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Height-related decreases in mesophyll conductance, leaf photosynthesis and compensating adjustments associated with leaf nitrogen concentrations in Pinus densiflora

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Hydraulic limitations associated with increasing tree height result in reduced foliar stomatal conductance (g_s) and light-saturated photosynthesis (A_max). However, it is unclear whether the decline in A_max is attributable to height-related modifications in foliar nitrogen concentration (N), to mesophyll conductance (g_m) or to biochemical capacity for photosynthesis (maximum rate of carboxylation, V_cmax). Simultaneous measurements of gas exchange and chlorophyll fluorescence were made to determine g_m and V_cmax in four height classes of Pinus densiflora Sieb. & Zucc. trees. As the average height of growing trees increased from 3.1 to 13.7 m, g_m decreased from 0.250 to 0.107 mol m^-2 s^-1, and the CO_2 concentration from the intercellular space (C_i) to the site of carboxylation (C_c) decreased by an average of 74 µmol mol^-1. Furthermore, V_cmax estimated from C_c increased from 68.4 to 112.0 µmol m^-2 s^-1 with the increase in height, but did not change when it was calculated based on C_i. In contrast, A_max decreased from 14.17 to 10.73 µmol m^-2 s^-1. Leaf dry mass per unit area (LMA) increased significantly with tree height as well as N on both a dry mass and an area basis. All of these parameters were significantly correlated with tree height. In addition, g_m was closely correlated with LMA and g_s, indicating that increased diffusive resistance for CO_2 may be the inevitable consequence of morphological adaptation. Foliar N per unit area was positively correlated with V_cmax based on C_c but negatively with A_max, suggesting that enhancement of photosynthetic capacity is achieved by allocating more N to foliage in order to minimize the declines in A_max. Increases in the N cost associated with carbon gain because of the limited water available to taller trees lead to a trade-off between water use efficiency and photosynthetic nitrogen use efficiency. In conclusion, the height-related decrease in photosynthetic performance appears to result mainly from diffusive resistances rather than biochemical limitations.

Keywords: assimilation, diffusive limitation, internal conductance, leaf dry mass per unit area, maximum rate of carboxylation, water use efficiency.

Introduction

Water and nitrogen are two key resources involved in the process of carbon fixation by plants. The majority of leaf nitrogen (N) is found in the photosynthetic complex, and its allocation between structural and functional components determines photosynthetic capacity (Evans and Seemann 1989, Takashima et al. 2004). When the stomata open to allow CO_2 to diffuse into the leaf, water is lost by transpiration. The parameters water use efficiency (WUE) and nitrogen use efficiency (NUE) are widely employed to describe the ratio of photosynthetic rate to transpiration rate and photosynthetic rate to leaf N concentration, respectively (Field et al. 1983). There is a general consensus that a trade-off exists between WUE and NUE (Field et al. 1983, Wright et al. 2003) under conditions of limited resource availability.

When trees grow taller, they experience increased leaf hydraulic limitation because of the effects of gravity and friction (0.0098 MPa m^-1; Zimmermann 1983). In most cases, taller trees exhibit structural and anatomical adaptations that minimize...
hydraulic limitations, including increased sapwood area relative to leaf area, greater sapwood hydraulic conductivity and cavitation resistance, and lower minimum leaf water potential (Mencuccini and Grace 1996, Hubbard et al. 1999, Hacke and Sperry 2001, Mencuccini 2002, Zimmermann et al. 2004, Ambrose et al. 2009). Difference in xylem structures between conifers and broad-leaved trees (e.g., tracheids and vessels) links to their functional difference in transporting water and hydraulic adaptations (Hacke and Sperry 2001). However, these adjustments cannot eliminate constant hydrostatic constraints imposed by gravity, which reduce xylem and leaf water potential (Hubbard et al. 1999, Ishii et al. 2008). This hydraulic limitation reduces stomatal conductance ($g_s$) and consequently photosynthetic rate (Yoder et al. 1994, Hubbard et al. 1999, Ambrose et al. 2009, Mullin et al. 2009). However, tree-height- or age-related variations in physiological characteristics are not yet fully understood. For example, reduced $g_s$ is not sufficient to explain the decline in photosynthesis in tall trees (Niinemets 2002). In addition to the decline in photosynthesis resulting from reduced $g_s$, recent studies indicate that mesophyll conductance ($g_n$) between the intercellular space and the site of carboxylation is sufficient to limit the rate of photosynthesis (Harley et al. 1992, Flexas et al. 2008, Warren 2008b, Evans et al. 2009, Niinemets et al. 2009). It is known that $g_n$ can change as a result of acclimation and can respond to both the long term (weeks and days) and the short term (hours and minutes) to external factors, including light, temperature, water, nitrogen and CO$_2$ concentrations (Flexas et al. 2008). For example, water stress causes a reduction in $g_n$ to a level similar to that found for a reduction in $g_s$ (Warren et al. 2004, Grassi and Magnani 2005). Moreover, internal factors such as leaf development and aging strongly influence $g_n$ (Hanba et al. 1999, Miyazawa and Terashima 2001, Warren 2006b, Flexas et al. 2008, Niinemets et al. 2009). Variations in leaf dry mass per unit area (LMA) are often inversely correlated with $g_m$ (Flexas et al. 2008, Hassiotou et al. 2009, Niinemets et al. 2009). Leaf dry mass per unit area is generally higher in tall trees because of reduced cell expansion and slower cell division (Niinemets 2002, Ishii et al. 2008, Cavaleri et al. 2010, Oldham et al. 2010). However, only a limited number of studies have examined tree-height-mediated variations in $g_m$ (Mullin et al. 2009, Woodruff et al. 2009). If $g_m$ varied with tree height, it would affect estimates of biochemical capacity for photosynthesis (maximum rate of carboxylation, $V_{\text{cmax}}$) according to the model of Farquhar et al. (1980), and thus the interpretation of the physiological response to tree height.

