

# **The biogeochemistry of regreened forests on a mining and smelting degraded landscape**

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## Thesis abstract

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Mining and smelting degraded landscapes are characterised by heavily eroded soils that are acidic, contaminated with toxic metals, and depleted of essential nutrients. Regreening degraded landscapes has been proposed to support global carbon (C) mitigation measures and protect biodiversity. One of the world's largest regreening programs in the City of Greater Sudbury, Canada has been ongoing since 1978 and involves liming and fertilizing selected areas followed by planting primarily jack pine (*Pinus banksiana* Lamb.) and red pine (*Pinus resinosa* Ait.) trees. The main objective of this thesis was to improve our understanding of biogeochemistry in the City of Greater Sudbury regreened forests, and to determine how nutrient pools and cycling change as stands age. I established a chronosequence of forested sites between 15–40 years-old and to account for the effects of erosion, each site was categorized as “stable” (<10% bedrock cover) or “eroded” (>30% bedrock cover). Individual tree growth and nutrient accumulation in aboveground biomass (AGB) did not differ between stable and eroded sites and were comparable to rates reported from pine plantations in similar ecozones. Aboveground nitrogen (N) pools were six times larger than N applied in fertilizer, suggesting N limitation is most likely not a concern. Rates of C cycling were generally similar to those measured at unimpacted jack and red pine plantations. The exception being a decrease in mineral soil and aggregate C concentrations. However, at the ecosystem-scale the loss of soil C is

trivial in comparison to increases in AGB C pools, leading to an overall increase in total ecosystem C following regreening (550,547 Mg in aboveground C across the 19,649 ha regreening landscape). Litter decomposition rates were higher at the regreening sites using a site-specific litter compared to a general common litter, indicating a home-field advantage for local decomposers. Soil temperature varied at the regreening sites and higher soil temperatures were related to higher rates of soil respiration. The regreening sites are rich in calcium (Ca) and magnesium (Mg); and while soils were generally poor in phosphorous (P) and potassium (K), foliar concentrations of P and K were comparable to those of “healthy” red pines. Overall, the regreening program appears to have increased tree growth and produced jack and red pine plantations that are biogeochemically similar to conifer plantations unimpacted by over a century of mining and smelting impacts.

Key words: degraded landscape; biogeochemistry; soil carbon; regreening; nutrient cycling; forests; tree growth

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## **Chapter 1: General introduction**

### *1.1. Forest degradation*

Globally, forests cover 31% of the world's land surface (FAO, 2020a), sequester 20% of the total carbon (C) emitted from anthropogenic sources (Friedlingstein et al., 2022), account for over 80% of terrestrial biodiversity (FAO, 2020b), directly support 87 million jobs (FAO, 2020b), and are widely used for recreational and spiritual purposes. Between 1990 and 2020 it is estimated that 420 million hectares of forests have been degraded. While the magnitude of forest degradation has decreased in the past decade it is estimated that 4.74 million hectares of forested land were degraded each year between 2010 and 2020 (FAO, 2020a). Forest degradation emits large amounts of C into the atmosphere (largely as carbon dioxide (CO<sub>2</sub>) but also as methane (CH<sub>4</sub>) in smaller quantities), exacerbating the effects of climate change (Pan, 2011). A decrease in forest cover alters evapotranspiration changing microclimate factors (Sampaio et al., 2007). An extreme example of this is the clear-cutting of the Amazon rainforest stimulating a positive feedback loop that decreases evapotranspiration and precipitation in the area – which some researchers believe will lead to the Amazon rainforest evolving into a savannah in the future (Lovejoy and Nobre, 2018). Hydrological pathways are also altered; degraded forests are generally less of a water sink leading to increased runoff and flooding events (van Dijk and Keenan, 2007). The alteration of hydrological pathways increases erosion, affecting the quantity and quality of water, sediment, and nutrients released to surface waters (Neary et al., 2009). Forest degradation is reported to have increased the likelihood

that 19,432 different vertebrate species will be listed as threatened or endangered (Betts et al., 2017).

### *1.2. Mining and smelting ecosystem impacts*

Humans have been extracting geological materials since the Paleolithic era when pre-human hominids mined flintstones to use as weapons for hunting large game (Mellars, 1996). Smelting practices are believed to have been innovated in the Old World in modern day Turkey, Iraq, and Iran where the refining of lead (Pb), tin (Sn), and copper (Cu) was well established by 5000 BC (Wertime, 1964). Mining in Canada has also been practiced for millennia. Recent radiocarbon dating found Cu artifacts in the Great Lakes region that were between 3500–8500 years old (Pompeani et al., 2021). The onset of the Industrial Revolution is largely thought to be the beginning of large-scale environmental damage caused by mining and smelting. Industrial mining in Canada began with the establishment of Cu mines on the north shore of Lake Huron at Bruce Mines, Ontario in 1848 (Cranstone, 2002).

Mining and smelting practices often entail the removal of overburden and vegetation to access ore bodies and use biomass as fuel for smelting. The removal of overburden and vegetation drastically reduces biomass, soil, and nutrients on a landscape, directly altering ecosystem structure and function. Soil structure is impacted through compaction and the mixing of soil horizons (Bradshaw, 1997; Indorante et al., 1981). The degradation of soil structure along with the removal of vegetation can greatly increase soil erosion by both wind and water, and increase soil export into surface waters (Bradshaw,



1997). Erosion can also expose soil organic matter otherwise not in contact with the atmosphere resulting in increased rates of organic matter mineralization which decreases soil nutrient pools, reduces soil biological communities, increases C emissions, and hinders the re-establishment of vegetation (Akala and Lal, 2000; Dudka and Adriano, 1997).

Mining and smelting operations are large point sources of sulphur dioxide (SO<sub>2</sub>) (Smith et al., 2011). Sulphur dioxide, along with nitrogen oxides (NO<sub>x</sub>), and ammonia (NH<sub>3</sub>) are the main contributors to acidic deposition and can be deposited as gases or particulates in dry deposition, or in precipitation as wet deposition (Reuss and Johnson, 1986). Acid deposition can negatively impact forest ecosystems directly through SO<sub>2</sub> toxicity in foliage (Knabe, 1976); as well as indirectly through soil acidification (Likens and Bormann, 1974). As concentrations of strong acids in soil increase, base cations (calcium (Ca), magnesium (Mg), and potassium (K)) can leach from soils; if the soil base cation pool is not sufficient to buffer the incoming acidity then soil pH decreases (Likens and Bormann, 1974). Decreasing soil pH can increase the solubility of metals such as aluminum (Al), and increase metal toxicity to biota (Cronan and Schofield, 1990). The increased leaching of base cations from soils and the increased mobility of inorganic Al can decrease tree productivity and stress tolerance (Driscoll et al., 2001; Tomlinson, 2003). Altering soil pH also has an impact on microbial communities and related processes such as litter decomposition (Sridhar et al., 2022).

Along with SO<sub>2</sub>, mining and smelting practices emit metal particulates that are deposited onto ecosystems in surrounding regions (Berg et al., 1991; Dudka and Adriano,

1997; Freedman and Hutchinson, 1980b). Metal toxicity and bioavailability within an ecosystem are influenced by multiple factors (Sauvé et al., 2000). Soil acidification increases the mobility of many metals, as increased hydrogen ions ( $H^+$ ) compete with organically bound metals freeing the metals into more toxic inorganic forms (McBride, 1989). The presence of soil organic matter can also control metal solubility by increasing the presence of negatively charged binding sites to immobilize metals in the soil solution (McBride et al., 1997). Increased loading of bioavailable metals in the soil solution can have direct toxic effects on vegetation and also compete with nutrients for plant uptake (Påhlsson, 1989). Mining and smelting practices have been found to decrease soil organic matter and soil pH, while increasing the concentration of metals in soils causing an increase in both the load and bioavailability of metals in an ecosystem with harmful effects to vegetation and soil biotic communities (Amiro and Courtin, 1981; Bradshaw, 1997; Freedman and Hutchinson, 1980b; Vindušková and Frouz, 2013; Whitby and Hutchinson, 1974).

### *1.3. Ecological restoration*

The restoration of degraded forests has been highlighted as an essential tool supporting global C mitigation measures and protecting biodiversity (Brancalion and Holl, 2020; Chazdon and Brancalion, 2019; Lewis et al., 2019; Strassburg et al., 2020). The goal of restoring degraded forests has been included in multiple international objectives and agreements including the Bonn Challenge, The Paris Agreement, the Convention on Biological Diversity, and REDD+ (Reducing Emissions from Deforestation and forest

Degradation) (IUCN, 2022). Despite these initiatives, nearly 5 million ha of forest are being degraded globally each year (FAO, 2020b). China's Grain for Green program is regarded as the world's largest forest restoration project; in the late 1990s the Loess Plateau was suffering from large-scale soil erosion but by 2019 trees and native grasses were planted over more than 34 million ha of China (Hua et al., 2022). While the Grain-for-Green project is the world's largest, massive forest restoration projects exist across the world in all types of forests (Lewis et al., 2019).

Forest restoration strategies are often focussed on planting trees. Well-planned tree planting efforts have been associated with mitigating climate change, conserving biodiversity, and providing food, wood, and income to small landowners (Holl and Brancalion, 2020). Planting trees increases aboveground C pools as trees sequester C from the atmosphere (Preston et al., 2020; Rumney et al., 2021). Tree planting has generally been thought to promote an increase in forest floor and mineral soil C (Ashwood et al., 2019; Laganière et al., 2010); but losses of soil C have also been reported (Friggens et al., 2020). The addition of new C into an ecosystem from tree planting can alter the structure and function of local decomposer communities (Kuzyakov, 2002). Planting trees can also increase the movement of nutrients and metals throughout the ecosystem (Kellaway et al., 2022).

The application of nutrients and liming agents are often used in forest restoration projects to increase soil nutrients and raise pH (Frouz et al., 2001; Lautenbach et al., 1995). Limestone has been used as a soil amendment to counteract soil acidity for over 100 years

(Saarsalmi et al., 2011). The chemical composition of limestone differs based on location, but generally limestone refers to either calcite ( $\text{CaCO}_3$ ) or dolomite ( $\text{CaMg}(\text{CO}_3)_2$ ). The application of limestone (or other high pH, nutrient rich amendments) has been found to reduce soil acidity, leading to the immobilization of metals and an increase in forest productivity (Lawrence et al., 2016). The increase in soil pH can also lead to unintended ecosystem changes such as altering litter decomposition by making soils more habitable for different soil biota (Persson et al., 2021). Applying bioavailable nutrients in the form of fertilizers (typically nitrogen (N), phosphorous (P), and K) have been shown to increase vegetation growth but may also alter other fundamental ecosystem processes (Pietrzykowski and Daniels, 2014).

Forest restoration strategies such as the application of limestone, fertilizers, and tree planting, to mining and smelting degraded landscapes is generally associated with repairing ecosystem structure and function. Vegetation becomes capable of establishing and growing (Frouz et al., 2009; Preston et al., 2020), forest floors begin to develop (Frouz et al., 2001; Rumney et al., 2021), and soil biotic communities begin to more closely reflect pre-degradation structure (Frouz et al., 2001). While the effects of forest restoration are largely positive, the long-term sustainability of restored forests and the effects of restoration on soil C are still unclear.

Studying biogeochemical processes within regreened forests will help to address uncertainties related to forest restoration. Currently, there are few examples of long-term studies (>25 years) in restored forests on mining and smelting degraded landscapes.

Ecological restoration is a relatively young field of study and forest restoration has only begun to be widely practiced in recent decades. A chronosequence study could be valuable to our scientific understanding of the biogeochemical processes within restored forests and how these processes change with stand age, will provide essential information towards the future management of these systems. Field experiments are a valuable tool to provide site-specific information that can be used to inform remote sensing models to project ecosystem properties such as aboveground biomass to the landscape scale (Lu et al., 2016). Similarly, data from the field studies can be used to amend existing temporal models to forecast forest dynamics such as C pools and fluxes (Metsaranta et al., 2017).

#### *1.4. The City of Greater Sudbury area*

The City of Greater Sudbury (UTM zone 17; Easting: 501620, Northing: 5148797) and surrounding region in northern Ontario, Canada, has been subject to legacy effects of long-term Cu and nickel (Ni) mining and smelting (1886 – present day). In 1960, the City of Greater Sudbury region was the world's largest point source of SO<sub>2</sub>, emitting 2.56 million tonnes of SO<sub>2</sub> per year – roughly 4% of global emissions at the time (Potvin and Negusanti, 1995). During that same time period, tens of thousands of tonnes of metal particulates were emitted from the region's smelters each year (Potvin and Negusanti, 1995). Smelting emissions and expansive clear-cutting created approximately ~20,000 ha of barren land with highly eroded, acidic soils enriched in toxic metal and depleted of essential nutrients, inhibiting the regrowth of vegetation (Freedman and Hutchinson, 1980a, 1980b) (Figure 1.1; Figure 1.2). Soil biological communities were devastated by

acidity and the presence of toxic metals leading to dramatic alterations of decomposition processes (Freedman and Hutchinson, 1980a).



**Figure 1.1:** City of Greater Sudbury barren areas prior to greening began. Large-scale clearcutting led to massive levels of soil erosion and acid and metal deposition prevented the re-establishment of vegetation (Winterhalder, 1995).



**Figure 1.2:** City of Greater Sudbury barren areas prior to greening. Acid and metal deposition devastated local decomposers leading to large amounts of old undecomposed material remaining on the landscape (Winterhalder, 1995).

### *1.5. The greening efforts of the City of Greater Sudbury area*

In 1978, the City of Greater Sudbury began a greening program that is regarded as one of the largest in the world. Since the advent of the greening program (which is still ongoing) SO<sub>2</sub> and particulate metal emissions have decreased by over 95%, more than 3,400 ha of land have been treated with lime, fertilizer, and a grass-legume seed mixture, and over 10 million trees have been planted (VETAC, 2021). The greening procedure includes the application of 10 Mg ha<sup>-1</sup> of crushed dolomitic limestone (CaMg(CO<sub>3</sub>)<sub>2</sub>), 390 kg ha<sup>-1</sup> of 6-24-24 N-P-K fertilizer, and 40 kg ha<sup>-1</sup> of a grass-seed mixture that included N-

fixing legumes (Figure 1.3). One-year following the amendment applications, trees are planted. Jack pine (*Pinus banksiana* Lamb.) and red pine (*Pinus resinosa* Ait.) were the most widely planted tree species because they are native to the Great Lakes-St. Lawrence region and are capable of surviving in acidic, sandy soils. Other native species were also planted including white pine (*Pinus strobus* L.), white spruce (*Picea glauca* Moench), and green alder (*Alnus viridis* Chaix) (VETAC, 2021). Areas for greening are selected each year based on multiple operational and resource factors and needs, resulting in a mosaic of forest stands of different ages in the barren and semi-barren areas of the region. The landscape remains highly heterogenous, with large areas of exposed bedrock with little-to no soil following massive erosion events that occurred after the onset of largescale mining and smelting practices. The greening program has led to an increase in soil nutrients (Kellaway et al., 2022; SARA Group, 2009), decreased metal bioavailability (Kellaway et al., 2022; Nkongolo et al., 2013), and increased aboveground C storage (Preston et al., 2020; Rumney et al., 2021). As one of the world's largest greening programs, the City of Greater Sudbury region provides a unique opportunity to investigate the biogeochemistry of the greened forests and how this changes as stands age.





**Figure 1.3:** Crushed dolomitic limestone was spread over 3,400 ha of land in the City of Greater Sudbury barren areas, largely done by hand by local volunteers (City of Greater Sudbury, 2023).

#### *1.6. Thesis objective*

The main objective of this thesis was to study the biogeochemistry of the City of Greater Sudbury regreened forests, and to determine how nutrient pools and cycling change as stands age. The patchwork nature of regreening, where different areas received regreening treatments at different times, provided us with the opportunity to develop a chronosequence of forested sites with differing stand ages (between 15–40 years). To account for the effects of erosion on biogeochemistry we established two series of sites:

eroded sites and stable sites. Eroded sites were heavily influenced by erosion and were largely covered by exposed bedrock (>30% bedrock cover). Stable sites were covered in a layer of soil (>90% soil cover) (Figure 1.4). Using a chronosequence approach that consisted of planted sites within the City of Greater Sudbury barren areas with consistent geologies, we assumed that the primary differences among sites were stand age and erosion class.



**Figure 1.4:** Field sites showing different site types in the current study. The left panel is a picture of an eroded site with an average mineral soil depth of 10.0 cm. The right panel is a picture of a stable site with an average mineral soil depth of 46.4 cm.

### *1.7. Thesis structure, chapter objectives and hypotheses*

This thesis is composed of five chapters with the intent to better understand the effects of greening on forest biogeochemistry in the City of Greater Sudbury. The first chapter is a general introduction while the research chapters (Chapters 2–4) are written as manuscripts. The final chapter (Chapter 5) is a conclusion chapter.

Chapter 2, “One of the world’s largest greening programs promotes healthy tree growth and nutrient accumulation up to 40-years post restoration” aimed to 1) assess how aboveground biomass (AGB) and AGB nutrient pools changed over time since greening at both highly eroded and stable sites using a space-for-time-approach; and 2) quantify AGB and AGB nutrient pools arising from the City of Greater Sudbury greening program. Aboveground biomass and aboveground nutrient pools were predicted to increase with stand age at both eroded and stable sites, but tree growth and aboveground nutrient increases were expected to be larger at the stable sites.

Chapter 3, “Soil carbon dynamics following the greening of a mining and smelting degraded landscape” aimed to determine how forest soil C dynamics change with stand age, and if forest soil C dynamics differ between highly eroded sites and sites with more stable soils. We tested the hypotheses that forest soil C cycling will change over stand age and with erosion class. Specifically we predicted that: 1) litterfall C inputs will increase with stand age and be higher at stable sites; 2) fine root production will decrease with stand age and there will be no difference between site types; 3) litter decomposition rates will increase with stand age and not differ between eroded and stable sites; 4) litter decomposition rates will be higher using site-specific versus common litter; 5) soil respiration will increase with stand age and be higher at stable sites; 6) forest floor C pools will increase with stand age and be higher at stable sites; 7) bulk mineral soil and aggregate C concentrations will increase with stand age and will be higher at stable sites; 8) total

ecosystem C pools will increase with stand age in our relatively young (~40 year old max) sites regardless of changes in soil C.

Chapter 4, “Nutrient cycling in jack and red pine forests following the regreening of a mining and smelting degraded landscape” aimed to assess how nutrient cycling differs with stand age following regreening. We tested the hypotheses that stand age following regreening affects forest nutrient cycling processes. Specifically, we predicted that: 1) concentrations of Ca, Mg, P, and K will decrease in the forest floor and increase in mineral soil and vegetation with stand age, while N concentrations in soils and trees will increase with age; 2) nutrient RE will increase with time since regreening while nutrient RP will decrease over time; 3) loss of litter nutrients through decomposition will increase as stands age; and 4) N mineralization in soil will increase with stand age.

#### *1.8. Authorship statements*

Chapter 2, “One of the world’s largest regreening programs promotes healthy tree growth and nutrient accumulation up to 40-years post restoration.”

Authors: *Patrick A. Levasseur, Jessica Galarza, Shaun A. Watmough*

**Patrick A. Levasseur** was responsible for: conceptualization, methodology, formal analysis, investigation, writing – original draft, writing – review & editing, visualization. **Jessica Galarza** was responsible for: methodology, and resources. **Shaun A. Watmough** was responsible for: conceptualization, methodology, resources, writing – review & editing, visualization, supervision, funding acquisition.

Chapter 3, “Soil carbon dynamics following the greening of a mining and smelting degraded landscape.”

Authors: *Patrick A. Levasseur, Julian Aherne, Nathan Basiliko, Erik J.S. Emilson, Michael D. Preston, Eric P.S. Sager, Shaun A. Watmough*

**Patrick A. Levasseur** was responsible for: conceptualization, methodology, formal analysis, investigation, writing – original draft, writing – review & editing, visualization. **Julian Aherne** was responsible for: writing – review & editing, supervision. **Nathan Basiliko** was responsible for: writing – review & editing, funding acquisition. **Erik J.S. Emilson** was responsible for: writing – review & editing, supervision. **Michael D. Preston** was responsible for: methodology, validation, writing – review & editing. **Eric P.S. Sager** was responsible for: writing – review & editing, supervision. **Shaun A. Watmough** was responsible for: conceptualization, methodology, validation, resources, writing – review & editing, visualization, supervision, funding acquisition.

Chapter 4, “Nutrient cycling in red and jack pine forests following the greening of a mining and smelting degraded landscape.”

Authors: *Patrick A. Levasseur, Shaun A. Watmough*

**Patrick A. Levasseur** was responsible for: conceptualization, methodology, formal analysis, investigation, writing – original draft, writing – review & editing, visualization. **Shaun A. Watmough** was responsible for: conceptualization, methodology, validation, resources, writing – review & editing, visualization, supervision, funding acquisition.

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## **Chapter 2: One of the world's largest greening programs promotes healthy tree growth and nutrient accumulation up to 40-years post restoration**

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### **2.0. Abstract**

Mining and smelting degraded landscapes are characterised by heavily eroded, acidic soils that are contaminated with toxic metals and depleted of essential nutrients. Increasing forest cover through greening of degraded landscapes has been highlighted to support carbon (C) mitigation measures and protect biodiversity. One of the world's largest greening programs in the City of Greater Sudbury, Ontario has been ongoing since 1978 and involves the liming and fertilization of selected areas followed by planting of primarily coniferous trees. In this study, we assessed how aboveground biomass (AGB) and aboveground nutrient (calcium (Ca), magnesium (Mg), nitrogen (N), phosphorous (P), potassium (K), and C) pools changed using a space-for-time approach. We established a series of sites ranging from 15 to 42 years since treatment. To determine the potential effects of erosion on AGB and AGB nutrient pools, each site was categorized as "stable" (less than 10% bedrock cover) or "eroded" (greater than 30% bedrock cover). Both AGB and AGB nutrient pools increased with time since greening at rates similar to conifer plantations grown in undisturbed regions. Individual tree growth and nutrient accumulation did not differ between stable and eroded sites; however, stable sites had a higher stem density leading to overall higher per hectare AGB and AGB nutrient pools.

Future N limitation of the regreening forests does not appear to be a concern as aboveground N pools were six times larger than applied N. Conversely, aboveground P concentrations decreased with time since tree planting and the 40-year-old study sites had aboveground P concentrations below values for “healthy” trees. This study shows that the regreening efforts have led to a massive addition of 1,144,588 Mg of AGB (550,547 Mg C) onto the landscape, and capable of sustaining healthy tree growth up to 40-years post regreening. However, as the regreening stands age, nutrient limitation may impact future tree growth and warrants further study.

### *2.1. Introduction*

Degraded landscapes with the potential to support forests are estimated to total 1.7–1.8 billion hectares and occur in almost every country in the world (Bastin et al. 2019). The Intergovernmental Panel on Climate Change (IPCC) and the International Union for the Conservation of Nature (IUCN) state that restoring forests on degraded landscapes will be essential to supporting global carbon (C) mitigation measures and protecting biodiversity (IUCN, 2020; Rogelj et al. 2018). However, concerns regarding planting forests on land that is needed for other purposes such as agriculture, limit the area of degraded land that is available for regreening; an exception to this is mining and smelting degraded landscapes where programs to increase forest cover are widely supported (Friedlingstein et al. 2019). Most regreening programs do not fully recover pre-degradation ecosystem services and successful programs require well-planned management (Holl & Brancalion, 2020).

Soils on mining and smelting impacted landscapes are often heavily eroded, acidic, contaminated with metals, depleted of essential nutrients, and require external nutrient inputs to support ecosystem function (Pietrzykowski & Daniels 2014). Applying crushed limestone and fertilizer prior to tree planting increases soil pH, microbial biomass, and nutrient levels and decreases the bioavailability of toxic metals (Kellaway et al. 2021; Narendrula-Kotha & Nkongolo, 2017). Tree growth has also been found to increase after lime and fertilizer application compared with unamended sites (Rumney et al. 2021). In forests, aboveground biomass (AGB) is a significant ecosystem pool of essential nutrients, such as calcium (Ca), magnesium (Mg), nitrogen (N), phosphorous (P), potassium (K), and C (Dielemen et al. 2020; Hooker & Compton 2003; Johnson & Todd 1998; Likens et al. 1994; Likens et al. 1998; Yanai 1992). Nutrients stored within AGB are not only essential for tree growth, the cycling of AGB nutrients to other ecosystem pools provides necessary nutrients to all other forest biota (Gordon & Jackson 2000; Gorgolewski et al. 2020; Neumann et al. 2018).

Aboveground biomass has traditionally been measured using field studies, which is regarded as the most accurate way to estimate AGB at small scales (Lambert et al. 2005; Lu 2005). Landscape scale AGB estimates are often conducted using a combination of field measurements and spatial characteristics collected from remote sensing such as Landsat (Seidel et al. 2011; Lu et al. 2016). High correlations between vegetation properties and spectral bands allow Landsat images to accurately estimate AGB across large areas (Lu 2005). Landsat images are free to access from the United States Geological Survey's (USGS)

EarthExplorer Program for any location across the globe, further increasing their popularity (USGS 2020). Many AGB predictive models using Landsat images are developed on fully forested sites (López-Serrano et al. 2020); or to assess the impacts of deforestation (Wu et al. 2016).

The City of Greater Sudbury, Ontario, Canada and surrounding region has been exposed to legacy effects of centuries of copper (Cu) and nickel (Ni) mining and smelting. Smelting emissions and expansive clear-cutting led to 20,000 ha of barren land with highly eroded soils depleted of essential nutrients (Gunn et al. 1995). Since 1978, sulphur dioxide and particulate metal emissions have been decreased by over 95%, and industry and government have invested over \$35 million (CAD) to begin one of the world's largest greening programs (City of Greater Sudbury 2020; VETAC 2020). The greening landscape remains highly heterogenous including large areas of exposed bedrock with little to no soil following massive erosion events. A recent study by Preston et al. (2020) measured AGB at forested sites (n=10) across the greening area. Preston et al. (2020) did not incorporate historic soil erosion into their site selection, but they highlight its potential to affect AGB pools.

The objectives of the current study were to: 1) assess how AGB and AGB nutrient pools changed over time since greening at both highly eroded and stable sites using a space-for-time approach. 2) quantify AGB and AGB nutrient pools arising from the City of Greater Sudbury greening program. To account for the effects of erosion on AGB growth and AGB nutrient accumulation we established two series of sites ranging in time since

regreening treatment was applied. One series of sites was heavily influenced by erosion and largely covered by exposed bedrock (>30% bedrock cover) (referred to as “eroded sites”); the other series was covered with a layer of soil (>90% soil cover) (referred to as “stable sites”) (Figure 2.1). Aboveground biomass and AGB nutrient pools were expected to increase with time since tree planting at both stable and eroded sites, but tree growth and AGB increases were expected to be larger at stable sites.



**Figure 2.1:** The left panel is a picture of CON4 an eroded site with an average mineral soil depth of 4.9 cm. The right panel is a picture of CON1 a stable site with an average mineral soil depth of 32.6 cm.

