

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

By

Bilei Gao

A Thesis Submitted in
Partial Fulfillment of the Requirements for
the Doctor of Philosophy in Forest Sciences

Faculty of Natural Resources Management

Lakehead University

April 2018

1 **Abstract**

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

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

23 Based on the results of the first study, I then quantified forest C storage and examined
24 long-term C storage dynamics by using a replicated chronosequence design that covered a
25 wide range of stand ages (from 8 to 210 years old), following stand-replacing fire,

26 undergoing multiple succession pathways in the boreal forest of central Canada. I found that
27 total ecosystem C storage increased from 8 to 147 years old, but declined from 147 to 210
28 years old, coinciding with canopy transition from pioneer species to late succession species,
29 irrespective of succession pathway. Our result rejected the view that old forests continually
30 accumulate C as they age. These findings emphasize that inclusion of stands older than the
31 longevity of the pioneer cohort is critical to a better understanding of the contribution of old
32 forests to the global C cycle.

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

49 **Table of Contents**

50 Abstract I

51 Table of Contents III

52 List of Tables: VI

53 List of Figures VIII

54 Acknowledgments X

55 Notes to readers XI

56 Chapter One : General introduction 1

57 Chapter Two : Variation in total and volatile carbon concentration among the major tree
58 species of the boreal forest 3

59 Introduction 3

60 Materials and methods 6

61 Study area 6

62 Sampling design 7

63 Chemical analysis 8

64 Data analysis 9

65 Results 10

66 Discussion 16

67 Chapter Three : Carbon storage declines in old boreal forests irrespective of succession
68 pathway 21

69 Introduction 21

70 Materials and Methods 24

71 Study area.....24

72 Sampling design.....24

73 Data collection27

74 Aboveground live biomass carbon.....27

75 Belowground live biomass carbon.....29

76 Aboveground deadwood carbon30

77 Belowground deadwood carbon32

78 Forest floor and mineral soil carbon33

79 Data analysis34

80 Results.....36

81 Discussion.....41

82 Carbon dynamics among overstory types41

83 Carbon dynamics during late succession44

84 Chapter Four : The relationship between tree growth and species diversity change with
85 succession and relative size in the boreal forest47

86 Introduction.....47

87 Materials and Methods.....50

88 Study area.....50

89 Sampling design.....50

90 Data collection54

91 Data analysis56

92 Results.....58

93	Tree growth-diversity relationships in relation to stand age.....	58
94	Tree growth-diversity relationships in relation to relative size	58
95	Tree growth-diversity relationships in relation to disturbance origin.....	59
96	Discussion	63
97	Successional change and the tree growth-diversity relationship	63
98	Impact of relative size on tree growth-diversity relationship	65
99	Tree growth-diversity relationship following fire vs. harvesting	66
100	Chapter Five: General conclusion.....	68
101	References.....	71
102	Appendix I. Supplemental information for Chapter Two	85
103	Appendix II. Supplemental information for Chapter Three.....	88
104	Appendix III. Supplemental information for Chapter Four	94
105		
106		

107 **List of Tables:**

108 **Table 2-1** Results from paired *t*-tests comparing differences in total and volatile C
109 concentrations between bark and stemwood tissues for each tree species. The columns
110 give the degrees of freedom (d.f.), *t* values, and *P* values. Significant terms (*P* < 0.05)
111 are in bold. 11

112 **Table 2-2** Means and standard errors (S.E.) of total and volatile C concentrations of six tree
113 species in bark and stemwood tissues, respectively..... 12

114 **Table 2-3** The effects of shade tolerance, species, and DBH on total and volatile C
115 concentrations of each woody tissue type. Tree species was nested within shade
116 tolerance. The columns give the degrees of freedom (d.f.), sum of squares (SS), *F*
117 values, *P* values, and % deviance (in brackets) explained by the explanatory variables.
118 Significant terms (*P* < 0.05) are in bold..... 13

119 **Table 3-1** Characteristics of the 43 sample stands in the boreal forests of Ontario, Canada. .27

120 **Table 3-2** Results of stand age (*A_i*) and overstorey type (*T_j*) on total ecosystem C and
121 individual C pools following fire. The columns give the degrees of freedom (d.f.), *F*
122 value, and *P* value. Statistically significant terms (*p* < 0.05) are in bold.....36

123 **Table 4-1** Characteristics of the 80 sample stands in the northwest boreal forests of Ontario,
124 Canada.....53

125 **Table 4-2** The effects (*p* values) of stand age (*A*), diversity (*D*), relative size (*RS*) and their
126 two-way interactions on annual tree growth rate for five major tree species following
127 fire and harvesting disturbance, respectively. Statistically significant terms (*p* < 0.05)
128 are in bold.59

129 **Table 4-3.** The effects (*p* values) of stand age (*A*), diversity (*D*), relative size (*RS*),
130 disturbance origin (*O*), and their interactions on annual tree growth rate of two

131	dominated tree species following fire and harvesting disturbance, respectively.	
132	Statistically significant terms ($p < 0.05$) are in bold.....	60
133		

134 **List of Figures**

135 **Figure 2-1** Total and volatile C concentrations in relation to wood tissue type and DBH for
136 jack pine, trembling aspen, white birch. Closed and open circles represent C
137 concentrations of bark and stemwood, respectively. Solid lines represent the
138 significant relationship between DBH and total or volatile C concentration of bark (P
139 < 0.05), whereas short dashed lines represent the significant relationship between
140 DBH and total or volatile C concentration of stemwood ($P < 0.05$). Dotted lines
141 represent a marginal relationship between DBH and total or volatile C concentration
142 of bark ($0.05 < P < 0.3$), whereas dash-dot lines represent a marginal relationship
143 between DBH and total or volatile C concentration of stemwood ($0.05 < P < 0.3$). ... 14

144 **Figure 2-2** Total and volatile C concentrations in relation to wood tissue type and DBH for
145 black spruce, white spruce, and balsam fir. Closed and open circles represent C
146 concentration of bark and stemwood, respectively. Solid lines represent the significant
147 relationship between DBH and total or volatile C concentration of bark ($P < 0.05$),
148 whereas short dashed lines represent the significant relationship between DBH and
149 total or volatile C concentration of stemwood ($P < 0.05$). Dotted lines represent a
150 marginal relationship between DBH and total or volatile C concentration of bark (0.05
151 $< P < 0.3$), whereas dash-dot lines represent a marginal relationship between DBH
152 and total or volatile C concentration of stemwood ($0.05 < P < 0.3$). 15

153 **Figure 3-1** Carbon storage in relation to time since stand-replacing fire and overstorey type
154 for a) total ecosystem; and b) components of the four individual pools. Symbols and
155 vertical lines represent sample means and standard errors, respectively, and ribbons
156 represent the 95% confidence interval of sample means. 38

157 **Figure 3-2** Carbon storage in relation to time since stand-replacing fire and overstorey type
158 for a) live biomass; b) deadwood. Symbols and vertical lines represent sample means

159	and standard errors, respectively, and ribbons represent the 95% confidence interval	
160	of sample means.....	39
161	Figure 3-3 Carbon storage in relation to time since stand-replacing fire and overstorey type	
162	for a) forest floor; and b) mineral soil. Symbols and vertical lines represent sample	
163	means and standard errors, respectively, and ribbons represent the 95% confidence	
164	interval of sample means.	40
165	Figure 4-1 Effect of Shannon index on the annual basal area growth rate of five tree species	
166	in relation to stand age after accounting for relative size and stand level basal area.	
167	Colors indicate species. Lines with shading are means and 95% bootstrapped	
168	confidence intervals of the slope fitted by linear mixed-effect models.	61
169	Figure 4-2 Effect of Shannon index on the annual basal area growth rate of five tree species	
170	in relation to relative size and stand age. Colors indicate relative size. Values (means	
171	and 95% bootstrapped confidence intervals) represent the diversity effect (diversity	
172	slopes) for given age class and relative size..	62
173		

174 **Acknowledgments**

175 I would like to give my sincere gratitude to my supervisor Dr. Han Chen for his patient
176 guidance, support, and mentorship through the development of my thesis. I would also like to
177 thank my committee members, Dr. Jian Wang and Dr. Anthony Taylor for their
178 encouragement and insightful comments.

179 I am grateful to Frank Luckai, Hua Liu, Wanwen Yu, Siyao Yang, Allan Chen,
180 Xiaoping Guo, Magali Furlan Nehemy, and all my lab colleagues for their help with field
181 work and lab work. I would also like to extend my thanks to Dr. Yu Zhang and Eric Searle
182 for their constructive comments and help with statistics. Financial support from the Natural
183 Sciences and Engineering Research Council of Canada Strategic Grant Project
184 (STPGP428641) is greatly acknowledged.

185 Finally, I would like to thank my parents Qingchang Gao and Jiafeng Sun, my sister
186 Weijiao Gao and my brother-in-law Mocong Yang for their encouragement and moral
187 support, and special thanks to my beloved wife, Le Wang, for her unconditional love which
188 makes this happen.

189

190 **Notes to readers**

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

194 Chapters:

195 2. Gao B, Taylor AR, Chen HYH, *et al.* 2016. Variation in total and volatile carbon
196 concentration among the major tree species of the boreal forest. *Forest Ecology and*
197 *Management* 375: 191-199.

198 3. Gao B, Taylor AR, Searle EB, *et al.* 2017. Carbon Storage Declines in Old Boreal Forests
199 Irrespective of Succession Pathway. *Ecosystems*. [https://doi.org/10.1007/s10021-017-](https://doi.org/10.1007/s10021-017-0210-4)
200 0210-4.

201 4. Gao B, Taylor AR, Chen HYH. 2018. The relationship between tree growth and species
202 diversity changes with succession and relative tree size in the boreal forest (In
203 preparation)

204

205 **Chapter One: General introduction**

206 The world's boreal forests play an important role in regulating atmospheric carbon dioxide
207 because they account for 49% of global forest vegetation and soil carbon (C) ([Dixon et al.,](#)
208 [1994](#)). Wildfire is the predominant form of natural stand-replacing disturbance in the boreal
209 forest and exerts strong impacts on C sequestration and storage ([Bond-Lamberty et al., 2007](#)).
210 Over the past several decades, fire activity has increased in many regions to levels
211 unprecedented since the last ice age ([Kelly et al., 2013](#)), highlighting the importance of
212 understanding C flux in the boreal forest ([Canadell and Raupach, 2008](#); [Bellassen and](#)
213 [Luyssaert, 2014](#); [Gauthier et al., 2015](#)). In particular, accurately quantifying C concentration
214 of live trees in the boreal forest has important implications for the estimation of C storage and
215 validation of global C accounting models used to predict future C emission scenarios
216 ([McKinley et al., 2011](#); [Metsaranta et al., 2011](#)), as trees generally comprise the large and
217 most dynamic C pool in forest ecosystems ([Pan et al., 2011](#)). However, despite increased
218 accuracy of C concentration estimates for tropical and temperate tree species ([Thomas and](#)
219 [Malczewski, 2007](#); [Martin and Thomas, 2011](#); [Martin et al., 2013](#); [Martin et al., 2015](#)), few
220 studies have examined the variation in C concentration of boreal tree species.

221 Although advances have been made in understanding carbon storage changes as forest
222 stands age ([Taylor et al., 2014](#)), the long-term recovery of forest C storage following stand-
223 replacing fire, remains unclear. Uncertainty surrounding C storage in old forests largely stems
224 from both a lack of repeated measurements in forest stands older than the longevity of the
225 pioneer cohort and a lack of consideration of multiple succession pathways. Forest net
226 ecosystem production (NEP) increases rapidly during early stand development, peaks as
227 stands mature, and then declines as stands age ([Ryan et al., 1997](#)). As a result, forest C
228 storage decreases slightly during the early stand recovery stage as forest NEP is negative due
229 to rate of decomposition exceeding production, and then increases with stand development as

230 NEP turns positive. However, previous studies conflict with regards to whether old-growth
231 stands continue to accumulate C or whether total C eventually peaks and then declines.
232 ([Bond-Lamberty et al., 2004](#); [Harmon et al., 2004](#); [Goulden et al., 2011](#); [Kashian et al., 2013](#);
233 [Taylor et al., 2014](#)). The divergent findings may have resulted from the lack of consideration
234 of forest canopy transition, from dominance of early to late-succession species ([Taylor and](#)
235 [Chen, 2011](#)).

236 The past several decades have seen a sharp increase in the number of studies
237 investigating the relationship between species diversity and forest ecosystem functions.
238 Positive relationships between diversity and forest productivity tend to be predominant in
239 global forests ([Zhang et al., 2012](#); [Liang et al., 2016](#)). However, most studies have focused on
240 examining the effect of species diversity on community-level productivity, often overlooking
241 variability in the response of individual species to diversity at the neighbourhood level.
242 Because diversity-productivity relationships are largely realized through the growth response
243 of individual tree species ([Barrufol et al., 2013](#); [Chamagne et al., 2017](#)), a closer examination
244 of species-specific growth responses to neighbourhood diversity is warranted ([Fichtner et al.,](#)
245 [2017](#)).

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

254 **Chapter Two: Variation in total and volatile carbon concentration among** 255 **the major tree species of the boreal forest**

256 **Introduction**

257 The world's boreal forests play a critical role in the global carbon (C) cycle, containing
258 approximately 49% of all forest ecosystem C ([Dixon et al., 1994](#)), largely due to the slow rate
259 of decomposition and high accumulation of dead organic matter at high northern latitudes
260 ([Bonan and Shugart, 1989](#); [Dixon et al., 1994](#)). However, northern ecosystems are expected
261 to experience the greatest warming over the coming century, potentially affecting the
262 capacity of the boreal forest to sequester and store C ([Gauthier et al., 2015](#)). A better
263 understanding of C flux in the boreal forest is becoming increasingly imperative given its
264 strong impact on global C dynamics ([Canadell and Raupach, 2008](#); [Bellassen and Luysaert,](#)
265 [2014](#)). In particular, accurately quantifying C stocks of live trees in the boreal forest has
266 important implications for the verification and validation of global C accounting models used
267 to predict future C emission scenarios ([McKinley et al., 2011](#); [Metsaranta et al., 2011](#)) as
268 trees generally comprise the large and most dynamic C pool in forest ecosystems ([Pan et al.,](#)
269 [2011](#)).

270 The C contained in live trees is most often calculated by converting biomass estimates to
271 C stocks using a C concentration value, which is widely assumed to be 50% ([Chave et al.,](#)
272 [2008](#); [Saatchi et al., 2011](#); [Taylor et al., 2014](#); [Grunzweig et al., 2015](#)). However, recent
273 studies have shown that C concentration varies substantially among tree species and
274 assuming 50% C concentration for all species may significantly over- or underestimate forest
275 C stocks by as much as 5% ([Lamlom and Savidge, 2003](#); [Thomas and Malczewski, 2007](#);
276 [Thomas and Martin, 2012](#)), which, when scaled-up through biome- or nation-wide C
277 accounting schemes can lead to global-level errors in terrestrial C stock estimates. Although
278 the accuracy of C concentration has been improving among tropical and temperate tree

279 species ([Thomas and Malczewski, 2007](#); [Martin and Thomas, 2011](#); [Martin et al., 2013](#);
280 [Martin et al., 2015](#)), few studies have examined variation in C concentration among boreal
281 species.

282 Variation in tree C concentration is primarily attributable to the physical and chemical
283 properties of their woody tissues ([Savidge, 2000](#); [Elias and Potvin, 2003](#); [Bert and Danjon,
284 2006](#); [Thomas and Malczewski, 2007](#)). Recent progress on tissue-specific C concentration
285 suggests that C concentration derived from stemwood can be used to represent other major
286 tissues that account for important biomass fractions in trees (i.e., coarse roots and branches),
287 with the exception of bark ([Thomas and Martin, 2012](#); [Martin et al., 2015](#)). Studies of
288 temperate tree species have shown that bark has a significantly higher C concentration than
289 stemwood ([Bert and Danjon, 2006](#); [Martin et al., 2015](#)) due to higher concentrations of C-rich
290 lignin and suberin compounds associated with the bark's roles in limiting water loss and as a
291 protective layer against insect and pathogen attack ([Hengst and Dawson, 1994](#); [Franceschi et
292 al., 2005](#)). It is, therefore, reasonable to expect that similar differences in C concentration
293 between bark and stemwood tissues also exist in boreal tree species ([Liebhold et al., 1995](#);
294 [Lovett et al., 2006](#)). However, previous attempts to examine C concentration for boreal tree
295 species have mainly focused on radial and vertical variation of C concentration in stemwood
296 tissue only ([Lamlom and Savidge, 2003, 2006](#))

297 In addition, C concentration may decrease with increasing tree size. Higher C
298 concentration in small trees has recently been reported for 16 tropical tree species by
299 comparing C concentration of saplings with conspecific large trees ([Martin et al., 2013](#)). The
300 proposed explanation for this is that smaller trees require higher C-rich lignin concentrations
301 to support wood resistance to insect and pathogen attack ([Vance et al., 1980](#); [Wainhouse et
302 al., 1990](#)) and for improving stem mechanical stability ([Voelker et al., 2011](#)). However,
303 [Martin and Thomas \(2013\)](#) found a linear increase in C concentration with tree size for two

304 other tropical tree species (*Dacryodes excelsa* Vahl. and *Miconia mirabilis* (Aubl.) L.O.
305 Williams). Discrepancies among studies remain unexplained but may be caused by neglecting
306 the important role of volatile C compounds on total C concentration ([Martin et al., 2013](#)) or
307 failing to consider the effect of life-history traits, such as shade tolerance, on tissue C content
308 ([Thomas and Malczewski, 2007](#); [Pons and Poorter, 2014](#)).

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

321 Life-history traits, such as shade tolerance, may also affect C concentration due to its
322 important role in plant C balance ([Pons and Poorter, 2014](#)). For instance, shade tolerance
323 affects the survival and growth of plants by influencing C uptake (i.e., photosynthesis) and C
324 release (i.e., respiration) in response to limited light availability ([Hillebrand and Matthiessen,](#)
325 [2009](#)). Yet, empirical evidence is still scarce on the influence of contrasting shade tolerance
326 on plant tissue C concentration. Moreover, previous studies have detected diverse
327 relationships between tree C concentration and other traits related to shade tolerance,
328 including wood density and growth rate ([Elias and Potvin, 2003](#); [Martin and Thomas, 2011](#);

329 [Becker et al., 2012](#)). These mixed results, however, may be attributable to lack of
330 consideration of tree ontogenetic variation on C concentration ([Martin and Thomas, 2013](#)).
331 Further examination of the interaction between tree size and shade tolerance may help in
332 understanding the role of plant life-history adaptations on tissue C concentration ([Niinemets,
333 2006](#)).

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

346 **Materials and methods**

347 *Study area*

348 Our study was conducted in the boreal forest, approximately 150 km north of Thunder Bay,
349 Ontario, Canada, between 49°44' to 49°65' N and 89°16' to 90°13' W. This area is
350 characterized by warm summers and cold, snowy winters. Mean annual temperature of 1.9 °C
351 and mean annual precipitation of 824.8 mm were recorded at the closest meteorological
352 station in Cameron Falls, Ontario, Canada ([Environment Canada, 2015](#)). Soils in our study
353 area largely originated from the Wisconsinan glaciation, which ended approximately 9,500

354 years ago in this region. Stand-replacing wildfire is the most common natural disturbance in
355 our study area, with an average fire-return interval of approximately 100 years for the past
356 century, resulting in mosaic of stand ages across the landscape ([Senici et al., 2010](#)).

357 Commercial logging began in our study area in the 1970s. Dominant overstory tree species
358 include jack pine, trembling aspen, white birch, black spruce, white spruce, and balsam fir.

359 *Sampling design*

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

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

379 *Chemical analysis*

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

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

400
$$VMF = (M_{freeze} - M_{heat}) / M_{freeze} \quad (1)$$

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

402
$$C_{freeze-corr} = C_{freeze} \times (1 / (1 - VMF)) \quad (2)$$

403 Volatile C concentration (C_{vol} , %) was calculated as,

404 $C_{vol} = C_{freeze-corr} - C_{heat}$ (3)

405 *Data analysis*

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

413
$$Y_{ijk} = \mu + T_i + S_{(i)j} + D_k + (T \times D)_{ik} + (S \times D)_{(i)jk} + \varepsilon_{(ijk)} \quad (4)$$

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

424 We then used the results from our nested ANOVA to inform a more species-specific
 425 analysis, whereby, if tree size in Eq. (4) was significant, a simple linear regression was then
 426 used to more specifically describe the size-associated relationship between total and volatile
 427 C concentrations and DBH for each woody tissue type and for each tree species. We

428 compared linear and logarithmic functions to select the best bivariate relationships based on
429 Akaike's Information Criterion (AIC), whereby the simplest model that explained the most
430 variation was selected when the difference in AICs between alternative models was less than
431 2 ([Burnham and Anderson, 2002](#)). All statistical analyses were conducted using the R 3.2.3
432 ([R Development Core Team, 2017](#)).

433 **Results**

434 Total C concentration was significantly higher in bark tissue than in stemwood tissue for all
435 species (Table 2-1). Bark tissues were, on average, 6.2% and 5.0% higher in total C
436 concentration than stemwood for all shade-intolerant and shade-tolerant tree species,
437 respectively (Table 2-2). The minimum difference between mean total C concentration of
438 bark and stemwood tissue was 3.6% in black spruce, whereas the maximum difference was
439 11.0% in white birch (Table 2-2). Volatile C concentration was also significantly higher in
440 bark than in stemwood tissue for all species (Table 2-1). The average difference in mean
441 volatile C concentration between bark and stemwood was 2.0% and 3.8% for all shade-
442 intolerant and shade-tolerant species, respectively. Balsam fir had the maximum difference in
443 mean volatile C concentration between bark and stemwood of 5.7%, whereas trembling aspen
444 showed the least difference of 0.9% (Table 2-2).

445 Total C concentration of bark tissue differed significantly with shade tolerance, tree
446 species, and tree size and showed marginally ($P = 0.096$ and 0.079 , respectively) significant
447 interactions (Table 2-3). Mean total C concentration of bark was higher in shade-intolerant
448 species than in shade-tolerant species. Furthermore, total C concentration in bark
449 significantly increased with tree size for shade-intolerant jack pine and trembling aspen (Fig.
450 2-1), but significantly and marginally decreased with tree size for shade-tolerant balsam fir
451 and white spruce, respectively (Fig. 2-2). Similarly, volatile C concentration of bark was also
452 significantly influenced by shade tolerance, tree species, and tree size (as indicated by the

453 significant interaction between tree size and species) (Table 2-3). The mean volatile C
 454 concentration of bark was generally lowest for shade-intolerant tree species; however, this
 455 was mainly due to the low C concentration observed in aspen and birch, not jack pine, which
 456 had the highest mean volatile C concentration of all species at $7.5 \pm 0.2\%$ (S.E.) (Table 2-2).
 457 In addition, interactions between shade tolerance and tree size, and between species and tree
 458 size, had significant effects on volatile C concentration (Table 2-3). For shade-intolerant
 459 trembling aspen and white birch, volatile C concentration in bark showed a marginal ($P =$
 460 0.092 and 0.062 , respectively) positive trend with tree size (Fig. 2-1). Volatile C of bark
 461 marginally decreased with DBH for shade-tolerant white spruce, and significantly decreased
 462 with DBH for balsam fir (Fig. 2-2).

