CARBON STORAGE DYNAMICS AND TREE GROWTH RESPONSE TO SPECIES DIVERSITY IN BOREAL FORESTS

By
Bilei Gao

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Abstract

The boreal forest plays a critical role in regulating the global carbon (C) cycle. Both wildfire and harvesting affect ecosystem C. However, the long-term recovery of forest C storage with a diverse range of overstory composition following wildfire remains unclear. The uncertainties associated with long-term C storage dynamics include accurate C concentration data of woody tissues and total ecosystem C inventory, particularly for stands older than the longevity of the pioneer cohort. Moreover, despite major advances demonstrating positive relationships between species diversity and forest productivity at the community level, little is known about the responses of individual tree growth to species diversity in relation to succession, tree size, and disturbance origin. The objective of this dissertation is to improve the understanding of long-term C storage dynamics following fire and the relationship between species diversity and tree growth in relation to forest succession, size, and disturbance origin.

In the first study, I examined the variation of carbon concentration for six major tree species of the boreal forest. I found bark had significantly higher total C and volatile C concentrations than stemwood and that both total C and volatile C concentration significantly varied among tree species. Furthermore, total C and volatile C concentration in stemwood and bark of shade-intolerant species increased with tree size, whereas those of shade-tolerant species showed negative or neutral size-associated changes. These results suggest that volatile C concentration is a key driver of the variation in total C concentration and highlight the importance of considering the variation in C concentration when estimating live tree C storage.

Based on the results of the first study, I then quantified forest C storage and examined long-term C storage dynamics by using a replicated chronosequence design that covered a wide range of stand ages (from 8 to 210 years old), following stand-replacing fire.
undergoing multiple succession pathways in the boreal forest of central Canada. I found that total ecosystem C storage increased from 8 to 147 years old, but declined from 147 to 210 years old, coinciding with canopy transition from pioneer species to late succession species, irrespective of succession pathway. Our result rejected the view that old forests continually accumulate C as they age. These findings emphasize that inclusion of stands older than the longevity of the pioneer cohort is critical to a better understanding of the contribution of old forests to the global C cycle.

Finally, I investigated the relationships between tree growth of five major tree species and species diversity in central boreal forests of Canada in relation to succession, tree size, and disturbance origins. I found that significant relationships between tree growth and species diversity occurred at canopy transition stage. Species diversity positively correlated with the growth rate of spruce (*Picea* spp.) but negatively affected the growth rate of trembling aspen (*Populus tremuloides* Michx.) and white birch (*Betula papyrifera* Marsh.) during early canopy transition stage (99 years old). However, when stand entered the late canopy transition stage (147 years old), species diversity promoted tree growth for both trembling aspen and spruce. The positive diversity effect on tree growth increased with relative size for jack pine (*Pinus banksiana* Lamb), at age 99 and spruce at age 147, and the negative diversity effect increased with relative size for trembling aspen at age 99, 147, and 210. Moreover, there was no difference in the tree growth-diversity relationships between post-fire and post-harvest stands during early successional stages. These results emphasize the importance of considering species-specific, size-dependent growth response to species diversity related to forest succession to better understand the relationships between biodiversity and forest ecosystem functions.
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Notes to readers

The various Chapters were written individually to meet the submission requirements of the peer-reviewed journals. Thus, the styles for writing, references, tables, and figures may slightly differ.

Chapters:


Chapter One: General introduction

The world’s boreal forests play an important role in regulating atmospheric carbon dioxide because they account for 49% of global forest vegetation and soil carbon (C) (Dixon et al., 1994). Wildfire is the predominant form of natural stand-replacing disturbance in the boreal forest and exerts strong impacts on C sequestration and storage (Bond-Lamberty et al., 2007). Over the past several decades, fire activity has increased in many regions to levels unprecedented since the last ice age (Kelly et al., 2013), highlighting the importance of understanding C flux in the boreal forest (Canadell and Raupach, 2008; Bellassen and Luysaart, 2014; Gauthier et al., 2015). In particular, accurately quantifying C concentration of live trees in the boreal forest has important implications for the estimation of C storage and validation of global C accounting models used to predict future C emission scenarios (McKinley et al., 2011; Metsaranta et al., 2011), as trees generally comprise the large and most dynamic C pool in forest ecosystems (Pan et al., 2011). However, despite increased accuracy of C concentration estimates for tropical and temperate tree species (Thomas and Malczewski, 2007; Martin and Thomas, 2011; Martin et al., 2013; Martin et al., 2015), few studies have examined the variation in C concentration of boreal tree species.

Although advances have been made in understanding carbon storage changes as forest stands age (Taylor et al., 2014), the long-term recovery of forest C storage following stand-replacing fire, remains unclear. Uncertainty surrounding C storage in old forests largely stems from both a lack of repeated measurements in forest stands older than the longevity of the pioneer cohort and a lack of consideration of multiple succession pathways. Forest net ecosystem production (NEP) increases rapidly during early stand development, peaks as stands mature, and then declines as stands age (Ryan et al., 1997). As a result, forest C storage decreases slightly during the early stand recovery stage as forest NEP is negative due to rate of decomposition exceeding production, and then increases with stand development as
NEP turns positive. However, previous studies conflict with regards to whether old-growth stands continue to accumulate C or whether total C eventually peaks and then declines. (Bond-Lamberty et al., 2004; Harmon et al., 2004; Goulden et al., 2011; Kashian et al., 2013; Taylor et al., 2014). The divergent findings may have resulted from the lack of consideration of forest canopy transition, from dominance of early to late-succession species (Taylor and Chen, 2011).

The past several decades have seen a sharp increase in the number of studies investigating the relationship between species diversity and forest ecosystem functions. Positive relationships between diversity and forest productivity tend to be predominant in global forests (Zhang et al., 2012; Liang et al., 2016). However, most studies have focused on examining the effect of species diversity on community-level productivity, often overlooking variability in the response of individual species to diversity at the neighbourhood level. Because diversity-productivity relationships are largely realized through the growth response of individual tree species (Barrufol et al., 2013; Chamagne et al., 2017), a closer examination of species-specific growth responses to neighbourhood diversity is warranted (Fichtner et al., 2017).

The objective of this thesis is to improve the understanding of C dynamics over long-term succession following fire, and tree growth response to species diversity in boreal forests. In order to achieve these goals, I first quantified the variation in total and volatile C concentration of the major tree species in the boreal forest, in Chapter two. In Chapter three, based on the species-specific C concentration data, I examined whether overstory type influenced forest C storage as stands age following stand-replacing fire. In Chapter four, I investigated the relationship between tree growth of five tree species and species diversity of individual tree neighbours in relation to succession, tree size, and disturbance origin.
Chapter Two: Variation in total and volatile carbon concentration among the major tree species of the boreal forest

Introduction

The world's boreal forests play a critical role in the global carbon (C) cycle, containing approximately 49% of all forest ecosystem C (Dixon et al., 1994), largely due to the slow rate of decomposition and high accumulation of dead organic matter at high northern latitudes (Bonan and Shugart, 1989; Dixon et al., 1994). However, northern ecosystems are expected to experience the greatest warming over the coming century, potentially affecting the capacity of the boreal forest to sequester and store C (Gauthier et al., 2015). A better understanding of C flux in the boreal forest is becoming increasingly imperative given its strong impact on global C dynamics (Canadell and Raupach, 2008; Bellassen and Luyssaert, 2014). In particular, accurately quantifying C stocks of live trees in the boreal forest has important implications for the verification and validation of global C accounting models used to predict future C emission scenarios (McKinley et al., 2011; Metsaranta et al., 2011) as trees generally comprise the large and most dynamic C pool in forest ecosystems (Pan et al., 2011).

The C contained in live trees is most often calculated by converting biomass estimates to C stocks using a C concentration value, which is widely assumed to be 50% (Chave et al., 2008; Saatchi et al., 2011; Taylor et al., 2014; Grunzweig et al., 2015). However, recent studies have shown that C concentration varies substantially among tree species and assuming 50% C concentration for all species may significantly over- or underestimate forest C stocks by as much as 5% (Lamlom and Savidge, 2003; Thomas and Malczewski, 2007; Thomas and Martin, 2012), which, when scaled-up through biome- or nation-wide C accounting schemes can lead to global-level errors in terrestrial C stock estimates. Although the accuracy of C concentration has been improving among tropical and temperate tree
species (Thomas and Malczewski, 2007; Martin and Thomas, 2011; Martin et al., 2013; Martin et al., 2015), few studies have examined variation in C concentration among boreal species.

Variation in tree C concentration is primarily attributable to the physical and chemical properties of their woody tissues (Savidge, 2000; Elias and Potvin, 2003; Bert and Danjon, 2006; Thomas and Malczewski, 2007). Recent progress on tissue-specific C concentration suggests that C concentration derived from stemwood can be used to represent other major tissues that account for important biomass fractions in trees (i.e., coarse roots and branches), with the exception of bark (Thomas and Martin, 2012; Martin et al., 2015). Studies of temperate tree species have shown that bark has a significantly higher C concentration than stemwood (Bert and Danjon, 2006; Martin et al., 2015) due to higher concentrations of C-rich lignin and suberin compounds associated with the bark’s roles in limiting water loss and as a protective layer against insect and pathogen attack (Hengst and Dawson, 1994; Franceschi et al., 2005). It is, therefore, reasonable to expect that similar differences in C concentration between bark and stemwood tissues also exist in boreal tree species (Liebhold et al., 1995; Lovett et al., 2006). However, previous attempts to examine C concentration for boreal tree species have mainly focused on radial and vertical variation of C concentration in stemwood tissue only (Lamlom and Savidge, 2003, 2006)

In addition, C concentration may decrease with increasing tree size. Higher C concentration in small trees has recently been reported for 16 tropical tree species by comparing C concentration of saplings with conspecific large trees (Martin et al., 2013). The proposed explanation for this is that smaller trees require higher C-rich lignin concentrations to support wood resistance to insect and pathogen attack (Vance et al., 1980; Wainhouse et al., 1990) and for improving stem mechanical stability (Voelker et al., 2011). However, Martin and Thomas (2013) found a linear increase in C concentration with tree size for two
other tropical tree species (Dacryodes excelsa Vahl. and Miconia mirabilis (Aubl.) L.O. Williams). Discrepancies among studies remain unexplained but may be caused by neglecting the important role of volatile C compounds on total C concentration (Martin et al., 2013) or failing to consider the effect of life-history traits, such as shade tolerance, on tissue C content (Thomas and Malczewski, 2007; Pons and Poorter, 2014).

When measuring the total concentration of C in trees, the concentration of volatile C compounds is often overlooked (Thomas and Martin, 2012; Martin et al., 2013). These compounds of low molecular weight, such as alcohols, phenols, terpenoids, and aldehydes, may be volatilized and lost when using the traditional method of oven-drying wood samples before elemental analysis (Lamlom and Savidge, 2003). Indeed, recent studies in temperate trees have suggested that volatile C concentration is non-negligible and varies substantially among tree species (Thomas and Malczewski, 2007; Martin and Thomas, 2011; Thomas and Martin, 2012). Furthermore, Martin et al. (2013) have also hypothesized that volatile C concentration may influence size-associated changes in total C concentration by offsetting size-related decreases in C-rich lignin. They suggest that volatile C concentration may increase with tree size due to a shift in allocation from lignin to secondary volatile C compounds for supporting plant defense functions.

Life-history traits, such as shade tolerance, may also affect C concentration due to its important role in plant C balance (Pons and Poorter, 2014). For instance, shade tolerance affects the survival and growth of plants by influencing C uptake (i.e., photosynthesis) and C release (i.e., respiration) in response to limited light availability (Hillebrand and Matthiessen, 2009). Yet, empirical evidence is still scarce on the influence of contrasting shade tolerance on plant tissue C concentration. Moreover, previous studies have detected diverse relationships between tree C concentration and other traits related to shade tolerance, including wood density and growth rate (Elias and Potvin, 2003; Martin and Thomas, 2011;
These mixed results, however, may be attributable to lack of consideration of tree ontogenetic variation on C concentration (Martin and Thomas, 2013). Further examination of the interaction between tree size and shade tolerance may help in understanding the role of plant life-history adaptations on tissue C concentration (Niinemets, 2006).

In this study, we examine variation in C concentration, including the total and the volatile fraction, of bark and stemwood tissues across a range of tree sizes for the major tree species of North America's boreal forest, including jack pine (Pinus banksiana Lamb.), trembling aspen (Populus tremuloides Michx.), white birch (Betula papyrifera Marsh.), black spruce (Picea mariana [Mill.] B.S.P), white spruce (Picea glauca [Moench] Voss), and balsam fir (Abies balsamea [L.] Mill.). We sought to test the following hypotheses concerning C concentrations in our target tree species: 1) bark tissue has higher total and volatile C concentration than stemwood tissue; 2) total and volatile C concentrations vary among tree species; 3) total and volatile C concentrations change with tree size, but this size-dependent effect in total C concentration is influenced by size-related changes in volatile C concentration; 4) the size-associated relationships of total and volatile C concentrations are dependent on shade tolerance.

Materials and methods

Study area

Our study was conducted in the boreal forest, approximately 150 km north of Thunder Bay, Ontario, Canada, between 49°44' to 49°65' N and 89°16' to 90°13' W. This area is characterized by warm summers and cold, snowy winters. Mean annual temperature of 1.9 °C and mean annual precipitation of 824.8 mm were recorded at the closest meteorological station in Cameron Falls, Ontario, Canada (Environment Canada, 2015). Soils in our study area largely originated from the Wisconsinan glaciation, which ended approximately 9,500
years ago in this region. Stand-replacing wildfire is the most common natural disturbance in
our study area, with an average fire-return interval of approximately 100 years for the past
century, resulting in mosaic of stand ages across the landscape (Senici et al., 2010).
Commercial logging began in our study area in the 1970s. Dominant overstory tree species
include jack pine, trembling aspen, white birch, black spruce, white spruce, and balsam fir.