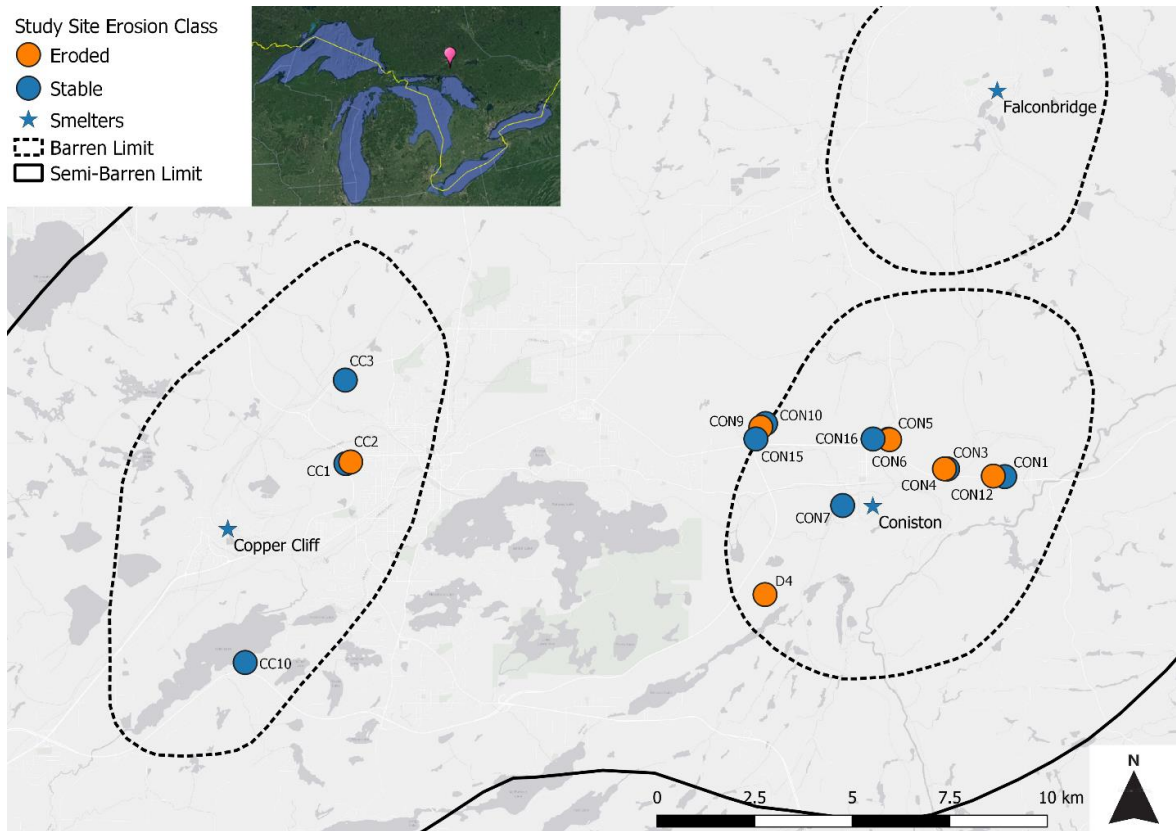
## 2.2. Materials and Methods

### 2.2.1. Study area and regreening process

The City of Greater Sudbury (UTM zone 17; Easting: 501620, Northing: 5148797) is in northern Ontario, Canada on the southern portion of the Precambrian Shield. Average monthly temperature ranges from -13.0 °C in January to 19.1 °C in July, and average precipitation is 904 mm per year (Environment Canada 2019). The City of Greater Sudbury



lies in the vegetation transition zone between the Great Lakes – St. Lawrence Forests regions of the south and the boreal forest to the north (Rowe 1972). The topography of the region is undulating and dominated by rocky outcrops overlain by soils that are typically shallow and sandy (Pearson & Pitbaldo 1995). Prior to regreening efforts, the soils within the barrens of the City of Greater Sudbury were highly acidic (pH <4.3), devoid of nutrients (particularly P, K, Ca, and Mg) and contained high concentrations of bioavailable Ni and Cu (Winterhalder 1995). In order to increase pH and soil nutrient concentrations over 3,400 ha of land were treated with 10 Mg ha<sup>-1</sup> of crushed dolomitic limestone (CaMg(CO<sub>3</sub>)<sub>2</sub>), 390 kg ha<sup>-1</sup> of 6-24-24 N-P-K fertilizer. A grass-seed mixture, that included N-fixing legumes, was applied at a rate of 40 kg ha<sup>-1</sup> after liming and fertilizing. Across the entire City of Greater Sudbury 9.9 million trees have been planted with tree species that are native to the region, predominantly: jack pine (*Pinus banksiana* Lamb.) and red pine (*Pinus resinosa* Ait.), but also included white pine (*Pinus strobus* L.), white spruce (*Picea glauca* Moench) and green alder (*Alnus viridis* Chaix) (VETAC, 2020). Areas selected for regreening are randomly selected each year resulting in a mosaic of forest stands of different ages.



**Figure 2.2:** Map of the City of Greater Sudbury, Ontario, Canada including sixteen study sites, the outlines for the barren and semi-barren regions, and the location of the three main smelters.

### 2.2.2. Site Selection

Sixteen sites varying in time since the greening treatment were established within the barrens of the Coniston and Copper Cliff smelters (Figure 2.2). The barrens were defined as areas that were devoid of plant life and had soil pH below 4.3 before greening efforts took place (Lautenbach et al. 1995). All sites were limed, fertilized, seeded, and planted with either red pine and/or jack pine. Planting dates ranged from 1979 to 2006, and sites that only received planting at one time were specifically selected. Sites were split

into two erosion classes based on visually estimated bedrock cover; highly eroded sites had greater than 30% bedrock cover and typically had very little soil. Sites with less than 10% bedrock cover and deeper soils were considered “stable”. Six eroded sites and ten stable sites were established. Each site was 30 m x 30 m in size and located within a larger forested area with similar characteristics.

### *2.2.3. Field sampling*

Field sampling was conducted in June 2021. At each site diameter at breast height (dbh) and tree species were recorded for every tree. Trees were categorized as woody plants with a dbh greater than 2.5 cm. Soil depth was measured at nine points at each site using a soil auger. Forest floor depths were taken at nine points using a 30 cm ruler after separating the forest floor from mineral soil using a knife. Elevation was measured using a Garmin eTrex Legend H GPS and slope was measured using a Suunto PM-5/1520 clinometer. Tree cores were taken from three trees at each site to verify age and used for wood nutrient analysis. Two cores were taken from each sampled tree, one at breast height, and the second 20 cm below at a 90° angle from the entrance point of the first core. Cores were stored in plastic straws with the ends taped. Bark samples were taken from these same trees at breast height by scraping bark off the tree using a knife and composited from the three trees in a polyethylene bag. Foliage samples were taken by hand from the highest possible branch from each sampled tree and composited into paper bags. Current year needles were targeted for foliage sampling. Most sites were monocultures of jack or red pine, and the three vegetation sampling trees were selected

to be the dominant species (greater than 33% of stems) for each site. On the occasion that both jack and red pine comprised greater than 33% of stems per site vegetation samples were taken from three trees of both species. All vegetation (cores, bark, and foliage) samples were stored in a refrigerator (4 °C) upon return from the field. Vegetation samples were only collected for eleven of the sixteen study sites.

#### *2.2.4. Chemical analysis*

All vegetation samples were oven-dried at 105 °C for 24-hours and then milled to a fine powder using a coffee grinder. Milled samples were analyzed for Ca, Mg, K and P by digesting 0.2 g of sample in 2.5 mL of concentrated nitric acid (trace grade 70% nitric acid (HNO<sub>3</sub>)) at 100 °C for eight hours followed by a digestion at room temperature for eight hours. The digested material was filtered through a 0.45 µm pore filter paper and diluted using B-pure until total volume reached 25 mL. The digest samples were then diluted 1:10 using B-pure and analyzed using a Perkin Elmer Inductively Ion Coupled Optical Emission Spectrometer (7000 DV). Carbon and N values were determined for the milled vegetation samples using an Elementar Macro CNS analyzer. Quality control was confirmed by running all samples in triplicate and including experimental blanks as well as a NIST-1515 apple leaf standard every 25 samples.

#### *2.2.5. Aboveground biomass and nutrient pools*

Aboveground biomass was calculated for each site using dbh-based allometric equations from Lambert et al. (2005). Aboveground nutrient (Ca, Mg, N, P, K, and C) pools were determined using measured nutrient concentrations for each biomass component

(bark, foliage, wood + branches) and the mass of each biomass component from the allometric equations. Tree core chemistry was used for both wood and branches biomass components. For sites where both jack and red pine made up greater than 33% of stems nutrient concentrations for both species were averaged. Aboveground biomass pools were calculated for all sixteen study sites, aboveground nutrient pools were calculated for the eleven sites where chemical analyses on vegetation samples were conducted. Aboveground biomass and nutrient accumulation rates were determined by dividing AGB or nutrient pool size by years since tree planting, which was one year after the application of lime and fertilizer.

#### *2.2.6. Landsat image processing*

The Landsat image was acquired from USGS Landsat 8 OLI program from July 9, 2021 (path: 19 and row: 28). A level 1T (L1T) image was selected that had less than 5% cloud cover. Landsat Level-1 products are geometrically corrected by orthorectification of ground control points and digital elevation models (USGS 2019). The image was radiometrically and atmospherically corrected using the semi-classification plugin based on the dark object subtraction (DOS1) technique (Chavez 1996) on Quantam GIS (QGIS) Version 3.2.1 (QGIS.org 2020).

#### *2.2.7. Random forest model development and application*

A random forest (RF) model was developed using Landsat 8 OLI bands 2–7, and ten vegetation indices (VIs) (Table 2.1) at 35 sites within the City of Greater Sudbury barren areas where AGB was measured. Each remote sensing variable included in model

development has previously been shown to reflect various components of AGB (Chenge & Osho, 2018; López-Serrano et al. 2020; Zhang et al. 2019). The 35 sites used for model development included: the sixteen study sites, ten sites planted with red and/or jack pine from Rumney et al. (2021), and nine sites that had an AGB of zero (three areas with bare exposed bedrock, three roads, and three lakes). The study sites and zero AGB sites were 30 m x 30 m squares while the Rumney et al. (2021) sites were circular plots with a diameter of 35.68 m.

To assess AGB and AGB nutrient pools for the regreening areas the RF model was applied to the 19,649 ha where red and/or jack pine had been planted across the barren areas of the City of Greater Sudbury. Magnesium, K, and C AGB concentrations were determined by averaging the AGB nutrient concentrations from the study sites. Calcium, P, and N AGB concentrations were determined using a linear regression based on the results from the study sites where nutrient concentration changed with time since tree planting. Nutrient pools were determined for 7,216 ha of the barren areas where red and/or jack pine were planted, and liming and fertilizing took place. Location and dosage rate of lime and fertilizer applications were available from the City of Greater Sudbury regreening data (City of Greater Sudbury 2020).

**Table 2.1:** Vegetation indices included in random forest to develop predictive model for aboveground biomass using remotely sensed data. Landsat 8 OLI band number depicted using *b*. *L* is soil brightness correction factor and its value is 0.5.

Vegetation Index	Equation	Reference
Brightness index (BI)	$b2 * 0.3029 + b3 * 0.2786 + b4 * 0.4733 + b5 * 0.5599 + b6 * 0.508 + b7 * 0.1872$	Baig et al. (2014)
Green normalized difference vegetation index (GNDVI)	$(b5 - b3)/(b5 + b3)$	Gitelson et al. (1996)
Greenness vegetation index (GVI)	$b2 * -0.2941 + b3 * -0.243 + b4 * -0.5424 + b5 * 0.7276 + b6 * 0.0713 + b7 * -0.1608$	Baig et al. (2014)
Land surface water index (LSWI)	$(b5 - b6)/(b5 + b6)$	Xiao et al. (2004)
Modified soil adjusted vegetation index (MSAVI)	$((b5 - b4)/(b5 + b4)) * (1 + L)$	Qi et al. (1994)
Normalized difference infrared index (NDII)	$(b5 - b6)/(b5 + b6)$	Kimes et al. (1981)
Normalized difference infrared index with SWIR2 (NDII7)	$(b5 - b7)/(b5 + b7)$	Kimes et al. (1981)
Normalized difference vegetation index (NDVI)	$(b5 - b4)/(b5 + b4)$	USGS, (2016)
Normalized difference water index (NDWI)	$(b3 - b6)/(b3 + b6)$	Gao, (1996)
Wetness index (WI)	$b2 * 0.1511 + b3 * 0.1973 + b4 * 0.3283 + b5 * 0.3407 + b6 * -0.7117 + b7 * -0.4559$	Baig et al. (2014)

### *2.2.8. Statistical analysis*

The relationship between AGB (at the hectare and stem scale), and AGB nutrient concentrations, with time since tree planting was assessed using regression coefficient ( $R^2$ ) values from linear regression. Normality was tested for using the Lilliefors corrected Kolmogorov-Smirnov test, variables were considered normally distributed if  $p$  was greater than 0.05. Differences in AGB and nutrient accumulation rates between erosion classes were compared using an independent two-group Mann-Whitney-Wilcoxon test. Differences and relationships were determined to be significant if  $p$  was less than 0.05, except for differences in nutrient accumulation rates between erosion classes where differences were significant if  $p$  was less than 0.008 following a Bonferroni correction for multiple comparisons. The AGB predictive model was developed using RF regression for seventeen remotely sensed predictor variables and AGB measurements from 35 sites. Random forest was conducted through the Forest-based Classification and Regression function in ArcGIS Pro version 2.8.2. (ArcGIS Pro, 2021). The training of the RF model was conducted on 90% of the available data with 10% being held back for validation. The selected RF model included 1,500 decision trees, 5 randomly selected variables, and 4 nodes per tree. The RF model was evaluated on the training data set using residual mean squared error (RMSE) and regression coefficient ( $R^2$ ) values and validated using the  $R^2$  value from the validation data set as well as out-of-bag RMSE. All statistical analysis was done on R version 4.0.2 (Rstudio Team 2020). All maps were created using Quantam GIS (QGIS) Version 3.2.1 (QGIS.org 2020).



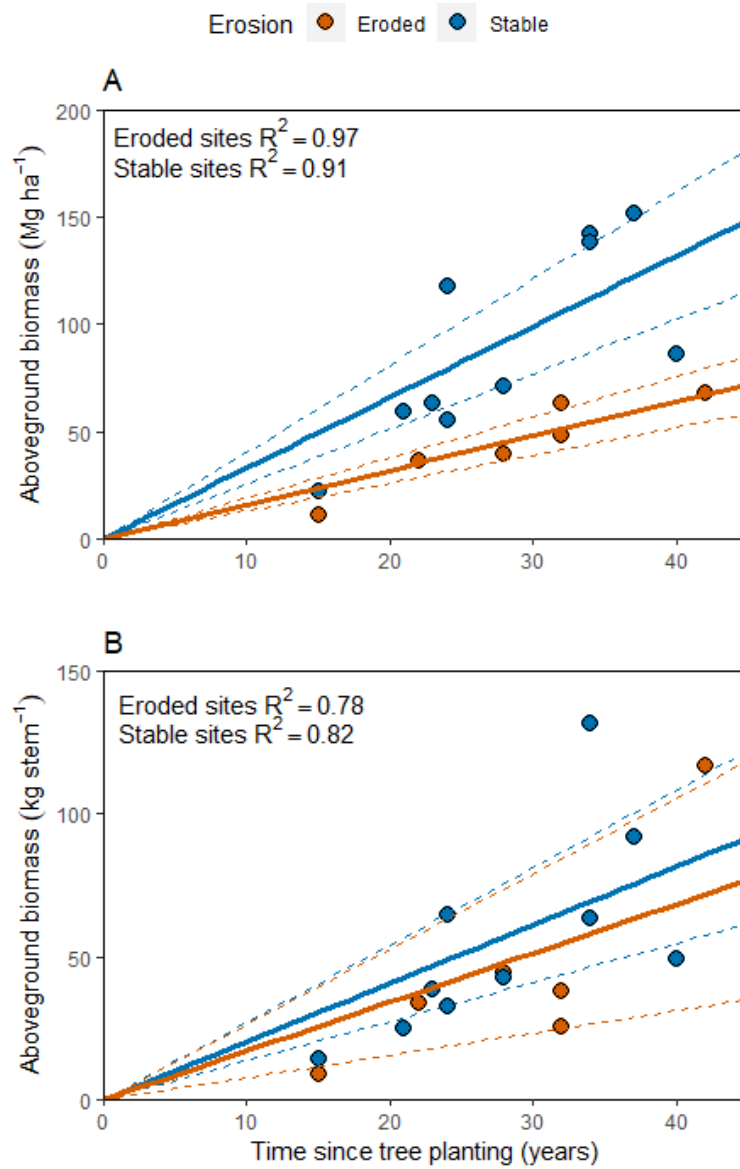
## 2.3. Results

### 2.3.1. Site characteristics

Stand age ranged from 15 to 42 years old with a median age of 27 years old (Table 2.2). Median eroded site age was 30 years old while median stable site age was 26 years old. Fifty percent of the total sites were dominated by jack pine, 25% by red pine and 25% were a mix of red and jack pine (Table 2.2). Stem density ranged from 578 to 2,378 stems  $\text{ha}^{-1}$  with a median stem density of 1,660 stems  $\text{ha}^{-1}$ . Eroded sites had a median stem density of 1,144 stems  $\text{ha}^{-1}$  while stable sites had a stem density of 1,668 stems  $\text{ha}^{-1}$ . Average tree dbh ranged from 5.1 cm to 16.0 cm with a median of 9.5 cm. Both eroded and stable sites had a median tree dbh of 9.5 cm. Soil depth was a median of 25.1 cm deep for stable sites and 6.0 cm for eroded sites.

**Table 2.2:** Site characteristics of sixteen study sites within the barren areas of the City of Greater Sudbury, Ontario, Canada used to develop relationship between aboveground biomass and stand age.

Site	Easting	Northing	Age (years)	Erosion class	Elevation (m)	Slope (°)	Dominant species	Tree density (stems ha <sup>-1</sup> )	DBH (cm)	Forest floor depth (cm)	Mineral soil depth (cm)
CC1	498390	5148618	24	Stable	309	4	Jack pine	1,822	14.3	4.58	15.06
CC2	498513	5148661	32	Eroded	300	15	Jack pine	1,866	8.1	1.07	3.56
CC3	498375	5150759	23	Stable	298	0	Red pine	1,644	10.9	1.32	25.87
CC10	495810	5143521	37	Stable	215	8	Red pine	1,655	14.7	5.00	23.33
CON1	515267	5148285	34	Stable	270	15	Jack pine	1,078	14.3	4.35	32.62
CON3	513804	5148482	40	Stable	211	8	Jack pine	1,744	8.1	1.78	14.31
CON4	513724	5148484	32	Eroded	258	13	Jack pine	1,666	8.8	0.88	4.94
CON5	512289	5149244	28	Stable	268	3	Mixed	1,666	7.4	4.25	22.94
CON6	512320	5149231	28	Eroded	281	6	Red pine	889	13.2	1.63	10.06
CON7	511116	5147545	21	Stable	241	0	Mixed	2,378	7.0	1.37	46.40
CON9	509020	5149553	15	Eroded	304	6	Jack pine	1,211	5.1	1.03	6.00
CON10	509140	5149646	15	Stable	290	14	Mixed	1,500	6.5	0.92	25.06
CON12	513979	5148306	42	Eroded	278	17	Jack pine	578	16.0	3.50	6.22
CON15	508894	5149251	24	Stable	215	14	Mixed	1,711	7.7	5.50	23.33
CON16	511895	5149249	34	Stable	278	2	Red pine	2,178	14.5	7.00	24.33
D1	509127	5145262	22	Eroded	295	2	Jack pine	1,078	10.2	1.33	4.87



**Figure 2.3:** Relationship between aboveground biomass (AGB) and time since tree planting for both highly eroded sites and stable sites. Figure 2.3A depicts relationship at the hectare scale; Figure 2.3B is per individual stem. Dashed lines represent 95% confidence interval.

### 2.3.2. Aboveground biomass pools

Aboveground biomass ( $\text{Mg ha}^{-1}$ ) had a significant positive linear relationship with time since tree planting at the site scale for both the eroded ( $p < 0.01$ ) and stable sites ( $p < 0.01$ ) (Figure 2.3A). Stable sites ( $2.78 \pm 1.05 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ) had a significantly larger ( $p < 0.01$ ) AGB growth rate compared with eroded sites ( $1.55 \pm 0.38 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ). Aboveground biomass ( $\text{kg stem}^{-1}$ ) also had a significant positive linear relationship with time since tree planting at the stem scale for eroded ( $p < 0.01$ ) and stable sites ( $p < 0.01$ ), however, AGB growth rates per stem were not significantly different ( $p = 0.30$ ) between eroded ( $1.34 \pm 0.71 \text{ kg stem}^{-1} \text{ year}^{-1}$ ) and stable sites ( $1.59 \pm 0.85 \text{ kg stem}^{-1} \text{ year}^{-1}$ ) (Figure 2.3B). Relationships between AGB and time since tree planting at both the hectare and stem scale were also significant when no intercept was included ( $p < 0.01$ ).

**Table 2.3:** Nutrient concentrations for each biomass component. Both the measured concentrations from the study sites and the concentrations listed for red pine from the Tree Chemistry Database (TCD) (Pardo et al. 2005) are included.

Nutrient	Bark		Foliage		Wood	
	Study sites ( $\text{g kg}^{-1}$ )	TCD ( $\text{g kg}^{-1}$ )	Study sites ( $\text{g kg}^{-1}$ )	TCD ( $\text{g kg}^{-1}$ )	Study sites ( $\text{g kg}^{-1}$ )	TCD ( $\text{g kg}^{-1}$ )
Ca	$3.78 \pm 2.17$	$7.65 \pm 3.91$	$3.67 \pm 1.81$	$4.17 \pm 1.60$	$0.80 \pm 0.15$	$1.09 \pm 0.35$
Mg	$0.31 \pm 0.14$	$0.46 \pm 0.13$	$1.27 \pm 0.62$	$0.86 \pm 0.34$	$0.19 \pm 0.05$	$0.19 \pm 0.12$
N	$4.82 \pm 1.28$	$3.10 \pm 0.38$	$10.46 \pm 1.16$	$11.49 \pm 1.30$	$1.24 \pm 0.40$	$0.82 \pm 0.11$
P	$0.33 \pm 0.20$	$0.44 \pm 0.08$	$1.27 \pm 0.33$	$1.28 \pm 0.16$	$0.03 \pm 0.03$	$0.08 \pm 0.02$
K	$0.47 \pm 0.47$	$0.88 \pm 0.27$	$4.36 \pm 1.59$	$3.67 \pm 1.00$	$0.35 \pm 0.12$	$0.24 \pm 0.09$
C	$525.92 \pm 26.15$	Not available	$492.50 \pm 15.73$	Not available	$480.59 \pm 5.90$	Not available

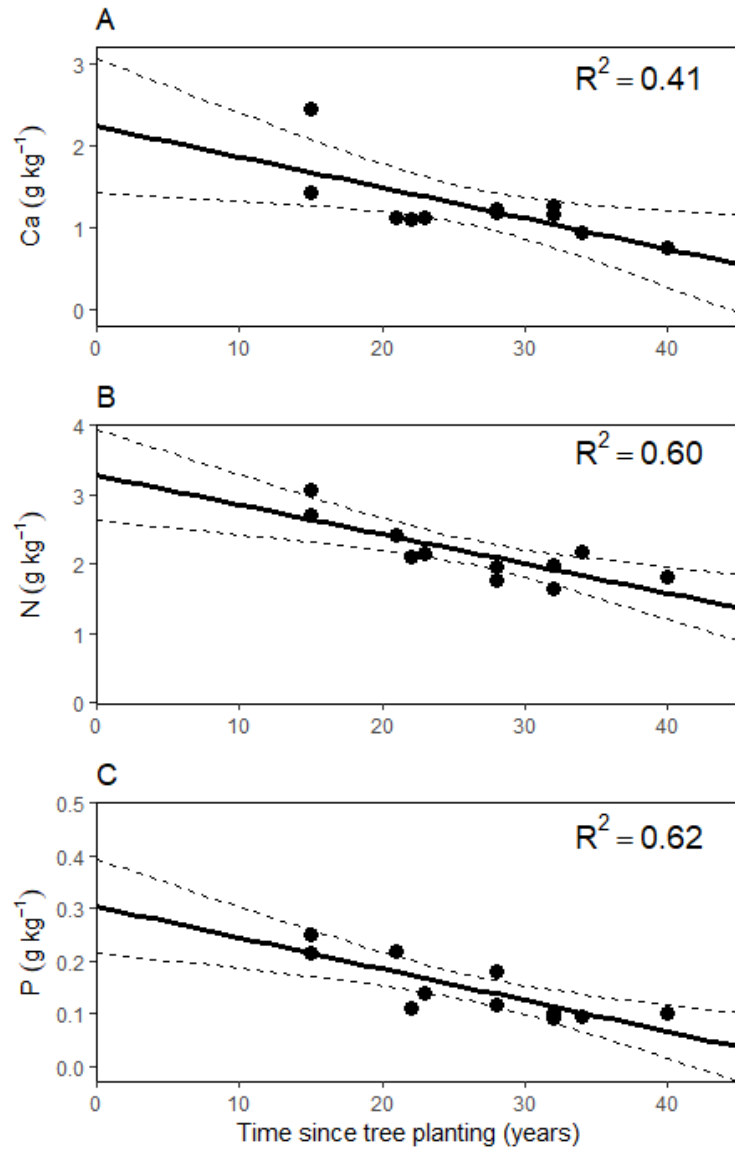
### 2.3.3. Aboveground nutrients

Aboveground Ca, Mg, N, P, and K concentrations did not differ between eroded and stable sites and median concentrations were within a standard deviation of healthy red

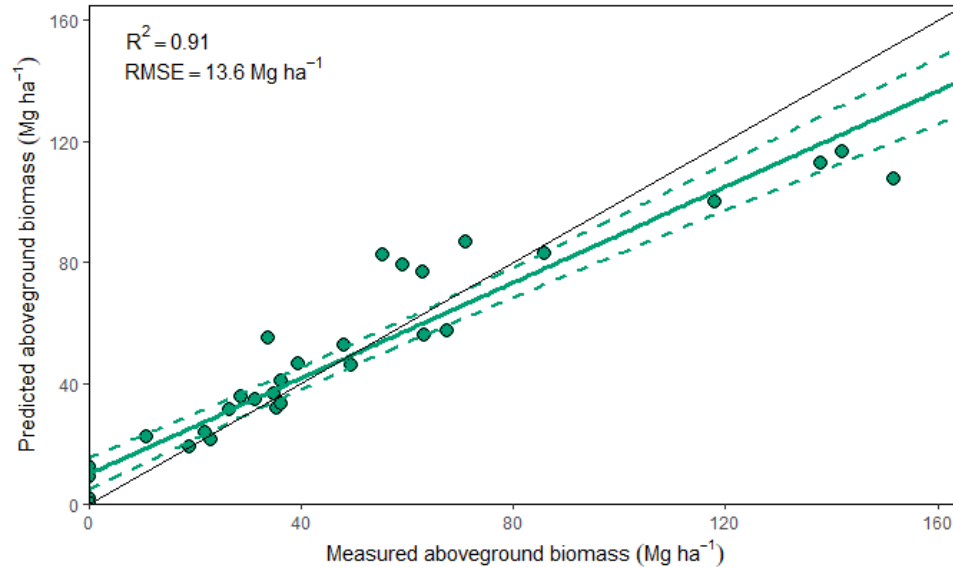
pine nutrient concentrations included in the United States Department of Agriculture Tree Chemistry Database (TCD) (Pardo et al. 2005) (Table 2.3). Carbon concentration for wood at the study sites was  $48.1 \pm 0.6\%$ , firmly in between the Martin et al. (2018) values for boreal ( $46.8 \pm 0.6\%$ ) and temperate conifers ( $50.1 \pm 0.4\%$ ). Aboveground nutrient concentrations were not significantly different between eroded or stable sites ( $p > 0.05$ ) (Table 2.4). Accumulation rates of N ( $p < 0.008$ ), P ( $p < 0.008$ ), K ( $p < 0.008$ ), and C ( $p < 0.008$ ) within AGB were significantly higher for stable versus eroded sites at the hectare scale. However, at the stem scale there was no significant difference in nutrient accumulation rates between site erosion classes ( $p > 0.05$ ) (Table 2.4). There was a significant negative linear relationship between AGB concentration of Ca ( $p < 0.05$ ) (Figure 2.4A), N ( $p < 0.01$ ) (Figure 2.4B), and P ( $p < 0.01$ ) (Figure 2.4C) and time since tree planting and no relationship between concentration of Mg, K, or C in AGB and time since tree planting ( $p > 0.05$ ).

**Table 2.4:** Aboveground biomass nutrient concentrations and accumulation rates (at both the hectare and stem scale). Asterix denotes significant difference in nutrient concentration, or accumulation rate between eroded and stable erosion classes ( $p < 0.05$ ).

Nutrient	Erosion Class	Nutrient		
		concentrations ( $\text{g kg}^{-1}$ )	Accumulation Rate ( $\text{kg ha}^{-1} \text{ year}^{-1}$ )	Accumulation Rate ( $\text{g stem}^{-1} \text{ year}^{-1}$ )
Calcium	Eroded	$1.44 \pm 0.51$	$1.80 \pm 0.29$	$1.49 \pm 0.34$
	Stable	$1.10 \pm 0.21$	$3.06 \pm 0.76$	$1.61 \pm 0.88$
Magnesium	Eroded	$0.31 \pm 0.08$	$0.48 \pm 0.16$	$0.26 \pm 0.20$
	Stable	$0.26 \pm 0.05$	$0.74 \pm 0.23$	$0.41 \pm 0.19$
Nitrogen	Eroded	$2.07 \pm 0.35$	$2.77 \pm 0.69^*$	$2.34 \pm 0.76$
	Stable	$2.22 \pm 0.43$	$5.18 \pm 1.77^*$	$2.90 \pm 2.10$
Phosphorous	Eroded	$0.14 \pm 0.05$	$0.18 \pm 0.04^*$	$0.13 \pm 0.07$
	Stable	$0.15 \pm 0.06$	$0.37 \pm 0.12^*$	$0.24 \pm 0.07$
Potassium	Eroded	$0.61 \pm 0.17$	$0.75 \pm 0.16^*$	$0.67 \pm 0.18$
	Stable	$0.67 \pm 0.15$	$1.51 \pm 0.38^*$	$0.90 \pm 0.34$
Carbon	Eroded	$483.53 \pm 3.16$	$726.67 \pm 199.51^*$	$574.17 \pm 188.70$
	Stable	$485.62 \pm 6.36$	$1279.70 \pm 402.59^*$	$669.08 \pm 475.10$



**Figure 2.4:** Relationship between aboveground nutrient concentrations and time since tree planting for calcium (Ca) (Figure 2.4A), nitrogen (N) (Figure 2.4B), and phosphorous (P) (Figure 2.4C). Dashed lines represent 95% confidence intervals. Regression coefficient ( $R^2$ ) of relationship between time since tree planting and nutrient concentration is included.