463 **Table 2-1** Results from paired *t*-tests comparing differences in total and volatile C
 464 concentrations between bark and stemwood tissues for each tree species. The columns give
 465 the degrees of freedom (d.f.), *t* values, and *P* values. Significant terms ($P < 0.05$) are in bold.
 466

Species	Total C concentration			Volatile C concentration		
	d.f.	t value	P value	d.f.	t value	P value
Jack pine	23	12.8	<0.001	23	15.4	<0.001
Trembling aspen	35	22.1	<0.001	35	4.4	<0.001
White birch	26	7.3	<0.001	26	5.0	<0.001
Black spruce	32	10.3	<0.001	32	7.2	<0.001
White spruce	20	19.0	<0.001	20	17.7	<0.001
Balsam fir	23	21.1	<0.001	23	14.8	<0.001

467
 468 Total C concentration of stemwood differed significantly with shade tolerance, tree
 469 species, tree size, and their interactions (Table 2-3). Shade-intolerant tree species had a higher
 470 (0.5%) overall mean total C concentration in stemwood than shade-tolerant tree species, with
 471 a maximum mean C concentration of stemwood observed in jack pine at $53.0 \pm 0.3\%$ (S.E.),
 472 and a minimum value of $49.0 \pm 0.1\%$ (S.E.) in shade-tolerant white spruce (Table 2-2). The
 473 total C concentration of stemwood significantly increased with tree size for all shade-
 474 intolerant species (Table 2-3, Fig. 2-1), but no general tree size pattern was observed for total

475 C concentration in stemwood for the shade-tolerant species (Fig. 2-2). Similarly, shade
476 tolerance, tree species, tree size, and their interactions also had significant effects on volatile
477 C concentration of stemwood (Table 2-3). Higher mean volatile C concentration of stemwood
478 was found in shade-intolerant tree species compared with shade-tolerant tree species (Table
479 2-2). At the species level, jack pine had the highest mean volatile C concentration of
480 stemwood ($4.2 \pm 0.2\%$ S.E.), and white spruce had the lowest mean value ($1.4 \pm 0.1\%$ S.E.).
481 The relationship between volatile C concentration in stemwood and tree size was
482 significantly positive for all three shade-intolerant tree species (Fig. 2-1), but no tree size-
483 associated pattern was observed for shade-tolerant tree species, except a marginal negative
484 trend observed for white spruce (Fig. 2-2).

485 **Table 2-2** Means and standard errors (S.E.) of total and volatile C concentrations of six tree
486 species in bark and stemwood tissues, respectively.
487

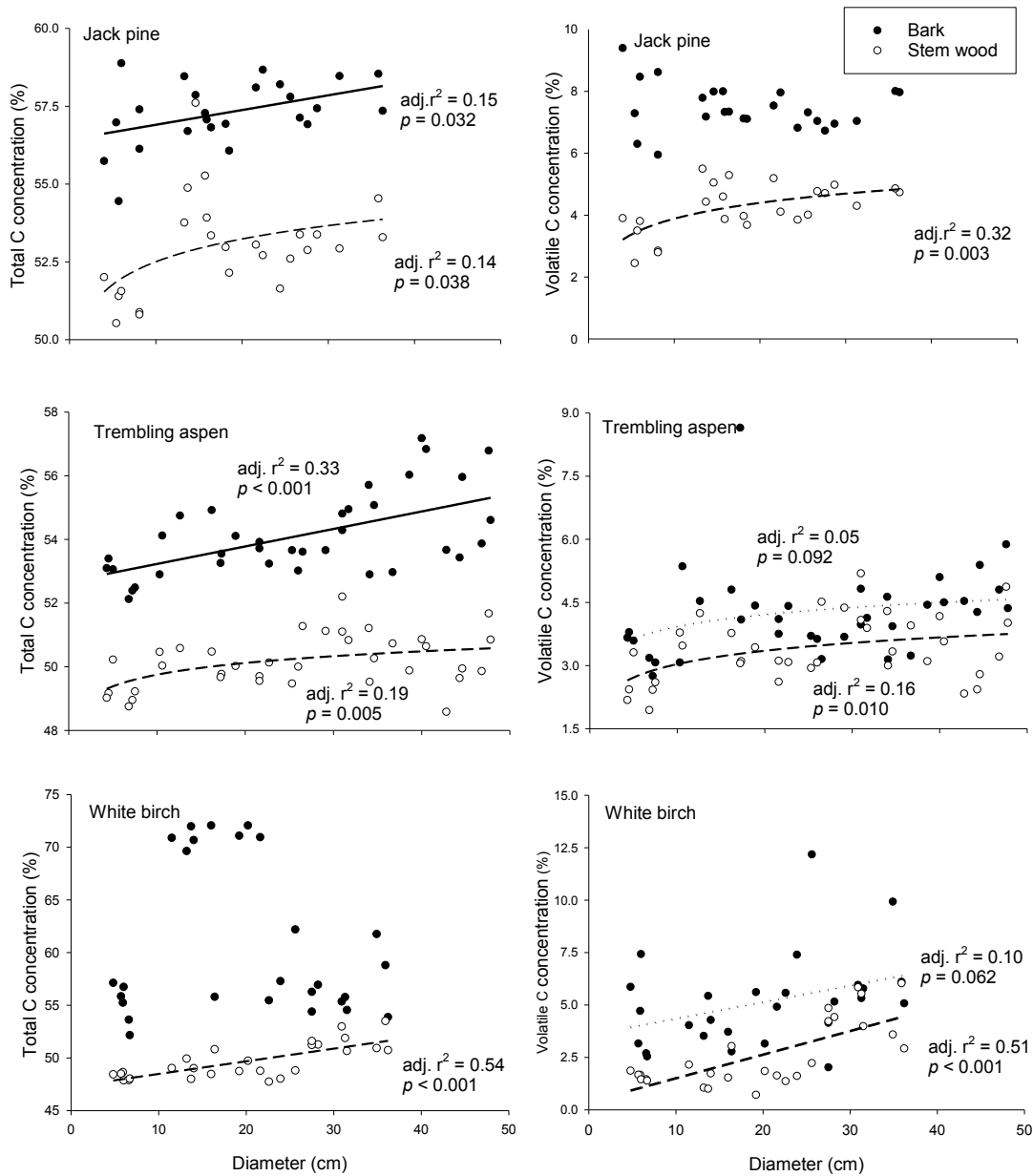
Species	Total C concentration (%)		Volatile C concentration (%)	
	Bark	Stemwood	Bark	Stemwood
<i>Shade-intolerant species</i>				
Jack pine	57.3 ± 0.2	53.0 ± 0.3	7.5 ± 0.2	4.2 ± 0.2
Trembling aspen	54.1 ± 0.2	50.2 ± 0.1	4.2 ± 0.2	3.4 ± 0.1
White birch	60.7 ± 1.4	49.7 ± 0.3	5.1 ± 0.4	2.6 ± 0.3
All shade-intolerant species	57.0 ± 0.5	50.8 ± 0.2	5.4 ± 0.2	3.4 ± 0.1
<i>Shade-tolerant species</i>				
Black spruce	55.1 ± 0.3	51.7 ± 0.2	6.2 ± 0.2	4.0 ± 0.2
White spruce	54.1 ± 0.3	49.0 ± 0.1	5.6 ± 0.2	1.4 ± 0.1
Balsam fir	56.6 ± 0.2	49.5 ± 0.2	7.2 ± 0.3	1.5 ± 0.2
All shade-tolerant species	55.3 ± 0.2	50.3 ± 0.2	6.3 ± 0.2	2.5 ± 0.2
All species	56.2 ± 0.3	50.5 ± 0.1	5.8 ± 0.1	3.0 ± 0.1

488

489 **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
 490 nested within shade tolerance. The columns give the degrees of freedom (d.f.), sum of squares (SS), *F* values, *P* values, and % deviance (in
 491 brackets) explained by the explanatory variables. Significant terms ($P < 0.05$) are in bold.
 492

Source	d.f.	Total C concentration				Volatile C concentration						
		Bark		Stemwood		Bark			Stemwood			
		P (% deviance explained)		SS	F	P	SS	F	P	SS	F	P
Shade	1	0.007 (5.29)		10.5	9.9	0.002	35.1	21.0	<0.001	28.9	36.6	<0.001
Species	4	<0.001 (86.48)		275.0	64.6	<0.001	181.8	27.2	<0.001	133.8	42.3	<0.001
DBH	1	0.003 (0.55)		26.5	24.9	<0.001	3.7	2.2	0.136	25.5	32.3	<0.001
Shade × DBH	1	0.096 (1.92)		10.6	10.0	0.002	18.4	11.0	0.001	14.8	18.8	<0.001
Species × DBH	4	0.079 (5.76)		18.5	4.3	0.002	39.1	5.9	<0.001	17.1	5.4	<0.001
Residual				162.9			255.3			120.9		

493



494

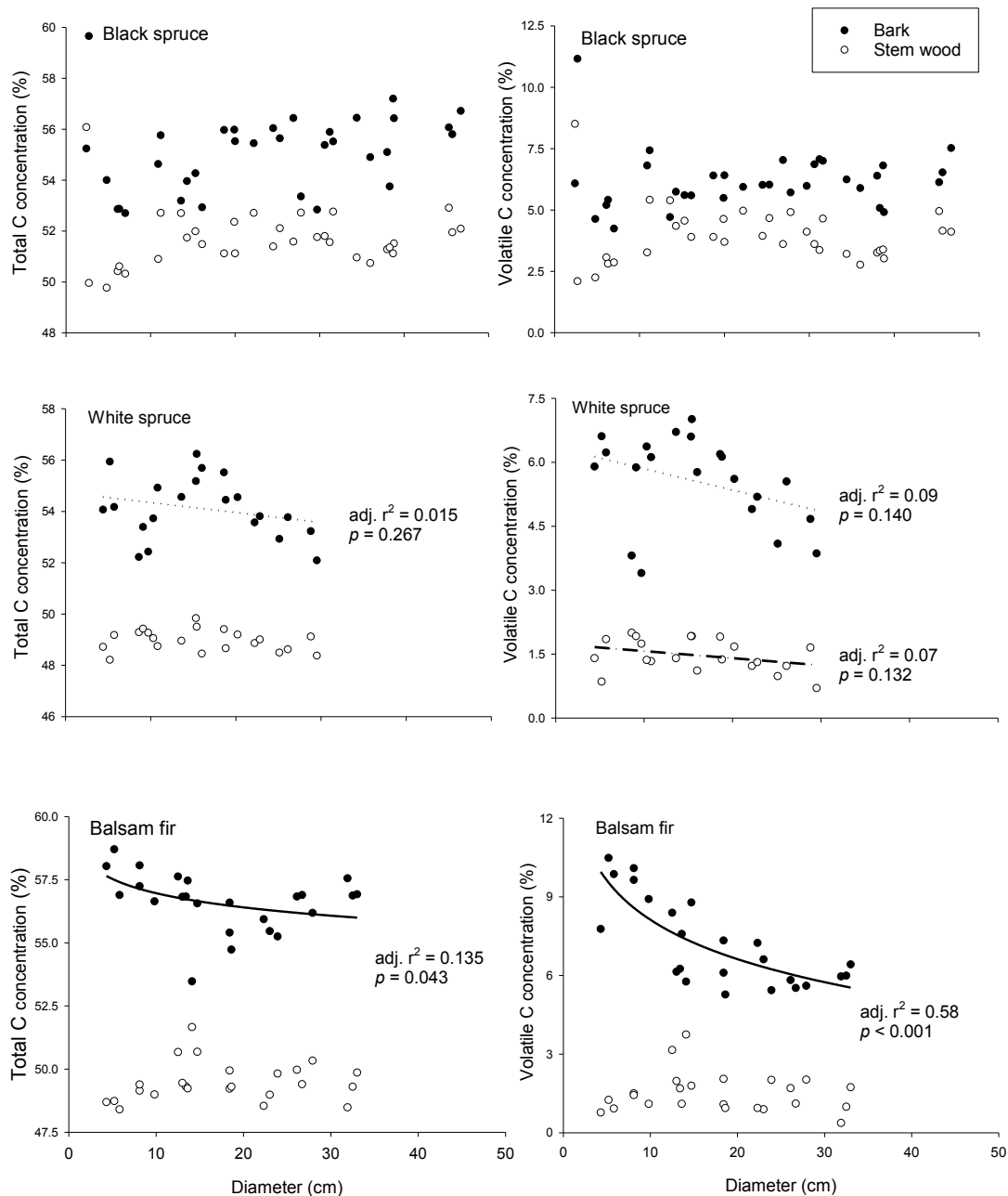
495 **Figure 2-1** Total and volatile C concentrations in relation to wood tissue type and DBH
 496 for jack pine, trembling aspen, white birch. Closed and open circles represent C
 497 concentrations of bark and stemwood, respectively. Solid lines represent the significant
 498 relationship between DBH and total or volatile C concentration of bark ($P < 0.05$),
 499 whereas short dashed lines represent the significant relationship between DBH and total
 500 or volatile C concentration of stemwood ($P < 0.05$). Dotted lines represent a marginal
 501 relationship between DBH and total or volatile C concentration of bark ($0.05 < P < 0.3$),
 502 whereas dash-dot lines represent a marginal relationship between DBH and total or
 503 volatile C concentration of stemwood ($0.05 < P < 0.3$).

504

505

506

507



508

509 **Figure 2-2** Total and volatile C concentrations in relation to wood tissue type and DBH
 510 for black spruce, white spruce, and balsam fir. Closed and open circles represent C
 511 concentration of bark and stemwood, respectively. Solid lines represent the significant
 512 relationship between DBH and total or volatile C concentration of bark ($P < 0.05$),
 513 whereas short dashed lines represent the significant relationship between DBH and total
 514 or volatile C concentration of stemwood ($P < 0.05$). Dotted lines represent a marginal
 515 relationship between DBH and total or volatile C concentration of bark ($0.05 < P < 0.3$),
 516 whereas dash-dot lines represent a marginal relationship between DBH and total or
 517 volatile C concentration of stemwood ($0.05 < P < 0.3$).
 518

519 **Discussion**

520 We found that C concentration significantly differed between bark and stemwood tissue
521 and varied significantly among tree species for each woody tissue type. The observed
522 dependence of C concentration on woody tissue type is consistent with our hypothesis
523 that C concentration in bark is greater than that of stemwood. Our findings agree with
524 results from previous studies in temperate forests ([Bert and Danjon, 2006](#); [Martin et al.,
525 2015](#)), which show strong differences in C concentration between bark and other tree
526 tissues. The divergence of C concentration between bark and stemwood is hypothesized
527 to be related to the functional adaptations of bark tissue for water storage and protection
528 ([Franceschi et al., 2005](#); [Rosell et al., 2014](#)). More specifically, previous studies suggest
529 that higher C concentration in bark, compared with stemwood, is caused by higher
530 concentrations of C-rich elements (e.g., lignin, tannins, and suberin), which help limit
531 water loss and defend against insects and pathogens ([Franceschi et al., 2005](#)) and fire
532 ([Hengst and Dawson, 1994](#)).

533 Volatile C concentration was also higher in bark tissue compared with stemwood
534 and also significantly varied among tree species, agreeing with previous studies of
535 temperate tree species ([Thomas and Malczewski, 2007](#)). However, our data suggest that
536 volatile C concentration, instead of C-rich elements like lignin, is primarily responsible
537 for the observed difference in C concentration between bark and stemwood tissue. Indeed,
538 we found that the difference between volatile C concentration in bark and stemwood
539 accounted for a large fraction (e.g., 80% for balsam fir) of the overall difference in total C
540 concentration between bark and stemwood. Furthermore, we also found that the
541 difference in C concentration between bark and stemwood is generally higher for boreal
542 tree species than previous findings for temperate tree species. Boreal tree species had, on
543 average, 5.7% higher total C concentration in bark than in stemwood based on our data,

544 whereas the difference was less than 3% for temperate tree species ([Martin et al., 2015](#)).
545 This difference may be related to differences in the severity and type of insect herbivory
546 experienced by boreal tree species compared with temperate species. For instance, the
547 boreal forest is subject to higher incidences of widespread insect infestations ([Liebhold et](#)
548 [al., 1995](#); [Lovett et al., 2006](#)), including mountain pine beetle (*Dendroctonus ponderosae*
549 Hopkins) and spruce beetle (*Dendroctonus rufipennis* Kirby), which can infect large areas
550 of pine and spruce–fir forests by feeding within the phloem tissue and killing trees ([Hicke](#)
551 [et al., 2012](#)). Volatile compounds are hypothesized to help in plant defences ([Martin et](#)
552 [al., 2013](#)) and may aid in inhibiting bark beetle infection. Furthermore, boreal forests
553 experience more frequent wildfire disturbance than other biomes ([Pan et al., 2011](#)), and
554 higher C concentration of bark of boreal tree species may also relate to the role of bark in
555 fire resistance ([Hengst and Dawson, 1994](#)).

556 Our data yielded mixed results for tree size effects on total C concentration of bark
557 and stemwood. We found both neutral and positive relationships between tree size and
558 total C concentration of stemwood, partially in agreement with [Martin and Thomas](#)
559 [\(2013\)](#) whose study in tropical forest found C concentration increases with tree size. In
560 addition, we also found negative, neutral, and positive relationships between tree size and
561 total C concentration in bark tissue. The reason for the diverse relationships is likely
562 attributable to size-associated changes in volatile C concentration, which parallel total C
563 concentration. Traditional views suggest that total C concentration is mostly dominated
564 by C-rich elements like lignin ([Lamlom and Savidge, 2003](#)). It is, thus, reasonable to
565 assume that small trees have higher C concentrations than large trees because small trees
566 generally contain higher lignin concentrations ([Martin et al., 2013](#)), supporting
567 mechanical stability ([Alvarez-Clare and Kitajima, 2007](#)) and defense mechanisms
568 ([Wainhouse et al., 1990](#)). However, our findings suggest that the size-associated trend of

569 total C concentration is likely led by change in the concentration of volatile C compounds
570 (Figs. 2-1 and 2-2). These results support [Martin et al. \(2013\)](#), who hypothesized that
571 volatile C concentration plays an important role in size-related changes in total C
572 concentration. Furthermore, when considering our observation that the difference in
573 volatile C concentration between bark and stemwood tissue accounts for a large
574 proportion of the difference in total C concentration between bark and stemwood tissue,
575 we speculate that the fraction of volatile C concentration plays a key role in driving
576 overall variation in total tree C concentration.

577 Moreover, size-associated trends in stemwood C and bark C differed according to
578 shade tolerance. All shade-intolerant tree species had positive relationships between
579 stemwood C concentration and tree size, whereas no general size-associated trend was
580 found in stemwood for shade-tolerant tree species. Similarly, shade-intolerant tree species
581 also showed positive size-associated changes in C concentration in bark tissue, with the
582 exception of white birch, where we observed some individuals with exceptionally high C
583 values. These high C values in white birch are likely related its high concentration of C-
584 rich betulinic acid ([O'Connell et al., 1988](#)). The observed difference in trends between
585 shade-tolerant and intolerant species supports our hypothesis that size-associated changes
586 in total C concentration depend on shade tolerance. The potential reason for this size-
587 associated interaction with contrasting shade tolerance may be attributable to species' life
588 history strategy under low light availability in the understorey. Shade-tolerant seedlings
589 and saplings that can tolerate low light levels tend to grow slower and have denser wood,
590 resulting in higher C concentration ([Elias and Potvin, 2003](#); [Poorter et al., 2005](#)).
591 However, the generality of these results is still uncertain, given that previous studies also
592 found positive relationships between C concentration and tree size for shade-tolerant tree
593 species in tropical forests ([Martin et al., 2013](#)). Further empirical study of woody tissue C

594 concentration, that explicitly accounts for volatile C, is needed from different biomes to
595 further test the underlying effects of shade tolerance (or other functional traits) on tree C
596 concentration.

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

$$613 \quad C_{freeze-corr} = 1.115 \times C_{heat} \quad (5)$$

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

$$615 \quad C_{freeze-corr} = 1.063 \times C_{heat} \quad (6)$$

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

617 Our study highlights the importance of considering variation in C concentration
618 when estimating live tree C stocks, which has broad implications for global C accounting

619 and validation of C accounting models. Carbon concentration is significantly higher in
620 bark than in stemwood tissue, and varies among tree species. Tree size and shade
621 tolerance also have effects on C concentration, but more empirical studies are needed to
622 identify the generality of these effects. Understanding variation in total C concentration
623 within and among tree species and incorporating it when quantifying forest C stocks and
624 validating C accounting models is important to successfully predict future global C
625 emission scenarios. Moreover, we observed that much of the variation in total C
626 concentration is attributed to volatile C concentration, providing new insight that volatile
627 C plays a key role in variation of total C concentration. Our results indicate that volatile C
628 concentration, rather than C-rich elements (e.g., lignin), is the dominant driver behind
629 size-associated changes in tree C concentration, and the dominant factor underlying the
630 difference in total C concentration between bark and stemwood tissue for major boreal
631 tree species. However, the underlying mechanisms of why volatile C concentration
632 showed different size-associated relationships between species is not clear, and whether
633 these results can be generalized to other species, especially tree species in other biomes,
634 requires further testing.
635

636 **Chapter Three: Carbon storage declines in old boreal forests**

637 **irrespective of succession pathway**

638 *Running head: Carbon storage dynamics in the boreal forest*

639 **Introduction**

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

652 Carbon storage changes as forest stands age. It has long been recognized that
653 forest net ecosystem production (NEP) increases rapidly during early stand development,
654 peaks as stands mature, and then declines as stands age ([Ryan et al., 1997](#)). During
655 periods of positive NEP, total ecosystem C increases, but during periods of negative NEP
656 (i.e., when gross primary production is less than total ecosystem respiration), total
657 ecosystem C decreases. Across diverse forest types, whether forest ecosystems continue
658 to accumulate C, decline, or stabilize as they transition from mature, even-aged stands to
659 structurally complex old-growth stands is unclear, with many studies reporting conflicting
660 results ([Bond-Lamberty et al., 2004](#); [Harmon et al., 2004](#); [Goulden et al., 2011](#); [Kashian](#)

661 [et al., 2013](#); [Taylor et al., 2014](#)). One possible explanation for this discrepancy may be
662 lack of consideration of forest canopy transition, from dominance of early to late-
663 succession species, on C dynamics. Most studies that have examined forest C storage in
664 relation to stand aging in boreal forests have focused on stands that have not yet
665 transitioned from dominance of individuals established immediately after fire and are
666 typically restricted to stands less than 160 years old. The transition from early to late-
667 succession species in boreal stands generally does not occur until early succession species
668 have reached their average maximum lifespan, which can be in excess of 150 years ([Chen](#)
669 [and Popadiouk, 2002](#)).