**Sampling design**

Woody tissue samples were collected in July and August of 2015 from forest stands located
on upland, mesic site types, with slope ≤5%, and underlain by moderately deep (≥50 cm)
glacial tills, belonging to the Brunisolic soil order, according to the Canadian system of soil
classification (Soil Classification Working Group, 1998). To sample a wide range of tree
sizes, we used stratified random sampling to select trees of varying diameter at breast height
(DBH, 1.3 m above root collar), from tree stems with a minimum DBH of 2 cm to the
maximum DBH that could be found in the study stand. Tree size was broken into 4 cm DBH
intervals, resulting in 8, 12, 9, 11, 7, and 8 diameter classes for jack pine, trembling aspen,
white birch, black spruce, white spruce, and balsam fir, respectively. Three individual trees
were randomly selected from each diameter class of each species, resulting in 165 tree
samples in total for stemwood and bark tissue extraction.

For trees <10 cm DBH, tissue extraction consisted of cutting stem disks at DBH, which
provided both bark and stemwood. For trees ≥10 cm DBH, we used an increment core borer
with a 5.15 mm diameter bit to extract both stemwood and bark tissue samples. All samples
were sealed in plastic bags or straws and placed in a cooler with ice to minimize loss of
volatiles during transportation from the field to the laboratory. Similar to previous studies
(Martin and Thomas, 2011; Martin et al., 2015), trees with crooked stems, substantial heart-
rot, or other forms of stem damage, such as stem abrasion, fungal infections, or major branch
losses, were not sampled.
Chemical analysis

In the laboratory, the outer edges of each core and disk were cut away, using razor blade utility knives, to remove oxidized tissue that may have lost volatiles or that may have been contaminated by the surfaces of the increment core borers. Each of the individual stemwood and bark tissue samples were cut into small pieces and ground into a homogenous powder using a Wiley Mill (no. 40 mesh). We then divided each sample into two subsamples: one for C concentration determination by the freeze-dried method, and the other for C determination using the oven-dried method. The freeze-dried subsample was freeze-dried under a vacuum for 7 days using a Labconco 8-L freeze drying system (Labconco Co., Kansas City, MO, USA). Then, 40 mg of each freeze-dried subsample was analyzed for C concentration using a LECO CNS-2000 analyzer and recorded as $C_{freeze}$ (%). The oven-dried subsample was first freeze-dried for 7 days and then placed in a forced-air oven at 65 °C for 48 h. Each oven-dried subsample was analyzed for C concentration using the LECO CNS-2000 analyzer and recorded as $C_{heat}$ (%). The mass of the oven-dried subsample was weighed twice, i.e., directly after freeze drying ($M_{freeze}$, g) and directly after oven drying ($M_{heat}$, g).

The value of $C_{freeze}$, which is determined from the freeze-dried-only subsample, cannot be directly used to convert tree biomass to C stock mass because tree biomass is normally estimated from the oven-dried mass produced by convection drying. Therefore, to derive total C concentration estimates from $C_{freeze}$ that represent C mass as a percentage of oven-dried biomass, we corrected $C_{freeze}$ to $C_{freeze-corr}$ (Total C concentration; %) by applying the volatile mass fraction (VMF) described by Martin and Thomas (2011), where

$$VMF = \left( M_{freeze} - M_{heat} \right) / M_{freeze}$$

(1)

The $C_{freeze-corr}$ is then expressed as,

$$C_{freeze-corr} = C_{freeze} \times \left( 1 / (1 - VMF) \right)$$

(2)

Volatile C concentration ($C_{vol}$, %) was calculated as,
Data analysis

We performed individual paired $t$-test to assess whether significant differences could be detected between the total and volatile C concentrations in the bark and stemwood tissue for each tree species. We also used a partially nested analysis of variance (ANOVA) to examine the effect of shade tolerance, species, and tree size (DBH) on total and volatile C concentrations. Each tissue type was analyzed separately using the following statistical model, including two interaction terms, and tree species were nested within shade tolerance, such as:

$$Y_{ijk} = \mu + T_i + S_{(i)j} + D_k + (T \times D)_{ik} + (S \times D)_{(i)jk} + \varepsilon_{(ijk)}$$

where $Y_{ijk}$ is the measured total C concentration or volatile C concentration of the bark or stemwood tissue, $\mu$ is the overall mean, $T_i$ (shade intolerant vs. shade tolerant) represents shade tolerance with two levels (determined by Burns and Honkala (1990)), $S_{(i)j}$ (j = 1, 2, 3) is tree species (nested within shade tolerance), $D_k$ is DBH (a continuous variable), and $\varepsilon_{(ijk)}$ is random sampling error. We tested the assumption of normality by Shapiro’s test, and that of homogeneity of variance by Levene’s test. We used the rank transformation method from the “GenABEL” R software package (GenABEL project developers, 2013) to transform the total C concentration of bark data as the untransformed data did not meet the assumptions of normality and homogeneity. We used a generalized linear model with the Gaussian family error distribution and an identity link function for analyzing our rank-transformed data.

We then used the results from our nested ANOVA to inform a more species-specific analysis, whereby, if tree size in Eq. (4) was significant, a simple linear regression was then used to more specifically describe the size-associated relationship between total and volatile C concentrations and DBH for each woody tissue type and for each tree species. We
compared linear and logarithmic functions to select the best bivariate relationships based on Akaike’s Information Criterion (AIC), whereby the simplest model that explained the most variation was selected when the difference in AICs between alternative models was less than 2 (Burnham and Anderson, 2002). All statistical analyses were conducted using the R 3.2.3 (R Development Core Team, 2017).

Results

Total C concentration was significantly higher in bark tissue than in stemwood tissue for all species (Table 2-1). Bark tissues were, on average, 6.2% and 5.0% higher in total C concentration than stemwood for all shade-intolerant and shade-tolerant tree species, respectively (Table 2-2). The minimum difference between mean total C concentration of bark and stemwood tissue was 3.6% in black spruce, whereas the maximum difference was 11.0% in white birch (Table 2-2). Volatile C concentration was also significantly higher in bark than in stemwood tissue for all species (Table 2-1). The average difference in mean volatile C concentration between bark and stemwood was 2.0% and 3.8% for all shade-intolerant and shade-tolerant species, respectively. Balsam fir had the maximum difference in mean volatile C concentration between bark and stemwood of 5.7%, whereas trembling aspen showed the least difference of 0.9% (Table 2-2).

Total C concentration of bark tissue differed significantly with shade tolerance, tree species, and tree size and showed marginally ($P = 0.096$ and 0.079, respectively) significant interactions (Table 2-3). Mean total C concentration of bark was higher in shade-intolerant species than in shade-tolerant species. Furthermore, total C concentration in bark significantly increased with tree size for shade-intolerant jack pine and trembling aspen (Fig. 2-1), but significantly and marginally decreased with tree size for shade-tolerant balsam fir and white spruce, respectively (Fig. 2-2). Similarly, volatile C concentration of bark was also significantly influenced by shade tolerance, tree species, and tree size (as indicated by the
significant interaction between tree size and species) (Table 2-3). The mean volatile C
centration of bark was generally lowest for shade-intolerant tree species; however, this
was mainly due to the low C concentration observed in aspen and birch, not jack pine, which
had the highest mean volatile C concentration of all species at 7.5 ± 0.2% (S.E.) (Table 2-2).
In addition, interactions between shade tolerance and tree size, and between species and tree
size, had significant effects on volatile C concentration (Table 2-3). For shade-intolerant
trembling aspen and white birch, volatile C concentration in bark showed a marginal ($P =
0.092$ and $0.062$, respectively) positive trend with tree size (Fig. 2-1). Volatile C of bark
marginally decreased with DBH for shade-tolerant white spruce, and significantly decreased
with DBH for balsam fir (Fig. 2-2).

**Table 2-1** Results from paired $t$-tests comparing differences in total and volatile C
concentrations between bark and stemwood tissues for each tree species. The columns give
the degrees of freedom (d.f.), $t$ values, and $P$ values. Significant terms ($P < 0.05$) are in bold.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total C concentration</th>
<th></th>
<th>Volatile C concentration</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>$t$ value</td>
<td>$P$ value</td>
<td>d.f.</td>
</tr>
<tr>
<td>Jack pine</td>
<td>23</td>
<td>12.8</td>
<td>$&lt;0.001$</td>
<td>23</td>
</tr>
<tr>
<td>Trembling aspen</td>
<td>35</td>
<td>22.1</td>
<td>$&lt;0.001$</td>
<td>35</td>
</tr>
<tr>
<td>White birch</td>
<td>26</td>
<td>7.3</td>
<td>$&lt;0.001$</td>
<td>26</td>
</tr>
<tr>
<td>Black spruce</td>
<td>32</td>
<td>10.3</td>
<td>$&lt;0.001$</td>
<td>32</td>
</tr>
<tr>
<td>White spruce</td>
<td>20</td>
<td>19.0</td>
<td>$&lt;0.001$</td>
<td>20</td>
</tr>
<tr>
<td>Balsam fir</td>
<td>23</td>
<td>21.1</td>
<td>$&lt;0.001$</td>
<td>23</td>
</tr>
</tbody>
</table>

Total C concentration of stemwood differed significantly with shade tolerance, tree
species, tree size, and their interactions (Table 2-3). Shade-intolerant tree species had a higher
(0.5%) overall mean total C concentration in stemwood than shade-tolerant tree species, with
a maximum mean C concentration of stemwood observed in jack pine at 53.0 ± 0.3% (S.E.),
and a minimum value of 49.0 ± 0.1% (S.E.) in shade-tolerant white spruce (Table 2-2). The
total C concentration of stemwood significantly increased with tree size for all shade-
intolerant species (Table 2-3, Fig. 2-1), but no general tree size pattern was observed for total
C concentration in stemwood for the shade-tolerant species (Fig. 2-2). Similarly, shade tolerance, tree species, tree size, and their interactions also had significant effects on volatile C concentration of stemwood (Table 2-3). Higher mean volatile C concentration of stemwood was found in shade-intolerant tree species compared with shade-tolerant tree species (Table 2-2). At the species level, jack pine had the highest mean volatile C concentration of stemwood (4.2 ± 0.2% S.E.), and white spruce had the lowest mean value (1.4 ± 0.1% S.E.).

The relationship between volatile C concentration in stemwood and tree size was significantly positive for all three shade-intolerant tree species (Fig. 2-1), but no tree size-associated pattern was observed for shade-tolerant tree species, except a marginal negative trend observed for white spruce (Fig. 2-2).

**Table 2-2** Means and standard errors (S.E.) of total and volatile C concentrations of six tree species in bark and stemwood tissues, respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total C concentration (%)</th>
<th>Volatile C concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bark</td>
<td>Stemwood</td>
</tr>
<tr>
<td><strong>Shade-intolerant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jack pine</td>
<td>57.3 ± 0.2</td>
<td>53.0 ± 0.3</td>
</tr>
<tr>
<td>Trembling aspen</td>
<td>54.1 ± 0.2</td>
<td>50.2 ± 0.1</td>
</tr>
<tr>
<td>White birch</td>
<td>60.7 ± 1.4</td>
<td>49.7 ± 0.3</td>
</tr>
<tr>
<td>All shade-intolerant</td>
<td>57.0 ± 0.5</td>
<td>50.8 ± 0.2</td>
</tr>
<tr>
<td><strong>Shade-tolerant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black spruce</td>
<td>55.1 ± 0.3</td>
<td>51.7 ± 0.2</td>
</tr>
<tr>
<td>White spruce</td>
<td>54.1 ± 0.3</td>
<td>49.0 ± 0.1</td>
</tr>
<tr>
<td>Balsam fir</td>
<td>56.6 ± 0.2</td>
<td>49.5 ± 0.2</td>
</tr>
<tr>
<td>All shade-tolerant</td>
<td>55.3 ± 0.2</td>
<td>50.3 ± 0.2</td>
</tr>
<tr>
<td>species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All species</td>
<td>56.2 ± 0.3</td>
<td>50.5 ± 0.1</td>
</tr>
</tbody>
</table>
Table 2-3 The effects of shade tolerance, species, and DBH on total and volatile C concentrations of each woody tissue type. Tree species was nested within shade tolerance. The columns give the degrees of freedom (d.f.), sum of squares (SS), $F$ values, $P$ values, and % deviance (in brackets) explained by the explanatory variables. Significant terms ($P < 0.05$) are in bold.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Total C concentration</th>
<th></th>
<th>Volatile C concentration</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bark</td>
<td>Stemwood</td>
<td>Bark</td>
<td>Stemwood</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P (% deviance explained)</td>
<td>SS</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Shade</td>
<td>1</td>
<td>0.007 (5.29)</td>
<td>10.5</td>
<td>9.9</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Species</td>
<td>4</td>
<td>&lt;0.001 (86.48)</td>
<td>275.0</td>
<td>64.6</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>DBH</td>
<td>1</td>
<td>0.003 (0.55)</td>
<td>26.5</td>
<td>24.9</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Shade × DBH</td>
<td>1</td>
<td>0.096 (1.92)</td>
<td>10.6</td>
<td>10.0</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Species × DBH</td>
<td>4</td>
<td>0.079 (5.76)</td>
<td>18.5</td>
<td>4.3</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Residual</td>
<td></td>
<td>162.9</td>
<td>255.3</td>
<td>120.9</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2-1 Total and volatile C concentrations in relation to wood tissue type and DBH for jack pine, trembling aspen, white birch. Closed and open circles represent C concentrations of bark and stemwood, respectively. Solid lines represent the significant relationship between DBH and total or volatile C concentration of bark (P < 0.05), whereas short dashed lines represent the significant relationship between DBH and total or volatile C concentration of stemwood (P < 0.05). Dotted lines represent a marginal relationship between DBH and total or volatile C concentration of bark (0.05 < P < 0.3), whereas dash-dot lines represent a marginal relationship between DBH and total or volatile C concentration of stemwood (0.05 < P < 0.3).
Figure 2-2 Total and volatile C concentrations in relation to wood tissue type and DBH for black spruce, white spruce, and balsam fir. Closed and open circles represent C concentration of bark and stemwood, respectively. Solid lines represent the significant relationship between DBH and total or volatile C concentration of bark ($P < 0.05$), whereas short dashed lines represent the significant relationship between DBH and total or volatile C concentration of stemwood ($P < 0.05$). Dotted lines represent a marginal relationship between DBH and total or volatile C concentration of bark ($0.05 < P < 0.3$), whereas dash-dot lines represent a marginal relationship between DBH and total or volatile C concentration of stemwood ($0.05 < P < 0.3$).
Discussion

We found that C concentration significantly differed between bark and stemwood tissue and varied significantly among tree species for each woody tissue type. The observed dependence of C concentration on woody tissue type is consistent with our hypothesis that C concentration in bark is greater than that of stemwood. Our findings agree with results from previous studies in temperate forests (Bert and Danjon, 2006; Martin et al., 2015), which show strong differences in C concentration between bark and other tree tissues. The divergence of C concentration between bark and stemwood is hypothesized to be related to the functional adaptations of bark tissue for water storage and protection (Franceschi et al., 2005; Rosell et al., 2014). More specifically, previous studies suggest that higher C concentration in bark, compared with stemwood, is caused by higher concentrations of C-rich elements (e.g., lignin, tannins, and suberin), which help limit water loss and defend against insects and pathogens (Franceschi et al., 2005) and fire (Hengst and Dawson, 1994).

