



Variation in total and volatile carbon concentration among the major tree species of the boreal forest



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ABSTRACT

Understanding variation in carbon (C) concentration of live trees is essential for quantifying forest C stocks and validating forest C accounting models. Previous studies in boreal forests have assumed 50% C concentration or focused on species-specific C concentration estimation based on samples taken mostly from stemwood tissue of large trees. Yet, little is known about differences in C concentration between woody tissues or among trees of different sizes nor about the effects of life-history traits, such as shade tolerance and the role of volatile C on total C concentration in live trees. In this study, we examined variation in total and volatile C concentration in bark and stemwood tissues for trees of different sizes for six major North American boreal tree species. We found that bark had significantly higher total C and volatile C concentrations than stemwood and that both total C and volatile C concentration significantly varied among tree species. The average total C concentrations were 56.2% in the bark and 50.5% in the stemwood, and the average volatile C concentration were 5.8% and 3.0% for bark and stemwood, respectively. Furthermore, total C and volatile C concentration in stemwood and bark of almost all shade-intolerant species increased with tree size, whereas those of shade-tolerant species showed negative or neutral size-associated change. Our results show that volatile C concentration is a key driver of variation in total C concentration and highlights the importance of considering variation in C concentration when quantifying forest C stocks, which has important consequences for predicting future global C emissions scenarios.

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1. Introduction

The world's boreal forests play a critical role in the global carbon (C) cycle, containing approximately 32% of all forest ecosystem C (Pan et al., 2011), largely due to the slow rate of decomposition and high accumulation of dead organic matter at high northern latitudes (Bonan and Shugart, 1989; Dixon et al., 1994). However, northern ecosystems are expected to experience the greatest warming over the coming century, potentially affecting the capacity of the boreal forest to sequester and store C (Gauthier et al., 2015). A better understanding of C flux in the boreal forest is becoming increasingly imperative given its strong impact on global C dynamics (Canadell and Raupach, 2008; Bellassen and Luyssaert, 2014). In particular, accurately quantifying C stocks of live trees in the boreal forest has important implications for the verification and validation of global C accounting models used to predict future

C emission scenarios (McKinley et al., 2011; Metsaranta et al., 2011) as trees generally comprise the largest and most dynamic C pool in forest ecosystems (Pan et al., 2011).

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

Variation in tree C concentration is primarily attributable to the physical and chemical properties of their woody tissues (Savidge,

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

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

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

Life-history traits, such as shade tolerance, may also affect C concentration due to its important role in plant C balance (Pons and Poorter, 2014). For instance, shade tolerance affects the survival and growth of plants by influencing C uptake (i.e., photosynthesis) and C release (i.e., respiration) in response to limited light availability (Hillebrand and Matthiessen, 2009). Yet, empirical evidence is still scarce on the influence of contrasting shade tolerance on plant tissue C concentration. Moreover, previous studies have detected diverse relationships between tree C concentration and other traits related to shade tolerance, including wood density and growth rate (Elias and Potvin, 2003; Martin and Thomas, 2011; Becker et al., 2012). These mixed results, however, may be attributable to lack of consideration of tree ontogenetic variation on C concentration (Martin and Thomas, 2013). Further examination of the interaction

between tree size and shade tolerance may help in understanding the role of plant life-history adaptations on tissue C concentration (Niinemets, 2006).

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

2. Materials and methods

2.1. Study area

Our study was conducted in the boreal forest, approximately 150 km north of Thunder Bay, Ontario, Canada, between 49°44' to 49°65'N and 89°16' to 90°13'W. This area is characterized by warm summers and cold, snowy winters. Mean annual temperature of 1.9 °C and mean annual precipitation of 824.8 mm were recorded at the closest meteorological station in Cameron Falls, Ontario, Canada (Environment Canada, 2015). Soils in our study area largely originated from the Wisconsinian glaciation, which ended approximately 9500 years ago in this region. Stand-replacing wildfire is the most common natural disturbance in our study area, with an average fire-return interval of approximately 100 years for the past century, resulting in mosaic of stand ages across the landscape (Senici et al., 2010). Commercial logging began in our study area in the 1970s. Dominant overstory tree species include jack pine, trembling aspen, white birch, black spruce, white spruce, and balsam fir.