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

686 (*Choristoneura fumiferana* Clemens) or bark beetle (e.g., *Dendroctonus ponderosae*
687 Hopkins) can promote the persistence of broadleaf species in old stands, forming complex
688 mixedwood stands ([Kneeshaw and Bergeron, 1998](#)). These multiple succession pathways
689 may have diverse effects on forest C storage as species have different growth rates and
690 maximum standing biomass. Furthermore, diverse species mixtures can have positive
691 effects on forest productivity relative to species-poor mixtures, depending on stand age
692 ([Zhang et al., 2012](#); [Liang et al., 2016](#)).

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

707

708 **Materials and Methods**

709 *Study area*

710 Our study was conducted in the boreal forest region, approximately 150 km north of
711 Thunder Bay, Ontario, Canada, between 49°44' to 49°65' N and 89°16' to 90°13' W. This
712 area is characterized by warm summers and cold, snowy winters. Mean annual
713 temperature is 1.9 °C and mean annual precipitation is 824.8 mm as measured by the
714 closest meteorological station in Cameron Falls, Ontario, Canada ([Environment Canada,
715 2017](#)). Soils in our study area were largely deposited by the Wisconsinan glaciation,
716 which ended approximately 9,500 years ago in this region ([Moran and Clayton, 1984](#)).
717 Stand-replacing wildfire is the most common natural disturbance in the study area, with
718 an average fire-return interval of approximately 100 years during the past century,
719 resulting in a mosaic of stand ages across the landscape ([Senici et al., 2010](#)). We
720 conducted our sampling in the Spruce Forest, which is approximately a million of
721 hectares. Dominant overstorey tree species in the study area, in order from least to most
722 shade tolerant, include jack pine, trembling aspen, white birch, black spruce, white
723 spruce, and balsam fir ([Burns and Honkala, 1990](#)).

724 *Sampling design*

725 To determine the effect of stand aging and succession on forest C storage, we constructed
726 a chronosequence of postfire stands. Although the use of the chronosequence method has
727 been criticized because it makes the assumption that sample stands along the temporal
728 sequence have followed the same developmental history ([Johnson and Miyanishi, 2008](#)),
729 given careful site selection, replication, and demonstration of developmental links, the
730 chronosequence method is appropriate for studying patterns of C storage over decadal to
731 centennial time scales ([Walker et al., 2010](#)). Based on local fire history and the
732 availability of different aged stands in our study area, we were able to sample five

733 different stand age classes, including 8, 34, 99, 147, and 210 years since fire, representing
734 the stand initiation, stem exclusion, early canopy transition, late canopy transition, and
735 gap dynamic stages of boreal forest development, respectively, modified from [Chen and](#)
736 [Popadiouk \(2002\)](#).

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

751 We attempted to select postfire stands that had not been managed, including three
752 replicates for each age class and overstorey type combination; however, two replicate
753 mixedwood stands, each at age 34 and 147 years, were accidentally damaged by
754 harvesting activities between consecutive years of forest inventory, resulting in a total of
755 43 stands sampled. Sample stands were positioned several kilometers away from each
756 other and selected from different road accesses to minimize the impact of spatial
757 autocorrelation. In order to minimize site variability, all selected stands were located on

758 mesic sites with flat midslope positions, with no slope exceeding 5%. All stands are
759 underlain by moderately deep (≥ 50 cm) glacial tills, belonging to the Brunisolic soil
760 order, according to the Canadian system of soil classification ([Soil Classification](#)
761 [Working Group, 1998](#)). To ensure that each sample stand met the selection criteria, soil
762 pits were dug in each candidate stand to verify whether the site was mesic, following the
763 procedures described in [Taylor et al. \(2000\)](#). Soil attribute data for all sites used in this
764 study have been previously reported by [Hume et al. \(2016\)](#)

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

778

779 **Table 3-1** Characteristics of the 43 sample stands in the boreal forests of Ontario,
 780 Canada.
 781

Age	Overstorey ^a	N	Stand density (stems ha ⁻¹) or basal area (m ² ha ⁻¹) ^b	Stand composition (%) ^c					
				Tremblin g aspen	White birch	Jack pine	Spruce spp.	Balsa m fir	Other s
8	B	3	5933 (581)	95 (5)		5 (5)			
	C	3	7067 (1551)	3 (3)		97 (3)			
	M	3	6933 (926)	45 (9)		55 (9)			
34	B	3	26 (1)	92 (3)	4 (4)	1 (1)	2 (1)		1 (1)
	C	3	28 (2)	4 (2)		95 (3)	1 (1)		
	M	2	19 (3)	52 (6)		35 (1)	11(7)		2 (2)
99	B	3	51 (7)	91 (2)	3 (2)		1 (1)	1 (1)	4 (1)
	C	3	52 (2)	3 (2)		43 (12)	50 (17)	4 (3)	
	M	3	43 (5)	40 (12)	16 (11)	9 (6)	15 (8)	18 (3)	2 (1)
147	B	3	58 (8)	85 (3)	7 (4)		5 (1)	2 (1)	1 (1)
	C	3	51 (9)	1 (1)	2 (2)	53 (27)	37 (26)	7 (1)	
	M	2	36 (3)	38 (2)	30 (1)		12 (5)	19 (4)	1 (1)
210	B	3	41 (3)	54 (22)	24 (18)		10 (6)	10 (4)	2 (1)
	C	3	40 (8)	5 (5)	7 (4)		36 (18)	50 (17)	2 (1)
	M	3	46 (3)	11 (4)	39 (5)	5 (3)	38 (7)	7 (3)	

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

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

785 ^b Values are means with 1 SE in parentheses. Stand density (stems ha⁻¹) was determined
 786 for the younger (8 years old) stands and basal area (m² ha⁻¹) for older stands.

787 ^c The 'Others' category includes *Salix* spp., *Acer spicatum*, *Alnus viridis*, *Sorbus decora*,
 788 *Corylus cornuta*, *Prunus pensylvanica*, and *Larix laricina*.

789

790 **Data collection**

791 *Aboveground live biomass carbon*

792 In each selected stand, we randomly established a 400 m² (11.28 m radius) circular

793 sampling plot. Within each plot, diameters at breast height (DBH; 1.3 m above the root

794 collar) of all overstorey trees (DBH \geq 9 cm) were measured. Trees with a DBH in the
795 range of 3–9 cm were measured within a 50 m² circular subplot (3.99 m radius; shared
796 plot center with the 400 m² plot). Aboveground biomass of all trees \geq 3 cm in DBH was
797 estimated as the sum of bark, stem, branch, and foliage biomass calculated by using
798 published Canadian allometric equations ([Lambert et al., 2005](#)). For tree saplings (DBH
799 <3 cm) and large shrubs (height \geq 50 cm), the base diameter of each individual was
800 measured in three randomly selected 2 \times 2 m plots within each 400 m² main plot.
801 Aboveground biomass of tree saplings and large shrubs was estimated by using allometric
802 equations developed for small trees and large shrubs ([Smith and Brand, 1983](#); [Bond-
803 Lamberty et al., 2002](#)).

804 Aboveground biomass of understorey vegetation, including short shrubs (height
805 <50 cm), forbs, graminoids (grass, sedge), bryophytes (mosses), ferns and lichens, was
806 estimated by two stages of sampling during July 2014, when annual plant cover was at or
807 near its maximum ([MacDonald et al., 2012](#)). Firstly, the percent cover of each
808 understorey species was estimated within each 400 m² plot. Secondly, the percent cover
809 of each understorey species was visually estimated again within twenty-one 2 \times 2 m
810 subplots, which were randomly located in the larger 400 m² main plot. All understorey
811 plants in the subplots were then harvested by species, stored in paper bags, and
812 transported to the laboratory for biomass determination. Samples were oven dried at 68°C
813 for 48 h and weighed. Data of all harvested samples were pooled together, and linear
814 regression was used to develop allometric relationships between understorey biomass and
815 percent coverage for each growth form ([MacDonald et al., 2012](#)). The whole plot biomass
816 was then estimated based on percent cover of each species from the first step by using
817 developed regressions from the second step.

818 *Belowground live biomass carbon*

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

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

839 In each 400 m² plot, seven soil cores (6.6 cm in diameter) were randomly selected
840 to collect fine roots using a power auger. Cores were collected monthly from June–
841 October 2014 and in May 2015, with six sampling times in total (we reported the average
842 of six measurements). The extracted soils were separated into a forest floor layer and two

843 mineral soil layers (0–15 and 15–30 cm) and transported in an ice-filled cooler to the
844 laboratory, where they were stored in a freezer until processing. In the laboratory,
845 samples were soaked in water to separate roots from the soil and then sieved (0.2 mm
846 mesh). Fine roots were sorted according to vitality (live or dead). Live roots were pale
847 colored on the exterior, elastic and flexible, free of decay, and had a whitish cortex,
848 whereas dead roots were brown or black, rigid and inflexible, in various stages of decay,
849 and had a darker cortex. The “live” and “dead” components were then oven dried
850 separately to a constant mass at 65°C and weighed.

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

857 *Aboveground deadwood carbon*

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

867
$$DWD = \frac{\pi^2 \sum d^2}{8L} \quad (1)$$

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

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

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

891 *Belowground deadwood carbon*

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

902
$$CRB_{adj} = \left(\frac{DW}{LW} \right) \times CRB_{unadj} \quad (2)$$

903 where CRB_{adj} is the coarse root necromass of a snag or stump after correcting for losses
904 associated with decay, LW is the density of the individual assuming it was still alive, DW
905 is the density of the individual in its current state of decay, and CRB_{unadj} is the calculated
906 coarse root biomass using the equations of [Bond-Lamberty et al. \(2002\)](#) or [Brassard et al.](#)
907 [\(2011\)](#) as if the individual were still alive. LW values were taken from [Alemdag \(1984\)](#),
908 who reports average stem wood density values for live jack pine, aspen, white birch,
909 black spruce, white spruce, and balsam fir from samples collected in the boreal forest of
910 Ontario, Canada. DW values were calculated using local equations from [Seedre et al.](#)
911 [\(2013\)](#), derived from the same study area.

912 Dead medium and fine root necromass was estimated using the same method
913 mentioned above for live medium and fine root biomass. Simple linear regressions were
914 developed between fine root necromass in mixedwood stands and in conifer stands, and

915 between fine root necromass in mixedwood stands and in broadleaf stands, respectively
916 (see Fig. S3-2). Dead medium and fine root necromass data for all mixedwood stands of
917 our chronosequence were derived from the chronosequence study of [Yuan and Chen](#)
918 [\(2012\)](#), and then we used locally developed fine root necromass equations (see Fig. S3-2)
919 to calculate medium and fine root necromass data in broadleaf and conifer stands for each
920 stand age.

921 Total aboveground tree biomass and tree coarse root biomass were converted to C
922 using locally developed C concentrations estimated by Chapter two. The C content of
923 aboveground understorey vegetation was calculated as 45% of dry biomass ([IPCC, 2013](#)).
924 Belowground medium and fine root C content was similarly assumed to be 45% of dry
925 biomass. The C content of aboveground deadwood and belowground deadwood was
926 assumed to be 50% of dry necromass, whereas C content of dead medium and fine roots
927 was assumed as 45% of dry necromass ([Garrett et al., 2012](#)). All live biomass and
928 deadwood C pools were scaled-up to $\text{Mg C}\cdot\text{ha}^{-1}$.

929 *Forest floor and mineral soil carbon*

930 Forest floor C and mineral soil C for all sites in this study have been previously reported
931 ([Hume et al., 2016](#)). In brief, ten soil cores were randomly collected per 400 m² main plot
932 using a Dutch soil auger. Soil cores were separated by forest floor (i.e., the fibric and
933 humus layers) and two mineral soil layers according to mineral soil depth, i.e., M1 (0–15
934 cm) and M2 (15–30 cm). Soil samples were stored in a cooler and immediately
935 transported to the laboratory for chemical analysis. Soil samples were air dried to constant
936 weight and sieved (2-mm mesh) to remove coarse fragments, and then ground to pass
937 through a 100-mesh (0.15 mm) sieve to ensure a uniform sample. Total C for each sample
938 was determined by the flash dynamic combustion method and converted to Mg C ha^{-1}
939 following the method described by [Wairiu and Lal \(2003\)](#).

940 We did not collect mineral soil samples >30 cm depth as C storage in the deep
941 mineral soil was expected to change little over the temporal range of our chronosequence
942 ([Harden et al., 1997](#); [Wirth et al., 2002](#); [Goulden et al., 2011](#)). Nonetheless, to provide a
943 more realistic estimate of total mineral soil C storage at each site, we used the average
944 value of deep (30–100 cm) mineral soil C mass derived by [Laganière et al. \(2012\)](#) from
945 six local 92-year-old stands from our study area to approximate mineral soil C deeper
946 than 30 cm in each of our study stands.

947 **Data analysis**

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

$$954 Y_{ijk} = \mu + A_i + T_j + A \times T_{ij} + \varepsilon_{(ij)k} \quad (3)$$

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

958 For our second question, we used Tukey's Honest Significant Difference (HSD)
959 test to examine if C storage increased, decreased, or showed no significant change as
960 postfire boreal stands transitioned from mature, even-aged stands to structurally complex
961 old-growth stands (i.e., from 147- to 210-year old stands), and whether this change in C
962 storage was influenced by successional pathways, characterized by overstorey types.
963 Furthermore, as individual C pools transfer C amongst each other and have potentially
964 different age-dependent C dynamics ([Seedre et al., 2011](#)), we also performed the two-way

965 ANOVA (Eq. 3) on each C pool component to test the influence of succession pathways
966 on individual C pools to help understand underlying changes in total ecosystem C storage.
967 We verified the ANOVA assumptions of normality and homogeneity of variance using
968 the Shapiro–Wilk test and Levene’s test, respectively. The tests revealed the assumptions
969 of normality and homogeneity were met for all analyses without data transformation. All
970 data analyses were performed in R 3.4.2 ([R Development Core Team, 2017](#)).

971 **Results**

972 Following stand-replacing fire, total ecosystem C changed significantly with stand age.
 973 Broadleaf stands on average had higher total ecosystem C than conifer and mixedwood
 974 stands; but age-related trends of total ecosystem C storage were similar for all three
 975 overstorey types (Table 3-2). Averaged across all overstorey types, total ecosystem C
 976 increased rapidly from 96.5 ± 5.1 (Mean \pm S.E.) Mg C ha⁻¹ in 8-year-old stands to
 977 300.4 ± 9.4 Mg C ha⁻¹ in 99-year-old stands, peaking at 327.9 ± 11.9 Mg C ha⁻¹ in 147-
 978 year-old stands, and then significantly decreasing to 271.1 ± 13.6 Mg C ha⁻¹ in 210-year-
 979 old stands (Fig. 3-1a).

980 **Table 3-2** Results of stand age (A_i) and overstorey type (T_j) on total ecosystem C and
 981 individual C pools following fire. The columns give the degrees of freedom (d.f.), F
 982 value, and P value. Statistically significant terms ($p < 0.05$) are in bold.
 983

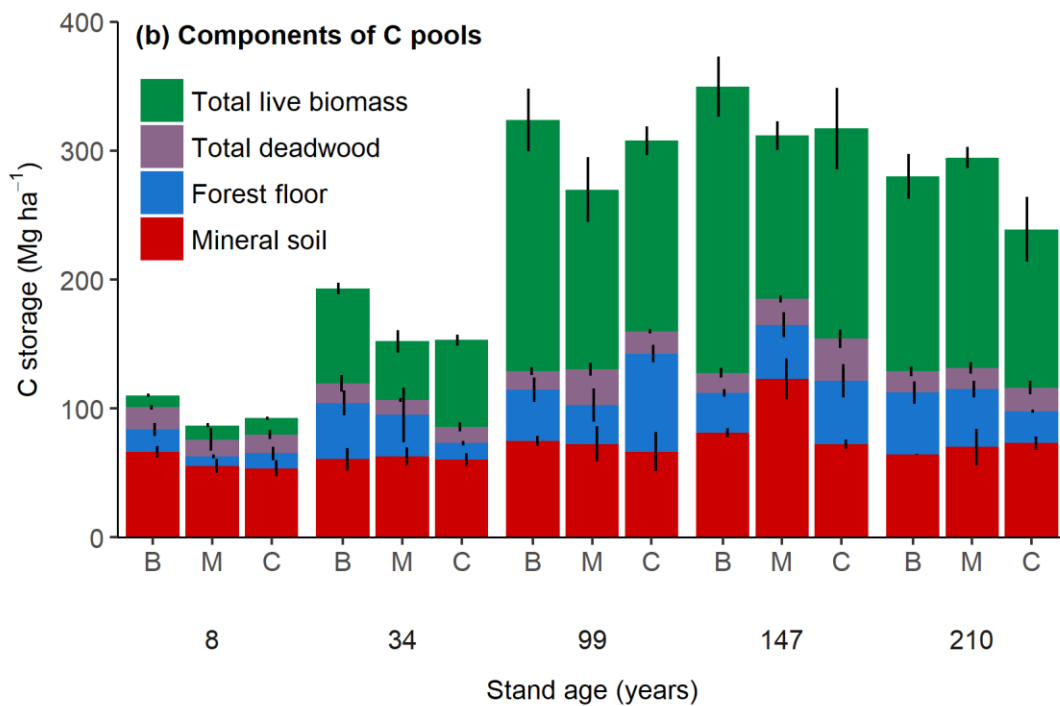
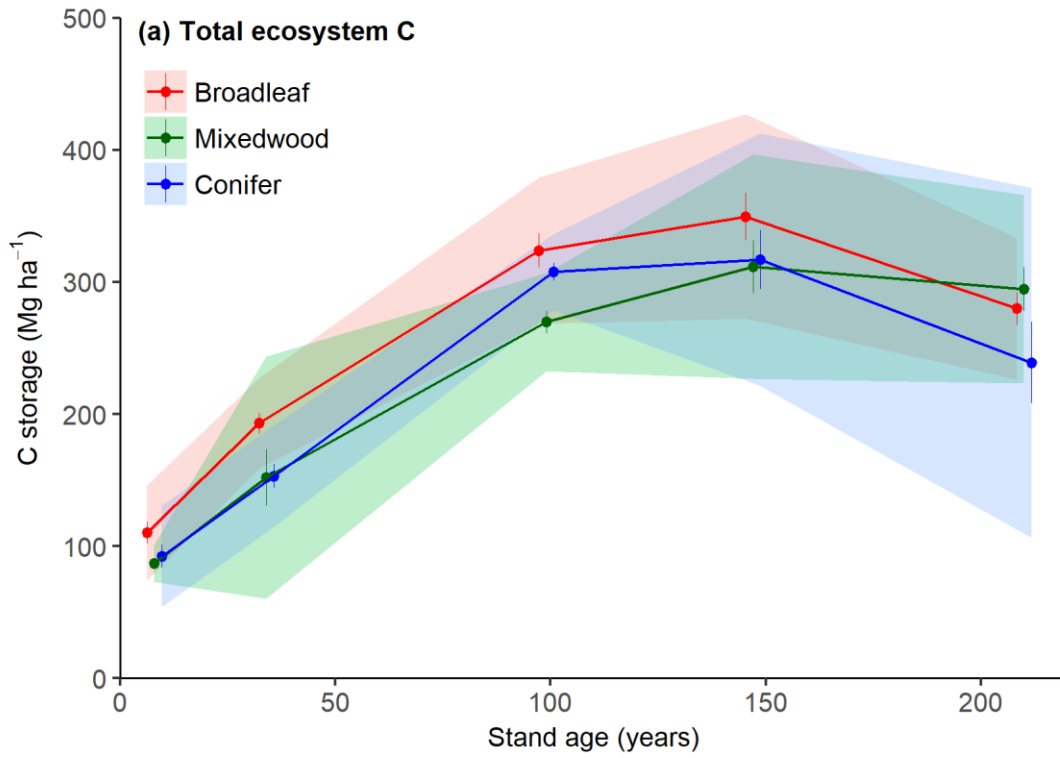
C Pools	A_i (d.f. = 4)		T_j (d.f. = 2)		$A \times T_{ij}$ (d.f. = 8)	
	F	P	F	P	F	P
Total ecosystem C	124.5	< 0.001	6.1	0.006	1.5	0.202
Total live biomass	50.8	< 0.001	4.7	0.017	1.8	0.112
Total deadwood	1.9	0.144	0.7	0.516	1.4	0.259
Forest floor	8.8	< 0.001	0.5	0.591	4.0	0.003
Mineral soil	5.5	0.002	1.6	0.215	1.9	0.102

984
 985 Among all C pools, the live biomass C pool had the strongest temporal dynamics
 986 (Fig. 3-1b). Total live biomass C was significantly influenced by stand age, overstorey
 987 type, and their interaction (Table 3-2, Fig. 3-2a). Similar to changes in total ecosystem C
 988 storage, live biomass C, averaged over all overstorey types, increased rapidly from $9.4 \pm$
 989 $1.3 - 12.6 \pm 1.1$ Mg C ha⁻¹ in 8-year-old stands to $139.5 \pm 25.1 - 195.1 \pm 24.5$ Mg C ha⁻¹
 990 in 99-year-old stands. However, age-related changes in live biomass C marginally
 991 differed among overstorey types thereafter (Table 3-2, Fig. 3-2a). Conifer and broadleaf
 992 stands continued to increase to a peak of 163.1 ± 8.3 and 221.9 ± 23.4 Mg ha⁻¹ in the 147-

993 year-old stands, respectively, then declined. In comparison, the mixedwood stands
994 showed a slight decrease, and then increased to a peak of $163.4 \pm 8.3 \text{ Mg ha}^{-1}$ in the 210-
995 year-old stands. Total deadwood C only demonstrated a marginally significant change
996 with stand age (Table 3-2, $P < 0.15$), and it did not change notably until 147 years old
997 (Fig. 3-2b).

998 Carbon storage in the forest floor changed significantly with stand age, and
999 showed a significant interaction effect between stand age and overstorey type (Table 3-2,
1000 Fig 3-3a). The forest floor C pool increased rapidly in the broadleaf and mixedwood from
1001 8 to 34 years old, but fluctuated thereafter. It remained relatively low in the conifer stands
1002 until it peaked in the 99-year-old stands, and then significantly declined in the 210-year-
1003 old stands (Fig. 3-3a). Similar to forest floor C storage, our ANOVA test indicated a
1004 significant age effect on mineral soil C storage, however, age-related changes in mineral
1005 soil C marginally differed among the three overstorey types (Table 3-2). Our data suggest
1006 that mineral soil C storage remained relatively stable throughout succession and that the
1007 age-related effect was mainly caused by the peak in mineral soil C observed in the 147-
1008 year-old mixedwood stands. During late succession, mixedwoods had a steeper decline in
1009 mineral soil C than other types from 147- to 210-year-old stands (Fig. 3-3b).

