the extraction pit with tailings sand (an end-product of the hydrocarbon removal process) and fragmented surface material (overburden). A clean cap of salvaged mineral soil and organic peat is typically placed on top of the overburden, and native plant species are planted to re-establish vegetation cover (Alberta Environment, 2009). Incorporation of peat into mineral soil is done out of necessity to supplement limited mineral soil, but it is also thought to improve the likelihood of successful vegetation establishment because it increases soil organic carbon (SOC), nutrient content, and water-holding capacity in reclaimed soils (Mackenzie, 2012).

In 2008, researchers from the University of British Columbia and the University of Alberta collaborated with six oil sands extraction companies to investigate soil biogeochemical processes in reclaimed and natural reference soils, and to assess whether reconstructed soils were functioning similarly to natural soils. Reclaimed ecosystems differed from natural ecosystems in nutrient availability, SOM composition, and microbial community composition, although there was overlap between reclaimed sites and some natural sites in nutrient availability and microbial composition (Hemstock et al., 2009; Turcotte et al., 2009; Rowland et al., 2009; Dimitriu et al., 2010). Rowland et al. (2009) found that at sites where a peat-mineral mix (PMM) had been used as a surface substrate, the reclaimed sites were on a trajectory towards becoming functioning forest soils. However, when natural and reclaimed sites were analyzed separately, natural sites tended to group together based on vegetation cover, whereas reclaimed soils could not be differentiated into clear groups based on the different vegetation treatments (Quideau et al., 2013). The results from Quideau et al. (2013) suggest that while soil processes were occurring at reclaimed sites, soils had not developed to the point where clear differences could be measured between vegetation types. Over time, the differentiation of soil properties based on vegetation type at reclaimed sites could indicate that ecosystem-specific soil processes have been re-established, and thus ecosystem reclamation has been achieved.

Soil carbon stabilization is an important ecosystem process that reflects the quality and quantity of organic inputs from vegetation and the ability of a soil to retain carbon and nutrients in short-term and long-term pools. Boreal ecosystems represent the second largest pool of SOC in the world, primarily due to carbon accumulation in peat-forming wetlands (Amundson, 2001). Therefore, losses of soil carbon from this biome as a result of oil sands extraction could have significant impacts on global carbon cycling, and atmospheric CO_2 levels (Ussiri and Lal, 2005). Carbon is stored in intermediate- and long-term soil pools through incorporation into the soil structure, resulting in physical protection from decomposition, and through forming organo-mineral complexes with clay and silt surfaces, resulting in chemical protection from decomposition. While several studies have investigated organic matter content and composition in reclaimed soils (Anderson, 2014; Turcotte et al., 2009), it is still unclear whether reclaimed soils are storing carbon in long-term pools, and how much of the carbon in reclaimed soils is a result of the initial peat amendment. This study contributes to our understanding of soil carbon storage and accumulation at reclaimed sites by comparing SOC in physically and chemically protected pools between reclaimed and natural soils. Peat contributions to total soil carbon are assessed by comparing biochemically resistant vs labile C pools between reclaimed and natural sites. Finally, this study explores how vegetation treatments are influencing carbon stabilization in reclaimed soils.

1.2 The scope of oil sands reclamation

1.2.1 Oil sands mining has shaped the landscape of northern Alberta since the 1970s

The Alberta oil sands is the third largest oil sands reserve in the world, and is composed of three separate deposits that collectively cover 142 000 km^2 of land (Alberta Energy Regulator, 2014). Oil sands, also known as bitumen, is a form of crude oil that contains sand, clay and water, and is extremely viscous. It does not flow easily through traditional pumping systems, and can only be extracted through surface mining or through *in situ* techniques that use steam to increase the flow rate of the material. Surface mining occurs when the quality and volume of the bitumen has greater economic value than the cost of removing the overburden above the deposit (Yarmuch, 2003). All of the reclaimed sites assessed in this study were surface-mined. Over 715 km^2 of boreal forest in northern Alberta have been disturbed by surface mining so far, and if it continues to be economically viable, more than 4700 km^2 of forest will be affected before the surface-mineable deposit is exhausted (Government of Alberta, 2013). As of 2012, 77 km^2 of the disturbed area was undergoing active reclamation, but only 1 km^2 has been certified reclaimed by the Alberta government (Government of Alberta, 2013). This has contributed to a negative public image, and growing concern that reclamation efforts cannot compete with the pace and scale of land disturbance caused by oil sands extraction (Gosselin et al., 2010).

1.2.2 Reclamation goals are continually evolving to reflect our growing understanding of ecosystem structure and function

Reclamation is defined by the Society for Ecological Restoration (2004) as a facet of restoration that includes terrain stabilization, aesthetic improvement, and the return of the land to what is considered a useful purpose within a regional context. In northern Alberta, commercial forest, wildlife habitat, traditional land use, and recreation have been identified as useful purposes for reclaimed land (Alberta Environment, 2009). An important distinction between reclamation and restoration is the implication that elements of the pre-disturbance ecosystem are present in restoration projects and thus, the same vegetation, landscape and soil processes can be returned. Reclamation is typically associated with more intense disturbance, and the goal is to return similar structure and function to the ecosystem, but likely not the same structure and function. In oil sands surface-mined areas, restoration is not the intended end result due to the magnitude of disturbance. Reclamation is viewed as an achievable way to return pre-disturbance structure and function, without necessarily recreating the ecosystem that was there before (Powter et al., 2012). However, measuring reclamation success can be challenging and requires a holistic approach.

Equivalent land capability (ELC) was first introduced in the 1980s and means that "the ability of the land to support various land uses after conservation and reclamation is similar to the ability that existed prior to an activity being conducted on the land, but that the individual land uses will not necessarily be identical" (Province of Alberta, 2014). Unfortunately, the practical application of ELC has caused considerable confusion over the past 30 years (Oil Sands Research and Information Network, 2011). Determining clear criteria that can be widely applied and measured at reclaimed sites is difficult when the final objective is so loosely defined. To add further complexity, each mining company has developed its own reclamation strategy independently, without considering how reclaimed areas in other mines will interact with their sites in the future landscape (for example, through the movement of water across the landscape). In some ways, this flexibility is beneficial in oil sands reclamation to create a diverse landscape that simultaneously meets the needs of multiple stakeholders. However, without clear, measurable indicators of successful reclamation, sites could be certified reclaimed that are not capable of sustained ecosystem functioning, or sites that are capable of ecosystem function may not be recognized as reclaimed. Once a company has obtained reclamation certification for a specific site by the Alberta Government, responsibility for that site falls to the government (Province of Alberta, 2014). It is therefore in the best interests of companies to reclaim sites quickly and be released from the monetary obligations of site upkeep, while it is in the public's best interests to ensure that reclamation is done well and sustainably.

There have been several attempts to standardize reclamation procedures. In 2006, the Alberta government created a field manual for land capability classification (Cumulative Environmental Management Association, 2006). The purpose of this document was to rate the capability of a variety of naturally-occurring soils to support vegetation based primarily on soil nutrient and moisture regimes, as well as other soil physical and chemical characteristics that could limit vegetation productivity such as salt concentrations. This field manual used Beckingham and Archibald's edatopic grid (1996) to predict the vegetation that could be supported by a specific site. Reclaimed sites were divided into eight possible ecosites based on the edatopic grid, and species that aligned with the site characteristics could then be planted according to the "Guidelines for Reclamation to Forest Vegetation in the Athabasca Oil Sands Region" (Alberta Environment, 2009).

In 2012, the Cumulative Environmental Management Association (CEMA) released a report that proposed a criteria and indicators framework designed to streamline the process of certifying reclaimed sites (Poscente and Charette, 2012). One of the three primary objectives to achieve successful reclamation was that "natural ecosystem functions are established on the reclaimed landscape" (Poscente and Charette, 2012). The criteria suggested to gauge the progress of this objective were water quality, water quantity, nutrient cycling, ecosystem productivity and the capacity for resilience. Of the 30 terrestrial indicators suggested to measure whether the criteria were met, six were soil properties, and three of those were accepted by the Reclamation Working Group. Soil quality indicators are under-represented in this framework, and it is evident that there is a bias towards measuring reclamation success based on the productivity of the aboveground component of the ecosystem. Ensuring that soil ecosystem functions are established on reclaimed landscapes is less straightforward to measure than site productivity, but could be more valuable in the long term.

1.2.3 Returning reclaimed areas to equivalent land capability requires a comprehensive understanding of the pre-disturbance ecosystem.

Ultimately, reclamation guidelines and end goals must reflect the ecosystem that existed prior to disturbance to some extent. Even if reclamation results in a novel ecosystem, it must be adapted to survive local climate and weather conditions. The Boreal ecozone is a severe environment, characterized by long, cold winters and short, mild summers (Lorenz and Lal, 2010). Plant growth is limited by low minimum temperatures and nutrients, thus boreal plant species are typically cold tolerant, have low nutrient requirements and contribute low-quality litter to the soil (high C:N ratios), such as coniferous species (De Deyn et al., 2008). In regions where winters are less severe, deciduous species also appear. Dominant tree species in the oil sands region include trembling aspen (*Populus tremuloides*), balsam poplar (*Populus balsamifera*), white spruce (*Picea glauca*) and jack pine (*Pinus banksiana*). Black spruce (*Picea mariana*) and tamarack (*Larix laricina*) are common in low, water-logged areas. The successional sequence of these ecosystems is slower than temperate ecosystems, and is predominately controlled by fire regimes, which can result in a significant loss of carbon, particularly in dry upland forests (Harden et al., 2000). Some boreal tree species such as jack pine require fire as a precursor to seed germination and establishment. Disturbance regimes are therefore an important consideration for reclaimed areas because fire disturbance is suppressed for the lifetime of the mine, and cannot be used as an ecosystem-shaping process during reclamation. This could make reclaimed ecosystems more vulnerable to severe fire events in the future.

Boreal forest soils in the Fort McMurray area are predominantly Orthic Brunisols, Orthic or Gleyed Luvisols, and Organic soils. These soils are not strongly weathered due to the short time since the last glaciation event and the limited impact of vegetation due to the previously mentioned growing restrictions. Boreal ecosystems play an important role in global carbon storage. Boreal SOC relative to vegetation C has an average ratio of 5:1, compared to 2:1 in temperate forests, and 1:1 in tropical forests (Jarvis et al., 2005). Confounding factors like deep soil C that results from cryogenic mixing in the Boreal ecozone make it difficult to determine how much C is stored in this biome, but it is widely considered to be a net C sink (Lorenz and Lal, 2010). Organic soils from boreal peatlands alone are estimated to contain one-third of the world's soil C (Limpens et al., 2008). Boreal soil C stores are particularly vulnerable to global warming due to the effect of temperatures on permafrost depth and soil microbial activity, though there is great uncertainty about how the ecosystem will respond to a warming climate (Flato et al., 2013). Dry upland boreal forests do not accumulate as much carbon as peat wetlands, but it is unlikely that wetlands will represent as much of the post-mining footprint as the pre-mining footprint, because closure plans favour upland forest reclamation, due to operational constraints (Rooney et al., 2012). One study estimated that land-use changes associated with four of the smaller oil sands mines could release 11.4 - 47.3 million metric tons of stored C, and may reduce carbon sequestration potential by 5,734 - 7,241 metric tons C per year if peatlands cannot be successfully reclaimed (Rooney et al., 2012). Based on the ability of pre-disturbance ecosystems to store significant amounts of SOC, reclaimed ecosystems should be managed to promote SOC storage.

1.2.4 Reclaimed soil material is composed of a mix of mineral soil and organic soil salvaged from the mining footprint

Oil companies have been faced with many practical challenges over the course of reclamation that have shaped current reclamation practice. First, the overburden material used to backfill open pits is predominantly composed of fragmented Clearwater Shale, and is saline-sodic. Pore-water of this material has an electrical conductivity as high as 17dS m^{-1}, which inhibits plant growth (Kessler et al., 2010). Kessler et al. (2010) also found that salt concentrations reached inhibitory levels in cover materials 15 - 20 cm above overburden in reclaimed oil sands sites. To reduce salt leaching and prevent slumping and erosion due to dispersive sodium-clays, an 80-cm non-saline layer of overburden or till is often placed above the overburden as a subsoil cap (Rowland et al., 2009). The depth of the peat-mineral mix (PMM) above this subsoil layer varies from 0 to 70 cm based on the mining company, and the year the topsoil was applied (Rowland, 2008). An average depth of 20 cm is most common and in this study, only reclaimed sites with a minimum PMM depth of 10 cm above a subsoil layer were sampled.

Both mineral upland soil and peat organic soil are salvaged from the mining footprint and stored until they can be used as a surface substrate on reclaimed sites. Storage of this material can negatively affect soil quality by decreasing biological activity and aggregate stability, and increasing compaction (Abdul-Kareem and McRae, 1984). Soil can also be compacted when heavy machinery is used to apply the topsoil material during reclamation (Phellps and Holland, 1987). To limit compaction and to preserve some of the original soil structure, soil is commonly salvaged and replaced during the winter, and dozers are used rather than heavy machinery with large rubber tires (M. Yarmuch, pers. comm, 2013). Organic matter content, nutrient availability and soil physical characteristics are often limiting in topsoil materials used in reclamation (Chatterjee and Lal, 2009). In oil sands reclamation, the topsoil substrate used in upland sites is a mixture of upland mineral soil and organic soil from peatlands. Depending on the oil company, mixing of the mineral and peat soils can occur when organic soils are overstripped and the mineral soil beneath is mixed in, or upon placement, when machinery is used to manually mix the two materials (Yarmuch, 2003). The target peat-to-mineral ratio is between 50:50 and 70:30, and it is typically variable as a result of operational constraints (Mackenzie, 2012).

Peat is used as an organic amendment because it contains organic material and nutrients, and is thought to enhance water-holding capacity and facilitate soil structure formation (Rowland et al., 2009). It is also the dominant soil type in the mining footprint, so peat is abundant and necessary to supplement the limited mineral soil available (Mackenzie, 2012; Lindsay et al., 1962). Previous research assessing the use of organic amendments in mining reclamation suggested that using an organic amendment initially results in indirect benefits to such as faster establishment of ground cover, and reestablishing nutrient cycling between above- and belowground components (Vetterlein and Huttle, 1999). However, another mining reclamation study compared a control cover treatment of fine-textured overburden to a native soil cover, a sawdust cover, and sewage sludge covers and found that after 16 years, SOM content in the control was comparable to or better than all other cover treatments (Bendfelt et al., 2001). These results suggest that as long as vegetation cover can be established, it may be unnecessary to provide additional organic material for the purpose of enhancing soil quality.

1.2.5 Planting practices are designed to return pre-disturbance species faster than they would naturally regenerate

After topsoil has been placed at reclaimed sites, a stabilizing cover crop (typically barley) is sown to prevent soil erosion. Barley (*Hordeum vulgare*) is a beneficial cover crop because it contributes OM to the soil while preventing excessive leaching of nutrients, and it can be easily out-competed by native vegetation. Fertilization with nitrogen, phosphorus and potassium is common at most oil-sands mines, although frequency and duration vary among the mines. There is typically a heavy initial fertilization, after which point some mines will be re-fertilized in smaller doses on an annual or multi-year basis (Mackenzie, 2012). While older reclaimed sites were all fertilized, general practice is shifting to fertilizing only when needed due to the accidental effects of fertilizing non-target species such as herbaceous plants instead of tree and shrub seedlings at reclaimed upland forest sites (Staples et al., 1999). Nursery-grown trees also have restricted rhizospheres during the first few years of establishment and are not likely to benefit from surface application of fertilizer (Mackenzie, 2012).

Natural regeneration was initially suggested as an approach to re-establish native ecosystems, but establishment of woody shrubs and trees was unsuccessful under natural regeneration (Hardy BBT Ltd., 1990). The current practice is to plant tree and shrub seedlings during the next available planting season. Boreal tree species that are planted include trembling aspen, white spruce, black spruce, paper birch (*Betula papyrifera*) and jack pine. Planted shrub species include green alder (*Alnus cripsa*), redosier dogwood (*Cornus stolonifera*), low bush cranberry (*Viburnum edule*), buffaloberry (*Sherperdia canadensis*), pincherry (*Prunus pensylvanica*), prickly rose (*Rosa acicularis*), blueberry (*Vaccinium myrtilloides*) and Saskatoon berry (*Amelanchier alnifolia*). All seedlings are nursery-grown and seeds originated from within 80 km of the planting location. Planted species and their densities are assigned using the eight ecosites on the edatopic grid, with drier sites being planted with jack pine and trembling aspen, and wetter sites being planted with white spruce, black spruce, and trembling aspen (Alberta Environment, 2009). However, in older reclaimed sites, planting prescriptions were less strategic. This was primarily due to a lack of available tree and shrub species for planting, a lack of restrictions against planting non-native species, and a limited understanding of site capability (M. Yarmuch, pers. comm., 2013). This absence of strategy in matching soil properties with appropriate tree species in older sites is actually beneficial from a scientific perspective because it allows for a randomized design approach when studying the effect of plant species on soil properties.

1.3 Long-term carbon stabilization is an important process to promote in reclaimed soils

1.3.1 Soil carbon can be stabilized in chemically and physically protected soil pools

Large-scale disturbance causes severe soil degradation and loss of carbon from the system. Carbon stabilization is beneficial in an ecological context because soil carbon positively contributes to soil physical, chemical, and biological properties, as well as promoting ecosystem productivity. In a broader context, managing degraded ecosystems to sequester carbon in stable pools can help mitigate carbon losses from disturbance (Ussiri and Lal, 2005). If we consider the total carbon storage potential of an ecosystem, SOC is an important pool of stable C because it is less sensitive to land-use change and other environmental factors than aboveground biomass carbon (Lorenz and Lal, 2010).

The inherent molecular structure of litter inputs and the conversion of organic matter into humic polymers as a result of decomposition were thought to be the predominant mechanism behind soil C stabilization (von Lutzow et al., 2006). While complex chemical structure can hinder decomposition, recent research has suggested that soil physical and chemical protection may be more important in long-term C stabilization (Dungait et al., 2012). Therefore, while measuring total SOC content is useful for a coarse comparison between ecosystems, separating organic carbon into different pools based on the mechanisms through which each pool is stabilized provides a more precise estimate of long-term carbon storage. SOC content of each aggregate size fraction has been used to approximate C residence times and relative protection based on the size of the aggregate and the binding mechanisms associated with each size class (Six et al., 2002a). However, particularly in organic soils, light-fraction C that is physically unprotected can be measured along with each aggregate size class resulting in an overestimation of physically protected SOC. A combination of size and density fractionation techniques has been used to separate bulk soil into more exact structurally-relevant SOC fractions (Gregorich and Beare, 2006; von Lützow et al., 2006).

The three SOC fractions that result from this separation technique are the free light fraction (LF), occluded particulate organic matter (POM) and silt-and-clay associated OM (S&C). Free LF is characterized as relatively fresh plant litter (< 1.7 g cm^{-3}) that has been incorporated into the mineral soil, but is not protected from decomposition. A liquid density between 1.6 – 1.8 g cm^{-3} is commonly used to separate the free LF from the heavy fraction in soils (Sollins et al., 1999). POM and S&C fractions together make up the heavy fraction (> 1.7 g cm^{-3}). POM is partially decomposed organic material that has become incorporated into soil aggregates, and because of limited microbial access, is physically protected from further decomposition. The S&C fraction is highly decomposed OM that is typically the oldest and most stable pool of OM as a result of adsorption to the surface of clay and silt particles by chemical bonds. It is generally unaffected by land-use changes. In this study, occluded OC will be referred to as physically protected C (or POM) and silt- and clay-associated OC will be referred to as chemically protected C (or S&C). The LF and POM pools are collectively called physically uncomplexed OM, which is classified as the intermediate step between fresh inputs of plant residue and stabilized SOM (Gregorich and Beare, 2006). While POM is physically protected and is thus temporarily stabilized within the soil structure, it is still sensitive to aggregate disruption, whereas chemically protected C is strongly bonded to clay and silt, and has longer residence times (von Lützow et al., 2006; Eusterhues et al., 2003). Hence, POM is grouped with LF more than with S&C carbon. In this study, each size-density fraction is considered separately.

1.3.2 Chemical protection of soil carbon may be limited by the availability of clay surfaces

Hassink (1997) assessed the ability of clay and silt particles to preserve organic C in uncultivated temperate and tropical soils and found a strong positive correlation ($r=0.89$) between the percentage of clay and silt in the soil (<20 µm) and the amount of C within this pool, indicating enhanced stabilization of C as the percentage of silt and clay increased. Hassink's research, as well as several other studies published in the last 15 years (Six et al., 2002a; Gulde et al., 2008; Laganière et al., 2011), suggest that soils may have a maximum C content that can be stabilized within the soil, based on the amount of available binding sites on clay and silt surfaces. Modelling C within S&C fractions across a range of different soils has allowed Hassink (1997) to calculate a theoretical C saturation capacity for any soil based on total clay content, which Carter et al. (2003) extended to include silt and clay particles. The following equation was the result:

Capacity (g C kg^{-1} soil) = 9.04 + 0.27 (% clay + silt particles)

The theoretical C saturation capacity can then be compared to the actual amount of C associated with silt and clay surfaces to determine the proximity to saturation. Laganière et al. (2011)

applied this concept to boreal forest soils under aspen, black spruce and mixedwood stands and found that the black spruce and mixedwood soils were not significantly different from theoretical C saturation level, while the aspen soils had significantly less C than the saturation capacity. This correlated with significantly more uncomplexed OM at black spruce soils than at aspen soils, which suggests that after soils reach saturation capacity, carbon tends to accumulate in uncomplexed pools.