Compared with the well-documented compensatory adjustments linked to alleviating hydraulic limitation in taller trees, the results reported for changes in leaf N with increasing tree height are still contradictory. Although in conifers decreases in this value have been reported (Schoettle 1994, Niinemets 1997, Woodruff et al. 2009), in other instances no changes were observed (Mencuccini and Grace 1996, Hubbard et al. 1999, Niinemets 2002). In contrast, increases in leaf N with increasing tree height have been observed in a deciduous broad-leaf tree species (Nabeshima and Hiura 2004). It is possible that the variation in nutrient availability across stands on various soils and at different geographical locations plays a more important role than tree-height- or age-related adjustments in foliar chemistry. For these reasons, making valid comparisons between trees of vastly different size, without the confounding effects of differences in environmental factors (especially soil nutrient availability), is a particular challenge. In this study, simultaneous measurements of gas exchange and chlorophyll fluorescence were conducted in 5- and 15-year-old Pinus densiflora Sieb. & Zucc. trees in 2003 and the same individuals were revisited in 2009 to minimize the confounding abiotic effect. The main objective was to examine whether $g_m$ and $V_{\text{cmax}}$ decreased as $P$. densiflora trees became taller. In addition, since all the trees were grown under the same soil water and nutrient conditions, it was possible to examine how foliar N changed with increasing tree height and determine whether there was a trade-off between WUE and NUE as the trees grew taller.

**Materials and methods**

**Study site**

This study was carried out in $P$. densiflora stands of four age classes in a field in the grounds of the Forestry and Forest Products Research Institute, Japan (36°0′0″N, 140°7′E, 24 m above sea level (asl)). The soil in which the stands grow is an Andisol, developed from parent materials of volcanic ash, with total nitrogen and carbon concentrations in the top 20 cm layer of 0.29 and 3.68%, respectively. During the period 1961–2009, the mean annual precipitation and temperature were recorded, at a nearby meteorological station (36°3′N, 140°7′E, 25 m asl, Japanese Bureau of Meteorology), as 1240 mm and 13.5 °C, respectively. Forest floor vegetation was sparse. In February 2003, the 5-year-old stand had attained an average height of 3.5 m and a diameter at breast height (DBH) of 4.1 cm, and the 15-year-old stand had an average height of 8.0 m and a DBH of 9.3 cm. When the same stands were measured in February 2009, the average values were 9.0 m high with a DBH of 10.3 cm in the 11-year-old stand, and 13.5 m high with a DBH of 16.1 in the 21-year-old stand.

**Measurements of gas exchange and chlorophyll fluorescence**

Since in situ gas exchange measurements were impractical owing to technical difficulties in accessing the shoots, all measurements were made on excised branches collected on two separate occasions in June and August 2003. Branches, ~0.50 m in length, were cut from the upper crown of both
5- and 15-year-old trees, immediately re-cut under water and then transported to the laboratory where measurements were made within 12 h of excision. Preliminary experiments with needles in situ from 5-year-old trees indicate that detachment did not influence the results of gas exchange characteristics within 24 h (data not shown), as has been found in similar studies with other conifers (Lange et al. 1986, Warren et al. 2003, Warren 2006b).

