



**Needle age and season influence photosynthetic
temperature response in mature *Picea mariana* trees: Do we
overestimate the importance of new needles?**

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1 Needle age and season influence photosynthetic temperature response in mature *Picea*
2 *mariana* trees: Do we overestimate the importance of new needles?

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14 **Running head:** PHOTOSYNTHETIC TEMPERATURE RESPONSE IN BLACK SPRUCE.

15
16 **Keywords**

17 Black spruce; temperature adjustment; carbon assimilation; A/C_i curve; leaf age; Q10;
18 evergreen; SPRUCE project; STELLA model; respiration

Abstract

Using seasonal- and cohort-specific photosynthetic temperature response functions, we quantified the physiological significance of maintaining multiple foliar cohorts in mature (~40-45 year old) *Picea mariana* trees in an ombrotrophic *Sphagnum*-bog, northern Minnesota, USA. We measured photosynthetic capacity, foliar respiration (R_d), biochemistry and morphology to estimate annual carbon (C) uptake by cohort, season and canopy position. Temperature response of key photosynthetic parameters at 25°C (i.e., light-saturated rate of CO_2 assimilation (A_{sat}), light-saturated rate of Rubisco carboxylation (V_{cmax}), light-saturated electron transport rate (J_{max})) were clearly dependent on season and were generally less responsive in younger needles. Temperature optimums range between 18.7-23.7, 31.3-38.3 and 28.7-36.7°C for A_{sat} , V_{cmax} and J_{max} respectively. Current-year (Y0) foliage had lower photosynthetic capacities compared to one-year-old (Y1) and two-year-old (Y2) foliage. As Y0 needles matured, values of A_{sat} , V_{cmax} , J_{max} , foliar LMA and nitrogen increased. Values of V_{cmax} , J_{max} and R_d were related to foliar nitrogen but only in the youngest (Y0) cohort. Foliar ontogeny affected photosynthetic capacity more than growth temperature. Morphological and physiological cohort differences were reflected by their annual contribution to modeled C uptake, with a ~36% lower estimated annual C uptake by Y0 needles (LAI 0.52 m² m⁻²) compared to Y1&2 cohorts (LAI 0.67 m² m⁻²). Collectively, these results illustrate the physiological and ecological significance of characterizing multiple foliar cohorts during bud break and throughout the growth season, and for cumulative C uptake model estimates.

Abbreviations

A_{sat} , light-saturated rate of CO_2 assimilation at [CO_2] 400 ppm; g_m , mesophyll conductance; J_{max} , light-saturated electron transport rate; LAI, leaf area index; LMA, leaf mass per area; N_a , nitrogen content per unit leaf area; N_m , nitrogen content per unit leaf mass; PA, projected

leaf area; R_d , daytime respiration; T_{air} , air temperature; T_{growth} , growth temperature; T_{opt} , temperature optimum; TPU, triose phosphate utilization; V_{cmax} , light-saturated rate of Rubisco carboxylation; Y0, current year old; Y1, one-year-old; Y2, two-year-old.

Introduction

In *Picea mariana* (i.e., black spruce), as in many evergreen trees, the maintenance of multiple foliar cohorts is considered an adaptive trait to conserve nutrients but also to enable early season carbon (C) assimilation prior to and during shoot flushing (e.g. Troeng and Linder 1982, Öquist and Huner 2003). The latter may be especially important for boreal conifers where bud break occurs in May-June and new foliage is only fully matured late in the growing season (Teskey et al. 1984). For such conifers, moderate temperature increases associated with global warming may enhance total seasonal CO₂ assimilation as a result of earlier onset of bud break and photosynthesis in overwintering needles (Mäkelä et al. 2004, Goodine et al. 2008, Fløistad and Granhus 2010, Sutinen et al. 2012), thus potentially reducing the physiological/ecological significance of older cohorts. In addition, greater assimilation rates across a warmer growth season may also result from photosynthesis operating closer to a localized species-specific photosynthetic temperature optimum (T_{opt}) (Berry and Björkman 1980, Way and Sage 2008a,b, Yamori et al. 2013).

Springtime resumption of photosynthesis in overwintering needle cohorts and bud break is a function of soil and air temperature (T_{air}) in many conifer species (Bergh and Linder 1999, Tanja et al. 2003, Fréchette et al. 2011). The release of dormancy entails changes in chloroplast ultra-structure and elevated levels of chlorophyll, photosystem II, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and downstream co-/enzymes of the Calvin Cycle (see Öquist and Huner 2003). While, it is well documented that rates of

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3 71 photosynthetic recovery from dormancy and new foliar development increase with increasing
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5 72 air temperature (Troeng and Linder 1982, Goulden et al. 1997, Mäkelä et al. 2004), little is
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7 73 known about the seasonal and developmental dynamics of temperature response of
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9 74 photosynthetic biochemical parameters (e.g. the maximum Rubisco carboxylation rate (V_{cmax})
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11 75 and the maximum rate of electron transport (J_{max}) or the mesophyll conductance (g_m) across
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13 76 multiple foliar cohorts.

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16 77 Temperature not only affects the immediate biochemical and enzymatic activities
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18 78 involved in photosynthesis but also drives short- and long-term temperature acclimation (Lin
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20 79 et al. 2013, Way and Yamori et al. 2013). Higher growth temperatures (T_{growth}) often result in
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22 80 greater T_{opt} for key photosynthetic parameters, such as V_{cmax} and J_{max} (Berry and Björkman
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24 81 1980, Way and Sage 2008a). Several studies have looked at thermal regulation/adjustment
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26 82 between seasons and across a latitudinal temperature gradient (e.g. Way and Sage 2008a,
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28 83 Hikosaka et al. 2007, Dillaway and Kruger 2010), whereas few studies have investigated
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30 84 effects of ontogeny or seasonality on T_{opt} of these key photosynthetic parameters (Medlyn et
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32 85 al. 2002a, Gunderson et al. 2010).

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36 86 Identifying and quantifying seasonal, canopy and ontogenetic variation in
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38 87 photosynthetic parameters is essential for modeling species and ecosystem-specific
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40 88 sensitivities to climate changes, including important aspects related to warming. Whereas the
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42 89 seasonal and canopy variations in photosynthetic capacity (e.g. maximum assimilation rate
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44 90 (A_{sat}), V_{cmax} and J_{max}) have been intensely studied across multiple functional groups and
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46 91 species (e.g. Wullschleger 1993, Niinemets et al. 2004), the underlying photosynthetic
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48 92 temperature responses have often been assumed constant. This assumption persists due to
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50 93 lack of data; even though factors known to affect thermal adjustment of photosynthesis, such
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52 94 as T_{air} , foliar age, or C, nutrient or water availability, are known to vary between seasons,
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canopy position and foliar age (Niinemets 2002, Grassi et al 2005, Gunderson et al. 2010, Salmon et al. 2011).

Here we quantify the seasonal physiological significance of maintaining multiple foliar cohorts in *P. mariana* at the southern edge of its current natural range. Our study site is an ombrotrophic *Picea-Sphagnum* bog in northern Minnesota, USA, that is part of a long-term climate change experiment to study ecosystem response to elevated CO₂ and temperature: “Spruce and Peatland Responses Under Climatic and Environmental Change” (SPRUCE; <http://mnspruce.ornl.gov/>). *P. mariana* has wide distribution at high latitudes across North America and is considered to be sensitive to future elevated summer temperatures (Dang and Lieffers 1989, Way and Sage 2008b). Over the last century, mean annual T_{air} in the northern American boreal zone has increased by 1.75-2.5°C, and global atmospheric model simulations suggest this trajectory will continue (Christensen et al. 2007, IPCC 2013). For *P. mariana*, a combination of elevated air temperatures and altered precipitation regimes may reduce the capacity to maintain older foliar cohorts, which could affect seasonal physiological performance.

The purpose of the study was to identify and quantify seasonal, canopy and cohort C assimilation patterns of a *P. mariana* stand (the S1-Bog), using seasonal- and cohort-specific temperature responses. To meet these objectives we: 1) characterized seasonal temperature response of key photosynthetic parameters (i.e., V_{cmax} , J_{max} and triose phosphate utilization (TPU)) and daytime respiration (R_d) of two successive *P. mariana* needle cohorts, 2) measured temporal and spatial differences in photosynthetic capacity, R_d , foliar morphology and nitrogen (N) status and 3) characterized the canopy structure of the *P. mariana* trees of the S1-Bog. Finally, 4) data were used as model input to project annual tree canopy C uptake using dynamic cohort-specific photosynthetic temperature response functions.