Volatile C concentration was also higher in bark tissue compared with stemwood and also significantly varied among tree species, agreeing with previous studies of temperate tree species (Thomas and Malczewski, 2007). However, our data suggest that volatile C concentration, instead of C-rich elements like lignin, is primarily responsible for the observed difference in C concentration between bark and stemwood tissue. Indeed, we found that the difference between volatile C concentration in bark and stemwood accounted for a large fraction (e.g., 80% for balsam fir) of the overall difference in total C concentration between bark and stemwood. Furthermore, we also found that the difference in C concentration between bark and stemwood is generally higher for boreal tree species than previous findings for temperate tree species. Boreal tree species had, on average, 5.7% higher total C concentration in bark than in stemwood based on our data.
whereas the difference was less than 3% for temperate tree species (Martin et al., 2015). This difference may be related to differences in the severity and type of insect herbivory experienced by boreal tree species compared with temperate species. For instance, the boreal forest is subject to higher incidences of widespread insect infestations (Liebhold et al., 1995; Lovett et al., 2006), including mountain pine beetle (Dendroctonus ponderosae Hopkins) and spruce beetle (Dendroctonus rufipennis Kirby), which can infect large areas of pine and spruce–fir forests by feeding within the phloem tissue and killing trees (Hicke et al., 2012). Volatile compounds are hypothesized to help in plant defences (Martin et al., 2013) and may aid in inhibiting bark beetle infection. Furthermore, boreal forests experience more frequent wildfire disturbance than other biomes (Pan et al., 2011), and higher C concentration of bark of boreal tree species may also relate to the role of bark in fire resistance (Hengst and Dawson, 1994).

Our data yielded mixed results for tree size effects on total C concentration of bark and stemwood. We found both neutral and positive relationships between tree size and total C concentration of stemwood, partially in agreement with Martin and Thomas (2013) whose study in tropical forest found C concentration increases with tree size. In addition, we also found negative, neutral, and positive relationships between tree size and total C concentration in bark tissue. The reason for the diverse relationships is likely attributable to size-associated changes in volatile C concentration, which parallel total C concentration. Traditional views suggest that total C concentration is mostly dominated by C-rich elements like lignin (Lamlom and Savidge, 2003). It is, thus, reasonable to assume that small trees have higher C concentrations than large trees because small trees generally contain higher lignin concentrations (Martin et al., 2013), supporting mechanical stability (Alvarez-Clare and Kitajima, 2007) and defense mechanisms (Wainhouse et al., 1990). However, our findings suggest that the size-associated trend of
total C concentration is likely led by change in the concentration of volatile C compounds (Figs. 2-1 and 2-2). These results support Martin et al. (2013), who hypothesized that volatile C concentration plays an important role in size-related changes in total C concentration. Furthermore, when considering our observation that the difference in volatile C concentration between bark and stemwood tissue accounts for a large proportion of the difference in total C concentration between bark and stemwood tissue, we speculate that the fraction of volatile C concentration plays a key role in driving overall variation in total tree C concentration. Moreover, size-associated trends in stemwood C and bark C differed according to shade tolerance. All shade-intolerant tree species had positive relationships between stemwood C concentration and tree size, whereas no general size-associated trend was found in stemwood for shade-tolerant tree species. Similarly, shade-intolerant tree species also showed positive size-associated changes in C concentration in bark tissue, with the exception of white birch, where we observed some individuals with exceptionally high C values. These high C values in white birch are likely related its high concentration of C-rich betulinic acid (O’Connell et al., 1988). The observed difference in trends between shade-tolerant and intolerant species supports our hypothesis that size-associated changes in total C concentration depend on shade tolerance. The potential reason for this size-associated interaction with contrasting shade tolerance may be attributable to species’ life history strategy under low light availability in the understorey. Shade-tolerant seedlings and saplings that can tolerate low light levels tend to grow slower and have denser wood, resulting in higher C concentration (Elias and Potvin, 2003; Poorter et al., 2005). However, the generality of these results is still uncertain, given that previous studies also found positive relationships between C concentration and tree size for shade-tolerant tree species in tropical forests (Martin et al., 2013). Further empirical study of woody tissue C
concentration, that explicitly accounts for volatile C, is needed from different biomes to further test the underlying effects of shade tolerance (or other functional traits) on tree C concentration.

In agreement with previous findings (Lamlom and Savidge, 2003; Thomas and Malczewski, 2007; Zhang et al., 2009), our results reveal that assuming 50% C concentration without considering tree species, woody tissue type, and size may underestimate C stocks for major boreal tree species (see Appendix I). For example, a trembling aspen tree with a DBH of 48 cm would have 280 kg higher C mass using our C concentration data instead of using the standard assumed concentration of 50%. Although such a difference may seem trivial for an individual tree, when scaled across entire forest stands or landscapes, the discrepancy can be substantial, especially for forests where these species comprise a large amount of aboveground biomass, such as at intermediate stages of succession for boreal forests in Canada (Chen and Popadiouk, 2002). By applying the same tree inventory data as Taylor et al. (2014), we found that 92-year-old, fire-origin, mixedwood stands would have 7000 kg C ha⁻¹ higher C stocks using our C concentration values compared with using the standard 50%. Furthermore, we recommend that corrected C concentration (C_{freeze-corr}) should be used when converting conventional oven-dried C measures (i.e., C_{heat}) of tree biomass to C stocks by applying the following functions derived from our data:

\[ C_{freeze-corr} = 1.115 \times C_{heat} \]  

(5)

(For bark, linear regression constrained to have y intercept = 0; \( r^2 = 0.74; p < 0.001 \))

\[ C_{freeze-corr} = 1.063 \times C_{heat} \]  

(6)

(For stemwood, linear regression constrained to have y intercept = 0; \( r^2 = 0.34; p < 0.001 \))

Our study highlights the importance of considering variation in C concentration when estimating live tree C stocks, which has broad implications for global C accounting.
and validation of C accounting models. Carbon concentration is significantly higher in bark than in stemwood tissue, and varies among tree species. Tree size and shade tolerance also have effects on C concentration, but more empirical studies are needed to identify the generality of these effects. Understanding variation in total C concentration within and among tree species and incorporating it when quantifying forest C stocks and validating C accounting models is important to successfully predict future global C emission scenarios. Moreover, we observed that much of the variation in total C concentration is attributed to volatile C concentration, providing new insight that volatile C plays a key role in variation of total C concentration. Our results indicate that volatile C concentration, rather than C-rich elements (e.g., lignin), is the dominant driver behind size-associated changes in tree C concentration, and the dominant factor underlying the difference in total C concentration between bark and stemwood tissue for major boreal tree species. However, the underlying mechanisms of why volatile C concentration showed different size-associated relationships between species is not clear, and whether these results can be generalized to other species, especially tree species in other biomes, requires further testing.
Chapter Three: Carbon storage declines in old boreal forests

irrespective of succession pathway

Running head: Carbon storage dynamics in the boreal forest

Introduction

The world’s boreal forests play an important role in regulating atmospheric carbon dioxide through storage of 49% of global forest vegetation and soil carbon (C) (Dixon et al., 1994). Wildfire is the predominant form of natural stand-replacing disturbance in the boreal forest and exerts strong impacts on C sequestration and storage (Bond-Lamberty et al., 2007). Over the past several decades, fire activity has increased in many regions to levels unprecedented since the last ice age (Kelly et al., 2013), highlighting the importance of understanding its effects on forest C dynamics. Still, the long-term impact and recovery of forest C storage following stand-replacing fire, especially during late succession, remains unclear (Taylor et al., 2014). This is especially concerning because boreal forests are expected to experience warming at twice the global average rate over the coming century, increasing the frequency and intensity of fire disturbance (Gauthier et al., 2015).

Carbon storage changes as forest stands age. It has long been recognized that forest net ecosystem production (NEP) increases rapidly during early stand development, peaks as stands mature, and then declines as stands age (Ryan et al., 1997). During periods of positive NEP, total ecosystem C increases, but during periods of negative NEP (i.e., when gross primary production is less than total ecosystem respiration), total ecosystem C decreases. Across diverse forest types, whether forest ecosystems continue to accumulate C, decline, or stabilize as they transition from mature, even-aged stands to structurally complex old-growth stands is unclear, with many studies reporting conflicting results (Bond-Lamberty et al., 2004; Harmon et al., 2004; Goulden et al., 2011; Kashian et al., 2015).
et al., 2013; Taylor et al., 2014). One possible explanation for this discrepancy may be lack of consideration of forest canopy transition, from dominance of early to late-succession species, on C dynamics. Most studies that have examined forest C storage in relation to stand aging in boreal forests have focused on stands that have not yet transitioned from dominance of individuals established immediately after fire and are typically restricted to stands less than 160 years old. The transition from early to late-succession species in boreal stands generally does not occur until early succession species have reached their average maximum lifespan, which can be in excess of 150 years (Chen and Popadiouk, 2002).

The commonly accepted pathway of forest succession for mesic sites in the North American boreal forest is characterized by the sequential replacement of fast-growing, early succession colonizers, such as trembling aspen (Populus tremuloides Michx.) and jack pine (Pinus banksiana Lamb.), by slower-growing, shade-tolerant species, such as spruce (Picea spp.) and balsam fir (Chen and Popadiouk, 2002). Indeed, it has been postulated that the transition from large, fast-growing, early succession species to small, slow-growing, late-succession species may contribute to a reduction in NEP and loss of C in old boreal stands (Taylor et al., 2014). Furthermore, depending on pre-disturbance forest composition, disturbance type and severity, neighboring stands, and the influence of minor disturbances (Johnstone and Chapin, 2006; Chen et al., 2009), similar site types in the boreal forest may undergo multiple succession pathways (Taylor and Chen, 2011). For example, broadleaf stands dominated by shade-intolerant trembling aspen and white birch may self-replace in the absence of competition from shade-tolerant conifers (Kneeshaw and Bergeron, 1998; Brassard et al., 2008). Conifer stands dominated by shade-intolerant jack pine have a high probability of transitioning to shade-tolerant spruce and balsam fir (Taylor and Chen, 2011). Minor infestations by spruce budworm
Choristoneura fumiferana Clemens) or bark beetle (e.g., Dendroctonus ponderosae)

Hopkins) can promote the persistence of broadleaf species in old stands, forming complex mixedwood stands (Kneeshaw and Bergeron, 1998). These multiple succession pathways may have diverse effects on forest C storage as species have different growth rates and maximum standing biomass. Furthermore, diverse species mixtures can have positive effects on forest productivity relative to species-poor mixtures, depending on stand age (Zhang et al., 2012; Liang et al., 2016).

In this study, we used a replicated chronosequence design that covered a wide range of stand age classes (from 8 to 210 years old), following stand-replacing fire, undergoing multiple succession pathways in the boreal forest of central Canada, to examine C storage dynamics. We selected stands of different overstorey types (i.e., broadleaf, conifer, or mixedwood) for each age class in order to address how forest C storage changed in relation to stand aging and succession. Specifically, we sought to address the following questions: 1) does the direction of forest succession (as represented by different overstorey types at each stand age) influence forest C storage as stands age following stand-replacing fire? and 2) does C storage increase, decrease, or show no significant change in the late successional stage as stands transition from mature, even-aged stands to structurally complex old-growth stands following different successional trajectories? To understand the age-dependent total C storage dynamics, we determined the dynamics of individual C pools including live biomass, dead wood, forest floor and mineral soil.
Materials and Methods

Study area

Our study was conducted in the boreal forest region, approximately 150 km north of Thunder Bay, Ontario, Canada, between 49°44′ to 49°65′ N and 89°16′ to 90°13′ W. This area is characterized by warm summers and cold, snowy winters. Mean annual temperature is 1.9 °C and mean annual precipitation is 824.8 mm as measured by the closest meteorological station in Cameron Falls, Ontario, Canada (Environment Canada, 2017). Soils in our study area were largely deposited by the Wisconsinan glaciation, which ended approximately 9,500 years ago in this region (Moran and Clayton, 1984). Stand-replacing wildfire is the most common natural disturbance in the study area, with an average fire-return interval of approximately 100 years during the past century, resulting in a mosaic of stand ages across the landscape (Senici et al., 2010). We conducted our sampling in the Spruce Forest, which is approximately a million of hectares. Dominant overstorey tree species in the study area, in order from least to most shade tolerant, include jack pine, trembling aspen, white birch, black spruce, white spruce, and balsam fir (Burns and Honkala, 1990).

Sampling design

To determine the effect of stand aging and succession on forest C storage, we constructed a chronosequence of postfire stands. Although the use of the chronosequence method has been criticized because it makes the assumption that sample stands along the temporal sequence have followed the same developmental history (Johnson and Miyanishi, 2008), given careful site selection, replication, and demonstration of developmental links, the chronosequence method is appropriate for studying patterns of C storage over decadal to centennial time scales (Walker et al., 2010). Based on local fire history and the availability of different aged stands in our study area, we were able to sample five
different stand age classes, including 8, 34, 99, 147, and 210 years since fire, representing the stand initiation, stem exclusion, early canopy transition, late canopy transition, and gap dynamic stages of boreal forest development, respectively, modified from Chen and Popadiouk (2002).