1.3.3 Microbial biomass is the fastest cycling pool of soil carbon

Another important carbon pool to consider is microbial biomass carbon (MBC). Microbial biomass is included in total SOC, and is a measure of mostly bacterial and fungal biomass (Singh and Singh, 1995). It is the most active pool of C, with a turnover rate of 1-3 years, which means that this pool responds rapidly to changes in the soil environment (Paul and Voroney, 1980). MBC is closely correlated to changes in organic matter. In forest soils, MBC ranged from 1.6 – 3.6 % of SOC, although wider ratios of MBC:SOC were observed where the soil LF accumulated (Wardle et al., 1992). Microbial biomass is also correlated with soil structure and soil texture, as a result of well-aggregated, clayey soils providing greater protection from predation by restricting the access of microbial feeders (Wardle et al., 1992).

1.3.4 Soil structure plays an important role in carbon stabilization

The development of soil structure results in physically protected carbon and can lead to chemically protected carbon through facilitating greater contact between OM and clay surfaces. Soil structure is defined as the shape, size and arrangement of aggregates and pore spaces within the soil matrix (Brady and Weil, 2008). Aggregates are the building blocks of soil structure. They are composed of organic matter and particles of sand, silt and clay that are held together by cohesive forces, either temporarily through the action of roots and fungi, or more long-term through organic and inorganic cementing agents (Brady and Weil, 2008). Aggregate stability is empirically measured by applying a disruptive force to the soil, typically immersion in water, and capturing any aggregates that withstand this pressure, which are called water-stable aggregates. Aggregate dynamics have been extensively studied in agricultural soils because of the importance of soil structure in stabilizing carbon, improving the physical soil environment for microbes, increasing aeration and water movement through the soil, and contributing to soil quality by storing nutrients (Karlen et al., 1997). More recently, silviculturalists have begun using soil quality measurements like soil structure as indicators of sustainable forest management (Schoenholtz et al., 2000).

The method used in this study to separate aggregates in to four size classes is by Six et al. (2000). The smallest size class (< 53 µm in diameter) is composed of silt- and clay-sized particles that are too small to be considered part of aggregated soil. This size class is similar to the S&C fraction isolated in the size-density separation, except that the S&C fraction includes all silt and clay minerals, whereas silt and clay fraction from the aggregate analysis include only the silt and clay that are not included in aggregates. Microaggregates are 53 - 250 µm in diameter and tend to be strongly resistant to external disruptive forces. Macroaggregates are broken down into small (250-2000 µm) and large (>2000 µm) size classes. Large macroaggregates are the most influential size class in determining aeration and water holding capacity, and they tend to have the highest concentration of carbon, but they are also the most easily disturbed (Jastrow et al., 1996).

1.3.5 The presence of key binding agents facilitate structural development

Texture and organic matter play an important role in soil structural development. A well-structured soil typically occurs when there is sufficient OM content, and an even mixture of sand, silt and clay, resulting in a wide size range and abundance of stable aggregates. While clay is not categorized as an aggregate binding agent, the adsorption of organic matter to mineral surfaces is essential for the chemical protection of soil C and results in the formation of aggregates. Binding agents such as microbial and root exudates facilitate micro- and macroaggregate formation (Jastrow, 1996). Glomalin, for example, is a glycoprotein secreted by arbuscular mycorrhizal fungi that has been strongly correlated with water-stable aggregate stability (Rillig and Steinberg, 2002). The presence of exchangeable cations such as calcium and magnesium also facilitate aggregate formation through cation bridges between clay surfaces and organic matter (Muneer and Oades, 1989). At a larger scale, the temporary actions of binding agents like roots and fungi facilitate large and small unstable macroaggregate formation, which can lead to the formation of stable aggregates over time.

Particularly in younger, less weathered soils, SOM and soil biotic influences are the driving force of soil aggregation (Oades and Waters, 1991). SOM contributes to structural stability are by forming strong bonds with mineral particles, by physically enmeshing particles into loose macroaggregates, and by reducing the wettability of aggregates which can lead to the breakdown of bonds holding aggregates together (Chenu et al., 2000). SOM typically has a high cation exchange capacity that allows this material to form multiple bonds. In older, more weathered soils, the abundance of weathered clay minerals and Fe and Al oxides overpower the role of organic binding agents in determining aggregate stability (Denef and Six, 2005; Hassink, 1993). Thus, it is important to consider site characteristics when attempting to predict what mechanisms are facilitating soil structure formation.

1.3.6 A well-structured reclaimed soil should have both a high aggregate stability and a wide distribution of aggregate sizes

The concept of "good" soil structure is difficult to capture in one representative term. A soil structure composed of a distribution of macro- and microaggregates ensures that small pores form in which organic carbon can be occluded, and microorganisms are protected from predation, as well as large pores that can quickly drain during rainfall events. Mean weight diameter (MWD) is the sum of each size-class weight divided by the average diameter for that size class, and was initially proposed by van Bavel (1949) as a way of assessing aggregate stability (Marquez et al., 2004). A high MWD infers that a significant amount of the soil is aggregated, and that macroaggregates predominate. However, long-term carbon sequestration is dependent on the formation of microaggregates and clay- and silt-associated C, which is not necessarily captured in MWD (Verchot et al., 2011). The formation of microaggregates within macroaggregates is thought to contribute highly stable microaggregates with strongly protected C to the soil (Oades, 1984; Six et al., 2002b). It is important to consider both MWD and the distribution of aggregate size-classes, as well as the distribution of soil C in different soil fractions, to assess the contribution of soil structure in soil C stabilization. The overall benefits of a well-structured soil at reclaimed sites go beyond soil carbon storage, as structure provides other benefits that increase the likelihood of establishing a functioning boreal forest soil such as providing a habitat for microorganisms and resistance to water erosion.

1.3.7 Vegetation can have significant effects on the mechanisms of soil carbon stabilization

Functional traits of plant species influence soil carbon stabilization in different ways, primarily through the interaction of roots with the soil, through the quantity and quality of organic inputs, and by creating microclimate conditions that support a specific soil microbial and macrofaunal community. Roots contribute to soil structure by pushing soil particles together as they grow through the soil and enmeshing organic material, facilitating the formation of macroaggregates (Bronick and Lal, 2005). Dead roots can also act as nuclei for aggregates, and as a result, become quickly incorporated into physically-protected C pool. Gale et al. (2000) found that stable macroaggregates had higher concentrations of root-derived carbon than unstable macroaggregates, so root litter may contribute to stable aggregate formation. The abundance of fine roots in particular is thought to play an important role in C stabilization, as the more closely roots associate with soil particles prior to senescence, the more likely that roots will become incorporated into aggregates after senescence (Bronick and Lal, 2005). More root carbon has been found to be incorporated into stable aggregates than shoot-derived carbon (Rasse et al., 2005).

One of the most influential ways that plants can affect soil C stabilization is through the quality and quantity of OM. Fast-growing species tend to contribute large amounts of easily decomposable material, whereas slow-growing species tend to contribute a more nutrient-poor plant material with a higher C:N ratio (De Deyn et al., 2008). Quideau et al. (2001) used solid state ^{13}C NMR spectroscopy to characterize the chemical structure of OM as it cycled from fresh litter to the LF pool to fine S&C fractions (2-5 µm) under several different vegetation types. Deciduous stands showed a high degree of oxidation between litter and the fine S&C fraction through a loss of carbohydrates and an accumulation of phenolic C signals in the fine S&C. Coniferous organic material showed little alteration in chemical structure from litter to the fine S&C fraction, with a prevalence of alkyl C throughout, demonstrating that inherent chemical composition of coniferous litter relative to deciduous litter resulted in a less processed end-product of decomposition. Increasing phenolic concentrations in plants has been correlated with increased aggregation and C sequestration (Martens, 2000). Laganière et al. (2011) found that in boreal forest stands, physically protected C was more affected by different stand types than chemically protected or free LF C, suggesting that effect of vegetation inputs on soil structure development and OM incorporation into aggregates was important in determining soil C stabilization among vegetation types.

The predominance of soil fungi vs bacteria between vegetation types will also play an important role in contributing to aggregate stability and carbon uptake in the soil structure. Fungal-dominated soils are associated with high organic matter content and low substrate quality, meaning that the C:N ratios of the OM is high (Deyn et al., 2008). The ratio of fungi to bacteria is typically wider in forest soils than in agricultural or grassland soils (Lauber et al., 2008); this is thought to be a result of differences in litter quality and quantity, with grasslands having more easily decomposable litter containing more nitrogen. Fungi facilitate physical protection of C through enmeshing soil and OM together and forming macroaggregates, whereas microbes release exudates that bond OM to minerals, which is more likely to lead to chemical protection. Fungi are also highly efficient at extracting nutrients from nutrient-poor substrates, and thus, can break down and release C from resistant soil C pools (Phillips et al., 2013). Grassland species also form symbiotic fungal associations, but with arbuscular mycorrhizal fungi, which release the glycoprotein glomalin. Glomalin has been suggested to be a binding agent for aggregates, and has been correlated with increased aggregation and greater carbon storage (Emran et al., 2012). The complex interactions of fungi and bacteria with organic and mineral soil constituents can have significant effects on soil C stabilization and should be taken into account when considering differences between vegetation types.

1.4 Reclaimed ecosystems differ from natural soils in ways that may influence C stabilization

1.4.1 The peat amendment could facilitate faster soil structure development and greater C storage at reclaimed sites

An important difference between reclaimed and naturally-occurring soils is the addition of peat to reclaimed soils. Organic amendments are often used in degraded soils to increase the initial availability of nutrients and improve soil physical properties (Larney and Angers, 2012). However, it is important to understand how these organic amendments affect long-term soil properties and processes compared to organic inputs from vegetation. As previously stated, SOM plays a major role in aggregate formation, and physical and chemical protection of C. Diacono and Montemurro (2010) found that the benefits of using organic matter as an amendment in reclamation included greater microbial biomass C, greater enzymatic activity, more carbon sequestration, greater aggregate stability, and lower soil bulk density, cumulating in enhanced biological functioning and greater crop yield. Based on this evidence, one could hypothesize that reclaimed soils may ultimately be capable of even greater physically and chemically protected C than naturally occurring soils as a result of the peat amendment.

Greater SOM content and differences in SOM quality have been found in reclaimed soils compared to natural soils (Anderson, 2014; Turcotte et al. 2009). However, determining whether soil carbon at reclaimed sites is a result of vegetation inputs or the persistence of peat from the initial peat amendment has proven to be very difficult and has been addressed in several different ways. Anderson (2014) found that at 40 cm, OM content was similar in all reclaimed vegetation types, and was similar to the OM content in the top 10 cm of coniferous sites. He proposed that this OM was the remains of the original peat amendment, whereas the excess OM in the top 30 cm had accumulated since reclamation.

Turcotte et al. (2009) used size and density fractionation to separate reclaimed and natural reference soils into three carbon pools similar to the pools isolated in this study. ^{13}C nuclear magnetic resonance (NMR) spectroscopy was also performed on the free light fraction OM to assess chemical composition. They found a strong macromolecular signal in the reclaimed soil LF pool that is associated with relatively unaltered peat, which suggests the light fraction at reclaimed sites may be predominately composed of residual peat material. One important distinction between the methods used by Turcotte et al. (2009) and the methods used in this study is that they separated the free LF from the heavy fraction using water, whereas I used a heavy liquid (1.7 g cm^{-3}), which is more commonly used to isolate the LF from the heavy fraction.

Free organic matter isolated by density separation (< 1.6 g cm^{-3}) has been identified as mostly large fragments of undecomposed or partially decomposed material with recognizable plant structures (Golchin et al., 1994). In contrast, occluded organic matter (> 1.6 g cm^{-3}) was found to be smaller particles of incompletely decomposed organic residues composed of more resistant chemical compounds. This suggests that the use of a heavy liquid is an appropriate method to separate OM into labile and intermediate/passive pools of soil carbon, and as a heavy liquid was used in this study, the free LF pool represents a different pool of OM than the one measured by Turcotte et al. (2009). Perhaps as a result of using water to separate the free LF, Turcotte et al. (2009) found no difference in LF carbon pools between reclaimed and natural sites, which represented less than 5 % of total OC in both cases. This was surprising because the LF pool has been shown to be a sensitive indicator of land-use change (Degryze et al., 2004), and thus, was expected to be significantly different between reclaimed and natural sites.

While size-density fractionation is useful in distinguishing unprotected carbon from physically and chemically protected carbon, this method does not address whether the carbon in each fraction is biochemically protected as a result of the inherent chemical properties of the material. Biochemical recalcitrance has been previously stated as being less important than chemical and physical protection in carbon stabilization (Dungait et al., 2012), yet an abundance of recalcitrant material such as peat will increase C residence times, regardless of the size-density pool in which it is located. Peat-forming wetlands have been accumulating carbon in organic material since the last glaciation event (Dioumaeva et al., 2002). Thus, peat C is probably significantly older and composed of more biochemically resistant material than the oldest carbon from vegetation inputs at reclaimed sites, which is less than 40 years.

One possible way to distinguish peat carbon from vegetation-input carbon is to use sodium hypo-chlorite (NaOCl) to oxidize labile C pools, leaving behind older, biochemically resistant pools. This method was initially proposed as a way of removing SOM from the soil to assess clay minerals. More recently, it has been used as a way of mimicking enzymatic breakdown of SOM, thus isolating a carbon pool composed of complex polymers that are resistant to microbial decomposition. The carbon pool that is oxidized is typically characterized as labile, rapidly-cycling carbon that is bound up in less complex forms (Zimmerman et al., 2007; Blair et al., 1995). One study found that the ^{14}C age of labile SOM that was removed during NaOCl oxidation was from recent to 2500 years old, whereas SOM from samples that survived oxidation was between 1500 – 7000 years old (von Lützow et al., 2007). It is possible that some peat C will not be highly complexed, and may break down with the labile C fraction. However, the majority of the biochemically resistant C at reclaimed sites is probably peat. Measuring C:N ratios before and after oxidation allows a comparison of relative nutrient content of resistant and labile fractions at reclaimed and natural sites.

1.4.2 Differences in exchangeable cations may also affect carbon stabilization between reclaimed and natural sites

Reclaimed soils may differ from natural soils in their exchangeable cation concentrations. Ca^{2+} and Mg^{2+} cations have been found in higher concentrations in reclaimed soils compared to natural soils (Rowland et al., 2009; Anderson, 2014). As previously mentioned, the presence of cations facilitates bridging between OM and mineral surfaces. Considering that texture is a measure of silt, sand, and clay, excluding SOM, and that mineral soil from the pre-mining footprint is used in the PMM, it is unlikely that texture would be significantly different from natural mineral soils in the top 10 cm. Therefore, high concentrations of exchangeable cations in reclaimed soils, and a similar clay and silt content compared to natural sites, could be facilitating aggregate formation and C storage.

1.4.3 Macrofauna have been found to stimulate soil functioning in reclaimed sites

Macro- and mesofauna have a dynamic relationship with soil structure, through facilitating macroaggregate formation and by increasing contact between organic material and clay surfaces during the ingestion of soil (Pulleman et al., 2005). Inoculation of earthworms in degraded grassland soils has initiated stable aggregate formation in restored mine sites in the UK through their addition of mucus, which acts as a binding agent (Marashi and Scullion, 2003). At post-mining sites that were not reclaimed in the Czech Republic, Frouz et al. (2008) found that earthworm activity played a principal role in mixing organic surface material into the mineral soil and creating a humus layer. Frouz et al. (2006) also found that earthworm abundances in mine-disturbed soils were correlated with greater water-holding capacity, which is likely due to improved soil aggregation. At the reclaimed oil sands sites that were sampled in this study, Anderson (2014) found higher bioturbation in reclaimed grassland and deciduous sites than in naturally fire-disturbed or reclaimed coniferous sites. McMillan et al. (2007) found lower microbial biomass C (MBC) and microbial biomass N (MBN) in reclaimed soils 5-6 years after reclamation compared to undisturbed aspen forest, indicating that lower microbial activity may restrict soil structural development initially. Thus, it could be interesting to see whether greater C stabilization correlates with greater macrofaunal and microbial activity among vegetation treatments.

1.4.4 Reclaimed vegetation treatments will probably vary in their ability to stabilize carbon

In a reclamation context, it is important to consider how different reclamation decisions will affect soil processes, particularly carbon storage. Several studies have found that reclaimed mine soils have the potential to stabilize large amounts of carbon, in some cases accumulating more than undisturbed soils in the same area (Ussiri and Lal, 2005; Vindušková and Frouz, 2012). The plant species selected for vegetation establishment in reclaimed ecosystems has important long-term effects on C stabilization. In a meta-analysis assessing what ecosystem type would maximize carbon sequestration on reclaimed sites, forests sequestered more C than grasslands, although this was primarily due to differences in aboveground biomass (Sperow, 2006). Grasslands tend to accumulate SOC more quickly than forests, which accumulate C in aboveground biomass and surface organic layers, as well as in the soil. Reclaimed grasslands reached SOC levels similar to undisturbed grasslands within 15 years, whereas forests took 20 years to reach undisturbed SOC levels (Akala and Lal, 2001). One mechanism that could facilitate faster SOC accumulation at grassland sites is the abundance of fine roots typically associated with grasslands, whereas coniferous and deciduous ecosystems tend to have a greater abundance of larger roots. Therefore, root activity at reclaimed forested sites may be more likely to influence C stabilization through the formation of macroaggregates in the upper soil layers, and through deep-rooting contributing to subsoil carbon storage (De Deyn et al., 2008), whereas the intimate association between fine roots and clay at grassland sites may result in greater physically and chemically protected carbon.

Poeplau and Don (2013) measured SOC from different size-density fractions and assessed the distribution and sensitivity of SOC to land-use changes between cropland, grassland, and forest ecosystems. Light fraction C increased under afforested grasslands, but the POM and S&C fractions both decreased, with the POM fraction showing the greatest decrease in SOC (Poeplau and Don, 2013). This is an intriguing result because it suggests that a decrease in soil structure from grassland to forested ecosystems was a significant contributor to the general loss of SOC stores. In a comparison of reclaimed deciduous and coniferous sites, deciduous sites had greater microbial biomass carbon and more enzymatic activity than coniferous stands, which resulted in greater accumulation of total soil C and N (Chodak and Niklinska, 2010). Therefore, in other reclaimed areas, the type of vegetation planted at reclaimed sites has had a significant impact on soil C dynamics, and thus, one would expect to find differences in soil C among vegetation types in this study.

1.4.5 Naturally wildfire-disturbed sites provide a better comparison for reclaimed sites than natural sites that have not experienced a disturbance

Fire has significant effects, both negative and positive, on soil properties such as soil aggregation and C storage (Mataix-Solera et al., 2011), and this should be taken into account before comparing between reclaimed and naturally fire-disturbed sites. The effect of fire on soil structure depends on the severity and intensity of the fire, and initial soil characteristics such as SOM content, texture, clay type, and soil water repellency. Boreal forest fires are highly variable in both severity and area burned (Harden et al., 2000). Summer wildfires in boreal forests can be severe, and may disrupt aggregate stability and decrease SOC by burning SOM and other organic binding agents that hold aggregates together (Certini, 2005). Unlike naturally fire-disturbed sites, initial SOC content was probably high at reclaimed sites due to the peat amendment. However, reclaimed sites would also have had higher SOC than any local natural analogue, except for peat organic soils. Fire has been found to increase soil pH and the availability of Ca, Mg, K, and Na (Arocena and Opio, 2003). Reclaimed soils have also been found to have a higher pH and greater exchangeable cation concentrations, so in this case, the effect of fire increased the similarity between reclaimed and naturally fire-disturbed sites (Rowland et al., 2009). Fire-disturbed sites were chosen as a useful comparison for reclaimed sites because they are common in the area surrounding oil-sands surface mining, and dates of fires have been documented. This allowed us to choose natural sites that were within the same age range as reclaimed sites, in which the vegetation was at a similar stage of development.

1.5 Summary and research objectives

The ability of reclaimed soils to stabilize carbon through physical protection in soil aggregates, and through chemical protection by adsorbing to clay surfaces is an important indicator of soil functioning and provides evidence that reclaimed sites are moving towards successful reclamation. Soil carbon is an integral component in soil biological, chemical, and physical properties, and is essential for aboveground vegetation. Moreover, promoting soil carbon stabilization in reclaimed landscapes could mitigate some of the carbon losses that occur as a result of oil sands extraction.

The persistence of soil carbon is now recognized as an ecosystem property, as opposed to solely a molecular property (Schmidt et al., 2011). Thus, carbon stabilization is directly related to the inherent properties of an ecosystem, and could be significantly affected by the plant species selected for reclaimed sites through their interactions with the soil environment. Organic matter accumulation and quality have been measured in reclaimed sites (Anderson, 2014; Turcotte et al., 2009). However, a comprehensive assessment of the distribution of soil organic carbon in free light fraction, physically protected, and chemically protected pools in the top 10 cm of reclaimed soil compared to natural soil has not been undertaken. Developing a better understanding of how carbon is stored between different reclaimed vegetation treatments, and how this compares to natural reference sites, could provide important insights into how to optimize soil carbon stabilization while maintaining similar land capability as pre-disturbance ecosystems. However, the contribution of peat carbon to total carbon must be considered in order to fully understand the stability of carbon pools, and the relative contributions of different vegetation types to soil C at reclaimed sites.

The objectives of this study were to compare carbon pools and other soil properties related to carbon stabilization between reclaimed and natural sites, and to explore how vegetation type affected carbon stabilization at reclaimed sites. Five naturally fire-disturbed sites were selected as natural reference sites, and will be referred to in future as natural sites. Three reclaimed vegetation treatments were selected (coniferous, deciduous, and grassland) and five sites were selected for each treatments. All 20 sites were reclaimed or disturbed 21 – 41 years ago, and the top 10 cm of mineral or equivalent reclaimed soil was sampled. The research questions that were addressed in this study are as follows:

1. Do reclaimed soils store more carbon in physically and chemically protected pools than natural boreal upland soils?
2. Do reclaimed sites have more biochemically resistant soil carbon compared to natural sites?
3. Does the reclaimed vegetation prescription affect how carbon is stabilized in reclaimed soils?