119 **Materials and methods**

120 *Study site*

121 The study was conducted in a mixed peatland *P. mariana* (Mill.) B.S.P. and *Larix laricina*
 122 (Du Roi) K. Koch) [tamarack; eastern larch] stand at the Marcell Experimental Forest in
 123 northern Minnesota, USA. Elevation is 410 m above sea level at N47°30'19" latitude and
 124 W93°27'18" longitude. The stand was established by natural regeneration following two strip
 125 cuttings in 1969 and 1974 (Sebestyen et al. 2011). The evaluated bog (S1-Bog) has an open
 126 canopy structure, with an average tree height of 4.5 m and a basal area of 5.62 cm² m⁻²,
 127 February 2013. The understory consists of woody shrubs species e.g. *Rhododendron*
 128 *groenlandicum* (Oeder) Kron and Judd) [Labrador tea], *Chamaedaphne calyculata* (L.)
 129 Moench) [leatherleaf], *Vaccinium oxycoccos* L. [bog cranberry], *V. angustifolium* Aiton)
 130 [blueberry] and a field layer of moss species including *Sphagnum* spp. and *Polytrichum* spp.,
 131 and herbaceous species such as *Eriophorum angustifolium* Honck. [cotton grass] and
 132 *Maianthemum trifolium* L. [three-leaf false Solomon's seal].

133 The climate is continental, with a 40-year average T_{air} of -15°C and 19°C during
 134 January and July, respectively, and annual precipitation averages at 780 mm (Sebestyen et al.
 135 2011). Site-specific climatic conditions were measured at the south end of the S1-Bog, 20-
 136 200 m from measurement trees (data available at <http://mnspruce.ornl.gov/>). T_{air} at 2 m and
 137 T_{soil} at multiple peat soil depths were measured using HMP45C temperature sensors and
 138 Type T sensors (Campbell Scientific, USA). Tree roots existed in the both the *Sphagnum*
 139 covered 'hummock' mounds and beneath the wetter 'hollows' between the mounds. Rooting
 140 depth was limited by a high but seasonally oscillating water table. Ground level is defined as
 141 the hollow surface. To evaluate the relationship between T_{growth} and V_{cmax} and J_{max} we
 142 calculated daily mean T_{air} of the 14 days prior to each field campaign.

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144 *Gas exchange*

145 Seasonal patterns of the photosynthetic capacity of current- (Y0) and one-year old (Y1)

146 foliage were measured in April, May, July, September, August and October between 2010

147 and 2013. Influences of canopy position and needle age on photosynthesis were measured for

148 three successive needle cohorts (Y0, Y1 and two-year old (Y2)) from top, middle and bottom

149 branches in July 2011. Photosynthetic response (A/C_i) curves were produced using portable

150 infrared gas analyzer systems (2x3 broadleaved chamber with a LED light source,

151 LI6400XT, Li-COR, USA). A/C_i curves were generated at saturating light levels ($PPFD >$

152 $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) by sequential adjustment of the reference CO_2 concentration between 50

153 and 1600 ppm (400, 300, 200, 100, 50, 400, 550, 650, 800, 950, 1200 and 1600 ppm) to

154 assess A_{sat} across a broad range of intercellular CO_2 concentrations.

155 Temperature response surfaces were generated by replication of the A/C_i curves at

156 multiple temperatures. Cuvette temperature was regulated from 2 to 50°C by Peltier

157 adjustment of the block temperature and by use of an external temperature controlled

158 circulating water bath attached to the cuvette with block water-jackets (LICOR model

159 number 6400-88). Rates were recorded 3-10 min. after target temperatures were reached.

160 Temperature responses of foliar R_d were measured in a similar fashion but using a conifer

161 chamber (April and September 2013). Maintaining a constant cuvette chamber vapor pressure

162 deficit (VPD) across temperature would was not possible during field measurements. We

163 minimized variations in VPD by regulating the humidity and for the October measurements

164 growth-chamber temperatures. Measurements were conducted on intact branches (attached to

165 the tree) or on cut branches that were re-cut under water prior to measurement. Most

166 measurements (>80%) on cut branches were completed the same day in the field, although

167 two sets of branches were shipped cold overnight back to Oak Ridge National Laboratory,

168 TN, USA for analysis of whole branch temperature responses in growth chambers (~8-44°C)

(Oct 2011) or to test impact of clipping. The potential reduction of the photosynthetic capacity due to clipping and shipping were tested by making point-measurements of A_{sat} in the field, then 1, 2, 4, 7, and 14 days after cutting and shipping to the lab (August 2012). No significant reduction in A_{sat} was observed after two days. However, after four days A_{sat} decreased by 43%. In total 308 A/C_i curves were used.

Following gas exchange measurements, foliage and branch material was shipped overnight to Tennessee for analysis of leaf area, leaf mass and N content. Shoots were defoliated and projected leaf area (PA) was estimated using WinRHIZO 2012b (Regent Instruments Canada Inc., Canada) and ImageJ 1.47 (Rasband 2012). Tissue was dried at 70°C then analyzed for leaf N content by mass (N_m) and carbon content using an elemental analyzer (Costech Analytical Technologies, Inc., USA). We calculated nitrogen per leaf area (N_a) as $N_a = N_m \times \text{LMA}$.

Tree allometry

To estimate canopy foliar distribution, eight 4-6 m tall trees were harvested in midsummer 2010 and 2011. Each tree was separated by one-meter stem increments and dried, such that foliar mass represented all cohorts of needles within each stem increment. The proportion of foliar mass per cohort was then determined in October 2012, by subsampling 15 trees between 4 and 7 m tall from three east-west transects spaced with 10 m distance, across the S1-Bog. A total of 45 branches were collected at the south side of the tree at three different positions within the canopy. Foliage on each branch was separated by cohort for analysis of relative foliar and woody mass, LMA and N content.

Data analysis

We obtained values for V_{cmax} , J_{max} and TPU for each individual A/C_i curve using the online available platform LeafWeb (<http://leafweb.ornl.gov>). LeafWeb utilizes the Farquhar-von Caemmerer-Berry method with a novel exhaustive dual optimization approach integrating g_m (Gu et al. 2010). Values of A_{sat} (PPFD > 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, CO_2 400 ppm) were obtained from individual A/C_i curve. We parameterized the temperature response of A_{sat} , V_{cmax} , J_{max} and TPU according to Medlyn et al. (2002b) by fitting eqn. 1 and 2 to gas exchange data collected from Y0 and Y1 needles in August and October.

$$f(T_k) = P_{(25^\circ\text{C})} \left(\exp\left[\frac{E_a(T_k - 298)}{298 R T_k}\right] \right) \left(\frac{1 + \exp\left(\frac{298 \Delta S - H_d}{298 R}\right)}{1 + \exp\left(\frac{T_k \Delta S - H_d}{T_k R}\right)} \right) \quad (1)$$

$$f(T_k) = P_{(opt)} \left(\frac{H_d \exp\left(\frac{H_a(T_k - T_{opt})}{T_k R T_{opt}}\right)}{H_d - H_a \left(1 - \exp\left(\frac{H_d(T_k - T_{opt})}{T_k R T_{opt}}\right) \right)} \right) \quad (2)$$

$P_{(25^\circ\text{C})}$ is the specific parameter (A_{sat} , V_{cmax} , J_{max} , and TPU) at 25°C (298 K), E_a describes the exponential part of the function or the activation energy, R is the gas constant ($8.3143 \text{ J K}^{-1} \text{ mol}^{-1}$), and T is leaf temperature in K, ΔS ($\text{J K}^{-1} \text{ mol}^{-1}$) is an entropy term, and H_d (J mol^{-1}) is the deactivation energy. The second part of eqn. 1 adjusts for loss of enzyme activity at higher temperatures (Leuning 2002). Equation 2 includes the parameter T_{opt} that is related to ΔS , see Dreyer et al. (2001) and Medlyn et al. (2002b) for full method description. Foliar R_d Q10 values were calculated according to Linder and Troeng (1981) using a linear regression model from the natural logarithm of R_d ; ($\ln(R_d) = \alpha + \beta T_{\text{air}}$).

$$Q_{10} = \exp^{(10 \beta)} \quad (3)$$

As Q_{10} values may be dependent on the underlying temperature range used (Atkin et al. 2005, Kruse et al. 2008), we normalized R_d values to 25°C ($R_{d25^\circ\text{C}}$) using eqn. 4, with a Q_{10} value calculated between 15 and 30°C using our September 2013 temperature response data.