To account for multiple succession pathways as stands age after fire, we carefully selected different overstorey types for each age class to account for multiple succession pathways. Broadleaf stands or conifer stands had >80% of their total stem density or basal area as broadleaf species or conifer species, respectively, whereas in mixedwood stands, stem density or basal area included relatively equal proportions of both broadleaf and conifer species (Table 1). More specifically, for stands in the stand initiation and stem exclusion stages of development (i.e., years 8, 34), we selected broadleaf stands that were dominated by trembling aspen, conifer stands that were dominated by jack pine, and mixedwood stands that contained a mixture of jack pine and trembling aspen. For stands in the canopy transition (i.e., years 99, 147) and gap dynamics stages (i.e., year 210), we selected broadleaf stands dominated by self-replacing trembling aspen and white birch, conifer stands that were transitioning from jack pine to spruce and balsam fir dominance, and mixedwood stands that were co-dominated by trembling aspen, white birch, spruce and balsam fir.

We attempted to select postfire stands that had not been managed, including three replicates for each age class and overstorey type combination; however, two replicate mixedwood stands, each at age 34 and 147 years, were accidentally damaged by harvesting activities between consecutive years of forest inventory, resulting in a total of 43 stands sampled. Sample stands were positioned several kilometers away from each other and selected from different road accesses to minimize the impact of spatial autocorrelation. In order to minimize site variability, all selected stands were located on...
mesic sites with flat midslope positions, with no slope exceeding 5%. All stands are
underlain by moderately deep (≥50 cm) glacial tills, belonging to the Brunisolic soil
order, according to the Canadian system of soil classification (Soil Classification
Working Group, 1998). To ensure that each sample stand met the selection criteria, soil
pits were dug in each candidate stand to verify whether the site was mesic, following the
procedures described in Taylor et al. (2000). Soil attribute data for all sites used in this
study have been previously reported by Hume et al. (2016)

Time since last stand-replacing fire (TSF) for sample stands less than 90 years old
was determined from detailed fire records (Senici et al., 2010). For stands greater than 90
years old, tree ages were used to estimate minimum TSF following the procedures
described in Senici et al. (2010). Of all sampled stands, we selected either jack pine or
trembling aspen trees to determine minimum TSF. In each stand, three canopy stems were
sampled by extracting a core or stem disk at breast height (1.3 m above root collar). The
cores and disks were transported to our laboratory, where the cores were mounted on
constructed core strips and sanded to make rings visible. Stem disks were cut
transversely, then mounted on constructed core strips and sanded to make rings visible.
Rings were counted using a handheld magnifier or a microscope until the same count was
obtained three successive times. Based on a locally derived age correction model
developed by Vasiliauskas and Chen (2002), 7 years were added to ring counts to
determine minimum TSF.
### Table 3-1 Characteristics of the 43 sample stands in the boreal forests of Ontario, Canada.

<table>
<thead>
<tr>
<th>Age</th>
<th>Overstorey</th>
<th>N</th>
<th>Stand density (stems ha(^{-1})) or basal area (m(^2) ha(^{-1}))</th>
<th>Stand composition (%)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trembling aspen</td>
<td>White birch</td>
</tr>
<tr>
<td>8</td>
<td>B</td>
<td>3</td>
<td>5933 (581)</td>
<td>95 (5)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3</td>
<td>7067 (1551)</td>
<td>3 (3)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>3</td>
<td>6933 (926)</td>
<td>45 (9)</td>
</tr>
<tr>
<td>34</td>
<td>B</td>
<td>3</td>
<td>26 (1)</td>
<td>92 (3)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3</td>
<td>28 (2)</td>
<td>4 (2)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2</td>
<td>19 (3)</td>
<td>52 (6)</td>
</tr>
<tr>
<td>99</td>
<td>B</td>
<td>3</td>
<td>51 (7)</td>
<td>91 (2)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3</td>
<td>52 (2)</td>
<td>3 (2)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>3</td>
<td>43 (5)</td>
<td>40 (12)</td>
</tr>
<tr>
<td>147</td>
<td>B</td>
<td>3</td>
<td>58 (8)</td>
<td>85 (3)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3</td>
<td>51 (9)</td>
<td>1 (1)</td>
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<tr>
<td></td>
<td>M</td>
<td>2</td>
<td>36 (3)</td>
<td>38 (2)</td>
</tr>
<tr>
<td>210</td>
<td>B</td>
<td>3</td>
<td>41 (3)</td>
<td>54 (22)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3</td>
<td>40 (8)</td>
<td>5 (5)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>3</td>
<td>46 (3)</td>
<td>11 (4)</td>
</tr>
</tbody>
</table>

**Notes:** Each age-overstorey combination has three replications, except 34- and 147-year-old mixedwood stands (two replicates).

\(^a\) Overstorey types: B = broadleaf, C = conifer, M = mixedwoods.

\(^b\) Values are means with 1 SE in parentheses. Stand density (stems ha\(^{-1}\)) was determined for the younger (8 years old) stands and basal area (m\(^2\) ha\(^{-1}\)) for older stands.

\(^c\) The ‘Others’ category includes Salix spp., Acer spicatum, Alnus viridis, Sorbus decora, Corylus cornuta, Prunus pensylvanica, and Larix laricina.

**Data collection**

**Aboveground live biomass carbon**

In each selected stand, we randomly established a 400 m\(^2\) (11.28 m radius) circular sampling plot. Within each plot, diameters at breast height (DBH; 1.3 m above the root
collar) of all overstorey trees (DBH ≥9 cm) were measured. Trees with a DBH in the range of 3–9 cm were measured within a 50 m² circular subplot (3.99 m radius; shared plot center with the 400 m² plot). Aboveground biomass of all trees ≥3 cm in DBH was estimated as the sum of bark, stem, branch, and foliage biomass calculated by using published Canadian allometric equations (Lambert et al., 2005). For tree saplings (DBH <3 cm) and large shrubs (height ≥50 cm), the base diameter of each individual was measured in three randomly selected 2 × 2 m plots within each 400 m² main plot. Aboveground biomass of tree saplings and large shrubs was estimated by using allometric equations developed for small trees and large shrubs (Smith and Brand, 1983; Bond-Lamberty et al., 2002).

Aboveground biomass of understorey vegetation, including short shrubs (height <50 cm), forbs, graminoids (grass, sedge), bryophytes (mosses), ferns and lichens, was estimated by two stages of sampling during July 2014, when annual plant cover was at or near its maximum (MacDonald et al., 2012). Firstly, the percent cover of each understorey species was estimated within each 400 m² plot. Secondly, the percent cover of each understorey species was visually estimated again within twenty-one 2 × 2 m subplots, which were randomly located in the larger 400 m² main plot. All understorey plants in the subplots were then harvested by species, stored in paper bags, and transported to the laboratory for biomass determination. Samples were oven dried at 68ºC for 48 h and weighed. Data of all harvested samples were pooled together, and linear regression was used to develop allometric relationships between understorey biomass and percent coverage for each growth form (MacDonald et al., 2012). The whole plot biomass was then estimated based on percent cover of each species from the first step by using developed regressions from the second step.
Belowground live biomass carbon

Coarse root (>10 mm diameter) biomass was calculated for the individual tree and large shrub species using published allometric equations. When a species-specific coarse root biomass equation was not available, we used the equation corresponding to the species with the most similar lifeform characteristics (Burns and Honkala, 1990). For jack pine, aspen, black spruce, and balsam fir ≥10 cm DBH, we used locally developed, species-specific equations (Brassard et al., 2011). For white spruce and white birch ≥10 cm DBH, equations developed by Brassard et al. (2011) for black spruce and aspen were used, respectively. There was no shrub ≥10 cm DBH present in any of our plots. For jack pine, aspen, black spruce, and white birch <10 cm DBH and Salix spp., we used the species-specific equations developed by Bond-Lamberty et al. (2002) for central Canadian boreal forests. For white spruce and balsam fir <10 cm DBH, we used the equation for black spruce, whereas the equation for trembling aspen was used for Acer spicatum, Alnus spp., Prunus pensylvanica, Castanea spp., Corylus cornuta, and Sorbus spp.

For medium (2–10 mm in diameter) and fine root (≤2 mm in diameter) biomass data, we used the medium and fine root biomass data from a similar chronosequence study conducted close to our study area (Yuan and Chen, 2012) due to time and resource limitations. As Yuan and Chen (2012) only focused on mixedwood stands, we measured fine root biomass in the 8- and 34-year-old stands across all overstorey types and developed linear regressions to estimate medium and fine root biomass for broadleaf and conifer stands.

In each 400 m² plot, seven soil cores (6.6 cm in diameter) were randomly selected to collect fine roots using a power auger. Cores were collected monthly from June–October 2014 and in May 2015, with six sampling times in total (we reported the average of six measurements). The extracted soils were separated into a forest floor layer and two...
mineral soil layers (0–15 and 15–30 cm) and transported in an ice-filled cooler to the 
laboratory, where they were stored in a freezer until processing. In the laboratory, 
samples were soaked in water to separate roots from the soil and then sieved (0.2 mm 
 mesh). Fine roots were sorted according to vitality (live or dead). Live roots were pale 
 colored on the exterior, elastic and flexible, free of decay, and had a whitish cortex, 
whereas dead roots were brown or black, rigid and inflexible, in various stages of decay, 
and had a darker cortex. The “live” and “dead” components were then oven dried 
separately to a constant mass at 65°C and weighed.

We segregated the measured fine root biomass data according to overstorey type 
(i.e., broadleaf, conifer, and mixedwood) and built linear regressions between fine root 
biomass in mixedwood stands and in conifer stands, and between fine root biomass in 
mixedwood stands and in broadleaf stands, respectively (see Fig. S3-1). For each stand’s 
age, medium and fine root biomass in broadleaf and conifer stands were then estimated 
by using mixedwood data from Yuan and Chen (2012) and locally developed regressions. 

Aboveground deadwood carbon

Aboveground deadwood included downed woody debris (DWD) and aboveground snags. 
Downed woody debris was defined as all dead wood lying or standing (with a zenith 
angle ≤45°) with a midlength diameter ≥2 cm and was measured using the line intercept 
method (Husch et al., 2002). Two 30-m transects were established in each sample stand, 
radiating out from the center of each 400 m² circular plot. The first transect was oriented 
randomly, whereas the second was at a 90° angle to the first. The diameter at transect, 
species, and decay class, according to Seedre et al. (2013), of all DWD pieces ≥2 cm 
occurring along the transect were recorded. The volume of DWD on an area basis (m³/ha) 
was calculated using the formula described by (Husch et al., 2002):
\[ DWD = \frac{\pi^2 \sum d^2}{8L} \]  
(1)

where \( d \) is the piece intercept diameter (cm), and \( L \) is the length (m) of the transect. The volume of \( DWD \) was converted to necromass using site-specific deadwood density values developed by Seedre et al. (2013).

Snags consisted of standing dead trees, with both intact tops and broken tops, and stumps, which consisted of broken top snags with heights less than 1.3 m. Standing dead trees included all trees that appeared dead with no green foliage remaining and stood with a zenith angle of >45° and were ≥1.3 m in height. The DBH of all standing dead trees (>2 cm DBH) was measured in each 400 m² plot. Aboveground necromass of standing dead trees was estimated similarly to live tree biomass, using collected DBH data and species-specific allometric equations (Lambert et al., 2005); however, to account for loss of bark and branches that accompany decay after death, all standing dead trees were grouped into one of the following three categories developed by Seedre et al. (2014), and their biomasses were reduced accordingly: (1) all bark and branches remained intact (no reduction); (2) only 50% of bark and 70% of branches remained intact (thus a 50% bark and 30% branch biomass reduction was applied); and (3) only 20% of bark and 50% of branches remained intact (thus an 80% bark and 50% branch biomass reduction was applied).

For each snag with a broken top, diameters both at the top and at the stem base, as well as the height, were measured in each 400 m² plot. Each snag or stump was assigned a locally derived deadwood decay class (Seedre et al., 2013). Snag and stump volume was estimated using a truncated cone formula (Husch et al., 2002) and was converted to necromass using the site-specific deadwood density values locally developed by Seedre et al. (2013).
Belowground deadwood carbon

Belowground deadwood consists of root necromass of snags and stumps. The coarse root necromass of snags and stumps was calculated similarly to live coarse roots. However, because the equations of Bond-Lamberty et al. (2002) and Brassard et al. (2011) were developed for live trees, dead coarse root necromass estimates have to be adjusted to reflect decay class (Seedre et al., 2013). For example, an individual tree that has been dead a long time would have a more decayed coarse root system and less root necromass than an individual with a shorter time-since-death. To calculate dead coarse root necromass and account for mass losses due to decay, we used the following equation, which takes advantage of the strong correlation between coarse root necromass and density during decay (Olajuyigbe et al., 2011):

\[
CRB_{\text{adj}} = \left( \frac{DW}{LW} \right) \times CRB_{\text{unadj}}
\]

(2)

where \( CRB_{\text{adj}} \) is the coarse root necromass of a snag or stump after correcting for losses associated with decay, \( LW \) is the density of the individual assuming it was still alive, \( DW \) is the density of the individual in its current state of decay, and \( CRB_{\text{unadj}} \) is the calculated coarse root biomass using the equations of Bond-Lamberty et al. (2002) or Brassard et al. (2011) as if the individual were still alive. \( LW \) values were taken from Alemdag (1984), who reports average stem wood density values for live jack pine, aspen, white birch, black spruce, white spruce, and balsam fir from samples collected in the boreal forest of Ontario, Canada. \( DW \) values were calculated using local equations from Seedre et al. (2013), derived from the same study area.

Dead medium and fine root necromass was estimated using the same method mentioned above for live medium and fine root biomass. Simple linear regressions were developed between fine root necromass in mixedwood stands and in conifer stands, and
between fine root necromass in mixedwood stands and in broadleaf stands, respectively
(see Fig. S3-2). Dead medium and fine root necromass data for all mixedwood stands of
our chronosequence were derived from the chronosequence study of Yuan and Chen
(2012), and then we used locally developed fine root necromass equations (see Fig. S3-2)
to calculate medium and fine root necromass data in broadleaf and conifer stands for each
stand age.

Total aboveground tree biomass and tree coarse root biomass were converted to C
using locally developed C concentrations estimated by Chapter two. The C content of
aboveground understorey vegetation was calculated as 45% of dry biomass (IPCC, 2013).
Belowground medium and fine root C content was similarly assumed to be 45% of dry
biomass. The C content of aboveground deadwood and belowground deadwood was
assumed to be 50% of dry necromass, whereas C content of dead medium and fine roots
was assumed as 45% of dry necromass (Garrett et al., 2012). All live biomass and
deadwood C pools were scaled-up to Mg C·ha⁻¹.