2.2. Sampling design

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

For trees <10 cm DBH, tissue extraction consisted of cutting stem disks at DBH, which provided both bark and stemwood. For trees ≥ 10 cm DBH, we used an increment core borer with a 5.15 mm diameter bit to extract both stemwood and bark tissue samples. All samples were sealed in plastic bags or straws and placed in a cooler with ice to minimize loss of volatiles during transportation from the field to the laboratory. Similar to previous

studies (Martin and Thomas, 2011; Martin et al., 2015), trees with crooked stems, substantial heart-rot, or other forms of stem damage, such as stem abrasion, fungal infections, or major branch losses, were not sampled.

2.3. Chemical analysis

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

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

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

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

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

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

$$C_{\text{vol}} = C_{\text{freeze-corr}} - C_{\text{heat}} \quad (3)$$

2.4. Data analysis

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

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

where Y_{ijk} is the measured total C concentration or volatile C concentration of the bark or stemwood tissue, μ is the overall mean, T_i (shade intolerant vs. shade tolerant) represents shade tolerance with two levels (determined by Burns and Honkala (1990)), $S_{(ij)}$ ($j = 1, 2, 3$) is tree species (nested within shade tolerance), D_k is DBH (a continuous variable), and $\varepsilon_{(ijk)}$ is random sampling error. We tested the assumption of normality by Shapiro's test, and that

of homogeneity of variance by Levene's test. We used the rank transformation method from the "GenABEL" R software package (GenABEL project developers, 2013) to transform the total C concentration of bark data as the untransformed data did not meet the assumptions of normality and homogeneity. We used a generalized linear model with the Gaussian family error distribution and an identity link function for analyzing our rank transformed data.

We then used the results from our nested ANOVA to inform a more species-specific analysis, whereby, if tree size in Eq. (4) was significant, a simple linear regression was then used to more specifically describe the size-associated relationship between total and volatile C concentrations and DBH for each woody tissue type and for each tree species. We compared linear and logarithmic functions to select the best bivariate relationships based on Akaike's Information Criterion (AIC), whereby the simplest model that explained the most variation was selected when the difference in AICs between alternative models was <2 (Burnham and Anderson, 2002). All statistical analyses were conducted using the R 3.2.3 (R Development Core Team, 2015).

3. Results

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

Total C concentration of bark tissue differed significantly with shade tolerance, tree species, and tree size and showed marginally ($P = 0.096$ and 0.079 , respectively) significant interactions (Table 3). Mean total C concentration of bark was higher in shade-intolerant species than in shade-tolerant species. Furthermore, total C concentration in bark significantly increased with tree size for shade-intolerant jack pine and trembling aspen (Fig. 1), but significantly and marginally decreased with tree size for shade-tolerant balsam fir and white spruce, respectively (Fig. 2). Similarly, volatile C concentration of bark was also significantly influenced by shade tolerance, tree species, and tree size (as indicated by the significant interaction between tree size and species) (Table 3). The mean volatile C concentration of bark was generally lowest for shade-intolerant tree species; however, this was mainly due to the low

Table 1

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

Species	Total C concentration			Volatile C concentration		
	d.f.	<i>t</i> value	<i>P</i> value	d.f.	<i>t</i> value	<i>P</i> 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

Table 2

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

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

C concentration observed in aspen and birch, not jack pine, which had the highest mean volatile C concentration of all species at $7.5 \pm 0.2\%$ (S.E.) (Table 2). In addition, interactions between shade tolerance and tree size, and between species and tree size, had significant effects on volatile C concentration (Table 3). For shade-intolerant trembling aspen and white birch, volatile C concentration in bark showed a marginal ($P = 0.092$ and 0.062 , respectively) positive trend with tree size (Fig. 1). Volatile C of bark marginally decreased with DBH for shade-tolerant white spruce, and significantly decreased with DBH for balsam fir (Fig. 2).