$$R_{d25^\circ\text{C}} = R_{dT} * Q_{10}^{[(25-T)/25]} \quad (4)$$

Seasonal, canopy and cohort specific values of A_{sat} , V_{cmax} , J_{max} , and TPU were normalized to 25°C ($A_{\text{sat}25^\circ\text{C}}$, $V_{\text{cmax}25^\circ\text{C}}$, $J_{\text{max}25^\circ\text{C}}$, and $\text{TPU}_{25^\circ\text{C}}$) based on temperature response data according to season and needle age. Photosynthetic parameters for Y2 needles were normalized using temperature response of Y1 needles. Effects of needle age (Y), canopy position (C), seasonality (M) and sampling year (2010-2013), were evaluated using an ANCOVA model, using a Tukey–Kramer's HSD test to separate means. We excluded sampling year from the analysis, as its effect was not significant for any of the variables. Pseudoreplication was accounted for when repeated measurement occurred (for example needle cohorts from the same branch or tree). We used OLS models to test relationships between temperature, foliar N, needle age and R_d , $V_{\text{cmax}25^\circ\text{C}}$, $J_{\text{max}25^\circ\text{C}}$, foliar mass, LMA, C:N ratios. Model assumptions were tested using a Shapiro-Wilk normality test and a Bartlett's test of homogeneity of variances. Test outputs are given as R^2 and $F_{(\text{df}, n)}$ -values with the following significant levels: ($P > 0.05^{\text{ns}}$, $P < 0.05^*$, $P < 0.01^{**}$ and $P < 0.001^{***}$). Data analyses were carried out in R, version 3.0 (R Core Team 2013).

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Estimating annual carbon uptake by foliar cohorts

We estimated the annual total C uptake contribution of each individual foliar cohorts of the S1-Bog *P. mariana* trees by using the derived seasonal and cohort specific temperature response functions applied to a simple model of canopy photosynthesis. Annual C uptake was estimated using an hourly time step model for *P. mariana* cohorts coded for each cohort class using STELLA 10.0.3 modeling software (ISEE Systems Inc., NH, USA). This photosynthetic module has been successfully used as a component of an ecosystem level C and water cycle model (INTRASTAND; Hanson et al. 2004, 2005) and was used here to estimate net C uptake based on photosynthetic measurements and site-specific environmental drivers. The module estimated annual C uptake from the coupled Farquhar/Ball–Berry photosynthetic and stomatal conductance model as described by Harley et al. (1992), using the temperature response formulations (equation 1) summarized by Medlyn et al. (2002b). Farquhar model and R_d variables were obtained from our gas exchange measurements described above. Given the open nature of the S1-Bog forest, all foliage was assumed to be exposed to full sun for the purposes of annual C gain calculations. There certainly was some shading of the older cohorts, especially those >3 years old which were assumed not to be a significant component of C uptake due to their low residual biomass. Measured meteorological conditions at 2 m in air (CO_2 T, relative humidity, PAR) were applied as the effective conditions at the leaf surface. Daytime wind velocities at the site were $\sim 1.2\text{ m s}^{-1}$, which reduced the boundary layer conductance sufficiently to support this assumption. Canopy foliar biomass per unit ground area was derived from mass allometric relationships developed specific for the SPRUCE site for trees >1.3 m at breast height (dbh). Site-specific tree diameter data for 21 defined plot areas (113 m^2) combined with the allometric relationships data provided a ground-area based estimate of total standing canopy, February 2013.

Live foliar mass data multiplied by corresponding leaf mass per unit area yielded the leaf area index (LAI) for the defined annual cohort(s). At the SPRUCE site, foliage older than three cohorts only makes up a small fraction of *P. mariana* foliar mass (7%). Because such older foliage has a reduced photosynthetic capacity (see also Niinemets 2002) those cohorts were not considered an important contribution to *P. mariana* annual C uptake.

Results

Climate and shoot phenology

Climatic conditions between 2010 and 2013 were similar to the 40-year monthly means reported by Sebestyen et al. (2011). The measured monthly mean temperatures, ranged from -9.9 ± 0.2 to $-16.7 \pm 0.2^\circ\text{C}$ and from 19.3 ± 0.2 to $28.1 \pm 0.2^\circ\text{C}$ in January and July, respectively. Growing season length averaged 175 days. Bud break for *P. mariana* occurred during June and the newly developed foliage (Y0), was visually classified as mature (fully expanded) towards the end of July and beginning of August.

Seasonal temperature response of photosynthesis

Season and needle age affected photosynthetic (A_{sat} , V_{cmax} and J_{max}) responsiveness to temperature and CO_2 , typically displaying flat response surfaces early in the season and in younger foliage (Fig. 1A-I). Table 1 gives the estimated parameters of the temperature response of A_{sat} , V_{cmax} , J_{max} and TPU according to season and needle age. In April, photosynthetic capacity (A_{sat} , V_{cmax} , J_{max} and TPU) of overwintering needles (Y1) showed a low responsiveness to CO_2 and temperature, compared to later in the season (Fig. 1 and Table 1). On projected leaf area values of A_{sat} and its response to temperature were similar for Y0 and Y1 needles but different between season, averaging around 9.2 ± 0.3 , 8.9 ± 0.4 and $6.4 \pm$

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0.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 25°C for Y1-August, Y0-August and Y0-October, respectively (Fig. 1B and C, Table 1). In contrast, both rates and responses of the biochemical parameters (V_{cmax} , J_{max} and TPU) depended on foliar age and season. In August, values of $P_{25^\circ\text{C}}$ and P_{opt} rates for V_{cmax} , J_{max} and TPU were much more responsive to CO_2 and temperature in Y1 needles compared to the new (Y0) foliage (Fig. 1E, H and Table 1). For Y0 foliage, values of $P_{25^\circ\text{C}}$ of V_{cmax} , J_{max} (Fig. 1E-F, H-I) and TPU increased with time (Table 1). Values of the activation energies (E_a) ranged between 18.3 and 49.8 kJ mol^{-1} for V_{cmax} , and 14.4 and 32.5 kJ mol^{-1} for J_{max} , generally greater as needles aged. For example, E_a values for V_{cmax} , J_{max} and TPU were 2-3 times greater in October compared to August (Table 1), in Y0 foliar cohorts. Temperature optima (T_{opt}) differed between cohorts and across season (Table 1). In August T_{opt} for V_{cmax} and J_{max} were 3.4 and 1.2°C lower in Y0 foliage compared to Y1 foliage, in contrast T_{opt} for V_{cmax} , J_{max} and TPU decreased in Y0 needles by 3.7, 6.8 and 7.7°C, respectively between August and October.

Temperature response of foliar R_d , on a leaf area basis, differed seasonally ($M \times T_{\text{air}}$: $F_{(1, 8)} = 24.6^{***}$), with Q10 values of 1.89 and 2.25 (between 15 and 30°C) during April and September 2013, respectively (Fig. 2). While LMA was greater in September ($371 \pm 19 \text{ g m}^{-2}$) compared to April ($274 \pm 18 \text{ g m}^{-2}$), temperature response on a mass basis still differed ($M \times T_{\text{air}}$: $F_{(1, 8)} = 13.1^{**}$). No sharp R_d reductions were observed at higher temperatures, and Q10 values (calculated in steps of 10°C) were similar over the used range of temperatures.

Seasonal patterns in needle photosynthetic capacity and morphology

The photosynthetic capacity at 25°C ($A_{\text{sat}25^\circ\text{C}}$, $V_{\text{cmax}25^\circ\text{C}}$, $J_{\text{max}25^\circ\text{C}}$), N_a and LMA was affected both by foliar age and season (Fig. 3). Mean ($\pm$ SE) values of $A_{\text{sat}25^\circ\text{C}}$ ranges from 0.1 ± 0.2 to 11.2 ± 2.3 and from 5.8 ± 0.2 to $9.1 \pm 0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ for Y1 and Y0 needles, respectively. Whereas rate of $A_{\text{sat}25^\circ\text{C}}$ different between foliar age (part of the season (Y: $F_{(1, 210)} = 13.2^{***}$),

311 M: $F_{(5, 210)} = 20.1^{***}$, Y×M: $F_{(1, 210)} = 12.0^{***}$), similar rates were observe for July and August
 312 (Fig. 3A).