Forest floor and mineral soil carbon
Forest floor C and mineral soil C for all sites in this study have been previously reported
(Hume et al., 2016). In brief, ten soil cores were randomly collected per 400 m² main plot
using a Dutch soil auger. Soil cores were separated by forest floor (i.e., the fibric and
humus layers) and two mineral soil layers according to mineral soil depth, i.e., M1 (0–15
cm) and M2 (15–30 cm). Soil samples were stored in a cooler and immediately
transported to the laboratory for chemical analysis. Soil samples were air dried to constant
weight and sieved (2-mm mesh) to remove coarse fragments, and then ground to pass
through a 100-mesh (0.15 mm) sieve to ensure a uniform sample. Total C for each sample
was determined by the flash dynamic combustion method and converted to Mg C ha⁻¹
following the method described by Wairiu and Lal (2003).
We did not collect mineral soil samples >30 cm depth as C storage in the deep mineral soil was expected to change little over the temporal range of our chronosequence (Harden et al., 1997; Wirth et al., 2002; Goulden et al., 2011). Nonetheless, to provide a more realistic estimate of total mineral soil C storage at each site, we used the average value of deep (30–100 cm) mineral soil C mass derived by Laganière et al. (2012) from six local 92-year-old stands from our study area to approximate mineral soil C deeper than 30 cm in each of our study stands.

Data analysis

To address our first research question of whether the direction of forest succession influences forest C storage as stands age following stand-replacing fire, we used a two-way analysis of variance (ANOVA) to test the effects of stand age and overstorey type on total ecosystem C storage. Specifically, we tested whether age-related changes in forest C storage differed among three overstorey types through examination of the interaction term as shown in the following statistical model.

\[
Y_{ijk} = \mu + A_i + T_j + A \times T_{ij} + \varepsilon_{(ij)k}
\]

(3)

where \(Y_{ijk}\) is forest C storage, \(\mu\) is the overall mean, \(A_i\) (\(i = 1, 2, \ldots 5\)) represents stand age classes, \(T_j\) (\(j = 1, 2, 3\)) is the overstorey type, and \(\varepsilon_{(ij)k}\) is random sampling error within stand age class and overstorey type.

For our second question, we used Tukey’s Honest Significant Difference (HSD) test to examine if C storage increased, decreased, or showed no significant change as postfire boreal stands transitioned from mature, even-aged stands to structurally complex old-growth stands (i.e., from 147- to 210-year old stands), and whether this change in C storage was influenced by successional pathways, characterized by overstorey types.

Furthermore, as individual C pools transfer C amongst each other and have potentially different age-dependent C dynamics (Seedre et al., 2011), we also performed the two-way
ANOVA (Eq. 3) on each C pool component to test the influence of succession pathways on individual C pools to help understand underlying changes in total ecosystem C storage. We verified the ANOVA assumptions of normality and homogeneity of variance using the Shapiro–Wilk test and Levene’s test, respectively. The tests revealed the assumptions of normality and homogeneity were met for all analyses without data transformation. All data analyses were performed in R 3.4.2 (R Development Core Team, 2017).
Results

Following stand-replacing fire, total ecosystem C changed significantly with stand age. Broadleaf stands on average had higher total ecosystem C than conifer and mixedwood stands; but age-related trends of total ecosystem C storage were similar for all three overstorey types (Table 3-2). Averaged across all overstorey types, total ecosystem C increased rapidly from 96.5 ± 5.1 (Mean ± S.E.) Mg C ha\(^{-1}\) in 8-year-old stands to 300.4 ± 9.4 Mg C ha\(^{-1}\) in 99-year-old stands, peaking at 327.9 ± 11.9 Mg C ha\(^{-1}\) in 147-year-old stands, and then significantly decreasing to 271.1 ± 13.6 Mg C ha\(^{-1}\) in 210-year-old stands (Fig. 3-1a).

Table 3-2 Results of stand age (A\(_i\)) and overstorey type (T\(_j\)) on total ecosystem C and individual C pools following fire. The columns give the degrees of freedom (d.f.), F value, and P value. Statistically significant terms (\(p < 0.05\)) are in bold.

<table>
<thead>
<tr>
<th>C Pools</th>
<th>A(_i) (d.f. = 4)</th>
<th>T(_j) (d.f. = 2)</th>
<th>A × T(_j) (d.f. = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Total ecosystem C</td>
<td>124.5</td>
<td>&lt; 0.001</td>
<td>6.1</td>
</tr>
<tr>
<td>Total live biomass</td>
<td>50.8</td>
<td>&lt; 0.001</td>
<td>4.7</td>
</tr>
<tr>
<td>Total deadwood</td>
<td>1.9</td>
<td>0.144</td>
<td>0.7</td>
</tr>
<tr>
<td>Forest floor</td>
<td>8.8</td>
<td>&lt; 0.001</td>
<td>0.5</td>
</tr>
<tr>
<td>Mineral soil</td>
<td>5.5</td>
<td>0.002</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Among all C pools, the live biomass C pool had the strongest temporal dynamics (Fig. 3-1b). Total live biomass C was significantly influenced by stand age, overstorey type, and their interaction (Table 3-2, Fig. 3-2a). Similar to changes in total ecosystem C storage, live biomass C, averaged over all overstorey types, increased rapidly from 9.4 ± 1.3 – 12.6 ± 1.1 Mg C ha\(^{-1}\) in 8-year-old stands to 139.5 ± 25.1 – 195.1 ± 24.5 Mg C ha\(^{-1}\) in 99-year-old stands. However, age-related changes in live biomass C marginally differed among overstorey types thereafter (Table 3-2, Fig. 3-2a). Conifer and broadleaf stands continued to increase to a peak of 163.1 ± 8.3 and 221.9 ± 23.4 Mg ha\(^{-1}\) in the 147-
year-old stands, respectively, then declined. In comparison, the mixedwood stands showed a slight decrease, and then increased to a peak of $163.4 \pm 8.3 \text{ Mg ha}^{-1}$ in the 210-year-old stands. Total deadwood C only demonstrated a marginally significant change with stand age (Table 3-2, $P < 0.15$), and it did not change notably until 147 years old (Fig. 3-2b).

Carbon storage in the forest floor changed significantly with stand age, and showed a significant interaction effect between stand age and overstorey type (Table 3-2, Fig 3-3a). The forest floor C pool increased rapidly in the broadleaf and mixedwood from 8 to 34 years old, but fluctuated thereafter. It remained relatively low in the conifer stands until it peaked in the 99-year-old stands, and then significantly declined in the 210-year-old stands (Fig. 3-3a). Similar to forest floor C storage, our ANOVA test indicated a significant age effect on mineral soil C storage, however, age-related changes in mineral soil C marginally differed among the three overstorey types (Table 3-2). Our data suggest that mineral soil C storage remained relatively stable throughout succession and that the age-related effect was mainly caused by the peak in mineral soil C observed in the 147-year-old mixedwood stands. During late succession, mixedwoods had a steeper decline in mineral soil C than other types from 147- to 210-year-old stands (Fig. 3-3b).
Figure 3-1 Carbon storage in relation to time since stand-replacing fire and overstorey type for a) total ecosystem; and b) components of the four individual pools. Symbols and vertical lines represent sample means and standard errors, respectively, and ribbons represent the 95% confidence interval of sample means.
Figure 3-2 Carbon storage in relation to time since stand-replacing fire and overstorey type for a) live biomass; b) deadwood. Symbols and vertical lines represent sample means and standard errors, respectively, and ribbons represent the 95% confidence interval of sample means.
Figure 3-3 Carbon storage in relation to time since stand-replacing fire and overstorey type for a) forest floor; and b) mineral soil. Symbols and vertical lines represent sample means and standard errors, respectively, and ribbons represent the 95% confidence interval of sample means.
Discussion

Carbon dynamics among overstory types

Overall, our results show a strong relationship between total ecosystem C and stand age following fire, similar to previous studies (Pregitzer and Euskirchen, 2004; Goulden et al., 2011; Taylor et al., 2014). We found broadleaf stands had, on average, higher total ecosystem C and live biomass C than the other types, supporting a previous hypothesis that suggests aspen abundance is a key driver of ecosystem C storage in Canadian boreal forest (Pare and Bergeron, 1995; Laganière et al., 2015). However, despite that boreal forest stands may follow multiple succession pathways as they age, our study did not detect any significant difference in total ecosystem C dynamics between the three overstorey types over the span of our chronosequence (i.e., 210 years). This suggests that succession trajectory may have little effect on total ecosystem C storage as forests age and that succession only accounts for minor variations in total ecosystem C storage dynamics as forest stands transition from mature, even-aged stands to structurally complex old-growth stands.

Although total ecosystem C storage had similar age-dependent trends among the three overstorey types, we did observe significant differences among stand types in the dynamics of individual C pools that comprise total ecosystem C, corroborating previous reports (Pregitzer and Euskirchen, 2004; Seedre et al., 2011). The live biomass C pool increased sharply from age 8 to age 99 years for all stand types, driving the rapid recovery of total ecosystem C storage (90% within 99 years) relative to the mean fire interval of 100 years, which agrees with a previous chronosequence study of Pinus contorta-dominated forests (Kashian et al., 2013). However, during the late successional stage, the live biomass C pool declined substantially from age 147 to 210 years in the broadleaf and conifer-dominated overstorey types, whereas the mixedwoods continued to
accumulate live biomass C, peaking in the oldest measured stands. This divergent pattern may be attributable to differences in tree mortality rates experienced among stand types. For instance, the negative effects of conspecific neighbors have been reported to increase tree mortality rates, especially for trembling aspen, white birch, and spruce (Luo and Chen, 2011), which dominated our broadleaf and conifer stand types, potentially causing higher tree mortality in old broadleaf and conifer stands compared with mixedwood stands. Furthermore, due to the potential effects of resource partitioning and niche complementary, mixtures of fast-growing, shade-intolerant species (e.g., trembling aspen and white birch) and slower-growing, shade-tolerant species (e.g., spruce and fir) may have a positive effect on the long-term productivity of old mixedwood stands compared with less diverse broadleaf and conifer-dominated stands (Zhang et al., 2012).

We also found conifer stands differ from broadleaf and mixedwood stands in the way they accumulate forest floor C, in agreement with previous reports of tree species composition effects on forest floor C storage (Laganière et al., 2012; Laganière et al., 2013). During early stand development (i.e., 8 and 34 years), conifer stands contained less forest floor C than broadleaf or mixedwood stands. This may be because in young conifer stands, the main input of C to the forest floor is slow-decomposing needle leaf litter. Conversely, young mixedwood and broadleaf stands support a higher density and diversity of understory shrub and herb species (Hart and Chen, 2008). The contribution of the understory vegetation combined with higher annual turnover of broadleaf litterfall (Chen et al., 2016) may lead to higher C accumulation in the forest floor. However, as conifer stands age, their understory becomes increasingly dominated by shade-tolerant, bryophyte species (Hart and Chen, 2008). Furthermore, cool, damp conditions, typical of conifer understory environments, impede decomposition and contribute to the accumulation of dead organic matter (Lang et al., 2009). This is supported in Fig. 3a by
the large hump in forest floor C in the conifer stands during the intermediate stages of stand development (i.e., the 99-year-old stands). Eventually, as conifer stands undergo canopy break-up and transition to structurally complex old-growth stands, increased light to the forest floor can enhance forest floor decomposition and alter understorey vegetation composition (Laganière et al., 2012; Kumar et al., 2017).

Correspondingly, we observed marginal differences in mineral soil C dynamics among the three overstorey types. Mixedwood stands had higher mineral soil C in 147-year-old stands, but then declined more sharply, thereafter, compared with the other overstorey types. High mineral soil C storage in the 147-year-old mixedwood stands may relate to the positive contribution of species diversity on fine root productivity and mortality (Jacob et al., 2014; Ma and Chen, 2016), which is the major input of C to the mineral soil layer in boreal forests (Clemmensen et al., 2013). The potential increase in soil organic matter decomposition in the oldest mixedwood stands (>147 years old) may be attributable to the effects of the litter mixture on local decomposers, relative to broadleaf and conifer stands. For instance, chemically divergent litter quality combined with increased surface temperature, due to late-succession canopy break-up, can promote the abundance and activities of decomposers (Gartner and Cardon, 2004), and thus accelerate decomposition in late succession.

Despite that other similar chronosequence studies have observed “U” shaped trends in deadwood abundance over time, with high deadwood abundance immediately following disturbance and again during late succession (e.g., Kashian et al., 2013; Taylor et al., 2014), we observed only marginal age-related changes in total deadwood C for all three overstorey types over the span of our chronosequence. The abundance of deadwood in younger forests is largely dependent on the intensity and severity of fire as well as pre-fire site condition such as stand density (Flannigan et al., 2000; Wang et al., 2003). The
low deadwood C we observed in the 8 years old stands may have resulted from high
intensity and severity fire and/or pre-fire stands were dominated by small-sized, young
trees, thus leading to low post-fire deadwood abundance on our sites (see Fig. S3-3).
Further, we observed relatively stable deadwood C throughout late succession, consistent
with previous studies in our area (Brassard and Chen, 2008). Despite the potential for
high inputs of mortality originated deadwood during the canopy transition stage of stand
development (i.e., the 99-147 year age cohorts), most of this deadwood would decompose
over 60 years (Angers et al., 2010), leading to low deadwood abundance between in the
210 years old stands. Further, the oldest stands were also dominated by faster
decomposing balsam fir and white birch stems, which may also have contributed to low
observed deadwood.

Carbon dynamics during late succession
Overall, within the timeframe of our chronosequence, our results showed a significant
decline in total ecosystem C storage (i.e., negative NEP) as stands transitioned into late
succession. Our findings conflict with the view, based largely on chronosequence studies
that do not consider forest succession, that old forests may slow in their rate of C
accumulation, but still continually accumulate C (Odum, 1969; Luyssaert et al., 2008;
Goulden et al., 2011; Kashian et al., 2013; Gough et al., 2016). Our observed decline in
total ecosystem C storage during late succession was previously reported by Taylor et al.
(2014) in the same study area, but this study only considered a single succession pathway,
with limited sample plots. By expanding the sample size to include different forest
overstorey types to account for multiple succession pathways, our study adds further
support for the hypothesis that as boreal forests age and transition into late succession,
they undergo a decline in total ecosystem C storage. Of course, it is reasonable to point
out that old stands cannot decline in perpetuity, or else they would disappear. Thus, it
remains to be observed how long this decline phase persists and at what point do old
boreal forests achieve a steady C state balance (if ever) since plant diversity is expected to
increase after canopy transition (Roberts and Gilliam, 1995), likely promoting C uptake
and storage in forests (Liang et al., 2016; Zhang et al., 2017).