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

pattern was observed for shade-tolerant tree species, except a marginal negative trend observed for white spruce (Fig. 2).

4. Discussion

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

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

Table 3

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

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		

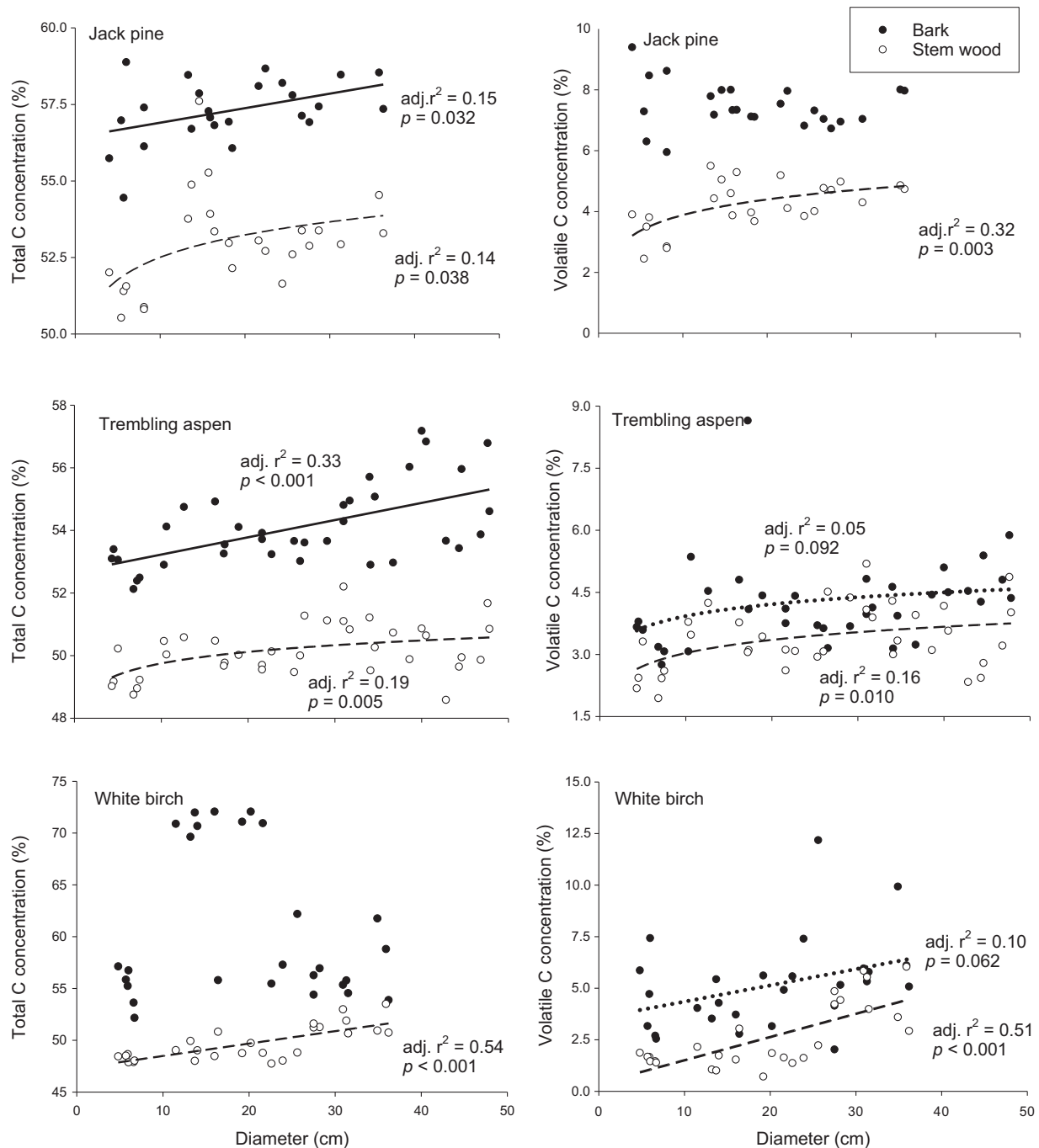


Fig. 1. Total and volatile C concentrations in relation to wood tissue type and DBH for jack pine, trembling aspen, white birch. Closed and open circles represent C concentrations of bark and stemwood, respectively. Solid lines represent the significant relationship between DBH and total or volatile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