313 For overwintering needles (Y1) mean values of $V_{\text{cmax}25^{\circ}\text{C}}$ increased from 5.1 ± 0.5 to
 314 $66.0 \pm 4.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ between April and May, and were stable around $66\text{--}76 \mu\text{mol m}^{-2} \text{s}^{-1}$
 315 until August. Current year (Y0) needles had 67% and 58% lower $V_{\text{cmax}25^{\circ}\text{C}}$ values compared
 316 to Y1 foliage in July and August, respectively. While $V_{\text{cmax}25^{\circ}\text{C}}$ of Y0 needles increased from
 317 21.0 ± 5.9 to $45.8 \pm 7.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ between July and September, they remained well below
 318 $V_{\text{cmax}25^{\circ}\text{C}}$ of Y1 needles during the growing season (Fig. 3B). For Y1 foliage $J_{\text{max}25^{\circ}\text{C}}$ and
 319 $\text{TPU}_{25^{\circ}\text{C}}$ increased significantly between April and May from 14.5 ± 0.9 to 90 ± 5.1 (Fig. 3C)
 320 and from 1.3 ± 0.1 to $6.6 \pm 0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ (data not shown), respectively. Seasonal $J_{\text{max}25^{\circ}\text{C}}$
 321 maximum values were 129.2 ± 0.6 and $93.8 \pm 4.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ in Y1 and Y0 foliage,
 322 occurring in August and October respectively (Fig. 3C). Growth temperatures (T_{growth} , daily
 323 mean temperature 14 days prior) at the site ranged from -1.4 to 20.0°C between April and
 324 July, and declined to 12.6°C by October. When normalized using cohort specific
 325 photosynthetic temperature response current-year needles (Y1) values of $V_{\text{cmax}25^{\circ}\text{C}}$ were
 326 positively related to T_{growth} ($y = 24.0 + 2.17x$, $R^2 = 0.652^{**}$). In contrast, and as the Y0
 327 needles developed, values of $V_{\text{cmax}25^{\circ}\text{C}}$ decreased with increasing T_{growth} ($y = 100.1 - 3.5x$, R^2
 328 $= 0.680^{**}$) (data not shown).

329 Values of N_a differed between cohort and season (Y: $F_{(1, 62)} = 44.5^{***}$, M: $F_{(5, 62)} =$
 330 5.4^{***}), and increased over time from 158.6 ± 4.3 to 200.3 ± 4.8 and 130.4 ± 7.3 to $177.3 \pm$
 331 17.2 mmol m^{-2} in Y1 and developing Y0 needles, respectively (Fig. 3D). Similarly, LMA was
 332 affected by cohort and season (Y: $F_{(1, 63)} = 12.3^{***}$, M: $F_{(5, 63)} = 41.2^{***}$), with average values
 333 ranging from 274.1 ± 5.4 to 364.4 ± 10.9 and 229.9 ± 10.8 to $355.2 \pm 13.6 \text{ g m}^{-2}$ in Y1 and
 334 Y0 needles, respectively (Fig. 3E).

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336 *Photosynthetic capacity and R_d by needle age and canopy position*

337 In July of 2011, needle age (Y: $F_{(2, 22)} = 5.9^*$, $F_{(2, 20)} = 4.1^*$), canopy position (C: $F_{(df, 22)} =$
338 6.1^* , $F_{(2, 20)} = 4.4^*$) but not their interaction affected $V_{cmax25^\circ C}$ and $J_{max25^\circ C}$, whereas $R_{d25^\circ C}$ was
339 only affected by needle age (Y: $F_{(2, 28)} = 12.9^{***}$) (Fig. 4A-C). Low rates of $V_{cmax25^\circ C}$ and
340 $J_{max25^\circ C}$ were generally observed in Y0 needles and in lower parts of the canopy (Fig. 4A, B),
341 although there was substantial variation in values due to environmental conditions such as
342 periodic shading from adjacent trees. Y0 foliage exhibited variation in $V_{cmax25^\circ C}$ and $J_{max25^\circ C}$
343 that was at least partially the result of differences in N_a content, although linear regressions
344 only explained 25% of the variation ($V_{cmax25^\circ C}$ $R^2 = 0.24^{***}$, $P < 0.001$, $J_{max25^\circ C}$, $R^2 = 0.25^{***}$).
345 Foliar respiration ($R_{d25^\circ C}$) was 73-84, 61-69 and 75-75% greater in Y0 than Y1-Y2 needles
346 for branches at the top, mid and bottom of the canopy, respectively (Fig. 4C). N_a was
347 negatively associated with $R_{d25^\circ C}$ ($R^2 = 0.41^{**}$) but only in Y0 foliage. As LMA was 14-18%
348 greater in Y1 and Y2 needles, this relationship is positive on a leaf mass basis (data not
349 shown).

351 *Canopy allometric and foliar morphology*

352 Needle mass distribution varied according to canopy position and cohort, as represented by a
353 6 m tall *P. mariana* tree whose foliage was sequentially harvested in 2012 (Fig. 5). For the
354 eight *P. mariana* harvested in June-July 2010/2011; 2-30%, 19-67% and 25-32% of the total
355 needle mass (3.39 ± 1.02 kg) was distributed in bottom-, mid-, and top-canopy layers,
356 respectively. Toward the end of the 2012 growing season approximately 80% of the total
357 foliar mass belonged to the three youngest foliar cohorts (Y0, Y1 and Y2) (Fig. 6A),
358 displayed towards the tips of the branches especially in upper tree locations. Branches in the
359 top of the canopy had a significantly greater proportion of foliar mass in the Y0 cohort, while
360 the proportion of foliar mass in other cohorts was not affected by height, and declined with

needle age (Fig. 6A). Older needle cohorts were observed to have greater LMA ($Y: F_{(5, 238)} = 2.4^*$) and C:N ratios ($Y: F_{(5, 194)} = 2.9^*$) as compared to Y0 needles, whereas canopy position had no significant effect (Fig. 6B,C).

Annual carbon uptake components by foliar cohorts

Photosynthetic temperature response measurements showed that annual leaf cohort contributions to total annual C uptake were different and must be estimated independently by cohort groups having similar characteristics. We estimated the annual C uptake (NPP) for the S1-Bog *P. mariana* stand to be 558 and 514 gC m⁻² yr⁻¹ for 2011 and 2012, respectively. Although, Y0 LAI was relative large 0.52 m² m⁻² compared to 0.67 m² m⁻² for Y1&2, current year foliar contributed with only 36% of the total annual C uptake.

Discussion

We evaluated the physiological significance of maintaining multiple foliar cohorts in mature (~40-45 year old) *P. mariana* trees of the S1-Bog in northern Minnesota, USA. Results presented here demonstrate; that estimates of canopy C uptake without integrating seasonal and cohort specific photosynthetic temperature functions would result in an overestimation of the annual C uptake, relative to each cohort's LAI contribution.

Seasonal dynamics of photosynthetic capacity

The cohort-specific seasonal patterns of $A_{\text{sat}25^\circ\text{C}}$ -values were similar to earlier reported rates in *P. mariana* and other boreal conifers, such as *Picea abies* and *Pinus sylvestris* (Fig. 3A)(Troeng and Linder 1982, Bergh and Linder 1999). While, our $A_{\text{sat}25^\circ\text{C}}$ estimations on average were greater than earlier published values for *P. mariana* (Goulder et al. 1997, Way

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and Sage 2008a, b, Hébert et al. 2010, Bronson and Gower 2010), it may reflect site-specific differences in growth temperature and resource availabilities. For example, values of $A_{\text{sat}25^{\circ}\text{C}}$ presented here are 2-2.5 times greater than values reported by Bronson and Gower (2010), for *P. mariana* saplings grown in Manitoba, Canada (1300 km north of the S1-Bog). It is generally assumed that A_{sat} decline with needle age, at least on leaf area basis (e.g. Hébert et al. 2010). Our results contrast this, in general having lower values of A_{sat} in younger needles. This reduced photosynthetic capacity, is mainly a results of cohort-specific temperature response of the photosynthetic biochemical component (V_{cmax} and J_{max}), whereas folia morphological development partly may counter this effect in *P. mariana* (Fig. 1 and 3). This is illustrated by the relatively similar overall values and temperature response of A_{sat} between Y0 and Y1 needles, in spite of significant lower rates of V_{cmax} and J_{max} in Y0 needles (Fig. 1B, E, H and 3A, B, C), a discrepancy that can be explained by shift in mesophyll conductance (g_m) with leaf maturation. Developing needles may therefore, at least to some extent, be able to compensate for this reduced capacity by adjusting g_m (Busch et al. 2013).