It is worth noting, that forest companies in our study area preferentially harvest
mature, “productive” stands. Consequently, this may induce bias in our sample of old
stands since it is conceivable that the old, 210 years stands (ca. 3000 ha of our study area,
which is approximately one million ha) represent less desirable, low productivity stands
left unharvested, which could have artificially driven the decline in C storage we
observed in late succession. However, commercial forestry in our study area did not begin
until the mid-1970s and targeted mostly pure jack pine stands less than 120 years old. Our
oldest stands, which were almost 160 years old in the 1970s, were beyond the preferred
age for harvesting in the area at that time and, consequently, were left unharvested. Thus,
the possibility of “cherry-picking” low productivity, 210-year-old stands is low and
confirmed from our personal communications with the long-term District Forester in this
area, Paul Poschmann, who has been working as the area forester since the mid 1970s.

Although our results show an overall decline in total ecosystem C storage, the
mechanisms that drive this decline seemed to differ according to overstorey type. For
instance, a reduction of live biomass C, mainly resulting from increased tree mortality
during canopy transition, led to total ecosystem C decline after year 147 in broadleaf and
conifer stands. However, the decrease of total ecosystem C observed in mixedwood
stands was largely driven by decreases in mineral soil C, suggesting that an increase in
decomposition may be an important driver of total C decline. Nonetheless, the generality
of these hypotheses needs further investigation that explicitly examines C flux (e.g.,
forest production, tree mortality rate, and decomposition) while also considering multiple
succession pathways.

By accounting for multiple succession pathways, our results show a strong
relationship between total ecosystem C storage and stand age following fire. Broadleaf
stands had on average higher total ecosystem C; however, temporal dynamics of total
ecosystem C were similar among all three overstorey types. Moreover, we show that total
ecosystem C storage declines during canopy transition to late-succession forests,
irrespective of successional pathway, conflicting traditional views that old forests
continually accumulate C as they age. However, further studies that include stands older
than 200 years must be conducted to determine how long this decline phase persists and
at what point do old boreal forests achieve a steady C state.
Chapter Four: The relationship between tree growth and species
diversity change with succession and relative size in the boreal forest

Introduction

The past several decades have seen a sharp increase in the number of studies investigating the relationship between species diversity and forest ecosystem functions. Major advances have demonstrated positive correlations between diversity and a wide range of ecosystem processes, such as productivity (Liang et al. 2016). Nonetheless, most diversity-productivity studies have tended to focus on the effect of species diversity on community-level productivity, often overlooking variability in the response of individual species to diversity at the neighbourhood level. Because diversity-productivity relationships are largely realized through the growth response of individual tree species (Barrufol et al. 2013, Chamagne et al. 2017), a closer examination of species-specific growth responses to species diversity is warranted (Fichtner et al. 2017).

The positive effect of species diversity on forest productivity is widely considered to be driven by niche complementarity (i.e., resource partitioning) and interspecific facilitation (Tilman 1999, Hooper et al. 2005, Williams et al. 2017). Therefore, it is not unreasonable to expect that the relationship between species diversity and tree growth may vary as forests undergo succession leading to changes in site conditions and resources over time (Forrester 2014, Mina et al. 2017). For example, during early succession, following stand-replacing disturbance, resources such as light and soil nutrients are plentiful and the role of niche complementarity and interspecific facilitation in governing productivity may be less influential. However, resources become more limited as forests develop and competition intensifies. This could amplify positive diversity effects since species interactions may increase under harsher, resource-limited conditions, as postulated by the stress gradient hypothesis (Bertness and Callaway 1994).
In addition, as tree species composition transitions from fast-growing, early-succession colonizers to slower-growing, shade-tolerant, late succession species (Chen and Popadiouk 2002, Taylor and Chen 2011), overlapping mixtures of early and late succession species may exhibit stronger niche complementarity effects due to higher diversity of contrasting life history traits in the community (Coomes et al. 2009). Nonetheless, despite efforts to investigate the relationship between species diversity and tree growth (Cavard et al. 2010, Chamagne et al. 2017, Fichtner et al. 2017), few studies have attempted to examine how this relationship may vary as forests undergo succession. The relationship between species diversity and tree growth may also be influenced by individual tree size as tree size strongly affects its ability to compete for site resources (Coomes et al. 2011). For example, competition for light among trees is size-asymmetric, in that larger trees capture disproportionately greater amount of light when interacting with smaller ones. As a result, positive diversity effects may be stronger for trees of relatively larger size. This has been indirectly supported by a recent study that showed overstorey tree species diversity has a positive effect on canopy trees but negligible or negative effect on understorey trees (Zhang et al. 2016). However, previous studies have also reported conflicting results, observing stronger complementarity effect on smaller rather than larger trees (Madrigal-González et al. 2016), or no size-dependent effect at all (Báez and Homeier 2018). Such differences in results may be because past studies have not included a wide enough range of tree sizes, with most focusing on trees greater than 10 cm in diameter at breast height (DBH). Moreover, directly using DBH to represent tree size neglects the important role of stand age on tree size, as trees of the same size may constitute different forest strata (e.g., understorey vs. overstorey) depending on stand development stage. For example, a trembling aspen tree of 20 cm DBH can be the largest
tree in the forest canopy during early to mid-succession, but may only represent a mid-
size tree as the forest transitions into late-succession.

It has also been hypothesized that the effect of species diversity on tree growth
may differ according to the type of disturbance from which the forest ecosystem
originates (Forrester and Bauhus 2016), possibly due to the different effects of
disturbances on soil nutrient availability (Thiffault et al. 2008, Hume 2016). For example,
historically, fire is considered the predominant form of stand-replacing disturbance in the
boreal forest, but harvesting increasingly affects millions of hectares of boreal forests
each year (Pan et al. 2013). Although leaching of soil nutrients occurs following both fire
and harvesting, fire-driven volatilization of nutrients results in generally higher net losses
of nutrients than harvesting immediately after disturbance (Thiffault et al. 2007). This
divergence is likely to dissipate as forests age (Hume 2016), but it is possible that the
effect of diversity on tree growth may be stronger in more resource-limited, young post-
fire stands than that of post-harvest stands.

In this study, we used a chronosequence sampling design to examine the
relationship between species diversity and tree growth rate of five major tree species in
the boreal forests of central Canada. Our chronosequence covered a wide range of forest
stand ages, following stand-replacing fire (from 8 to 210 years old) and harvesting (from
8 to 34 years old). We hypothesized that (1) the effect of species diversity on tree growth
rate would change as forest stands undergo succession and become stronger when
competition for resources intensifies or as functional diversity increases when early and
late succession species co-dominate; (2) the effect of species diversity would increase
with relative tree size since trees have size-asymmetric competitive ability for resources;
(3) the effect of species diversity on tree growth rate would be stronger in more resource-
limited, post-fire stands than that of post-harvest stands.
**Materials and Methods**

**Study area**

Our study was conducted in the boreal forest region, approximately 150 km north of Thunder Bay, Ontario, Canada, between 49°44′ to 49°65′ N and 89°16′ to 90°13′ W. This area is characterized by warm summers and cold, snowy winters. Mean annual temperature is 1.9 ºC and mean annual precipitation is 824.8 mm as measured by the closest meteorological station in Cameron Falls, Ontario, Canada (Environment Canada 2017). Soils in our study area were largely deposited by the Wisconsinan glaciation, which ended approximately 9,500 years ago in this region. Stand-replacing wildfire is the most common natural disturbance in the study area, with an average fire-return interval of approximately 100 years during the past century, resulting in a mosaic of stand ages across the landscape (Senici et al. 2010). Commercial harvesting began in our study area in the 1970s. Dominant tree species in our study area, in order from least to most shade tolerant, include jack pine, trembling aspen, white birch, spruce, and balsam fir.

**Sampling design**

We employed the chronosequence sampling method to study how species diversity effects vary as forest stands develop through succession and following different disturbance origins. Although the use of the chronosequence method has been criticized due to its assumption that sample stands along the temporal sequence have followed the same developmental history (Johnson and Miyashita 2008), given careful site selection, replication, and demonstration of developmental links, the chronosequence method is appropriate for studying successional dynamics over decadal to centennial time scales (Walker et al. 2010).

Based on local fire history and the availability of different aged stands in our study area, we were able to sample six different stand age classes (i.e., time since last
stand-replacing disturbance), including 8, 16, 34, 99, 147, and 210 years since fire,
representing the stand initiation, late stand initiation, stem exclusion, early canopy
transition, late canopy transition, and gap dynamic stages of boreal forest development,
respectively (Chen and Popadiouk, 2002). Harvest-origin stands were only sampled for
the stand initiation and stem exclusion stages (i.e., 8, 16, 34 years since harvesting)
because commercial harvesting did not begin in our study area until the early 1970s. To
ensure we included a wide variety of forest overstorey types and to account for the fact
that similar boreal forest sites may undergo multiple successional pathways, we carefully
selected three different overstorey types (i.e., broadleaf, conifer and mixedwood) at each
age class. More specifically, for stands in the stand initiation and stem exclusion stages of
development (i.e., years 8, 16, and 34), we selected broadleaf stands that were dominated
by trembling aspen, conifer stands that were dominated by jack pine, and mixedwood
stands that contained a mixture of jack pine and trembling aspen. For stands in the canopy
transition (i.e., years 99, 147) and gap dynamics stages (i.e., year 210), we selected
broadleaf stands dominated by self-replacing trembling aspen and white birch, conifer
stands that were transitioning from jack pine to spruce and balsam fir dominance, and
mixedwood stands that were co-dominated by trembling aspen, white birch, spruce and
balsam fir.

We selected post-disturbance stands that had not been managed (e.g., planted,
sprayed or thinned), including three replicates for each age class and overstorey type
combination; however, one replicate mixedwood stand at age 147 years was accidentally
damaged by harvesting activities between consecutive years of sampling, resulting in a
total of 80 stands measured overall. Sample stands were positioned several kilometers
away from each other and selected from different road accesses to minimize the impact of
spatial autocorrelation. In order to minimize site variability, all selected stands were
located on mesic sites with flat mid-slope positions, with no slope exceeding 5%. All stands are underlain by moderately deep (≥50 cm) glacial tills, belonging to the Brunisolic soil order, according to the Canadian system of soil classification (Soil Classification Working Group 1998). To ensure that each sample stand met the selection criteria, soil pits were dug in each candidate stand to verify whether the site was mesic, following the procedures described in Taylor et al. (2000). Soil attribute data for all sites used in this study have been previously reported by Hume et al. (2016).

Stand age for sample stands less than 90 years old was determined from detailed fire and harvesting records (Hart and Chen 2008, Senici et al. 2010). For stands greater than 90 years old, tree ages were used to estimate minimum stand age following the procedures described in Senici et al. (2010). Of all sampled stands, we selected either jack pine or trembling aspen trees to determine minimum stand age. In each stand, three canopy stems were sampled by extracting a core or stem disk at breast height (1.3 m above root collar). The cores and disks were transported to our laboratory, where the cores were mounted on constructed core strips and sanded to make rings visible. Stem disks were cut transversely, then mounted on constructed core strips and sanded to make rings visible. Rings were counted using a handheld magnifier or a microscope until the same count was obtained three successive times. Based on a locally derived age correction model developed by Vasiliauskas and Chen (2002), 7 years were added to ring counts to determine minimum stand age.
Table 4-1 Characteristics of the 80 sample stands in the northwest boreal forests of Ontario, Canada.

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<tr>
<th>Origin</th>
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<th>Overstoreya</th>
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<th>Basal area (m² ha⁻¹)</th>
<th>Stand composition (%)b</th>
<th>Spruce spp.</th>
<th>Balsam fir</th>
<th>Others</th>
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Notes: Each age-overstorey combination has three replications, values are means with 1 SE in parentheses.

a Overstorey types: B = broadleaf, C = conifer, M = mixedwoods.

b The ‘Others’ category includes *Salix* spp., *Acer spicatum*, *Alnus viridis*, *Sorbus decora*, *Corylus cornuta*, *Prunus pensylvanica*, and *Larix laricina*.
Data collection

Within each of the 80 selected stands, we randomly located and established a 0.04 ha (11.28 m radius) fixed area circular plot, approximately 50 or more meters from the forest edge, to represent the stand. We recorded species identity and diameter at breast height (DBH, 1.3 m above root collar) for all trees larger than 9 cm DBH within each plot. Overstory types were assigned based on the relative basal area of broadleaf and conifer tree species in a plot. Broadleaf and conifer stands were defined as having > 80% broadleaf or conifer tree species composition by stem density or basal area while all other stands were classified as mixedwood stands (Table 1).

To determine tree growth, we used stratified random sampling to select trees of varying diameter at breast height, from tree stems with a minimum DBH of 2 cm to the maximum DBH that could be found for each species in a given sample plot. Because species composition changes with succession, we sampled jack pine from the 8-, 16-, 34-, 99-, and 147-year-old age classes in the fire-origin stands and all three age classes in the harvest-origin stands; trembling aspen from all age classes in fire- and harvest-origin stands; white birch, spruce, and balsam fir from the 99-, 147-, and 210-year-old age classes in the fire-origin stands. For each species within each sample plot, tree size was grouped into 4 cm DBH intervals and we randomly sampled up to three trees from each DBH group if available. Trees with crooked stems, substantial heart-rot, or other forms of stem damage, such as stem abrasion, fungal infections, or major branch losses were not sampled. In total, 255, 395, 136, 167, and 135 trees for jack pine, trembling aspen, white birch, spruce, and balsam fir, respectively, were sampled across all overstory type, stand ages, and stand origins.