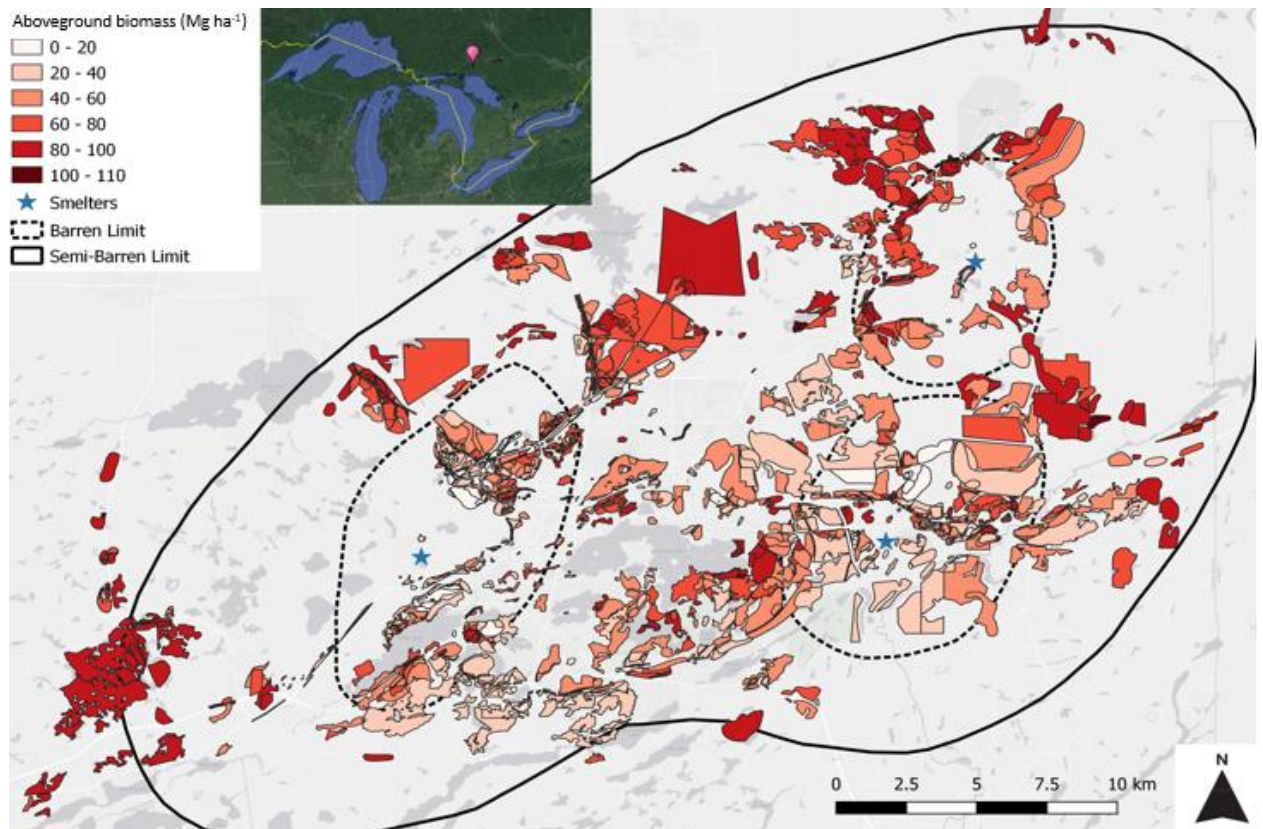
**Figure 2.5:** Predicted aboveground biomass (AGB) values compared with measured AGB for 35 sites where AGB was calculated. Black solid line represents 1:1 line. Solid green line represents regression line. Dashed green lines represent 95% confidence intervals. Relationship between measured and predicted AGB assessed using root mean square error (RMSE) and regression coefficient ( $R^2$ ).

#### 2.3.4. Random forest model and landscape-scale dynamics

Aboveground biomass predictions from the RF model had a  $R^2$  of 0.91 and RMSE of  $13.6 \text{ Mg ha}^{-1}$  when compared with AGB measurements from the training data set (Figure 2.5). Validation of the RF model revealed a  $R^2$  of 0.91 when compared with AGB measurements of the validation data set and an out-of-bag RMSE of  $31.35 \text{ Mg ha}^{-1}$ . Total AGB predicted using the RF model for red and jack pine planted sites (19,649 ha) of the City of Greater Sudbury is 1,144,588 Mg (550,547 Mg C) (Figure 2.6). The mass of applied Ca and Mg was 20–100 times greater than the mass of applied N, P, or K (in the form of 6-



24-24 fertilizer) and Ca and Mg pools in AGB amounted to less than 5% of the applied Ca and Mg (Table 2.5). In contrast, the mass of N stored in AGB was over six times higher than the amount of N applied in fertilizer and the proportion of P and K stored within AGB compared relative to applied nutrients was also substantial (Table 2.5).



**Figure 2.6:** Aboveground biomass for red pine and jack pine planting sites across the City of Greater Sudbury, Ontario, Canada.

**Table 2.5:** Comparison of applied nutrients (applied as either lime or fertilizer) with nutrients stored in aboveground biomass (AGB). Aboveground biomass nutrient pools are for all sites in the City of Greater Sudbury that were limed, fertilized, and planted with red and/or jack pine (7,216 ha).

Nutrients	Applied (Mg)	Nutrient concentration (g kg <sup>-1</sup> )	Proportion of applied nutrients stored in AGB (%)
Calcium	12,707	1.25 ± 0.41	3.74
Magnesium	7707	0.28 ± 0.07	1.17
Nitrogen	126	2.16 ± 0.40	614.41
Phosphorous	239	0.14 ± 0.06	24.84
Potassium	454	0.64 ± 0.16	45.15

## 2.4. Discussion

### 2.4.1. Aboveground biomass pools and accumulation rates

Greater AGB pools at stable versus eroded sites was initially assumed to be due to deeper soils leading to increased stem growth, as both jack pine and red pine have been found to be more productive in deeper soils (Rudolph & Laidly 1990; Rudolph 1990). However, this was not the case as individual stem growth did not differ between the two erosion classes and differences in hectare scale AGB totals is driven by stem density. Initial planting densities reported by the City of Greater Sudbury (2020) were similar to measured stem density at the study sites, suggesting that the higher stem density on stable sites is likely due to increased planting density as opposed to other factors such as increased survivorship. Previous studies assessing C accumulation rates of jack and red pine plantations in northern Ontario, Canada (Hunt et al. 2010), and Manitoba, Canada (Park, 2015), converted AGB growth to C accumulation rates assuming C comprised 48%

of AGB for red pine and 50% for jack pine (Hunt et al. 2010; Park 2015). The individual stem growth rates from both Hunt et al. (2010) ( $1.33 \pm 0.33 \text{ kg stem}^{-1} \text{ year}^{-1}$ ) and Park (2015) ( $0.92 \pm 0.53 \text{ kg stem}^{-1} \text{ year}^{-1}$ ) were very similar to values measured in the current study. Despite the history of highly acidified soils and high total metal concentrations, the trees in the regreening sites of the City of Greater Sudbury are growing at similar rates to those of jack and red pine plantations in relatively undisturbed areas regardless of erosion class.

#### *2.4.2. Aboveground nutrient concentrations and accumulation rates*

While the median AGB nutrient concentrations for the study sites are like the healthy values from the TCD, concentrations of Ca, P, and N decreased with increasing time since tree planting. A decrease in total AGB concentration of Ca, N, and P with increasing stand age has been documented previously as these nutrients are stored in high concentrations in foliage which accumulates mass very quickly in young trees as opposed to the slower growing, more nutrient poor wood (MacLean and Wein, 1977). The magnitude of the decrease for Ca and P at the study sites is cause for some concern as trees at the two oldest study sites (CON12 and CON3) have bark and wood Ca and P concentrations well below TCD values. Conversely, despite the decreasing concentration of N with time since tree planting AGB N concentration at the oldest sites exceed TCD values.

Aboveground nutrient accumulation rates did not differ between erosion classes once stem density is controlled for. Accumulation rates of Ca, Mg, N, P, K, and C for the study sites increase over time with a pattern similar to AGB growth and closely reflect values from coniferous plantations in similar climates (Alban et al. 1978; Hunt et al., 2010; MacLean and Wein, 1977; Morrison, 1973; Park, 2015). Morrison (1973) assessed nutrient accumulation in a 30-year-old jack pine plantation in northern Ontario, Canada at both high- and low-quality sites defined by biomass yield. The accumulation rates of AGB Ca, Mg, P, and K at the City of Greater Sudbury greening study sites closely reflected stands from Morrison (1973) grown on poorer quality soils, while AGB N accumulation rates were comparable with the higher quality sites. The similarities of tree growth rates and aboveground nutrient pools between the greening sites and plantations unimpacted by mining and smelting practices suggest soil amelioration followed by tree planting can lead to successful restoration goals on acidified and heavy metal contaminated soils.

#### *2.4.3. Random Forest estimates of aboveground biomass pools in greening area*

A RF model incorporating ten VIs and Landsat 8 OLI bands 2–7 was a successful predictor of AGB at the current study's greening sites, further supporting the potential of RF models using Landsat data to predict AGB pools (Gao et al. 2018; Jiang et al. 2021; López-Serrano et al. 2020; Zhang et al. 2019). The strong relationship and low error for the RF model in the current study could be attributed to the greening forests low complexity and low AGB. The oldest greening site is only 42 years old and all the sites are often a monoculture with limited understory. It has been reported that models using VIs have

decreased accuracy with high complexity forests and saturate at AGB values greater than 150 Mg ha<sup>-1</sup> (Lu 2005; Lu et al. 2016). The limited sample size of the current study could also be cause for the high R<sup>2</sup> and low RMSE values; few studies have investigated AGB pools within the study area, leading to limited access to AGB measurements.

The total AGB for red and jack pine stands across 19,649 ha within the City of Greater Sudbury is 1,144,588 Mg. Using an average of the eroded and stable site growth-curves the average AGB of 58.25 Mg ha<sup>-1</sup> implies that the average site is 27.3 years old. As the remediated stands continue to age, we expect AGB to increase as jack pine accumulates biomass up to 100 years old and red pines can accumulate biomass until up to 300 years old (Rudolph & Laidly 1990; Rudolph 1990). The total AGB C pool of 550,547 Mg C is the equivalent to C emissions from 319,158 cars over one year (3.67% of the registered vehicles in the Province of Ontario) (NRCAN 2014). Applying the Canadian government's price for C at \$50 CAD per Mg, the AGB C pools of the greening sites of the City of Greater Sudbury is equal to \$27.53 million CAD. The current study's AGB C projections only include sites planted with jack and red pine, while jack and red pine were the most planted tree species, there are regions planted with other conifer and deciduous species that are not included in this total (VETAC, 2020).

#### *2.4.4. Landscape-scale aboveground nutrient pools*

A concern for long-term forest growth on degraded landscapes is lack of nutrients. To overcome the low nutrient concentrations of the City of Greater Sudbury's barren soils the greening program applied massive quantities of nutrients (particularly Ca and Mg)

to the landscape (Winterhalder 1996). Only 4% of applied Ca and 1% of applied Mg were stored within AGB, this is most likely due to large-scale erosion and run-off removing Ca and Mg from the landscape. A recent study conducted on the same sites as the current study found that large quantities of applied Ca and Mg were unaccounted for in nutrient budgets (Kellaway et al. 2021). High percentages of P, and K were accumulated within AGB which could also be an indication of nutrient limitation and P concentrations in biomass decrease as stands age. Phosphorous has often been reported to be the primary limiting nutrient within the barrens of the City of Greater Sudbury (Beckett & Negusanti 1990; Lautenbach et al. 1995; Munford et al. 2021). The limited soil organic matter and slow decomposition rates caused a limited source of available P and the low soil pH caused large proportions of available P to be bound to inorganic complexes (Marschner 1991). Previous studies on the City of Greater Sudbury barrens found that when liming and fertilizer has been applied vegetation rapidly accumulates P in biomass (Winterhalder 1996). Little work has been done on K limitation in Sudbury, however, liming and fertilizing has been found to either not impact or decrease bioavailable K in soils within the City of Greater Sudbury (Nkongolo et al. 2013; SARA Group 2009). Despite the limited bioavailability of K in soils, Kellaway et al. (2021) found that the equivalent of 95% of applied K at the study sites was stored in biomass.

Limited work has been done on N dynamics in the regreening forests within the City of Greater Sudbury, but this study showed that N pools stored within AGB were found to be over six times as high as the amount applied in fertilizer, implying trees were

accumulating N from additional sources outside of fertilizer. The SARA Group (2009) found that total N concentrations in forest soils of the City of Greater Sudbury barrens were lower than those of reference forests, however, residual organic matter in the forest soils of the barrens has been proposed to be a sufficiently large pool to sustain vegetation communities (Winterhalder 1995). The pre-regreening forest soil N pools may make up the difference between N applied as fertilizer and N stored in AGB. Additionally, N-fixing species such as *Lotus corniculatus* (Bird's-foot trefoil), *Trifolium hybridum* (Alsike clover) were planted widely during regreening, and the native *Comptonia peregrina* (Sweet fern), and *Pleurozium sp.* (Feather moss) have become common in the recovering jack pine stands (Lautenbach et al. 1995).

## 2.5. Conclusions

The City of Greater Sudbury regreening program is regarded as one of the world's largest and has led to over one million tonnes of AGB (over 500,000 tonnes of C) establishing in the 19,649 ha of regreening area over 40 years. Regreening has led to individual trees growing and accumulating nutrients at rates like jack and red pine plantations grown in areas unimpacted by mining and smelting practices. Aboveground nutrient concentrations exceeding TCD values (particularly N) similarly suggest that up to 40 years post regreening, the soil amendments applied to the regreening areas appear to sufficiently support healthy tree growth. This finding emphasizes the ability of regreening treatments to successfully restore tree growth up to 42-years following treatment on highly eroded, acidified, and metal contaminated soils. Some cause for concern going

forward is aboveground P concentrations are decreasing as trees age, and 40-year-old study sites already have aboveground P concentrations below “healthy” tree values. Phosphorous limitation has been previously documented in the greening areas and future studies should continue to investigate potential future nutrient limitation.

## *2.6. Acknowledgements*

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## **Chapter 3: Soil carbon dynamics following the greening of a mining and smelting degraded landscape**

### *3.0. Abstract*

Increasing forest cover through the greening of mining and smelting degraded landscapes provides an opportunity for global carbon (C) sequestration. However, the reported effects of greening on soil C processes are mixed. One of the world's largest greening programs is in the City of Greater Sudbury, Canada and has been ongoing since 1978. Prior to greening, the soils in the City of Greater Sudbury area were highly eroded, acidic, rich in bioavailable metals, and poor in nutrients. This study used a chronosequence approach to investigate how forest soil C dynamics have changed with stand age in highly "eroded" sites with minimal soil cover (n=6) and "stable" sites covered by soil (n=6). The relationship between stand age and soil C processes (litterfall, litter decomposition, soil respiration, fine root growth) at both stable and eroded sites, were comparable to observations reported for jack pine (*Pinus banksiana* Lamb.) and red pine (*Pinus resinosa* Ait.) plantations that have not been subject to over a century of industrial impacts. There was a "home-field advantage" with respect to litter decomposition, where litter decomposition rates were higher using a site-specific litter compared with a common litter. Higher soil respiration at eroded sites was due to higher soil temperature likely because of a more open canopy. Mineral soil C and aggregate C concentration decreased with stand age. Soil C – particularly forms protected in aggregates – is generally considered the most stable terrestrial C pool and the effects of greening on soil C (while still poorly

understood) have been found to be highly dependent on local environmental conditions. While the effects of greening on soil C warrants further investigation, at the City of Greater Sudbury greening sites the loss of soil C is small relative to the substantial increases in aboveground tree and forest floor C pools leading to a sizeable increase in total ecosystem C pool following application of greening methods.

### *3.1. Introduction*

Soils are one of the largest global carbon (C) pools, with more C stored in soils than the atmosphere and vegetation combined (Scharlemann et al., 2014). Soil C is made up of both inorganic and organic forms; soil organic C comprises two thirds of global soil C and is derived from residual decaying vegetation and the metabolic activities of living organisms, including fungal and bacterial growth (Batjes, 1996). Further, soil organic C stored in forest soils makes up roughly 45% of the global soil organic C pool (Jobbágy and Jackson, 2000; Lal, 2004), with the remainder mostly stored in grasslands and wetlands. Increasing forest soil C stocks has been targeted as a global climate mitigation measure, however, practices to increase forest soil C pools are controversial and results greatly differ based on ecosystem properties (Mayer et al., 2020; Schmidt et al., 2011).

Degraded landscapes with the potential for forest restoration cover approximately 1.7–1.8 billion hectares of land worldwide, and increasing forest soil C pools on these landscapes represents an opportunity for global C sequestration (Bastin et al., 2019). The greening of mining and smelting degraded landscapes is of particular interest as soils on these landscapes are generally low in C, and their potential for C accumulation is high



(Ussiri and Lal, 2005). Soils in mining and smelting degraded landscapes are often impacted by erosion, and characterized by low pH, heavy metal contamination, and low nutrients – inhibiting or retarding the growth and establishment of vegetation (Akala and Lal, 2001; Driscoll et al., 2001; Hutchinson and Whitby, 1977; Šourková et al., 2005). Regreening degraded landscapes can include the application of soil amendments such as crushed dolomitic limestone, nitrogen (N) – phosphorous (P) – potassium (K) fertilizers, as well as planting native vegetation (Frouz et al., 2001; Lautenbach et al., 1995; Pietrzykowski and Daniels, 2014). Regreening has been found to increase tree growth (Frouz et al., 2009; Levasseur et al., 2022; Moore et al., 2012), however, the effects on soil C are mixed with studies reporting that regreening can increase (Jandl et al., 2003; Pietrzykowski and Daniels, 2014; Sridhar et al., 2022), decrease (Kreutzer, 1995; Persson et al., 2021), or have no effect (Preston et al., 2020) on soil C pools through the alteration of soil respiration and litter decomposition processes. The effects of regreening on soil C dynamics are governed by site-specific factors and increasing the context on these effects will be beneficial to future regreening projects.

The City of Greater Sudbury, Ontario, Canada, and surrounding region has been exposed to legacy effects of well over one century of copper (Cu) and nickel (Ni) mining and smelting. Smelting emissions and expansive clear-cutting led to ~20,000 ha of barren land with highly eroded, acidic soils enriched in metal toxicants and depleted of essential nutrients, inhibiting the regrowth of vegetation (Freedman and Hutchinson, 1980a, 1980b). Ecosystem processes such as litter decomposition, soil respiration, and root

growth were highly impaired (Amiro and Courtin, 1981; Hutchinson and Whitby, 1977). However, the City of Greater Sudbury's greening program is one of the world's largest; more than 3,400 ha of land has been limed and fertilized and over 10 million trees have been planted since 1978 while sulphur dioxide (SO<sub>2</sub>) and particulate metal emissions have decreased by over 95% (VETAC, 2021).

The City of Greater Sudbury's landscape provides a unique location to investigate the effects of greening on forest soil C dynamics as treatments were, and continue to be, applied in a patchwork approach, allowing us to develop a chronosequence of sites varying in time since greening treatment. To account for the effects of erosion on soil C processes we established two series of sites ranging in stand age. One series of sites was heavily influenced by erosion and largely covered by exposed bedrock (>30% bedrock cover) (referred to as "eroded sites"); the other series was covered with a layer of soil (>90% soil cover) (referred to as "stable sites"). The objective of the current study was to determine how forest soil C dynamics change with stand age, and if forest soil C dynamics differ between highly eroded and sites with more stable soils. We tested the hypotheses that forest soil C cycling will change over stand age and with erosion class. Specifically we predicted that: 1) litterfall C inputs will increase with stand age and be higher at stable sites; 2) fine root production will decrease with stand age and there will be no difference between site types; 3) litter decomposition rates will increase with stand age and not differ between eroded and stable sites; 4) litter decomposition rates will be higher using site-specific versus common litter; 5) soil respiration will increase with stand age and be higher

at stable sites; 6) forest floor C pools will increase with stand age and be higher at stable sites; 7) bulk mineral soil and aggregate C concentrations will increase with stand age and will be higher at stable sites; 8) total ecosystem C pools will increase with stand age in our relatively young (~40 year old max) sites regardless of changes in soil C.

### *3.2. Materials and Methods*

#### *3.2.1. Study area and greening process*

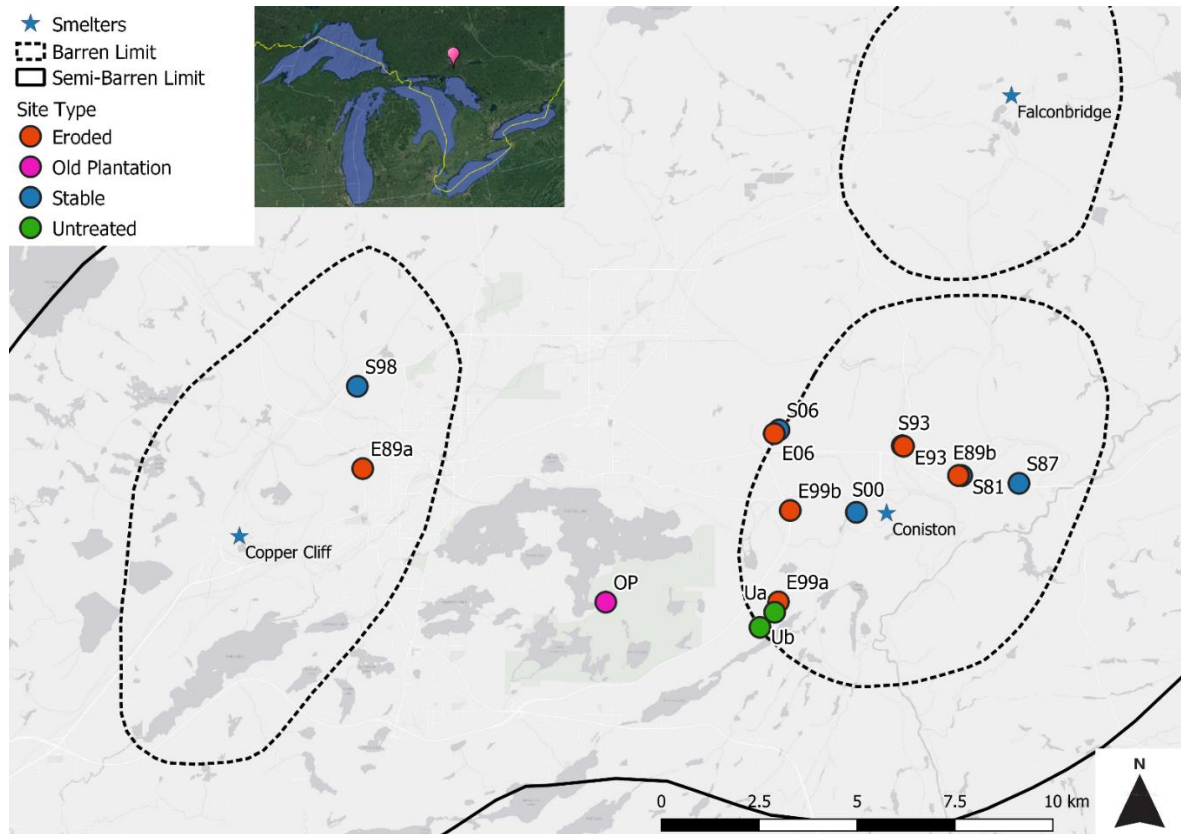
The City of Greater Sudbury (UTM zone 17; Easting: 501620, Northing: 5148797) is in northern Ontario, Canada, on the southern portion of the Precambrian Shield at an elevation of 347 m. Thirty-year (1981–2010) average monthly temperature ranges from -13.0 °C in January to 19.1 °C in July, and average precipitation is 904 mm per year (Environment Canada, 2022). The City of Greater Sudbury lies in the vegetation transition zone between the Great Lakes – St. Lawrence forest regions of the south and the boreal forest to the north (Rowe, 1972). The topography of the region is undulating and dominated by rocky outcrops overlain by soils that are typically shallow and sandy (Pearson and Pitblado, 1995). Prior to greening efforts, the soils within the barrens of the City of Greater Sudbury were highly acidic (pH <4.3), devoid of nutrients, and contained high concentrations of bioavailable Cu and Ni (Freedman and Hutchinson, 1980b). Since 1978, more than 3,400 ha of land have been treated with 10 Mg ha<sup>-1</sup> of crushed dolomitic limestone (CaMg(CO<sub>3</sub>)<sub>2</sub>), 390 kg ha<sup>-1</sup> of 6-24-24 N-P-K fertilizer, and 40 kg ha<sup>-1</sup> of a grass-seed mixture, that included N-fixing legumes. One-year after the application of lime, fertilizer, and grass-seed, trees were planted and across the entire City

of Greater Sudbury region 10 million trees have been planted with species that are native to the region. Planted tree species were predominantly jack pine (*Pinus banksiana* Lamb.) and red pine (*Pinus resinosa* Ait.), but also included white pine (*Pinus strobus* L.), white spruce (*Picea glauca* Moench) and green alder (*Alnus viridis* Chaix) (VETAC, 2021). Areas for greening are selected each year based on multiple operational and resource factors and needs, resulting in a mosaic of forest stands of different ages in the barren and semi-barren areas of the region.

### 3.2.2. Site Selection

Fifteen study sites were established within the barrens of the Coniston and Copper Cliff smelters (Figure 3.1) The barrens were defined as areas that were largely devoid of plant life and had soil pH below 4.3 before greening efforts took place (Lautenbach et al., 1995). We established two series of sites ranging in stand age, six “eroded” sites (>30% bedrock cover); and six “stable sites” (>90% soil cover) (Figure 1). Both eroded and stable sites (collectively referred to as “greening sites”) were limed, fertilized, seeded, and planted with either jack pine and/or red pine. Planting dates ranged from 1981 to 2006, and sites that only received planting at one time were specifically selected. Two untreated sites were also established within the barrens that received no greening treatment, untreated sites included highly coppiced paper birch (*Betula papyrifera* Marshall), trembling aspen (*Populus tremuloides* Michx.), and red maple (*Acer rubrum* L.). Additionally, an old plantation site was established where red pines were planted in 1960 prior to the beginning of the greening program; the old plantation was used as a

benchmark to compare future forest soil C processes within the regreening sites. All study plots were 10 m x 10 m in size and located within a larger forested area with similar characteristics.



**Figure 3.1:** Map of the City of Greater Sudbury region in Ontario, Canada, including fifteen study sites, the outlines for the barren and semi-barren regions, and the locations of the three main smelters (City of Greater Sudbury, 2023).

### 3.2.3. Field sampling

#### 3.2.3.1. Soil sampling

Soil sampling was conducted between May–August 2019. At each site, total mineral soil depth to bedrock was measured at sixteen grid points using a soil auger (20 cm

length by 5 cm width). Both forest floor and mineral soil samples were taken at nine points within each site (three horizontally along both the north and south borders of the plot and three running horizontally through the center). Mineral soils were sampled using a soil auger for 0–5 cm depths at eroded and stable sites, and 5–10 cm depths at only stable sites as no eroded sites had mineral soils deeper than 5 cm. Forest floor samples were collected by cutting a 10 cm x 10 cm square using a knife and separating the forest floor into L (litter) and fibric-humic (FH) layers (Soil Classification Working Group, 1998). The depth of the L and FH layers at each sampling location were measured. Mineral soil bulk density (BD) samples were also taken using a BD hammer for the 0–5 cm and 5–10 cm (when mineral soils reached 10 cm) depths at three points in each plot (north corner, plot center, and south corner). Forest floor and mineral soil samples were immediately placed in sealable polyethylene bags. All soil samples were stored in a refrigerator (4 °C) upon return from the field.

#### *3.2.3.2. Litter traps*

Three litter traps were established in July 2018 at each site (north corner, plot center, and south corner). Litter traps were created using a milk crate with a 30 cm x 40 cm opening at the top. A mesh hammock (made using 2 mm mesh) was suspended within the trap so that no litter was sitting at the bottom of the trap. Large branches were removed from litter traps, but foliage, small twigs, and seeds were all collected as litter. Litter was collected 3 times a year (in both 2018 and 2019) between July and November. Litter was

immediately placed in sealable polyethylene bags. All litter samples were stored in a refrigerator (4 °C) upon return from the field.