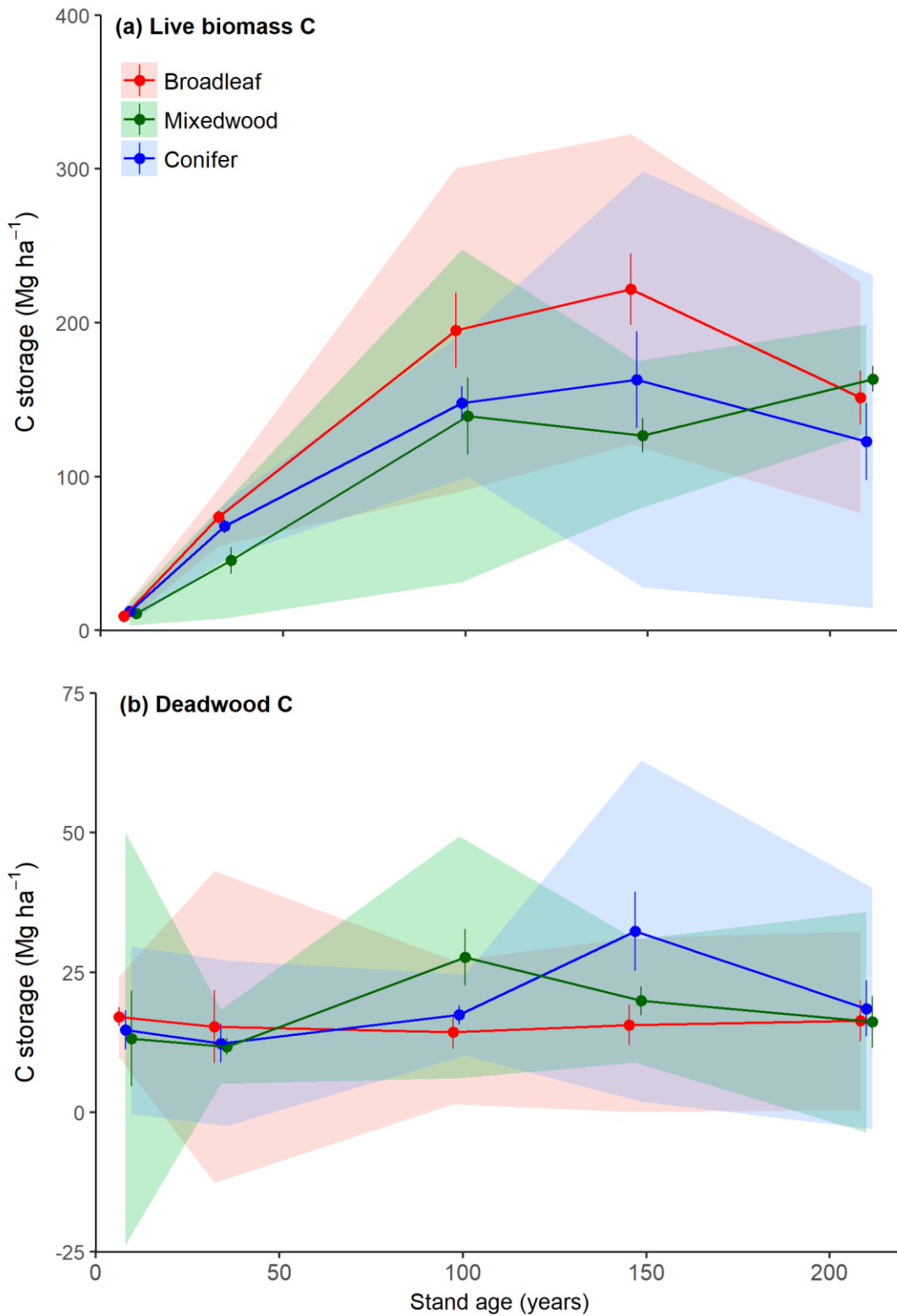
1010



1011

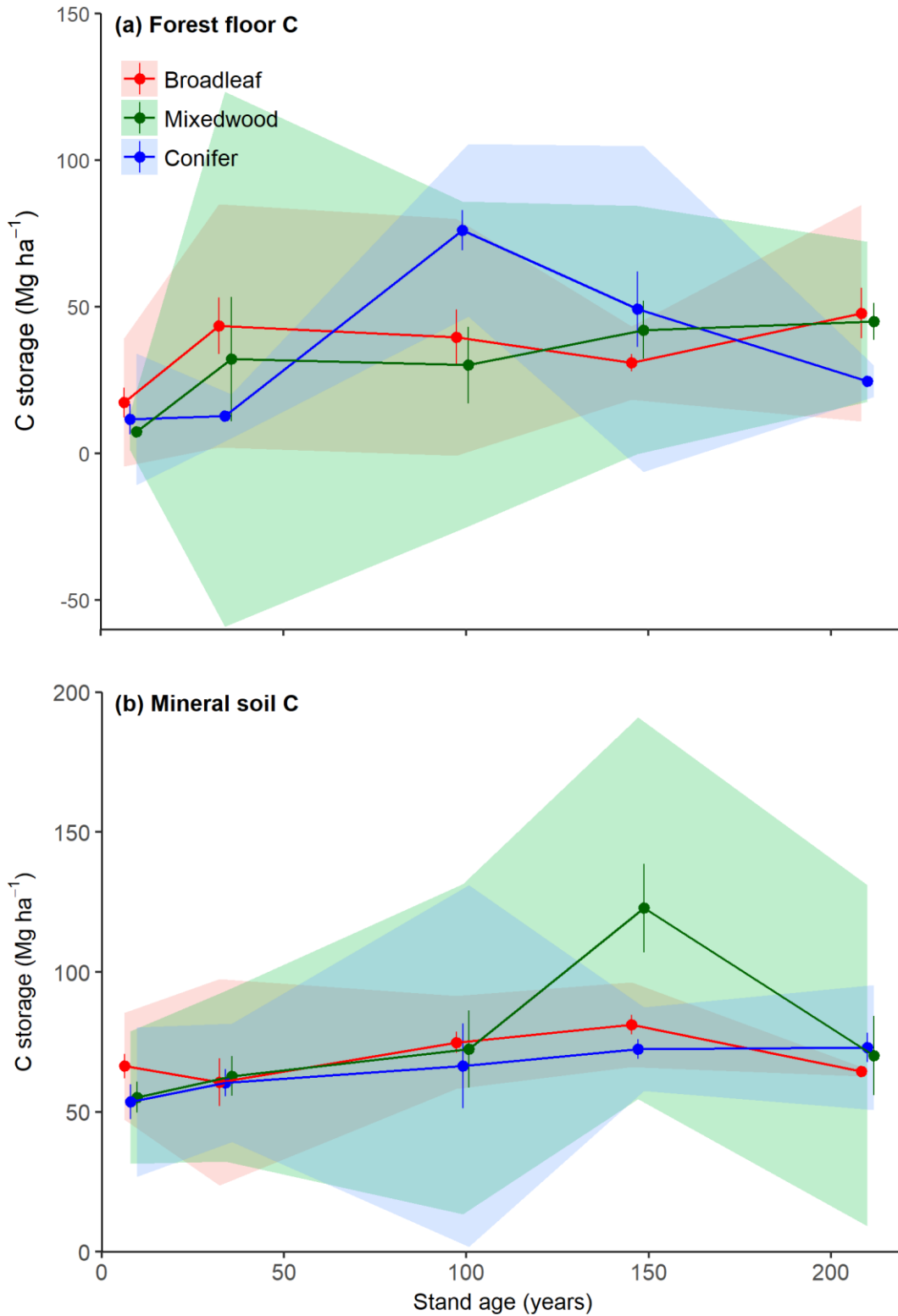
1012 **Figure 3-1** Carbon storage in relation to time since stand-replacing fire and overstorey
 1013 type for a) total ecosystem; and b) components of the four individual pools. Symbols and
 1014 vertical lines represent sample means and standard errors, respectively, and ribbons
 1015 represent the 95% confidence interval of sample means.

1016



1017

1018 **Figure 3-2** Carbon storage in relation to time since stand-replacing fire and overstorey
 1019 type for a) live biomass; b) deadwood. Symbols and vertical lines represent sample means
 1020 and standard errors, respectively, and ribbons represent the 95% confidence interval of
 1021 sample means.



1022

1023 **Figure 3-3** Carbon storage in relation to time since stand-replacing fire and overstorey
 1024 type for a) forest floor; and b) mineral soil. Symbols and vertical lines represent sample
 1025 means and standard errors, respectively, and ribbons represent the 95% confidence
 1026 interval of sample means.

1027 **Discussion**

1028 *Carbon dynamics among overstorey types*

1029 Overall, our results show a strong relationship between total ecosystem C and stand age
1030 following fire, similar to previous studies ([Pregitzer and Euskirchen, 2004](#); [Goulden et al.,
1031 2011](#); [Taylor et al., 2014](#)). We found broadleaf stands had, on average, higher total
1032 ecosystem C and live biomass C than the other types, supporting a previous hypothesis
1033 that suggests aspen abundance is a key driver of ecosystem C storage in Canadian boreal
1034 forest ([Pare and Bergeron, 1995](#); [Laganière et al., 2015](#)). However, despite that boreal
1035 forest stands may follow multiple succession pathways as they age, our study did not
1036 detect any significant difference in total ecosystem C dynamics between the three
1037 overstorey types over the span of our chronosequence (i.e., 210 years). This suggests that
1038 succession trajectory may have little effect on total ecosystem C storage as forests age
1039 and that succession only accounts for minor variations in total ecosystem C storage
1040 dynamics as forest stands transition from mature, even-aged stands to structurally
1041 complex old-growth stands.

1042 Although total ecosystem C storage had similar age-dependent trends among the
1043 three overstorey types, we did observe significant differences among stand types in the
1044 dynamics of individual C pools that comprise total ecosystem C, corroborating previous
1045 reports ([Pregitzer and Euskirchen, 2004](#); [Seedre et al., 2011](#)). The live biomass C pool
1046 increased sharply from age 8 to age 99 years for all stand types, driving the rapid
1047 recovery of total ecosystem C storage (90% within 99 years) relative to the mean fire
1048 interval of 100 years, which agrees with a previous chronosequence study of *Pinus*
1049 *contorta*-dominated forests ([Kashian et al., 2013](#)). However, during the late successional
1050 stage, the live biomass C pool declined substantially from age 147 to 210 years in the
1051 broadleaf and conifer-dominated overstorey types, whereas the mixedwoods continued to

1052 accumulate live biomass C, peaking in the oldest measured stands. This divergent pattern
1053 may be attributable to differences in tree mortality rates experienced among stand types.
1054 For instance, the negative effects of conspecific neighbors have been reported to increase
1055 tree mortality rates, especially for trembling aspen, white birch, and spruce ([Luo and](#)
1056 [Chen, 2011](#)), which dominated our broadleaf and conifer stand types, potentially causing
1057 higher tree mortality in old broadleaf and conifer stands compared with mixedwood
1058 stands. Furthermore, due to the potential effects of resource partitioning and niche
1059 complementary, mixtures of fast-growing, shade-intolerant species (e.g., trembling aspen
1060 and white birch) and slower-growing, shade-tolerant species (e.g., spruce and fir) may
1061 have a positive effect on the long-term productivity of old mixedwood stands compared
1062 with less diverse broadleaf and conifer-dominated stands ([Zhang et al., 2012](#)).

1063 We also found conifer stands differ from broadleaf and mixedwood stands in the
1064 way they accumulate forest floor C, in agreement with previous reports of tree species
1065 composition effects on forest floor C storage ([Laganière et al., 2012](#); [Laganière et al.,](#)
1066 [2013](#)). During early stand development (i.e., 8 and 34 years), conifer stands contained less
1067 forest floor C than broadleaf or mixedwood stands. This may be because in young conifer
1068 stands, the main input of C to the forest floor is slow-decomposing needle leaf litter.
1069 Conversely, young mixedwood and broadleaf stands support a higher density and
1070 diversity of understory shrub and herb species ([Hart and Chen, 2008](#)). The contribution of
1071 the understory vegetation combined with higher annual turnover of broadleaf litterfall
1072 ([Chen et al., 2016](#)) may lead to higher C accumulation in the forest floor. However, as
1073 conifer stands age, their understory becomes increasingly dominated by shade-tolerant,
1074 bryophyte species ([Hart and Chen, 2008](#)). Furthermore, cool, damp conditions, typical of
1075 conifer understory environments, impede decomposition and contribute to the
1076 accumulation of dead organic matter ([Lang et al., 2009](#)). This is supported in Fig. 3a by

1077 the large hump in forest floor C in the conifer stands during the intermediate stages of
1078 stand development (i.e., the 99-year-old stands). Eventually, as conifer stands undergo
1079 canopy break-up and transition to structurally complex old-growth stands, increased light
1080 to the forest floor can enhance forest floor decomposition and alter understory vegetation
1081 composition ([Laganière et al., 2012](#); [Kumar et al., 2017](#)).

1082 Correspondingly, we observed marginal differences in mineral soil C dynamics
1083 among the three overstorey types. Mixedwood stands had higher mineral soil C in 147-
1084 year-old stands, but then declined more sharply, thereafter, compared with the other
1085 overstorey types. High mineral soil C storage in the 147-year-old mixedwood stands may
1086 relate to the positive contribution of species diversity on fine root productivity and
1087 mortality ([Jacob et al., 2014](#); [Ma and Chen, 2016](#)), which is the major input of C to the
1088 mineral soil layer in boreal forests ([Clemmensen et al., 2013](#)). The potential increase in
1089 soil organic matter decomposition in the oldest mixedwood stands (>147 years old) may
1090 be attributable to the effects of the litter mixture on local decomposers, relative to
1091 broadleaf and conifer stands. For instance, chemically divergent litter quality combined
1092 with increased surface temperature, due to late-succession canopy break-up, can promote
1093 the abundance and activities of decomposers ([Gartner and Cardon, 2004](#)), and thus
1094 accelerate decomposition in late succession.

1095 Despite that other similar chronosequence studies have observed “U” shaped
1096 trends in deadwood abundance over time, with high deadwood abundance immediately
1097 following disturbance and again during late succession (e.g., [Kashian et al., 2013](#); [Taylor
1098 et al., 2014](#)), we observed only marginal age-related changes in total deadwood C for all
1099 three overstorey types over the span of our chronosequence. The abundance of deadwood
1100 in younger forests is largely dependent on the intensity and severity of fire as well as pre-
1101 fire site condition such as stand density ([Flannigan et al., 2000](#); [Wang et al., 2003](#)). The

1102 low deadwood C we observed in the 8 years old stands may have resulted from high
1103 intensity and severity fire and/or pre-fire stands were dominated by small-sized, young
1104 trees, thus leading to low post-fire deadwood abundance on our sites (see Fig. S3-3).
1105 Further, we observed relatively stable deadwood C throughout late succession, consistent
1106 with previous studies in our area ([Brassard and Chen, 2008](#)). Despite the potential for
1107 high inputs of mortality originated deadwood during the canopy transition stage of stand
1108 development (i.e., the 99-147 year age cohorts), most of this deadwood would decompose
1109 over 60 years ([Angers et al., 2010](#)), leading to low deadwood abundance between in the
1110 210 years old stands. Further, the oldest stands were also dominated by faster
1111 decomposing balsam fir and white birch stems, which may also have contributed to low
1112 observed deadwood.

1113 *Carbon dynamics during late succession*

1114 Overall, within the timeframe of our chronosequence, our results showed a significant
1115 decline in total ecosystem C storage (i.e., negative NEP) as stands transitioned into late
1116 succession. Our findings conflict with the view, based largely on chronosequence studies
1117 that do not consider forest succession, that old forests may slow in their rate of C
1118 accumulation, but still continually accumulate C ([Odum, 1969](#); [Luyssaert et al., 2008](#);
1119 [Goulden et al., 2011](#); [Kashian et al., 2013](#); [Gough et al., 2016](#)). Our observed decline in
1120 total ecosystem C storage during late succession was previously reported by [Taylor et al.](#)
1121 [\(2014\)](#) in the same study area, but this study only considered a single succession pathway,
1122 with limited sample plots. By expanding the sample size to include different forest
1123 overstorey types to account for multiple succession pathways, our study adds further
1124 support for the hypothesis that as boreal forests age and transition into late succession,
1125 they undergo a decline in total ecosystem C storage. Of course, it is reasonable to point
1126 out that old stands cannot decline in perpetuity, or else they would disappear. Thus, it

1127 remains to be observed how long this decline phase persists and at what point do old
1128 boreal forests achieve a steady C state balance (if ever) since plant diversity is expected to
1129 increase after canopy transition ([Roberts and Gilliam, 1995](#)), likely promoting C uptake
1130 and storage in forests ([Liang et al., 2016](#); [Zhang et al., 2017](#)).

1131 It is worth noting, that forest companies in our study area preferentially harvest
1132 mature, “productive” stands. Consequently, this may induce bias in our sample of old
1133 stands since it is conceivable that the old, 210 years stands (*ca.* 3000 ha of our study area,
1134 which is approximately one million ha) represent less desirable, low productivity stands
1135 left unharvested, which could have artificially driven the decline in C storage we
1136 observed in late succession. However, commercial forestry in our study area did not begin
1137 until the mid-1970s and targeted mostly pure jack pine stands less than 120 years old. Our
1138 oldest stands, which were almost 160 years old in the 1970s, were beyond the preferred
1139 age for harvesting in the area at that time and, consequently, were left unharvested. Thus,
1140 the possibility of “cherry-picking” low productivity, 210-year-old stands is low and
1141 confirmed from our personal communications with the long-term District Forester in this
1142 area, Paul Poschmann, who has been working as the area forester since the mid 1970s.

1143 Although our results show an overall decline in total ecosystem C storage, the
1144 mechanisms that drive this decline seemed to differ according to overstorey type. For
1145 instance, a reduction of live biomass C, mainly resulting from increased tree mortality
1146 during canopy transition, led to total ecosystem C decline after year 147 in broadleaf and
1147 conifer stands. However, the decrease of total ecosystem C observed in mixedwood
1148 stands was largely driven by decreases in mineral soil C, suggesting that an increase in
1149 decomposition may be an important driver of total C decline. Nonetheless, the generality
1150 of these hypotheses needs further investigation that explicitly examines C flux (e.g.,

1151 forest production, tree mortality rate, and decomposition) while also considering multiple
1152 succession pathways.

1153 By accounting for multiple succession pathways, our results show a strong
1154 relationship between total ecosystem C storage and stand age following fire. Broadleaf
1155 stands had on average higher total ecosystem C; however, temporal dynamics of total
1156 ecosystem C were similar among all three overstorey types. Moreover, we show that total
1157 ecosystem C storage declines during canopy transition to late-succession forests,
1158 irrespective of successional pathway, conflicting traditional views that old forests
1159 continually accumulate C as they age. However, further studies that include stands older
1160 than 200 years must be conducted to determine how long this decline phase persists and
1161 at what point do old boreal forests achieve a steady C state.

1162

1163 **Chapter Four: The relationship between tree growth and species**
1164 **diversity change with succession and relative size in the boreal forest**

1165 **Introduction**

1166 The past several decades have seen a sharp increase in the number of studies investigating
1167 the relationship between species diversity and forest ecosystem functions. Major
1168 advances have demonstrated positive correlations between diversity and a wide range of
1169 ecosystem processes, such as productivity (Liang et al. 2016). Nonetheless, most
1170 diversity-productivity studies have tended to focus on the effect of species diversity on
1171 community-level productivity, often overlooking variability in the response of individual
1172 species to diversity at the neighbourhood level. Because diversity-productivity
1173 relationships are largely realized through the growth response of individual tree species
1174 (Barrufol et al. 2013, Chamagne et al. 2017), a closer examination of species-specific
1175 growth responses to species diversity is warranted (Fichtner et al. 2017).

1176 The positive effect of species diversity on forest productivity is widely considered
1177 to be driven by niche complementarity (i.e., resource partitioning) and interspecific
1178 facilitation (Tilman 1999, Hooper et al. 2005, Williams et al. 2017). Therefore, it is not
1179 unreasonable to expect that the relationship between species diversity and tree growth
1180 may vary as forests undergo succession leading to changes in site conditions and
1181 resources over time (Forrester 2014, Mina et al. 2017). For example, during early
1182 succession, following stand-replacing disturbance, resources such as light and soil
1183 nutrients are plentiful and the role of niche complementarity and interspecific facilitation
1184 in governing productivity may be less influential. However, resources become more
1185 limited as forests develop and competition intensifies. This could amplify positive
1186 diversity effects since species interactions may increase under harsher, resource-limited
1187 conditions, as postulated by the stress gradient hypothesis (Bertness and Callaway 1994).

1188 In addition, as tree species composition transitions from fast-growing, early-succession
1189 colonizers to slower-growing, shade-tolerant, late succession species (Chen and
1190 Popadiouk 2002, Taylor and Chen 2011), overlapping mixtures of early and late
1191 succession species may exhibit stronger niche complementarity effects due to higher
1192 diversity of contrasting life history traits in the community (Coomes et al. 2009).
1193 Nonetheless, despite efforts to investigate the relationship between species diversity and
1194 tree growth (Cavard et al. 2010, Chamagne et al. 2017, Fichtner et al. 2017), few studies
1195 have attempted to examine how this relationship may vary as forests undergo succession.

1196 The relationship between species diversity and tree growth may also be influenced
1197 by individual tree size as tree size strongly affects its ability to compete for site resources
1198 (Coomes et al. 2011). For example, competition for light among trees is size-asymmetric,
1199 in that larger trees capture disproportionately greater amount of light when interacting with
1200 smaller ones. As a result, positive diversity effects may be stronger for trees of relatively
1201 larger size. This has been indirectly supported by a recent study that showed overstorey
1202 tree species diversity has a positive effect on canopy trees but negligible or negative
1203 effect on understorey trees (Zhang et al. 2016). However, previous studies have also
1204 reported conflicting results, observing stronger complementarity effect on smaller rather
1205 than larger trees (Madrigal-González et al. 2016), or no size-dependent effect at all (Báez
1206 and Homeier 2018). Such differences in results may be because past studies have not
1207 included a wide enough range of tree sizes, with most focusing on trees greater than 10
1208 cm in diameter at breast height (DBH). Moreover, directly using DBH to represent tree
1209 size neglects the important role of stand age on tree size, as trees of the same size may
1210 constitute different forest strata (e.g., understorey vs. overstorey) depending on stand
1211 development stage. For example, a trembling aspen tree of 20 cm DBH can be the largest

1212 tree in the forest canopy during early to mid-succession, but may only represent a mid-
1213 size tree as the forest transitions into late-succession.

1214 It has also been hypothesized that the effect of species diversity on tree growth
1215 may differ according to the type of disturbance from which the forest ecosystem
1216 originates (Forrester and Bauhus 2016), possibly due to the different effects of
1217 disturbances on soil nutrient availability (Thiffault et al. 2008, Hume 2016). For example,
1218 historically, fire is considered the predominant form of stand-replacing disturbance in the
1219 boreal forest, but harvesting increasingly affects millions of hectares of boreal forests
1220 each year (Pan et al. 2013). Although leaching of soil nutrients occurs following both fire
1221 and harvesting, fire-driven volatilization of nutrients results in generally higher net losses
1222 of nutrients than harvesting immediately after disturbance (Thiffault et al. 2007). This
1223 divergence is likely to dissipate as forests age (Hume 2016), but it is possible that the
1224 effect of diversity on tree growth may be stronger in more resource-limited, young post-
1225 fire stands than that of post-harvest stands.