2 METHODS

2.1 Site description

The oil sands are located within the North American Boreal Plain in the Central Mixed Wood sub region. The Boreal plains reach from Manitoba to northeastern British Columbia and are the dominant ecozone in Alberta. Uplands in this region are formed on glaciofluvial, glaciolacustrine and glacial till deposits, and lowlands are formed on top of fine glacial sediment. Within the region surrounding oil sands development, upland soils are mostly Grey Luvisols that have developed on medium- to fine-textured lacustrine deposits, or Dystric Brunisols that have developed on coarser glacial deposits and aeolian sands (Natural Regions Committee, 2006). The dominant tree species present in upland areas are jack pine on well-drained soils, and trembling aspen and white spruce on wetter soils.

The poorly drained lowlands, which originally covered over 60% of the landscape overlying the oil sands deposit, are composed of Organic soils (Rooney et al., 2012). Peat from these soils is salvaged from the mining footprint and used as an organic amendment in soil reclamation (Mackenzie, 2012). Peatlands are dominated by peat moss (*Sphagnum sp.*). Schreber's moss (*Pleurozium schreberi*), golden moss (*Tomentypnum nitens*) and tree species such as black spruce (*Picea mariana*) and larch (*Larix laricina*). Seasonally, this region exhibits long, cold winters and short, warm summers, with average monthly temperatures ranging from -17.4 ^{o}C in January to 17.1 ^{o}C in July (Environment Canada, 2015). Average annual precipitation is 418.6 mm and mostly falls in the summer months. Average annual snowfall is 133.8 cm and falls over 7-9 months of the year. The region sits at 370 m above sea level.

Reclaimed research sites are located on Syncrude and Suncor mining leases north of Fort McMurray, AB (56°39'12" N, 111°13'24" W). In 2012, 20 research sites were established to compare organic matter accumulation and biogeochemical processes among three reclaimed vegetation treatments (5 sites/treatment) and naturally wildfire-disturbed forest (5 sites). The same sites were sampled again in 2013 for this study to further contribute to the understanding of biogeochemical processes at these sites. After collecting the 2012 data, two of the reclaimed sites were eliminated due to thin topsoil layers, and two of the natural sites were excluded due to the proximity to human activity. Replacement sites were found during the 2013 field season to ensure that there were five sites for each reclamation treatment and five natural sites. The reclaimed sites selected for this study are all classified as upland sites, and were selected based on their age and whether a peat-mineral mix (PMM) above overburden cover treatment was used.

Reclaimed sites were established 21-41 years ago, and each treatment includes a range of site ages to limit age as a confounding factor. The three reclaimed vegetation treatments that were selected for comparison are coniferous forest, deciduous forest, and grassland ecosystems. The coniferous and deciduous sites were previously established as part of a study to investigate forest-floor development in reclaimed areas (Sorenson et al., 2011). Forested sites were selected based on whether > 80 % of the trees were either white spruce trees at coniferous sites, or trembling aspen at deciduous sites. Understory species at deciduous sites were mostly grasses (20.8 % ±26.53) and shrubs (14.7 % ±15.61), with only 3.5 % bryophytes, whereas bryophytes dominated the understory cover at coniferous sites (46.0 % ±23.44), with some forbs (11.1 % ±11.74) and shrubs (5.1 % ±3.38). The most common shrubs at deciduous sites were *Rubus idaea* and *Rosa acicularis*, and cumulative understory cover was 41.9 % on average. The most common shrub at coniferous sites was *Rosa acicularis*, and understory cover was 17.1 %. Grassland sites had < 5 % tree cover and were dominated by grasses (75.3 % ±22.08) and some forbs (18.3 % ±19.73) (Anderson, 2014). Natural sites were selected based on their age since the previous forest fire burn using historical fire data. Fire-disturbed sites were selected as an ideal reference condition because fire is a common occurrence in the boreal biome so there was an abundance of sites to choose from within the designated age range. Further, ecosystem functioning was expected to be more similar between reclaimed and natural sites that were disturbed 20-40 years ago, than if natural sites had not experienced any recorded disturbance. Natural sites within the age range of 18-41 years were selected if they fell within similar moisture and nutrient regimes as reclaimed sites according to Beckingham and Archibald's Ecotypes of Northern Alberta (1996). Specifically ecosystems that were classified as edatopic type b, c, or d, which range from sub-mesic to mesic, and poor to rich nutrient regimes, were chosen. All of the natural sites contained a mixture of trembling aspen and jack pine, following the successional sequence of burned sites in this region. Shrubs covered 30.5 % of the plot at natural sites, and the dominant species present included *Ledum groenlandicum*, *Vaccinium myrtiloides*, *Vaccinium vitis-idaea* and *Viburnum edule.* The cumulative understory cover was 37.3 %, and bryophytes, predominantly feather mosses, covered 42 % of the plots on average (Anderson, 2014). No natural analogs of a comparative age were available for the reclaimed grassland treatments. Individual site characteristics can be found in Table 1.

Table 1. Characteristics of the 20 study sites

Site ID	Vegetation type	Location	Reclamation cover /soil type	Depth of PMM	Soil texture class	Years since reclamation/ disturbance
C18	Coniferous	Syncrude	PMM/Overburden	100	Sandy Loam	23
C29	Coniferous	Suncor	PMM/Overburden	25	Sandy Loam	22
C33	Coniferous	Suncor	PMM/Overburden	10	Sandy Loam	29
C37	Coniferous	Suncor	PMM/Overburden	25	Sandy Loam	28
C43	Coniferous	Suncor	PMM/Overburden	10	Sandy Loam	21
D7	Deciduous	Syncrude	PMM/Secondary	20	Sandy Loam	21
D12	Deciduous	Syncrude	PMM/Overburden	100	Loamy Sand	29
D34	Deciduous	Suncor	PMM/Overburden	15	Sandy Loam	28
D38	Deciduous	Suncor	PMM/Overburden	20	Loamy Sand	29
D42	Deciduous	Syncrude	PMM/Direct Placement	20	Sandy Loam	21
G1	Grassland	Syncrude	PMM/Overburden	20	Loamy Sand	41
G2	Grassland	Suncor	PMM/Overburden	10	Sandy Loam	28
G3	Grassland	Suncor	PMM/Overburden	10	Sandy Loam	28
G4	Grassland	Suncor	PMM/Overburden	100	Loamy Sand	35
G6	Grassland	Syncrude	PMM/Overburden	30	Loamy Sand	30
NS2	Naturally fire-disturbed	South of Fort McMurray	Gleyed Eluviated Eutric Brunisol	--	Loamy Sand	18
NS3	Naturally fire-disturbed	South of Fort McMurray	Brunisolic Gray Luvisol	--	Silt Loam	41
NS4	Naturally fire-disturbed	South of Fort McMurray	Brunisolic Gray Luvisol	--	Silt Loam	32
NS5	Naturally fire-disturbed	South of Fort McMurray	Brunisolic Gray Luvisol	--	Silt	32
NS6	Naturally fire-disturbed	South of Fort McMurray	Eluviated Eutric Brunisol*	--	Sand	18

*NS6 may also have been gleyed, but it was difficult to assess at time of sampling because it had rained and the soil pits had water ponding at the bottom.

The reasoning behind planting different species at different sites throughout the reclaimed areas is not well documented for older sites. According to current reclamation guidelines for revegetation, the edatopic grid is loosely used to determine individual site capability based on soil moisture and nutrient regimes, and vegetation prescriptions are selected using these parameters (Alberta Environment, 2009). It is likely that in older reclaimed sites, vegetation prescriptions were assigned randomly, or were based on the tree species available for planting and not on specific site capability (Yarmuch, personal communication, May 15, 2013). Grassland sites are not typically a reclamation goal for upland areas because the equivalent land capability in upland boreal ecosystems was forest. However, unplanted areas can occur during reclamation due to operational constraints. In this study, grassland sites occurred beside rarely used access roads, next to a radio tower, and beside a low-lying wetland area. Therefore, it is reasonable to assume that these sites were never planted, and are not the result of trees being unable to grow on them. A full vegetation description was completed for each site when they were established (Anderson, 2014). Other site parameters measured were slope, aspect, slope position, and direction of drainage.

2.2 Soil sampling

Soil samples were taken in July, 2013 and where possible, the same 10 m x 10 m plot that had been established in 2012 for previous research was sampled again (Anderson, 2014; Masse, unpublished data). Three subplots were selected for sampling at each site. To determine the location of the subplots, the plot was divided into 1 m by 1 m squares that were given grid coordinates. Random number generation was used to select three sets of coordinates. If a selected subplot was located on top of a tree or shrub, a new subplot was selected. At each subplot, a soil pit (approx. 40 cm deep) was dug and a soil profile assessment was conducted. This consisted of identifying the soil horizons, recording observations of texture and visible structure, and recording evidence of macrofauna. Soil samples were taken from the top 10 cm of each subplot, excluding surface organic horizons, and homogenized on-site for future chemical and microbial analysis.

Soil structure, soil carbon pools, bulk density, and root biomass were analyzed from each subplot individually, resulting in three subsamples for each site. The mixing of peat and mineral soil into a reclaimed soil substrate sometimes created macro-scale clumps of peat or mineral soil at reclaimed sites, which were observed during sampling. The sporadic arrangement of these clumps makes it unlikely that they would have significantly affected overall soil functioning, yet they can significantly affect mean values of soil properties at each site. Thus, for the soil properties listed above, measurements were recorded for all three subplots, but only the two most similar of the three subplots were used to determine sites averages.

To extract soil for structural analysis, surface organic horizons on top of the mineral soil were scraped away, and a long-tipped trowel was used to cut a triangular section of soil 10 cm deep. The sample was carefully extracted by cutting away soil around the section, lifting it out using the trowel, and placing it in a 1-L plastic container. Root samples were extracted by cutting a 10-cm^3 block of soil from one of the faces of the soil pit. The distance from the soil pit to the three closest trees was measured at forested sites to help explain root sample variation within a site that may have resulted from close proximity to a tree. After the root sample was removed, the dimensions of the extracted area were measured to determine the volume of extracted soil so that bulk density could be determined. All soil samples were stored at 4^oC until they could be further processed.

2.3 Total SOC and SOC pool separation by size-density fractionation

The soil samples collected for structure analysis were also used for carbon analysis to allow for a better comparison between the abundance and size distribution of aggregates and carbon content in physically and chemically protected fractions. Field-moist soil blocks were gently broken apart by hand and passed through an 8-mm sieve to homogenize the soil and remove large roots and litter fragments. A 5-g subsample was weighed to determine initial water content. Samples were then air-dried at room temperature and stored in sealed plastic containers for no longer than 30 days.

To determine the location of organic carbon within the soil structure, density and size separation techniques were used to separate the soil into three pools: the free light fraction (free LF), physically protected carbon (POM) and chemically protected carbon (S&C) (Figure 1). Fractionation was performed on the air-dried soil samples using the method described in Langanière et al. (2011) and adapted from Gregorich and Beare (2006). To separate the free LF from the heavy fraction (HF), a 25-g soil sample was gently shaken with 50 mL of sodium iodide (NaI, mixed to a specific gravity of 1.7 g cm^{-3}) on a reciprocal shaker for 20 minutes, or until the entire sample had been wetted. Soaked samples were allowed to settle for 48 h. The suspended free LF was then aspirated from the surface of each sample and vacuum-filtered with a 0.45-μm nylon filter. Any remaining NaI was washed from the sample using a 0.01-M $CaCl_2$ solution and distilled water. Due to the high OM content in reclaimed soils, this procedure was repeated for each sample to ensure that all of the free LF was removed from the heavy fraction (HF). Free LF samples were oven-dried at 60°C and weighed.

Size separation was used to separate the HF into POM and S&C fraction. The remaining sample including residual NaI was washed with the 0.01-M $CaCl_2$ solution and centrifuged for 15 min at 2000 rpm. The supernatant was poured off and the process was repeated three times to ensure that no NaI remained. The washed HF was then added to 100 mL of distilled water and shaken on a reciprocal shaker with 10 glass beads overnight, to disperse aggregates. It is important to note that aggregates were disturbed prior to sieving, so POM carbon is not the same as aggregate-associated carbon. Some of the silt- and clay-associated carbon contained within aggregates would have been dispersed, and ended up in S&C fraction. However, all of the POM carbon is physically protected or else it would have remained suspended in a heavy liquid, hence the terms physically and chemically protected carbon pools are used in this study. The dispersed sample was wet-sieved through a 53-μm sieve. The sample remaining on the sieve (POM) and the sample that passed through the sieve (S&C) were oven-dried at 60°C and weighed.

Each size-density fraction was ground by hand with a mortar and pestle and analyzed for organic carbon concentrations using the loss-on-ignition method (Rabenhorst, 1988). Subsamples from each size fraction (2 g) were oven-dried at 105°C overnight to remove hygroscopic water content, and then weighed (w_i). Samples were then heated to 400°C for 20 min to burn off the organic matter, and weighed again (w_f). Soil sample standards with a known weight of OM were used to test the accuracy of this method and all standards fell within the 95 % confidence interval. To determine the organic C content per gram of dried soil, the following equation was used:

$$\text{OC content} = \left(\frac{w_f - w_i}{w_i}\right) /1.724$$

Total SOC was determined by summing mean OC concentrations from the three carbon pools at each subplot. Results were expressed in g C kg soil^{-1}. Carbon pools were also expressed as a percentage of total SOC. Inorganic carbon content can be determined by further burning the same soil samples to 950°C and weighing them again (Rabenhorst, 1988). This additional procedure was not performed on the samples in this study, but another study using soil samples from the same sites found that while inorganic carbon was significantly greater in reclaimed soils than in natural soils, it was less than 1 % at all sites and was therefore contributing negligible amounts to total soil carbon (Masse, unpublished data).

Hassink's (1997) equation was used to calculate carbon saturation capacity for each vegetation type using % silt and % clay at each site to find a site C capacity level, and averaging these values to find the mean for each vegetation type. Carbon saturation capacity is defined as the silt- and clay-associated C content at which point all reactive mineral surface sites are occupied, and the mineral adsorption of a soil has reached its maximum.

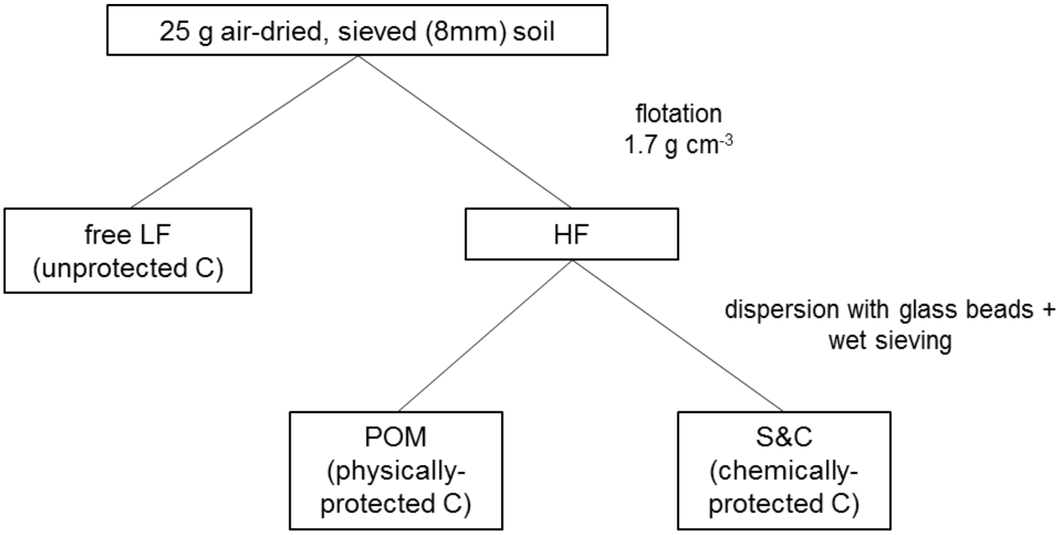

Figure 1. Fractionation method used to isolate free LF, POM and S&C carbon pools

2.4 Biochemically labile vs biochemically resistant carbon pools by NaOCl oxidation

The method used to separate soil carbon into labile vs biochemically resistant pools is described by Zimmerman et al. (2007). Due to time constraints, one subplot from each site was selected for analysis based on whether it represented the average SOC concentrations found at that site. A 25 g sample of air-dried, sieved (2 mm) soil was added to 50 mL of 6 % (wt/wt) sodium hypochlorite (NaOCl). Samples were oxidized for 16 hrs at 25°C. The residue was centrifuged at 1000 rpm for 20 minutes and decanted. A rubber policeman was used to re-suspend the sample in NaOCl, and to disperse aggregates. This process was repeated twice and then the sample was washed three times with distilled water to remove any NaOCl residue. Samples were dried at 60°C and weighed. Dried NaOCl-treated samples and untreated samples were then ground using a mortar and pestle, and carbon and nitrogen were measured by dry combustion using an elemental analyzer (Elementar Vario EL Cube; in C, N mode, Hanau, Germany). Carbon and nitrogen were expressed as total % C and N content.

Total % carbon using this method includes both organic and inorganic C. As stated previously, inorganic carbon was present at reclaimed sites, although in negligible amounts. Therefore, total SOC and total carbon determined from LOI and dry combustion methods, respectively, should produce similar results. A strong positive correlation between SOC and % C (r^2 = 0.68) was found. One site (D42) was identified as an outlier in the % C data, and after this site was removed, the r^2 rose to 0.91 (Appendix A). Despite the total carbon results being similar between these two analyses, different C pools were isolated, addressing different research questions. Therefore, SOC pools will refer to physically protected, chemically protected, and unprotected C, whereas total % C will refer to labile and biochemically resistant C.

Carbon to nitrogen (C:N) ratios provide an indication of the degree of organic matter decomposition. Most soils range from 9.9 – 17.1 in the top 30 cm, with Podzols and Organic soils having the widest ratios at 23.8 and 25.8 respectively (Batjes, 1996). The wide ratios in these soils reflect the accumulation of low-quality plant litter with high carbon content relative to nitrogen content. The C:N ratio of plant material narrows as it decomposes, thus biochemically resistant SOM tends to have a narrow C:N ratio compared to bulk soil, unless a wide initial ratio made the plant material resistant to decomposition (Paul, 2007).

2.5 Extractable microbial biomass carbon

Two subsamples of 10 g of homogenized fresh soil were taken from soil samples collected that same day to measure microbial biomass C and N using the chloroform-fumigation direct-extraction method from Tate et al. (1988). The first subsample was extracted with 50 mL of 0.5 M K_2SO_4 to determine salt-extractable C and N contents. The second subsample was fumigated with 10 mL of ethanol free chloroform in a darkened desiccator jar for 24 hrs at room temperature. This subsample was then extracted in a similar manner to the first, to determine chloroform-susceptible C and N contents.

Extractions were performed after shaking the soil sample in K_2SO_4 multiple times over the course of an hour, and then filtering the sample through a 1.2-µm pore-size glass fiber filter. Extracted samples were then frozen, stored at -20°C, and sent to the Analytical Service Laboratory of the University of Alberta for further chemical analyses.

Total organic carbon (TOC) in extracted samples were determined by oxidative combustion and infra-red analysis using a TOC-TN autoanalyzer (Shimadzu, Kyoto, Japan). Extractable microbial biomass C (MBC) was calculated by determining the difference between fumigated and non-fumigated salt-extractable C. Carbon concentrations were corrected for dilution as a result of moisture content in initial soil samples. Microbial biomass nitrogen (MBN) were also measured, but were very low for all sites, resulting in inexplicably high C:N ratios (200:1). A lab error was suspected, so this data has been excluded from this study. Results are presented in grams of C per kg of soil dry weight.

A calibration factor (k_{EC}) is typically applied to extractable MBC to estimate total biomass C (Sparling et al., 1990). No constant was used in this study because reclaimed soils are different from natural soils, particularly due to the peat amendment, and thus applying a k_{EC} that is based on natural soils would probably produce an inaccurate result. Sparling et al. (1990) found that soils with high and low organic matter content had similar calibration constants, but without calculating the constant at reclaimed sites, it is unclear whether the k_{EC} applied to natural soils would be sufficient for reclaimed sites. Therefore, extractable microbial biomass C, as opposed to total microbial biomass C, was compared among sites, and is referred to as microbial biomass C in this study.

2.6 Soil Texture

Organic matter and soil carbonates were removed from soil samples using the methods described in Konert and Vandenberghe (1997). Samples were then sent to the Environmental Geoscience Research Centre at Trent University for textural analysis, using a laser diffraction with a Partica LA-950 (Horiba

Ltd., Kyoto, Japan). Three replicates were measured after homogenizing the sample, and the mean was used to describe the % weight of particles in clay (<2 µm), silt (2-60 µm), sand (60-2.0 mm), fine sand (60-250 µm), coarse sand (0.25-2.0 mm) and pebble (>2.0 mm) size classes. Size classes that were analyzed during this study were clay, silt and sand.

2.7 Aggregate separation

The method for aggregate separation was adapted from Elliot (1986) and Six et al. (2000) and used to separate bulk soil into four aggregate size classes: large macroaggregates (2000 – 8000 µm), small macroaggregates (250 – 2000 µm), microaggregates (53 – 250 µm) and silt and clay particles (< 53 µm). As previously stated, prior to aggregate analysis, refrigerated soil structure samples were gently broken apart, sieved through an 8-mm sieve, air-dried and stored for no longer than 30 days. A 50-g sample of this soil was slaked by immersing the sample in distilled water on a 2000-µm sieve inside a shallow basin for 5 min. It is important to note that slaking, as opposed to re-wetting, is more disruptive to soil aggregates, thus only strong aggregates were measured (Six et al., 2002). Re-wetting strengthens aggregates and applies less pressure to the bonds that hold aggregates together, but particularly for soils with high SOM content, once soils have been air-dried, it is very difficult to evenly moisten soil samples to field capacity. Since all aggregate soil samples were air-dried prior to analysis to prevent structural degradation during storage, slaking was used to measure water-stable aggregates.