et al., 2011), and higher C concentration of bark of boreal tree species may also relate to the role of bark in fire resistance (Hengst and Dawson, 1994).

Our data yielded mixed results for tree size effects on total C concentration of bark and stemwood. We found both neutral and positive relationships between tree size and total C concentration of stemwood, partially in agreement with Martin and Thomas (2013) whose study in tropical forest found C concentration increases with tree size. In addition, we also found negative, neutral, and positive relationships between tree size and total C

concentration in bark tissue. The reason for the diverse relationships is likely attributable to size-associated changes in volatile C concentration, which parallel total C concentration. Traditional views suggest that total C concentration is mostly dominated by C-rich elements like lignin (Lamloom and Savidge, 2003). It is, thus, reasonable to assume that small trees have higher C concentrations than large trees because small trees generally contain higher lignin concentrations (Martin et al., 2013), supporting mechanical stability (Alvarez-Clares and Kitajima, 2007) and defence mechanisms (Wainhouse et al., 1990). However, our findings suggest that the

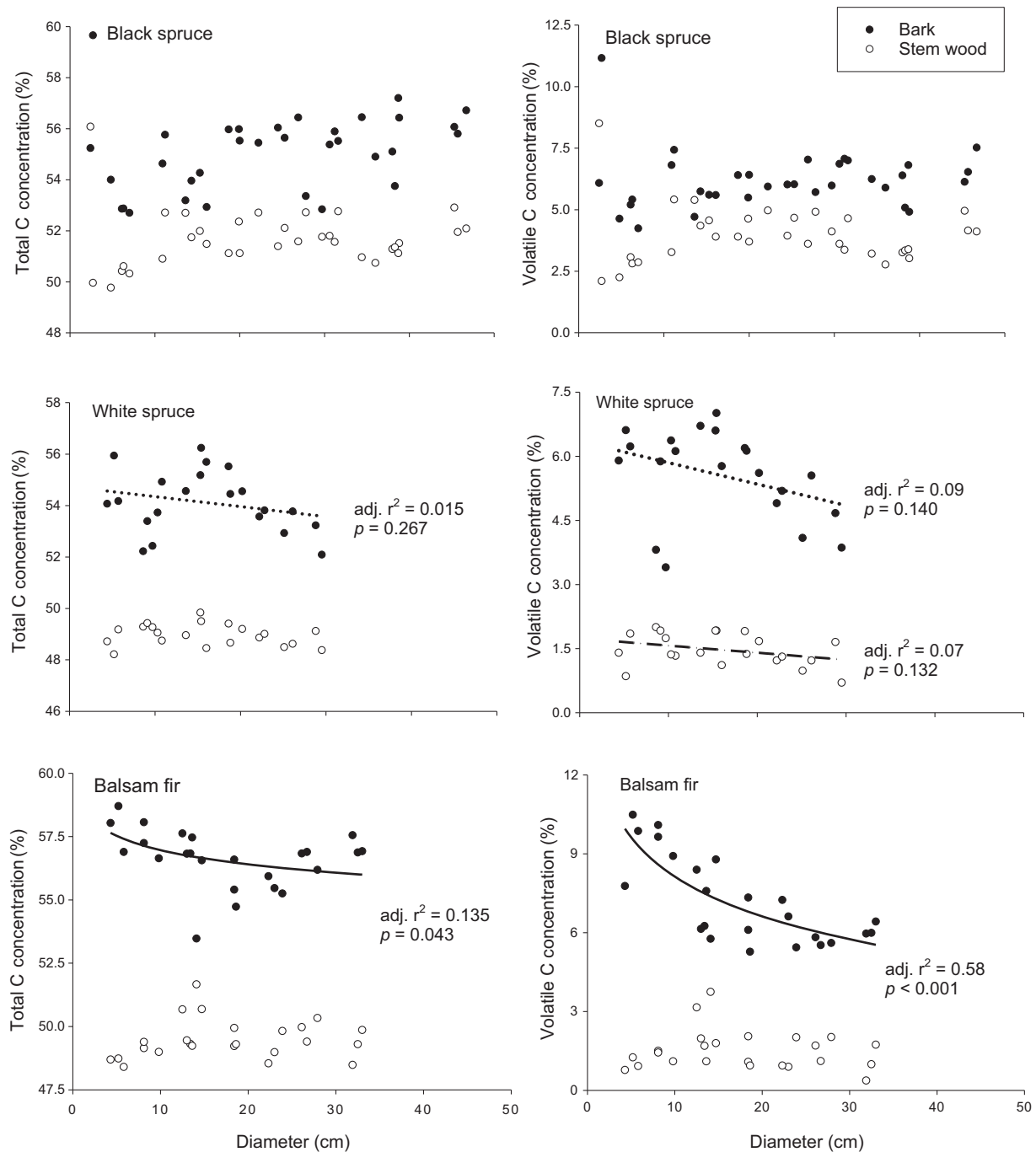


Fig. 2. Total and volatile C concentrations in relation to wood tissue type and DBH for black spruce, white spruce, and balsam fir. Closed and open circles represent C concentration of bark and stemwood, respectively. Solid lines represent the significant relationship between DBH and total or volatile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

size-associated trend of total C concentration is likely led by change in the concentration of volatile C compounds (Figs. 1 and 2). These results support Martin et al. (2013), who hypothesized that volatile C concentration plays an important role in size-related changes in total C concentration. Furthermore, when considering our observation that the difference in volatile C concentration between bark and stemwood tissue accounts for a large proportion of the difference in total C concentration between bark and stemwood tissue, we speculate that the fraction of volatile C

concentration plays a key role in driving overall variation in total tree C concentration.

Moreover, size-associated trends in stemwood C and bark C differed according to shade tolerance. All shade-intolerant tree species had positive relationships between stemwood C concentration and tree size, whereas no general size-associated trend was found in stemwood for shade-tolerant tree species. Similarly, shade-intolerant tree species also showed positive size-associated changes in C concentration in bark tissue, with the