1226 In this study, we used a chronosequence sampling design to examine the
1227 relationship between species diversity and tree growth rate of five major tree species in
1228 the boreal forests of central Canada. Our chronosequence covered a wide range of forest
1229 stand ages, following stand-replacing fire (from 8 to 210 years old) and harvesting (from
1230 8 to 34 years old). We hypothesized that (1) the effect of species diversity on tree growth
1231 rate would change as forest stands undergo succession and become stronger when
1232 competition for resources intensifies or as functional diversity increases when early and
1233 late succession species co-dominate; (2) the effect of species diversity would increase
1234 with relative tree size since trees have size-asymmetric competitive ability for resources;
1235 (3) the effect of species diversity on tree growth rate would be stronger in more resource-
1236 limited, post-fire stands than that of post-harvest stands.

1237 **Materials and Methods**

1238 *Study area*

1239 Our study was conducted in the boreal forest region, approximately 150 km north of
1240 Thunder Bay, Ontario, Canada, between 49°44' to 49°65' N and 89°16' to 90°13' W. This
1241 area is characterized by warm summers and cold, snowy winters. Mean annual
1242 temperature is 1.9 °C and mean annual precipitation is 824.8 mm as measured by the
1243 closest meteorological station in Cameron Falls, Ontario, Canada (Environment Canada
1244 2017). Soils in our study area were largely deposited by the Wisconsinan glaciation,
1245 which ended approximately 9,500 years ago in this region. Stand-replacing wildfire is the
1246 most common natural disturbance in the study area, with an average fire-return interval of
1247 approximately 100 years during the past century, resulting in a mosaic of stand ages
1248 across the landscape (Senici et al. 2010). Commercial harvesting began in our study area
1249 in the 1970s. Dominant tree species in our study area, in order from least to most shade
1250 tolerant, include jack pine, trembling aspen, white birch, spruce, and balsam fir.

1251 *Sampling design*

1252 We employed the chronosequence sampling method to study how species diversity
1253 effects vary as forest stands develop through succession and following different
1254 disturbance origins. Although the use of the chronosequence method has been criticized
1255 due to its assumption that sample stands along the temporal sequence have followed the
1256 same developmental history (Johnson and Miyanishi 2008), given careful site selection,
1257 replication, and demonstration of developmental links, the chronosequence method is
1258 appropriate for studying successional dynamics over decadal to centennial time scales
1259 (Walker et al. 2010).

1260 Based on local fire history and the availability of different aged stands in our
1261 study area, we were able to sample six different stand age classes (i.e., time since last

1262 stand-replacing disturbance), including 8, 16, 34, 99, 147, and 210 years since fire,
1263 representing the stand initiation, late stand initiation, stem exclusion, early canopy
1264 transition, late canopy transition, and gap dynamic stages of boreal forest development,
1265 respectively ([Chen and Popadiouk, 2002](#)). Harvest-origin stands were only sampled for
1266 the stand initiation and stem exclusion stages (i.e., 8, 16, 34 years since harvesting)
1267 because commercial harvesting did not begin in our study area until the early 1970s. To
1268 ensure we included a wide variety of forest overstorey types and to account for the fact
1269 that similar boreal forest sites may undergo multiple successional pathways, we carefully
1270 selected three different overstorey types (i.e., broadleaf, conifer and mixedwood) at each
1271 age class. More specifically, for stands in the stand initiation and stem exclusion stages of
1272 development (i.e., years 8, 16, and 34), we selected broadleaf stands that were dominated
1273 by trembling aspen, conifer stands that were dominated by jack pine, and mixedwood
1274 stands that contained a mixture of jack pine and trembling aspen. For stands in the canopy
1275 transition (i.e., years 99, 147) and gap dynamics stages (i.e., year 210), we selected
1276 broadleaf stands dominated by self-replacing trembling aspen and white birch, conifer
1277 stands that were transitioning from jack pine to spruce and balsam fir dominance, and
1278 mixedwood stands that were co-dominated by trembling aspen, white birch, spruce and
1279 balsam fir.

1280 We selected post-disturbance stands that had not been managed (e.g., planted,
1281 sprayed or thinned), including three replicates for each age class and overstorey type
1282 combination; however, one replicate mixedwood stand at age 147 years was accidentally
1283 damaged by harvesting activities between consecutive years of sampling, resulting in a
1284 total of 80 stands measured overall. Sample stands were positioned several kilometers
1285 away from each other and selected from different road accesses to minimize the impact of
1286 spatial autocorrelation. In order to minimize site variability, all selected stands were

1287 located on mesic sites with flat mid-slope positions, with no slope exceeding 5%. All
1288 stands are underlain by moderately deep (≥ 50 cm) glacial tills, belonging to the
1289 Brunisolic soil order, according to the Canadian system of soil classification (Soil
1290 Classification Working Group 1998). To ensure that each sample stand met the selection
1291 criteria, soil pits were dug in each candidate stand to verify whether the site was mesic,
1292 following the procedures described in Taylor et al. (2000). Soil attribute data for all sites
1293 used in this study have been previously reported by Hume et al. (2016).

1294 Stand age for sample stands less than 90 years old was determined from detailed
1295 fire and harvesting records (Hart and Chen 2008, Senici et al. 2010). For stands greater
1296 than 90 years old, tree ages were used to estimate minimum stand age following the
1297 procedures described in Senici et al. (2010). Of all sampled stands, we selected either jack
1298 pine or trembling aspen trees to determine minimum stand age. In each stand, three
1299 canopy stems were sampled by extracting a core or stem disk at breast height (1.3 m
1300 above root collar). The cores and disks were transported to our laboratory, where the
1301 cores were mounted on constructed core strips and sanded to make rings visible. Stem
1302 disks were cut transversely, then mounted on constructed core strips and sanded to make
1303 rings visible. Rings were counted using a handheld magnifier or a microscope until the
1304 same count was obtained three successive times. Based on a locally derived age
1305 correction model developed by Vasiliauskas and Chen (2002), 7 years were added to ring
1306 counts to determine minimum stand age.

1307

1308

1309

1310

1311

1312 **Table 4-1** Characteristics of the 80 sample stands in the northwest boreal forests of
 1313 Ontario, Canada.
 1314

Origin	Age	Overstorey ^a	N	Basal area (m ² ha ⁻¹)	Stand composition (%) ^b						
					Trembling aspen	White birch	Jack pine	Spruce spp.	Balsam fir	Others	
Fire	8	B	3	3 (1)	92 (3)	3 (3)	3 (3)			2 (1)	
		C	3	4 (1)			100 (0)				
		M	3	5 (1)	30 (6)	4 (2)	66 (4)				
	16	B	3	20 (2)	89 (6)	9 (5)	1 (1)			1 (1)	
		C	3	9 (1)	1 (1)	1 (1)	97 (2)			1 (1)	
	34	M	3	9 (1)	30 (3)		67(1)	2 (2)		1 (1)	
		B	3	25 (1)	94 (3)	4. (4)	1 (1)	1 (1)			
		C	3	28 (2)	4 (2)	1 (1)	94 (3)	1 (1)			
	99	M	3	17 (3)	50 (4)		41 (5)	8 (5)		1 (1)	
		B	3	51 (7)	91 (2)	3 (2)		1 (1)	1 (1)	4 (1)	
		C	3	52 (2)	3 (2)		43 (12)	50 (17)	4 (3)		
	147	M	3	43 (5)	40 (12)	16 (11)		9 (6)	15 (8)	18 (3)	2 (1)
		B	3	58 (8)	85 (3)	7 (4)			5 (1)	2 (1)	1 (1)
		C	3	51 (9)	1 (1)	2 (2)	53 (27)		37 (26)	7 (1)	
	210	M	2	36 (3)	38 (2)	30 (1)			12 (5)	19 (4)	1 (1)
B		3	41 (3)	54 (22)	24 (18)			10 (6)	10 (4)	2 (1)	
C		3	40 (8)	5 (5)	7 (4)			36 (18)	50 (17)	2 (1)	
Harvest	8	M	3	46 (3)	11 (4)	39 (5)	5 (3)	38 (7)	7 (3)		
		B	3	5 (2)	89 (6)	3 (2)	5 (3)			3 (3)	
		C	3	8 (1)		4 (4)	94 (5.9)	2 (2)			
	16	M	3	8 (1)	47 (14)		48 (10)			5 (5)	
		B	3	16 (4)	91 (8)	1 (1)	6 (6)			2 (2)	
		C	3	4 (1)			98 (2)	2 (2)			
	34	M	3	13 (2)	32 (3)		68 (2)				
		B	3	21 (1)	80 (5)	17 (5)	1 (1)	1 (1)		1 (1)	
		C	3	30 (2)	1 (1)		96 (2)	2 (1)		1 (1)	
		M	3	19 (2)	44 (6)	1 (1)	50 (4)	5 (4)			

1315 *Notes:* Each age-overstorey combination has three replications, values are means with 1
 1316 SE in parentheses.

1317 ^a Overstorey types: B = broadleaf, C = conifer, M = mixedwoods.

1318 ^b The 'Others' category includes *Salix* spp., *Acer spicatum*, *Alnus viridis*, *Sorbus decora*,
 1319 *Corylus cornuta*, *Prunus pensylvanica*, and *Larix laricina*.

1320 *Data collection*

1321 Within each of the 80 selected stands, we randomly located and established a 0.04 ha
1322 (11.28 m radius) fixed area circular plot, approximately 50 or more meters from the forest
1323 edge, to represent the stand. We recorded species identity and diameter at breast height
1324 (DBH, 1.3 m above root collar) for all trees larger than 9 cm DBH within each plot.
1325 Overstory types were assigned based on the relative basal area of broadleaf and conifer
1326 tree species in a plot. Broadleaf and conifer stands were defined as having > 80%
1327 broadleaf or conifer tree species composition by stem density or basal area while all other
1328 stands were classified as mixedwood stands (Table 1).

1329 To determine tree growth, we used stratified random sampling to select trees of
1330 varying diameter at breast height, from tree stems with a minimum DBH of 2 cm to the
1331 maximum DBH that could be found for each species in a given sample plot. Because
1332 species composition changes with succession, we sampled jack pine from the 8-, 16-, 34-,
1333 99-, and 147-year-old age classes in the fire-origin stands and all three age classes in the
1334 harvest-origin stands; trembling aspen from all age classes in fire- and harvest-origin
1335 stands; white birch, spruce, and balsam fir from the 99-, 147-, and 210-year-old age
1336 classes in the fire-origin stands. For each species within each sample plot, tree size was
1337 grouped into 4 cm DBH intervals and we randomly sampled up to three trees from each
1338 DBH group if available. Trees with crooked stems, substantial heart-rot, or other forms of
1339 stem damage, such as stem abrasion, fungal infections, or major branch losses were not
1340 sampled. In total, 255, 395, 136, 167, and 135 trees for jack pine, trembling aspen, white
1341 birch, spruce, and balsam fir, respectively, were sampled across all overstory type, stand
1342 ages, and stand origins.

1343 For each sample tree, increment cores or disk samples were collected to estimate
1344 annual tree radial growth increment. For trees <10 cm in DBH, we cut a stem disk at

1345 DBH, while for trees ≥ 10 cm in DBH, we used an increment core borer with a 5.15 mm
1346 diameter bit to extract stem core samples. All samples were sealed in plastic bags (for
1347 disks) or straws (for cores) and transported from the field to the laboratory. In the
1348 laboratory, all stem disk and core samples were sanded and annual increments were
1349 measured using a WinDENDRO measuring system.

1350 Tree annual growth rate was calculated as the average annual basal area increment
1351 of the last 5 years (2009 – 2014). Because tree-ring measurements were conducted for
1352 growth within tree bark, while tree basal area growth is calculated for DBH outside the
1353 bark, similar to [Chen and Klinka \(2003\)](#), we first developed species-specific relationships
1354 between DBH with and without bark. We then used these relationships to calculate DBH
1355 with bark from measured stemwood DBH in 2009 and 2014 for each sample.

1356 We calculated Shannon's diversity index (H') as a measure of species diversity for
1357 each sample plot, which accounts for both species richness and evenness:

$$1358 \quad H' = \sum_{i=1}^S P_i \ln(P_i) \quad (1)$$

1359 where S is the total species richness, P_i is the relative abundance of species i based on
1360 basal area of the i th species.

1361 To examine whether the effect of species diversity on tree growth rate varies with
1362 relative tree size in a stand, we also calculated the relative sizes for all individual trees by
1363 using basal area (BA) of each sample tree divided by the mean BA of the sample plot. We
1364 used relative tree size instead of the absolute tree size (i.e., DBH) because our study
1365 covered a wide range of stand ages and relative size better reflects the competitive
1366 position of individual trees when encountering other individuals in a stand ([Luo and
1367 Chen, 2015](#)).

1368 *Data analysis*

1369 To address our first and second hypotheses, we used linear mixed-effect models to test
1370 the effects of the Shannon index, stand age, relative size, and their two-way interactions
1371 on tree growth rate for each of the five tree species in our study:

$$1372 Y = \beta_0 + \beta_1 A + \beta_2 D + \beta_3 RS + \beta_4 A \times D + \beta_5 A \times RS + \beta_6 D \times RS + \beta_7 A \times D \times RS + \\ 1373 \beta_8 BA + \pi_{plot} + \varepsilon \quad (2)$$

1374 where Y is annual basal area growth rate ($\text{cm}^2 \text{ year}^{-1}$) for a given tree species, A
1375 represents stand age (years) following fire (a categorical variable), D is the Shannon
1376 index (a continuous variable), RS is relative tree size (a continuous variable), BA is plot-
1377 level basal area ($\text{m}^2 \text{ ha}^{-1}$; a continuous variable) which accounts for plot stand density,
1378 π_{plot} is the random effect of the sample plots which accounts for autocorrelation among
1379 trees sampled within each plot, and ε is the sampling error. We conducted the analysis
1380 using maximum likelihood estimation with the “lme4” package ([Bates et al., 2017](#)). All
1381 continuous variables were scaled prior to analysis to ease interpretation by using the
1382 “scale” function from the “data.table” R package ([Dowle et al., 2017](#)).

1383 To assess our third hypothesis, we included stand origin (O) and its interactions
1384 with the other factors in the Eq. 2, using the following full, linear mixed effect model:

$$1385 Y = \beta_0 + \beta_1 D + \beta_2 RS + \beta_3 A + \beta_4 O + \beta_5 BA + \beta_6 D \times RS + \beta_7 D \times A + \beta_8 RS \times A + \\ 1386 \beta_9 D \times O + \beta_{10} RS \times O + \beta_{11} A \times O + \beta_{12} D \times RS \times A + \beta_{13} D \times RS \times O + \\ 1387 \beta_{14} D \times A \times O + \beta_{15} RS \times A \times O + \beta_{16} D \times RS \times A \times O + \pi_{plot} + \varepsilon \quad (3)$$

1388 Shapiro-Wilk’s tests on model residuals showed some statistical models did not meet the
1389 assumption of normality at $\alpha = 0.05$. We used bootstrapped regression parameter
1390 estimates by 1000 iterations from package “boot” ([Canty and Ripley, 2017](#)) and
1391 compared the bootstrapped estimates with those of the linear models. Both methods

1392 yielded quantitatively similar trends, so we reported the linear model results for simplicity.

1393 All data analyses were performed in R 3.4.1 ([R Development Core Team, 2017](#)).

1394 **Results**

1395 *Tree growth-diversity relationships in relation to stand age*

1396 The relationship between tree growth and species diversity in postfire stands differed with
1397 tree species and among stand ages (Table 4-2, Fig.4-1). During the stand initiation stage
1398 of development, from age 8 to 16 years, there was no significant relationship between tree
1399 growth rate and species diversity for any of the species sampled (Fig. 4-1a, and 4-1b).
1400 When stands entered the stem exclusion stage (i.e., 34 years old), tree growth rate of
1401 trembling aspen marginally decreased with species diversity, while tree growth of jack
1402 pine marginally increased (Fig. 4-1c). As stands aged to early canopy transition stage
1403 (i.e., 99 years old), tree growth rate decreased significantly and marginally with species
1404 diversity for trembling aspen and white birch, whereas the growth rate of spruce and jack
1405 pine marginally increased. There was no relationship between tree growth rate of balsam
1406 fir and species diversity in the 99 years old stands (Fig. 4-1d). During the late canopy
1407 transition stage (i.e., 147 years old), tree growth rate increased significantly and
1408 marginally with species diversity for trembling aspen and spruce, respectively, while no
1409 growth-diversity relationship for jack pine, white birch, and balsam fir was observed (Fig.
1410 4-1e). In the 210 years old stands, there was no significant relationship between tree
1411 growth rate and species diversity except a marginally positive relationship for spruce
1412 (Fig. 4-1f).

1413 *Tree growth-diversity relationships in relation to relative size*

1414 The relationship between tree growth and diversity also changed with relative tree size,
1415 depending on species and stand age (Table 4-2, Fig. 4-2). The tree growth-diversity
1416 relationship was significantly positive for large sized trees of jack pine at age 99 and
1417 spruce at age 147 (Fig. 4-2a and 4-2d). The negative relationship between tree growth and
1418 diversity for trembling aspen significantly increased with relative tree size at age 99,

1419 whereas at age 147 and 210, tree growth of relatively small sized trembling aspen
 1420 positively related with species diversity (Fig. 4-2b). The relationship between tree growth
 1421 and species diversity did not change with relative size for white birch and balsam fir (Fig.
 1422 4-2c and 4-2e).

1423 *Tree growth-diversity relationships in relation to disturbance origin*

1424 Tree growth rate of jack pine and trembling aspen did not significantly differ according to
 1425 stand origin during early succession (age 8 to 34 years; Table 4-3). There was no overall
 1426 effect of species diversity on tree growth rate in either species in post-fire and post-
 1427 harvest stands during early successional stages. Further, differences in relative sizes and
 1428 stand ages did not produce significant diversity effects on tree growth (Table 4-3).

1429 **Table 4-2** The effects (*p* values) of stand age (A), diversity (D), relative size (RS) and
 1430 their two-way interactions on annual tree growth rate for five major tree species following
 1431 fire and harvesting disturbance, respectively. Statistically significant terms (*p* < 0.05) are
 1432 in bold.

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

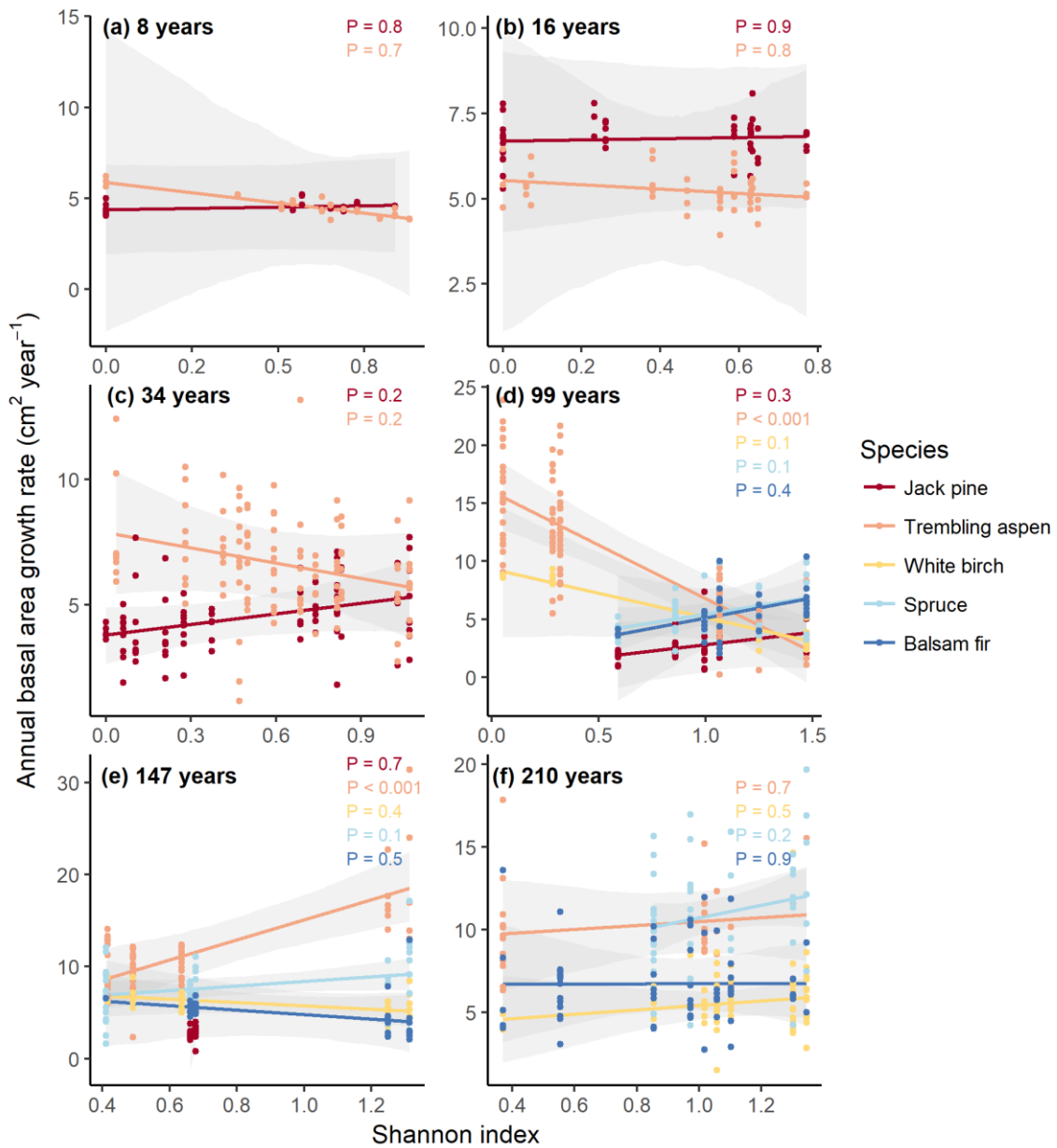
1434
 1435 Linear mixed effect model fit tests used Satterthwaite approximations of degrees of
 1436 freedom.

1437

1438 **Table 4-3.** The effects (*p* values) of stand age (A), diversity (D), relative size (RS),
 1439 disturbance origin (O), and their interactions on annual tree growth rate of two dominated
 1440 tree species following fire and harvesting disturbance, respectively. Statistically
 1441 significant terms (*p* < 0.05) are in bold.