Gas exchange and chlorophyll fluorescence were measured simultaneously in 1-year-old hemicylindrical needles developed in the previous year (Li-6400; Li-Cor, Lincoln, NE, USA). One leaf from three to five individuals was tested in both tree age classes. For each replicate, five fascicles, consisting of 10 needles, were mounted in the Li-6400-40 fluorescence chamber in a holder that kept the needles separated. The curved needle surfaces were exposed to a 6400-40 light source. For each of the photosynthesis response curves to intercellular CO₂ concentration (A/C), the needles were dark-adapted in the fluorescence chamber at a CO₂ concentration of 350 µmol mol⁻¹ for 1 h to measure the rate of dark respiration and the minimum and maximum fluorescence parameters. The needles were then exposed to photosynthetic photon flux densities (PPFDs) of 50, 100, 500 and 1100 µmol m⁻² s⁻¹ for 5 min at each step. After the rates of photosynthesis and transpiration were determined to be steady at PPFD 1100 µmol m⁻² s⁻¹ (equivalent to >95% of the threshold for light-saturated photosynthesis (Han et al. 2003)), the CO₂ concentration in the chamber was adjusted stepwise to 300, 250, 200, 150, 100, 50, 400, 500, 600, 700, 800, 1000, 1300, 1500 and 1800 µmol mol⁻¹, allowing at least 4 min for adjustment and stabilization at each step. Leaf temperatures were maintained at 25.8 ± 0.2 °C. The vapor pressure deficit in the chamber was <1.4 kPa for all measurements. To minimize leakage of CO₂ and H₂O into and out of the leaf chamber, new gaskets were changed, the chamber was enclosed in a holder that kept the needles separated. The curved needle surfaces were exposed to a 6400-40 light source. For each of the photosynthesis response curves to intercellular CO₂ concentration (A/C), the needles were dark-adapted in the fluorescence chamber at a CO₂ concentration of 350 µmol mol⁻¹ for 1 h to measure the rate of dark respiration and the minimum and maximum fluorescence parameters. The needles were then exposed to photosynthetic photon flux densities (PPFDs) of 50, 100, 500 and 1100 µmol m⁻² s⁻¹ for 5 min at each step. After the rates of photosynthesis and transpiration were determined to be steady at PPFD 1100 µmol m⁻² s⁻¹ (equivalent to >95% of the threshold for light-saturated photosynthesis (Han et al. 2003)), the CO₂ concentration in the chamber was adjusted stepwise to 300, 250, 200, 150, 100, 50, 400, 500, 600, 700, 800, 1000, 1300, 1500 and 1800 µmol mol⁻¹, allowing at least 4 min for adjustment and stabilization at each step. Leaf temperatures were maintained at 25.8 ± 0.2 °C. The vapor pressure deficit in the chamber was <1.4 kPa for all measurements. To minimize leakage of CO₂ and H₂O into and out of the leaf chamber, new gaskets were changed, the chamber was enclosed in a plastic bag and a moderate flow rate of 250 µmol s⁻¹ was selected (Flexas et al. 2007a, Pons et al. 2009).

A/C curves from the same trees were derived again in June and August 2009 when the trees were 11 or 21 years old. This unique experimental design minimized any confounding abiotic effects on leaf nitrogen and thus provided an ideal opportunity to investigate the compensating adjustments in taller trees associated with leaf nitrogen concentration and trade-off between WUE and NUE. Meanwhile, two separate campaigns within one growing season reduced the year-to-year variations in leaf morphological and photosynthetic parameters.

Analysis of leaf nitrogen concentration

Needles were harvested after performing the gas exchange measurements, and their projected leaf areas were measured using a scanner (LiDE200; Canon, Tokyo, Japan) and image analysis software (LIA32, K. Yamamoto of Nagoya University, Nagoya, Japan). They were then dried at 70 °C for 48 h and ground using a micro-homogenizing system (MS-100; Tomy, Tokyo, Japan). Approximately 25 mg of powdered bulk sample was combusted with circulating O₂ using an nitrogen carbon (NC) analyzer (Sumigraph NC-900; SCAS, Osaka, Japan), and the nitrogen concentration per unit dry mass (Nₜ) was determined using a gas chromatograph (GC-8A, Shimadzu, Kyoto, Japan).