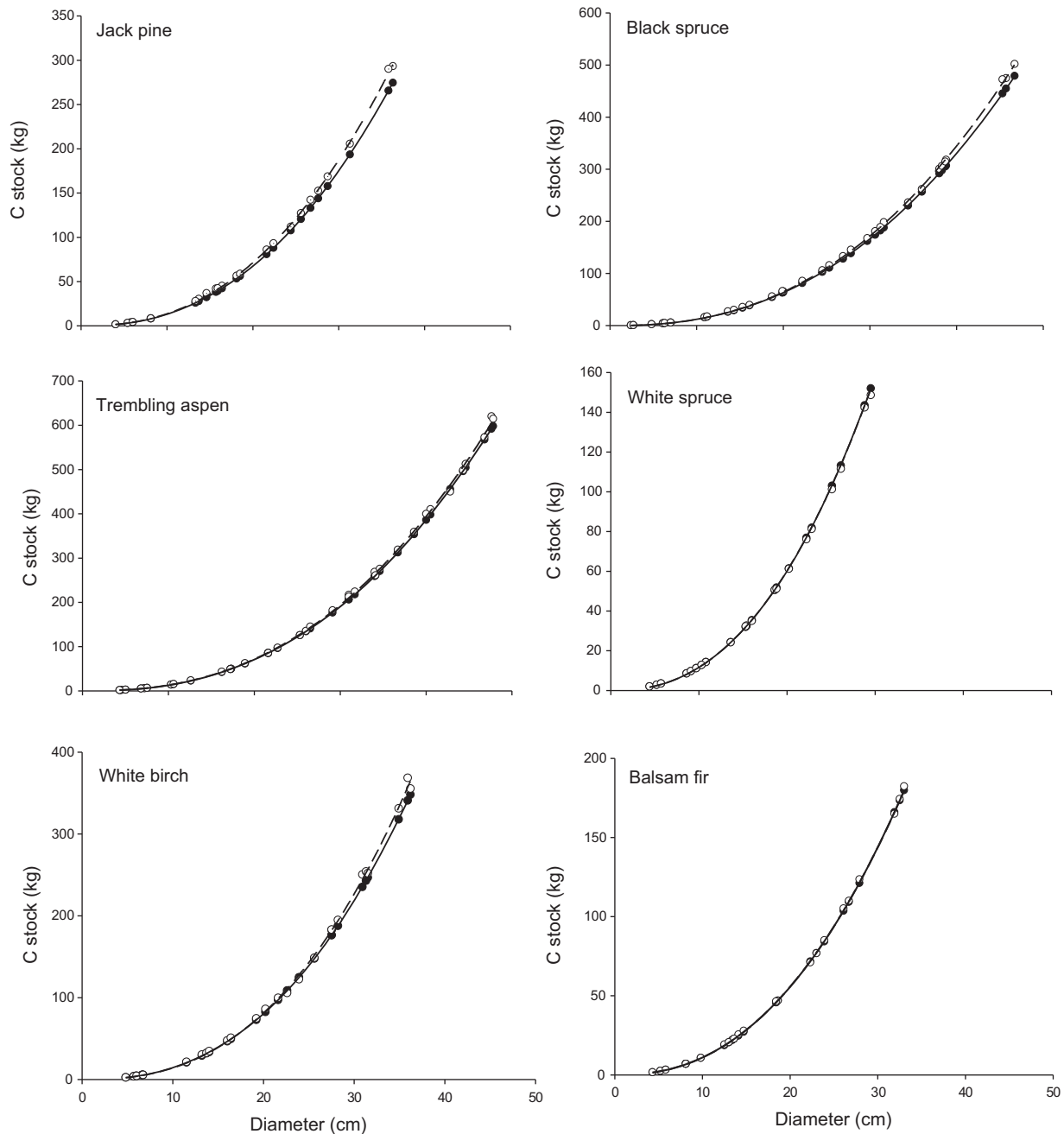


Fig. A.1. Aboveground carbon (C) stock estimates at the tree level for six boreal tree species. Open and closed circles represent C stocks calculated by our C concentration data and 50%, respectively. Regression relating DBH to total tree C stock was constructed and used to estimate species-level tree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

$$y = \beta_1 D^{\beta_2} + e \quad (\text{A.1})$$

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

exception of white birch, where we observed some individuals with exceptionally high C values. These high C values in white birch are likely related its high concentration of C-rich betulinic acid (O'Connell et al., 1988). The observed difference in trends between shade-tolerant and intolerant species supports our hypothesis that size-associated changes in total C concentration depend on shade tolerance. The potential reason for this size-associated interaction with contrasting shade tolerance may be

attributable to species' life history strategy under low light availability in the understorey. Shade-tolerant seedlings and saplings that can tolerate low light levels tend to grow slower and have denser wood, resulting in higher C concentration (Elias and Potvin, 2003; Poorter et al., 2005). However, the generality of these results is still uncertain, given that previous studies also found positive relationships between C concentration and tree size for shade-tolerant tree species in tropical forests (Martin et al.,

2013). Further empirical study of woody tissue C concentration, that explicitly accounts for volatile C, is needed from different biomes to further test the underlying effects of shade tolerance (or other functional traits) on tree C concentration.

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

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

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

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

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

5. Conclusion

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

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Table A.1

Model parameter estimates and their standard error (S.E.) for the DBH-based set 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

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Appendix A

See Fig. A.1 and Table A.1.

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