The 2000-µm sieve containing the slaked soil sample was moved up and down by hand for 50 repetitions over 2 min. The soil left on the sieve was back-washed in to an aluminum drying pan and any floating organic material was poured off and discarded because it is considered too large to be part of the SOM fraction (Six et al., 2002b). The soil that passed through the sieve was poured over a 250-µm sieve, and the sieving process was repeated. This process was repeated again using a 53-µm sieve. Soil particles that were small enough to pass through all of the sieves were collected in the shallow basin, and then transferred to large aluminum drying pans. All four aggregate size classes were dried in a forced-air oven at 60°C. Sand grains >1 mm in diameter that were caught on sieves, but identified as not bound up in macroaggregates were removed by hand from the dried samples of large macroaggregates (>2000 µm) and small macroaggregates (250 – 2000 µm), and the weight of the removed sand was subtracted from the total sample weight. Samples were then weighed.

Free organic matter (>53 µm) that was not incorporated into stable aggregates was removed by allowing samples to settle in water for 16 h, and then pouring off floating debris (modified from Turcotte et al., 2009). Floating LF that was collected on 53 and 250 µm sieves made up approximately 4 % of the sample weight at reclaimed sites, and < 1 % at natural sites. The aggregate samples were dried in a forced-air oven at 60°C and weighed. Individual fraction weights are expressed as a percentage of dry soil weight. Mean weight diameter for each sample was calculated as described in Kemper and Rosenau (1986). Mean weight diameter is frequently used as a proxy for aggregate stability, and as a summary statistic for aggregate size distribution (Marquez et al., 2004). It is calculated by summing the mean diameter of each size class multiplied by the proportion of total soil weight found in that size class (Kemper and Rosenau, 1986).

2.8 Bulk density and root biomass

All 10-cm^3 soil blocks were stored at 4°C. Field-moist samples were weighed, and a 5-g soil subsample was oven-dried at 105°C to determine gravimetric soil moisture content. Total bulk density (ρ_{bT}) in grams cm^{-3} was determined using soil sample volumes recorded in the field. The sample was then broken apart by hand, and roots were sorted into four size classes: very fine (<1 mm), fine (1-2 mm), medium (2-5 mm) and coarse (>5 mm). Any roots greater than 1 mm in diameter that lacked turgor and colour were considered dead and excluded from the sample. Roots less than 1 mm in diameter were too small to identify if they were dead, so all of these extracted roots were considered living. Root samples were extracted until it became difficult to separate fine roots from organic matter, or for 1 hr. The high organic matter content of reclaimed soils made it almost impossible to separate very fine roots from peat, and most of the reclaimed samples took the full hour to sort. Therefore, it is likely that the very fine root biomass at reclaimed sites is an underestimate. Root samples were then washed and dried in a forced-air oven at 60°C, and then weighed. Any rocks greater than 2 mm were separated from the sample, washed, air-dried, and weighed. Bulk density was corrected for gravel and coarse fragments by using the following equation to determine fine bulk density (ρ_{bs}) (Page-Dumroese et al.,1999):

$$\text{fine bulk density } (\rho_{bs}) = \rho_{bT}\,(1 - gr)/(1 - vr)$$

where gr is the gravimetric rock fragment content determined by dividing the mass of the rock fragments by the total sample mass. The volumetric rock fragment content (vr) was determined using the following equation:

$$vr = \rho_{bT}\,(gr/\,\rho_{br})$$

where rock fragment density (ρ_{br}) is a constant assumed to be 2.65 mg m^{-3}. Root biomass results were expressed as tons per hectare using fine-soil bulk density and a sampling depth of 10 cm.

2.9 CEC and exchangeable cation concentrations

The analysis of cation exchange capacity and exchangeable cation concentrations of Ca, Mg, Na, K, Al and Mn for all soil samples was performed at the University of Montreal using the procedure described by Sumner et al. (1996). CEC was determined as the sum of concentrations of Ca, Mg, K, Na, Fe, Al, and Mn. This study focussed on Ca and Mg because these cations are thought to play an important role in aggregate stability (Muneer and Oades, 1989; Zhang and Norton, 2002).

2.10 Conductivity and pH (w)

Soil electrical conductivity and pH (water) were measured using a ratio of 1:10 (soil:water). Samples were shaken for 30 minutes and left to sit for 4 hours. A Denver UB-10 instrument was used to measure pH. A radio Copenhagen CDM conductivity meter was used to measure electrical conductivity.

2.11 Statistical analysis

All statistical data analysis was performed using R (R Core Team, 2014). Measurements from two of the three subplot within a site were averaged to calculate total SOC and soil carbon fractions, aggregate size distribution and stability, root biomass distribution, and bulk density, as described earlier. Composite samples from the three subplots at each site were used to measure microbial biomass, CEC, exchangeable cations, conductivity, and pH. Each of the five sites within a treatment were then averaged to compare soil properties between treatments. One-way analysis of variance (ANOVA) was performed to determine whether treatments were significantly different from one another (n=5). All data met assumptions of normality, or were transformed to ensure that assumptions were met. If assumptions of normality were not met after transformations were applied, non-parametric Kruskal-Wallis rank sum tests were used to compare treatment means and the use of non-parametric tests was noted. Tukey HSD tests were used to compare differences among treatments. A p-value less than or equal to 0.10 was considered significant due to the high spatial variability in forest and grassland ecosystems. Student’s t-tests were used to test whether the mean calculated C saturation capacities were significantly different from S&C-associated carbon concentrations.

Redundancy analysis (RDA), a linear multivariate method, was used to explore relationships among selected response variables, explanatory variables, and sites. The first RDA explored how much of the variation in soil carbon pools could be explained by soil chemical, physical, and biological properties, and the second RDA explored relationships between aggregate size distributions and soil properties. All variables were assessed for normal distribution, and were transformed if they did not meet assumptions of normality prior to analysis. A forward step-wise discriminant analysis was used to test for collinearity among explanatory variables, and variables that were not contributing to the statistical power of the model were removed, thus maximizing the amount of variation in the response matrix that was explained by the explanatory variables. Permutation testing was used to test for significance of the model, and to test for significant axes and explanatory variables. Clustering of sites based on whether they were reclaimed and natural, or by vegetation type in the ordination space indicated that vegetation type was influencing soil properties.

3 RESULTS

3.1 Soil carbon pools were greater at reclaimed sites, and carbon distribution in soil fractions differed between reclaimed and natural sites

3.1.1 Soil organic carbon (SOC)

Concentrations of SOC were significantly greater at reclaimed sites than natural sites (Figure 2), ranging from 29.0 to 152.5 g C kg^{-1} at reclaimed sites, and 3.4 to 21.3 g C kg^{-1} at natural sites. Soil organic carbon content was also significantly greater at reclaimed sites, with an average of three times as much SOC in the top 10 cm per hectare as natural sites (Figure 3). Concentrations of SOC in reclaimed soils were not significantly affected by vegetation type, although deciduous sites had the greatest mean SOC content, and grassland and coniferous sites had similar SOC content. However, when soil bulk density was taken into account, grassland sites had significantly greater SOC content than coniferous sites (Figure 3). The variability in SOC concentrations at deciduous sites was high (40.9 – 152.5 g C kg^{-1}), while the variability in SOC content at deciduous sites was similar to the other reclaimed vegetation treatments, suggesting that differences in bulk densities between sites were contributing to the variation in SOC concentrations at deciduous sites.

3.1.2 SOC distribution within soil physical fractions

On average, reclaimed sites had more SOC than natural sites in all three soil fractions (Figure 4). Light fraction carbon concentrations were significantly greater at reclaimed sites than at natural sites. Interestingly, LF C was the largest C pool at reclaimed sites, and the smallest C pool at natural sites. Reclaimed site LF C concentrations ranged from 7.8 to 142.2 g kg^{-1}, while natural site LF C concentrations ranged from 0.3 to 1.7 g kg^{-1}. Mean POM C tended to be greater at reclaimed sites than at natural sites, and grasslands were significantly greater. Physically protected C pools ranged from 7.7 to 25.8 g kg^{-1} at reclaimed sites and 2.0 to 11.2 g kg^{-1} at natural sites. Mean chemically protected C was significantly greater in both grassland and coniferous sites, compared to natural sites. Deciduous sites did not differ from natural sites in chemically or physically protected C. Chemically protected C pools at reclaimed sites ranged from 2.0 to 30.0 g S&C C kg^{-1}, and 1.1 to 8.5 g S&C C kg^{-1} at natural sites. Few differences were found among reclaimed vegetation treatments in any of the three soil fractions, although grassland sites had greater physically protected C than coniferous sites, and deciduous sites had three times as much mean LF C concentrations as coniferous and grassland sites (not significant).

SOC content in the three soil fractions displayed similar trends as SOC concentrations between reclaimed and natural sites, although differences in reclaimed vegetation treatments were more distinct in some cases (Table 2). Grasslands had significantly greater SOC content in chemically and physically protected pools than deciduous sites, and the difference in LF C content between deciduous sites versus grassland and coniferous sites was smaller. The assumptions of normality for the LF C content data could not be met using transformations, so a non-parametric Kruskal-Wallis rank sum test was used to compare treatment means.

When SOC content in each soil fraction was considered proportional to total SOC, it became clear that C was distributed differently at reclaimed sites compared to natural sites (Figure 5). Reclaimed sites had significantly more C stored in the LF pool and significantly less C stored in the POM pool. Silt- and clay-associated C relative to total SOC was only significantly smaller at deciduous sites compared to natural sites, while grassland and coniferous sites were not significantly different than natural sites. Between 86 – 95 % of SOC at natural sites was either chemically or physically protected from decomposition, while 6 – 83 % of SOC at reclaimed sites was in protected pools. Within reclaimed treatments, significantly more SOC was found in the LF C pool at deciduous sites compared to grassland sites, and deciduous sites also tended to have more LF C than coniferous sites. No other differences between reclamation treatments were found.

3.1.3 Carbon saturation capacity

Because soil texture did not significantly differ between vegetation types, the C saturation capacity levels, which are based on clay and silt content, were not significantly different. Capacity levels ranged from 15.5 g C kg^{-1} to 23.3 g C kg^{-1} (Figure 6). At reclaimed sites, the S&C-associated carbon was not significantly different from the estimated saturation capacities, signifying that there are no available clay surfaces left for interactions with OM. In contrast, natural sites were significantly below saturation capacity, with the S&C fraction saturated at only 21 % of the capacity level. While saturation capacity was reached across all reclaimed sites, grassland sites in particular showed signs of surpassing C saturation, which is surprising if the C saturation theory is accurate (Hassink, 1997; Carter et al., 2003). Thus, natural sites have the potential to accumulate 79 % more OC within the strongly protected S&C pool before saturation is reached, whereas reclaimed sites appear to have minimal potential to accumulate additional OC in this pool.

3.1.4 Extractable microbial biomass carbon (MBC) and MBC:SOC

Data from two sites were eliminated from MBC analysis because the C concentration was so low that a lab fumigation error was suspected (G4), and because the MBC:SOC ratio was greater by 20 % than all other sites (NS2). Reclaimed sites had greater mean MBC concentrations than natural sites, although only the deciduous mean was significantly greater (Table 3). MBC ranged from 0.57 to 2.84 g kg^{-1} at reclaimed sites, and from 0.23 to 0.73 g kg^{-1} at natural sites. MBC content did not significantly differ between reclaimed and natural sites, and ranged from 0.3 to 1.3 t ha^{-1} across all sites (Table 3). Reclaimed vegetation treatments had very similar mean microbial biomass concentrations and contents. When MBC content was compared to total SOC content at each site, reclaimed sites had a narrower

MBC:SOC ratio than natural sites, and deciduous and grassland sites had a significantly narrower MBC:SOC (Table 3). MBC made up 1 – 3 % of total OC content at reclaimed sites, and 1 – 5 % of total OC at natural sites. Reclaimed vegetation treatments were not significantly different, although coniferous sites tended to have more microbial biomass, relative to total SOC content, than deciduous or grassland sites.

3.1.5 Total % carbon, nitrogen, and C:N ratios of bulk soil, biochemically resistant organic matter, and labile organic matter

Reclaimed sites had significantly more mean total % C and % N than natural sites, although C:N ratios were similar (Figure 7). Total C was 4 – 17 % of soil dry weight in reclaimed sites, and 0.6 – 1.9 % of soil dry weight in natural sites. Total N was 0.18 – 0.89 % of soil dry weight in reclaimed sites, and 0.02 – 0.06 % in natural soils. Within reclaimed vegetation types, deciduous sites had the highest mean % C, and had significantly more than grassland sites, although similar to SOC concentrations, this difference may be a result of different soil bulk densities between grassland and deciduous sites, which is not taken into account with total % carbon and nitrogen. Coniferous and grassland sites were not significantly different. Deciduous sites also had the highest mean total % N, but there were no significant differences in mean total N among reclaimed treatments. C:N ratios ranged from 12 to 29 across all sites.

Carbon and nitrogen that remain in the soil after oxidation is characterized as oxidation-resistant OM that is biochemically protected from decomposition (Mikutta et al., 2005). After chemical oxidation, % C dropped to 0.8 – 2.4 % at reclaimed sites, and 0.04 – 0.11 % at natural sites. Nitrogen dropped to 0.03 – 0.07 % at reclaimed sites, and 0.01 – 0.02 % at natural sites. Similar to total soil C and N, reclaimed sites had significantly higher mean % C and % N than natural sites after oxidation. Unlike untreated soils, the mean C:N ratio of this oxidation-resistant material was significantly greater at reclaimed sites than at natural sites. On average, reclaimed sites lost 72 – 80 % of total C and 82 – 87 % of total N during oxidation, while natural sites lost 90 % of total C and only 63 % of total N. At reclaimed sites, C:N ratios increased to 18 – 55, while at natural sites, C:N dropped to 2 – 8. Within reclamation treatments, % C, % N, and C:N ratios after oxidation were similar in all three vegetation treatments.

Organic matter that was oxidized is associated with a more active, labile organic matter pool (Mikutta et al., 2005). During oxidation, reclaimed sites lost more organic matter than natural sites, although only deciduous and grassland sites lost significantly more OM (as a % of sample dry weight) (Figure 8). Reclaimed sites lost 9 – 28 % of the sample weight, while natural sites lost 4 – 11 % of the sample weight. C:N ratios of oxidizable OM were lower at reclaimed sites than at natural sites, although coniferous sites were not significantly lower. Ratios ranged from 15 to 24 at reclaimed sites, and 21 to 48 at natural sites. Deciduous sites had the highest mean % loss, and lost significantly more than coniferous sites. Grassland sites were not significantly different from any reclaimed treatment. The C:N ratios of this active organic matter pool more closely resembled the C:N ratios of vegetation inputs. Coniferous sites had the highest mean C:N ratio, which was significantly higher than C:N at deciduous sites. Grassland sites were not significant different from either coniferous or deciduous sites in C:N ratios.

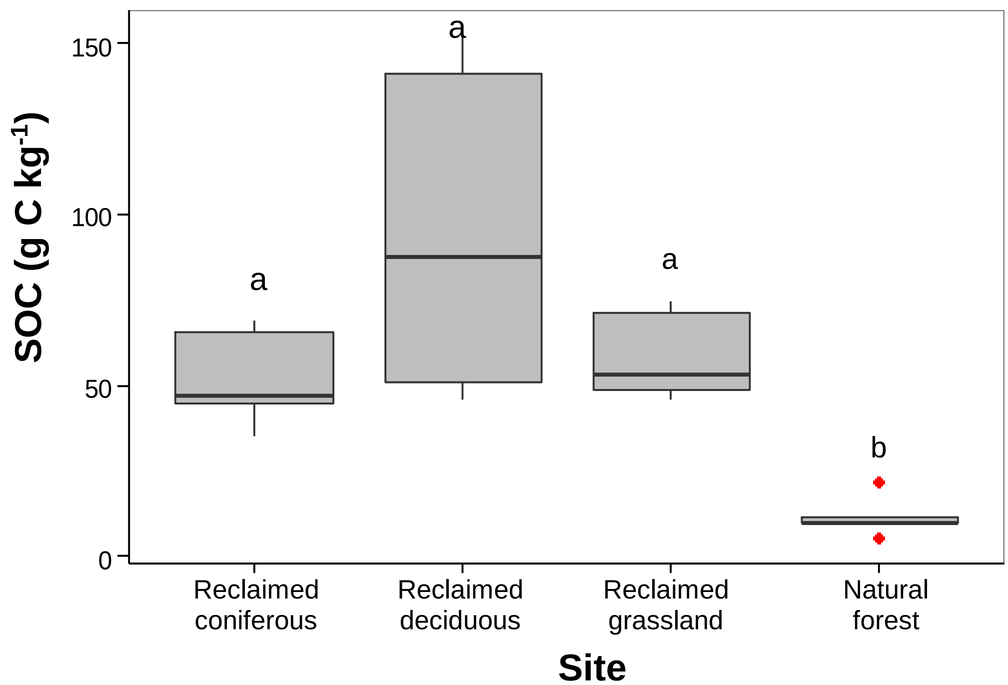

Figure 2. Mean SOC concentrations at reclaimed and natural sites (n=5). Different letters denote significant differences at $p < 0.10$ between vegetation types. Outliers are represented by red points.

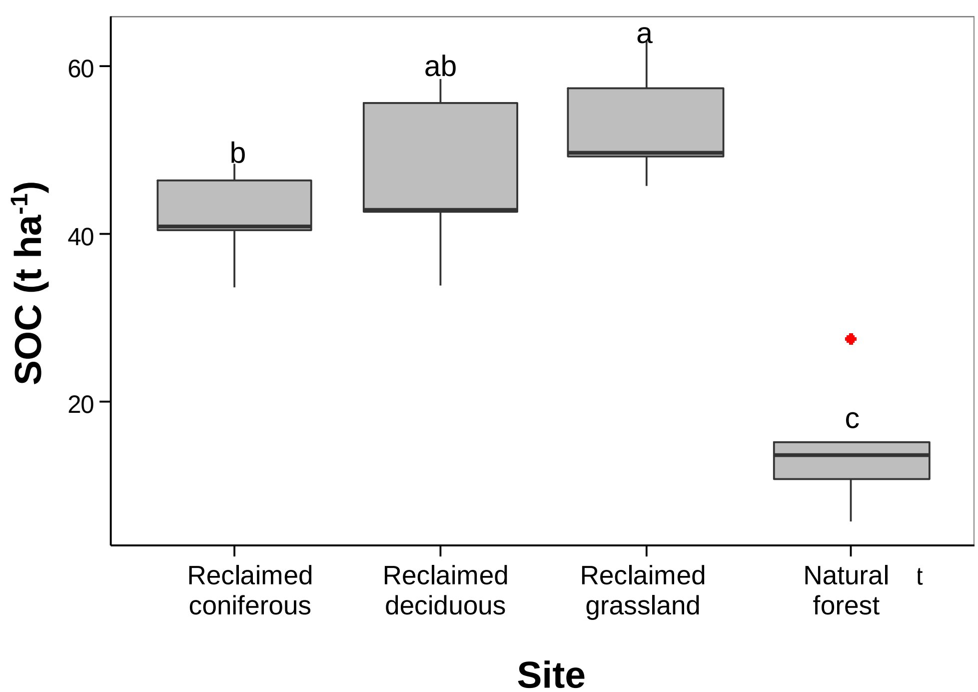

Figure 3: Mean SOC content at reclaimed and natural sites (n=5). Different letters denote significant differences at $p < 0.10$ between vegetation types. Outliers are represented by red points.

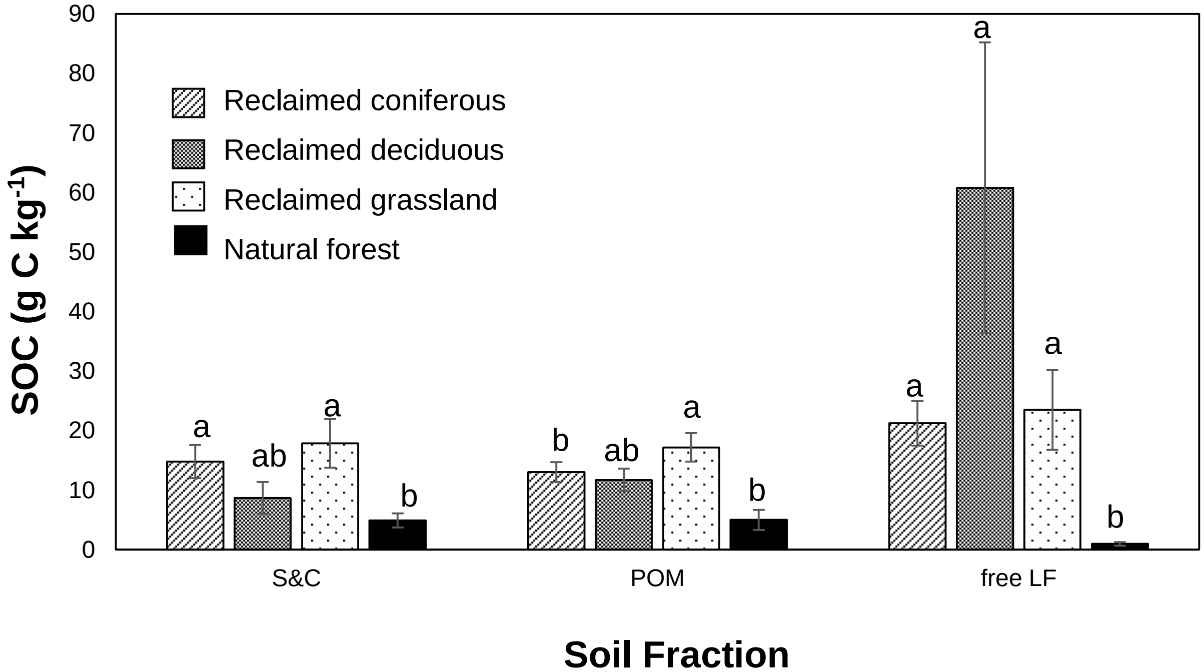

Figure 4. Mean C concentrations in three fractions of soil organic carbon (SOC) at reclaimed and natural sites (n=5). Different letters denote significant differences at p < 0.10 between vegetation types. Error bars represent standard error of the mean. S&C = chemically protected OC; POM = physically protected OC; free LF = free light-fraction OC.