#### *3.2.3.3. Litter decomposition bags*

Eighteen 20 cm x 20 cm litter decomposition bags (made using 2 mm fiberglass mesh) were placed at each site. Nine litter bags were filled with 3 g of oven-dried non-milled litter collected from the respective sites' litter traps (referred to as "site-specific litter") and nine were filled with oven-dried red pine and jack pine litter (paper birch litter for the untreated sites) collected adjacent to one specific site (E89a) to control for litter quality (referred to as "common litter"). In April 2019, the nine site-specific litter bags were tied together, and left on top of the forest floor secured to the base of a tree at plot center. The same procedure was performed for the common litter bags. In November 2019, 2020, and 2021 (0.5, 1.5, 2.5 years after deployment), three site-specific and three common litter bags were removed from each site. The litter bags were immediately placed in sealable polyethylene bags in the field (Bocock and Gilbert, 1957).

#### *3.2.3.4. Fine root ingrowth cores*

Fine root ingrowth cores were constructed using a polyethylene mesh with 2 mm openings. The mesh was formed into an open-ended cylinder 10 cm in height and 3 cm in diameter. Ingrowth cores were placed 10 cm deep in the soil where possible; where soil was less than 10 cm deep, cores were buried as deep as possible and depth was recorded. The cores were filled with dried and sieved (to 2 mm) soil from the respective site, cores were filled until soil in the core was at the same height as surrounding mineral soil. At each

site ten ingrowth cores were installed in April 2019, two cores were placed at each corner of the site and plot center, ingrowth cores retrieved in November 2020 (Persson, 1979).

#### *3.2.3.5. Soil CO<sub>2</sub> respiration*

Soil CO<sub>2</sub> respiration was measured every three weeks between April and September 2019. Five PVC soil collars with a radius of 5 cm and a height of 20 cm were installed at each site at a soil depth ranging from 3–7 cm. Total soil respiration of CO<sub>2</sub> was measured using a Gaset DX-4040 Trace Gas Analyzer. The chamber was placed on top of the collar and measured flux chamber concentrations of CO<sub>2</sub> for 5 minutes with 20 second measurement times. Prior to soil CO<sub>2</sub> measurements, soil temperature was measured at 5 cm depth using a soil thermometer adjacent to each respiration collar.

#### *3.2.3.6. Tree surveys and vegetation sampling*

Tree surveys were conducted July 2018, and vegetation sampling was conducted July 2019. Diameter at breast height (dbh) and tree species were recorded for every tree within each of the 10 m x 10 m sites. Trees were categorized as woody plants with a dbh greater than 2.5 cm. Tree cores were taken from three trees in each site to verify age and used for wood C analysis. Two cores were taken from each sampled tree, one at breast height, and the second 20 cm below at a 90° angle from the entrance point of the first core. Cores were stored in plastic straws with the ends taped. Stand age was not measured at the untreated sites as sprout and root system of paper birch and trembling aspen are potentially much older than individual stems (Courtin, 1995). Bark samples were taken from the three trees where tree cores were taken at all sites these same trees at breast



height by scraping bark off the tree using a knife and composited from the three trees in a polyethylene bag. Foliage samples were taken by hand from the highest possible branch from each sampled tree and composited into paper bags. Current year needles were targeted for foliage sampling. Most sites were monocultures of jack or red pine, and the three vegetation sampling trees were selected to be the dominant species (greater than 33% of stems) for each site. On the occasion that both jack and red pine comprised greater than 33% of stems per site vegetation samples were taken from three trees of both species. All vegetation (tree cores, bark, and foliage) samples were stored in a refrigerator (4°C) upon return from the field.

#### *3.2.4. Sample preparation and laboratory analysis*

All samples were oven-dried at 105°C for 24-hours once returned from the field. Bulk densities were calculated for the L, FH, 0–5 cm, and 5–10 cm samples by dividing the oven-dry mass by the sample volume. Litter from litterfall traps and litter bags were weighed after drying. Litter, forest floor, and vegetation samples were milled to a fine powder using a grinder. Mineral soil samples were sieved to 2 mm. Fine roots were picked out of soils in the fine root ingrowth cores by hand and weighed. A subset of mineral soil samples (n=36) were separated into three different aggregate size fractions (>200 µm, 63–200 µm, and <63 µm) through wet-sieving using a 5 g L<sup>-1</sup> hexametaphosphate and reverse-osmosis water solution (Cambardella and Elliott, 1993). This method avoids aggregates from breaking down into primary particles (von Lütow et al., 2007). Litterfall, litter decomposition, vegetation, forest floor, bulk mineral soils, and aggregates were analyzed

for C and N concentrations using an Elementar Macro CNS analyzer. Quality control was confirmed by running all samples in triplicate and including three NIST-1515 apple leaf standards and one control sample (16 mg sulfadiazine) every 25 samples.

### *3.2.5. Calculations*

#### *3.2.5.1. Carbon pools*

Forest floor and mineral soil C pools were determined for each layer by multiplying the median bulk density ( $\text{g cm}^{-3}$ ) by the median layer depth (cm); the mass of each layer (per ha) was then multiplied by the layer's median C concentrations (%). Aboveground biomass pools were calculated using the dbh-based allometric equations developed by Lambert et al. (2005). Aboveground C pools were determined using measured C concentrations (%) for each biomass component (bark, foliage, wood + branches) and the mass of each biomass component from the allometric equations ( $\text{Mg ha}^{-1}$ ). Tree core C concentrations (%) were used for both wood and branches biomass components. For sites where both jack pine and red pine made up greater than 33% of stems, C concentrations (%) for both species were averaged.

#### *3.2.5.2. Litterfall carbon*

The mass of litterfall collected in the three traps per site (g) was summed and extrapolated to the hectare scale using the area of the three litter traps combined ( $0.36 \text{ m}^2$ ). Litterfall mass was then multiplied by measured litterfall C concentrations (%).

#### *3.2.5.3. Fine root production*

Fine root production was calculated by extrapolating the mass of roots in each

ingrowth core (g) to a m<sup>2</sup> and dividing by years spent in field. Carbon concentrations were not measured for fine roots due to limited sample (Persson, 1979).

#### 3.2.5.4. Decomposition calculations

Litter decomposition rate constants were determined using the Olson (1963) equation:

$$k = -\ln \left( \frac{X_t}{X_0} \right) / t \quad \text{[Equation 3.1]}$$

where  $k$  is the decomposition rate constants,  $X_t$  is the mass of litter C (g) remaining in each litter bag after  $t$  time (years), and  $X_0$  is the initial mass of litter C (g) in each bag.

#### 3.2.5.5. Soil carbon respiration

Ten to 15 measurements of CO<sub>2</sub> concentration (ppm) were taken over 5 minutes (one reading every 20–30 seconds), the first CO<sub>2</sub> measurement was subtracted from the last CO<sub>2</sub> measurement. Rates of soil respiration (FCO<sub>2</sub>) (μmol m<sup>-2</sup> s<sup>-1</sup>) were calculated using the following two equations:

$$n = PV / RT_{atm} \quad \text{[Equation 3.2]}$$

$$FCO_2 = n * [\Delta CO_2] / t / A \quad \text{[Equation 3.3]}$$

where  $n$  is the number of moles of gas,  $P$  is pressure measured in the Gasmeter chamber in Pa,  $R$  is the ideal gas constant (8.314 m<sup>3</sup> Pa mol<sup>-1</sup> K<sup>-1</sup>),  $T_{atm}$  is the temperature measured in the Gasmeter chamber in K,  $V$  is the combined volume of the Gasmeter chamber and the soil collar headspace in m<sup>3</sup>,  $[\Delta CO_2]$  is the change in CO<sub>2</sub> concentration during each measurement period in ppm,  $t$  is the sample period time in s, and  $A$  is the area of each collar in m<sup>2</sup>.

The temperature sensitivity parameter ( $Q_{10}$ ) was calculated by applying the coefficients from the relationship between soil temperature and  $CO_2$  emission rates,  $Q_{10}$  was calculated for each site type using:

$$\ln(FCO_2) = B_0 + B_1 * T_{soil} \quad [\text{Equation 3.4}]$$

$$Q_{10} = e^{(B_1 * 10)} \quad [\text{Equation 3.5}]$$

where  $B_0$  and  $B_1$  are coefficients, and  $T_{soil}$  is soil temperature in °C. To standardize soil temperature among site types,  $Q_{10}$  was used to calculate soil  $CO_2$  emission rates adjusted to a soil temperature of 10°C ( $FCO_2@10^\circ C$ ) for each individual soil collar at each measurement period using:

$$FCO_2@10^\circ C = FCO_2 / (Q_{10}^{\frac{T_{soil}-10}{10}}) \quad [\text{Equation 3.6}]$$

### 3.2.6. Statistical analyses

Logarithmic and linear regressions were used to assess the relationship between stand age and a variety of variables including litterfall rates, fine root production, litter decomposition k-values, litter C:N, forest floor C pools, bulk and aggregate mineral soil C concentrations, and soil  $CO_2$  emissions (both temperature adjusted and unadjusted). Normality of the error terms was assessed using quantile-quantile plots. Relationships were determined to be significant if p was less than 0.05. Normality of data used for comparisons were tested using the Lilliefors corrected Kolmogorov-Smirnov test, variables were considered normally distributed if p was greater than 0.05. Differences between site types were tested using Kruskal-Wallis tests for several variables including: litterfall rates, fine-root production, litter decomposition k-values, litter C:N ratios, forest floor C

accumulation rates, bulk and aggregate mineral soil C concentration, soil temperature, and soil CO<sub>2</sub> emissions (both adjusted and unadjusted); differences were considered significant if p was less than 0.05. Site types that were significantly different using the Kruskal-Wallis tests were tested using the Mann-Whitney U-test to determine which groups were significantly different from one another; differences were considered significant based on Bonferroni corrections. The Kruskal-Wallis, Mann-Whitney U-test with Bonferroni correction procedure was also followed to test differences in k-values and C:N ratios between litter in decomposition bags that had been deployed in the field for varying amounts of time as well as common and site-specific litter. All statistical analyses were completed using R version 4.0.5 (RStudio, 2020). All maps were created using Quantam GIS version 3.2.1 (QGIS.org, 2022).

### *3.3. Results*

#### *3.3.1. Site characteristics*

Jack pine was the dominant tree species at 67% of the eroded sites and 33% of the stable sites; red pine dominated the remaining 33% of eroded sites and 17% of the stable sites; 50% of the stable sites were dominated by a mix of jack and red pine. The old plantation was a red pine monoculture. Both untreated sites were dominated by paper birch (Table 3.1). Median stem density was higher at stable sites (1655 stems ha<sup>-1</sup>) compared with eroded sites (1355 stems ha<sup>-1</sup>) but stem density at the regreening sites was considerably lower than the untreated sites (3000 stems ha<sup>-1</sup>) and considerably higher than the old plantation (800 stems ha<sup>-1</sup>). Median forest floor depth decreased in the order

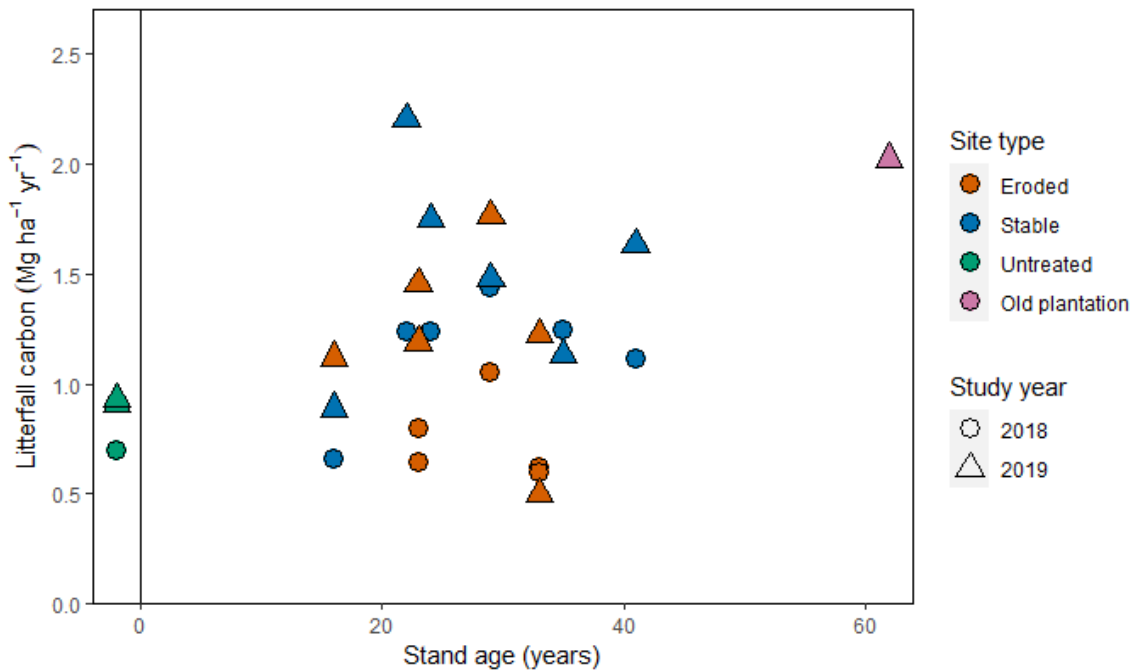
of old plantation (6 cm) > untreated (4.1 cm) > stable (2.1 cm) > eroded (1.5 cm). While median mineral soil depth decreased in the order of old plantation (>100 cm) > untreated (40 cm) > stable (25.1 cm) > eroded (6 cm) (Table 3.1). Median mineral soil bulk density decreased in the order of stable ( $0.91 \pm 0.27 \text{ g cm}^{-3}$ ), OP ( $0.87 \pm 0.22 \text{ g cm}^{-3}$ ), eroded ( $0.81 \pm 0.14 \text{ g cm}^{-3}$ ), and untreated ( $0.78 \pm 0.27 \text{ g cm}^{-3}$ ).

**Table 3.1:** Site characteristics of the 15 study sites within the barren and semi-barren areas of the City of Greater Sudbury, Canada.

Site	Easting	Northing	Stand age (years)	Site type	Elevation (m)	Slope (°)	Dominant species	Tree density (stems ha <sup>-1</sup> )	DBH (cm)	Forest floor depth (cm)	Mineral soil depth (cm)	Mineral soil bulk density (g cm <sup>-3</sup> )
E06	509020	5149553	15	Eroded	304	6	Jack pine	1,211	5.5	0.5	6.0	0.62
E99a	509127	5145262	22	Eroded	295	2	Jack pine	1,078	11.1	3.3	4.9	0.84
E99b	509428	5147592	22	Eroded	276	1	Red pine	1,500	9.8	2.1	4.8	0.87
E93	512320	5149231	28	Eroded	281	6	Red pine	889	12.6	1.6	10.1	0.73
E89a	498513	5148661	32	Eroded	300	15	Jack pine	1,866	10.6	1.3	3.6	0.89
E89b	513724	5148484	32	Eroded	258	13	Jack pine	1,666	13.1	1.4	4.9	0.95
S06	509140	5149646	15	Stable	290	14	Mixed	1,500	5.4	0.4	25.1	0.54
S00	511116	5147545	21	Stable	241	0	Mixed	2,378	8.6	4.2	46.4	0.70
S98	498375	5150759	23	Stable	298	0	Red pine	1,644	9.6	2.7	25.9	0.83
S93	512289	5149244	28	Stable	268	3	Mixed	1,666	10.7	3.0	22.9	1.19
S87	515267	5148285	34	Stable	270	15	Jack pine	1,078	15.5	1.6	32.6	0.88
S81	513804	5148482	40	Stable	211	8	Jack pine	1,744	10.9	1.2	14.3	1.05
Ua	509030	5144997	NA	Untreated	267	11	Paper birch	2,600	5.5	5.3	32.0	0.64
Ub	508655	5144612	NA	Untreated	260	6	Paper birch	3,400	6.4	2.9	48.0	0.89
OP	504718	5145254	61	Old plantation	267	4	Red pine	800	24.5	6.0	>100.0	0.87

### 3.3.2. Litterfall carbon

Annual litterfall C flux and stand age were not significantly related for either the 2018 or 2019 sampling period ( $p>0.05$ ) (Figure 3.2) and there were no significant differences in annual litterfall C flux among site types ( $p>0.05$ ) (Appendix). While not significantly different, the old plantation had higher annual litterfall C ( $2.02 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) than stable ( $1.33 \pm 0.39 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ), eroded ( $1.00 \pm 0.38 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ), or untreated ( $0.91 \pm 0.10 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) sites. For stable and eroded sites, the 2019 ( $1.34 \pm 0.43 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) sampling season had a marginally higher litterfall C compared with 2018 ( $0.96 \pm 0.29 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) ( $p=0.04$ ) (Appendix).

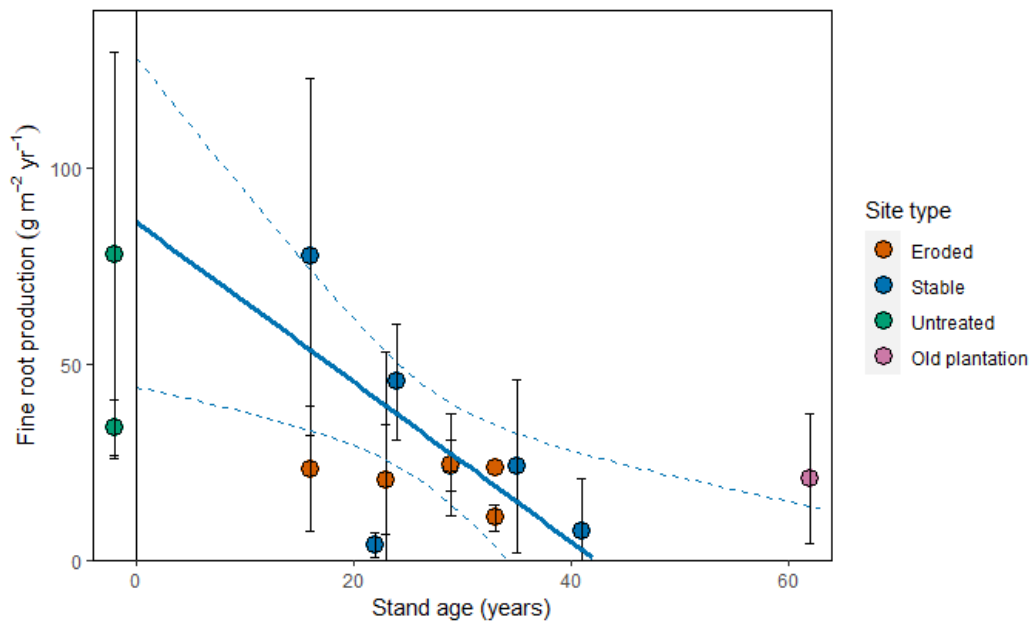


**Figure 3.2:** Annual litterfall (mean) carbon for the 2018 and 2019 field seasons and stand age for eroded, stable, untreated, and old plantation sites.



### 3.3.3. Fine root production

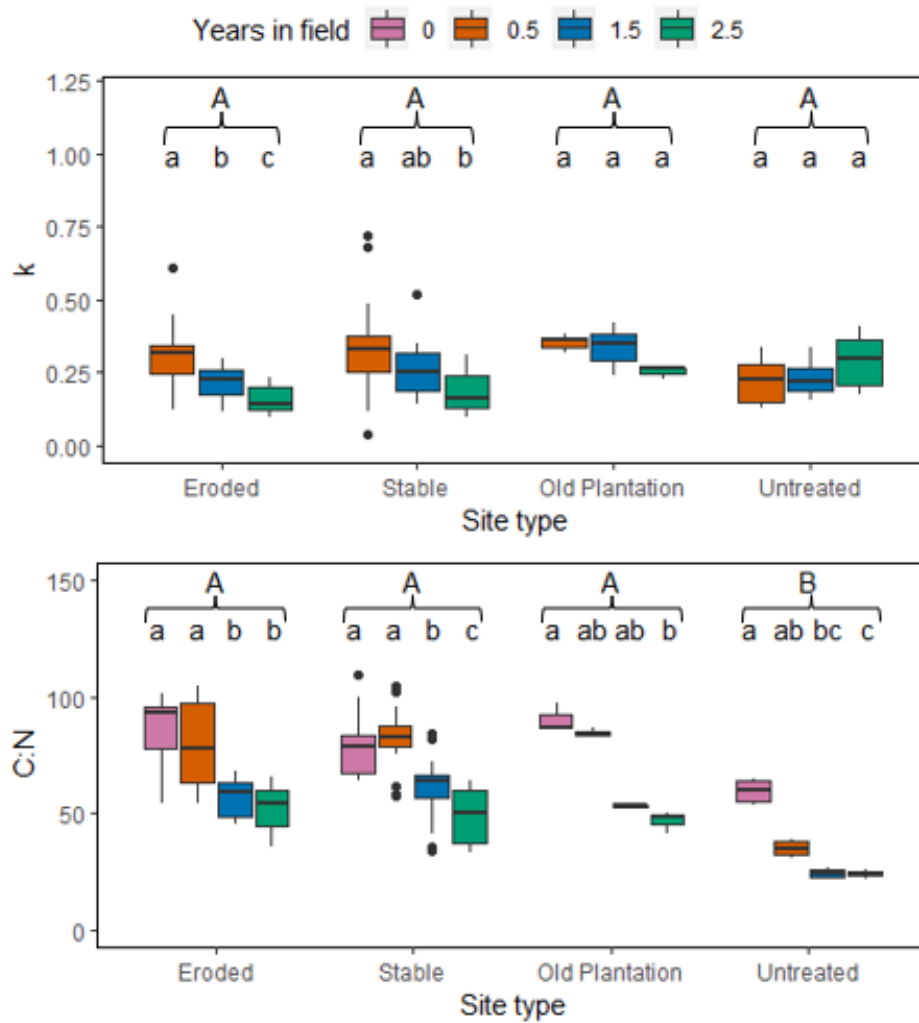
Fine root production measured after 18 months in the 0–10 cm soil layer decreased significantly ( $77.35 \pm 40.49 \text{ g m}^{-2} \text{ yr}^{-1}$  to  $7.79 \pm 11.34 \text{ g m}^{-2} \text{ yr}^{-1}$ ) with stand age at stable sites ( $R^2=0.22$ ,  $p<0.01$ ) but not at eroded sites ( $p>0.05$ ) (Figure 3.3). Eroded sites ( $21.21 \pm 17.39 \text{ g m}^{-2} \text{ yr}^{-1}$ ) had significantly lower fine root production compared with untreated sites ( $55.80 \pm 39.63 \text{ g m}^{-2} \text{ yr}^{-1}$ ) ( $p<0.05$ ); however, there were no other significant differences in fine root production among site types ( $p>0.05$ ) (Appendix).



**Figure 3.3:** Fine root production (mean  $\pm$  standard deviation) after 18-months deployment (April 2019–October 2020) and stand age for eroded, stable, untreated, and old plantation sites. Solid line is linear regression line for the relationship between fine root production and stand age at stable sites. Dashed lines are 95% confidence intervals.

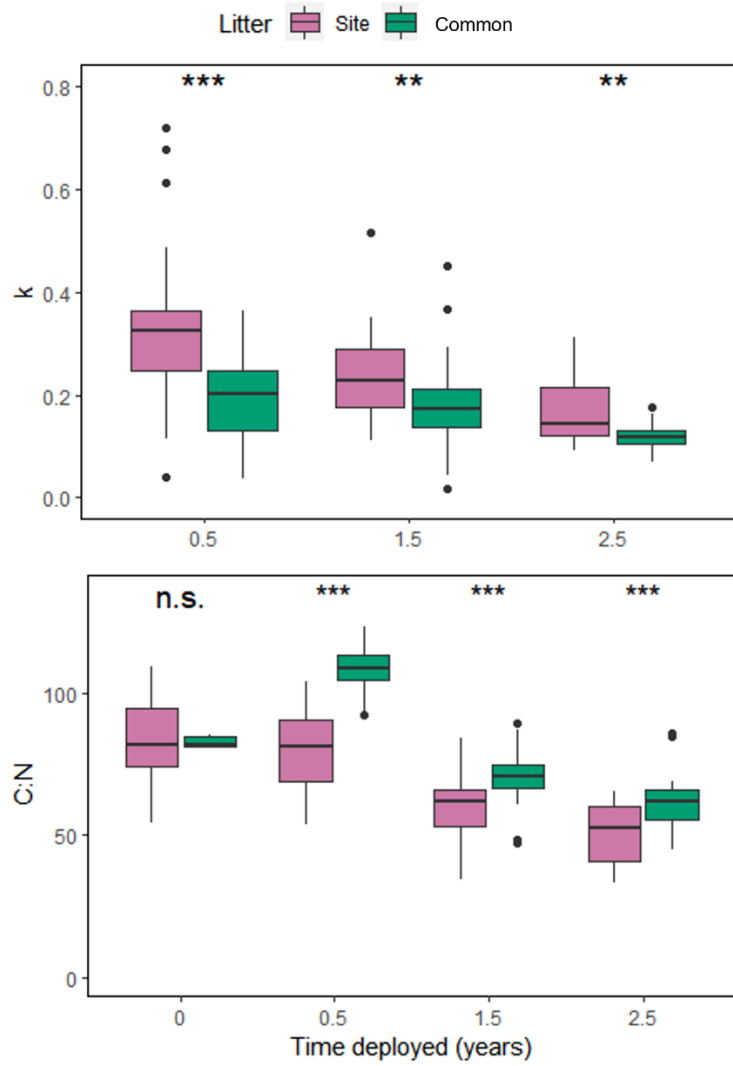
#### 3.3.4. Litter decomposition

The  $k$  values of site-specific litter did not differ among site types regardless of litterbag exposure period ( $p>0.05$ ) (Figure 3.4). Using site-specific litter,  $k$  values were significantly lower after 2.5-years compared with 0.5-years at eroded ( $0.31 \pm 0.11$  versus  $0.15 \pm 0.04$ ) and stable sites ( $0.34 \pm 0.17$  versus  $0.18 \pm 0.07$ ) ( $p<0.05$ ); there were no significant differences in  $k$  values and length of exposure at old plantation and untreated sites ( $p>0.05$ ) (Figure 3.4). Litter C:N ratios were significantly lower at untreated sites ( $35.7 \pm 15.0$ ) compared with all other site types ( $p<0.0001$ ). Median  $k$  values were significantly higher using site-specific litter compared with common litter at eroded ( $0.22 \pm 0.10$  versus  $0.17 \pm 0.06$ ) and stable sites ( $0.27 \pm 0.14$  versus  $0.17 \pm 0.09$ ) ( $p<0.01$ ) (Figure 3.5). Median C:N ratios were significantly lower in site-specific versus common litter for all deployment times except prior to exposure (0 years) coinciding with lower rates of decomposition. There were no changes in  $k$ -value or C:N ratio with stand age for eroded or stable sites.



**Figure 3.4:** Comparison between decomposition k-values (**top panel**) and carbon:nitrogen ratios (C:N) (**lower panel**) for litter bags after 0, 0.5, 1.5, and 2.5-years deployment in the field at eroded, stable, untreated, and old plantation sites using site-specific litter. Box center lines are medians, box limits are 25<sup>th</sup> and 75<sup>th</sup> percentiles, whiskers are at most 1.5 times the inter-quartile range, and dots beyond whiskers are outliers. Significant differences ( $p < 0.05$ ) in k-values and C:N ratios with varying times in the field are denoted

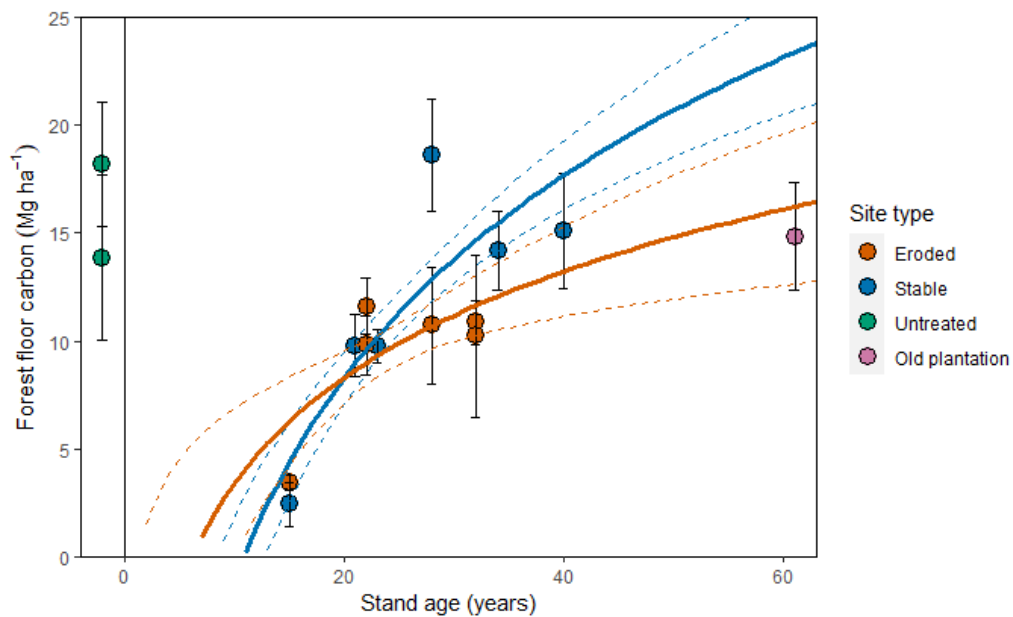
by differing letters under brackets; significant differences ( $p < 0.05$ ) in k-values and C:N ratios between site types are denoted by differing letters above brackets.