Carbon isotope analysis

The combined system of an elemental analyzer (NC 2500; CE Instruments, Milan, Italy) and an isotope ratio mass spectrometer (MAT252; Thermo Electron, Bremen, Germany) was used for analysis of foliar carbon isotope composition (δ¹³C). Approximately 1 mg of powdered bulk sample was weighed into tin cups for each analysis. All δ¹³C values were expressed relative to the international standard Vienna Pee Dee Belemnite (VPDB): δ¹³C = (Rsample/RVPDB − 1) × 1000, with R being the ¹³C/¹²C ratio of the sample or the VPDB standard. The measured isotope ratio was expressed in δ notation in ‰. dl-α-Alanine (δ¹³C = −23.50‰) was used as the running standard for data calibration. The standard deviation for four replicate combustions of the dl-α-alanine standard was 0.02‰.

Calculation of photosynthetic parameters

Projected needle area was used in the photosynthetic parameters but not surface area. Photosynthetic electron transport (J) was calculated according to the default program installed in Li-6400, assuming a leaf absorptance of 0.85. Mesophyll conductance was estimated using the ‘variable J method’ of Harley et al. (1992):

\[ g_m = \frac{A}{C_i - \left( (\Gamma^* \left[ J + 8(A + R_d) \right]) / (J - 4(A + R_d)) \right)} \] (1)

where the CO₂ compensation point in the absence of mitochondrial respiration (\(\Gamma^*\)) was taken from Bernacchi et al. (2002), and the rate of non-photorespiratory respiration continuing in the light (\(R_d\)) was taken to be half of the rate of respiration measured in the dark. This simplification is supported by several experimental observations (Villar et al. 1995, Niinemets et al. 2005). \(g_m\) values were calculated for each step of the A/C curves. Since CO₂ concentration may affect \(g_m\) (Flexas et al. 2007b), data in the range 10 < \(dC_i/dA\) < 50 were averaged to estimate a constant \(g_m\) value (Harley et al. 1992):

\[ \frac{dC_i}{dA} = \frac{12 \times \Gamma^* \times J}{(J - 4 \times (A + R_d))^2} \] (2)

From this constant \(g_m\), the CO₂ concentration at the site of carboxylation (\(C_c\)) was calculated as

\[ C_c = C_i - \frac{A}{g_m} \] (3)

This simplification is supported by several experimental observations (Villar et al. 1995, Niinemets et al. 2005). \(g_m\) values were calculated for each step of the A/C curves. Since CO₂ concentration may affect \(g_m\) (Flexas et al. 2007b), data in the range 10 < \(dC_i/dA\) < 50 were averaged to estimate a constant \(g_m\) value (Harley et al. 1992):
The maximum rate of carboxylation and the maximum rate of electron transport \((I_{\text{max}})\) were estimated from \(A/C_i\) curves based on the model of Farquhar et al. (1980) using a non-linear curve-fitting routine available for use in Microsoft Excel (Sharkey et al. 2007). Limiting factors (the properties of ribulose 1,5-bisphosphate carboxylase/oxygenase, the rate of ribulose 1,5-bisphosphate regeneration and the rate of use of triose phosphates) were determined from \(J\) by fluorescence measurements. The values of \(V_{\text{cmax}}\) and \(I_{\text{max}}\) were estimated from \(A/C_i\) curves using the same non-linear curve-fitting routine. All \(V_{\text{cmax}}\) and \(I_{\text{max}}\) values were standardized to a common temperature of 25 °C.

Errors in the estimation of \(g_m\) by the ‘variable \(J\) method’ result from uncertainties in the calculation of \(R_\alpha\) and \(J\) (Warren 2006a, Pons et al. 2009). Although some uncertainty surrounds the true value of \(R_\alpha\), realistic changes in \(g_m\) have a minimal effect on \(g_m\) estimations. In the current study, sensitivity analysis demonstrated that \(g_m\) increased by only 7% when \(R_\alpha\) was assumed to be 200% greater, consistent with previous studies (Warren 2006a, Warren 2008a, Hassiotou et al. 2009, Pons et al. 2009). In the case of \(J\), a sensitivity analysis was also done to examine the effect of different leaf absorption (\(\alpha\)) on \(g_m\). When \(\alpha\) changed from 0.75 to 0.95, \(g_m\) varied from 14 to −8.2% compared with the value estimated at \(\alpha = 0.85\). Nevertheless, bias resulted from \(\alpha\) and \(R_\alpha\) unaffected tree-height/age-related variation in \(g_m\) and its correlations with leaf nitrogen and LMA.