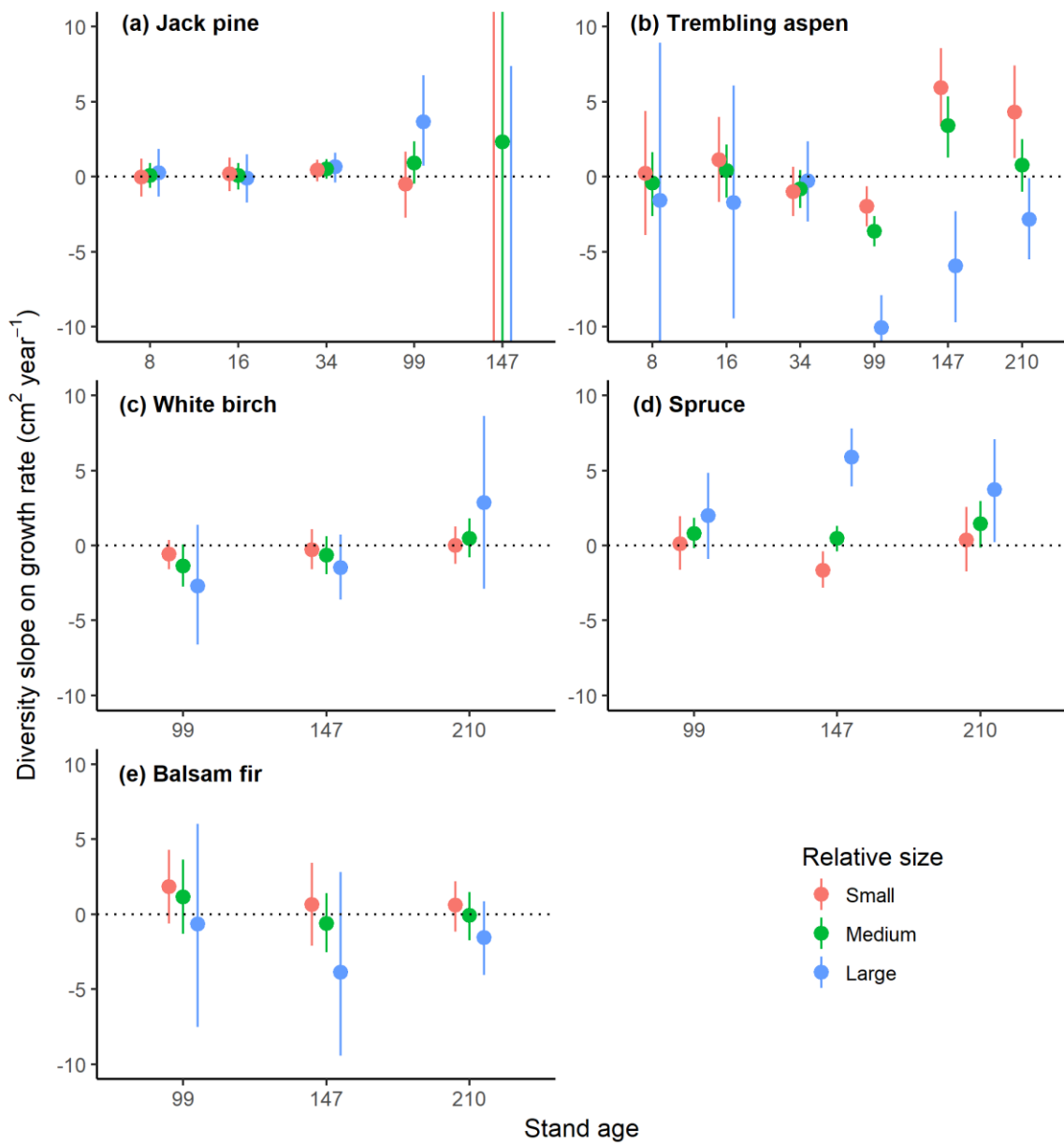
	Jack pine	Trembling aspen
D	0.437	0.495
RS	<0.001	<0.001
A	0.002	0.061
O	0.607	0.722
BA	0.469	0.347
D × RS	0.955	0.768
D × A	0.906	0.989
RS × A	0.025	0.376
D × O	0.235	0.675
RS × O	0.715	0.670
A × O	0.064	0.877
D × RS × A	0.703	0.627
D × RS × O	0.109	0.413
D × A × O	0.400	0.818
RS × A × O	0.035	0.240
D × RS × A × O	0.668	0.847

1442 Linear mixed effect model fit tests used Satterthwaite approximations of degrees of
 1443 freedom.



1444

1445 **Figure 4-1** Effect of Shannon index on the annual basal area growth rate of five tree
 1446 species in relation to stand age after accounting for relative size and stand level basal
 1447 area. Colors indicate species. Lines with shading are means and 95% bootstrapped
 1448 confidence intervals of the slope fitted by linear mixed-effect models.



1449

1450

1451

1452

1453

1454

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..

1455 **Discussion**

1456 *Successional change and the tree growth-diversity relationship*

1457 Using a long-term, replicated chronosequence approach, our results showed that the
1458 relationship between tree growth and species diversity varied throughout forest
1459 succession. During stand initiation stages (8 and 16 years old), we found no strong
1460 evidence for a relationship between tree growth rate and species diversity, which is
1461 consistent with our hypothesis that weak species interactions during early succession,
1462 driven by high resources availability following disturbance, would lessen the effect of
1463 niche complementarity and facilitation on tree growth. However, when stands entered the
1464 more intense competition, stem exclusion stage (34 years old), there were marginally
1465 positive and negative relationships between tree growth and species diversity for jack
1466 pine and trembling aspen, respectively, partially supporting our hypothesis that the effect
1467 of species diversity becomes stronger when competition for resources intensifies. This
1468 marginally, positive diversity effect may have resulted from improved soil conditions for
1469 jack pine by inputs from nutrient-rich broadleaf litter ([Cote et al., 2000](#); [Hume et al.,
1470 2016](#)); whereas tree growth rate of trembling aspen can be negatively affected by the
1471 alteration of soil chemistry over time generated by conifer litter ([Prescott et al., 2000](#);
1472 [Calder et al., 2011](#); [Cavard et al., 2011](#)), despite less intense competition for light when
1473 mixed with conifers.

1474 As stands developed into the canopy transition stage (99 years old) of succession,
1475 our results show significant and marginal relationships between tree growth and diversity
1476 for trembling aspen, white birch, and spruce, supporting our hypothesis that species
1477 diversity effects on tree growth become stronger at key transition stages during forest
1478 succession ([Zhang et al., 2012](#)). When stands entered the early canopy transition stage (99
1479 years old), we observed a significantly negative relationship between species diversity

1480 and tree growth rate for trembling aspen. The stronger negative effect in 99 years old
1481 stands compared with that of 34 years old stands suggests that soil limitation may be
1482 more influential during the early canopy transition stage than stem exclusion stage. This
1483 is partially supported by the marginally negative diversity effect on tree growth of
1484 broadleaf white birch and marginally positive diversity effect on conifer species jack pine
1485 and spruce, in that negative effect of conifers on tree growth of broadleaf species through
1486 decrease in fertility became noticeable when soil nutrient is limited ([Calder et al., 2011](#)).
1487 Whereas, conifers could benefit from improved soil condition generated by nutrient-rich
1488 broadleaf litters ([Cote et al., 2000](#)). However, we did not find any positive diversity effect
1489 on tree growth of balsam fir in 99 years old stands, despite the potential facilitation of soil
1490 nutrient availability by mixing with broadleaf species. An absence of positive diversity
1491 effect for balsam fir may be caused by its functional traits: trees with conservative
1492 strategies, such as slow-growing and shade-tolerant ([Reich, 2014](#)), respond less to the
1493 change of resources availability compared to fast-growing and shade-intolerant tree
1494 species ([Báez and Homeier, 2018](#)).

1495 During the late canopy transition stage (147 years old), the relationship between
1496 tree growth and diversity for trembling aspen changed from negative to positive. This
1497 change, corresponding with soil nutrients increase from age 99 to 147 years old ([Hume et
1498 al., 2016](#)), suggest that the limitation of soil nutrients may be less important for tree
1499 growth at this stage and tree growth of trembling aspen may have started to benefit from
1500 reduced aboveground competition for light when mixing with conifers ([Williams et al.,
1501 2017](#)). This could also explain the negligible diversity effect on the tree growth rate of
1502 white birch, where the negative diversity effect on tree growth of white birch may be
1503 limited during this stage and can be offset by benefiting from less competition for light.

1504 At the late-successional, gap dynamic stage (210 years old), there was marginal or
1505 no significant relationship between tree growth and diversity for any species. Negligible
1506 tree growth-diversity relationships at this stage may be attributable to weak facilitation
1507 and competition among species, largely because light and soil nutrients become less
1508 limiting at the gap dynamics stage compared with earlier stages ([Hume et al., 2016](#);
1509 [Kumar et al., 2017](#)). Moreover, trees in the late-successional stage are mostly large in size
1510 and old in age, which may also lead to less obvious diversity effect on tree growth since
1511 large trees allocate more production to self-maintenance than to radial growth
1512 ([Mencuccini et al., 2005](#)).

1513 *Impact of relative size on tree growth-diversity relationship*

1514 Our data yielded mixed results for the influence of relative tree size on the relationship
1515 between the tree growth and species diversity. We found that diversity effects on tree
1516 growth increased with relative size for jack pine at age 99 and spruce at age 147, which
1517 agrees with our hypothesis, suggesting that the diversity effect is shaped by the size-
1518 asymmetric competition of individual trees. Moreover, we found negative diversity effect
1519 on trembling aspen increased with relative size in 99 years old stands, while at age 147
1520 and 210, tree growth of relatively small-sized aspen positively correlated with species
1521 diversity. This negative diversity effect for all sized trees of trembling aspen at age 99
1522 further supports our speculation that soil limitation plays a key role in driving the effect
1523 of diversity on tree growth during the early canopy transition stage. While as stands
1524 entered the late canopy transition stage, correspondingly, soil fertility also increased from
1525 age 99 to age 147 ([Hume et al., 2016](#)), soil nutrient limitation may be less influential on
1526 relative small-sized trees compared with large trees since smaller trees require less soil
1527 nutrient to support growth. Therefore, relatively small trees of trembling aspen started to
1528 benefit from increased light availability when mixing with conifer trees. Moreover, small

1529 trees grow faster than relatively larger trees under improved conditions ([Wright et al.,](#)
1530 [2011](#); [Alvarez-Clare et al., 2013](#)). As a result, complementarity effects on the growth rate
1531 of small trees can be greater than that of large trees, despite size-asymmetric competition
1532 leading to more resources allocated to large trees ([Weiner, 1990](#)). Our results also showed
1533 that the influence of species diversity on tree growth of white birch and balsam fir did not
1534 change with relative size, which may suggest the growth of these species is less sensitive
1535 to changes in resources availability.

1536 *Tree growth-diversity relationship following fire vs. harvesting*

1537 Contrary to our hypothesis, we found no significant difference of species diversity effects
1538 on tree growth rate between our two stand origins during early-successional stages for
1539 any of the dominant tree species, despite the different effect of fire on soil nutrients
1540 compared with harvesting ([Thiffault et al., 2007](#); [Thiffault et al., 2008](#); [Hume, 2016](#)). This
1541 may be attributable to weak species interactions during the early stand initiation stage,
1542 where the limitation of soil nutrients on tree growth is less important. Our results suggest
1543 that disturbance origin had limited effect on the relationship between tree growth and
1544 species diversity and provide further evidence that common forest management practices
1545 (i.e., harvesting) and fire may have a similar impact on ecosystem functions in young
1546 boreal forests ([Seedre et al., 2014](#)). However, it is unclear whether the similar growth
1547 responses to species diversity in post-fire and post-harvest stands can be extrapolated to
1548 older ages. Future studies should examine whether differences in the tree growth rate-
1549 species diversity effect between stand origins become more apparent at older ages.

1550 In summary, by studying the relationships between species diversity and tree
1551 growth rate of five species, we find that the relationship is largely dependent on tree
1552 species, forest successional stage, and relative tree size. There was no relationship
1553 between diversity and tree growth during the early successional stages (from 8 to 34 years

1554 old), whereas both positive and negative diversity effects were observed at the canopy
1555 transition stage. Species diversity was positively related to tree growth rate of spruce but
1556 negatively related to tree growth rate of trembling aspen and white birch during the early
1557 canopy transition stage (99 years old). While in the late canopy transition stage (147 years
1558 old), tree growth was positively related to species diversity for both trembling aspen and
1559 spruce. At the late successional stage (210 years old), there was a negligible relationship
1560 between tree growth rate and species diversity for any species. Moreover, species
1561 diversity effects on tree growth rates increased with relative size for trembling aspen and
1562 spruce according to stand age but there was no association between diversity effects and
1563 relative size for white birch. The relationship between tree growth and species diversity
1564 did not differ between post-fire and post-harvest stands during early successional stages.
1565 Our findings emphasize the importance of considering species-specific, size-dependent
1566 growth related to forest succession when examining relationships between biodiversity
1567 and forest ecosystem functions.

1568

1569 **Chapter Five: General conclusion**

1570 By using chronosequence design that covered a wide range of stands ages, undergoing
1571 multiple successional pathways following stand-replacing fire and harvesting, in the
1572 boreal forest of central Canada, I quantified the variation of carbon concentration for six
1573 major tree species of the boreal forest in Chapter two, I then examined the long-term C
1574 storage dynamics following fire in Chapter three based on the species-specific C
1575 concentration data from Chapter two. Finally, I investigated the relationship between tree
1576 growth and species diversity in relation to succession, relative tree size, and disturbance
1577 origin for five species in Chapter four. A summary of key findings of this dissertation are
1578 as follows:

- 1579 1. Bark had significantly higher total C and volatile C concentrations than stemwood
1580 and that both total C and volatile C concentration significantly varied among tree
1581 species. The average total C concentrations were 56.2% in the bark and 50.5% in
1582 the stemwood, and the average volatile C concentration were 5.8% and 3.0% for
1583 bark and stemwood, respectively. Furthermore, total C and volatile C
1584 concentration in stemwood and bark of almost all shade-intolerant species
1585 increased with tree size, whereas those of shade-tolerant species showed negative
1586 or neutral size-associated change. Our results show that volatile C concentration is
1587 a key driver of variation in total C concentration.
- 1588 2. There was a strong relationship between total ecosystem C storage and stand age
1589 following fire. Broadleaf stands had on average higher total ecosystem C;
1590 however, the inferred temporal dynamics of total ecosystem C were similar among
1591 all three overstorey types. Importantly, we found that total ecosystem C storage
1592 declined from canopy transition to late succession stages, irrespective of

1593 succession pathway, contradicting views that old forests continually accumulate C
1594 as they age.

1595 3. During long-term succession following fire, significant relationships between tree
1596 growth and species diversity at the canopy transition stage for trembling aspen,
1597 white birch, and spruce. Species diversity positively correlated with the growth
1598 rate of spruce but negatively affected the growth rate of trembling aspen and white
1599 birch during early the canopy transition stage (99 years old). However, when
1600 stands entered the late canopy transition stage (147 years old), species diversity
1601 promoted tree growth for both trembling aspen and spruce. Diversity had a
1602 positive effect on the growth of larger trees for jack pine at age 99, and spruce at
1603 age 147, but, had negative effect with increased size of trembling aspen at age 99,
1604 147, and 210. Furthermore, there was no difference in the tree growth-diversity
1605 relationships between post-fire and post-harvest stands during early successional
1606 stages.

1607 Overall, the findings of this dissertation improve our understanding of long-
1608 term C storage dynamics following wildfire by providing accurate C concentration
1609 data and including a diverse range of overstorey composition to account for multiple
1610 succession pathways. In Chapter two, the results highlight the importance of
1611 considering the species-specific C concentration when estimating live tree C storage
1612 and emphasize that variation of C concentration related to tree size and woody tissue
1613 (e.g., bark vs. stemwood) need to be accounted for in national, biome, or global C
1614 models. In Chapter three, the results indicate that total ecosystem C storage declines
1615 during canopy transition stage irrespective of successional pathway and highlight the
1616 importance of including stands older than 200 years to capture the canopy transition
1617 stage to determine the long-term C storage dynamics in boreal forests. Moreover,

1618 results of Chapter four suggest that knowledge of species-specific, size-dependent tree
1619 growth response to species diversity related to forest succession is critical to better
1620 understand the relationships between biodiversity and forest ecosystem functions.

1621 **References**

- 1622 Alemdag IS. 1984. *Wood density variation of 28 tree species from Ontario*. Chalk River,
1623 ON: Petawawa National Forestry Institute, Natural Resources Canada
- 1624 Alvarez-Clare S, Kitajima K. 2007. Physical defence traits enhance seedling survival of
1625 neotropical tree species. *Functional Ecology* 21: 1044-1054.
- 1626 Alvarez-Clare S, Mack MC, Brooks M. 2013. A direct test of nitrogen and phosphorus
1627 limitation to net primary productivity in a lowland tropical wet forest. *Ecology* 94:
1628 1540-1551.
- 1629 Angers VA, Drapeau P, Bergeron Y. 2010. Snag degradation pathways of four North
1630 American boreal tree species. *Forest Ecology and Management* 259: 246-256.
- 1631 Báez S, Homeier J. 2018. Functional traits determine tree growth and ecosystem
1632 productivity of a tropical montane forest: Insights from a long-term nutrient
1633 manipulation experiment. *Global Change Biology* 24: 399-409.
- 1634 Barrufol M, Schmid B, Bruelheide H, *et al.* 2013. Biodiversity Promotes Tree Growth
1635 during Succession in Subtropical Forest. *PLoS One* 8: e81246.
- 1636 Bates D, Maecler M, Bolker B, *et al.* 2017. Package "lme4". [https://cran.r-](https://cran.r-project.org/web/packages/lme4/lme4.pdf)
1637 [project.org/web/packages/lme4/lme4.pdf](https://cran.r-project.org/web/packages/lme4/lme4.pdf).
- 1638 Becker GS, Braun D, Gliniars R, *et al.* 2012. Relations between wood variables and how
1639 they relate to tree size variables of tropical African tree species. *Trees-Structure*
1640 *and Function* 26: 1101-1112.
- 1641 Bellassen V, Luyssaert S. 2014. Managing forests in uncertain times. *Nature* 506: 153-
1642 155.
- 1643 Bert D, Danjon F. 2006. Carbon concentration variations in the roots, stem and crown of
1644 mature *Pinus pinaster* (Ait.). *Forest Ecology and Management* 222: 279-295.

- 1645 Bonan GB, Shugart HH. 1989. Environmental factors and ecological processes in boreal
1646 forests. *Annual review of ecology and systematics*. Vol. 20: 1-28.
- 1647 Bond-Lamberty B, Peckham SD, Ahl DE, *et al.* 2007. Fire as the dominant driver of
1648 central Canadian boreal forest carbon balance. *Nature* 450: 89-92.
- 1649 Bond-Lamberty B, Wang C, Gower ST. 2002. Aboveground and belowground biomass
1650 and sapwood area allometric equations for six boreal tree species of northern
1651 Manitoba. *Canadian Journal of Forest Research* 32: 1441-1450.
- 1652 Bond-Lamberty B, Wang C, Gower ST. 2004. Net primary production and net ecosystem
1653 production of a boreal black spruce wildfire chronosequence. *Global Change*
1654 *Biology* 10: 473-487.
- 1655 Brant AN. 2014. Foliar nutrient resorption and litterfall production with stand age,
1656 overstory composition, and disturbance origin in boreal forests. Lakehead
1657 University.
- 1658 Brassard BW, Chen HYH. 2008. Effects of Forest Type and Disturbance on Diversity of
1659 Coarse Woody Debris in Boreal Forest. *Ecosystems* 11: 1078-1090.
- 1660 Brassard BW, Chen HYH, Bergeron Y, *et al.* 2011. Coarse root biomass allometric
1661 equations for *Abies balsamea*, *Picea mariana*, *Pinus banksiana*, and *Populus*
1662 *tremuloides* in the boreal forest of Ontario, Canada. *Biomass & Bioenergy* 35:
1663 4189-4196.
- 1664 Brassard BW, Chen HYH, Wang JR, *et al.* 2008. Effects of time since stand-replacing
1665 fire and overstory composition on live-tree structural diversity in the boreal forest
1666 of central Canada. *Canadian Journal of Forest Research* 38: 52-62.
- 1667 Burnham KP, Anderson DR. 2002. *Model selection and multimodel inference: a practical*
1668 *information-theoretic approach*: Springer Science & Business Media.

- 1669 Burns RM, Honkala BH. 1990. *Silvics of North America*. Washington, D.C., USA:
1670 United States Government Printing.
- 1671 Calder WJ, Horn KJ, St Clair SB. 2011. Conifer expansion reduces the competitive
1672 ability and herbivore defense of aspen by modifying light environment and soil
1673 chemistry. *Tree Physiology* 31: 582-591.
- 1674 Canadell JG, Raupach MR. 2008. Managing forests for climate change mitigation.
1675 *Science* 320: 1456-1457.
- 1676 Canty A, Ripley B. 2017. Package "boot". [https://cran.r-](https://cran.r-project.org/web/packages/boot/boot.pdf)
1677 [project.org/web/packages/boot/boot.pdf](https://cran.r-project.org/web/packages/boot/boot.pdf).
- 1678 Cavard X, Bergeron Y, Chen HYH, *et al.* 2011. Competition and facilitation between tree
1679 species change with stand development. *Oikos* 120: 1683-1695.
- 1680 Chamagne J, Tanadini M, Frank D, *et al.* 2017. Forest diversity promotes individual tree
1681 growth in central European forest stands. *Journal of Applied Ecology* 54: 71-79.
- 1682 Chave J, Condit R, Muller-Landau HC, *et al.* 2008. Assessing evidence for a pervasive
1683 alteration in tropical tree communities. *PLoS biology* 6: e45.
- 1684 Chen HYH, Brant AN, Seedre M, *et al.* 2016. The Contribution of Litterfall to Net
1685 Primary Production During Secondary Succession in the Boreal Forest.
1686 *Ecosystems*: 1-15.
- 1687 Chen HYH, Klinka K. 2003. Aboveground productivity of western hemlock and western
1688 redcedar mixed-species stands in southern coastal British Columbia. *Forest
1689 Ecology and Management* 184: 55-64.
- 1690 Chen HYH, Popadiouk RV. 2002. Dynamics of North American boreal mixedwoods.
1691 *Environmental Reviews* 10: 137-166.
- 1692 Chen HYH, Vasiliauskas S, Kayahara GJ, *et al.* 2009. Wildfire promotes broadleaves and
1693 species mixture in boreal forest. *Forest Ecology and Management* 257: 343-350.

1694 Clemmensen K, Bahr A, Ovaskainen O, *et al.* 2013. Roots and associated fungi drive
1695 long-term carbon sequestration in boreal forest. *science* 339: 1615-1618.

1696 Cote L, Brown S, Pare D, *et al.* 2000. Dynamics of carbon acid nitrogen mineralization in
1697 relation to stand type, stand age and soil texture in the boreal mixedwood. *Soil*
1698 *Biology & Biochemistry* 32: 1079-1090.

1699 Dixon RK, Solomon AM, Brown S, *et al.* 1994. Carbon Pools and Flux of Global Forest
1700 Ecosystems. *science* 263: 185-190.

1701 Dowle M, Srinivasan A, Gorecki J, *et al.* 2017. data.table: Extension of 'data.frame'. R
1702 package version 1.8-0. [https://cran.r-](https://cran.r-project.org/web/packages/data.table/index.html)
1703 [project.org/web/packages/data.table/index.html](https://cran.r-project.org/web/packages/data.table/index.html).

1704 Elias M, Potvin C. 2003. Assessing inter- and intra-specific variation in trunk carbon
1705 concentration for 32 neotropical tree species. *Canadian Journal of Forest*
1706 *Research-Revue Canadienne De Recherche Forestiere* 33: 1039-1045.

1707 Environment Canada. 2015. Canadian Climate Normals 1981-2010.
1708 http://climate.weather.gc.ca/climate_normals/index_e.html.

1709 Environment Canada. 2017. Canadian Climate Normals 1981-2010.
1710 http://climate.weather.gc.ca/climate_normals/index_e.html.