Resumption of photosynthesis in the spring entailed a substantial increase in $V_{\text{cmax}25^{\circ}\text{C}}$ and $J_{\text{max}25^{\circ}\text{C}}$ in Y1 needles. The rate of recovery of $V_{\text{cmax}25^{\circ}\text{C}}$ and $J_{\text{max}25^{\circ}\text{C}}$ (e.g. time period to seasonal optima) different; suggesting that growth temperatures and/or cumulative days of growth stimulate production and activation of Rubisco and the electron transport chain differently (Fig. 3B, C)(Sevanto et al. 2006). For the developing Y0 cohort the photosynthetic capacity ($V_{\text{cmax}25^{\circ}\text{C}}$ and $J_{\text{max}25^{\circ}\text{C}}$) of the Y0 cohort of needles increased during the growing season, but did not reach the capacity of the Y1 needles by October despite equal or greater LMA and N_a (Fig. 3A-C). Thus our results indicated that the photosynthetic apparatus of Y0 needles requires more than one growth season to mature, may explain difference in spring-time rate of recovery of $V_{\text{cmax}25^{\circ}\text{C}}$ and $J_{\text{max}25^{\circ}\text{C}}$.

In agreement with previously findings from boreal *Picea* trees (Temesgen and Weiskittel 2006) we found greater seasonal variation in leaf N and LMA in Y0 needles compared to Y1 needles (Fig. 3D and E). Although, the site is classified as ombrotrophic, and the vegetation potentially N restricted (Iversen et al. 2010, Sebestyen et al. 2011), we found no relationship between foliar N_a and $V_{cmax25^{\circ}C}$, $J_{max25^{\circ}C}$ or R_d in mature (Y1), indicating that the photosynthesis was not N limited (Fig. 3B, C and D). In contrast, and similar to findings by Greenway et al. (1992), N_a increased in Y0 foliar cohorts through time (Fig. 3D), followed by increased photosynthetic capacity (V_{cmax} and J_{max}) (Fig. 3B, C and D). Thus on a seasonal scale, total canopy C uptake may be N limited, as ~36% of the total C uptake are associated with Y0 needles.

Seasonality and needle age affected both the temperature optimum and temperature response curve shapes (Fig. 1 and Table 1). Temperature response curves for V_{cmax} and J_{max} were noticeably more bell-shaped with greater thermal optimums (T_{opt}) and greater activation energies (E_a) in one-year-old (Y1) needles than in new (Y0) needles. However, with time and towards the end of the growth season, Y0 needles' response curves became more similar to response curves observed for Y1 needles (Fig. 1 and Table 1). Observed values of T_{opt} in *P. mariana* are of the same magnitude as reported for *Quercus robur*, *Betula pendula* (Dreyer et al. 2001) and *Pinus radiata* but ~10°C greater than observed for *Pinus sylvestris* (Medlyn et al. 2002). Multiple factors are correlated with shifts in T_{opt} of V_{cmax} and J_{max} including growth temperature, ontogeny and leaf nutrient status (Berry and Björkman 1980, Martindale and Leegood 1997, Niinmets 1999, Medlyn et al. 2002a, Khaembah et al. 2013). While changes in growth temperatures and light availability may explain seasonal differences, foliar nutrient status and ontogeny may account for needle-age differences in T_{opt} of V_{cmax} and J_{max} . We note that, as field conditions made it difficult to maintain a consistent VPD across temperatures, A/C_i curves generated at higher temperatures are less reliable (Medlyn et al. 2002a).

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435 Thermal adjustment of the photosynthetic apparatus occurred in both old and
436 developing needles, sometimes in parallel with shifts in N allocations (Berry and Björkman
437 1980, Hikosaka 1997, Öquist and Huner 2003, Niinemets 2007). Whereas, changes in T_{growth}
438 likely induce similar physiological caused on a given tissue, its capacity to respond and thus
439 adjust would be dependent on the biochemical status of the tissue. In *P. mariana*, Y0 and Y1
440 foliage had contrasting response to T_{growth} , with positive relationships in older needles.
441 Further, Y0 *P. mariana* needles response to T_{growth} differed depending on parameter V_{cmax} and
442 J_{max} , together these results suggest ontogenic shifts in thermal adjustment capacity,
443 potentially linked to tissue-specific N availability and allocation patterns (Fig. 3D). To our
444 knowledge, ontogenic thermal adjustment capacity has never been described for the
445 temperature response of V_{cmax} or J_{max} . However, it is outside the scope of the study to
446 quantify the thermal adjustment capacity at different developmental stages.

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448 *Seasonal temperature adjustment of foliar R_d*

449 Adjustments of respiration to temperature in mature tissues are common and thought to lead
450 to plant C balance homeostasis (Reich et al. 1998). In contrast to Way and Sage (2008a), we
451 found seasonal adjustment of Q10 R_d in *P. mariana* needles between April ($T_{\text{growth}} = -1.4^{\circ}\text{C}$)
452 and September ($T_{\text{growth}} = 16.4^{\circ}\text{C}$) both when assessed on leaf area and mass basis. This
453 adjustment reflects differences in metabolic activity (mitochondrial activity and substrate/Co-
454 enzyme availability) and T_{growth} (e.g. Atkin et al. 2005, Kruse et al. 2008). Here we measured
455 instantaneous foliar R_d temperature response of multiple needle cohorts, but as metabolic
456 activity often is greater during rapid growth and expansion (Machado and Reich 2006), Q10
457 values are likely cohort specific. This is partly supported, by the 61-75% greater R_d values
458 observed during July (2011) for Y0 needles compared to Y1&2 needles (Fig. 5C)(Teskey et

al. 1984). In agreement with earlier findings (Way and Sage 2008a, Tjoelker et al. 1998) low levels of foliar N were associated with low rates of respiration per leaf mass in *P. mariana*.

Annual carbon uptake contribution by foliar cohorts and model implications

Our simulations differ from earlier studies (Girardin et al. 2008, Hall et al. 2009) in that we modeled total annual carbon uptake separately by leaf cohort contributions based on insights of seasonal and cohort specific photosynthetic temperature response. For the S1-Bog, this reduced the total annual C uptake running the model with a single cohort, as the Y0 needle cohort contributed ~36% (LAI: Y0 = 0.52 and Y1&2 = 0.67 m² m⁻²). Multi-cohort modeling illustrates the physiological/ecological significance of retaining several needle cohorts, not only by assimilating CO₂ earlier in the season (resumption of photosynthesis) (Öquist and Huner 2003, Tanja et al. 2003, Hall et al. 2009) but also by maintaining significantly greater photosynthetic capacity throughout the growth season.

For model simplification, we assumed homogeneous light availability (full sun) between needle cohorts and throughout the canopy, likely resulting in an overestimation of the total canopy C uptake. While, we observed little evidence of self-shading between Y0 and Y1 (Fig. 3A, B and Table 1) or between Y1 and Y2 at branch level. Vertical light stratification influenced within canopy photosynthesis capacity (Fig. 5A, B). Suggesting, that for this relatively open S1-Bog *P. mariana* secondary forest, within canopy self-shading induces greater C estimation errors compared to branch level self-shading. However, assuming homogeneous light availability across multiple needle cohorts may induce greater C estimation errors in other systems, as for example productive upland forests with denser canopies (Dewar et al. 2012).

Findings presented here have two major implications for C-assimilation modeling at canopy and stand level, especially for boreal conifers with prolonged foliar ontogeny (weeks

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3 484 to months). Firstly, it is evident that individual leaf cohort contributions to annual C uptake
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5 485 must be estimated for cohort groups having similar characteristics, requiring high-resolution
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7 486 species- and site-specific-allometric and physiological information at leaf-, canopy- and stand
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10 487 -levels. Secondly, that the underlying photosynthetic temperature response depends on foliar
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12 488 age (ontogeny) and seasonality. We suggest, in spite of being very labor intensive, that
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14 489 species-, seasonal- and age-specific temperature response dynamics should be integrated and
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16 490 linked to T_{growth} . Taken together our findings show the physiological and ecological
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18 491 significance of maintaining multiple foliar cohorts, not only during bud break but also
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For Peer Review

Figure Titles

Fig. 1. Seasonal temperature response of (A-C) A_{sat} , (D-F) V_{cmax} and (G-I) J_{max} in one-year old (Y1) and current year (Y0) *P. mariana* needles at the S1-Bog in northern Minnesota, USA. The scatter is individual values of A_{sat} , V_{cmax} and J_{max} and solid lines denote the curve fittings (Eqn. 1), see Table 1.

Fig. 2. Seasonal temperature response of foliar daytime respiration rates (R_d) in Y0-Y2 needle cohorts during April and September 2013.