**Figure 3.5:** Decomposition k-values (**top panel**) and carbon:nitrogen (C:N) ratios (**bottom panel**) for stable and eroded sites using site-specific versus common litter at various deployment lengths. Box center lines are medians, box limits are 25th and 75th percentiles, whiskers are outliers. Significant differences denoted as: \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ , n.s.  $p > 0.05$ .

### 3.3.5. Forest floor carbon

Forest floor C mass increased significantly with stand age at both eroded ( $3.49 \pm 0.36 \text{ Mg ha}^{-1}$  to  $10.45 \pm 3.06 \text{ Mg ha}^{-1}$ ) ( $R^2=0.27$ ,  $p<0.001$ ) and stable sites ( $2.46 \pm 1.02 \text{ Mg ha}^{-1}$  to  $15.12 \pm 2.65 \text{ Mg ha}^{-1}$ ) ( $R^2=0.63$ ,  $p<0.001$ ) (Figure 3.6). Forest floor C pools were similar between untreated sites ( $16.01 \pm 3.95 \text{ Mg ha}^{-1}$ ) and the old plantation site ( $14.85 \pm 2.49 \text{ Mg ha}^{-1}$ ).

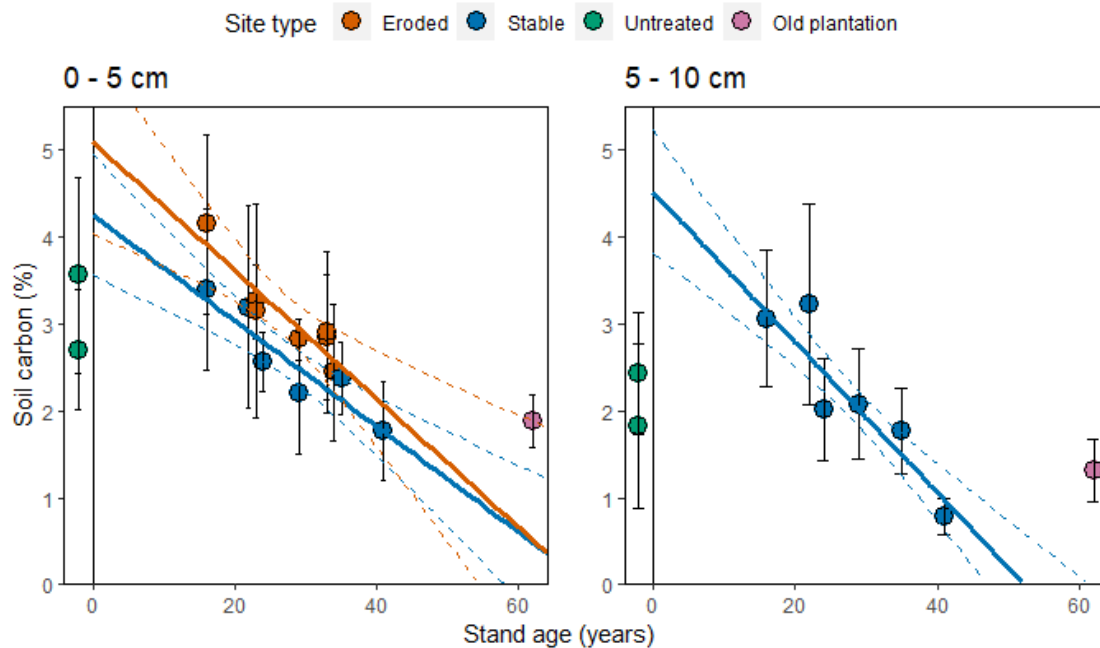


**Figure 3.6:** Forest floor carbon pool (mean  $\pm$  standard deviation) and stand age for eroded, stable, untreated, and old plantation sites. Solid lines are logarithmic regression line for the relationships between forest floor carbon and stand age at eroded and stable sites. Dashed lines are 95% confidence intervals.

### 3.3.6. Soil carbon

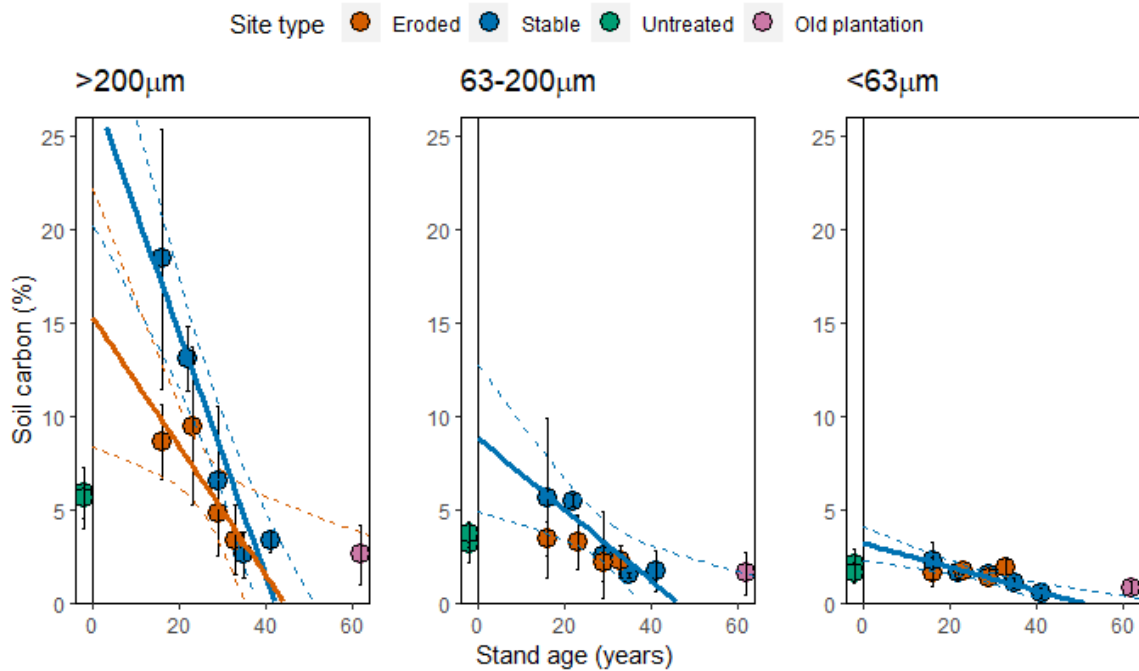
Soil C concentrations were low and decreased significantly with stand age at eroded ( $4.14 \pm 1.04\%$  to  $2.44 \pm 0.79\%$ ) ( $R^2=0.2$ ;  $p<0.01$ ) and stable sites ( $3.40 \pm 0.93\%$  to

1.76 ± 0.57%) ( $R^2=0.35$ ;  $p<0.0001$ ) in the 0–5 cm mineral soil depth and at stable sites in the 5–10 cm mineral soil depth (3.05 ± 0.79% to 0.78 ± 0.21%) ( $R^2=0.55$ ;  $p<0.0001$ ) (Figure 3.7). Soil C concentrations decreased significantly with stand age in all aggregate size fractions at stable sites (>200  $\mu\text{m}$  fraction (18.41 ± 5.66% to 3.37 ± 0.49%) ( $R^2=0.70$ ,  $p<0.001$ ), 63–200  $\mu\text{m}$  fraction (5.66 ± 3.51% to 1.74 ± 0.91%) ( $R^2=0.39$ ,  $p<0.001$ ), <63  $\mu\text{m}$  fraction (2.30 ± 0.80% to 0.56 ± 0.21%) ( $R^2=0.60$ ,  $p<0.001$ )) but only in the >200  $\mu\text{m}$  fraction (8.61 ± 1.63% to 3.42 ± 1.51%) ( $R^2=0.40$ ,  $p<0.05$ ) at eroded sites (Figure 3.8). In both bulk soils and aggregate fractions soil C concentrations at the untreated sites were more similar to the youngest regreening sites, while soil C at the old plantation were more similar to the oldest regreening sites.



**Figure 3.7:** Soil carbon concentration (mean ± standard deviation) and stand age for eroded, stable, untreated, and old plantation sites at the 0–5 cm depth (left panel) and 5–

10 cm depth (**right panel**). Solid lines are linear regression lines for relationship between aboveground carbon and stand age for both erosion classes. Dashed lines are 95% confidence intervals.



**Figure 3.8:** Soil carbon concentrations (mean  $\pm$  standard deviation) in the 0–5 cm depth and stand age for eroded, stable, untreated, and old plantation sites using the >200  $\mu\text{m}$  soil fraction (**left panel**) 63–200  $\mu\text{m}$  fraction (**center panel**) and <63  $\mu\text{m}$  fraction (**right panel**). Solid lines are linear regression lines for relationship between aboveground carbon and stand age for both erosion classes. Dashed lines are 95% confidence intervals.

### 3.3.7. Soil respiration

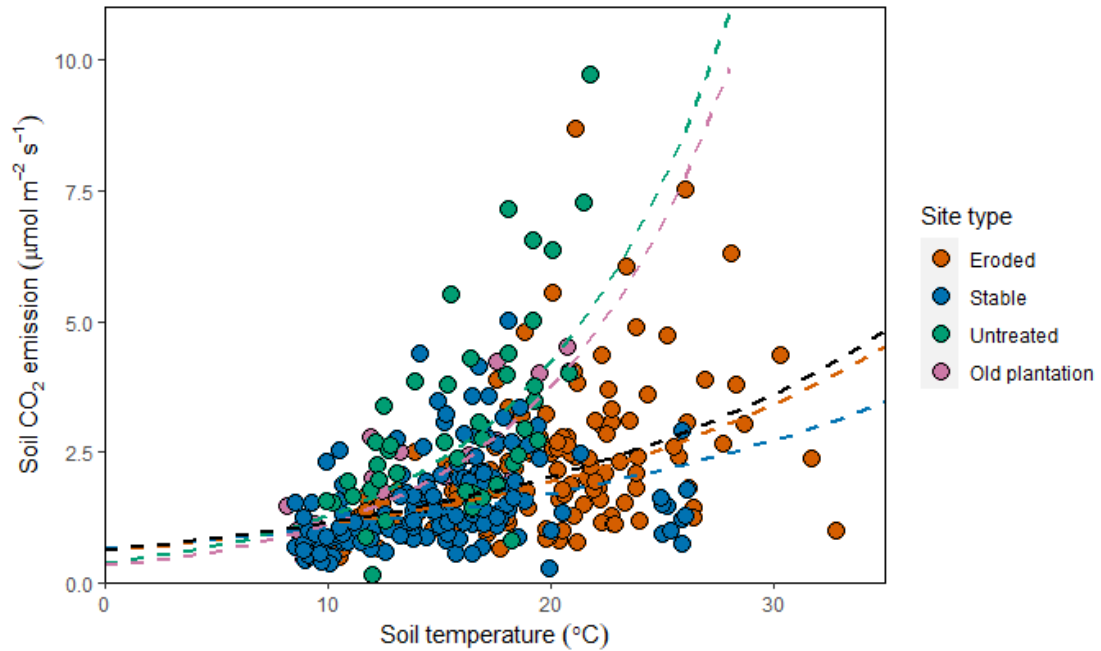
Soil temperature was significantly higher at eroded sites compared with all other site types ( $p < 0.0001$ ); there were no other significant differences in soil temperature among site types ( $p > 0.05$ ) (Table 3.2). Soil  $\text{CO}_2$  emissions were significantly related to soil

temperatures at all sites ( $R^2=0.21$ ;  $p<0.0001$ ) (Figure 3.9), and these relationships became stronger when separated into site types for eroded ( $R^2=0.24$ ;  $p<0.0001$ ), untreated ( $R^2=0.30$ ;  $p<0.0001$ ), and old plantation sites ( $R^2=0.63$ ;  $p<0.0001$ ); however, the relationships were weaker but still significant for stable sites ( $R^2=0.13$ ;  $p<0.0001$ ). Soil CO<sub>2</sub> emissions differed significantly among site types ( $p<0.0001$ ) with untreated sites having the highest CO<sub>2</sub> emission rate followed by eroded and then stable sites (Table 3.2). In contrast, soil CO<sub>2</sub> emissions at the old plantation were not significantly different to the other sites. When soil CO<sub>2</sub> emissions were adjusted to 10°C they were significantly higher at untreated sites ( $1.47 \pm 0.73 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) compared with stable sites ( $1.21 \pm 0.62 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) ( $p<0.05$ ) and there were no other significant differences in adjusted CO<sub>2</sub> emissions between site types ( $p>0.05$ ) (Appendix). Soil CO<sub>2</sub> emissions adjusted to 10°C were very weakly related to stand age at stable sites ( $R^2=0.03$ ;  $p=0.02$ ) and had no relationship with stand age at eroded sites ( $p>0.05$ ) (Figure 3.10).

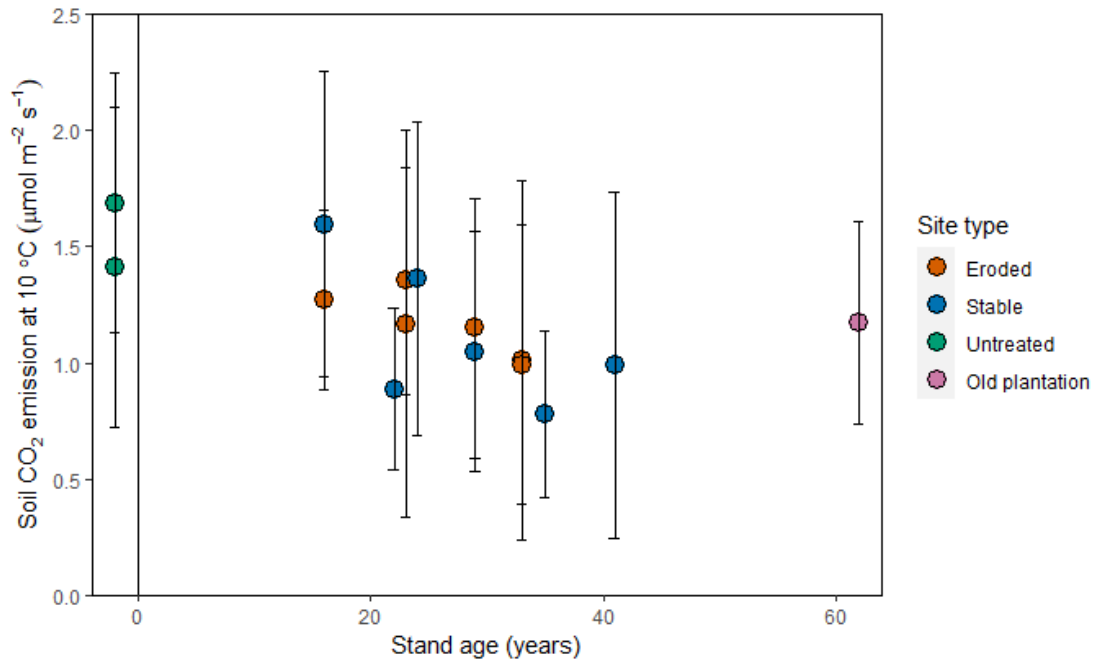
**Table 3.2:** Median soil carbon dioxide (CO<sub>2</sub>) emissions, median soil temperature, and the temperature sensitivity parameter (Q<sub>10</sub>) for all eroded, stable, untreated, and old plantation sites between April–September 2019. Significant differences ( $p<0.05$ ) in soil CO<sub>2</sub> emissions or soil temperature among site types are denoted using differing letters.

Site type	Soil CO <sub>2</sub> emission ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Soil temperature (°C)	Q <sub>10</sub>
Eroded	$2.22 \pm 1.31^a$	$19.8 \pm 5.1^a$	1.75
Stable	$1.52 \pm 0.98^b$	$15.5 \pm 3.8^b$	1.60
Untreated	$3.03 \pm 1.69^c$	$16.9 \pm 3.2^b$	3.26
Old plantation	$2.27 \pm 1.32^{abc}$	$14.2 \pm 4.2^b$	3.32





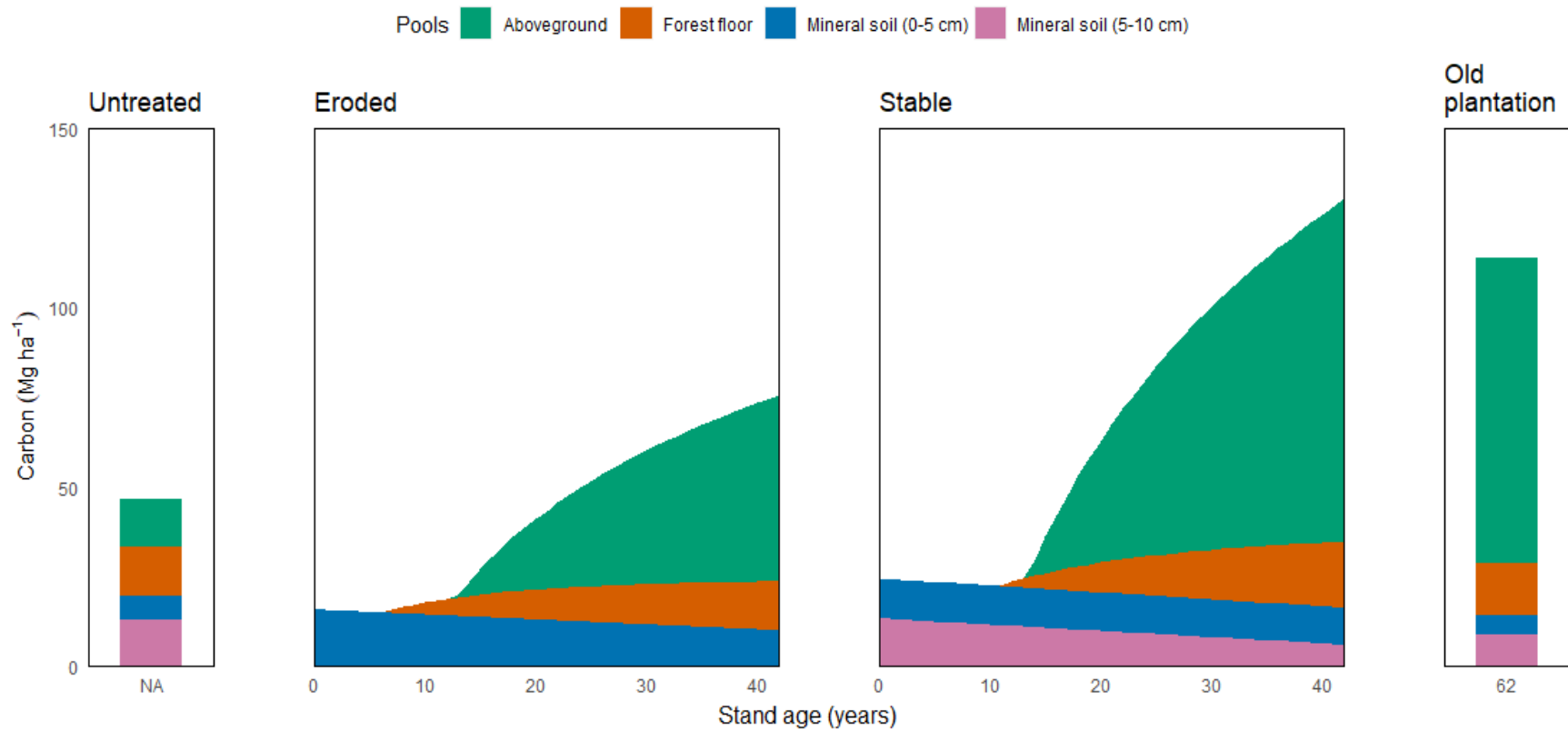
**Figure 3.9:** Soil carbon dioxide (CO<sub>2</sub>) emissions and soil temperature for all eroded, stable, untreated, or old plantation study sites from April–September 2019. Dashed lines are exponential regression line for relationship between soil CO<sub>2</sub> emissions and soil temperature for eroded (orange line), stable (blue line), untreated (green line), old plantation, (purple line), and all sites (black line).



**Figure 3.10:** Soil carbon dioxide (CO<sub>2</sub>) emissions (mean ± standard deviation) adjusted to 10°C and stand age for eroded, stable, untreated, and old plantation sites between April–September 2019.

### 3.3.8. Total ecosystem carbon pools

Total ecosystem C, aboveground C, and forest floor C pools increased with stand age at stable and eroded sites (Figure 3.11). In contrast, mineral soil C pools decreased with stand age at the 0–5 cm and 5–10 cm depth (in stable sites) and slightly decreased at the 0–5 cm depth in eroded sites. The oldest eroded and stable sites had higher total ecosystem C pools compared with the untreated sites largely due to the considerably larger aboveground C pools at regreening sites. Untreated sites had the largest forest floor C pools and mineral soil C pools were greater than those of the oldest stable sites but less than the oldest eroded sites in the 0–5 cm depths (Figure 3.11).



**Figure 3.11:** Total ecosystem carbon (C) pools over time for eroded (**center-left panel**) and stable sites (**center-right panel**), as well as total ecosystem C pools for untreated sites (**left panel**) and the old plantation (**right panel**).

### 3.4. Discussion

This study investigated soil C dynamics at a chronosequence of sites where regreening treatments were applied between 15–40 years ago, as well as an untreated site and an old plantation site treated prior to the regreening program. At the regreening sites there were no relationships with stand age and litterfall or between site types. Fine root production decreased with stand age at stable sites but did not change at eroded sites. Forest floor C pools increased with stand age at both eroded and stable sites. Litter decomposition rates were not significantly related to stand age; however, litter decomposition rates were significantly higher and litter C:N ratios were significantly lower when using site-specific litter compared with common litter. Bulk mineral soil C concentrations decreased with stand age in the 0–5 cm mineral soil horizon at both stable and eroded sites and in the 5–10 cm mineral soil horizon at stable sites. Mineral soil aggregate C concentrations decreased with stand age in all fractions at stable sites but only in the >200  $\mu\text{m}$  fraction at eroded sites. Both bulk mineral soil and aggregate fraction C concentrations at the youngest regreening sites were more like the untreated sites, while the oldest regreening sites were more like the old plantation site. Soil temperature and soil CO<sub>2</sub> emissions were significantly higher at eroded versus stable sites. However, once soil CO<sub>2</sub> emissions were adjusted for consistent temperature only untreated sites were significantly different from the other site types; there were also no relationships between adjusted soil CO<sub>2</sub> emissions and stand age. At the regreening sites total ecosystem C pools increased with stand age. The increase in total ecosystem C pool with stand age was largely

due to increasing aboveground C pools and both stable and eroded sites had larger ecosystem C pools than the untreated sites and similar to the old plantation.

#### *3.4.1. Litterfall*

We predicted that litterfall C would increase with stand age and would be higher at stable sites versus eroded sites due to higher aboveground tree biomass, however, there were no relationships between stand age or site type and litterfall C. Previous studies have found that following canopy closure, stand age and biomass have no influence on litterfall production and some studies have found that litterfall even decreases following canopy closure (Berg et al., 1999; Bray and Gorham, 1964; MacLean and Wein, 1978a; Starr et al., 2005). The lack of relationship between stand age and litterfall C following canopy closure in the reclaimed sites may be due to trees investing more energy into stem growth and less into producing foliage (Albrektson, 1988; Lehtonen, 2005; Vanninen and Mäkelä, 2000; Vogt et al., 1986). However, litterfall output can vary drastically from year to year and while we only found a marginal difference in litterfall between our two study years, Bray and Gorham (1964) found that litterfall within pine stands can vary up to 500% in a year. Nonetheless, annual litterfall C at the regreening sites ( $1.17 \pm 0.42 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) was similar to litterfall in the old plantation and to reported values from studies conducted in jack pine stands in Canada (Foster et al., 1995; MacLean and Wein, 1978a; Weber, 1987) and coniferous stands in boreal forest across the globe (Vogt et al., 1986).

### *3.4.2. Fine root production*

A decrease in fine root production with stand age, consistent with the regreening sites, has been documented previously in conifer stands (Persson, 1983). However, the mechanism behind this remains unclear and has been attributed to changes in successional vegetation communities (Finer et al., 1997) and with younger stands being more capable of recolonizing vacant soil space (Messier and Puttonen, 1993). Fine root growth at the youngest stable sites was more like the untreated sites, while fine root growth at the oldest regreening sites more closely resembled the old plantation site. Both Finer et al., (1997) and Messier and Puttonen (1993) highlight that fine root production is difficult to measure and that site-to-site variation can be very high. The absence of a relationship between fine root production and stand age at eroded sites could be due to the eroded sites having a smaller amount of soil space which becomes occupied sooner following regreening. Limited vacant soil space has been shown to lead to a decrease in investment in fine root production (Persson, 1983). Fine root production at regreening sites ranged from  $4.2 \pm 3.1 \text{ g m}^{-2} \text{ yr}^{-1}$  to  $77.4 \pm 45.3 \text{ g m}^{-2} \text{ yr}^{-1}$  which was low but comparable to measurements using a similar methodology in boreal conifer stands (Bond-Lamberty et al., 2004; Steele et al., 1997).

### *3.4.3. Litter decomposition*

Decomposition rates and C:N ratios did not differ between eroded or stable sites or change with stand age but there was a clear “home-field advantage” (HFA) using site-specific litter. We predicted that litter decomposition rates at the regreening sites would

increase with stand age as the local decomposer rebounded from historically low rates of litter decomposition. However, this recovery appears to have occurred more quickly than we anticipated as litter decomposition rates at the regreening sites for all stand ages ( $k = 0.22\text{--}0.26$  at 1.5 years) were in the range of other litterbag studies ( $k = 0.2\text{--}0.46$  at 1 year) conducted in jack and red pine stands (Bockheim et al., 1991; MacLean and Wein, 1978b; Moore et al., 1999; Weber, 1987). Historically, litter decomposition in the City of Greater Sudbury region had been evaluated as being very low (Amiro and Courtin, 1981; Freedman and Hutchinson, 1980a). Freedman and Hutchinson (1980a) found that areas closer to smelters with higher concentrations of metals in soils had significantly lower litter decomposition rates compared to control sites 30 km from the nearest smelter. Additionally both Freedman and Hutchinson (1980a) and Johnson and Hale (2004) found that litter decomposition rates were lower at metal contaminated sites regardless of litter metal concentrations suggesting that litter decomposition in the region is more affected by soil characteristics than by litter quality. The application of dolomitic limestone in the region has been found to increase microbial biomass for fungi, actinomycetes, and bacteria as well as increased levels of key decomposing enzymes (McKergow et al., 2021). The increase in decomposer biomass and activity following regreening has most likely stimulated litter decomposition to levels we would expect for a “natural” pine plantation in the region, and it appears that this increase in litter decomposition remains consistent up to 40-years post-regreening. However, despite increases in microbial biomass and activity, the microbial communities in the area have been found to still largely be made up

of bacteria (79% of total microbial biomass) indicating the region remains under environmental stress as acidic coniferous forests are typically dominated by fungal communities (McKergow et al., 2021).

We found that there was a HFA for local decomposer communities at the greening sites. Common litter was taken adjacent to one of the study sites where the greening treatment was applied that consisted of jack and red pine litter with a very similar C:N ratio to the site-specific litters prior to exposure. Despite these similarities, litter decomposition rate was higher (and C:N ratios lower) using site-specific litter at all greening sites. The combination of physiochemical properties and biotic interactions of both litter and soil is complex and the magnitude and direction of a HFA greatly relies on site-specific properties (Palozzi and Lindo, 2018; Veen et al., 2015). Home-field advantages have largely been documented in studies with drastic differences in litter types (Ayres et al., 2009; Chomel et al., 2015; Gholz et al., 2000) but have also been reported when using similar litter (Veen et al., 2019). The consistent presence of a HFA at the greening sites, despite the similarity between site-specific and common litter reinforces the heterogeneity of the City of Sudbury greening areas and suggests that local decomposer communities may be hyper-specialized to litter produced within their respective site. Previous studies in the City of Greater Sudbury area conducted in areas where greening did not occur suggest that initial litter quality did not affect decomposition rates (Freedman and Hutchinson, 1980a; Johnson and Hale, 2004).