1711 Fichtner A, Härdtle W, Li Y, *et al.* 2017. From competition to facilitation: how tree
1712 species respond to neighbourhood diversity. *Ecology Letters* 20: 892-900.

1713 Flannigan MD, Stocks BJ, Wotton BM. 2000. Climate change and forest fires. *Science of*
1714 *the Total Environment* 262: 221-229.

1715 Franceschi VR, Krokene P, Christiansen E, *et al.* 2005. Anatomical and chemical
1716 defenses of conifer bark against bark beetles and other pests. *New Phytologist*
1717 167: 353-375.

1718 Garrett LG, Kimberley MO, Oliver GR, *et al.* 2012. Decomposition of coarse woody
1719 roots and branches in managed *Pinus radiata* plantations in New Zealand - A time
1720 series approach. *Forest Ecology and Management* 269: 116-123.

1721 Gartner TB, Cardon ZG. 2004. Decomposition dynamics in mixed-species leaf litter.
1722 *Oikos* 104: 230-246.

1723 Gauthier S, Bernier P, Kuuluvainen T, *et al.* 2015. Boreal forest health and global change.
1724 *science* 349: 819-822.

1725 GenABEL project developers. 2013. GenABEL: genome-wide SNP association analysis.
1726 R package version 1.8-0. <http://genabel.r-forge.r-project.org/>.

1727 Gough CM, Curtis PS, Hardiman BS, *et al.* 2016. Disturbance, complexity, and
1728 succession of net ecosystem production in North America's temperate deciduous
1729 forests. *Ecosphere* 7.

1730 Goulden ML, McMillan AMS, Winston GC, *et al.* 2011. Patterns of NPP, GPP,
1731 respiration, and NEP during boreal forest succession. *Global Change Biology* 17:
1732 855-871.

1733 Grunzweig JM, Valentine DW, Chapin FS. 2015. Successional Changes in Carbon Stocks
1734 After Logging and Deforestation for Agriculture in Interior Alaska: Implications
1735 for Boreal Climate Feedbacks. *Ecosystems* 18: 132-145.

1736 Harden JW, O'Neill K, Trumbore S, *et al.* 1997. Moss and soil contributions to the annual
1737 net carbon flux of a maturing boreal forest. *Journal of Geophysical Research:*
1738 *Atmospheres* (1984–2012) 102: 28805-28816.

1739 Harmon ME, Bible K, Ryan MG, *et al.* 2004. Production, respiration, and overall carbon
1740 balance in an old-growth *Pseudotsuga-tsuga* forest ecosystem. *Ecosystems* 7: 498-
1741 512.

- 1742 Hart SA, Chen HY. 2008. Fire, logging, and overstory affect understory abundance,
1743 diversity, and composition in boreal forest. *Ecological Monographs* 78: 123-140.
- 1744 Hengst GE, Dawson JO. 1994. Bark Properties and Fire Resistance of Selected Tree
1745 Species from the Central Hardwood Region of North-America. *Canadian Journal*
1746 *of Forest Research-Revue Canadienne De Recherche Forestiere* 24: 688-696.
- 1747 Hicke J, Allen CD, Desai AR, *et al.* 2012. The effects of biotic disturbance on carbon
1748 budgets of North American forests. *Glob. Change Biol* 18: 7-34.
- 1749 Hillebrand H, Matthiessen B. 2009. Biodiversity in a complex world: consolidation and
1750 progress in functional biodiversity research. *Ecology Letters* 12: 1405-1419.
- 1751 Hume A, Chen HYH, Taylor AR, *et al.* 2016. Soil C:N:P dynamics during secondary
1752 succession following fire in the boreal forest of central Canada. *Forest Ecology*
1753 *and Management* 369: 1-9.
- 1754 Hume AM. 2016. Temporal trends in the effects of wildfire and harvesting on soil
1755 nutrients and tree growth in northern forests of varying overstorey types. Lakehead
1756 University.
- 1757 Husch B, Beers TW, Kershaw Jr JA. 2002. *Forest mensuration*. New Jersey: John Wiley
1758 & Sons Inc.
- 1759 IPCC. 2013. *Climate Change 2013: The Physical Science Basis. Contribution of Working*
1760 *Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate*
1761 *Change*. Cambridge University Press Cambridge, UK and New York, NY, USA
- 1762 Jacob A, Hertel D, Leuschner C. 2014. Diversity and species identity effects on fine root
1763 productivity and turnover in a species-rich temperate broad-leaved forest.
1764 *Functional Plant Biology* 41: 678-689.
- 1765 Johnson EA, Miyanishi K. 2008. Testing the assumptions of chronosequences in
1766 succession. *Ecology Letters* 11: 419-431.

- 1767 Johnstone J, Chapin FS, III. 2006. Effects of Soil Burn Severity on Post-Fire Tree
1768 Recruitment in Boreal Forest. *Ecosystems* 9: 14-31.
- 1769 Kashian DM, Romme WH, Tinker DB, *et al.* 2013. Postfire changes in forest carbon
1770 storage over a 300-year chronosequence of *Pinus contorta*-dominated forests.
1771 *Ecological Monographs* 83: 49-66.
- 1772 Kelly R, Chipman ML, Higuera PE, *et al.* 2013. Recent burning of boreal forests exceeds
1773 fire regime limits of the past 10,000 years. *Proceedings of the National Academy*
1774 *of Sciences* 110: 13055-13060.
- 1775 Kneeshaw DD, Bergeron Y. 1998. Canopy gap characteristics and tree replacement in the
1776 southeastern boreal forest. *Ecology* 79: 783-794.
- 1777 Kumar P, Chen HYH, Thomas SC, *et al.* 2017. Linking resource availability and
1778 heterogeneity to understorey species diversity through succession in boreal forest
1779 of Canada. *Journal of Ecology*: n/a-n/a.
- 1780 Laganière J, Cavard X, Brassard BW, *et al.* 2015. The influence of boreal tree species
1781 mixtures on ecosystem carbon storage and fluxes. *Forest Ecology and*
1782 *Management* 354: 119-129.
- 1783 Laganière J, Paré D, Bergeron Y, *et al.* 2012. The effect of boreal forest composition on
1784 soil respiration is mediated through variations in soil temperature and C quality.
1785 *Soil Biology and Biochemistry* 53: 18-27.
- 1786 Laganière J, Paré D, Bergeron Y, *et al.* 2013. Stability of Soil Carbon Stocks Varies with
1787 Forest Composition in the Canadian Boreal Biome. *Ecosystems* 16: 852-865.
- 1788 Lambert MC, Ung CH, Raulier F. 2005. Canadian national tree aboveground biomass
1789 equations. *Canadian Journal of Forest Research* 35: 1996-2018.

1790 Lamtom SH, Savidge RA. 2003. A reassessment of carbon content in wood: variation
1791 within and between 41 North American species. *Biomass & Bioenergy* 25: 381-
1792 388.

1793 Lamtom SH, Savidge RA. 2006. Carbon content variation in boles of mature sugar maple
1794 and giant sequoia. *Tree Physiology* 26: 459-468.

1795 Lang SI, Cornelissen JHC, Klahn T, *et al.* 2009. An experimental comparison of chemical
1796 traits and litter decomposition rates in a diverse range of subarctic bryophyte,
1797 lichen and vascular plant species. *Journal of Ecology* 97: 886-900.

1798 Liang J, Crowther TW, Picard N, *et al.* 2016. Positive biodiversity-productivity
1799 relationship predominant in global forests. *science* 354.

1800 Liebhold AM, Macdonald WL, Bergdahl D, *et al.* 1995. Invasion by Exotic Forest Pests -
1801 a Threat to Forest Ecosystems. *Forest Science* 41: 1-49.

1802 Lovett GM, Canham CD, Arthur MA, *et al.* 2006. Forest ecosystem responses to exotic
1803 pests and pathogens in eastern North America. *BioScience* 56: 395-405.

1804 Luo Y, Chen HYH. 2011. Competition, species interaction and ageing control tree
1805 mortality in boreal forests. *Journal of Ecology* 99: 1470-1480.

1806 Luo Y, Chen HYH. 2015. Climate change-associated tree mortality increases without
1807 decreasing water availability. *Ecology Letters* 18: 1207-1215.

1808 Luysaert S, Schulze ED, Borner A, *et al.* 2008. Old-growth forests as global carbon
1809 sinks. *Nature* 455: 213-215.

1810 Ma Z, Chen HYH. 2016. Effects of species diversity on fine root productivity in diverse
1811 ecosystems: a global meta-analysis. *Global Ecology and Biogeography*: 1387-
1812 1396.

1813 MacDonalD RL, Burke JM, Chen HYH, *et al.* 2012. Relationship between Aboveground
1814 Biomass and Percent Cover of Ground Vegetation in Canadian Boreal Plain
1815 Riparian Forests. *Forest Science* 58: 47-53.

1816 Martin AR, Gezahegn S, Thomas SC. 2015. Variation in carbon and nitrogen
1817 concentration among major woody tissue types in temperate trees. *Canadian*
1818 *Journal of Forest Research* 45: 744-757.

1819 Martin AR, Thomas SC. 2011. A reassessment of carbon content in tropical trees. *PLoS*
1820 *One* 6: e23533.

1821 Martin AR, Thomas SC. 2013. Size-dependent changes in leaf and wood chemical traits
1822 in two Caribbean rainforest trees. *Tree Physiology* 33: 1338-1353.

1823 Martin AR, Thomas SC, Zhao Y. 2013. Size-dependent changes in wood chemical traits:
1824 a comparison of neotropical saplings and large trees. *Aob Plants* 5.

1825 McKinley DC, Ryan MG, Birdsey RA, *et al.* 2011. A synthesis of current knowledge on
1826 forests and carbon storage in the United States. *Ecological Applications* 21: 1902-
1827 1924.

1828 Mencuccini M, Martínez-Vilalta J, Vanderklein D, *et al.* 2005. Size-mediated ageing
1829 reduces vigour in trees. *Ecology Letters* 8: 1183-1190.

1830 Metsaranta JM, Kurz WA, Neilson ET, *et al.* 2011. Implications of future disturbance
1831 regimes on the carbon balance of Canada's managed forest (2010–2100). *Tellus B*
1832 62.

1833 Moran SR, Clayton L. 1984. Chronology of Late Wisconsinan Glaciation in Middle
1834 North-America - Reply. *Quaternary Science Reviews* 3: R1-R6.

1835 Niinemets U. 2006. The controversy over traits conferring shade-tolerance in trees:
1836 ontogenetic changes revisited. *Journal of Ecology* 94: 464-470.

- 1837 O'Connell MM, Bentley MD, Campbell CS, *et al.* 1988. Betulin and lupeol in bark from
1838 four white-barked birches. *Phytochemistry* 27: 2175-2176.
- 1839 Odum EP. 1969. The strategy of ecosystem development. *Science* 164: 262-270.
- 1840 Olajuyigbe SO, Tobin B, Gardiner P, *et al.* 2011. Stocks and decay dynamics of above-
1841 and belowground coarse woody debris in managed Sitka spruce forests in Ireland.
1842 *Forest Ecology and Management* 262: 1109-1118.
- 1843 Pan Y, Birdsey RA, Fang J, *et al.* 2011. A large and persistent carbon sink in the world's
1844 forests. *science* 333: 988-993.
- 1845 Pare D, Bergeron Y. 1995. Above-ground biomass accumulation along a 230-year
1846 chronosequence in the southern portion of the Canadian boreal forest. *Journal of*
1847 *Ecology* 83: 1001-1007.
- 1848 Pons TL, Poorter H. 2014. The effect of irradiance on the carbon balance and tissue
1849 characteristics of five herbaceous species differing in shade-tolerance. *Frontiers in*
1850 *Plant Science* 5: 12.
- 1851 Poorter L, Bongers F, Sterck FJ, *et al.* 2005. Beyond the regeneration phase:
1852 differentiation of height–light trajectories among tropical tree species. *Journal of*
1853 *Ecology* 93: 256-267.
- 1854 Pregitzer KS, Euskirchen ES. 2004. Carbon cycling and storage in world forests: biome
1855 patterns related to forest age. *Global Change Biology* 10: 2052-2077.
- 1856 Prescott CE, Zabek LM, Staley CL, *et al.* 2000. Decomposition of broadleaf and needle
1857 litter in forests of British Columbia: influences of litter type, forest type, and litter
1858 mixtures. *Canadian Journal of Forest Research-Revue Canadienne De Recherche*
1859 *Forestiere* 30: 1742-1750.

1860 R Development Core Team. 2017. R: A language and environment for statistical
1861 computing. Vienna, Austria: R Foundation for Statistical Computing,
1862 <https://www.r-project.org/>.

1863 Reich PB. 2014. The world-wide ‘fast–slow’ plant economics spectrum: a traits
1864 manifesto. *Journal of Ecology* 102: 275-301.

1865 Roberts MR, Gilliam FS. 1995. Patterns and mechanisms of plant diversity in forested
1866 ecosystems - Implications for forest management. *Ecological Applications* 5: 969-
1867 977.

1868 Rosell JA, Gleason S, Méndez-Alonzo R, *et al.* 2014. Bark functional ecology: evidence
1869 for tradeoffs, functional coordination, and environment producing bark diversity.
1870 *New Phytologist* 201: 486-497.

1871 Ryan MG, Binkley D, Fownes JH. 1997. Age-related decline in forest productivity:
1872 Pattern and process. Begon M, Fitter AH editors. *Advances in Ecological*
1873 *Research*, Vol 27. London: Academic Press Ltd-Elsevier Science Ltd, p213-262.

1874 Saatchi SS, Harris NL, Brown S, *et al.* 2011. Benchmark map of forest carbon stocks in
1875 tropical regions across three continents. *Proc Natl Acad Sci U S A* 108: 9899-
1876 9904.

1877 Savidge RA. 2000. Biochemistry of seasonal cambial growth and wood formation - an
1878 overview of the challenges. *Cell and Molecular Biology of Wood Formation*: 1-
1879 30.

1880 Seedre M, Shrestha BM, Chen HYH, *et al.* 2011. Carbon dynamics of North American
1881 boreal forest after stand replacing wildfire and clearcut logging. *Journal of Forest*
1882 *Research* 16: 168-183.

1883 Seedre M, Taylor AR, Brassard BW, *et al.* 2014. Recovery of Ecosystem Carbon Stocks
1884 in Young Boreal Forests: A Comparison of Harvesting and Wildfire Disturbance.
1885 *Ecosystems* 17: 851-863.

1886 Seedre M, Taylor AR, Chen HYH, *et al.* 2013. Deadwood Density of Five Boreal Tree
1887 Species in Relation to Field-Assigned Decay Class. *Forest Science* 59: 261-266.

1888 Senici D, Chen HYH, Bergeron Y, *et al.* 2010. Spatiotemporal variations of fire
1889 frequency in central boreal forest. *Ecosystems* 13: 1227-1238.

1890 Smith WB, Brand GJ. 1983. Allometric biomass equations for 98 species of herbs,
1891 shrubs, and small trees.

1892 Soil Classification Working Group. 1998. The Canadian System of Soil Classification:
1893 NRC Research Press.

1894 Taylor AR, Chen HYH. 2011. Multiple successional pathways of boreal forest stands in
1895 central Canada. *Ecography* 34: 208-219.

1896 Taylor AR, Seedre M, Brassard BW, *et al.* 2014. Decline in Net Ecosystem Productivity
1897 Following Canopy Transition to Late-Succession Forests. *Ecosystems* 17: 778-
1898 791.

1899 Taylor KC, Arnup RW, Meredith MP, *et al.* 2000. A Field Guide to Forest Ecosystems of
1900 Northeastern Ontario: NEST Field Guide FG-01, Northeast Science and
1901 Technology, Ontario Ministry of Natural Resources, Timmins, Ontario, Canada.

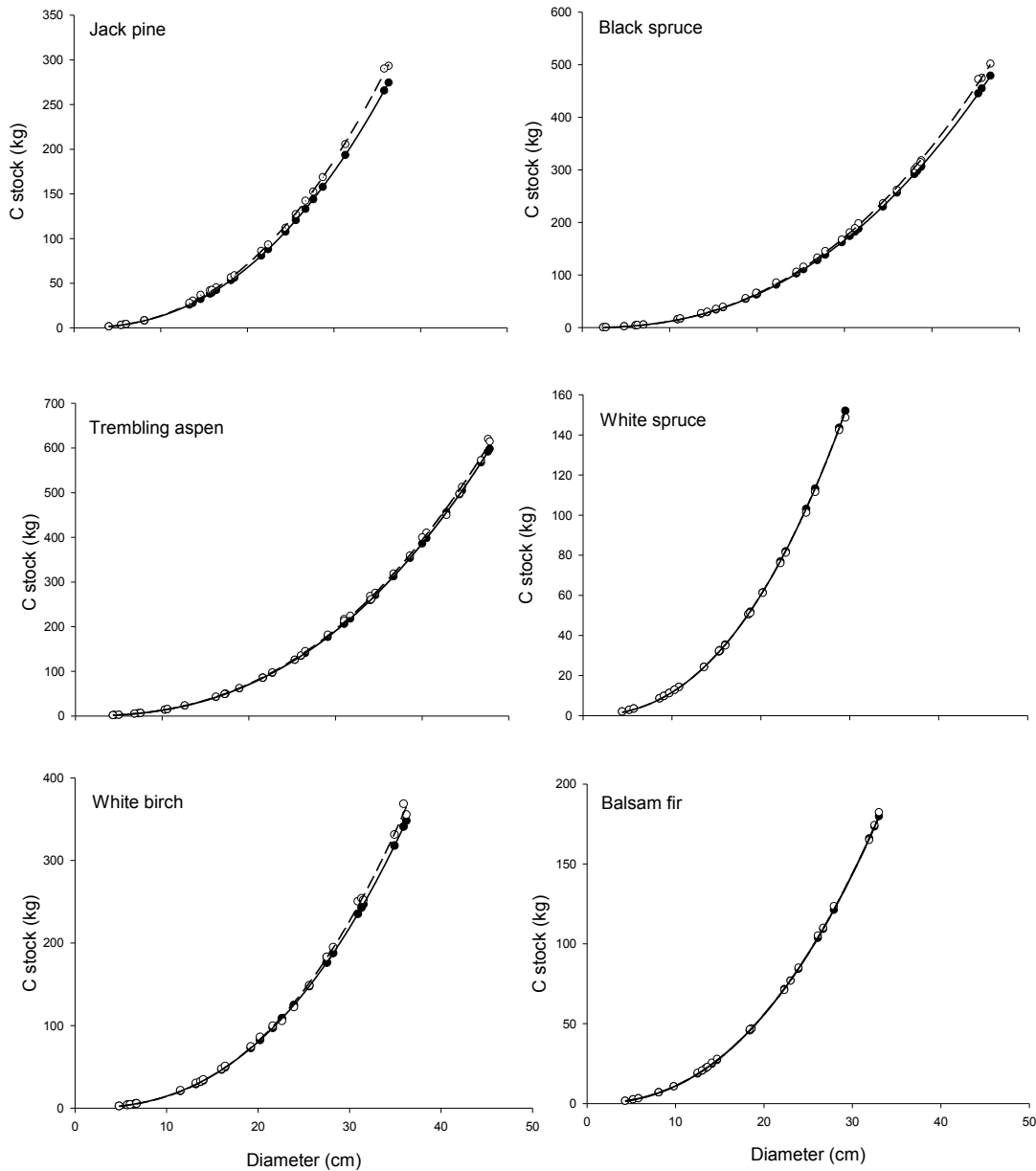
1902 Thiffault E, Belanger N, Pare D, *et al.* 2007. How do forest harvesting methods compare
1903 with wildfire ? A case study of soil chemistry and tree nutrition in the boreal
1904 forest. *Canadian Journal of Forest Research* 37: 1658-1668.

1905 Thiffault E, Hannam KD, Quideau SA, *et al.* 2008. Chemical composition of forest floor
1906 and consequences for nutrient availability after wildfire and harvesting in the
1907 boreal forest. *Plant and soil* 308: 37-53.

- 1908 Thomas SC, Malczewski G. 2007. Wood carbon content of tree species in Eastern China:
1909 Interspecific variability and the importance of the volatile fraction. *J Environ*
1910 *Manage* 85: 659-662.
- 1911 Thomas SC, Martin AR. 2012. Carbon Content of Tree Tissues: A Synthesis. *Forests* 3:
1912 332-352.
- 1913 Vance C, Kirk T, Sherwood R. 1980. Lignification as a mechanism of disease resistance.
1914 *Annual Review of Phytopathology* 18: 259-288.
- 1915 Vasiliauskas S, Chen HYH. 2002. How long do trees take to reach breast height after fire
1916 in northeastern Ontario? *Canadian Journal of Forest Research-Revue Canadienne*
1917 *De Recherche Forestiere* 32: 1889-1892.
- 1918 Voelker SL, Lachenbruch B, Meinzer FC, *et al.* 2011. Reduced wood stiffness and
1919 strength, and altered stem form, in young antisense 4CL transgenic poplars with
1920 reduced lignin contents. *New Phytologist* 189: 1096-1109.
- 1921 Wainhouse D, Cross DJ, Howell RS. 1990. The Role of Lignin as a Defense against the
1922 Spruce Bark Beetle *Dendroctonus-Micans* - Effect on Larvae and Adults.
1923 *Oecologia* 85: 257-265.
- 1924 Wairiu M, Lal R. 2003. Soil organic carbon in relation to cultivation and topsoil removal
1925 on sloping lands of Kolombangara, Solomon Islands. *Soil & Tillage Research* 70:
1926 19-27.
- 1927 Walker LR, Wardle DA, Bardgett RD, *et al.* 2010. The use of chronosequences in studies
1928 of ecological succession and soil development. *Journal of Ecology* 98: 725-736.
- 1929 Wang CK, Bond-Lamberty B, Gower ST. 2003. Carbon distribution of a well- and
1930 poorly-drained black spruce fire chronosequence. *Global Change Biology* 9:
1931 1066-1079.