Fig. 3. Seasonal patterns in (A-C) photosynthetic capacity, (D) N_a and (E) LMA, in one-year (Y1) and current (Y0) *P. mariana* needles. Values of A_{sat} , V_{cmax} and J_{max} are normalized to 25°C depending on season and needle age (Table 1). Data were collected and combined over a four-year period (2010-2013); April (Y1: 2013), May (Y1: 2011, 2012), June (Y1: 2010), July (Y0&1: 2011), August (Y0&1: 2013), September (Y0: 2010) and August and (Y0&Y1: 2013) and October (Y0: 2011, 2012). Values are means ($\pm$ 1SE), and ANCOVA output ($F_{(\text{df})}$ -values and n (total number of A/C_i curves across cohorts and months)), with significance levels for cohort (Y), month (M) and their interaction (YxM) estimates.

Fig. 4. Photosynthetic capacity ((A) $V_{\text{cmax}25^\circ\text{C}}$ and (B) $J_{\text{max}25^\circ\text{C}}$) and (C) foliar day-respiration rates ($R_{d25^\circ\text{C}}$) by needle age and canopy position measured over a four day period in July 2011. Values were normalized to 25°C, according to season and cohort (Table 1). Values are means ($\pm$ 1SE), and ANCOVA output ($F_{(\text{df})}$ -values and n), with significance levels for cohort (Y), canopy position (C) and their interaction (YxC) estimates.

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761 Fig. 5. Needle mass distribution within the canopy (m) of a 6 m tall representative *P. mariana*
762 tree, according to needle age. Whole trees were harvested in June/July 2010-2011 whereas
763 cohort proportions were estimated in October 2012.

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765 Fig. 6. (A) Proportion of total needle mass distribution, (B) LMA and (C) C:N ratios by
766 cohort and canopy position, October 2013. Values are means ($\pm$ 1SE), regression lines and
767 the ANCOVE output ($F_{(df)}$ -values and n), with significance levels for cohort (Y), canopy
768 position (C) and their interaction (YxC) estimates.

Table 1. Parameters of the temperature response of A_{sat} , V_{cmax} , J_{max} and TPU temperature response by cohort and season¹.
 Estimations on projected leaf area rates were generated using Equation 1 and 2. All estimated parameters, with exception of A_{sat} E_a of Y1
 needles, were significant ($P < 0.01$).

	$P_{25^\circ\text{C}}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	P_{opt} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	H_d (kJ mol^{-1})	E_a (kJ mol^{-1})	ΔS (J mol^{-1})	T_{opt} ($^\circ\text{C}$)	T_{growth} ($^\circ\text{C}$)	Residual SE ($P_{(25^\circ\text{C})} / P_{(\text{opt})}$)	df ($P_{(25^\circ\text{C})} / P_{(\text{opt})}$)	
A_{sat} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)										4
August										5
Y1	9.2 (0.3)	9.3 (0.3)	200	0.3 (2.5) ²	633.4 (3.1)	18.7	17.2	1.8/1.8	62/64	6
Y0	8.9 (0.4)	8.7 (0.2)	200	6.7 (3.1)	639.9 (2.3)	26.3	17.2	1.5/1.5	70/72	7
October										8
Y0	6.4 (0.4)	5.9 (0.2)	200	12.9 (4.9)	643.9 (2.4)	23.7	12.6	1.1/1.1	60/62	9
V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)										10
August										11
Y1	70.7 (2.3)	102.1 (2.8)	200	30.7 (3.2)	628.0 (2.3)	38.27	17.2	14.6/14.4	60/62	12
Y0	33.4 (1.3)	38.6 (1.2)	200	18.3 (3.4)	630.1 (2.7)	34.95	17.2	8.0/7.9	70/72	13
October										14
Y0	35.5 (2.0)	40.8 (1.4)	200	49.8 (7.8)	647.8 (2.5)	31.27	12.6	7.8/7.7	60/62	15
J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)										16
August										17
Y1	112.8 (2.2)	135.1 (2.7)	200	17.6 (2.1)	625.9 (2.3)	36.74	17.2	16.7/16.4	55/57	18
Y0	71.0 (2.0)	80.5 (1.8)	200	14.4 (2.3)	625.6 (2.8)	35.43	17.2	12.2/12.0	62/64	19
October										20
Y0	93.8 (4.8)	92.9 (2.57)	200	32.5 (5.7)	649.0 (2.5)	28.70	12.6	12.1/11.8	31/33	21
TPU ($\mu\text{mol m}^{-2} \text{s}^{-1}$)										22
August										23
Y1	8.5 (0.2)	9.9 (0.2)	200	15.9 (1.9)	623.8 (2.5)	37.33	17.2	1.2/1.2	55/57	24
Y0	5.2 (0.2)	6.2 (0.2)	200	16.1 (2.6)	624.0 (2.8)	37.28	17.2	1.1/1.1	70/72	25
October										26
Y0	7.0 (0.2)	6.2 (0.2)	200	33.2 (3.8)	647.2 (1.5)	29.59	12.6	0.9/0.9	60/62	27

¹Parameteres for April were not estimated, due to low responsiveness. ² P -values > 0.05

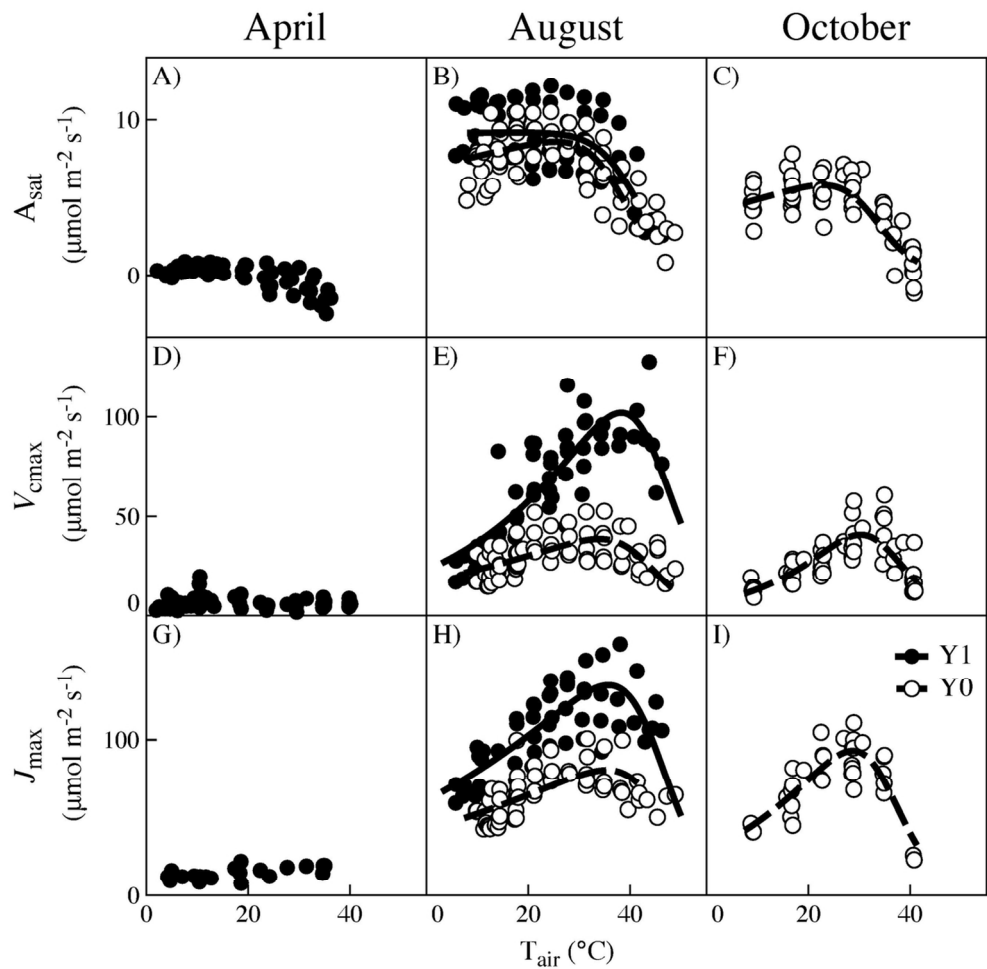


Fig. 1. Seasonal temperature response of (A-C) A_{sat} , (D-F) V_{cmax} and (G-I) J_{max} in one-year old (Y1) and current year (Y0) *P. mariana* needles at the S1-Bog in northern Minnesota, USA. The scatter is individual values of A_{sat} , V_{cmax} and J_{max} and solid lines denote the curve fittings (Eqn. 1), see Table 1.