#### *3.4.4. Soil respiration*

There were no clear relationships between soil CO<sub>2</sub> emission rates and stand age at the regreening sites. A lack of relationships has been previously reported in jack pine stands following forest fires (Yermakov and Rothstein, 2006). While there were no relationships between soil CO<sub>2</sub> emissions and stand age, eroded sites did have significantly higher unadjusted CO<sub>2</sub> emission rates and higher soil temperatures compared with stable sites. Tree density was substantially lower at the eroded sites likely leading to higher soil temperatures, stimulating increased rates of soil CO<sub>2</sub> emissions. Unadjusted soil CO<sub>2</sub> emission rates at untreated sites were significantly higher than at regreening sites contrary to the prediction that regreening increases soil CO<sub>2</sub> respiration rate. Mukerji (2020) reported soil CO<sub>2</sub> emissions were 2.9 times higher at reclaimed versus unreclaimed sites in the City of Greater Sudbury barrens. However, the untreated areas studied in Mukerji (2020) were largely devoid of vegetation aside from a few shrubs and grasses, while the untreated sites from the current study have a high density of established deciduous trees (average aboveground biomass 32.10 Mg ha<sup>-1</sup>) which tend to have high litterfall, and a relatively thick forest floor (average depth 4.1 cm) compared to our regreening sites. Prior to the beginning of the regreening program Amiro and Courtin (1981) defined two anthropogenic ecological communities in heavily impacted regions within the City of Greater Sudbury barren areas – barren communities, and birch transition communities. The dominant mechanism proposed by Amiro and Courtin (1981) for differing ecological structure was topography – birch transition communities occur lower in valleys where soil

and moisture conditions are more favorable than on eroded hill tops characteristic of barren communities. The establishment of paper birch and trembling aspen at the untreated sites appears to have increased soil CO<sub>2</sub> respiration rates compared with the totally barren sites. However, respiration rates at untreated sites in the current study were still considerably lower than paper birch and trembling aspen stands found in similar ecozones (Gaumont-Guay et al., 2009; Tang et al., 2009) while soil CO<sub>2</sub> emission rates at the regreening sites were similar to those from other jack pine stands (Striegl and Wickland, 1998; Weber, 1985). Soil CO<sub>2</sub> evolution within the City of Greater Sudbury barrens were reported as very low prior to regreening (Freedman and Hutchinson, 1980a); suggesting regreening has potentially led to an increase in soil respiration rates.

#### *3.4.5. Forest floor and mineral soil C*

At the regreening sites forest floor C pools increased with stand age while mineral soil C concentrations in bulk soils and aggregate fractions decreased. Soil C concentrations at the untreated sites were similar to the youngest regreening sites while the oldest regreening sites were more similar to the old plantation site. The reported effects of regreening on C pools in both organic and mineral soil horizons have been mixed (Jandl et al., 2007; Mayer et al., 2020; Paradelo et al., 2015). Many studies have reported that liming and fertilizing can both decrease (Emmerling and Eisenbeis, 1998; Forey et al., 2015; Kreutzer, 1995; Persson et al., 2021) or increase (McCay et al., 2013; Melvin et al., 2013; Sridhar et al., 2022) forest floor C pools by restructuring local decomposer communities. While there are generally less effects reported on the impact of lime and fertilizer addition

to mineral soil C compared with forest floor C – studies have also found that nutrient addition and increasing pH both increases (Jandl et al., 2003; Mäkipää, 1995; Nave et al., 2009) and decreases (Persson et al., 2021, 1995) mineral soil C, suggesting this is due to changes in decomposer communities or altered vegetation growth. Afforestation has generally been found to increase forest floor and mineral soil C pools through increased inputs of vegetation C (Ashwood et al., 2019; Frouz et al., 2009; Laganière et al., 2010) but losses of soil C have also been reported when afforestation occurs on C-rich soils such as grasslands or heathlands (Friggens et al., 2020; Guo et al., 2007).

Forest floor C pools of the regreening sites closely reflect accumulation patterns of jack pine wildfire chronosequences where forest floor C pools stabilize between 15–21 Mg ha<sup>-1</sup> up to 111-years post-fire (Nalder and Wein, 1999; Yermakov and Rothstein, 2006). Space-for-time studies assessing the effects of stand age and forest dynamics often use wildfires or clear-cutting as stand replacing events. While the importance of site history is not always discussed, the nature of the site replacing events dramatically alters forest floor accumulation rates and patterns. Following clear-cuts, the development of forest floor is influenced by large amounts of harvesting residue and the remnant forest floor, which complicates the forest floor development process (Covington, 1981; Yanai et al., 2000). Whereas following wildfires the forest floor is largely reduced, and in some instances >80% of the forest floor is consumed (Weber et al., 1987). Forest floor accumulation rates and patterns at the regreening sites more closely resembled those following wildfires compared with clearcuts. While the space-for-time chronosequence approach does not

allow us to have exact insight into the history of the regreening sites, the City of Greater Sudbury barren areas prior to regreening were reported to be largely devoid of any remaining forest floor following massive soil erosion events (Freedman and Hutchinson, 1980a).

The decrease in mineral soil C concentration with stand age in bulk mineral soils and aggregate fractions was potentially caused by regreening priming local decomposer communities and elevating C mineralization for both “new” C added from newly established vegetation, as well as “old” C remnant in soils before industrial degradation. Multiple mechanisms could be responsible for priming decomposers, such as increasing pH (McKergow et al., 2021), the addition of bio-available nutrients (Chen et al., 2014), or the introduction of novel sources of labile C such as plant litter (Kuzyakov, 2010). Older regreening stands would have elevated rates of CO<sub>2</sub> emissions for longer periods of time, leading to lower soil C concentrations. The decrease in the smallest measured aggregate fractions is cause for concern for long-term soil C sequestration, as turnover of soil C is much slower in smaller aggregates as the occlusion of soil C by aggregation limits accessibility to decomposers and their enzymes (Six et al., 2002; von Lützow et al., 2006). Additionally, long-term soil C decomposition is driven more by spatial accessibility and organo-mineral interactions than litter recalcitrance (Angst et al., 2022; Schmidt et al., 2011). Despite slower decomposition rates in smaller aggregates, the priming of decomposers has been reported to decrease labile C rates at similar rates to more stable C (Guenet et al., 2012).

We anticipate that the lost mineral soil C is most likely respired into the atmosphere as the regreening sites have soil CO<sub>2</sub> emissions similar to other jack and red pine stands compared with the very low soil CO<sub>2</sub> emission rates measured prior to regreening (Freedman and Hutchinson, 1980a). However, another potential pathway for lost mineral soil C is through DOC export. Increasing pH has been found to regulate the ability of organic C to associate with iron (Fe) and aluminum (Al) minerals, where a lower pH promotes the formation of organo-metal bonds (Ye et al., 2022). Lakes in the City of Greater Sudbury area have seen increases in DOC concentrations during regreening (Hall et al., 2021). The increase in lake DOC in the City of Greater Sudbury area has generally been assumed to be due to increasing surface water pH or internal DOC cycling export of terrestrial DOC could also be a factor (Meyer-Jacob et al., 2020).

#### *3.4.6. Total ecosystem C budgets*

Regreening increased total ecosystem C pools at both eroded and stable sites compared to the untreated sites up to 40-years after regreening largely due to increases in aboveground C. Previous studies have found similar results that regreening increases total ecosystem C pool particularly when applied to cropland or post-mining soils (Frouz et al., 2009; Ouimet et al., 2007; Thibault et al., 2022). Studies that included longer time periods found that aboveground C pools arising from natural succession eventually equal those from planted areas and that soil C pools following natural succession are equivalent or greater (Thibault et al., 2022). Additionally, ecosystem properties such as biodiversity are much higher following natural succession versus afforestation (Tremblay and Ouimet,

2013). However, regreening studies are generally not conducted on a region as denuded and toxic as the City of Greater Sudbury barrens where historically successful tree growth and natural succession processes were simply not possible without deliberate management. An example of this is the aboveground vegetation at this study's untreated sites that are dominated by paper birch but also included trembling aspen and red maple; early-succession tree species that dominate most of the unplanted and untreated regions across the City of Greater Sudbury (Munford et al., 2021; VETAC, 2021). The aboveground tree C pools at the untreated sites closely reflect those of the youngest treated sites, despite untreated sites having roughly double the stem density. Additionally, the paper birch and trembling aspen at the untreated sites were highly coppiced, with some individuals exceeding 10 stems. The heavy coppicing of paper birch and trembling aspen is linked to the continuous cycle of dieback and regrowth from sprouts (Safford et al., 1990). Aboveground C at our untreated sites may also be on the larger end for the region as our study specifically targeted untreated sites where there was tree growth. Both Preston et al. (2020) and Rumney et al. (2021) reported considerably lower aboveground C values at untreated areas. This could also be due to topography as previous studies conducted in the region found that exposed ridges had very little successful vegetation establishment compared to more protective areas (James and Courtin, 1985).

### *3.5. Conclusion*

The relationship between stand age and soil C processes at both stable and eroded sites after regreening in a severely impacted landscape closely reflected those of jack and

red pine stands in regions that have not been subject to over a century of industrial impacts. A major exception was a decrease in mineral soil and aggregate C concentration with stand age. The similarities between rates of soil CO<sub>2</sub> emissions and litter decomposition at the regreening sites to jack and red pine stands unimpacted by centuries of industrial degradation, as well as the reported repressed rates of soil respiration and litter decomposition prior to regreening suggest that regreening promoted increased mineralization of soil C up to 40-years post-regreening. Soil C – particularly forms protected in aggregates – is generally considered the most stable terrestrial C pool and the effects of regreening on soil C (while still poorly understood) have been found to be highly dependent on local environmental conditions. While the effects of regreening on soil C warrants further investigation, at the City of Greater Sudbury regreening sites, the loss of soil C is trivial in pool size relative to the substantial increases in aboveground tree and forest floor C pools – leading to a sizeable increase in total ecosystem C pool following application of regreening methods.

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## **Chapter 4: Nutrient cycling in jack and red pine forests following the greening of a mining and smelting degraded landscape**

### *4.0. Abstract*

Mining and smelting degraded landscapes are typically poor in nutrients, rich in toxic metals, with acidic and heavily eroded soils. The greening of these landscapes (application of lime and fertilizer followed by tree planting) can increase site productivity and ecosystem nutrient pools. However, uncertainties exist surrounding nutrient cycling, and long-term viability of restored forests. The current study aims to investigate how nutrient cycling processes of greened forests on a mining and smelting degraded landscape change with stand age using a chronosequence approach in the City of Greater Sudbury, Canada. The greening sites were rich in calcium (Ca) and magnesium (Mg), both in soils and trees. Soils were generally poor in phosphorous (P) and potassium (K), however foliar concentrations of all nutrients reflected those of “healthy” red pines. Nitrogen (N) mineralization rates and inorganic N concentrations were very low, however N stored in vegetation and total soil N are consistent with values expected for the region. Overall, there were few relationships between nutrient cycling processes and stand age. Nutrient cycling processes (such as nutrient resorption during translocation, litter decomposition) up to 40-years post-greening were like those of jack and red pine plantations that were not subject to over a century of mining and smelting impacts indicating no current effect of nutrient limitation or metal toxicity on forest biogeochemistry.

#### *4.1. Introduction*

Mining and smelting degraded landscapes are generally inhospitable to the vegetation. Limited nutrients inhibit the establishment and growth of vegetation and metals can have direct toxic effects on vegetation as well as compete with nutrients for biological uptake (Folkeson and Andersson-Bringmark, 1988; Pålsson, 1989; Whitby and Hutchinson, 1974). Due to the low amount of nutrients available on these landscapes, nutrient cycling is integral to long-term ecosystem sustainability. Therefore, studying processes such as nutrient resorption, litter decomposition, and nitrogen (N) mineralization can provide insight into overall ecosystem health (Cole and Rapp, 1980; Vitousek, 1982).

Limited nutrients and elevated metal concentrations in mining and smelting degraded landscapes can alter forest nutrient cycling processes in a number of ways (Šourková et al., 2005). Low nutrient availability may increase nutrient resorption as vegetation conserves a higher proportion of foliar nutrients during senescence (Munford et al., 2021). Nutrient resorption is measured as the proportion of nutrients retained in foliage during senescence (referred to as nutrient resorption efficiency (RE)), and as the concentration of nutrients in senesced foliage (resorption proficiency (RP)) (Killingbeck, 1996). Further, elevated metals which are toxic to soil organisms, can slow litter decomposition rates (Babich and Stotzky, 1985; Berg et al., 1991; Cotrufo et al., 1995) and the low levels of nutrients in litter available to soil organisms can also lead to a decrease in litter decomposition (Berg and Staaf, 1980; Coûteaux et al., 1995). Elevated metal

concentrations have been proposed to both increase (DeCatanzaro and Hutchinson, 1985) and decrease (Vásquez-Murrieta et al., 2006) N mineralization as the presence of toxic metals alters the local nitrifying community.

The City of Greater Sudbury, Ontario, Canada and surrounding region has been exposed to effects of over a century of copper (Cu) and nickel (Ni) mining and smelting. Smelting emissions and expansive clear-cutting resulted in ~20,000 ha of barren land with highly eroded, acidic soils enriched in metals, and depleted of essential nutrients inhibiting the regrowth of vegetation (Freedman and Hutchinson, 1980a, 1980b). Previous work on nutrient cycling in the City of Greater Sudbury barren areas found that nutrient RE was increased due to limited soil nutrients (Munford et al., 2021); litter decomposition was decreased due to low levels of nutrients and elevated metals (Freedman and Hutchinson, 1980a; Johnson and Hale, 2004; SARA Group, 2009); and N mineralization was increased due to elevated metal concentrations (DeCatanzaro and Hutchinson, 1985).

In 1978 the City of Greater Sudbury began a greening program that is regarded as one of the largest in the world (VETAC, 2021). Along with sulphur dioxide (SO<sub>2</sub>) and particulate metal emissions being decreased by over 95%, more than 3,400 ha of land has been limed and fertilized, and over 10 million trees have been planted (VETAC, 2021). The greening treatments have increased forest floor and mineral soil calcium (Ca) and magnesium (Mg) pools, increased soil pH, and decreased the bioavailability of metals (Kellaway et al., 2022; SARA Group, 2009) leading to increased tree growth and forest floor accumulation (Levasseur et al., 2022; Preston et al., 2020; Rumney et al., 2021). However,

the greening program in the City of Greater Sudbury (similar to many around the world, (Frouz et al., 2001; Persson et al., 1995; Pietrzykowski and Daniels, 2014)) currently applies nutrients as a one-time dose, with massive quantities of limestone ( $10 \text{ tonnes ha}^{-1}$ ), and lesser amounts of N-phosphorous (P)-potassium (K) fertilizers ( $390 \text{ kg ha}^{-1}$ ). Kellaway et al., (2022) found that much of the applied limestone was lost from the landscape, however, the status of other nutrients is unclear. Levasseur et al., (2022) found that some nutrients in trees decreased with stand age, so long term viability of the greening sites is unclear. Gaining a better understanding of the effects of greening on nutrient cycling and how these processes change over time is integral for future management planning.

We tested the hypothesis that stand age following greening affects forest nutrient cycling processes. The greening treatments have been applied in a patchwork approach allowing us to develop a chronosequence of sites to assess how nutrient cycling has changed with stand age following greening. Specifically, we predicted that: 1) concentrations of Ca, Mg, P, and K will decrease in the forest floor and increase in mineral soil and vegetation with stand age, while N concentrations in soils and trees will increase with age; 2) nutrient RE will increase with time since greening while nutrient RP will decrease over time; 3) loss of litter nutrients through decomposition will increase as stands age; and 4) N mineralization in soil will increase with stand age.

## 4.2. Materials and Methods

### 4.2.1. Study area and greening process

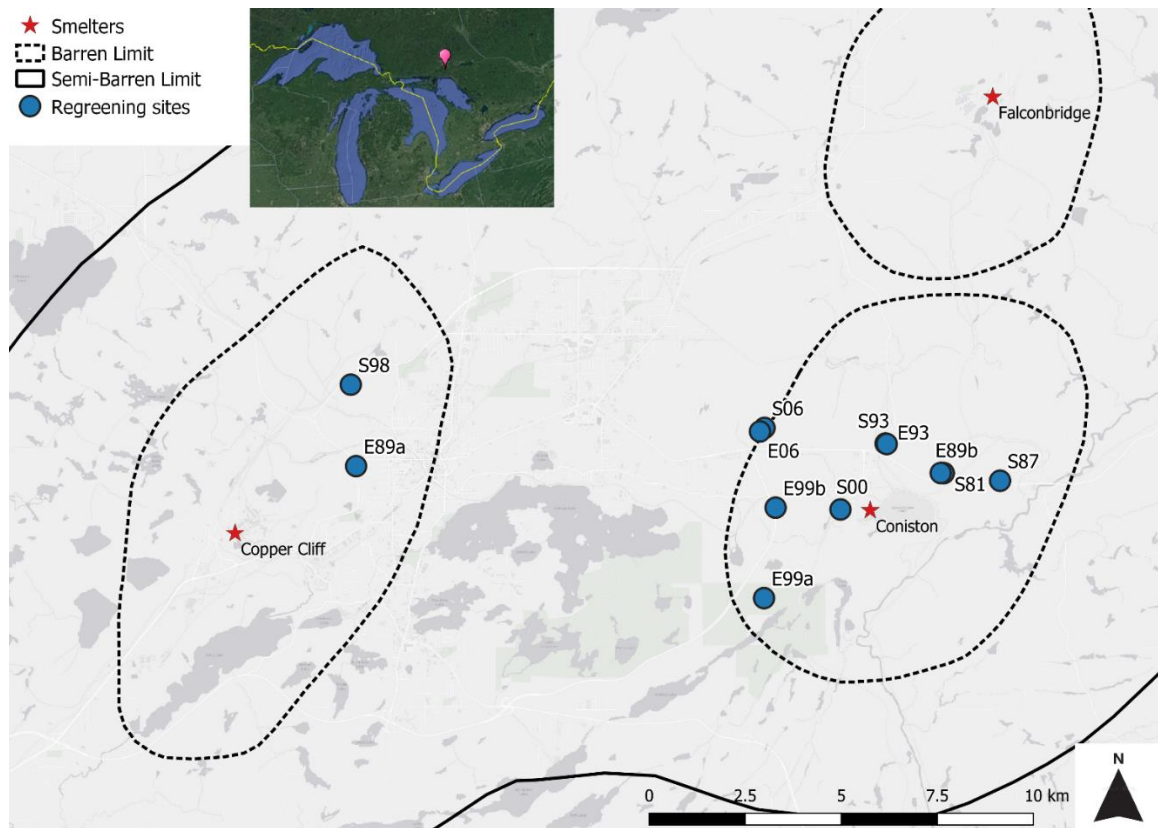
The City of Greater Sudbury (UTM zone 17; Easting: 501620, Northing: 5148797) is in northern Ontario, Canada on the southern portion of the Precambrian Shield. Thirty-year (1981–2010) average monthly temperatures range from -13.0 °C in January to 19.1 °C in July, and average precipitation is 904 mm per year (Environment Canada, 2022). The City of Greater Sudbury lies in the vegetation transition zone between the Great Lakes – St. Lawrence Forests regions of the south and the boreal forest to the north (Rowe, 1972). The topography of the region is undulating and dominated by rocky outcrops overlain by soils that are typically shallow and sandy (Pearson and Pitblado, 1995). Prior to greening efforts, the soils within the barrens of the City of Greater Sudbury were highly acidic (pH < 4.3), devoid of nutrients, and contained high concentrations of bioavailable aluminum (Al), Cu and Ni (Freedman and Hutchinson, 1980b). Since 1978, more than 3,400 ha of land have been treated with 10 Mg ha<sup>-1</sup> of crushed dolomitic limestone (CaMg(CO<sub>3</sub>)<sub>2</sub>), 390 kg ha<sup>-1</sup> of 6-24-24 N-P-K fertilizer, and 40 kg ha<sup>-1</sup> of a grass-seed mixture, that included N-fixing legumes. One-year after the application of lime, fertilizer, and grass-seed, trees were planted, and across the entire City of Greater Sudbury region more than 10 million trees have been planted with species that are native to the region. Planted tree species were predominantly jack pine (*Pinus banksiana* Lamb.) and red pine (*Pinus resinosa* Ait.), but also included white pine (*Pinus strobus* L.), white spruce (*Picea glauca* Moench) and green alder (*Alnus viridis* Chaix) (VETAC, 2021). Areas for greening are

selected each year based on multiple operational and resource factors and needs, resulting in a mosaic of forest stands of different ages in the barren and semi-barren areas of the region.

#### *4.2.2. Site Selection*

Twelve study sites were established within the barrens of the Coniston and Copper Cliff smelters (Figure 4.1). The barrens were defined as areas that were largely devoid of plant life and had soil pH below 4.3 before regreening took place (Lautenbach et al., 1995). To account for the effects of erosion on nutrient pools, Chapters 2 and 3 separated sites into “eroded” and “stable” classifications and found that there were few biogeochemical differences between the two site types, so in this study no distinction was made. The 12 “regreening sites” were limed, fertilized, seeded, and planted with either jack pine and/or red pine. Planting dates ranged from 1981 to 2006, and only sites that experienced a single planting occurrence were selected. All study sites were 10 m x 10 m in size and located within a larger forested area with similar characteristics.





**Figure 4.1:** Map of the twelve study sites, the outlines for the barren and semi-barren regions, and the location of the three main smelters (City of Greater Sudbury, 2023).

#### 4.2.3. Field sampling

##### 4.2.3.1. Soil sampling

Soil sampling occurred between May–August 2019. Both forest floor and mineral soil samples were taken at nine points within each site (three horizontally along both the north and south borders of the plot and three running horizontally through the center). Mineral soils were sampled by auger for 0–5 cm depths at all sites. Forest floor samples were collected by cutting a 10 cm x 10 cm square using a knife and separating the forest floor into L (litter) and fibric-humic (FH) layers (Soil Classification Working Group, 1998).

The depth of the L and FH layers at each sampling location were measured. Mineral soil bulk density (BD) samples were also taken using a BD hammer at three points in each plot (north corner, plot center, and south corner). Forest floor and mineral soil samples were immediately placed in sealable polyethylene bags. All soil samples were stored in a refrigerator (4°C) upon return from the field.

#### *4.2.3.2. Litter traps*

Three litter traps were established in July 2018 at each site (north corner, plot center, and south corner). Litter traps were created using milk crates with a 30 cm x 40 cm opening at the top. A mesh hammock (made using 2 mm mesh) was suspended within the trap so that no litter had contact with underlying soils. Large branches were removed from litter traps, but foliage, small branches, and seeds were all collected as litter. Litter was collected 3 times a year (in both 2018 and 2019) between July and November. Litter was immediately placed in sealable polyethylene bags and stored in a refrigerator (4°C) upon return from the field.

#### *4.2.3.3. Litter decomposition bags*

Eighteen 20 cm × 20 cm litter decomposition bags (made using 2 mm fibreglass mesh) were placed at each site. Nine litter bags were filled with 3 g of oven-dried non-milled litter collected from the respective sites' litter traps (referred to as "site-specific litter") and nine were filled with 3 g of oven-dried and homogenised red pine and jack pine litter collected adjacent to one specific site (E89a) to control for litter quality (referred to as "common litter"). In April 2019, the nine site-specific litter bags were tied together, and

left on top of the forest floor secured to the base of a tree at plot center. The same procedure was performed for the common litter bags. In November 2021 (2.5 years after deployment), three site-specific and three common litter bags were removed from each site. The litter bags were immediately placed in sealable polyethylene bags and were stored in a refrigerator (4 °C) upon return from the field (Bocock and Gilbert, 1957).

#### *4.2.3.4. Vegetation sampling*

Vegetation sampling was conducted July 2018 and 2019. Tree species (diameter at breast height >2.5cm) were recorded for every tree within each of the 10 m × 10 m sites. Tree cores were taken in July 2019 from three trees in each site, all sampled trees were >10 cm dbh and sampled trees within each site were of the same age. Tree cores were used to verify age and used for wood nutrient and metal analysis. Two cores were taken from each sampled tree, one at breast height, and the second 20 cm below at a 90° angle from the entrance point of the first core. Cores were stored in plastic straws with the ends taped. Bark samples were taken in July 2019 from these same trees at breast height by scraping bark off the tree using a knife and composited from the three trees in a polyethylene bag. Foliage samples were taken in both July 2018 and 2019 using telescoping pruners from the highest possible branch from each sampled tree and composited into paper bags. Current year needles were targeted for foliage sampling. Most sites were monocultures of jack or red pine, and the three vegetation sampling trees were selected to be the dominant species (greater than 33% of stems) for each site. On the occasion that both jack and red pine comprised greater than 33% of stems per site,

vegetation samples were taken from three trees of both species. All vegetation (tree cores, bark, and foliage) samples were stored in a refrigerator (4°C) upon return from the field.

#### *4.2.3.5. Buried-bag experiments*

Nitrogen mineralization rates were assessed using the buried-bag method for three separate 30-day incubation periods (May, July, and September) in the 2019 field season. For each incubation experiment two soil cores were taken at each site using a BD hammer. One sample was placed in a polyethylene bag and brought back to the lab. The other sample was placed in a polyethylene bag and placed in the hole made by the corer and incubated in the field for 30-days. After removal from the field, all samples were immediately placed in a freezer (−18°C) and frozen until analysis (Westermann and Crothers, 1980).

#### *4.2.4. Sample preparation and chemical analysis*

All samples (excluding the mineralization samples) were oven-dried at 105°C for 24-hours prior to analysis. Bulk densities were calculated for the L, FH, and 0–5 cm samples by dividing the oven-dry mass by the sample volume. Litter bags were weighed after drying. Litter, forest floor, and vegetation samples (including entire tree cores) were milled to a fine powder using a grinder. Mineral soil samples were sieved to 2 mm. Milled litter, vegetation, and forest floor samples were analyzed for acid-digestible Ca, Mg, K, P, Cu, and Ni by digesting 0.2 g of sample in 2.5 mL of concentrated nitric acid (trace grade 70% nitric acid (HNO<sub>3</sub>)) at 100°C for eight hours followed by a digestion at room temperature for eight hours. The digested material was filtered through a 0.45 µm pore filter paper and

diluted with B-pure deionized water until total volume reached 25 mL. The digest samples were then diluted 1:10 and analyzed using a Perkin Elmer (7000 DV) Inductively Ion Coupled Optical Emission Spectrometer (ICP-OES). Total N concentrations were measured for the sieved mineral soils, milled litter, vegetation, and forest floor samples using an Elementar Macro CNS analyzer. Sieved (< 2 mm) mineral soil samples were analyzed for extractable Ca, Mg, P, and K concentrations in a 1:10 soil-solution ratio with 1 M ammonium chloride (NH<sub>4</sub>Cl) after shaking for 2 hours on an oscillating shaker table. Samples were vacuum filtered, diluted, acidified, and analyzed by ICP-OES (Carter and Gregorich, 2007). Sieved mineral soil samples were analyzed for dissolved orthophosphate (extractable P) using a Mehlich-3 extraction method followed by filtering with 0.45 µm syringe filters. Samples were analyzed using a Perkin Elmer Lambda XLS+ UV-spectrophotometer at 845 nm (Mehlich, 1984). Extractable Cu and Ni was measured in sieved mineral soil samples using deionized water extractions by shaking 5 g of sample in 25 mL of B-Pure deionized water overnight, samples were then syringe filtered (0.45 µm), acidified with 0.2 mL nitric acid, and analyzed via ICP-OES. Inorganic N (nitrate (NO<sub>3</sub>) and ammonium (NH<sub>4</sub>)) concentrations were analyzed on 5 g of field-moist mineral soil samples from the buried-bag experiment. Soils were mixed with 50 mL of 2 M potassium chloride (KCl) and shaken for 30 minutes on an oscillating shaker table. Samples were extracted through 0.45 µm pore filter paper using vacuums, the extracts were analyzed on an automated segmented flow analyzer (Bran and Luebbe, Autoanalyzer 3) (Carter and Gregorich, 2007). Quality control was confirmed by running all samples in triplicate and

including experimental blanks as well as a standard (NIST-1515 apple leaf for forest floor and vegetation samples, and SS-2 soil standard for mineral soil) every 25 samples.

#### 4.2.5. Calculations

Nutrient RE is defined as the amount of nutrients resorbed during senescence and is expressed as a percentage (Aerts, 1996). Nutrient RE is calculated for both the 2018 and 2019 field seasons as:

$$RE = ((Nu_{gn} - Nu_{sn})/Nu_{gn}) * 100\% \quad \text{[Equation 4.1]}$$

Where RE is resorption efficiency (%),  $Nu_{gn}$  is green leaf nutrient concentration ( $\text{mg kg}^{-1}$ ), and  $Nu_{sn}$  is senesced leaf nutrient concentration ( $\text{mg kg}^{-1}$ ). Resorption proficiency ( $\text{mg kg}^{-1}$ ) is determined as the total concentration ( $\text{mg kg}^{-1}$ ) of elements in senesced leaves.

Litter decomposition rate constants were determined using the Olson, (1963) equation:

$$k = -\ln \left( \frac{X_t}{X_0} \right) / t \quad \text{[Equation 4.2]}$$

where  $k$  is the decomposition rate constant,  $X_t$  is the mass of litter (g) remaining in each litter bag after  $t$  time in years, and  $X_0$  is the initial mass of litter (g) in each bag.

Nutrient mass lost during litter decomposition (%) was calculated using:

$$Nm_{loss} = \frac{m_i * Nc_i - m_f * Nc_f}{(m_i * Nc_i)} * 100 \quad \text{[Equation 4.3]}$$

where  $Nm_{loss}$  is proportion of nutrient mass lost during litter decomposition (%),  $m_i$  is initial total mass of litter (mg),  $Nc_i$  is initial concentration of nutrient in litter ( $\text{mg kg}^{-1}$ ),  $m_f$  is final total litter mass (mg), and  $Nc_f$  is final nutrient concentration in litter ( $\text{mg kg}^{-1}$ ).