### Data analyses

Values measured in June and August were averaged for each height class. The significance of the differences in leaf morphological and physiological characteristics between height/age classes was quantified by one-way analysis of variance using StatView (SAS Institute, Inc., Cary, NC, USA), and class means were compared using the Tukey–Kramer post hoc test. Linear and non-linear regressions were employed in the analysis of variable correlations (SPSS, Chicago, IL, USA).

### Results

Leaf structural traits and photosynthetic functions vary along the vertical canopy/crown profile in a phenomenon known as light acclimation. In the present study however, the older stand was located on the northern side of the younger stand and all of the branches were sampled from the upper crown. In this position the branches were exposed fully to the sun. The variations in both leaf structural traits and photosynthetic functions therefore resulted exclusively from tree height and/or tree age, independent of light availability.

### Leaf structure

Leaf dry mass per unit area increased significantly with tree height \((P < 0.001)\), from 215.6 g m\(^{-2}\) in 3.1 m trees to 265.7 g m\(^{-2}\) in 13.7 m trees (Table 1). Leaf nitrogen concentration

### Table 1. Summary of morphological and physiological characteristics for 1-year-old needles of each tree age class (each height class).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>5-year old (3.1 ± 0.1 m)</th>
<th>11-year old (9.2 ± 0.2 m)</th>
<th>15-year old (8.2 ± 0.3 m)</th>
<th>21-year old (13.7 ± 0.6 m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMA (g m(^{-2}))</td>
<td>215.6 ± 2.5</td>
<td>233.5 ± 4.0</td>
<td>251.7 ± 3.6</td>
<td>265.7 ± 4.7</td>
</tr>
<tr>
<td>(N_i) (g m(^{-2}))</td>
<td>2.9 ± 0.1</td>
<td>3.3 ± 0.2</td>
<td>3.6 ± 0.1</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>(N_m) (mg g(^{-1}))</td>
<td>13.28 ± 0.53</td>
<td>13.94 ± 0.47</td>
<td>14.2 ± 0.44</td>
<td>16.24 ± 0.91</td>
</tr>
<tr>
<td>(g_{\text{cmax}}) (mol m(^{-2}) s(^{-1}))</td>
<td>0.310 ± 0.035</td>
<td>0.186 ± 0.022</td>
<td>0.173 ± 0.015</td>
<td>0.134 ± 0.010</td>
</tr>
<tr>
<td>(g_m) (mol m(^{-2}) s(^{-1}))</td>
<td>0.250 ± 0.028</td>
<td>0.157 ± 0.015</td>
<td>0.173 ± 0.006</td>
<td>0.107 ± 0.007</td>
</tr>
<tr>
<td>(C_i) (µmol mol(^{-1}))</td>
<td>257.0 ± 4.4</td>
<td>228.9 ± 4.2</td>
<td>223.9 ± 5.0</td>
<td>195.6 ± 7.0</td>
</tr>
<tr>
<td>(C_c) (µmol mol(^{-1}))</td>
<td>192.9 ± 10.6</td>
<td>153.4 ± 9.0</td>
<td>140.2 ± 7.2</td>
<td>121.5 ± 8.6</td>
</tr>
<tr>
<td>(A_{\text{max}}) (µmol m(^{-2}) s(^{-1}))</td>
<td>14.17 ± 0.73</td>
<td>11.56 ± 1.19</td>
<td>11.25 ± 0.41</td>
<td>10.73 ± 0.53</td>
</tr>
<tr>
<td>(V_{\text{cmax}, C_i}) (µmol m(^{-2}) s(^{-1}))</td>
<td>65.76 ± 3.27</td>
<td>49.37 ± 4.84</td>
<td>44.88 ± 2.18</td>
<td>39.92 ± 2.39</td>
</tr>
<tr>
<td>(V_{\text{cmax}, C_c}) (µmol m(^{-2}) s(^{-1}))</td>
<td>68.4 ± 3.8</td>
<td>86.0 ± 12.1</td>
<td>75.6 ± 7.2</td>
<td>110.0 ± 10.2</td>
</tr>
<tr>
<td>(I_{\text{max}, C_i}) (nmol g(^{-1}) s(^{-1}))</td>
<td>316.78 ± 16.36</td>
<td>365.60 ± 47.96</td>
<td>299.19 ± 25.52</td>
<td>416.21 ± 39.15</td>
</tr>
<tr>
<td>(I_{\text{max}, C_c}) (µmol m(^{-2}) s(^{-1}))</td>
<td>112.7 ± 6.7</td>
<td>126.1 ± 7.0</td>
<td>103.0 ± 6.3</td>
<td>124.8 ± 6.0</td>
</tr>
<tr>
<td>(I_{\text{max}, C_c}) (µmol g(^{-1}) s(^{-1}))</td>
<td>453.49 ± 29.10</td>
<td>480.01 ± 49.18</td>
<td>404.77 ± 22.96</td>
<td>463.20 ± 22.04</td>
</tr>
<tr>
<td>(V_{\text{cmax}, C_i}) (µmol m(^{-2}) s(^{-1}))</td>
<td>61.6 ± 4.2</td>
<td>56.0 ± 5.9</td>
<td>64.9 ± 4.6</td>
<td>60.6 ± 5.9</td>
</tr>
<tr>
<td>(I_{\text{max}, C_c}) (µmol g(^{-1}) s(^{-1}))</td>
<td>91.0 ± 4.5</td>
<td>103.6 ± 10.4</td>
<td>95.0 ± 5.2</td>
<td>111.0 ± 11.5</td>
</tr>
<tr>
<td>NUE (µmol g(^{-1}) s(^{-1}))</td>
<td>5.0 ± 0.3</td>
<td>3.5 ± 0.3</td>
<td>3.2 ± 0.2</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>WUE (µmol mol(^{-1}))</td>
<td>5.0 ± 0.3</td>
<td>5.9 ± 0.3</td>
<td>5.4 ± 0.1</td>
<td>8.0 ± 0.9</td>
</tr>
<tr>
<td>(\delta^{13}C) (‰)</td>
<td>−30.11 ± 0.13</td>
<td>−29.43 ± 0.16</td>
<td>−28.78 ± 0.14</td>
<td>−28.94 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE \((n = 3–5\) trees per height class). Mean values with the same superscript do not differ significantly \((P < 0.05)\). LMA, leaf mass per area; \(N_i\) and \(N_m\), nitrogen concentration per unit area and dry mass, respectively; \(g_{\text{cmax}}\), mesophyll conductance; \(C_i\) and \(C_c\), CO\(_2\) concentration at intercellular space and at the site of carboxylation, respectively; \(A_{\text{max}}\), light-saturated photosynthetic rate; \(V_{\text{cmax}, C_i}\) and \(V_{\text{cmax}, C_c}\), maximum rate of carboxylation estimated from \(A/C_i\) and \(A/C_c\) curves, respectively; \(I_{\text{max}, C_i}\) and \(I_{\text{max}, C_c}\), maximum rate of electron transport estimated from \(A/C_i\) and \(A/C_c\) curves, respectively; NUE, photosynthetic nitrogen use efficiency estimated from \(A_{\text{max}}/N_i\); WUE, water use efficiency estimated as the ratio of photosynthesis to transpiration.
per dry mass increased noticeably with tree height (Figure 1, \(P = 0.013\)), from 13.28 mg g\(^{-1}\) in 3.1 m trees to 16.24 mg g\(^{-1}\) in 13.7 m trees (Table 1). Therefore, leaf \(N\) per unit area (\(N_a\)) increased from 2.9 g m\(^{-2}\) in 3.1 m trees to 4.3 g m\(^{-2}\) in 13.7 m trees (Table 1), exhibiting a significant increase with tree height (\(P < 0.001\)).