Table 2. Mean soil organic carbon (SOC) content in three soil fractions at reclaimed and natural sites (n=5). Different letters denote significant differences at p< 0.10 between vegetation types. S&C = chemically protected OC; POM = physically protected OC; free LF = free light-fraction OC.

Treatment	**S&C**	**POM**	**free LF**
	$t\ ha^{-1}$	$t\ ha^{-1}$	$t\ ha^{-1}$
Reclaimed coniferous	13.0 ± 2.55 ab	11.2 ± 0.76 ab	17.8 ± 1.6 a
Reclaimed deciduous	6.7 ± 2.44 b	8.2 ± 2.06 b	31.7 ± 8.61 a
Reclaimed grassland	17.1 ± 4.41 a	17.1 ± 4.14 a	20.3 ± 4.49 a
Natural forest	6.6 ± 1.50 b	6.6 ± 1.50 b	1.3 ± 0.36 b

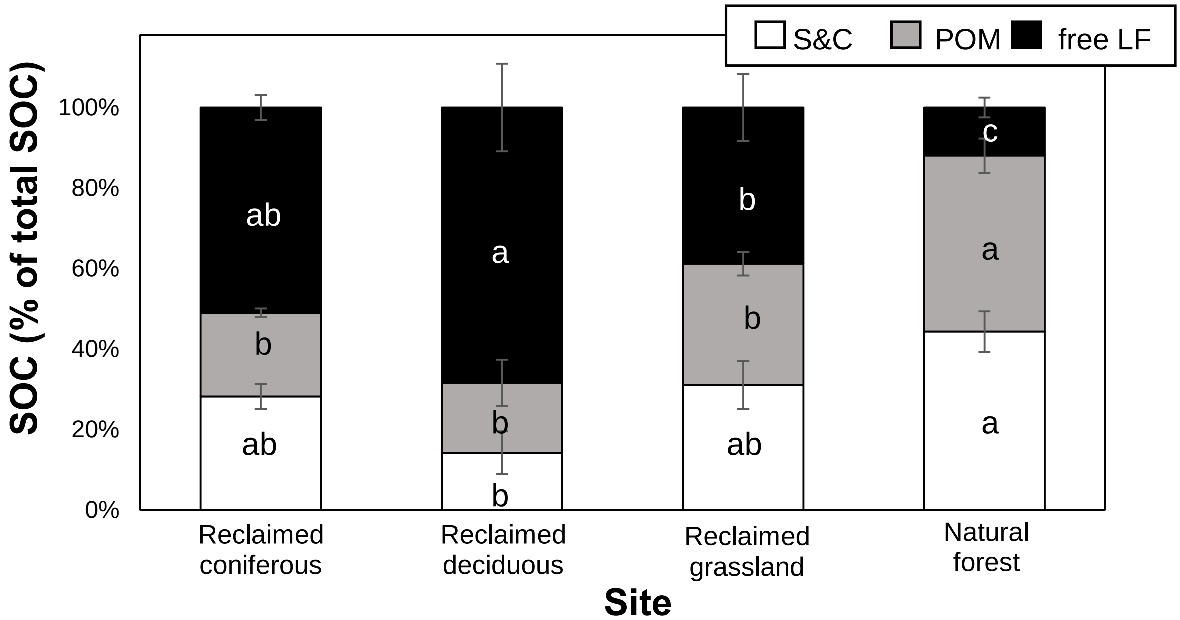

Figure 5. Contributions of each of the three soil fractions to total soil organic carbon (SOC) in reclaimed and natural sites (n=5). Different letters denote significant differences at $p < 0.10$. S&C = chemically protected OC; POM = physically protected OC; free LF = free light-fraction OC.

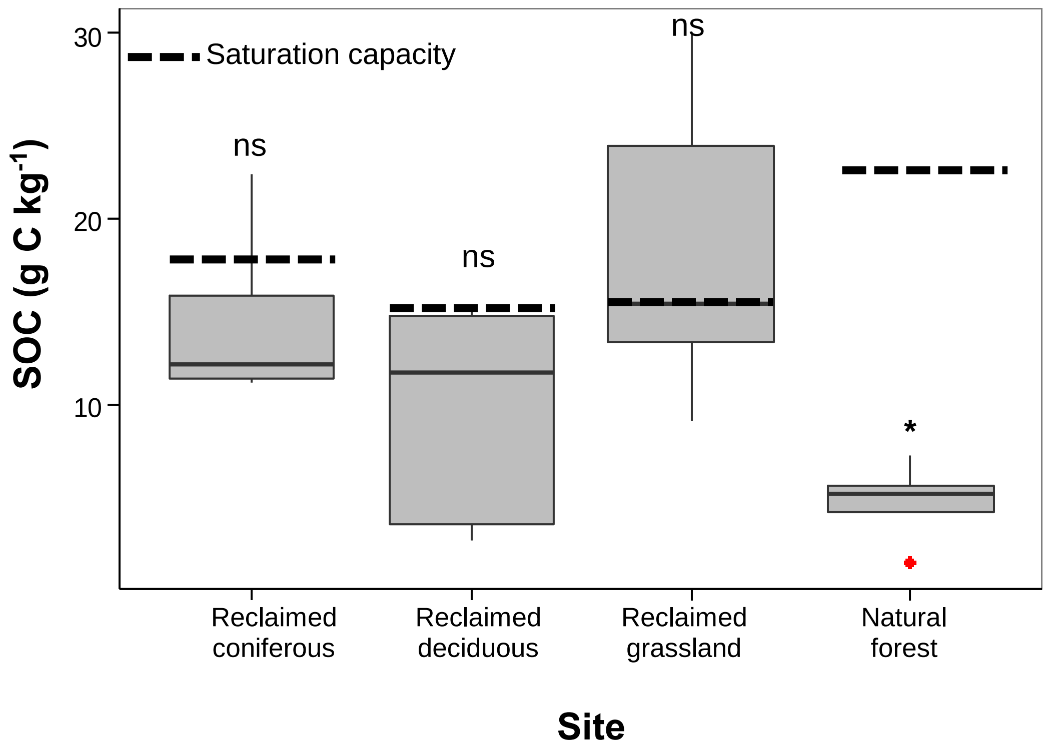

Figure 6. Silt- and clay-associated soil organic carbon (SOC) and calculated C saturation levels at reclaimed and natural sites. NS denotes no significance difference between SOC content and C saturation levels, and asterisk denotes significant differences at $p < 0.10$ according to Student's t-tests.

Table 3. Microbial biomass carbon (MBC) concentrations, content, and MBC:SOC ratios (based on contents) at reclaimed and natural sites (n=5,5,4,4). Means and standard errors; different letters denote significant differences at $p < 0.10$ between vegetation types according to one-way ANOVA.

Treatment	MBC concentrations	MBC content	MBC:SOC (contents)
	g C kg^{-1}	t ha^{-1}	%
Reclaimed coniferous	1.3 ± 0.17 ab	1.1 ± 0.08 a	2.7 ± 0.20 ab
Reclaimed deciduous	1.5 ± 0.38 a	0.9 ± 0.11 a	1.9 ± 0.21 b
Reclaimed grassland	1.1 ± 0.11 ab	1.0 ± 0.14 a	1.6 ± 0.49 b
Natural forest	0.4 ± 0.10 b	0.8 ± 0.23 a	3.5 ± 0.55 a

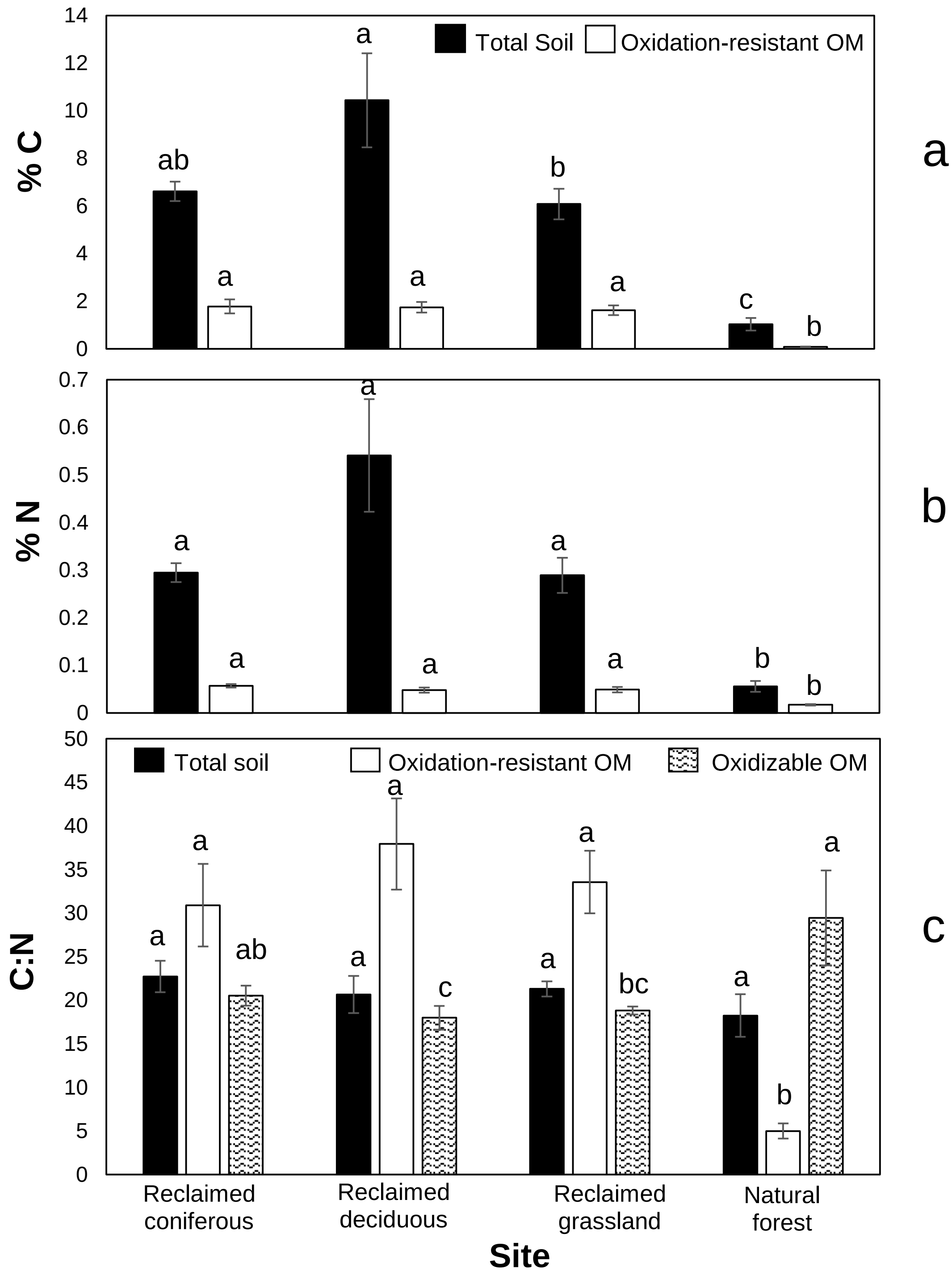

Figure 7. Mean % carbon (a), and % nitrogen (b) of untreated soil and soil treated with 6% NaOCl at reclaimed and natural sites (n=5). Mean C:N ratios (c) of total soil, oxidation-resistant soil, and oxidizable organic matter (OM) at reclaimed and natural sites. Letters denote significant differences between vegetation types at $p < 0.10$. Error bars represent standard error of the mean.

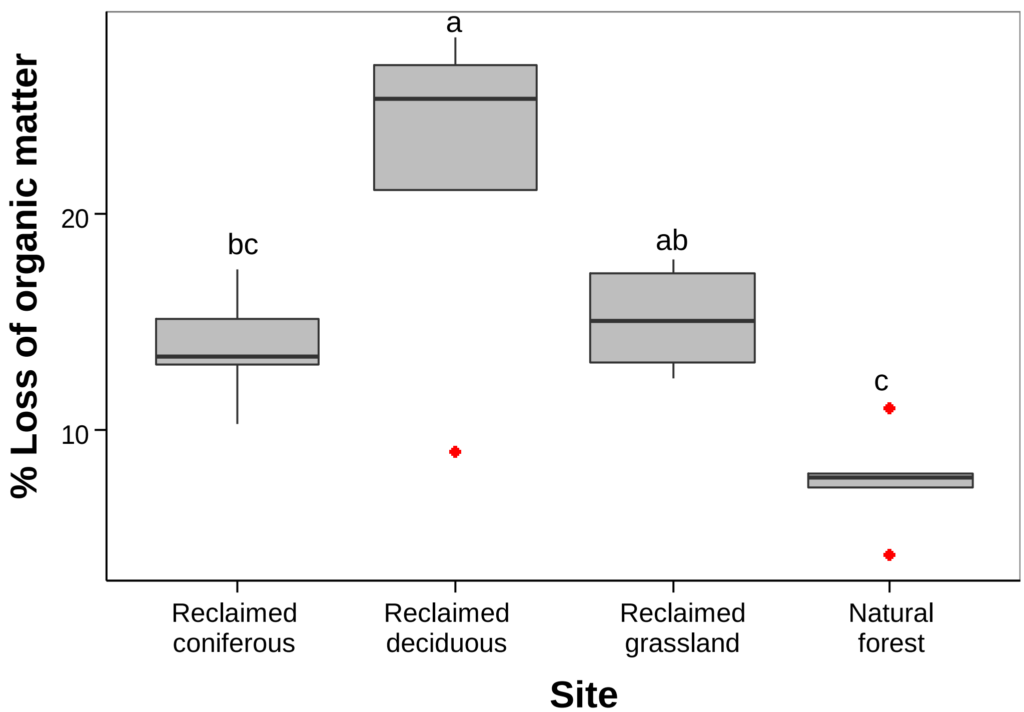

Figure 8. Mass lost from reclaimed and natural soils after treatment with 6% NaOCl (n=5). Different letters denote significant differences between vegetation types at $p < 0.10$. Red points represent outliers in the data.

3.2 Soil physical properties were similar at reclaimed and natural sites

3.2.1 Soil texture and bulk density

Soil texture was not significantly different between reclaimed and natural sites (Table 4). Soils at reclaimed sites were 53 – 76 % sand, 22 – 43 % silt, and 1 – 3 % clay. Likewise, there were no significant differences in soil texture among vegetation treatments. Ten of the fifteen reclaimed sites were classified as sandy loams, whereas the other five (two deciduous sites and three grassland sites) were loamy sands. Natural sites were more variable in texture, ranging from 6 to 88 % sand, 9 to 88 % silt, and 0.4 to 7 % clay. Natural sites also fell into a greater range of texture classes, and only NS3 and NS4 were in the same class. The three sites with luvisolic soils were fine-textured, and fell within silt and silty loam texture classes. The two sites identified as brunisolic soils were more coarse-textured and fell within loamy sand and sand texture classes. Unlike soil texture, fine bulk density was significantly lower at reclaimed sites compared to natural sites (Figure 9). Bulk density was 0.4 – 1.2 g cm^{-3} at reclaimed sites, and 1.2 – 1.7 g cm^{-3} at natural sites. Deciduous sites tended to have the lowest mean bulk density values, but no significant differences were found among reclamation treatments.

3.2.2 Aggregate stability

Mean weight diameters (MWD) ranged from 1.9 mm to 2.8 mm, and were not significantly different between reclaimed and natural sites (Figure 10). Likewise, no significant differences were found among reclaimed vegetation treatments, although grassland sites had the lowest mean aggregate stability and coniferous sites had the highest mean stability.

3.2.3 Aggregate size distribution

Data from one natural site was excluded from aggregate analysis because its textural class was identified as silt. As a result, the silt- and clay-sized fraction at this site was greater than any other natural or reclaimed site by an order of magnitude, and skewed the distribution of aggregate size classes at that site. Aggregate size distributions did not differ significantly between reclaimed and natural sites (Figure 11). On average, the soils all had 40 % small macroaggregates (250 – 2000 µm) and 30 % large macroaggregates (> 2000 µm), except for reclaimed coniferous sites, in which the reverse was true. Microaggregate (53 – 250 µm) comprised 14 – 24 % of soil dry weight, and the mean silt- and clay-sized soil fraction (<53 µm) was less than 6 % at all sites. There were also no significant differences among reclaimed vegetation treatments, although grassland sites had a higher average proportion of microaggregates and as mentioned, macroaggregate distribution at coniferous sites differed slightly from the other treatments.

Table 4. Percentages of clay, silt and sand at reclaimed and natural sites (n=5). Means and standard errors are shown; different letters denote significant differences ($p < 0.10$).

Treatment	% Clay	% Silt	% Sand
Reclaimed coniferous	2.6 ± 0.23 a	35.0 ± 2.77 a	62.3 ± 2.70 a
Reclaimed deciduous	1.9 ± 0.40 a	28.8 ± 1.56 a	69.3 ± 1.76 a
Reclaimed grassland	1.6 ± 0.22 a	29.9 ± 4.37 a	68.1 ± 4.46 a
Natural forest	3.5 ± 1.21 a	48.4 ± 13.81 a	47.5 ± 14.62 a

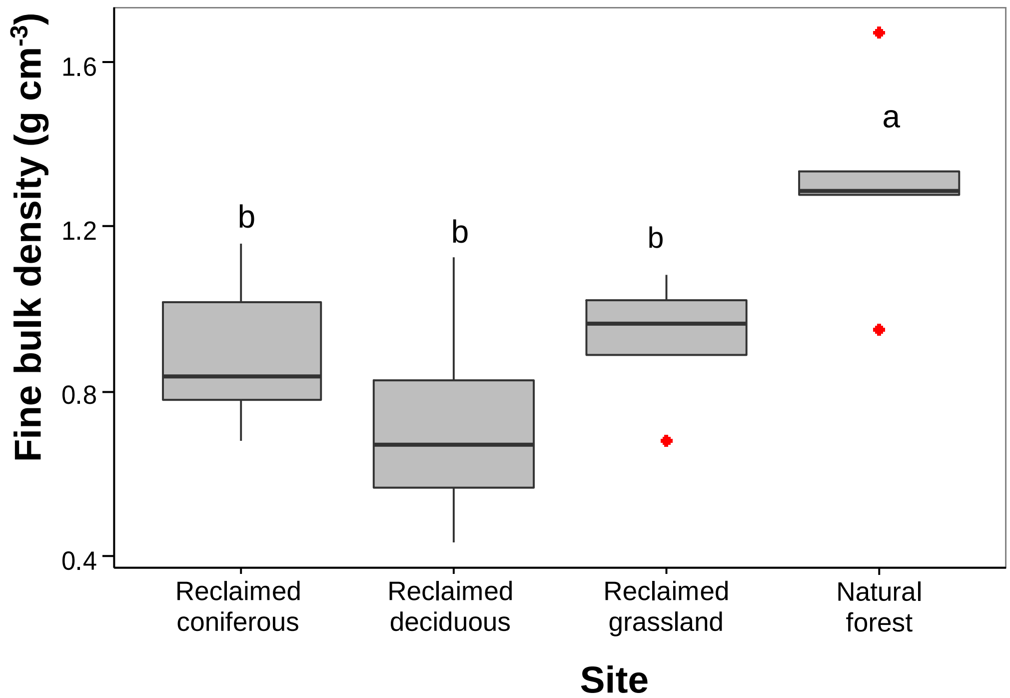

Figure 9. Fine bulk density of soil at reclaimed and natural sites (n=5). Different letters denote significant differences at $p < 0.10$ between vegetation types. Outliers are represented by red points.

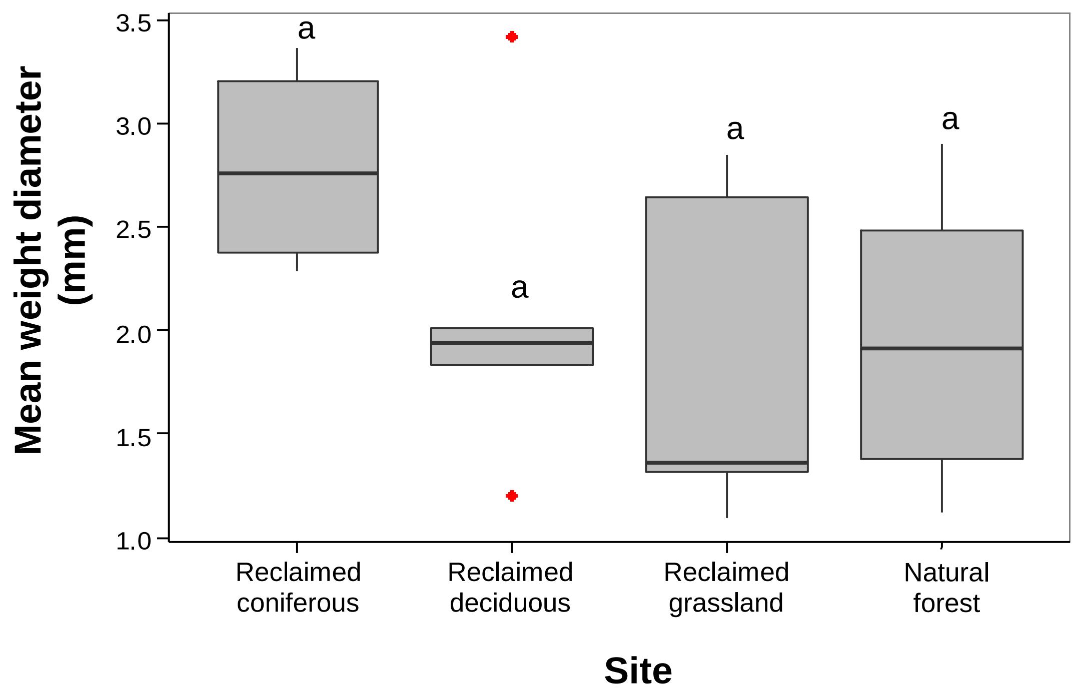

Figure 10. Mean weight diameter of soil at reclaimed and natural sites (n=5,5,5,4). Different letters denote significant differences at $p < 0.10$ between vegetation types. Outliers are represented by red points.