For each sample tree, increment cores or disk samples were collected to estimate annual tree radial growth increment. For trees <10 cm in DBH, we cut a stem disk at
DBH, while for trees ≥10 cm in DBH, we used an increment core borer with a 5.15 mm
diameter bit to extract stem core samples. All samples were sealed in plastic bags (for
disks) or straws (for cores) and transported from the field to the laboratory. In the
laboratory, all stem disk and core samples were sanded and annual increments were
measured using a WinDENDRO measuring system.

Tree annual growth rate was calculated as the average annual basal area increment
of the last 5 years (2009 – 2014). Because tree-ring measurements were conducted for
growth within tree bark, while tree basal area growth is calculated for DBH outside the
bark, similar to Chen and Klinka (2003), we first developed species-specific relationships
between DBH with and without bark. We then used these relationships to calculate DBH
with bark from measured stemwood DBH in 2009 and 2014 for each sample.

We calculated Shannon’s diversity index (H’) as a measure of species diversity for
each sample plot, which accounts for both species richness and evenness:

\[ H' = \sum_{i=1}^{S} P_i \ln(P_i) \]  

where S is the total species richness, P_i is the relative abundance of species i based on
basal area of the ith species.

To examine whether the effect of species diversity on tree growth rate varies with
relative tree size in a stand, we also calculated the relative sizes for all individual trees by
using basal area (BA) of each sample tree divided by the mean BA of the sample plot. We
used relative tree size instead of the absolute tree size (i.e., DBH) because our study
covered a wide range of stand ages and relative size better reflects the competitive
position of individual trees when encountering other individuals in a stand (Luo and
Chen, 2015).
Data analysis

To address our first and second hypotheses, we used linear mixed-effect models to test the effects of the Shannon index, stand age, relative size, and their two-way interactions on tree growth rate for each of the five tree species in our study:

\[ Y = \beta_0 + \beta_1 A + \beta_2 D + \beta_3 RS + \beta_4 A \times D + \beta_5 A \times RS + \beta_6 D \times RS + \beta_7 A \times D \times RS + \beta_8 BA + \pi_{plot} + \varepsilon \]  

(2)

where \( Y \) is annual basal area growth rate (cm\(^2\) year\(^{-1}\)) for a given tree species, \( A \) represents stand age (years) following fire (a categorical variable), \( D \) is the Shannon index (a continuous variable), \( RS \) is relative tree size (a continuous variable), \( BA \) is plot-level basal area (m\(^2\) ha\(^{-1}\); a continuous variable) which accounts for plot stand density, \( \pi_{plot} \) is the random effect of the sample plots which accounts for autocorrelation among trees sampled within each plot, and \( \varepsilon \) is the sampling error. We conducted the analysis using maximum likelihood estimation with the “lme4” package (Bates et al., 2017). All continuous variables were scaled prior to analysis to ease interpretation by using the “scale” function from the “data.table” R package (Dowle et al., 2017).

To assess our third hypothesis, we included stand origin (\( O \)) and its interactions with the other factors in the Eq. 2, using the following full, linear mixed effect model:

\[ Y = \beta_0 + \beta_1 D + \beta_2 RS + \beta_3 A + \beta_4 O + \beta_5 BA + \beta_6 D \times RS + \beta_7 D \times A + \beta_8 RS \times A + \beta_9 D \times O + \beta_{10} RS \times O + \beta_{11} A \times O + \beta_{12} D \times RS \times A + \beta_{13} D \times RS \times O + \beta_{14} D \times A \times O + \beta_{15} RS \times A \times O + \beta_{16} D \times RS \times A \times O + \pi_{plot} + \varepsilon \]  

(3)

Shapiro-Wilk’s tests on model residuals showed some statistical models did not meet the assumption of normality at \( \alpha = 0.05 \). We used bootstrapped regression parameter estimates by 1000 iterations from package “boot” (Canty and Ripley, 2017) and compared the bootstrapped estimates with those of the linear models. Both methods
yielded quantitively similar trends, so we reported the linear model results for simplicity.

All data analyses were performed in R 3.4.1 (R Development Core Team, 2017).
### Results

**Tree growth-diversity relationships in relation to stand age**

The relationship between tree growth and species diversity in postfire stands differed with tree species and among stand ages (Table 4-2, Fig.4-1). During the stand initiation stage of development, from age 8 to 16 years, there was no significant relationship between tree growth rate and species diversity for any of the species sampled (Fig. 4-1a, and 4-1b).

When stands entered the stem exclusion stage (i.e., 34 years old), tree growth rate of trembling aspen marginally decreased with species diversity, while tree growth of jack pine marginally increased (Fig. 4-1c). As stands aged to early canopy transition stage (i.e., 99 years old), tree growth rate decreased significantly and marginally with species diversity for trembling aspen and white birch, whereas the growth rate of spruce and jack pine marginally increased. There was no relationship between tree growth rate of balsam fir and species diversity in the 99 years old stands (Fig. 4-1d). During the late canopy transition stage (i.e., 147 years old), tree growth rate increased significantly and marginally with species diversity for trembling aspen and spruce, respectively, while no growth-diversity relationship for jack pine, white birch, and balsam fir was observed (Fig. 4-1e). In the 210 years old stands, there was no significant relationship between tree growth rate and species diversity except a marginally positive relationship for spruce (Fig. 4-1f).

**Tree growth-diversity relationships in relation to relative size**

The relationship between tree growth and diversity also changed with relative tree size, depending on species and stand age (Table 4-2, Fig. 4-2). The tree growth-diversity relationship was significantly positive for large sized trees of jack pine at age 99 and spruce at age 147 (Fig. 4-2a and 4-2d). The negative relationship between tree growth and diversity for trembling aspen significantly increased with relative tree size at age 99,
whereas at age 147 and 210, tree growth of relatively small sized trembling aspen positively related with species diversity (Fig. 4-2b). The relationship between tree growth and species diversity did not change with relative size for white birch and balsam fir (Fig. 4-2c and 4-2e).

Tree growth-diversity relationships in relation to disturbance origin

Tree growth rate of jack pine and trembling aspen did not significantly differ according to stand origin during early succession (age 8 to 34 years; Table 4-3). There was no overall effect of species diversity on tree growth rate in either species in post-fire and post-harvest stands during early successional stages. Further, differences in relative sizes and stand ages did not produce significant diversity effects on tree growth (Table 4-3).

Table 4-2 The effects (p values) of stand age (A), diversity (D), relative size (RS) and their two-way interactions on annual tree growth rate for five major tree species following fire and harvesting disturbance, respectively. Statistically significant terms (p < 0.05) are in bold.

<table>
<thead>
<tr>
<th>Tree species</th>
<th>A</th>
<th>D</th>
<th>RS</th>
<th>BA</th>
<th>A×D</th>
<th>A×RS</th>
<th>D×RS</th>
<th>A×D×RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jack pine</td>
<td>0.007</td>
<td>0.638</td>
<td>&lt;0.001</td>
<td>0.147</td>
<td>0.871</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>0.011</td>
</tr>
<tr>
<td>Trembling aspen</td>
<td>0.144</td>
<td>0.787</td>
<td>&lt;0.001</td>
<td>0.111</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.049</td>
<td>0.002</td>
</tr>
<tr>
<td>White birch</td>
<td>0.816</td>
<td>0.204</td>
<td>&lt;0.001</td>
<td>0.675</td>
<td>0.184</td>
<td>0.221</td>
<td>0.493</td>
<td>0.268</td>
</tr>
<tr>
<td>Spruce</td>
<td>0.004</td>
<td>0.045</td>
<td>&lt;0.001</td>
<td>0.393</td>
<td>0.893</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.050</td>
</tr>
<tr>
<td>Balsam fir</td>
<td>0.339</td>
<td>0.857</td>
<td>&lt;0.001</td>
<td>0.833</td>
<td>0.572</td>
<td>0.908</td>
<td>0.094</td>
<td>0.862</td>
</tr>
</tbody>
</table>

Linear mixed effect model fit tests used Satterthwaite approximations of degrees of freedom.
Table 4-3. The effects ($p$ values) of stand age (A), diversity (D), relative size (RS),
disturbance origin (O), and their interactions on annual tree growth rate of two dominated
tree species following fire and harvesting disturbance, respectively. Statistically
significant terms ($p < 0.05$) are in bold.

<table>
<thead>
<tr>
<th></th>
<th>Jack pine</th>
<th>Trembling aspen</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>0.437</td>
<td>0.495</td>
</tr>
<tr>
<td>RS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A</td>
<td><strong>0.002</strong></td>
<td><strong>0.061</strong></td>
</tr>
<tr>
<td>O</td>
<td>0.607</td>
<td>0.722</td>
</tr>
<tr>
<td>BA</td>
<td>0.469</td>
<td>0.347</td>
</tr>
<tr>
<td>D \times RS</td>
<td>0.955</td>
<td>0.768</td>
</tr>
<tr>
<td>D \times A</td>
<td>0.906</td>
<td>0.989</td>
</tr>
<tr>
<td>RS \times A</td>
<td><strong>0.025</strong></td>
<td>0.376</td>
</tr>
<tr>
<td>D \times O</td>
<td>0.235</td>
<td>0.675</td>
</tr>
<tr>
<td>RS \times O</td>
<td>0.715</td>
<td>0.670</td>
</tr>
<tr>
<td>A \times O</td>
<td>0.064</td>
<td>0.877</td>
</tr>
<tr>
<td>D \times RS \times A</td>
<td>0.703</td>
<td>0.627</td>
</tr>
<tr>
<td>D \times RS \times O</td>
<td>0.109</td>
<td>0.413</td>
</tr>
<tr>
<td>D \times A \times O</td>
<td>0.400</td>
<td>0.818</td>
</tr>
<tr>
<td>RS \times A \times O</td>
<td><strong>0.035</strong></td>
<td>0.240</td>
</tr>
<tr>
<td>D \times RS \times A \times O</td>
<td>0.668</td>
<td>0.847</td>
</tr>
</tbody>
</table>

Linear mixed effect model fit tests used Satterthwaite approximations of degrees of
freedom.
Figure 4-1 Effect of Shannon index on the annual basal area growth rate of five tree species in relation to stand age after accounting for relative size and stand level basal area. Colors indicate species. Lines with shading are means and 95% bootstrapped confidence intervals of the slope fitted by linear mixed-effect models.
Figure 4-2 Effect of Shannon index on the annual basal area growth rate of five tree species in relation to relative size and stand age. Colors indicate relative size. Values (means and 95% bootstrapped confidence intervals) represent the diversity effect (diversity slopes) for given age class and relative size.
Discussion

Successional change and the tree growth-diversity relationship

Using a long-term, replicated chronosequence approach, our results showed that the relationship between tree growth and species diversity varied throughout forest succession. During stand initiation stages (8 and 16 years old), we found no strong evidence for a relationship between tree growth rate and species diversity, which is consistent with our hypothesis that weak species interactions during early succession, driven by high resources availability following disturbance, would lessen the effect of niche complementarity and facilitation on tree growth. However, when stands entered the more intense competition, stem exclusion stage (34 years old), there were marginally positive and negative relationships between tree growth and species diversity for jack pine and trembling aspen, respectively, partially supporting our hypothesis that the effect of species diversity becomes stronger when competition for resources intensifies. This marginally, positive diversity effect may have resulted from improved soil conditions for jack pine by inputs from nutrient-rich broadleaf litter (Cote et al., 2000; Hume et al., 2016); whereas tree growth rate of trembling aspen can be negatively affected by the alteration of soil chemistry over time generated by conifer litter (Prescott et al., 2000; Calder et al., 2011; Cavard et al., 2011), despite less intense competition for light when mixed with conifers.

As stands developed into the canopy transition stage (99 years old) of succession, our results show significant and marginal relationships between tree growth and diversity for trembling aspen, white birch, and spruce, supporting our hypothesis that species diversity effects on tree growth become stronger at key transition stages during forest succession (Zhang et al., 2012). When stands entered the early canopy transition stage (99 years old), we observed a significantly negative relationship between species diversity...
and tree growth rate for trembling aspen. The stronger negative effect in 99 years old stands compared with that of 34 years old stands suggests that soil limitation may be more influential during the early canopy transition stage than stem exclusion stage. This is partially supported by the marginally negative diversity effect on tree growth of broadleaf white birch and marginally positive diversity effect on conifer species jack pine and spruce, in that negative effect of conifers on tree growth of broadleaf species through decrease in fertility became noticeable when soil nutrient is limited (Calder et al., 2011). Whereas, conifers could benefit from improved soil condition generated by nutrient-rich broadleaf litters (Cote et al., 2000). However, we did not find any positive diversity effect on tree growth of balsam fir in 99 years old stands, despite the potential facilitation of soil nutrient availability by mixing with broadleaf species. An absence of positive diversity effect for balsam fir may be caused by its functional traits: trees with conservative strategies, such as slow-growing and shade-tolerant (Reich, 2014), respond less to the change of resources availability compared to fast-growing and shade-intolerant tree species (Báez and Homeier, 2018).

During the late canopy transition stage (147 years old), the relationship between tree growth and diversity for trembling aspen changed from negative to positive. This change, corresponding with soil nutrients increase from age 99 to 147 years old (Hume et al., 2016), suggest that the limitation of soil nutrients may be less important for tree growth at this stage and tree growth of trembling aspen may have started to benefit from reduced aboveground competition for light when mixing with conifers (Williams et al., 2017). This could also explain the negligible diversity effect on the tree growth rate of white birch, where the negative diversity effect on tree growth of white birch may be limited during this stage and can be offset by benefiting from less competition for light.
At the late-successional, gap dynamic stage (210 years old), there was marginal or no significant relationship between tree growth and diversity for any species. Negligible tree growth-diversity relationships at this stage may be attributable to weak facilitation and competition among species, largely because light and soil nutrients become less limiting at the gap dynamics stage compared with earlier stages (Hume et al., 2016; Kumar et al., 2017). Moreover, trees in the late-successional stage are mostly large in size and old in age, which may also lead to less obvious diversity effect on tree growth since large trees allocate more production to self-maintenance than to radial growth (Mencuccini et al., 2005).