**Leaf gas exchange and carbon isotope**

Stomatal conductance decreased significantly with tree height (Figure 2a, \(P < 0.001\)). In addition, \(g_m\) decreased linearly with tree height (Figure 2b, \(P < 0.001\)) from 0.250 to 0.107 mol m\(^{-2}\) s\(^{-1}\) when trees increased in height from 3.1 to 13.7 m (Table 1). Consequently, both \(C_i\) and \(C_c\) decreased significantly with tree height (Table 1, \(P < 0.001\)), which resulted in a drawdown of CO\(_2\) concentration from \(C_i\) and \(C_c\) of \(-64–83\) \(\mu\)mol mol\(^{-1}\) (Table 1). The light-saturated photosynthetic rate (\(A_{\text{max}}\)) at ambient CO\(_2\) concentration declined significantly with tree height (Table 1, \(P = 0.016\)). In contrast, \(V_{\text{cmax}, cc}\) estimated from \(A/C_i\) curves increased significantly with tree height (Figure 3, \(P = 0.010\)), whereas \(V_{\text{cmax}, ci}\) estimated from \(A/C_c\) curves was unaffected by tree height (Table 1, \(P = 0.680\)). Compared with \(V_{\text{cmax}, i}\) \(V_{\text{max}}\) was unaffected by tree height whether this was estimated from \(A/C_i\) or \(A/C_c\) curves (Table 1, \(P > 0.145\)). As the trees grew taller, NUE decreased significantly and WUE increased significantly (Table 1). Increased WUE was consistent with a strong positive correlation between \(\delta^{13}\)C and tree height (Table 1, \(P < 0.001\)).