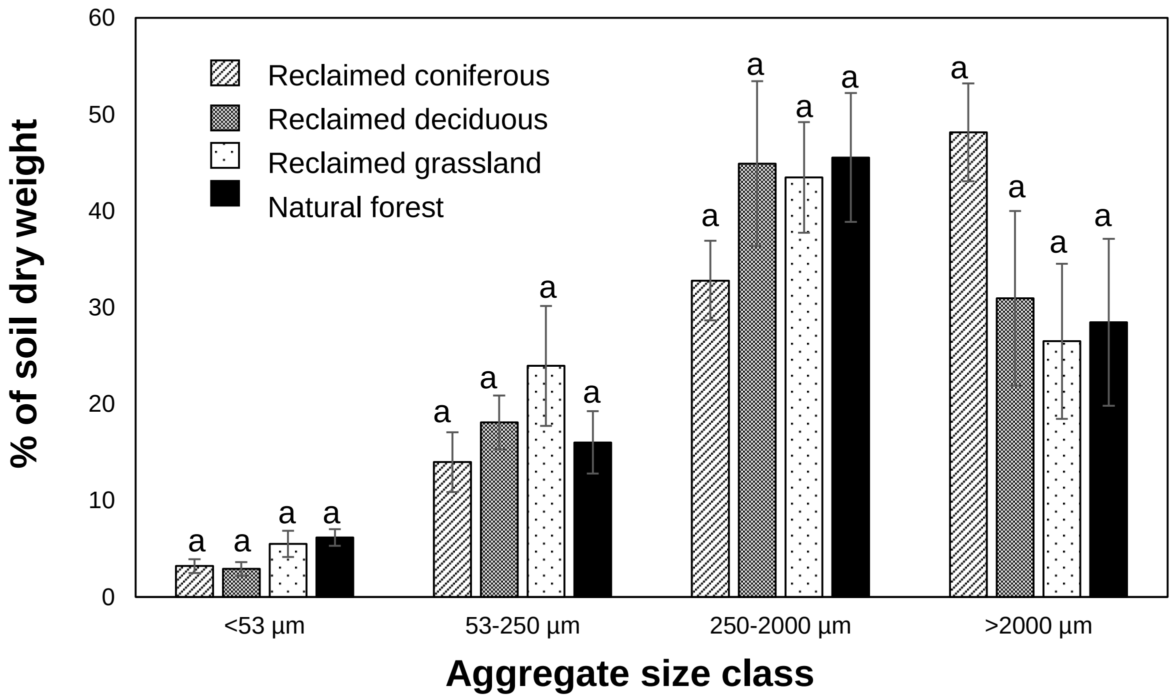

Figure 11. Mean aggregate size distribution, expressed as % of soil dry weight, at reclaimed and natural sites (n=5,5,5,4). Different letters denote significant differences at $p < 0.10$ between vegetation types. Error bars represent standard error.

3.3 Rooting activity and surface organic layer development were similar among most reclaimed and natural sites

3.3.1 Total root biomass and size distribution

Total root biomass was lower at reclaimed grassland sites than at natural sites, but coniferous and deciduous sites were not different from natural sites (Figure 12). Total root biomass was 2.3 – 10.5 t ha^{-1} at reclaimed sites, and 2.7 – 6.8 t ha^{-1} at natural sites. Among the reclaimed treatments, mean root biomass in grassland soils was significantly lower than deciduous and coniferous sites. At treed sites, the distance from the soil pit to the closest tree was measured, but no correlation was found between total root biomass and distance to the closest tree (data not shown, $r^2 < 0.20$). Therefore, total root biomass at treed sites was likely influenced by both tree roots and understory vegetation roots.

There were no significant differences in biomass of any root size class between reclaimed and natural sites, except that very-fine (< 1 mm-diameter) root biomass was greater at deciduous sites than at natural sites (Figure 13). Very-fine root biomass ranged from 0.5 to 4.1 T ha^{-1} across all sites; fine-root biomass (1 – 2 mm) was 0.1 – 1.6 T ha^{-1}; medium-root biomass (2 – 5 mm-diameter) was 0.03 – 2.8 T ha^{-1}, and coarse-root biomass (> 5 mm) was 0 – 5.3 T ha^{-1}. Reclaimed vegetation treatments did not significantly differ in size distribution of roots, although coniferous sites had greater mean medium-root biomass than grassland sites at $p < 0.10$. Deciduous sites tended to have the most very-fine root biomass and coniferous sites tended to have the most coarse-root biomass. As expected, there were few coarse roots at grassland sites. Coarse roots were infrequently found at all sites and tended to be heavy; as a result, coarse-root data were highly variable and could not be transformed to meet assumptions of normality. A Kruskal-Wallis test was therefore used to compare site means, and the conservative nature of this test may explain why no significant differences in coarse-root biomass were found between grasslands and the other vegetation types. There was also considerable heterogeneity among sites within a vegetation type, which may explain the lack of significant differences among vegetation types.

3.3.2 Surface organic horizon thickness

Surface organic horizon thickness did not significantly differ between reclaimed and natural sites, except at coniferous sites, where the organic horizons were significantly thinner than at natural sites (Figure 14). At reclaimed sites, organic horizons were 1.8 – 6.3 cm thick, while natural sites had litter layers 2.4 – 16.7 cm thick. Organic horizon thicknesses at reclaimed sites were not significantly different from one another, although coniferous sites tended to have slightly thinner horizons than the other reclaimed treatments, and more bare surface. Detailed descriptions of forest-floor classification and macrofaunal activity at all sites can be found in Anderson (2014).

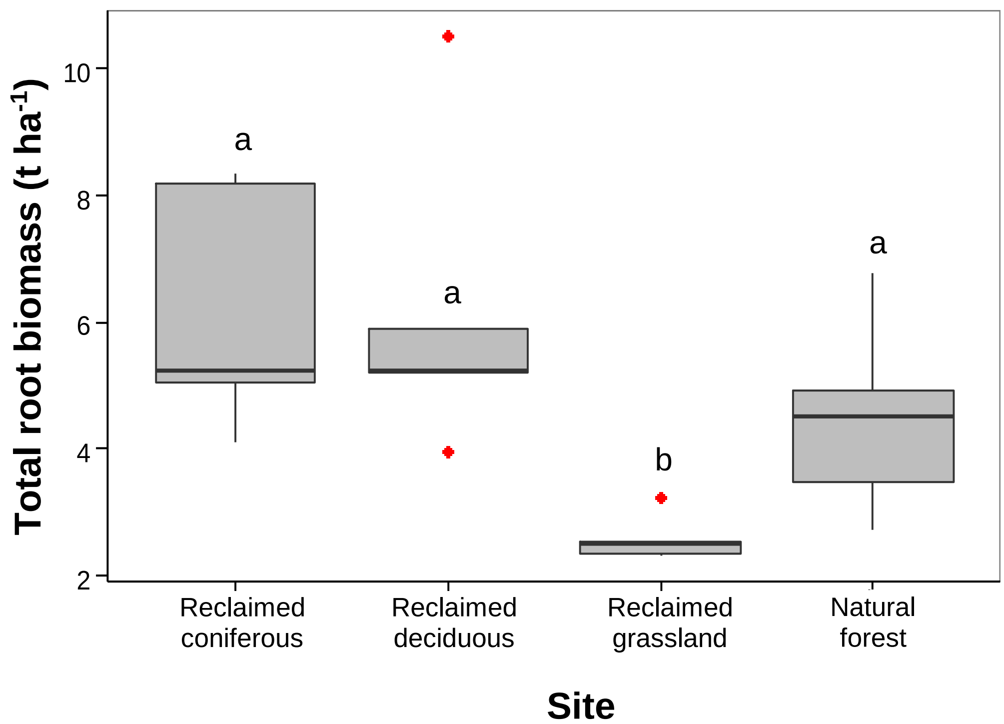

Figure 12. Total root biomass in the top 10 cm of mineral soil or reclaimed soil substrate at reclaimed and natural sites (n=5). Different letters denote significant differences at $p < 0.10$ between vegetation types. Outliers are represented by red points.

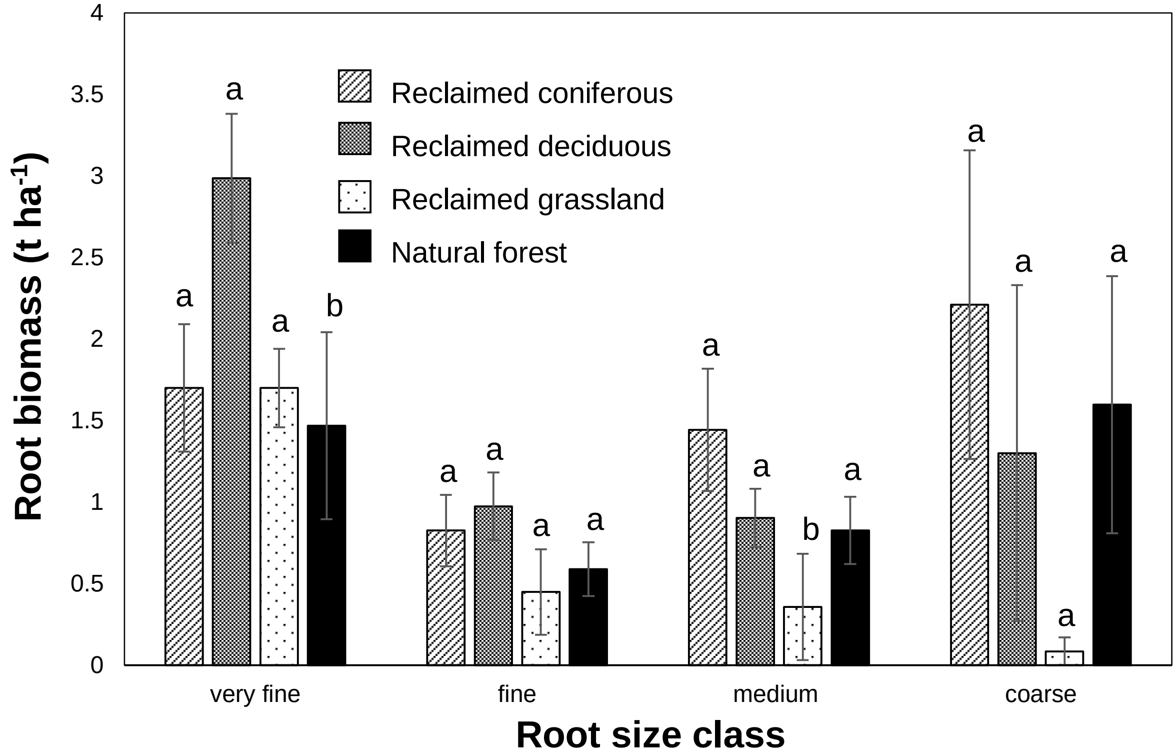

Figure 13. Root size distribution in the top 10 cm of mineral soil or reclaimed soil substrate, at reclaimed and natural sites (n=5). Different letters denote significant differences at $p < 0.10$ between vegetation types. Error bars represent standard error.

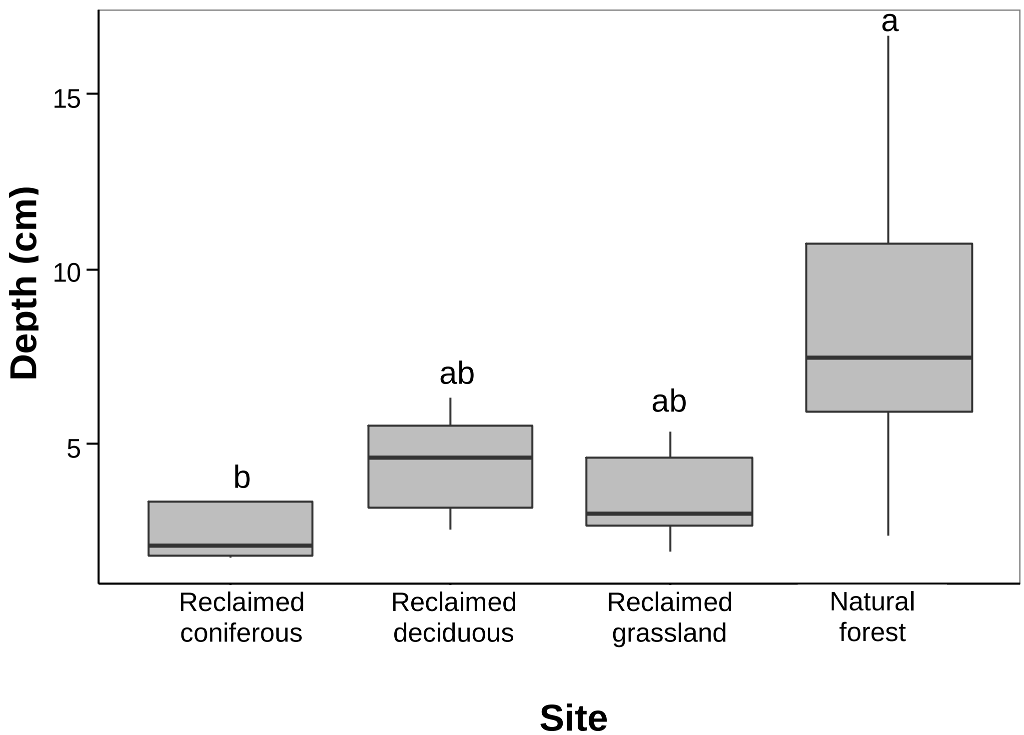

Figure 14. Surface organic horizons depth at reclaimed and natural sites (n=5). Different letters denote significant differences at $p < 0.10$ between vegetation types according to one-way ANOVA.

3.4 Cation concentrations and conductivity were greater at reclaimed sites, while pH was only greater at grassland sites

3.4.1 Cation exchange capacity (CEC) and exchangeable cation concentrations

Cation exchange capacity, Ca and Mg concentrations were higher at reclaimed sites than at natural sites by an order of magnitude (Table 5). Reclaimed sites had a CEC range of 31.3 – 69.8 cmol (+) kg^{-1}, while natural sites ranged from 1.3 – 3.7 cmol (+) kg^{-1}. Calcium concentrations ranged from 26.5 – 62 cmol (+) kg^{-1} at reclaimed sites, and 0.5 – 1.2 cmol (+) kg^{-1} at natural sites. Mg concentrations ranged from 1.7 – 10.7 cmol (+) kg^{-1} at reclaimed sites, compared to 0.1 – 0.4 cmol (+) kg^{-1} at natural sites. Reclaimed vegetation treatments did not significantly differ in Ca and Mg concentrations, or in CEC (Table 5). Grassland sites tended to have the highest mean CEC and Ca concentrations, while coniferous sites tended to have the highest Mg concentrations. Potassium and Na were only present in very low concentrations (between 0.06 – 0.93 cmol (+) kg^{-1} across all sites), and were not significantly different between reclaimed and natural sites, or within reclaimed vegetation treatments.

3.4.2 Conductivity and pH

Electrical conductivity was significantly higher at reclaimed sites than at natural sites (Table 6). Conductivity ranged from 70 – 143 µs cm^{-1} at reclaimed sites, and 8 – 17 µs cm^{-1} at natural sites. Conductivity was not significantly different between reclaimed vegetation types. Mean pH values were only significantly higher at grassland sites compared to natural sites (Table 6). Grassland sites also had a significantly higher mean pH than deciduous sites (7.0 and 6.4 respectively). No differences in pH were found between coniferous and deciduous sites. Across all sites, pH ranged from 5.8 – 7.1.

Table 5. Means and standard errors of CEC, Ca and Mg concentrations, in cmol(+) kg^{-1}, at reclaimed and natural sites (n=5). Different letters denote significant differences at $p < 0.10$ between vegetation types according to one-way ANOVA.

Treatment	CEC	Ca	Mg
	cmol (+) kg^{-1}	cmol (+) kg^{-1}	cmol (+) kg^{-1}
Reclaimed coniferous	40.0 ± 5.77 a	34.3 ± 9.25 a	5.3 ± 0.77 a
Reclaimed deciduous	48.1 ± 6.79 a	42.5 ± 14.11 a	4.9 ± 0.59 a
Reclaimed grassland	52.3 ± 2.86 a	47.5 ± 6.22 a	4.5 ± 0.53 a
Natural forest	2.63 ± 0.52 b	0.8 ± 0.33 b	0.24 ± 0.04 b

Table 6. Means and standard errors of conductivity and pH among vegetation types (n=5). Different letters denote significant differences at $p < 0.10$ between vegetation types according to one-way ANOVA.

Treatment	Conductivity	pH
	µS/cm	(water)
Reclaimed coniferous	102.7 ± 14.62 a	6.5 ± 0.09 ab
Reclaimed deciduous	108.6 ± 36.20 a	6.4 ± 0.15 b
Reclaimed grassland	107.2 ± 13.24 a	7.0 ± 0.14 a
Natural forest	14.4 ± 1.64 b	6.1 ± 0.14 b

3.5 Soil properties were important in explaining variation in soil carbon pools between reclaimed and natural sites, and among reclaimed vegetation treatments

3.5.1 Soil carbon pools and explanatory soil properties

Redundancy analysis was used to explore the relative importance of specific soil properties in explaining variation in carbon concentration distribution among different soil fractions (Figure 15). In this RDA model, the response variable matrix consisted of five different soil C pools measured in this study: chemically protected C, physically protected C, total SOC, MBC, and oxidation-resistant C (ORC). The environmental variables thought to play an important role in explaining variation in soil carbon pools at these sites were as follows: clay content, silt content, surface organic horizon depth, very-fine roots, total roots, microaggregates, small macroaggregates, pH, CEC, site age, and soil C:N ratios. Sites that were found to be outliers in any of the other data sets were excluded from this analysis in order to prevent these outlying points from skewing the data within the RDA. As a result, one coniferous site, one grassland site and one natural site were excluded (C33 due to high Ca and Mg concentrations; G4 NS5 was excluded due to inaccurate MBC data), leaving a total of 17 sites. Age, pH, and silt content were excluded after discriminant analysis revealed that these variables were not contributing to a greater adjusted R^2. Permutation testing indicated that the relationship between explanatory variables and response variables was highly significant (999 permutations, $p=0.002$). Using the same permutation testing, the first three axes were significant ($p=0.001$, $p=0.001$, and $p=0.003$ respectively), and all explanatory variables were significant, except for very-fine roots and soil C:N ratios.

Scaling 2 was selected as a visual display of the data to further explore the correlations between variables. The first axis explained 48.31 % of the total variation in the data. CEC, surface organic horizon depth, and % clay content, which were all closely correlated with the first axis, and played an important role in separating reclaimed sites from natural sites along this axis. These explanatory variables were also closely correlated with ORC and total SOC pools, which were both greater at reclaimed sites than at natural sites. The second axis explained 21.62 % of the total variation in the data, most of which was variation in soil C pools among reclaimed sites. While some overlap among the three reclaimed vegetation types was apparent, reclaimed deciduous and grassland sites showed greater overall variation than the reclaimed coniferous sites, which clustered in the centre. Deciduous and grassland sites were both positioned along the second axis, but dispersed in opposite directions, indicating that different explanatory variables were responsible for variations in carbon distribution between these vegetation types. Total root biomass, small macroaggregates, very-fine roots and microaggregates were positively correlated with the distribution of deciduous sites along the second axis, while C:N ratios were positively correlated with grassland sites.

3.5.2 Aggregate size classes and explanatory soil properties

Redundancy analysis was also used to explore the relative importance of specific soil properties in explaining variation in aggregate size-class distribution (Figure 16). A response variable matrix, consisting of the proportional weight of microaggregates, small macroaggregates, and large macroaggregates at each site, was created. To prevent the variables in this matrix from being directly related to one another (eg. adding up to a total of 100 % of soil dry weight), the silt-and clay-sized fraction was removed as this fraction does not contribute to soil structure. Variables in the second matrix were chosen based on hypotheses generated from literature concerning important soil properties that drive soil structure formation, and also were measured in this study. The variables selected for analysis were SOC, MBC, very-fine root biomass, surface organic horizon depth, % clay, % silt, and Ca and Mg concentrations. Again, only 17 sites were included in this analysis.