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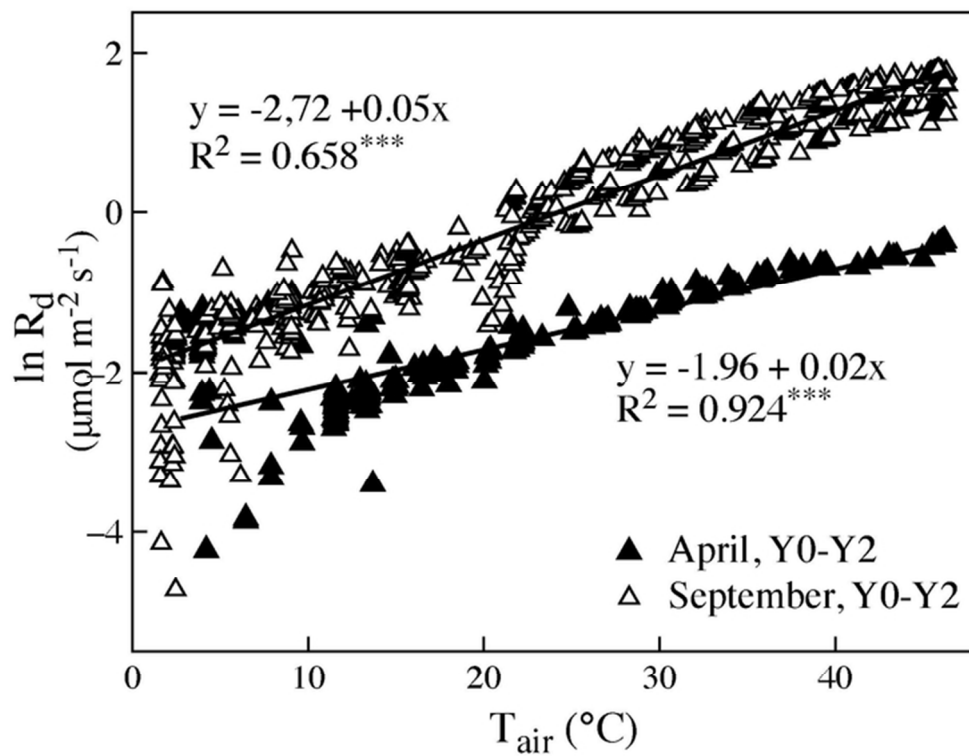


Fig. 2. Seasonal temperature response of foliar daytime respiration rates (R_d) in Y0-Y2 needle cohorts during April and September 2013.
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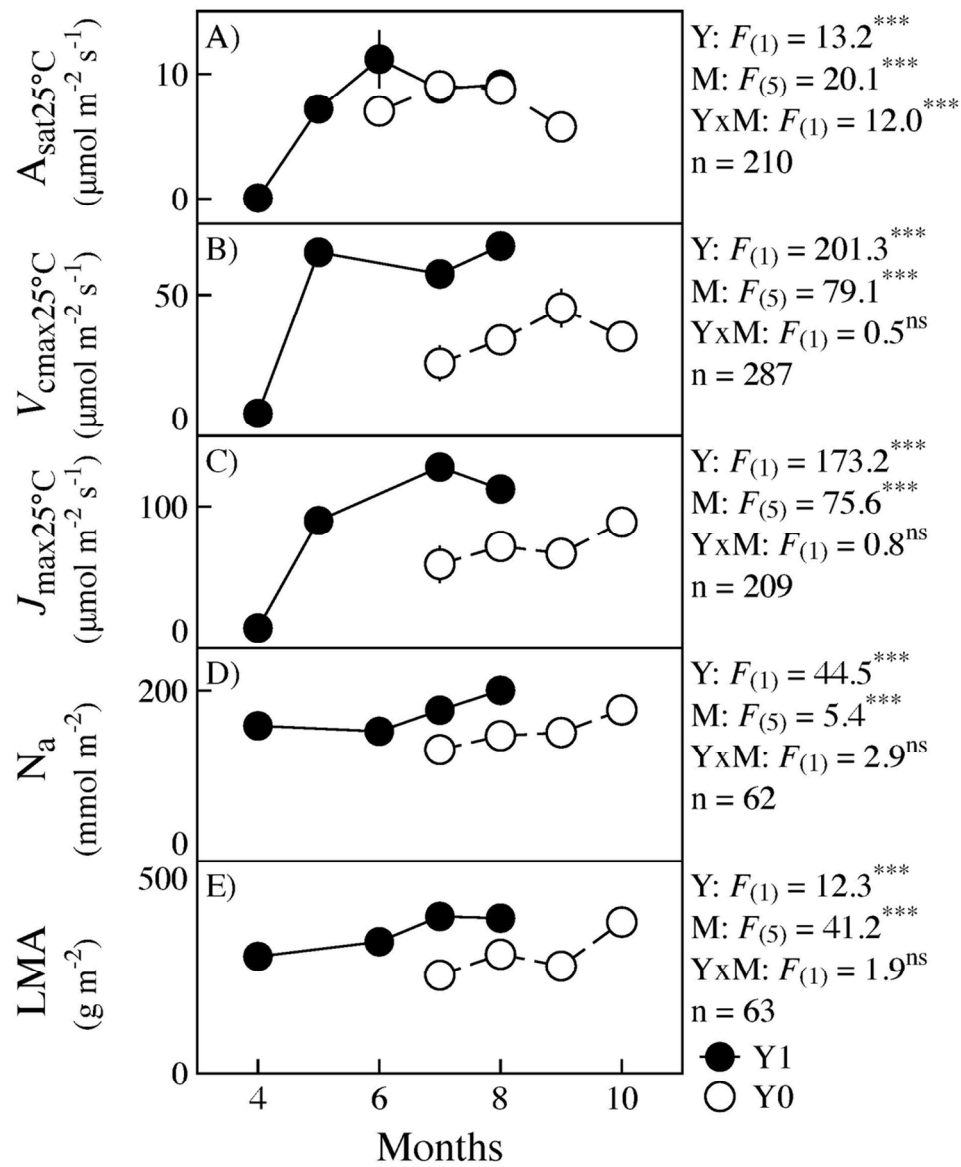


Fig. 3. Seasonal patterns in (A-C) photosynthetic capacity, (D) N_a and (E) LMA, in one-year (Y1) and current (Y0) *P. mariana* needles. Values of A_{sat} , V_{cmax} and J_{max} are normalized to 25°C depending on season and needle age (Table 1). Data were collected and combined over a four-year period (2010-2013); April (Y1: 2013), May (Y1: 2011, 2012), June (Y1: 2010), July (Y0&1: 2011), August (Y0&1: 2013), September (Y0: 2010) and August and (Y0&Y1: 2013) and October (Y0: 2011, 2012). Values are means ($\pm$ 1SE), and ANCOVA output ($F(df)$ -values and n (total number of A/Ci curves across cohorts and months)), with significance levels for cohort (Y), month (M) and their interaction (YxM) estimates.