**Relationship between leaf structure and gas exchange**

There was a negative correlation between LMA and \(g_m\) (Figure 4a, \(r^2 = 0.80\)), as well as between LMA and \(g_s\) (data not shown; \(r^2 = 0.86\), \(P = 0.049\)). Therefore, \(g_m\) and \(g_s\) decreased proportionally as LMA increased with tree height (Figure 4b, \(r^2 = 0.93\)). Although LMA was negatively correlated with \(A_{\text{max}}\) (Figure 5a, \(r^2 = 0.82\)), it tended to be positively correlated with \(V_{\text{cmax}, cc}\) (Figure 5b, \(r^2 = 0.62\), \(P = 0.105\)).

There was a positive correlation between \(N_a\) and \(V_{\text{cmax}, cc}\) (Figure 6a, \(r^2 = 0.81\), but a negative correlation between \(N_a\) and \(A_{\text{max}}\) (Figure 6b, \(r^2 = 0.99\)). Therefore, \(A_{\text{max}}\) was non-linearly correlated with \(V_{\text{cmax}, cc}\) (Figure 6c, \(r^2 = 0.94\)). As the
Height-related changes in mesophyll conductance and photosynthesis

Figure 4. Relationship between (a) leaf dry mass per unit area (LMA) and mesophyll conductance (gₘ) and (b) stomatal conductance (gₛ) and gₘ in 1-year-old needles in the upper crown of 5- (filled circle), 11- (open circle), 15- (filled triangle) and 21- (open triangle) year-old P. densiflora trees. The same symbols represent the same individuals revisited in the summer of 2009 after the first campaign in the summer of 2003. Each value is the mean ± SE of three to five trees. The asterisk beside the regression coefficient (r²) indicates the significance of the correlation coefficient (P) at P < 0.05.

Discussion

The mean value of gₘ across all tree heights of P. densiflora was 0.177 mol m⁻² s⁻¹, which is similar to the reported value of 0.17 mol m⁻² s⁻¹ for gₘ for Pinus pinaster Ait. (Warren 2006b) and 0.160 mol m⁻² s⁻¹ for Pinus radiata D. Don (Bown et al. 2009). Overall, the values of gₘ in P. densiflora are consistent with the values obtained from 11 coniferous species (Manter and Kerrigan 2004, Mullin et al. 2009, Woodruff et al. 2009). Furthermore, the mean drawdown from Cᵢ to Cₑ of 75 µmol mol⁻¹ in P. densiflora was very similar to the mean value of 91 µmol mol⁻¹ from 20 woody evergreens (Warren 2008b). The low gₘ and large drawdown from Cᵢ to Cₑ underestimated Vₘₚₙₐₓ, especially in 13.7 m trees, by up to 46%. These findings affect the interpretation of the photosynthetic response in relation to tree height.

Tree-height-related decrease in photosynthesis

Both gₘ and gₛ decreased proportionally with tree height, and therefore the drawdown in CO₂ concentration from Cᵢ to Cₑ exhibited the same magnitude as from ambient air to Cᵢ. These results indicate that internal resistance to CO₂ diffusion is as important as stomatal adjustment in determining the photosynthetic response to tree height. Photosynthetic capacity (i.e., Vₘₚₙₐₓ), estimated from A/Cₑ curves, increased with increasing tree height. In contrast, when the estimation was derived from A/Cᵢ curves, tree height had no influence. These results imply that gₘ and the resultant CO₂ drawdown from Cᵢ to Cₑ should be taken into account when estimating photosynthetic capacity (i.e., Vₘₚₙₐₓ). The decrease in photosynthetic performance (e.g., Aₘₚₙₐₓ) appears to result mainly from increasing CO₂ diffusive resistance between ambient air and the site of carboxylation, rather than from biochemical limitation.