Very-fine root biomass, % silt and surface organic horizon depth were removed to increase the adjusted R^2 of the model. Significance of the model was tested using a permutation test (999 permutations), and it revealed that the model was significant (p=0.036). The first axis explained 40.59 % of the total variation of the data, and together, the first two axes explained 52.21 %. Only the first two axes were significant (p=0.003 and p=0.045 respectively). Figure 16 illustrates the correlation bi-plot that resulted from this analysis, in which the angle between response and explanatory variables reflects their correlation to each other, and to the RDA axes. The correlation bi-plot (scaling 2) was chosen over the distance bi-plot (scaling 1) because very little clustering occurred among sites, so the relative distances between sites did not provide any new insights on why different aggregate size classes were occurring. Clay, Ca, and SOC were significant explanatory variables (p= 0.001, p = 0.081 and p = 0.085 respectively). Microaggregates were associated with the first axis, while large macroaggregates and small macroaggregates were more closely related to the second axis, although they were inversely related to each other.

Based on the direction of the explanatory variable vectors, none of the explanatory variables was strongly correlated with axis 1 or 2 specifically. Each variable probably explains some of the variation in both axes. Microbial biomass C was most closely related to RDA 1 and was strongly correlated with small macroaggregates, while being inversely related to large macroaggregates. Soil organic C was also correlated with small macroaggregates, and was inversely related to % clay content. Magnesium was closer to the second axis, and is probably most correlated with the variation in microaggegates. Calcium and microaggregates were also closely related, indicating that calcium and magnesium may play an important role in microaggregate abundances among sites. Large macroaggregates were not strongly correlated with any of the explanatory variables in this analysis, although this variable was closely correlated with RDA 1, indicating that a combination of variables could likely explain the variance in this response variable. The lack of clustering by vegetation type indicates that the vegetation prescription at reclaimed sites did not strongly influence aggregate development.

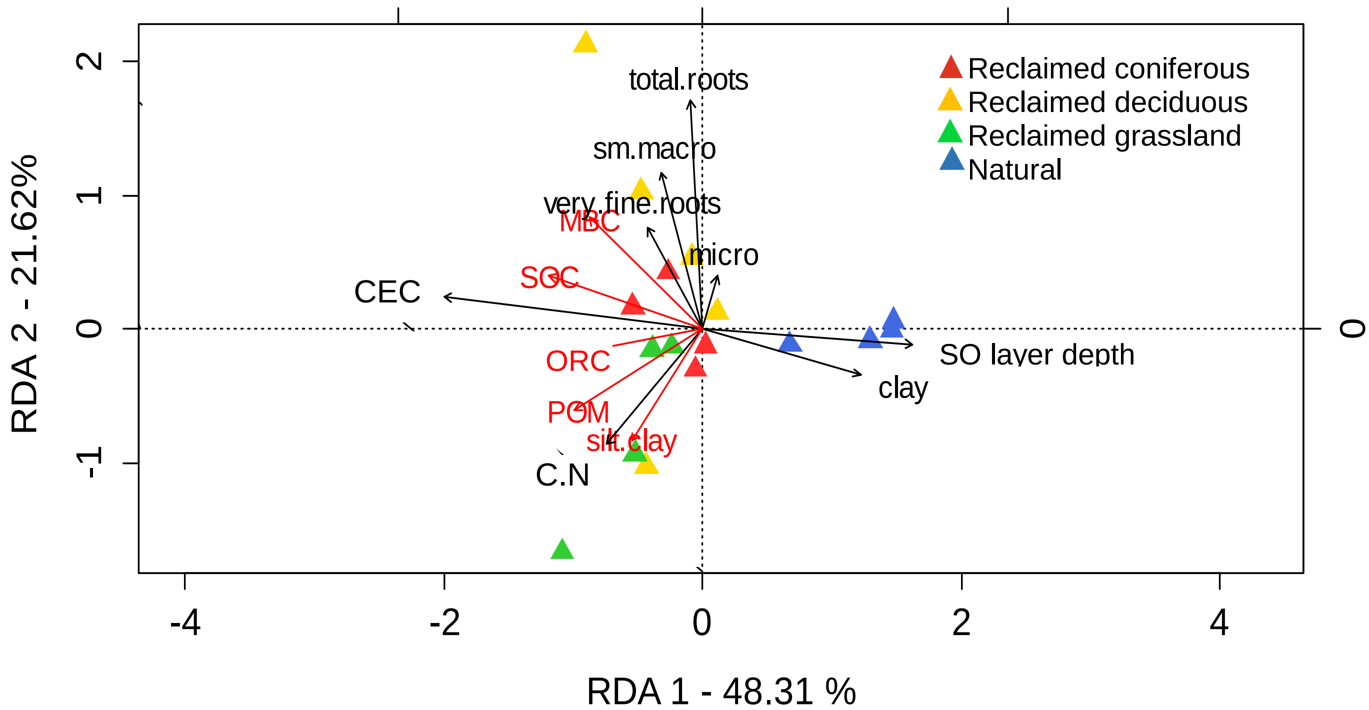

Figure 15. Redundancy analysis (RDA) testing the importance of soil properties (in black) in explaining variation in soil carbon concentrations (in red) across 17 sites. Explanatory variables explained significant variation in the response variables ($p < 0.01$). Soil properties selected for this analysis were as follows: CEC, clay, soil C:N ratios, total root biomass, very fine root biomass, small macroaggregates, microaggregates, and soil organic (SO) layers depth. Abbreviations of soil carbon pools were as follows: MBC = microbial biomass carbon; SOC = soil organic carbon; ORC = oxidation-resistant carbon (old carbon); POM = physically protected carbon; silt.clay = chemically protected carbon.

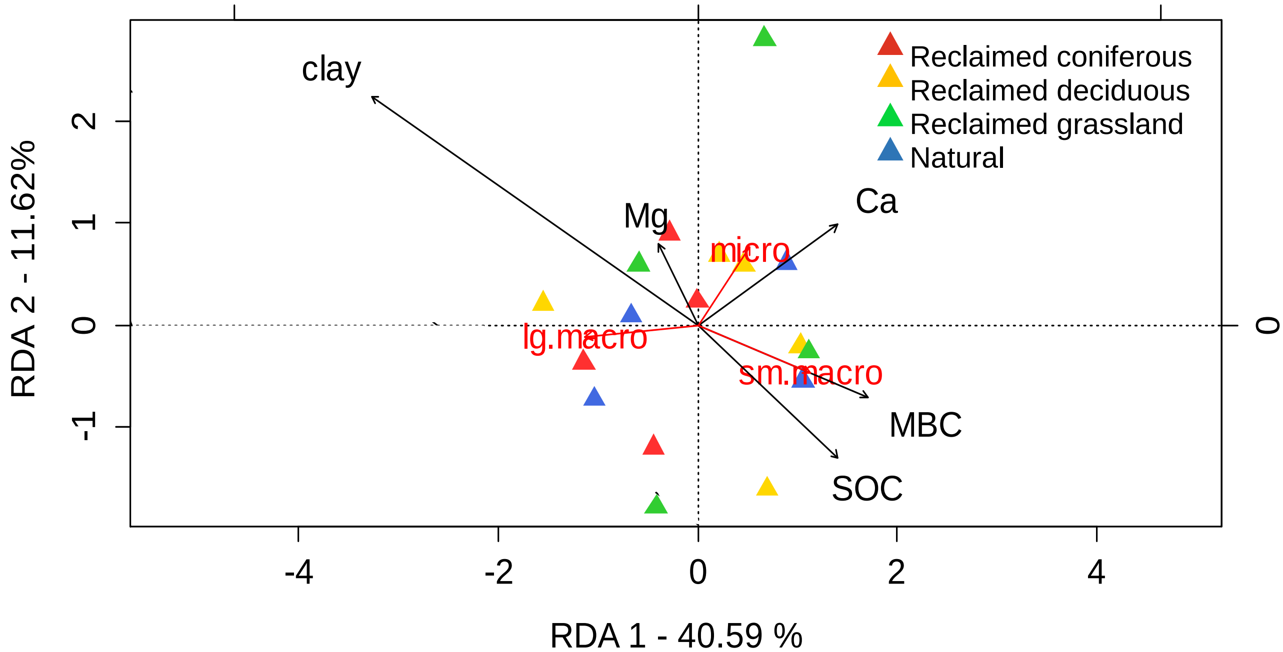

Figure 16. Redundancy analysis (RDA) testing the importance of five soil properties (in black) in explaining variation in aggregate size distribution (in red) across 17 sites. Explanatory variables explained significant variation in the response variables (p=0.035). Response variable abbreviations are as follows: micro = microaggregates (53- 250 µm); sm.macro = small macroaggregates (250 – 2000 µm); lg.macro = large macroaggregates (2000 – 8000 µm). MBC = microbial biomass carbon; SOC = soil organic carbon. Explanatory variables selected for this analysis were clay content, Mg, Ca, MBC, and total SOC.

4 DISCUSSION

4.1 Total and protected soil carbon pools were greater at reclaimed sites than at natural sites

In addition to having greater soil organic matter content than natural sites, as reported in other studies (Anderson, 2014), the soils at the reclaimed sites also had greater total C content, and similar or greater concentrations of physically and chemically protected C. Compared to natural sites, grasslands had more than three times as much C associated with silt and clay minerals, and more than three times as much C occluded within aggregates. Coniferous sites tended to have more chemically and physically protected C than natural sites, and deciduous sites were not significantly different from natural in either protected C pool.

4.1.1 Greater protected soil carbon at reclaimed sites was not related to differences in soil structure

The greater amounts of physically and chemically protected carbon was not associated with greater abundance of stable aggregates in reclaimed sites, as the stability and size distribution of water-stable aggregates was similar in reclaimed and natural sites. Laganière et al. (2011) also found that while total C and C distribution in size-density fractions differed among forest types, aggregate size distribution and aggregate C content did not differ. Size-density separation may therefore provide a more sensitive indicator of changes in soil C storage as a result of land-use change than size separation alone.

Initial soil structure at reclaimed sites is assumed to have been weaker than at natural sites as a result of disruption during the salvage and replacement process. However, two decades following reclamation, soil aggregate abundance was comparable at the reclaimed and natural sites in this study. Across all sites, the majority of soil dry weight (excluding sand grains > 1 mm) was found in stable macroaggregates (> 250 µm), which accounted for 70 – 80 % of the soil weight. Between 15 and 25 % of the soil dry weight was in microaggregates (53 – 250 µm), and less than 7 % was in non-aggregated soil (< 53 µm) on average, across all sites. These results are comparable to stable-aggregate size distributions found in other grassland and forested areas (Marquez et al., 2004; Six et al., 2002b; Laganière et al., 2011), although it is difficult to directly compare with other studies because of the wide range of soil types, and different methods used to assess aggregation. Therefore, soil aggregates were either maintained during removal, stockpiling and reclamation, or have developed in the two decades since reclamation (or both).

4.1.2 Clay content was important in explaining variation in both aggregate size classes and soil C pools

Soil texture was not significantly different between reclaimed and natural sites, or among the vegetation treatments. Clay content was between 0.9 and 3.1 % in reclaimed soils, and between 0.4 and 6.6 % in natural soils. Despite representing a consistently small portion of the soil texture, variations in clay content had a significant effect on aggregate stability and soil C pools. Clay content was the only soil property that significantly explained variation in aggregate size classes, and also explained variation in soil C pools. Conversely, silt content was between 24.0 and 43.9 % in reclaimed soils, and between 9.3 and 87.7 % in natural soils, but it was removed as an explanatory variable in both aggregate size distribution and soil C pool analysis because it did not explain any additional variation in the two response matrices. The availability of binding sites on clay surfaces, and to a lesser extent on silt surfaces, ultimately determines how many organo-mineral complexes can be formed. As a result, clay content, as well as clay + silt content, is often closely correlated with chemically protected carbon (Hassink, 1997).

For all sites, carbon saturation capacity (based on the equation derived by Hassink (1997) and modified by Carter et al., (2003)) was similar as a result of their similar clay contents, although the natural sites had slightly higher saturation capacity due to slightly higher clay and silt contents. At reclaimed sites, chemically protected C had already reached the calculated saturation capacity, with grasslands showing signs of surpassing saturation capacity. Soils at natural sites had significantly less C adsorbed to clay and silt surfaces than the amount of binding sites available for adsorption, and were estimated to be able to store an additional 79 % in the silt- and clay-associated pool. Despite storing more C in physically and chemically protected pools, the largest C pool at reclaimed sites was unprotected C. These results suggest that soils at reclaimed sites have reached the maximum carbon content that can be stored in chemically protected pools. After C saturation has been reached, carbon begins to accumulate in uncomplexed OM pools (LF and POM) (Laganière et al., 2011; Gulde et al., 2008). This may be why reclaimed sites had greater unprotected C pools. Physically protected carbon is also considered to be uncomplexed OM, however, the size of this pool may also be limited by available binding sites on clay and silt surfaces.

4.1.3 Texture was more important than biotic properties in determining soil structure

Sandy soils are typically associated with poor structures, whereas loams tend to be well aggregated due to the abundance of clay, and the pore size diversity facilitated by silt and sand particles. Oades (1993) theorized that in sandy soils with little or no clay, aggregate stabilization is uncomplicated by abiotic factors, and is influenced only by biotic components such as organic matter, microbes, roots, and the exudates that they release. Organic matter is usually the primary cementing agent for aggregates in poorly-to-moderately weathered soils, whereas clay type and abundances of Fe and Al take precedence in strongly-weathered soils (Denef et al., 2005). Considering the importance of OM and the young age of soils in northern Alberta, it was expected that greater SOC would correlate with greater aggregate stability and carbon stabilization, and that this relationship would be present across reclaimed and naturally fire-disturbed sites. Although reclaimed sites did have greater SOC than natural sites, aggregate stability and size distribution did not differ between reclaimed and natural sites. SOC was important in explaining variation in aggregate size distributions, especially small macroaggregate abundance, but no clear trends linking SOC with differences in vegetation treatments emerged, except that SOC was greater at reclaimed sites than at natural sites. More SOC was found in physically protected pools at reclaimed sites on average compared to natural sites, which suggests that it was being incorporated into aggregates, but proportional to total SOC, there was significantly less SOC in physically protected pools at reclaimed sites. Therefore, organic matter was probably acting as a binding agent, to some extent, at reclaimed sites, but not as much as expected, considering the amount of total SOC that was present, and the abundance of LF carbon that had not formed aggregates, or chemically bonded to clay and silt particles.

4.1.4 Microbial biomass did not explain aggregate size-class variation

Microbial biomass was significantly greater at reclaimed deciduous sites than at natural sites. However, MBC represented 1 – 4 % of SOC across all sites. Considering the abundance of SOC at reclaimed sites, this suggests that MBC was not very different between reclaimed and natural sites. It was also not a significant variable in the RDA model explaining aggregate size distribution, although MBC and small macroaggregates were closely related. MBC was also not strongly correlated with any explanatory variables in the model explaining soil carbon pools, except for being negatively correlated with clay content. This is more likely an artifact of higher clay content and lower microbial biomass at natural sites than a direct negative relationship between microbial abundance and clay. Biotic soil properties such as MBC, root biomass, and SOC may be more closely correlated with the abundance of unstable aggregates, as opposed to the stable aggregates measured in this study (Marquez et al., 2004). While abiotic soil properties like cation abundances result in the formation of very stable aggregates, aggregates formed through biotic processes, such as root enmeshment and microbial exudates, tend to be held together more loosely. An assessment of unstable aggregates (re-wetted) might provide evidence that biotic factors are influencing aggregate size distributions under field conditions at reclaimed and natural sites, but these aggregates are disrupted by the action of slaking prior to aggregate analysis. The lack of a strong relationship between soil biotic components and physically and chemically protected carbon suggest that a minimum clay content is needed for organo-mineral interactions to occur, and that below this level, additional biotic binding agents such as microbial exudates and organic matter will not result in additional stable aggregates because binding sites on mineral surfaces are not available.

4.1.5 Roots were not strongly correlated with soil aggregation or protected carbon pools

Live roots have been found to affect soil carbon stabilization through facilitating macroaggregates formation, which results in the occlusion of OM in physically protected pools (Tisdall and Oades, 1982; Six et al., 2002a). However, root biomass was poorly correlated with aggregate size-class abundances, and both total root biomass and very-fine root biomass (< 1 mm) were dropped as explanatory variables in the RDA model because they did not explain additional variation in the distribution of aggregate size classes. Compared to coniferous and deciduous sites, total root biomass was significantly lower at grassland sites, where physically and chemically protected pools were greatest, suggesting that the presence of more root biomass did not appear to be facilitating protected soil C at reclaimed sites. The lack of difference in root biomass between natural sites and reclaimed sites indicates that the peat mineral mix is neither hindering nor promoting rooting in the top 10 cm of soil.

4.2 Reclaimed sites had more biochemically resistant soil carbon, and more labile soil carbon than natural sites

Reclaimed sites had on average seven times more total % carbon in the top 10 cm of reclaimed soil material than the top 10 cm of mineral soil at natural sites. Even taking into account differences in bulk density between reclaimed and natural sites, reclaimed sites still had three times as much SOC content as natural sites. Previous studies on the oil sands (McMillan et al., 2007; Turcotte et al., 2009) reported that total soil C and total soil N at reclaimed sites was less than or only slightly more than at natural sites. The greater differences in soil C and N between reclaimed and natural sites in this study is attributable to the soil samples being taken exclusively from the mineral soil at natural sites, and the equivalent soil layer at reclaimed sites, whereas earlier investigators included F and H layers in the soil samples.

In temperate regions, reclaimed sites in which topsoil was used accumulated up to 200 % of pre-mining SOC in less than 40 years (Vindušková and Frouz, 2012; Ussiri and Lal, 2005; Akala and Lal, 2001). While I found that reclaimed sites demonstrated a similar trend, the magnitude of the difference between reclaimed and natural sites suggested that the total soil C measured in this study encompassed more than just SOC accumulation at reclaimed sites. One missing link in our understanding of carbon accumulation and storage in reclaimed oil sands sites is the exact carbon content of oil sands reclaimed soils at year zero. We could measure the C content of peat-mineral mixes that were recently used as a reclamation topsoil material to estimate initial C content of older reclaimed soils, but C contents are known to be highly variable. Moskal et al, (2001) created peat-mineral mixes that mimicked those used in oil sands reclamation and found C contents ranging from 7 to 18.9 %, which is comparable to the 4.8 – 16.7 % C that I found at the reclaimed sites. In contrast, % C at natural sites in my study (0.5 – 1.8 %) are much lower than the lowest C content of the peat-mineral mixes created by Moskal et al. (2001), indicating that initially, C content of reclaimed soils was much higher than natural soils, and is still contributing to elevated soil carbon concentrations at reclaimed sites.

4.2.1 High C:N of biochemically resistant carbon at reclaimed sites compared to natural sites indicate that residual peat is present

Despite reclaimed soils having higher concentrations of both C and N than natural soils, mean soil C:N ratios ranged from 18 – 23 across reclaimed and natural sites. While these C:N ratios are somewhat high for Luvisols and Brunisols, they are still within the range of forest soil C:N ratios, which suggests that reclaimed soil C:N ratios have stabilized similar to that of natural soils (Batjes, 1996). In contrast, C:N ratios of biochemically resistant organic matter were 18 – 55 at reclaimed sites, but only 2 – 8 at natural sites. Biochemically resistant soil carbon is typically associated with highly processed organic material that has a low C:N ratio (Paul, 2007). The natural sites reflected this trend, indicating that the pool of biochemically resistant OM at natural sites is likely composed of highly processed OM. The higher C:N ratio of old soil carbon at reclaimed sites suggests that this pool is at least partially composed of a very different material, probably residual peat. Malmer and Holm (1984) found C:N ratios as high as 107 in the top of *Sphagnum sp.* peat bogs and low C:N ratios (25-30) in the most humified samples at the bottom of the profile. Cabral (2012) found a mean C:N ratio of 35 in the least humified peat in Swedish peatlands, and a mean of 20 in the most humified peat. The relatively high C:N ratios of old carbon at reclaimed sites suggests that it is fibric or hemic peat, which are over two-thirds, or between one-third and two-thirds fiber respectively (Boelter, 1969). Sapric peat is the most humified form with less than one third fiber content, and has the narrowest C:N ratio, so it is unlikely that peat at most reclaimed sites has reached this humified state. Overall, between 20 and 28 % of the soil carbon at reclaimed sites was biochemically resistant to oxidation, compared to 10 % at natural sites.

Reclaimed soils not only had more biochemically resistant C, but more mass was lost during oxidation compared to natural soils, although it was not significantly more at coniferous sites. Organic material that can be oxidized is considered to be a younger, more labile pool of C. These results suggest that soil carbon is greater at reclaimed sites both as a result of biochemically resistant C pools, and through the accumulation of labile C from vegetation inputs. High C:N ratios of biochemically resistant C pools suggest that fibric or hemic peat is persisting at reclaimed sites, while greater labile C pools suggest that reclaimed sites are also accumulating more C, resulting in greater total soil carbon compared to natural sites.

4.2.2 Carbon is accumulating in the soil light fraction rather than in surface organic horizons at reclaimed sites

The largest difference in soil C pools between reclaimed and natural sites was in the light fraction. Reclaimed sites had 7 – 140 times as much C in this pool as natural sites. Boreal forest soils typically have very small pools of light-fraction C that represent less than 10 % of total SOC (Laganière et al., 2011), and large accumulations of organic C in surface organic layers. For example, Laganière et al., (2013) found more C in the surface organic layer than in the top 15 cm of mineral soil in coniferous and mixedwood boreal forests. The natural sites in this study were comparable to this, having thicker surface organic layers than reclaimed sites and soil light-fraction C pools averaging only 8.6 % of total SOC.