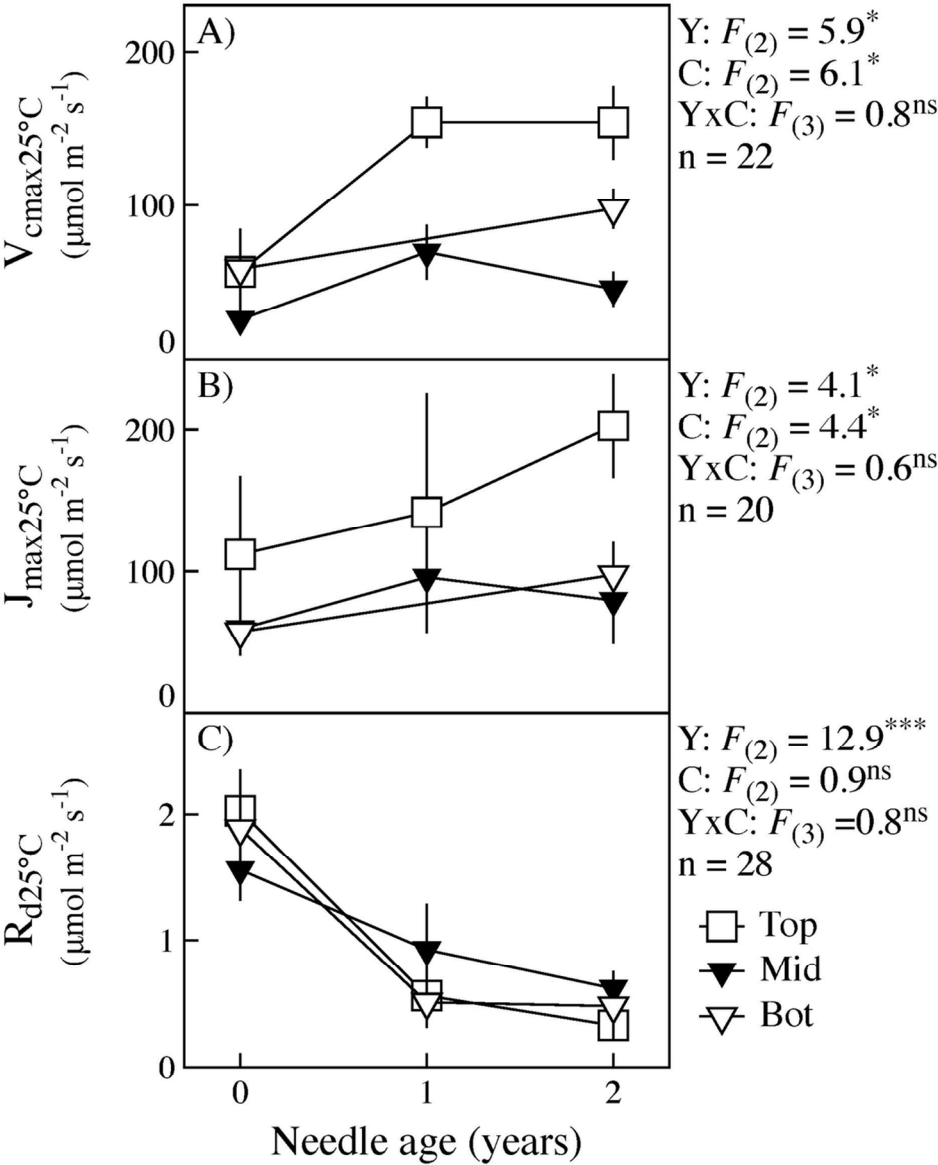


Fig. 4. Photosynthetic capacity ((A) $V_{cmax25^\circ C}$ and (B) $J_{max25^\circ C}$) and (C) foliar day-respiration rates ($R_{d25^\circ C}$) by needle age and canopy position measured over a four day period in July 2011. Values were normalized to $25^\circ C$, according to season and cohort (Table 1). Values are means (± 1 SE), and ANCOVA output ((F(df)-values and n), with significance levels for cohort (Y), canopy position (C) and their interaction (YxC) estimates.

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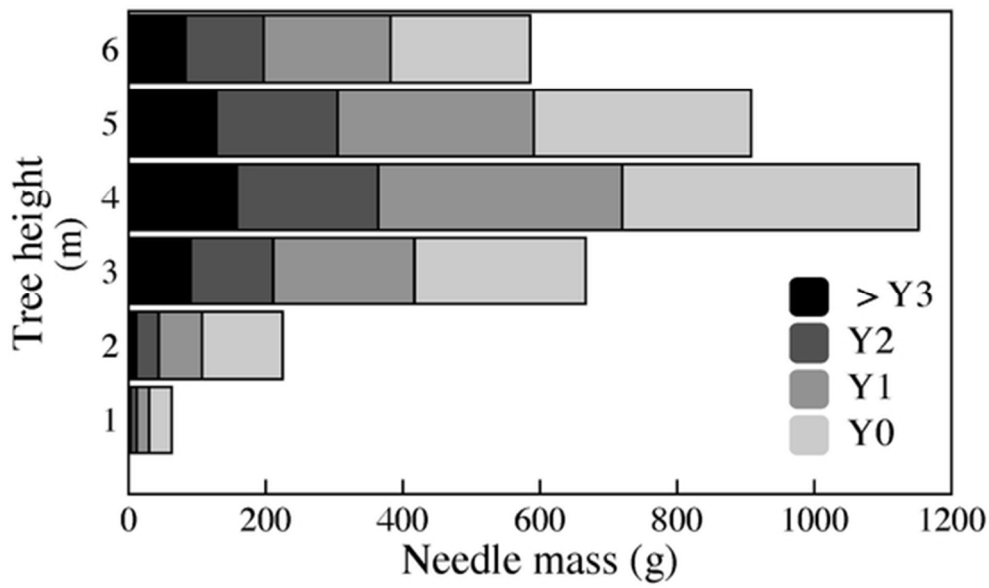


Fig. 5. Needle mass distribution within the canopy (m) of a 6 m tall representative *P. mariana* tree, according to needle age. Whole trees were harvested in June/July 2010-2011 whereas cohort proportions were estimated in October 2012.
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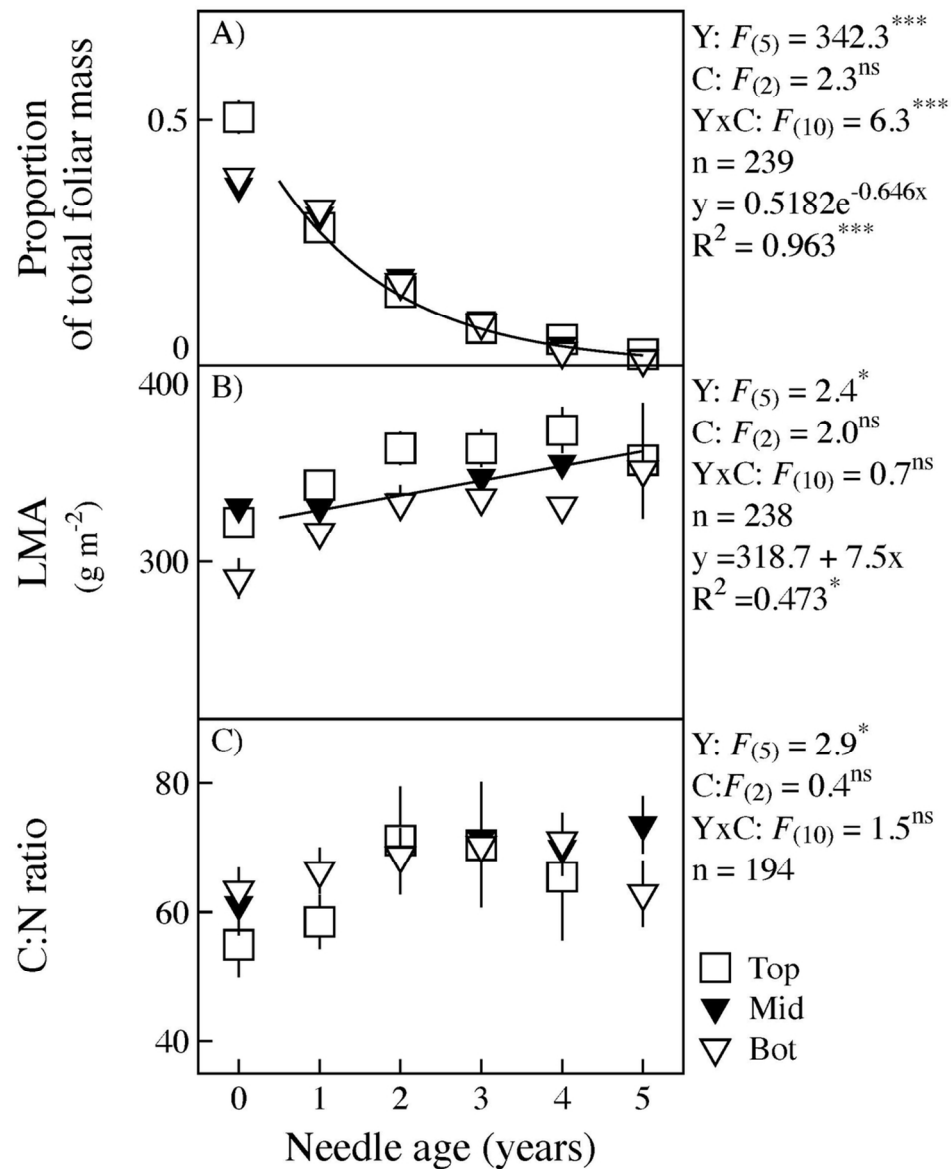


Fig. 6. (A) Proportion of total needle mass distribution, (B) LMA and (C) C:N ratios by cohort and canopy position, October 2013. Values are means ($\pm$ 1SE), regression lines and the ANCOVE output (F(df)-values and n), with significance levels for cohort (Y), canopy position (C) and their interaction (YxC) estimates.

101x125mm (300 x 300 DPI)