Ammonification rates were calculated by subtracting initial  $\text{NH}_4$  concentrations from final

NH<sub>4</sub> concentrations and dividing value by days incubated in field.

#### *4.2.6. Statistical analyses*

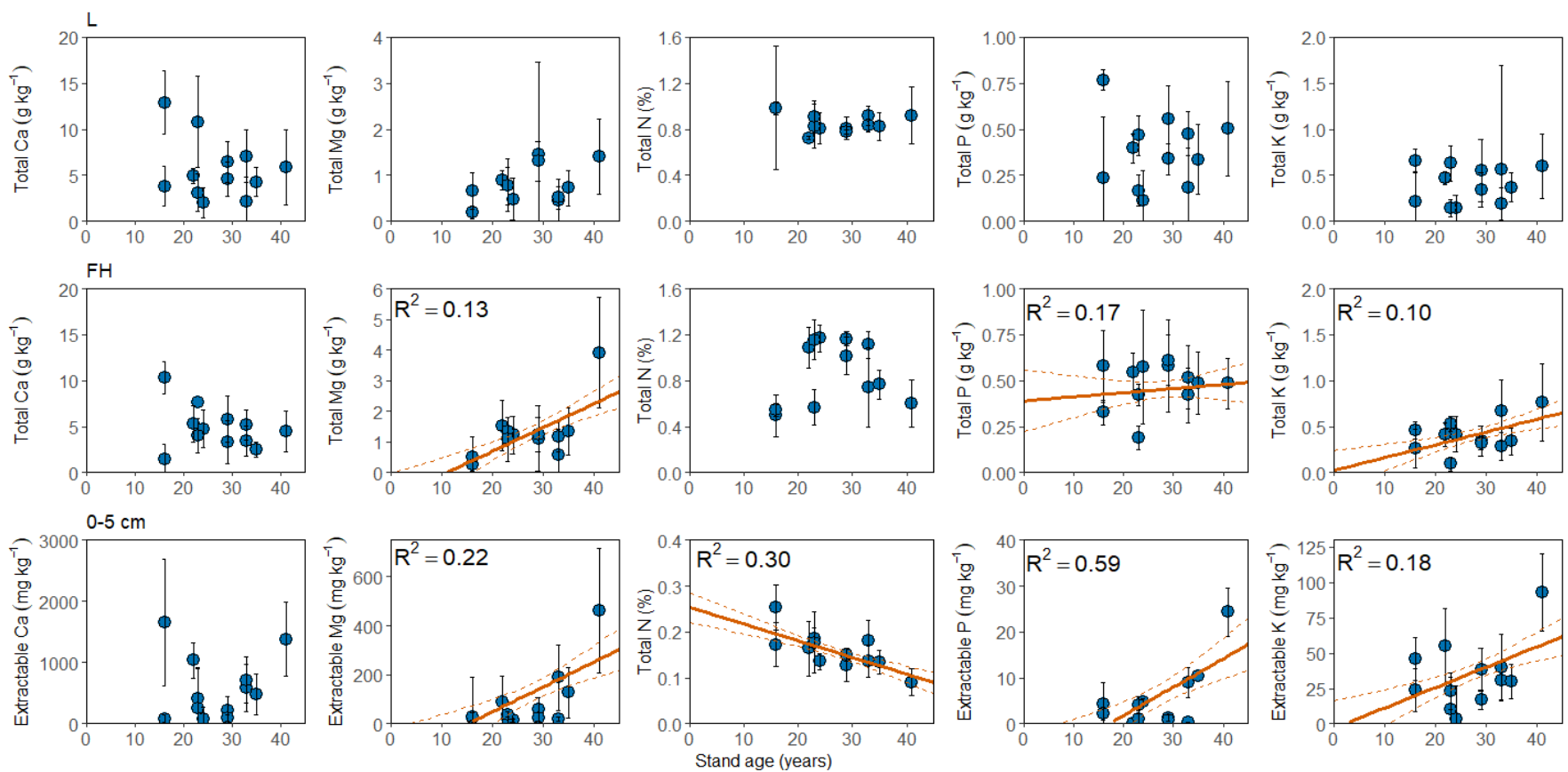
Linear regressions were used to assess the relationships between stand age and a variety of parameters including soil and vegetation nutrient concentrations, RP, RE, nutrient loss during litter decomposition, and ammonification rates. Linear regression was also used to assess the relationship between litter nutrients and litter decomposition rates. Normality of the error terms was assessed using quantile-quantile plots. Logarithmic regression was used to assess the relationship between foliar nutrients and nutrient RP. Relationships were determined to be statistically significant using Bonferroni corrections. Differences in nutrients lost between litter types were tested using Mann-Whitney U-test. Groups were considered significantly different using Bonferroni corrections. A correlation matrix using Spearman correlations was created to assess associations between RE, foliar nutrients, and soil metal nutrient concentrations; relationships were considered statistically significant using Bonferroni corrections. Prior to analysis normality of the data were tested using the Lilliefors corrected Kolmogorov-Smirnov test, variables were considered normally distributed if p was greater than 0.05. All statistical analysis was completed using R version 4.0.5 (RStudio Team, 2020). All maps were created using Quantam GIS version 3.2.1 (QGIS.org, 2022).

### *4.3. Results*

#### *4.3.1. Soil nutrients*

The nutrient content of the L horizon was unaffected by stand age (Figure 4.2). In contrast, soil nutrient concentrations largely increased in the FH and 0–5 cm horizon with stand age (Figure 4.2). Total Mg, P, and K increased in the FH horizon ( $p < 0.001$ ). Similarly, extractable Mg, P, and K increased in the 0–5 cm mineral soil horizon ( $p < 0.0001$ ). The one exception was total N concentrations that decreased with stand age in the 0–5 cm horizon of the mineral soil ( $p < 0.0001$ ).

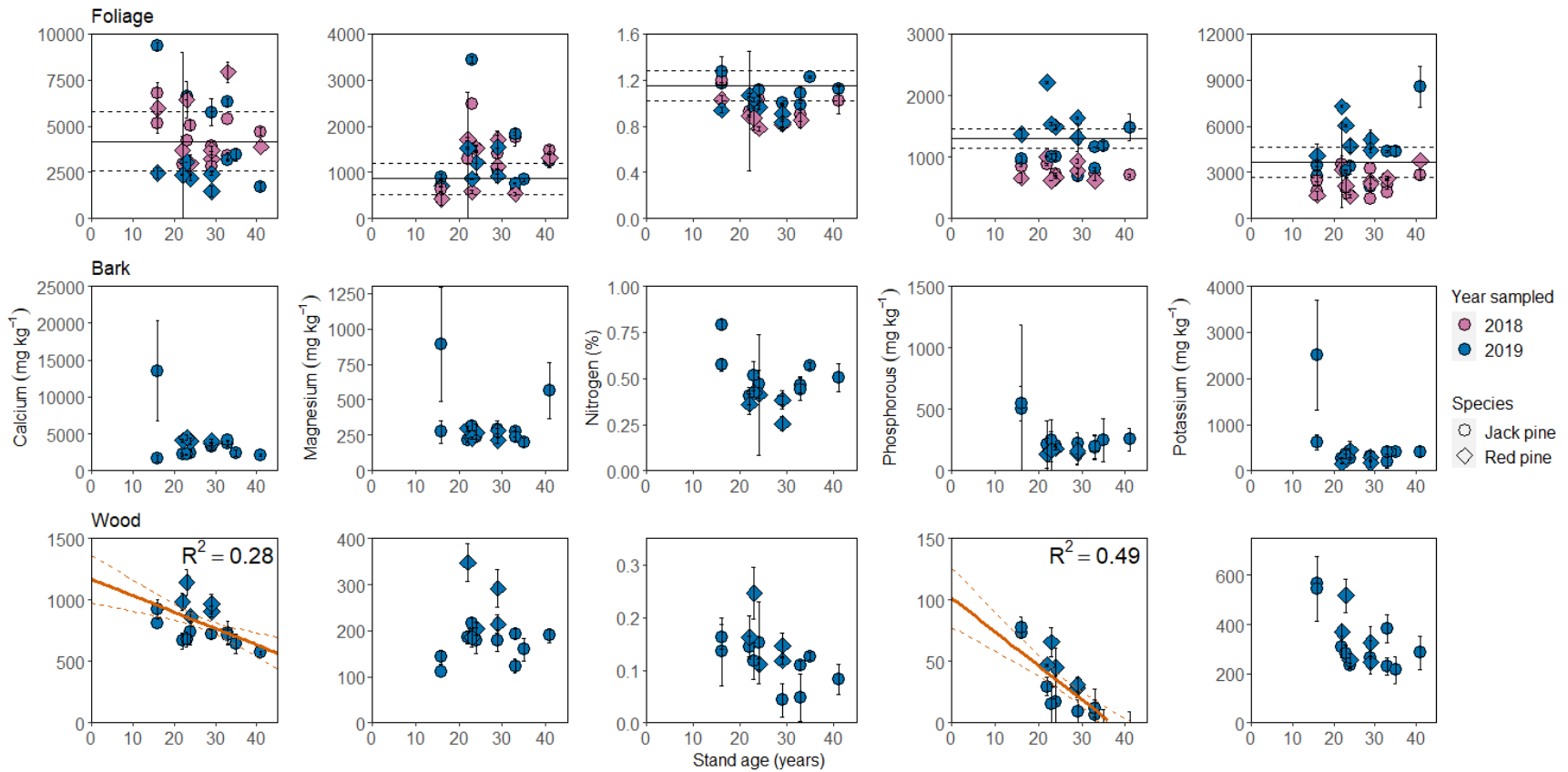




**Figure 4.2:** Total and extractable nutrient concentrations (mean  $\pm$  standard deviation) and stand age in the litter (L) (**top row**), fibric-humic (FH) (**middle row**), and 0–5 cm mineral soil (**bottom row**) horizons for regreening sites. Solid orange lines are significant ( $p < 0.003$ ) regression relationships between nutrient concentrations and stand age; dashed orange lines are 95% confidence intervals. Regression coefficients ( $R^2$ ) are included for statistically significant linear relationships.

#### 4.3.2. Aboveground tree nutrients

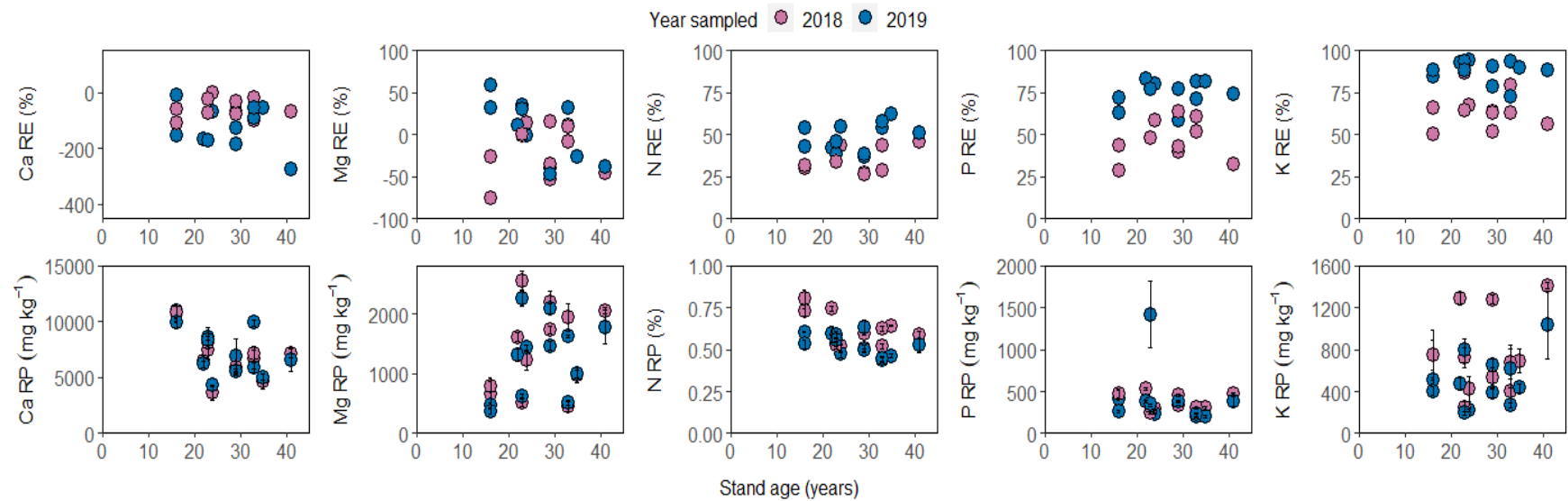
There were a few changes in aboveground tree nutrient concentrations over time (Figure 4.3). Specifically, wood Ca and P decreased with stand age ( $p < 0.0001$ ), while wood N and K did appear to decrease with stand age, these decreases were not statistically significant using Bonferroni corrections (N ( $R^2 = 0.09$ ;  $p = 0.03$ ); K ( $R^2 = 0.16$ ;  $p = 0.005$ )). Foliar and bark nutrient concentrations were unaffected by stand age ( $p > 0.05$ ). Foliage collected in the 2018 sampling season had significantly higher Ca concentrations, and significantly lower N, P, and K concentrations compared with foliage collected in the 2019 season ( $p < 0.0001$ ). The current study was not designed to compare differences in jack pine and red pine chemistry, however foliar nutrient concentrations were within one standard deviation of one another for both species.



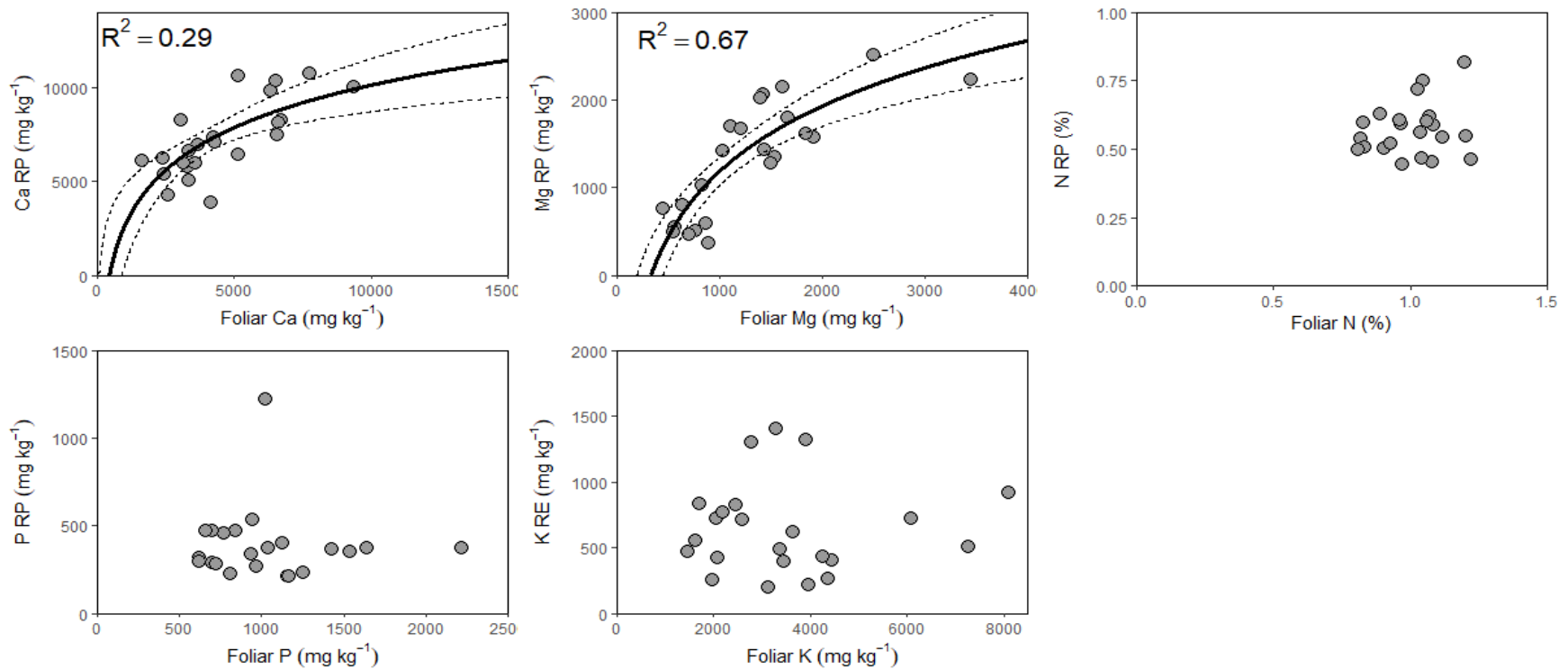
**Figure 4.3:** Nutrient concentrations (mean  $\pm$  standard deviation) and stand age in tree foliage (both 2018 and 2019 samples) (**top row**), tree bark (**middle row**), and tree wood (**bottom row**) for regreening sites. Solid orange lines are significant ( $p < 0.003$ ) regression relationships between nutrient concentrations and stand age; dashed orange lines are 95% confidence intervals. Regression coefficients ( $R^2$ ) are included for statistically significant linear relationships. Solid black line represents average foliar nutrient concentration for “healthy” red pine in the Tree Chemistry Database (Pardo et al., 2005); dashed black lines represent standard deviations of these values.

#### 4.3.3. Nutrient resorption efficiency and proficiency

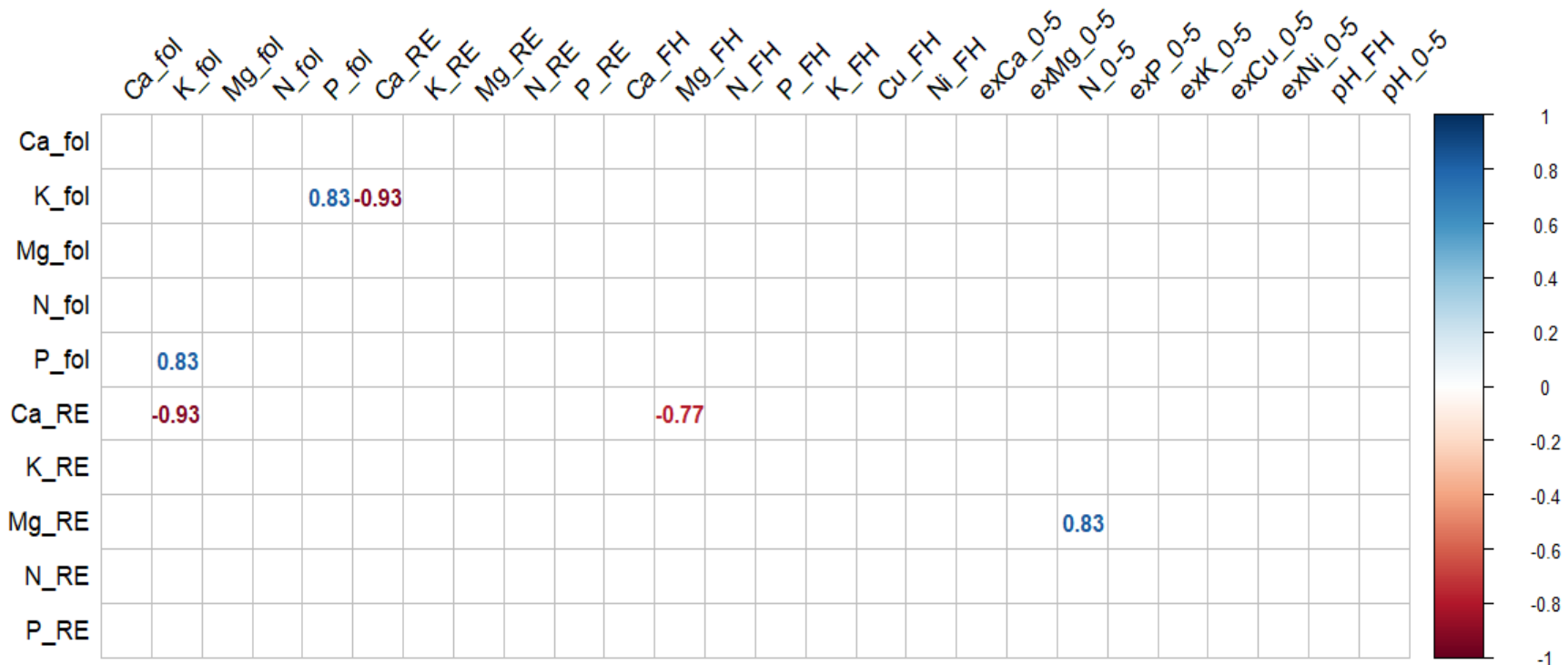
There were no significant relationships between RE or RP and stand age across the 12 regreening sites in both years (Figure 4.4). Resorption efficiencies for N, P, and K were significantly higher in the 2019 sampling season compared with the 2018 sampling season. Resorption proficiencies of Ca ( $R^2=0.29$ ) and Mg ( $R^2=0.67$ ) increased in a logarithmic pattern with foliar Ca and Mg; while there was no relationship between foliar N, P, and K and RE (Figure 4.5). There were few relationships between nutrient RE and RP and soil and foliage nutrient concentrations (Figure 4.6). Calcium RE was negatively correlated with foliar K ( $r=0.93$ ) and total Mg concentration in the FH ( $r=-0.77$ ) horizon. Magnesium RE was positively correlated with N concentration in the 0–5 cm horizon ( $r=0.83$ ). There was no association with foliar nutrients or RP and soil or foliar metals ( $p>0.05$ ) (Figure 4.6).



**Figure 4.4:** Nutrient resorption efficiencies (RE) (mean) (**top row**) and nutrient proficiencies (RP) (mean  $\pm$  standard deviation) (**bottom row**) by stand age for calcium (Ca), magnesium (Mg), nitrogen (N), phosphorous (P), and potassium (K) during both the 2018 and 2019 field season.



**Figure 4.5:** Foliar nutrient concentrations and nutrient resorption efficiencies (RP) for calcium (Ca), magnesium (Mg), nitrogen (N), phosphorous (P), and potassium (K). Solid black lines are significant ( $p < 0.01$ ) logarithmic relationships between foliar nutrient concentrations nutrient RP for both the 2018 and 2019 field seasons; dashed lines are 95% confidence intervals. Regression coefficients ( $R^2$ ) are included for statistically significant relationships.

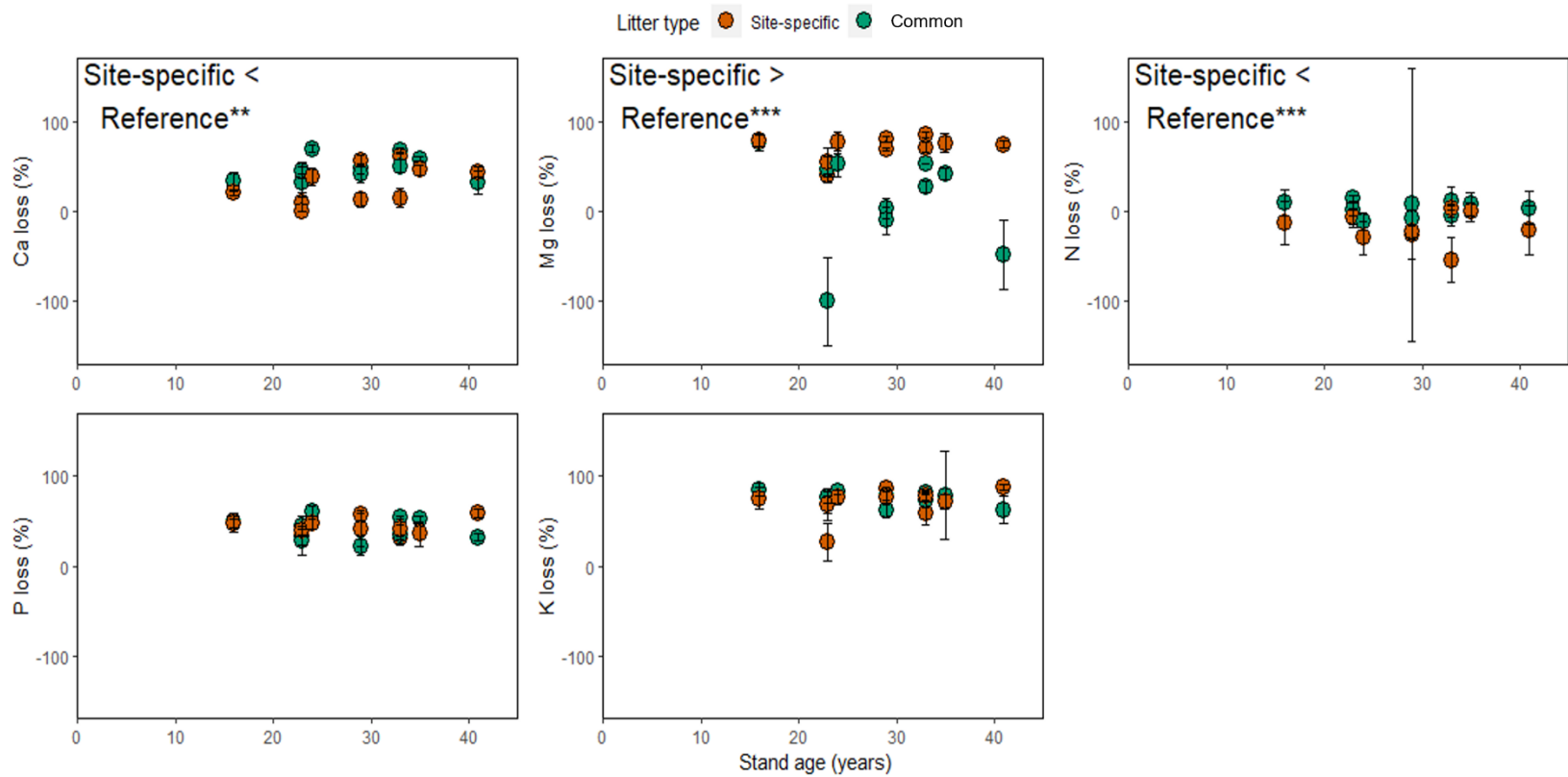


**Figure 4.6:** Correlation plot for foliar nutrient concentrations and nutrient resorption efficiencies (RE) and a variety of foliar and soil parameters. Significant correlations ( $p < 0.005$ ) are represented by shade and size of dots.

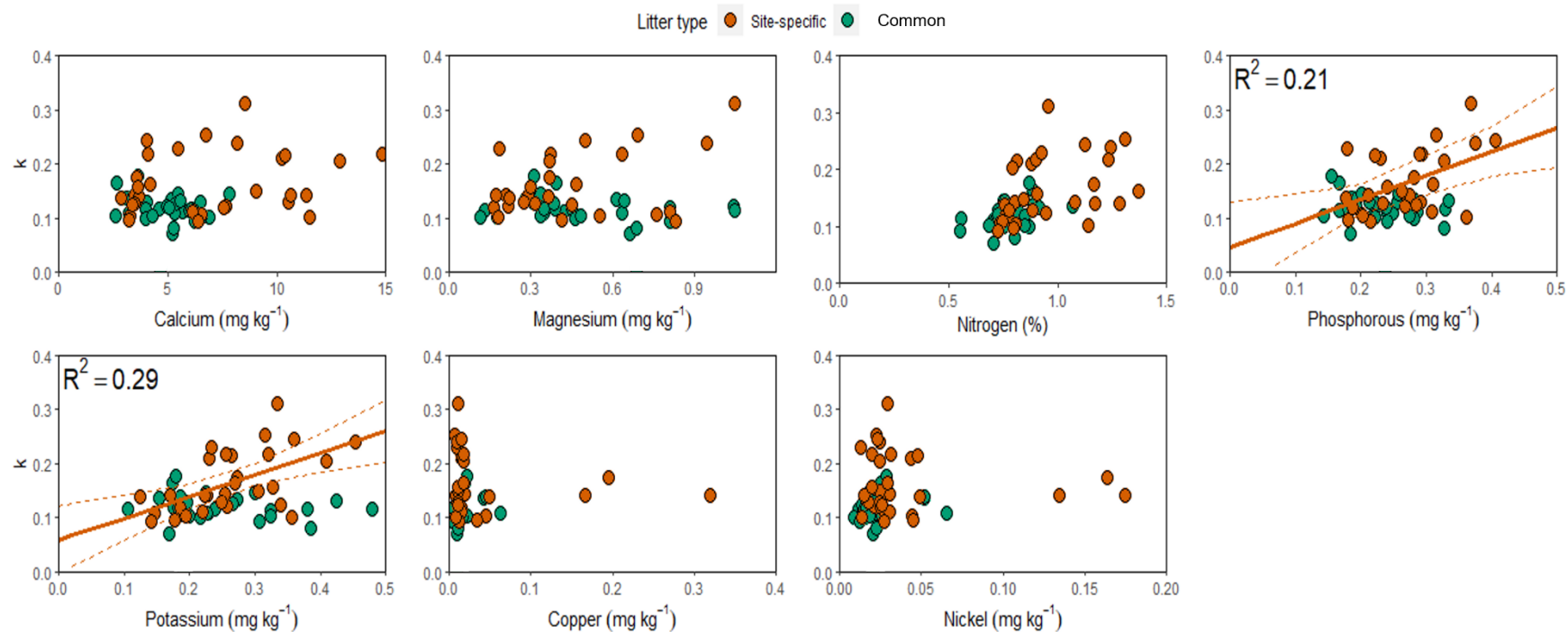
#### *4.3.4. Litter decomposition*

There were no significant relationships between stand age and litter nutrients lost from the litter decomposition bags (Figure 4.7). After 2.5 years of exposure, nutrients lost from the litter bags decreased in the order of Mg, K >> P, Ca >>> N, where litter generally increased in N (Figure 4.7). Site-specific litter lost significantly more Mg than common litter, while common litter lost more Ca and N (Figure 4.7). Decomposition k-values increased with litter concentrations of P and K using site-specific litter (Figure 4.8).