Impact of relative size on tree growth-diversity relationship

Our data yielded mixed results for the influence of relative tree size on the relationship between the tree growth and species diversity. We found that diversity effects on tree growth increased with relative size for jack pine at age 99 and spruce at age 147, which agrees with our hypothesis, suggesting that the diversity effect is shaped by the size-asymmetric competition of individual trees. Moreover, we found negative diversity effect on trembling aspen increased with relative size in 99 years old stands, while at age 147 and 210, tree growth of relatively small-sized aspen positively correlated with species diversity. This negative diversity effect for all sized trees of trembling aspen at age 99 further supports our speculation that soil limitation plays a key role in driving the effect of diversity on tree growth during the early canopy transition stage. While as stands entered the late canopy transition stage, correspondingly, soil fertility also increased from age 99 to age 147 (Hume et al., 2016), soil nutrient limitation may be less influential on relative small-sized trees compared with large trees since smaller trees require less soil nutrient to support growth. Therefore, relatively small trees of trembling aspen started to benefit from increased light availability when mixing with conifer trees. Moreover, small
trees grow faster than relatively larger trees under improved conditions (Wright et al., 2011; Alvarez-Clare et al., 2013). As a result, complementarity effects on the growth rate of small trees can be greater than that of large trees, despite size-asymmetric competition leading to more resources allocated to large trees (Weiner, 1990). Our results also showed that the influence of species diversity on tree growth of white birch and balsam fir did not change with relative size, which may suggest the growth of these species is less sensitive to changes in resources availability.

Tree growth-diversity relationship following fire vs. harvesting

Contrary to our hypothesis, we found no significant difference of species diversity effects on tree growth rate between our two stand origins during early-successional stages for any of the dominant tree species, despite the different effect of fire on soil nutrients compared with harvesting (Thiffault et al., 2007; Thiffault et al., 2008; Hume, 2016). This may be attributable to weak species interactions during the early stand initiation stage, where the limitation of soil nutrients on tree growth is less important. Our results suggest that disturbance origin had limited effect on the relationship between tree growth and species diversity and provide further evidence that common forest management practices (i.e., harvesting) and fire may have a similar impact on ecosystem functions in young boreal forests (Seedre et al., 2014). However, it is unclear whether the similar growth responses to species diversity in post-fire and post-harvest stands can be extrapolated to older ages. Future studies should examine whether differences in the tree growth rate-species diversity effect between stand origins become more apparent at older ages.

In summary, by studying the relationships between species diversity and tree growth rate of five species, we find that the relationship is largely dependent on tree species, forest successional stage, and relative tree size. There was no relationship between diversity and tree growth during the early successional stages (from 8 to 34 years
old), whereas both positive and negative diversity effects were observed at the canopy transition stage. Species diversity was positively related to tree growth rate of spruce but negatively related to tree growth rate of trembling aspen and white birch during the early canopy transition stage (99 years old). While in the late canopy transition stage (147 years old), tree growth was positively related to species diversity for both trembling aspen and spruce. At the late successional stage (210 years old), there was a negligible relationship between tree growth rate and species diversity for any species. Moreover, species diversity effects on tree growth rates increased with relative size for trembling aspen and spruce according to stand age but there was no association between diversity effects and relative size for white birch. The relationship between tree growth and species diversity did not differ between post-fire and post-harvest stands during early successional stages. Our findings emphasize the importance of considering species-specific, size-dependent growth related to forest succession when examining relationships between biodiversity and forest ecosystem functions.
Chapter Five: General conclusion

By using chronosequence design that covered a wide range of stands ages, undergoing multiple successional pathways following stand-replacing fire and harvesting, in the boreal forest of central Canada, I quantified the variation of carbon concentration for six major tree species of the boreal forest in Chapter two, I then examined the long-term C storage dynamics following fire in Chapter three based on the species-specific C concentration data from Chapter two. Finally, I investigated the relationship between tree growth and species diversity in relation to succession, relative tree size, and disturbance origin for five species in Chapter four. A summary of key findings of this dissertation are as follows:

1. Bark had significantly higher total C and volatile C concentrations than stemwood and that both total C and volatile C concentration significantly varied among tree species. The average total C concentrations were 56.2% in the bark and 50.5% in the stemwood, and the average volatile C concentration were 5.8% and 3.0% for bark and stemwood, respectively. Furthermore, total C and volatile C concentration in stemwood and bark of almost all shade-intolerant species increased with tree size, whereas those of shade-tolerant species showed negative or neutral size-associated change. Our results show that volatile C concentration is a key driver of variation in total C concentration.

2. There was a strong relationship between total ecosystem C storage and stand age following fire. Broadleaf stands had on average higher total ecosystem C; however, the inferred temporal dynamics of total ecosystem C were similar among all three overstorey types. Importantly, we found that total ecosystem C storage declined from canopy transition to late succession stages, irrespective of
succession pathway, contradicting views that old forests continually accumulate C as they age.

3. During long-term succession following fire, significant relationships between tree growth and species diversity at the canopy transition stage for trembling aspen, white birch, and spruce. Species diversity positively correlated with the growth rate of spruce but negatively affected the growth rate of trembling aspen and white birch during early the canopy transition stage (99 years old). However, when stands entered the late canopy transition stage (147 years old), species diversity promoted tree growth for both trembling aspen and spruce. Diversity had a positive effect on the growth of larger trees for jack pine at age 99, and spruce at age 147, but, had negative effect with increased size of trembling aspen at age 99, 147, and 210. Furthermore, there was no difference in the tree growth-diversity relationships between post-fire and post-harvest stands during early successional stages.

Overall, the findings of this dissertation improve our understanding of long-term C storage dynamics following wildfire by providing accurate C concentration data and including a diverse range of overstorey composition to account for multiple succession pathways. In Chapter two, the results highlight the importance of considering the species-specific C concentration when estimating live tree C storage and emphasize that variation of C concentration related to tree size and woody tissue (e.g., bark vs. stemwood) need to be accounted for in national, biome, or global C models. In Chapter three, the results indicate that total ecosystem C storage declines during canopy transition stage irrespective of successional pathway and highlight the importance of including stands older than 200 years to capture the canopy transition stage to determine the long-term C storage dynamics in boreal forests. Moreover,
results of Chapter four suggest that knowledge of species-specific, size-dependent tree
growth response to species diversity related to forest succession is critical to better
understand the relationships between biodiversity and forest ecosystem functions.
References

Alemdag IS. 1984. *Wood density variation of 28 tree species from Ontario*. Chalk River, ON: Petawawa National Forestry Institute, Natural Resources Canada


Brant AN. 2014. Foliar nutrient resorption and litterfall production with stand age, overstory composition, and disturbance origin in boreal forests. Lakehead University.


Hume AM. 2016. Temporal trends in the effects of wildfire and harvesting on soil nutrients and tree growth in northern forests of varying overstorey types. Lakehead University.


Martin AR, Thomas SC, Zhao Y. 2013. Size-dependent changes in wood chemical traits: a comparison of neotropical saplings and large trees. Aob Plants 5.


Fig. S2-1 Aboveground carbon (C) stock estimates at the tree level for six boreal tree species. Open and closed circles represent C stocks calculated by our C concentration data and 50%, respectively. Regression relating DBH to total tree C stock was constructed and used to estimate species-level tree C stocks. Short dashed lines and solid lines represent the relationship between DBH and aboveground C stocks calculated by our C concentration data and the standard assumed 50%, respectively. We estimated tree biomass based on species-specific allometric equations developed in Canada (Lambert et al., 2005). Carbon concentration of stemwood was also used for tree branches (Martin et al., 2015). Carbon concentration of foliage was measured in 2013 by a previous study in the same area (Brant, 2014). Equation related aboveground whole-tree C stock with DBH was used as

\[ y = \beta_1 D^\beta_2 + e \]  

Eq. (S1)
where $y$ is the aboveground C stock of a living tree (kilograms), $D$ is the DBH (centimeters), $\beta_1$, $\beta_2$ are estimated coefficient; $e$ is the error term (showed in Table S2-1).
Table S2-1. Model parameter estimates and their standard error (S.E.) for the DBH-based set of equations per species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Parameter</th>
<th>Estimate</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jack pine</td>
<td>$\beta_1$</td>
<td>0.0572</td>
<td>0.0027</td>
</tr>
<tr>
<td></td>
<td>$\beta_2$</td>
<td>2.3799</td>
<td>0.0135</td>
</tr>
<tr>
<td>Trembling aspen</td>
<td>$\beta_1$</td>
<td>0.0452</td>
<td>0.0029</td>
</tr>
<tr>
<td></td>
<td>$\beta_2$</td>
<td>2.4595</td>
<td>0.0172</td>
</tr>
<tr>
<td>White birch</td>
<td>$\beta_1$</td>
<td>0.0416</td>
<td>0.0039</td>
</tr>
<tr>
<td></td>
<td>$\beta_2$</td>
<td>2.5294</td>
<td>0.0271</td>
</tr>
<tr>
<td>Black spruce</td>
<td>$\beta_1$</td>
<td>0.0472</td>
<td>0.0019</td>
</tr>
<tr>
<td></td>
<td>$\beta_2$</td>
<td>2.4113</td>
<td>0.0110</td>
</tr>
<tr>
<td>White spruce</td>
<td>$\beta_1$</td>
<td>0.0521</td>
<td>0.0014</td>
</tr>
<tr>
<td></td>
<td>$\beta_2$</td>
<td>2.3520</td>
<td>0.0084</td>
</tr>
<tr>
<td>Balsam fir</td>
<td>$\beta_1$</td>
<td>0.0511</td>
<td>0.0018</td>
</tr>
<tr>
<td></td>
<td>$\beta_2$</td>
<td>2.3367</td>
<td>0.0104</td>
</tr>
</tbody>
</table>
Fig. S3-1 Linear regressions used to predict fine root biomass in broadleaf stands (a) and conifer stands (b) by fine root biomass in mixedwood stands, respectively.
Fig. S3-2 Linear regressions used to predict fine root necromass in broadleaf stands (a) and conifer stands (b) by fine root necromass in mixedwood stands, respectively.

(a) Broadleaf fine root necromass (Mg ha$^{-1}$) vs. Mixedwood fine root necromass (Mg ha$^{-1}$)

- $y = 0.08x + 0.89$
- $R^2 = 0.01$

(b) Conifer fine root necromass (Mg ha$^{-1}$) vs. Mixedwood fine root necromass (Mg ha$^{-1}$)

- $y = 0.32x + 0.5$
- $R^2 = 0.16$
Fig. S3-3 Distribution of mid-point diameters of down woody debris (DWD) in 8 years old stands.
Table S3-1 Results of stand age (A<sub>i</sub>) and overstorey type (T<sub>j</sub>) on total ecosystem C and individual C pools during late succession following fire (i.e., 147 and 210 years old stands). The columns give the degrees of freedom (d.f.), F value, and P value. Statistically significant terms (p < 0.05) are in bold.

<table>
<thead>
<tr>
<th>C Pools</th>
<th>A&lt;sub&gt;i&lt;/sub&gt; (d.f. = 1)</th>
<th></th>
<th>T&lt;sub&gt;j&lt;/sub&gt; (d.f. = 2)</th>
<th></th>
<th>A × T&lt;sub&gt;ij&lt;/sub&gt; (d.f. = 2)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Total ecosystem C</td>
<td>10.7</td>
<td>0.007</td>
<td>1.8</td>
<td>0.219</td>
<td>1.1</td>
<td>0.364</td>
</tr>
<tr>
<td>Total live biomass</td>
<td>2.8</td>
<td>0.125</td>
<td>2.2</td>
<td>0.153</td>
<td>2.8</td>
<td>0.103</td>
</tr>
<tr>
<td>Total deadwood</td>
<td>2.2</td>
<td>0.167</td>
<td>2.1</td>
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<td>1.2</td>
<td>0.332</td>
</tr>
<tr>
<td>Forest floor</td>
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<td>0.4</td>
<td>0.684</td>
<td>3.8</td>
<td>0.057</td>
</tr>
<tr>
<td>Mineral soil</td>
<td>8.4</td>
<td>0.014</td>
<td>4.0</td>
<td>0.049</td>
<td>5.3</td>
<td>0.025</td>
</tr>
<tr>
<td>Plot ID</td>
<td>Age</td>
<td>Overstorey type</td>
<td>Latitude</td>
<td>Longitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-----</td>
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<td>---------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>Broadleaf</td>
<td>49.531879</td>
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<td></td>
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<tr>
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<td>8</td>
<td>Broadleaf</td>
<td>49.572458</td>
<td>-90.135925</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>Broadleaf</td>
<td>49.532888</td>
<td>-90.075315</td>
<td></td>
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<tr>
<td>4</td>
<td>8</td>
<td>Conifer</td>
<td>49.547507</td>
<td>-90.132973</td>
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</tr>
<tr>
<td>5</td>
<td>8</td>
<td>Conifer</td>
<td>49.546296</td>
<td>-90.108515</td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>Conifer</td>
<td>49.553823</td>
<td>-90.129597</td>
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<td></td>
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<tr>
<td>7</td>
<td>8</td>
<td>Mixedwood</td>
<td>49.545906</td>
<td>-90.106947</td>
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<td></td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>Mixedwood</td>
<td>49.544349</td>
<td>-90.105379</td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>Mixedwood</td>
<td>49.553199</td>
<td>-90.127932</td>
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<td></td>
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<tr>
<td>10</td>
<td>34</td>
<td>Broadleaf</td>
<td>49.658629</td>
<td>-89.899643</td>
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<td></td>
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<tr>
<td>11</td>
<td>34</td>
<td>Broadleaf</td>
<td>49.658048</td>
<td>-89.900028</td>
<td></td>
<td></td>
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<tr>
<td>12</td>
<td>34</td>
<td>Broadleaf</td>
<td>49.65796</td>
<td>-89.89953</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>34</td>
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Fig. S4-1 Effect of richness on the annual basal area growth rate of five tree species in relation to stand age after accounting for relative size and stand level basal area. Colors indicate species. Lines with shading are means and 95% bootstrapped confidence intervals of the slope fitted by linear mixed-effect models.
Fig. S4-2 Effect of richness on the annual basal area growth rate of five tree species in relation to relative size and stand age. Colors indicate relative size. Values (means and 95% bootstrapped confidence intervals) represent the diversity effect (diversity slopes) for given age class and relative size.
Fig. S4-3 The relationship between relative size and the annual basal area growth rate of five tree species after accounting for Shannon index and stand-level basal area. Colors indicate species. Lines with shading are means and 95% bootstrapped confidence intervals of the slope fitted by linear mixed-effect models.