- 1932 Weiner J. 1990. Asymmetric competition in plant populations. *Trends in Ecology &*
1933 *Evolution* 5: 360-364.
- 1934 Williams LJ, Paquette A, Cavender-Bares J, *et al.* 2017. Spatial complementarity in tree
1935 crowns explains overyielding in species mixtures. *Nature Ecology &*
1936 *Evolution* 1: 0063.
- 1937 Wirth C, Schulze ED, Lühker B, *et al.* 2002. Fire and site type effects on the long-term
1938 carbon and nitrogen balance in pristine Siberian Scots pine forests. *Plant and Soil*
1939 242: 41-63.
- 1940 Wright SJ, Yavitt JB, Wurzburger N, *et al.* 2011. Potassium, phosphorus, or nitrogen
1941 limit root allocation, tree growth, or litter production in a lowland tropical forest.
1942 *Ecology* 92: 1616-1625.
- 1943 Yuan ZY, Chen HYH. 2012. Fine root dynamics with stand development in the boreal
1944 forest. *Functional Ecology* 26: 991-998.
- 1945 Zhang QZ, Wang CK, Wang XC, *et al.* 2009. Carbon concentration variability of 10
1946 Chinese temperate tree species. *Forest Ecology and Management* 258: 722-727.
- 1947 Zhang Y, Chen HYH, Reich PB. 2012. Forest productivity increases with evenness,
1948 species richness and trait variation: a global meta-analysis. *Journal of Ecology*
1949 100: 742-749.
- 1950 Zhang Y, Chen HYH, Taylor AR. 2017. Positive species diversity and above-ground
1951 biomass relationships are ubiquitous across forest strata despite interference from
1952 overstorey trees. *Functional Ecology* 31: 419-426.
- 1953

1954 **Appendix I. Supplemental information for Chapter Two**



1955

1956 **Fig. S2-1** Aboveground carbon (C) stock estimates at the tree level for six boreal tree species.
 1957 Open and closed circles represent C stocks calculated by our C concentration data and 50%,
 1958 respectively. Regression relating DBH to total tree C stock was constructed and used to
 1959 estimate species-level tree C stocks. Short dashed lines and solid lines represent the
 1960 relationship between DBH and aboveground C stocks calculated by our C concentration data
 1961 and the standard assumed 50%, respectively. We estimated tree biomass based on species-
 1962 specific allometric equations developed in Canada ([Lambert et al., 2005](#)). Carbon
 1963 concentration of stemwood was also used for tree branches ([Martin et al., 2015](#)). Carbon
 1964 concentration of foliage was measured in 2013 by a previous study in the same area ([Brant,](#)
 1965 [2014](#)). Equation related aboveground whole-tree C stock with DBH was used as

1966
$$y = \beta_1 D^{\beta_2} + e$$

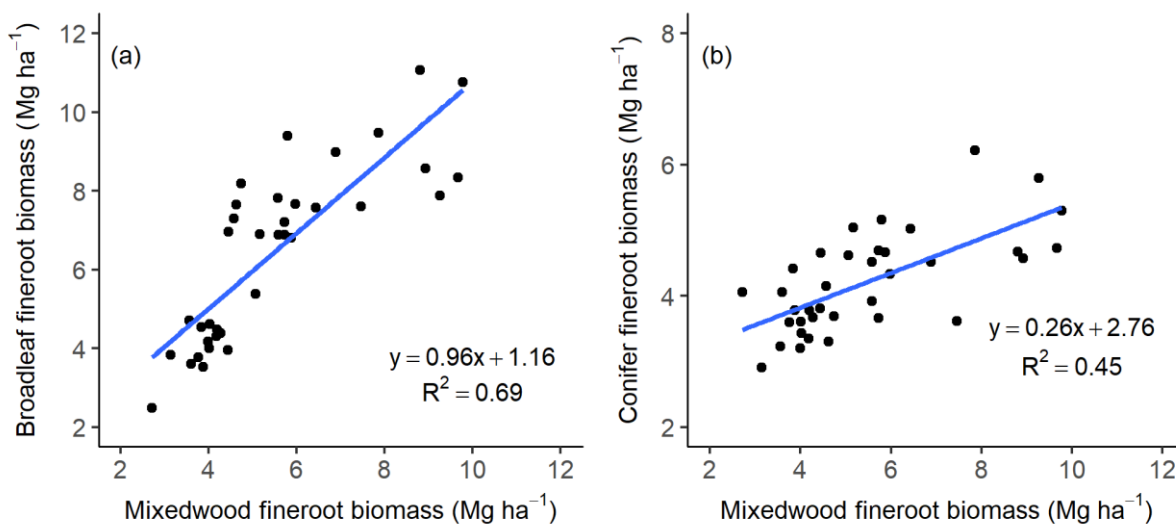
Eq. (S1)

1967 where y is the aboveground C stock of a living tree (kilograms), D is the DBH (centimeters),
1968 β_1 , β_2 are estimated coefficient; e is the error term (showed in Table S2-1).
1969

1970 **Table S2-1.** Model parameter estimates and their standard error (S.E.) for the DBH-based set
 1971 of equations per species.

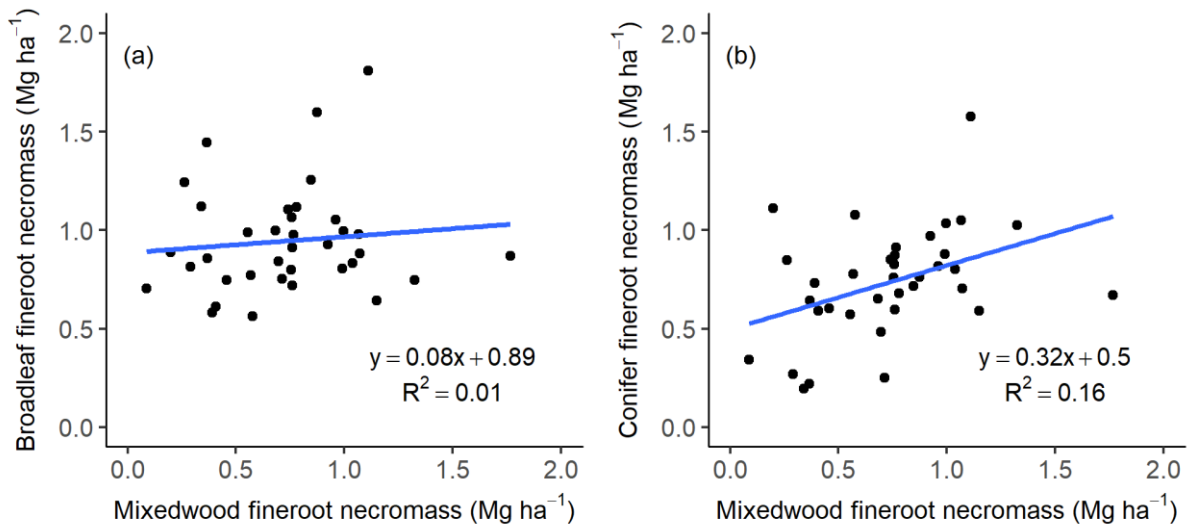
Species	Parameter	Estimate	S.E.
Jack pine	β_1	0.0572	0.0027
	β_2	2.3799	0.0135
Trembling aspen	β_1	0.0452	0.0029
	β_2	2.4595	0.0172
White birch	β_1	0.0416	0.0039
	β_2	2.5294	0.0271
Black spruce	β_1	0.0472	0.0019
	β_2	2.4113	0.0110
White spruce	β_1	0.0521	0.0014
	β_2	2.3520	0.0084
Balsam fir	β_1	0.0511	0.0018
	β_2	2.3367	0.0104

1972
 1973



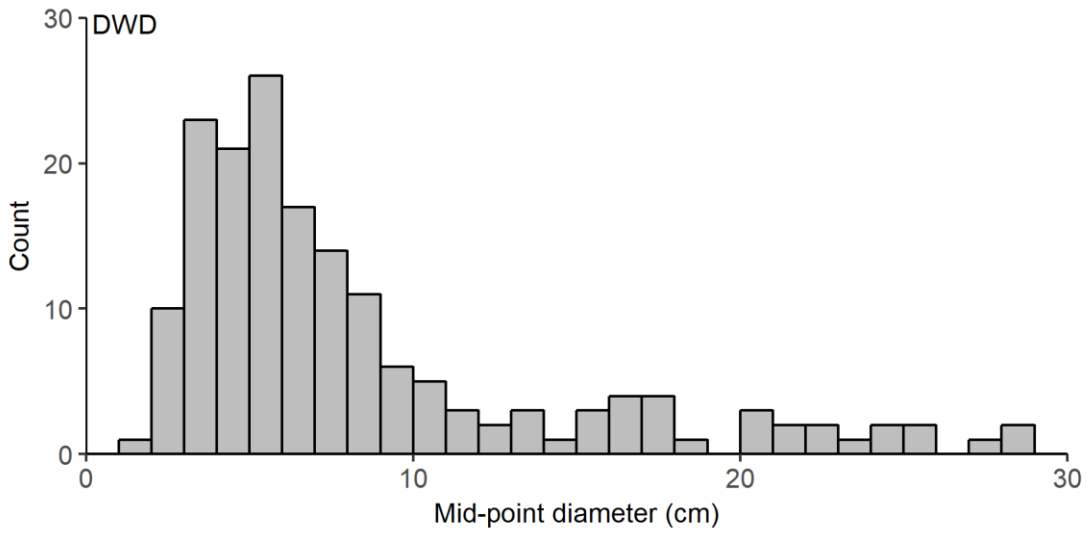
1975
1976
1977
1978

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.



1979
 1980
 1981
 1982

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.



1983
 1984
 1985
 1986

Fig. S3-3 Distribution of mid-point diameters of down woody debris (DWD) in 8 years old stands.

1987 **Table S3-1** Results of stand age (A_i) and overstorey type (T_j) on total ecosystem C and
 1988 individual C pools during late succession following fire (i.e., 147 and 210 years old stands).
 1989 The columns give the degrees of freedom (d.f.), F value, and P value. Statistically significant
 1990 terms (p < 0.05) are in bold.

C Pools	A _i (d.f. = 1)		T _j (d.f. = 2)		A × T _{ij} (d.f. = 2)	
	F	P	F	P	F	P
Total ecosystem C	10.7	0.007	1.8	0.219	1.1	0.364
Total live biomass	2.8	0.125	2.2	0.153	2.8	0.103
Total deadwood	2.2	0.167	2.1	0.165	1.2	0.332
Forest floor	0.1	0.833	0.4	0.684	3.8	0.057
Mineral soil	8.4	0.014	4.0	0.049	5.3	0.025

1991
 1992

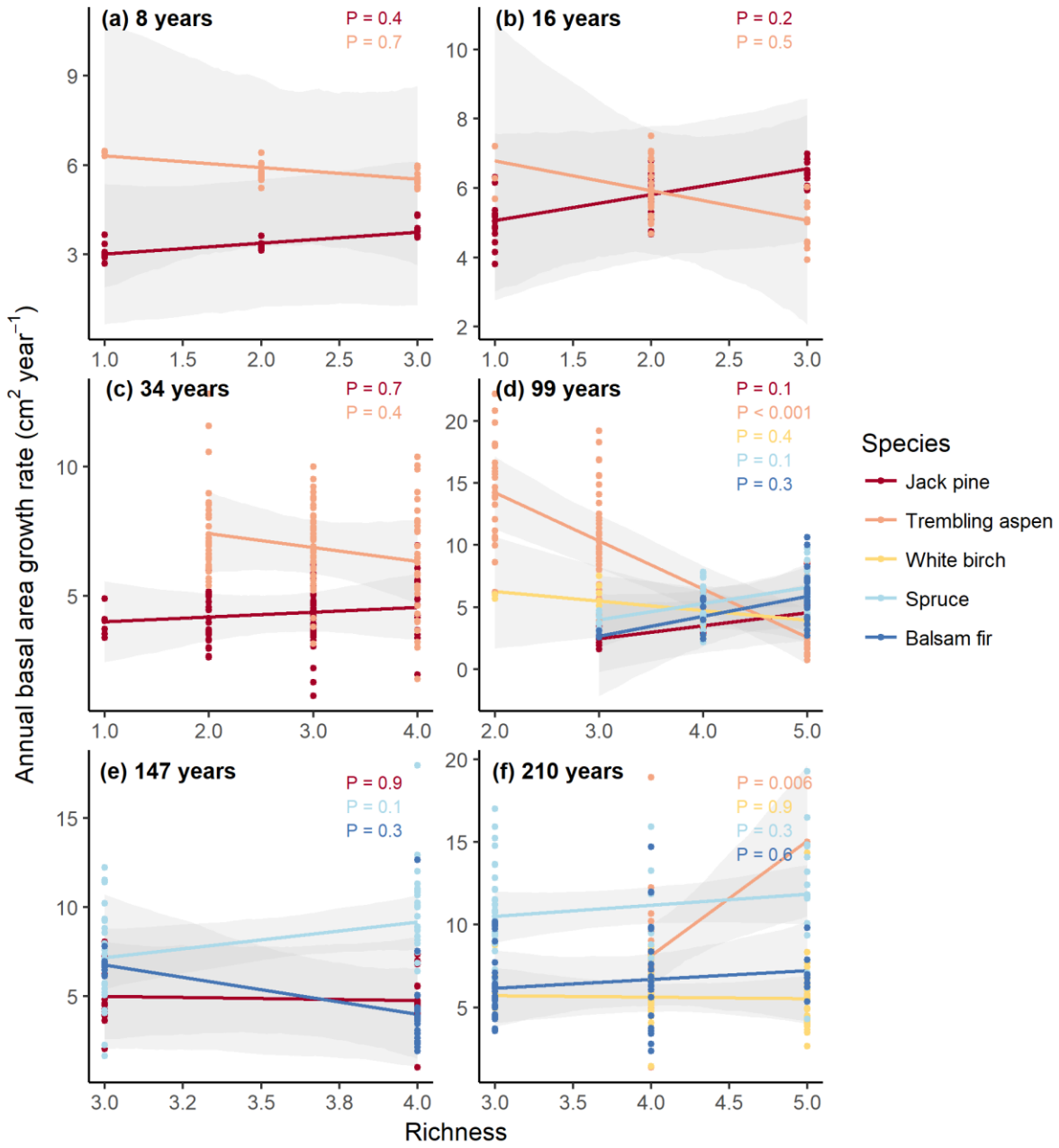
1993
1994

Table S3-2 Location information of the 43 sample stands in the boreal forests of Ontario, Canada.

Plot ID	Age	Overstorey type	Latitude	Longitude
1	8	Broadleaf	49.531879	-90.051382
2	8	Broadleaf	49.572458	-90.135925
3	8	Broadleaf	49.532888	-90.075315
4	8	Conifer	49.547507	-90.132973
5	8	Conifer	49.546296	-90.108515
6	8	Conifer	49.553823	-90.129597
7	8	Mixedwood	49.545906	-90.106947
8	8	Mixedwood	49.544349	-90.105379
9	8	Mixedwood	49.553199	-90.127932
10	34	Broadleaf	49.658629	-89.899643
11	34	Broadleaf	49.658048	-89.900028
12	34	Broadleaf	49.65796	-89.89953
13	34	Conifer	49.641328	-89.875006
14	34	Conifer	49.643216	-89.877531
15	34	Conifer	49.644124	-89.876205
16	34	Mixedwood	49.6493	-89.476667
17	34	Mixedwood	49.649008	-89.476988
18	99	Broadleaf	49.497406	-89.626369
19	99	Broadleaf	49.497178	-89.628086
20	99	Broadleaf	49.492973	-89.624802
21	99	Conifer	49.533161	-89.645825
22	99	Conifer	49.536469	-89.641447
23	99	Conifer	49.575998	-89.478897
24	99	Mixedwood	49.463255	-89.587389
25	99	Mixedwood	49.534551	-89.644994
26	99	Mixedwood	49.536015	-89.641007
27	147	Broadleaf	49.475949	-89.613288
28	147	Broadleaf	49.472183	-89.615002
29	147	Broadleaf	49.463667	-89.583075
30	147	Conifer	49.550147	-89.672792
31	147	Conifer	49.470409	-89.565161
32	147	Conifer	49.478641	-89.561113
33	147	Mixedwood	49.444005	-89.616536
34	147	Mixedwood	49.444975	-89.615036
35	210	Broadleaf	49.465436	-89.582612

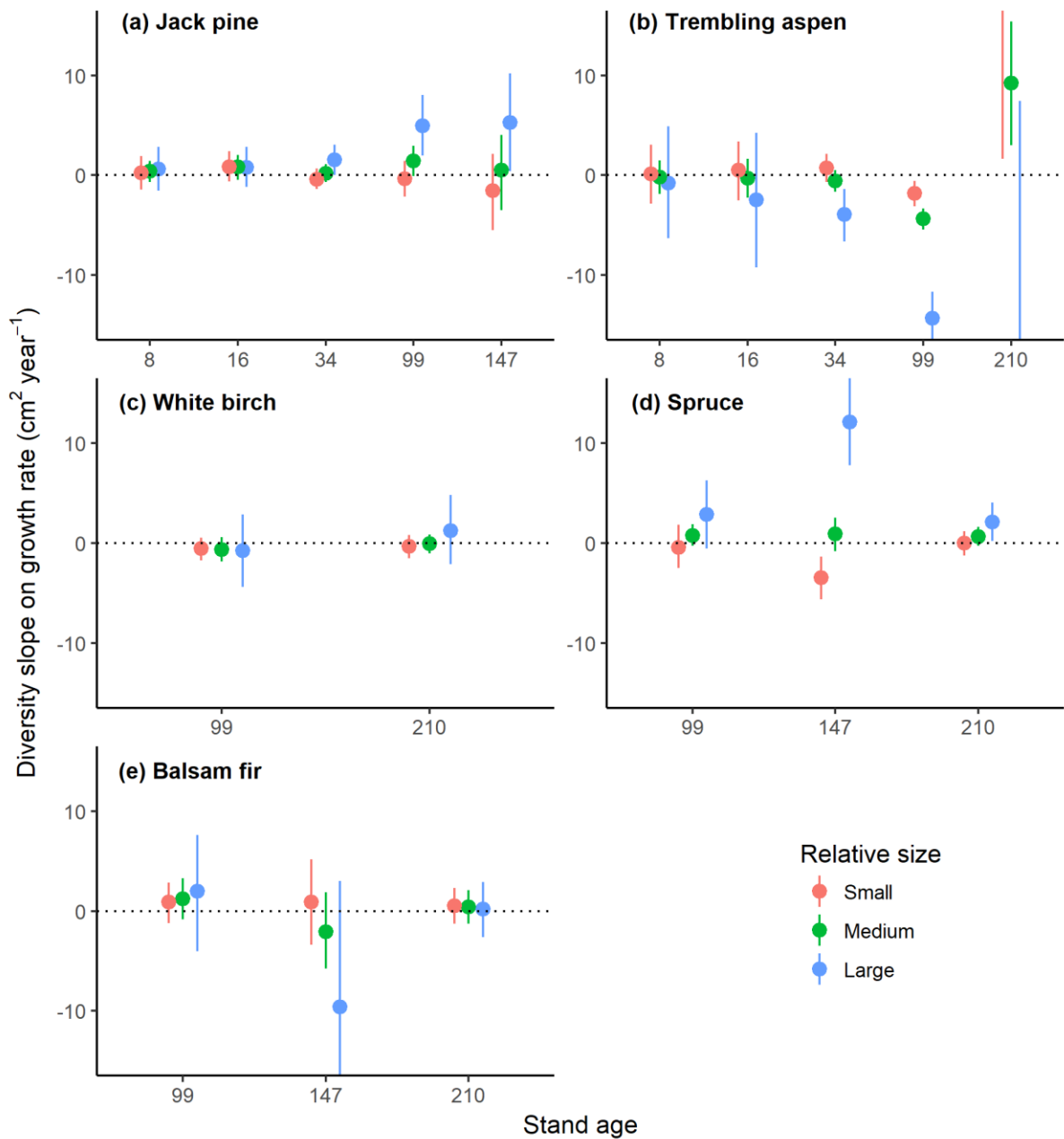
36	210	Broadleaf	49.464171	-89.582669
37	210	Broadleaf	49.467189	-89.582153
38	210	Conifer	49.458084	-89.601152
39	210	Conifer	49.45721	-89.601545
40	210	Conifer	49.463303	-89.599938
41	210	Mixedwood	49.460433	-89.599376
42	210	Mixedwood	49.467414	-89.580383
43	210	Mixedwood	49.46654	-89.580075

1995



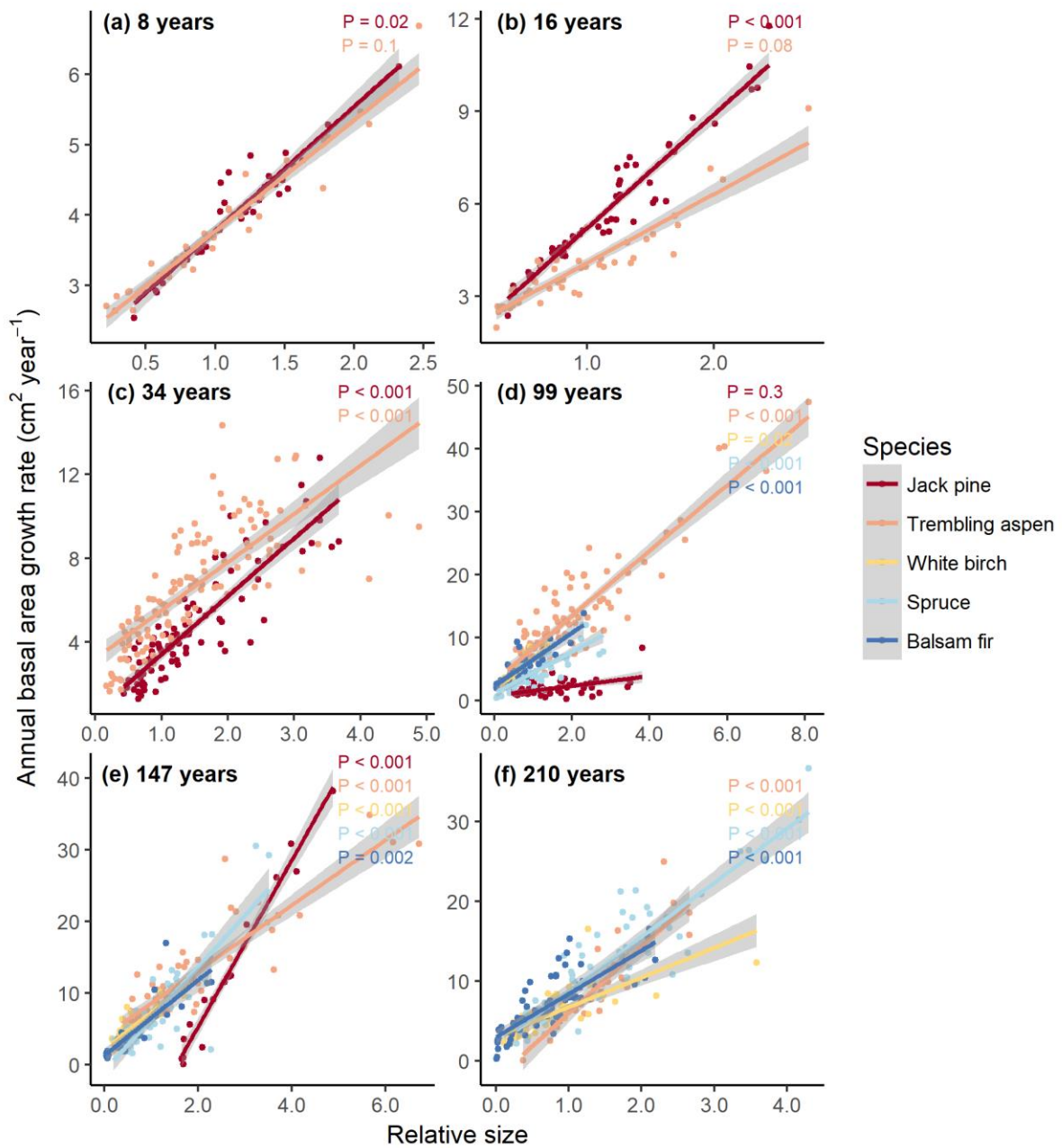
1997
1998
1999
2000
2001

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.



2002
 2003
 2004
 2005
 2006

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.



2007
 2008
 2009
 2010
 2011

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.