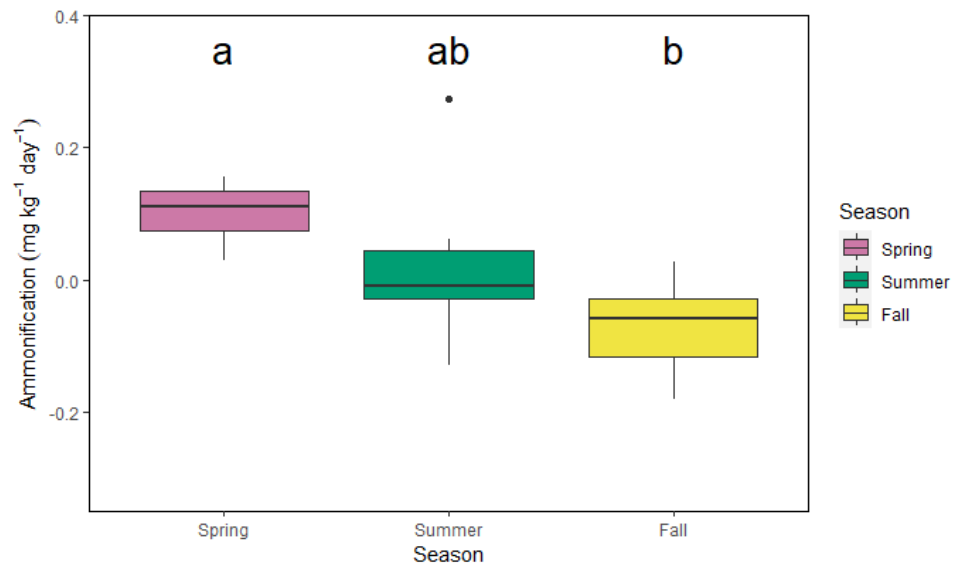
**Figure 4.7:** Nutrient loss in litter by stand age for site-specific and common litter. Nutrients analyzed includes calcium (Ca), magnesium (Mg), nitrogen (N), phosphorous (P), and potassium (K). Instances where percent of initial nutrients lost were significantly different between site-specific and common litter were denoted using: \*\*  $p < 0.005$ ; \*\*\*  $p < 0.0001$ .



**Figure 4.8:** Decomposition k-values and litter nutrient concentrations after 2.5-year exposure period for site-specific and common litter. Solid lines are significant ( $p < 0.007$ ) linear relationships between k and litter nutrient concentrations; dashed lines are 95% confidence intervals. Regression coefficients ( $R^2$ ) are included for statistically significant linear relationships.

#### 4.3.5. Nitrogen mineralization

Ammonification rates and  $\text{NH}_4$  concentrations did not vary with stand age for any season ( $p > 0.05$ ). Rates of ammonification were not significantly related with Cu or Ni concentrations in the 0–5 cm layer ( $p > 0.05$ ). Nitrate concentrations were undetectable using our instruments (detection limit =  $0.14 \text{ mg kg}^{-1} \text{ NO}_3\text{-N}$ ). Rates of ammonification were significantly higher in the spring compared with the fall ( $p = 0.0003$ ) (Figure 4.9) but there were no significant differences in  $\text{NH}_4$  concentrations among seasons ( $p > 0.05$ ).



**Figure 4.9:** Rates of ammonification during the growing seasons. Significant differences ( $p < 0.05$ ) in ammonification rates among seasons are denoted using letters. Box center lines are medians, box limits are 25th and 75th percentiles, whiskers are at most 1.5 times the inter-quartile range, and dots beyond whiskers are outliers.

#### *4.4. Discussion*

Overall, there were few relationships between nutrient cycling processes and stand age and there was no evidence that future productivity will be affected by nutrient limitation. Foliar nutrient levels were within TCD values, however, there were decreases in concentrations of P and K in wood across the chronosequence we sampled. Likely explaining why aboveground nutrient pools have been reported to decrease at regreened sites.

##### *4.4.1. Soil nutrients*

Extractable Mg, P, and K concentrations increased with stand age in the 0–5 cm mineral soil layer and total Mg, P, and K increased in the FH horizon. The increase in concentration of these nutrients in the upper layer of the mineral soil confirms our hypothesis that as stands age applied nutrients continue to work their way down the soil profile. Contrary to our hypotheses Ca concentrations did not change with stand age in the FH or 0–5 cm mineral soil horizon, potentially due to Ca being retained in organic horizons or lost through erosion. However, one of the younger sites had elevated Ca concentrations in the 0–5 cm horizon potentially obscuring a trend of increasing Ca in mineral soil. The continued increase in concentrations of Mg in the FH could be due to the slow dissolution of Mg from crushed limestone that can continue for up to 70-years post application (Schaffner et al., 2012). Phosphorous and K on the other hand were applied in plant-accessible forms – and in much smaller quantities than Ca and Mg (VETAC, 2021) – P and K were most likely quickly taken up by vegetation as has been found in previous

studies (Munford et al., 2021). Phosphorous and K are more strongly cycled through ecosystems in vegetation by litterfall and fine root turnover. The uptake into vegetation potentially allows for continued increase in P and K concentrations in the FH following turnover of fine roots, which are generally rich in P and K (Foster and Morrison, 1976).

In contrast to the other nutrients, mineral soil N concentrations decreased with stand age, conflicting with our hypothesis. Nitrogen in the mineral soil was largely in an organic form with minimal pools of inorganic N. Similar to the decrease in total soil C concentrations discussed Chapter 3, the decrease in soil N is related to a decrease in organic matter content of soils over time most likely caused by nutrient application increasing soil pH and stimulating decomposer communities increasing the decomposition of organic matter. Mechanisms for this were discussed in detail in Chapter 3.

Calcium and Mg concentrations at the regreening sites were highly elevated in the mineral soils compared with jack and red pine stands grown on “healthy” soils in similar ecoregions. Mineral soil concentrations of extractable Ca and Mg at the youngest regreening sites were similar to previously reported values – however the oldest sites Ca and Mg concentrations were 200% and 600% higher respectively (Foster and Morrison, 1976; Tappeiner and Alm, 1975). Forest floor Ca concentrations were similar to other studies, however forest floor Mg at the regreening sites were up to 500% higher than previously reported values (Krause, 1998; Shepard and Mitchell, 1990). Conversely, soil K concentrations were very low at the regreening sites with the highest concentrations of K only having 50% of other reported values (Ste-Marie et al., 2007; Tappeiner and Alm,

1975). On average, total P concentrations at the regreening sites were also 50% of “healthy” stands in the forest floor but comparable in extractable P concentrations in the mineral soil (Foster and Morrison, 1976; Shepard and Mitchell, 1990). Total N concentrations in the forest floor were comparable to other studies; and mineral soil N concentrations at the oldest regreening sites were similar to previously published results while the younger sites were highly elevated (Foster and Morrison, 1976; Ste-Marie et al., 2007). These findings from the regreening sites were like those of Schaffner et al., (2012), where after 70-years post application of limestone and fertilizer Ca concentrations remained elevated however N, P, and K were no longer elevated in the soil.

#### *4.4.2. Aboveground tree nutrients*

Foliar chemistry at the regreening sites did not change with stand age and was similar to values reported from the Tree Chemistry Database (TCD) for red pine (Pardo et al., 2005). The lack of a decrease in foliar nutrients over time at the regreening sites is promising as foliar nutrients are assumed to be indicative of tree health and function (Chapin, 1980). Levasseur et al., (2022) reported a decrease in weight-adjusted aboveground concentrations of Ca, N, and P. However, isolating the tree components it is evident that these decreases are largely driven by changes in wood chemistry as the oldest regreening sites have wood P that are 10% of the TCD values. This is potentially caused by the proportion of sapwood decreasing as trees age, as trees grow, more energy is invested into increasing low nutrient density wood biomass (MacLean and Wein, 1977). The tree cores used for nutrient analysis for our oldest sites were longer (sample trees had larger

dbh for older sites (Chapter 2); and may have been made of a lower proportion of sapwood artificially suppressing our wood P and K concentrations. The regreening sites have bark N concentrations 150% greater than TCD values, while bark K concentrations were 50% of TCD values (Pardo et al., 2005).

#### *4.4.3. Nutrient resorption*

Both RE and RP at the regreening sites (which included jack and red pine) were unaffected by stand age, further indicating that nutrients are most likely not limiting as older trees are not retaining higher concentrations of nutrients during senescence. However, RP of Ca and Mg at the regreening sites were considerably higher – while N, P, and K RP were substantially lower – than values reported from studies conducted on jack pine stands in Ontario, Canada (~70-years-old; Morrison, 2003) and Wisconsin, USA (~50-years-old; Bockheim and Leide, 1991). Comparing RE at the regreening sites to global conifer RE values, P and K RE at the regreening sites were highly elevated, while RE of Ca, Mg, and N were considerably lower (Vergutz et al., 2012). Nutrient RP for mobile nutrients N, P, and K (which are generally considered easier for trees to translocate) were unrelated to foliar concentrations; whereas nutrients such as Ca, and Mg generally aren't translocated (Fife et al., 2008) and exhibited logarithmic increase in RP with foliar concentrations.

#### *4.4.4. Litter decomposition*

Litter decomposition rates were not affected by stand age. Relative loss of nutrients from litter during decomposition decreased in the order of Mg, K >> Ca, P >>N

and litter decomposition rates were positively correlated with final litter concentrations of P and K in site-specific litter, suggesting that these litter nutrients were removed from the litter in a similar pattern to C. This pattern is similar to that reported by Freedman and Hutchinson (1980a) who proposed that this was due to K being lost through leaching, while Ca more closely reflected mass loss, as Ca makes up structural components of litter. While occasionally proposed to be a predictor of litter decomposition rate (Coûteaux et al., 1995), litter nutrients and metals were not correlated with litter decomposition rates at the regreening sites, which has been found in other jack and red pine stands (Bockheim et al., 1991; MacLean and Wein, 1978b). Previous studies conducted in the City of Greater Sudbury area similarly found that litter metal concentrations did not affect litter decomposition rates (Freedman and Hutchinson, 1980b; Johnson and Hale, 2004).

The rates of loss of Ca, Mg, and K at the regreening sites were similar to values from the City of Greater Sudbury area prior to regreening (Freedman and Hutchinson, 1980a), and P loss was similar to jack pine stands in eastern Ontario, Canada (Morrison, 2003). Nitrogen increased in the litter decomposition bags after 2.5-years of exposure. The increase in litter N could be from microorganisms importing additional N from lower in the soil profile (Berg and Söderström, 1979). Generally, the early phases of litter decomposition are limited by N (Moore, 1984) and in order to address low N, decomposer communities have been found to import N from lower in the soil profile, promoting the immobilization of N in litter over mineralization (Schlesinger and Hasey, 1981). This is



potentially occurring in litter decomposition at the regreening sites as litter C:N ratios were very high (~100) early in decomposition.

#### *4.4.5. Nitrogen mineralization*

Neither inorganic N ( $\text{NO}_3 + \text{NH}_4$ ) concentrations or mineralization rates at the regreening sites were related to stand age. Inorganic N concentrations were very low;  $\text{NO}_3$  concentrations were undetectable with our instruments (detection limits of  $0.14 \text{ mg kg}^{-1} \text{ NO}_3\text{-N}$ ). Low inorganic N and N mineralization rates are expected in acidic conifer forests, however,  $\text{NH}_4$  concentrations and ammonification rates at the regreening sites were considerably lower than those reported in previous studies in uncontaminated soils (DeLuca et al., 2007; McMillan et al., 2007). However, the rates of ammonification at the regreening sites are very similar to those reported in DeCatanzaro and Hutchinson (1985), which only tested metal contaminated soils in the City of Greater Sudbury area prior to regreening, suggesting that regreening has had no effect on N-mineralization. Previous studies have suggested that soil Ni concentration can increase N mineralization (DeCatanzaro and Hutchinson, 1985). While we did not observe a relationship with Ni (or Cu) concentration, all the regreening sites have highly elevated total soil Ni concentrations, so it is possible that within the tested range of soil Ni there were no differences in mineralization because the threshold that causes this increase has already been exceeded. The low concentrations of inorganic N and low mineralization rates do not appear to be affecting tree N concentrations, suggesting trees are potentially taking up organic forms of N (Näsholm et al., 1998). Seasonal patterns of both  $\text{NH}_4$  concentrations and rates of

ammonification decreased in the order of spring > summer > fall similar to previous reporting (Stottlemyer and Toczydlowski, 1999).

#### *4.5. Conclusion*

Overall, there were few relationships between nutrients and stand age at the City of Greater Sudbury regreening sites. In fact, nutrient cycling processes overall appear to be similar to those in jack and red pine plantations that have not been exposed to centuries of industrial impacts. The regreening sites are rich in Ca and Mg; previous Ca and Mg budget analyses have found that much of the added Ca and Mg was unaccounted for, however, these nutrients were applied in such massive quantities (10 Mg ha<sup>-1</sup> of dolomitic limestone) that the regreening sites are still enriched. Nitrogen, P, and K were applied in much smaller quantities (390 kg ha<sup>-1</sup>) and remnant concentrations are not distinguishable in the soils. Potassium, as well as P to a lesser extent, potentially may be a limiting nutrient in the future as the regreening stands continue to age as regreened soils are very low in K, and K RE is much higher than expected. That being said – foliar P and K concentrations remain at expected values, and tree growth rate is in line with expectations. The very low inorganic N concentrations and N mineralization rates are similar to those reported in the City of Greater Sudbury area prior to regreening efforts, suggesting that regreening had no effect on these processes. Despite the low inorganic N and decreasing total N concentrations, soil and vegetation N concentrations remain above what is expected in the region.

#### *4.6. Acknowledgements*

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## Chapter 5: Conclusion

Forest restoration is increasingly being targeted as a strategy to achieve global climate and biodiversity goals. Understanding the biogeochemistry of restored forests is important to inform management practices and assess long-term forest sustainability. The City of Greater Sudbury greening areas provided an incredible opportunity to study the biogeochemistry of regreened forests on a landscape degraded by over a century of mining and smelting practices. The regreening program of the City of Greater Sudbury is one of the longest running and most expansive in the world. The patchwork nature of the treatments provided a unique location to determine how forest biogeochemistry changes with stand age.

Overall, the regreening program has increased tree growth and produced jack and red pine plantations that are biogeochemically similar to pine plantations unimpacted by over a century of mining and smelting practices. The loss of soil carbon (C) remains concerning, however, previous studies that have found regreening to decrease soil C reported that this trend only continues for so much time before soil C pools either flatline or begin to increase. The literature on the effects of regreening on soil C is incredibly mixed with large support for regreening both increasing and decreasing soil C concentrations. We believe that the decrease in soil C with stand age at the regreening sites was due to regreening stimulating decomposer communities. Carbon mineralization rates were elevated from the very low rates that were measured prior to regreening, to rates that are more typical of an acidic conifer forest. Increasing pools of soil C has been a particularly

popular topic in recent literature as soil C is generally regarded as one of the more stable pools of C. The decrease in soil C measured at the regreening sites provides caution that while portions of soil C can be more stable than C stored in biomass, the long-term storage of C in soil is not guaranteed.

Future management should consider reducing the application rate of crushed dolomitic limestone at the regreening sites. Despite large quantities of lime being lost through erosion the regreening sites remain incredibly rich in Ca and Mg. The 10 Mg ha<sup>-1</sup> application rate used in the City of Greater Sudbury regreening program is similar to other liming studies, however lower application rates (< 5 Mg ha<sup>-1</sup>) have been found to increase soil pH and replenish Ca and Mg pools, while limiting the loss of soil C. The topography of the area should also play a role in application rate, where denuded hilltops potentially require a larger dose of lime anticipating more loss through erosion, in comparison to areas lower in a catchment.

A critical question for future management is whether the regreening program should focus efforts on untreated areas or return to previously regreened sites to apply additional treatments. I believe at this point the regreening program should focus on applying treatments to previously untreated areas. The current liming dosage appears to be more than sufficient and there does not currently appear to be a need to reapply P and K rich fertilizers. Foliar nutrients and resorption efficiencies did not change with stand age, and trees are growing at a healthy rate. However, soils in the regreening sites are low in P

and K. So while fertilizer reapplication does not appear to be necessary at this point, P and K may become limiting in the future.

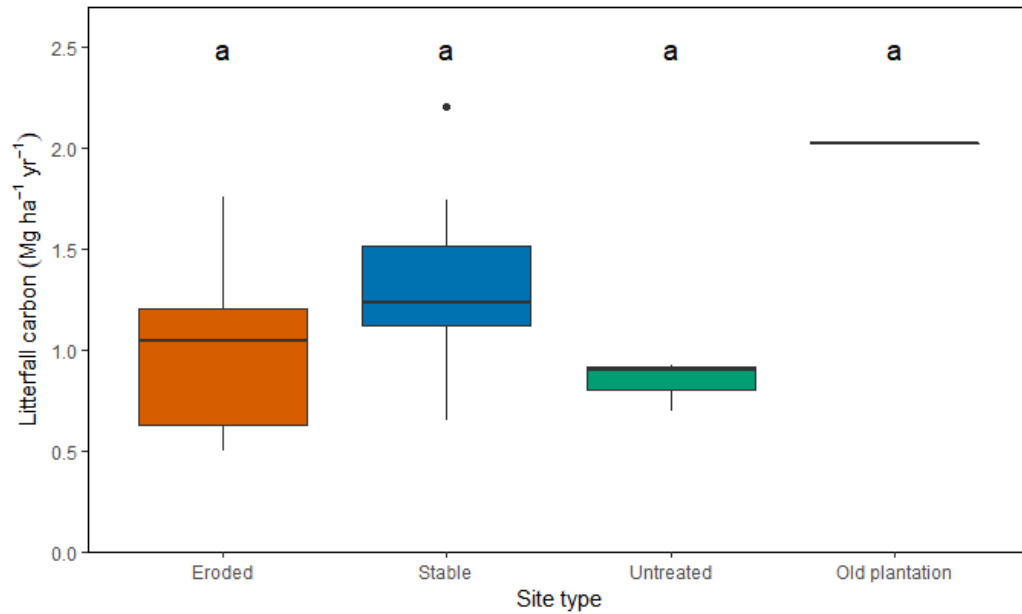
Total ecosystem C at the greening sites increased with stand age despite the decrease in soil C – largely due to increases in aboveground tree C. Fast-growing tree species such as jack pine and red pine were selected early in the greening program, however, as these trees die what will replace them is an interesting question. Jack pines have an average lifespan of 60 years and currently the oldest greening stands are just over 40-years-old. The understory at the greening sites is generally not very diverse (as has been reported in many other pine plantations) with effectively zero jack or red pine seedlings. Saplings in the greening sites are largely paper birch and trembling aspen, suggesting that as the older planted pines die-back they will potentially be replaced by pioneering deciduous species. The succession of these pine plantations will have dramatic effects on biogeochemical properties.

Current greening practices in the City of Greater Sudbury barrens have focussed on planting more diverse species including under-story trees, and shrubs. The inclusion of more diverse species and prioritization on including multiple layers of vegetation, shifts the greening programs goals to focus more on ecological restoration as opposed to the pine plantation style reclamation used in the early days of the program. My PhD thesis focussed solely on jack and red pine plantations in order to develop the longest chronosequence possible, but future studies investigating the biogeochemical implications of the more recent greening strategies would be very relevant. Another

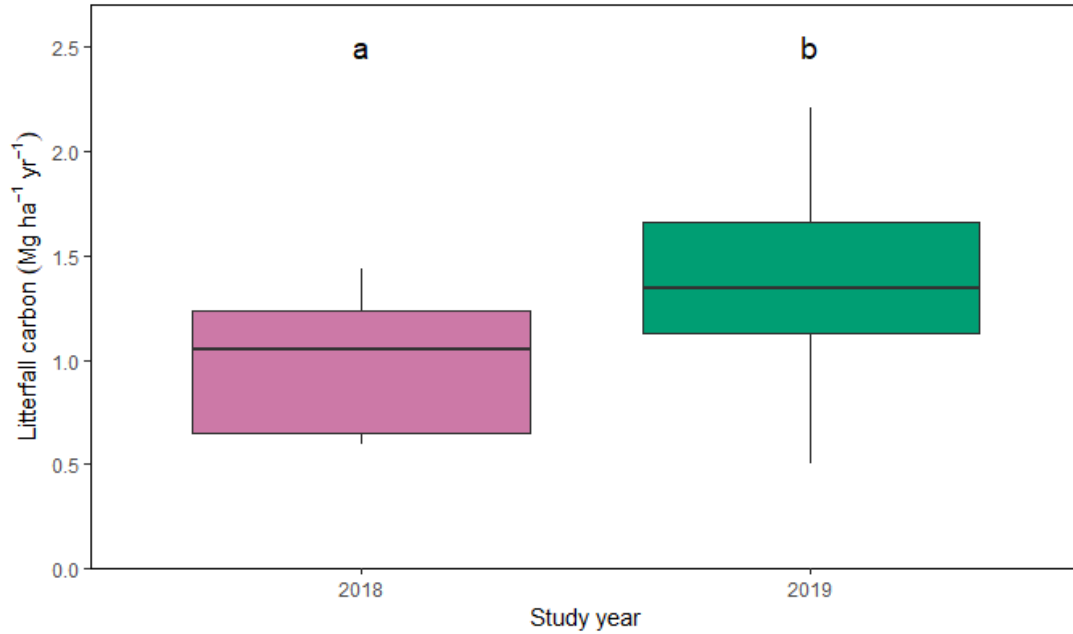
mechanism for restoration that I believe should be investigated further and over a longer period of time, is applying soil amendments but not planting any trees. Natural ecological succession in the region has been disturbed by industrial damage but improved air quality and addition of soil amendments can potentially support colonisation and successful growth of early successional species. Natural regeneration on disturbed landscapes has largely been found to increase biodiversity but also create larger C pools – particularly in soils – over long periods of time compared to conifer plantations. Prior to large-scale mining and smelting, forests in the City of Greater Sudbury barren areas were replaced every 100 years or so by fire. These systems have been undergoing successional patterns for millenia, I believe a worthwhile venture for academic and management purposes would be to determine whether improved air and soil quality is sufficient to restart these processes.

The City of Greater Sudbury greening areas have already hosted countless undergraduate, Masters, and PhD theses, not to mention industrial, academic, and government research projects; I hope that the findings of my PhD thesis inspire future research on one of the most unique and fascinating locations in the world.

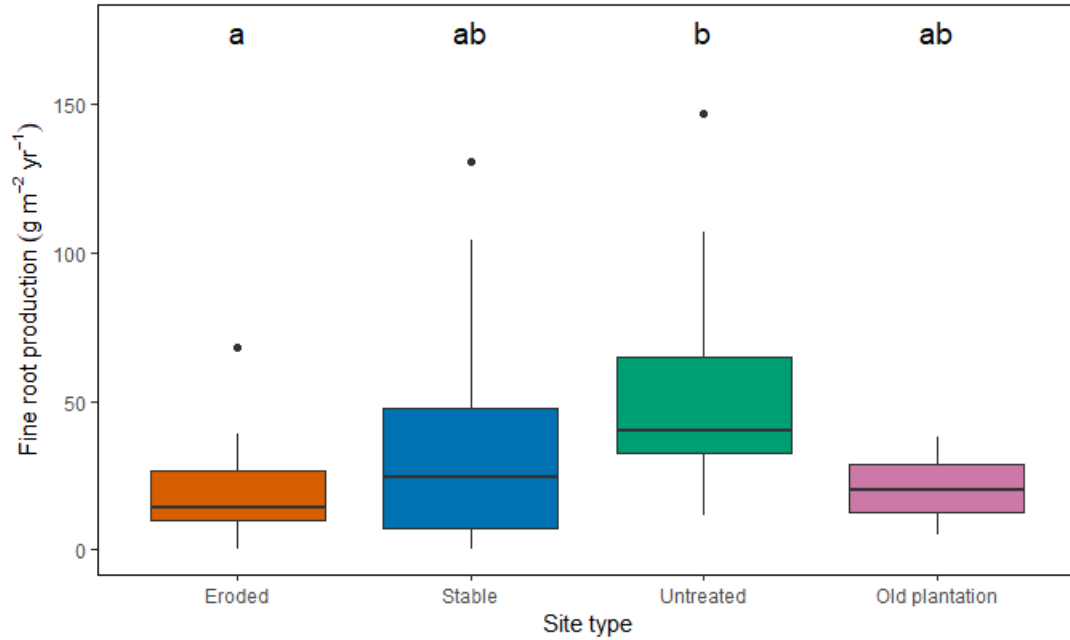
## Appendix



**Figure A.1:** Litterfall carbon transfer rates compared between eroded, stable, untreated, and old plantation sites. Box center lines are medians, box limits are 25<sup>th</sup> and 75<sup>th</sup> percentiles, whiskers are at most 1.5 times the inter-quartile range, and dots beyond whiskers are outliers. Significant differences ( $p < 0.05$ ) are denoted by differing letters.

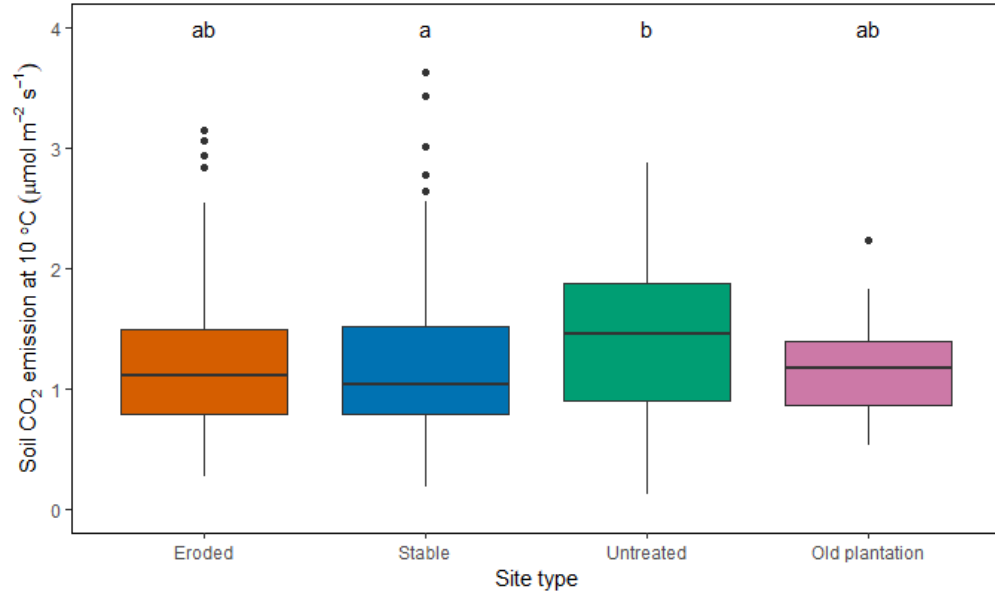


**Figure A.2:** Litterfall carbon transfer rates compared between 2018 and 2019 sampling season at eroded and stable sites. Box center lines are medians, box limits are 25<sup>th</sup> and 75<sup>th</sup> percentiles, whiskers are at most 1.5 times the inter-quartile range, and dots beyond whiskers are outliers. Significant differences ( $p < 0.05$ ) are denoted by differing letters.



**Figure A.3:** Fine root production rates compared between eroded, stable, untreated, and old plantation sites. Box center lines are medians, box limits are 25<sup>th</sup> and 75<sup>th</sup> percentiles, whiskers are at most 1.5 times the inter-quartile range, and dots beyond whiskers are outliers. Significant differences ( $p < 0.05$ ) are denoted by differing letters.





**Figure A.4:** Soil carbon dioxide (CO<sub>2</sub>) emission rates adjusted to 10°C compared between eroded, stable, untreated, and old plantation sites. Box center lines are medians, box limits are 25<sup>th</sup> and 75<sup>th</sup> percentiles, whiskers are at most 1.5 times the inter-quartile range, and dots beyond whiskers are outliers. Significant differences ( $p < 0.05$ ) are denoted by differing letters.