Mesophyll conductance is determined by the cell wall thickness of the mesophyll, the surface area of chloroplasts facing the intercellular airspace per unit leaf area, and the abundance and/or conductivity of aquaporins (Terashima et al. 2006, Flexas et al. 2008). Variation in LMA is often inversely correlated with gₘ (Flexas et al. 2008, Hassiotou et al. 2009). This LMA–gₘ pattern was also found in tree-height-mediated variation in P. densiflora. Therefore, increased diffusive limitation from Cᵢ to Cₑ may be the inevitable consequence of morphological adaptation to greater water stress in needles of taller trees.
Both $N_a$ and $N_m$ increased when trees grew taller, indicating that more $N$ was allocated to the needles. Despite the large body of evidence showing a decrease in $N_m$ with increasing tree height or age (Schoettle 1994, Niinemets 1997, Woodruff et al. 2009), some studies have demonstrated that $N_m$ varies independently of tree height or age (Mencuccini and Grace 1996, Hubbard et al. 1999, Niinemets 2002) or even that there is an increase in $N_m$ with tree size (Nabeshima and Hiura 2004). It is possible that the variation in nutrient availability across stands on various soils and at different geographical locations is of greater importance than tree-height- and age-related differences in foliar chemistry. In the present study, all of the height/age classes were present at the same site and the same specimens were revisited for sampling after a number of years to minimize any confounding abiotic effects. Therefore, the height-related increase in $N_m$ in $P. \text{densiflora}$ may be a compensating adjustment to increased CO$_2$ diffusive resistance. This is consistent with evidence that nitrogen fertilization enhances $g_m$ (Warren 2004, Bown et al. 2009) and WUE (Martin et al. 2010). Increased needle $N$ resulted in enhancement of photosynthetic capacity (e.g., $V_{cmax}$). However, enhancement of $V_{cmax}$ could not completely offset the decline in photosynthetic performance (e.g., $A_{max}$). Thus, it appears that constraints on water uptake and photosynthetic performance with increasing tree height could be overcome to some extent by investing more $N$ in leaves and reducing the water cost of carbon gain at the leaf.

Compensating adjustment

Both $N_a$ and $N_m$ increased when trees grew taller, indicating that more $N$ was allocated to the needles. Despite the large body of evidence showing a decrease in $N_m$ with increasing tree height or age (Schoettle 1994, Niinemets 1997, Woodruff et al. 2009), some studies have demonstrated that $N_m$ varies independently of tree height or age (Mencuccini and Grace 1996, Hubbard et al. 1999, Niinemets 2002) or even that there is an increase in $N_m$ with tree size (Nabeshima and Hiura 2004). It is possible that the variation in nutrient availability across stands on various soils and at different geographical locations is of greater importance than tree-height- and age-related differences in foliar chemistry. In the present study, all of the height/age classes were present at the same site and the same specimens were revisited for sampling after a number of years to minimize any confounding abiotic effects. Therefore, the height-related increase in $N_m$ in $P. \text{densiflora}$ may be a compensating adjustment to increased CO$_2$ diffusive resistance. This is consistent with evidence that nitrogen fertilization enhances $g_m$ (Warren 2004, Bown et al. 2009) and WUE (Martin et al. 2010). Increased needle $N$ resulted in enhancement of photosynthetic capacity (e.g., $V_{cmax}$). However, enhancement of $V_{cmax}$ could not completely offset the decline in photosynthetic performance (e.g., $A_{max}$).

### Trade-off between WUE and NUE

Maintenance of high WUE necessarily reduces NUE (Wright et al. 2003), and hence the $N$ cost of carbon gain increases because height-related limitations in water availability require greater WUE. Indeed, this trade-off between WUE and NUE has also been observed under salinity-induced limitations of water availability (Martin et al. 2010). Higher leaf $N_m$ usually correlates with higher rates of photosynthesis, because most of the nitrogen is associated with photosynthetic proteins (Evans and Seemann 1989). In the present study, this was in agreement with $N_a$-$V_{cmax}$ relationships. However, $N_a$ was even negatively correlated with $A_{max}$. Thus, it appears that constraints on water uptake and photosynthetic performance with increasing tree height could be overcome to some extent by investing more $N$ in leaves and reducing the water cost of carbon gain at the leaf.

![Figure 6](http://treephys.oxfordjournals.org/)

![Figure 7](http://treephys.oxfordjournals.org/)
level. Enrichment of the bulk of leaf $^{13}\text{C}$ with tree height provides more evidence for this conservative water use strategy.

In conclusion, mesophyll conductance and stomatal conductance decreased with increasing height of $P. \text{densiflora}$. The decline in photosynthetic performance with increasing tree height appeared to be caused by increased diffusive resistance for $\text{CO}_2$ but not by biochemical limitations. In contrast, photosynthetic capacity was enhanced as a compensating adjustment through allocation of more nitrogen to leaves. However, enhancement of photosynthetic capacity could not completely offset the decline in photosynthetic performance. These photosynthetic responses resulting from hydraulic limitation resulted in a trade-off between WUE and NUE.

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