Light-fraction C can be to be incorporated into mineral soil without being bound by soil particles through macrofaunal bioturbation, although processing of OM and mineral soil by earthworms and other macrofauna facilitates the formation of macro- and microaggregates, which still results in physically protected OM (Pulleman et al., 2005). In a previous study at the same sites, Anderson (2014) found that macrofaunal activity in surface organic layers was greatest at grassland sites, followed by deciduous sites, which were both significantly greater than coniferous and natural sites. In 22 - 32-year-old post-mining sites, Frouz et al. (2009) found that soil carbon storage was strongly and positively correlated with the abundance of earthworms and the presence of their casts. Thus, bioturbation at the reclaimed sites in this study could be facilitating greater incorporation of OM into the mineral soil. Grasslands had the most physically and chemically protected carbon, as well as the highest macrofaunal activity. In contrast, deciduous sites had the most unprotected soil carbon, and the second highest macrofaunal activity. This suggests that if macrofauna are influencing the distribution of soil carbon at reclaimed sites, there may be different species of macrofauna present between grassland and deciduous sites, and these macrofauna incorporate OM into the soil in different ways. No differences were found in light-fraction C between reclaimed vegetation treatments, although deciduous sites tended to have the highest mean light-fraction C, and were also the most variable. These results suggest that vegetation type may contribute to differences in light-fraction C among reclaimed vegetation treatments, although a lack of distinct trends suggest that a combination of factors are influencing light-fraction C pools.

4.2.3 Root distribution did not explain differences in light-fraction carbon between reclaimed and natural sites, but may be contributing to differences among reclaimed vegetation treatments

Total root biomass was important in explaining the variation in soil carbon pools, and was closely aligned with the distribution of deciduous sites. Very-fine roots were also positively correlated with the deciduous sites, although they were not significant in explaining variation in soil carbon pools. Since the light-fraction C pool was greatest at deciduous sites, total root biomass could be directly contributing to light-fraction C at these sites. Light-fraction distribution has been found to be strongly correlated with root biomass (Schrumpf et al., 2013). Compared to deciduous site means, coniferous sites had similar total root biomass, yet tended to have a smaller light-fraction C pool. The distribution of root biomass in different size fractions varied between coniferous and deciduous sites, although not significantly so due to the high variability in root biomass across all sites. The most abundant root size-class at deciduous sites was very-fine roots, whereas at coniferous sites, it was coarse roots, so it is possible that the size class of root is important in determining what soil carbon pool it ends up in. Rasse et al. (2005) found that the maximum age of fine roots in the soil was 20 years, suggesting that fine roots are more likely contributing to the quickly cycling light-fraction pool than contributing to older carbon pools, and this may be evident at deciduous sites. Thus, the direct contribution of root C to light-fraction C pools, as opposed to roots actively forming soil aggregates, is the more probable pathway through which rooting activity is influencing C stabilization at reclaimed sites.

4.2.4 The free light-fraction carbon pool at reclaimed sites may not be as vulnerable to land-use change as a typical natural light-fraction pool

By definition, the free light-fraction is freely accessible to microbial decomposition. It is typically associated with a young carbon pool composed of relatively fresh litter that is more sensitive to land-use changes and microclimatic differences than carbon that is protected (Gregorich et al., 1998). Therefore, it is possible that the majority of soil carbon at reclaimed sites could be more sensitive to changes in land-use and climate. However, light-fraction C may be predominantly peat as opposed to fresh litter inputs, and therefore might behave differently than the typical light fraction at a natural site.

While the LF pool is considered to be a younger pool of C that is sensitive to changes in land use, some researchers have found that this pool can be older than physically protected C pools (Six et al., 1998). Six et al. (1998) proposed that if inert material is present in the free light-fraction, then it is not likely to form a nucleation site for aggregation because little microbial activity is associated with this material. Thus, if light-fraction organic matter reaches a state of inertness without becoming incorporated into aggregates, or binding to silt and clay surfaces, then it is unlikely to ever transition into these pools. If this theory is applied to reclaimed soils, it could explain why peat that was incorporated into mineral soil 20 – 40 years ago may still be present in the light fraction, although our analysis did not allow us to determine if the light fraction is predominantly peat, or if peat has been incorporated into physically or chemically protected C pools. Ultimately, light-fraction C is more vulnerable to combustion during fire than physically and chemically protected pools, so significantly greater free LF C pools at reclaimed sites could result in significant carbon losses in the long-term in response to fire.

4.3 Few differences in soil properties were found between reclaimed vegetation treatments

Some soil properties were distinctly different between reclaimed and natural sites, but differences among reclaimed vegetation treatments were few and inconsistent. . Alaka and Lal (2001) found that under forested reclaimed sites where topsoil was used, SOC content increased to 120 % of natural reference sites after 21 years, and under pasture, SOC content increased to 180 % of natural reference sites after 25 years. Similarly, I found that total SOC content at reclaimed grassland sites was the highest, and was significantly greater than coniferous sites, while deciduous sites did not differ from either treatment. This difference between grassland and coniferous sites may reflect the quality and quantity of litter inputs between vegetation types, with conifer needles contributing less litter, with wider C:N ratios than grass litter. This is supported by coniferous sites having the highest C:N ratios of labile, oxidizable C, and losing the least OM during oxidation, although neither result was significantly different from grassland sites.

Concentrations of soil total C were significantly greater under deciduous sites than grassland sites, despite having similar SOC content. This may reflect the nature of the SOC present at deciduous sites compared to grassland sites. Grasslands had significantly greater C content in chemically and physically protected pools than deciduous sites, while proportionally more C at deciduous sites was in the low-density light fraction compared to grassland sites. This suggests that soil processes at deciduous sites, such as soil mixing by macrofauna or rooting activity, may be facilitating the incorporation of OM into the soil, but not into aggregates or organo-mineral complexes. On the other hand, soil processes at grassland sites, such as the formation of microaggregates, may be contributing to the chemically and physically protected C pools. Six et al. (2002a) plotted silt + clay content against silt- and clay-associated C concentrations (as per Hassink (1997)), and derived C-saturation equations for different ecosystem types. They found that forest soils had greater saturation capacity than grassland soils with similar clay contents. In my study, I would have suspected that grassland soils would have greater saturation capacity due to their greater chemically protected C. Moreover, some grassland sites had accumulated even more chemically protected C than the estimated capacity level, suggesting that the saturation capacity may be higher than what was estimated at grassland sites. The soils used by Six et al. (2002a) were from a sub-tropical region, which may explain the difference in trends compared to the forested and grassland sites in this study.

Peat can be patchy in the peat-mineral mix, which could explain some of the variability in SOC between reclamation treatments. However, concentrations of biochemically resistant C were very similar among all vegetation treatments (mean of 1.7 ± 0.2 %), suggesting that the difference in soil C concentrations between deciduous and grassland sites was not caused by variations in residual peat C. Deciduous litter, as opposed to coniferous and grassland litter, can be easily dispersed and gathered into clumps by the wind, which may also explain variability in C concentrations among deciduous sites.

Coniferous sites had significantly higher oxidizable C:N ratios than deciduous sites. Conifer litter is more resistant to decomposition than deciduous litter initially (Prescott et al., 2004), and annual litter fall under deciduous trees is typically greater than under conifers (Kavaadias et al., 2001) which could be resulting in a slower accumulation of young C at coniferous sites compared to deciduous sites.

Reclaimed coniferous sites have been found to reach a maximum C accumulation rate after 35 years, while deciduous sites reached a maximum after 10 – 20 years after which time they declined, and grasslands maintained a constant SOC accumulation in sites aged 0 – 30 years (Vindušková and Frouz, 2012). Thus, accumulation rates will probably continue to increase at coniferous sites, whereas grassland and deciduous sites have probably already reached maximum C accumulation rates. This could explain why coniferous sites had significantly lower SOC content than grassland sites. However, while Vindušková and Frouz (2012) compared reclaimed sites in the temperate zone of the Northern Hemisphere, oil sands reclaimed sites are located further north where decomposition is more restricted. It is possible that the reclaimed sites in this study will take longer to reach maximum C accumulation than the age ranges suggested.

4.3.1 Microaggregates and Ca bridging may be contributing to chemically protected C at grassland sites

Grassland sites tended to have the most microaggregates, higher pH than deciduous sites, and higher CEC and exchangeable Ca concentrations than coniferous sites. Lehmann et al. (2007) found that stabilization of carbon within microaggregates is associated with the adsorption of organic matter onto clay surfaces, as opposed to the occlusion of organic matter. Calcium is typically associated with microaggregates because cation bridging is a micro scale process that is thought to be important in the formation of smaller aggregates, whereas root enmeshment and macrofaunal activity are more commonly associated with the formation of larger aggregates (Muneer and Oades, 1989). Redundancy analysis of aggregate size classes revealed that Ca was an important explanatory variable ($p < 0.10$), and that microaggregates and Ca abundances were correlated with one another. Thus, microaggregate formation could be increasing contact between clay surfaces and organic matter, and greater Ca concentrations could be facilitating cation bridging, resulting in more chemically protected C at grassland sites.

4.3.2 Different rooting strategies between reclaimed vegetation types were not strongly evident

General trends suggest that grasslands and deciduous sites had predominantly very-fine and fine roots, whereas coniferous sites and natural sites had a more even distribution of root biomass in each category. Grasslands had significantly lower total root biomass than coniferous and deciduous sites, and significantly fewer medium roots than coniferous sites. This reflects the rooting strategies of grasses versus tree and shrub species to some extent. However, forbs and grasses were consistently found at grassland sites, and in the understory at deciduous sites, which may explain why root biomass was not more distinct among vegetation types. The difficulty in extracting very-fine roots from the peat-mineral mix at reclaimed sites may also have resulted in lower total root biomass at grassland sites. Over time, greater differences in total root biomass and size distribution are expected to develop between forested and grassland sites in particular, with grasslands having more fine and very-fine root biomass than the other vegetation treatments.

4.4 Conclusions and recommendations

The use of peat as an organic amendment in reclaimed oil-sands soil was initially suggested as a way of supplementing the limited availability of mineral soil in the mining footprint, while also improving soil conditions for vegetation establishment by increasing soil C, nutrient availability, and water-holding capacity. However, it was unclear whether this abundant organic matter was being incorporated into soil structure and protected. In other words, was the greater SOM at reclaimed sites resulting in more C in physically and chemically protected pools compared to natural sites? Further, residual peat in reclaimed soils potentially represented a third pool of protected C, as a result of being biochemically protected from decomposition, and this pool size had not been quantified. The influence of reclaimed vegetation type on C stabilization was also not addressed previously and could play an important role in future reclamation decisions if soil carbon storage is considered to be important for successful reclamation.

My results show that reclaimed sites had significantly more soil carbon in the top 10 cm than natural sites, and that reclaimed sites had at least as much C stored in physically and chemically protected pools as natural sites. This suggests that C stabilization mechanisms that result in protected C pools are occurring at both natural and reclaimed sites. Almost half of the SOC at reclaimed sites was stored in the free light fraction compared to less than 10 % at natural sites, which would typically signify that a large pool of reclaimed SOC was vulnerable to land-use change. However, 20 – 28 % of reclaimed soil carbon was found to be biochemically resistant, which was significantly more than at natural sites. The high C:N ratios of this resistant material at reclaimed sites suggested that it was probably residual fibric or hemic peat, and thus, not vulnerable to decomposition. While I did not directly measure the biochemical resistance of the free LF in this study, other studies have shown that this C pool is the most likely to be affected by land-use change, such as adding an organic amendment (Gulde et al., 2008; Degryze et al., 2004). Therefore, it is probable that at least some of the light fraction C is from residual peat, and thus, measuring chemically and physically protected C pools could be an underestimate of long-term soil C storage at reclaimed sites without taking into account biochemical protection.

Within vegetation treatments, reclaimed grassland soils tended to have the greatest SOC content, and the most chemically and physically protected SOC. Reclaimed deciduous soils had similar SOC content to grassland sites, but the majority of the carbon was in unprotected pools. Reclaimed coniferous soils tended to have more C in chemically and physically protected pools than deciduous sites, but also appeared to have smaller, low-quality litter inputs compared to the other vegetation treatments, resulting in less total SOC content. Moreover, coniferous sites may be further from reaching maximum C accumulations rates than grassland or deciduous sites. Therefore, 20 – 40 years after reclamation, the greatest physically and chemically protected carbon pools were found under grasslands, although coniferous sites may eventually surpass grasslands if C continues to accumulate in chemically and physically protected C pools at these sites. As a result of reclamation practices, post-mining upland areas may be able to store even more soil carbon in long-term pools than pre-mining upland areas, which has important implications for offsetting some of the C losses that have resulted from oil sands mining.

4.5 Project limitations and future research

4.5.1 Experimental Design

As with any scientific study, having a larger sample size would have provided a clearer picture of differences between reclaimed and natural, and reclaimed vegetation treatments. I would sample more natural sites or possibly stratify sites into soil texture classes to reduce the variability that results from sampling different soils and grouping them together. I would also increase my sample size for each of the vegetation treatments, and possibly sample more subplots at each reclaimed site. Using the data from two subplots and discarding the data from one did reduce sampling of the inherent variability of the peat-mineral mix, but it also meant that mean values for each site were based on the average of only two soil samples.

While all sites within a reclaimed vegetation treatment are theoretically more similar to each other than to other treatments, this was not quantified in this experiment. Site productivity measurements could provide important information on the similarity between sites within reclaimed vegetation treatments, and conversely, why a specific site may be stabilizing carbon in different ways. For example, it would be interesting to determine whether the variability in carbon pools at deciduous sites is a result of site productivity. Linking aboveground productivity to SOC storage could also be important to establish why facilitating soil functioning is so important.

Each site was only sampled once, therefore it is difficult to predict carbon accumulation at each site over time. It was also unclear how close each reclaimed vegetation treatment was to reaching maximum C accumulation, or in the case of deciduous and grassland sites, whether this stage had already been surpassed. Repeated measures of the distribution of carbon in physically and chemically protected pools 10 to 15 years from now could provide evidence of whether reclaimed ecosystems will differentiate based on vegetation type, and whether one reclaimed vegetation type results in greater soil carbon storage. Moreover, measuring carbon content of newly reclaimed soils under different vegetation types, and then returning in 20 years for further carbon measures will provide a more accurate analysis of how much carbon has accumulated, and how this differs between vegetation types.

4.5.2 Carbon analysis

While the chemical composition of the free light-fraction has been previously assessed at reclaimed oil-sands sites (Turcotte et al., 2009), key differences in the fractionation method used in this study resulted in different free light-fraction results at reclaimed sites. An analysis of the chemical composition of the free light-fraction from this study would clarify whether the free LF C pool at reclaimed sites is more vulnerable to changing land-use or climate, or whether it is primarily composed of biochemically resistant peat residue. One important consideration is that methods used to identify a specific soil property in a natural soil may not be relevant in a reclaimed soil. The presence of peat had confounding effects on several analyses, including being able to use size-density fractionation to separate soil carbon into pools that were relevant to C residence times.

Instead of measuring aggregate size distribution and soil carbon pools separately, I would use a combination of the two in future. Initially, I would remove the free LF from the soil samples, and then separate soil aggregates into different size classes, and as a final analysis, I would isolate physically and chemically protected C from each aggregate size class, to obtain a better understanding of how physical protection of OM within aggregates was facilitating chemical protection over time. Further analysis of the chemical composition and age of C in the light-fraction fraction isolated in this study is needed to identify whether the free light-fraction in reclaimed soils will behave in a similar way to land-use change as natural free light-fraction. This is an important question when assessing the carbon stabilization potential of reclaimed soils because at some of the deciduous sites, up to 93 % of SOC was present as free light-fraction. I would also be interested in determining how much of the physically and chemically protected C is peat versus vegetation inputs, because this would provide some indication of whether the peat amendment is contributing to soil structure development at reclaimed sites. In future analysis, I would choose either loss on ignition or dry combustion analysis to measure soil carbon to simplify comparison.

4.5.3 Microbial biomass and root biomass

The presence of abundant light-fraction OM at reclaimed sites also made it more difficult to determine total microbial biomass, and extract very-fine roots from the soil. A k_{EC} constant is typically used to determine the total microbial biomass from the extractable flush that was measured during chloroform fumigation. However, organic soils sometimes have different constants than mineral soils, and it is recommended that you determine your own constant when working with a unique soil (Sparling et al., 1992). I was not able to calculate my own constant because I did not measure microbial biomass C using any other method, so I was unable to translate the extractable flush to total microbial biomass. While I was still able to compare between treatments, I could not compare my results with other studies. One possible method for a more comprehensive root analysis could be to leave vegetation intact on top of the soil sample, and leave samples in storage for a limited amount of time. Soil could be more easily shaken off of roots, although this may still result in very-fine roots breaking off in the free light-fraction and being underestimated.

4.5.4 Aggregate analysis

The lack of differentiation in soil structure between reclaimed and natural sites, and among reclamation treatments was surprising. It may be that there are no differences among sites, but it might also indicate that the selected method was not able to capture subtle differences in soil structure between sites. There are many variations in methods of measuring aggregate abundance and stability, and even small differences in the method can provide different results. In future soil-structure assessments, I would carefully characterize soil structure in the field. I would then use this field-based knowledge of soil structure and soil texture to guide my decision on the most appropriate method to compare soil structure in the lab. For example, I noticed that soil at reclaimed sites felt “fluffy” because of the high organic matter content. Therefore, separating the free light-fraction from the aggregated soil should be the first step in aggregate analysis because the abundant light-fraction will contribute to aggregate size-class weights and possible carbon analysis. Collecting small soil monoliths and thin sectioning them in the lab could be an interesting method to explore major structural components in reclaimed soils, and to compare them to natural soils (Frouz et al., 2008). Air-drying soils as a pre-treatment was beneficial because it prevented the structural degradation that can occur during storage in field-moist condition. However, soil with high organic-matter content was very difficult to re-wet evenly, and re-wetting was eventually discarded as an option because of the time required. In future, I would perform wet-sieving on re-wetted and air-dried soils, to see how these methods contributed to differences in aggregate size distributions and stabilities.

4.5.5 Other differences in vegetation types that could be explored

One explanation for greater chemically and physically protected soil carbon at grassland sites that was not explored in this study is the presence of glomalin, which is a glycoprotein that acts as an aggregate binding agent, and has been associated with greater soil aggregation and carbon storage (Emran et al., 2012; Rillig et al., 2002). Glomalin is produced by arbuscular mycorrhizal fungi, which form symbiotic relationships with plants that would be consistent with grassland-associated species, whereas the tree species present at reclaimed deciduous and coniferous sites would form relationships with ectomycorrhizal fungi. I did not measure glomalin because I thought I would only be measuring it at one of the treatment types. However, forbs and grasses were also present in the understory at deciduous and to a lesser extent, coniferous sites, so it would be interesting to determine whether the presence of glomalin facilitated greater protection of carbon at reclaimed grassland sites, and whether it contributed to carbon stabilization at any of the other reclaimed or natural sites. Coniferous and deciduous species tend to extend roots deeper into the soil than grassland species, and this can facilitate accumulation of deep soil C. It would be interesting to investigate how deep roots are growing at reclaimed sites compared to natural sites, and whether this is facilitating deep soil C accumulation at reclaimed sites.

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APPENDIX

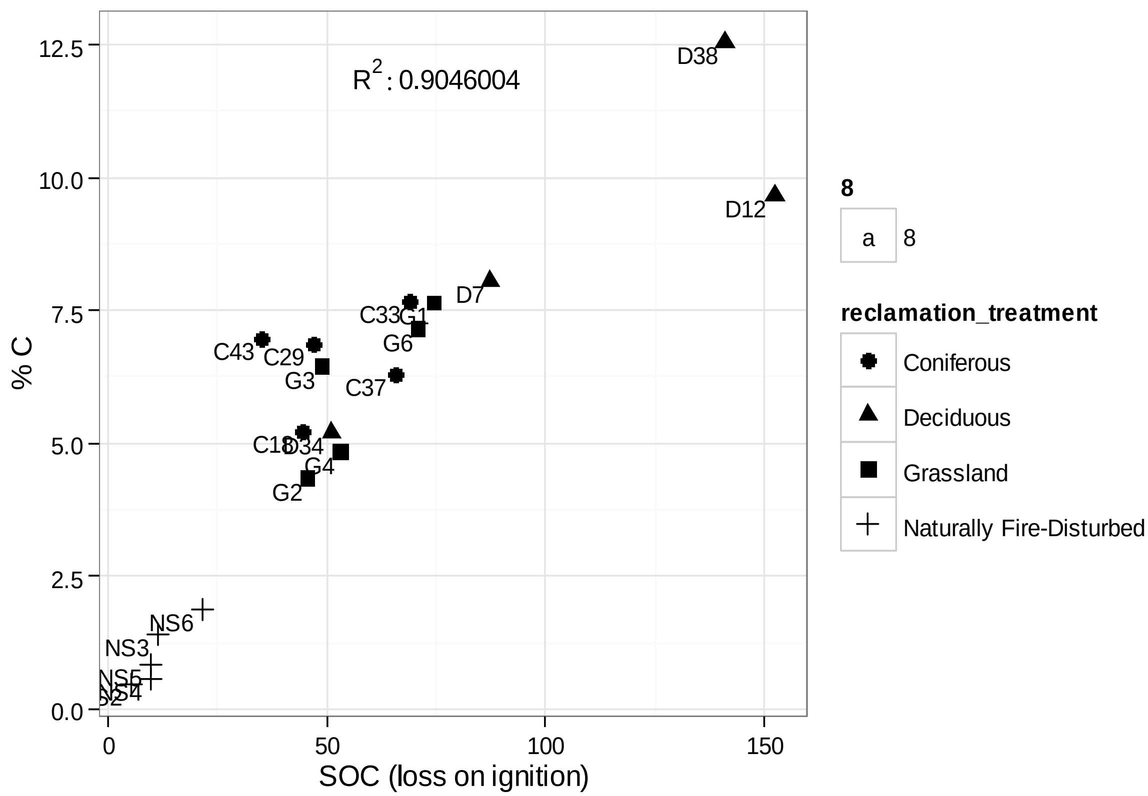

Figure 17. The correlation between total soil organic carbon from the loss-on-ignition method and total % carbon from the dry combustion method, excluding site D42. A correlation coefficient of 0.904 was found.