Summary  We examined the effects of leaf age and mutual shading on the morphology, photosynthetic properties and nitrogen (N) allocation of foliage of an evergreen understory shrub, Daphniphyllum humile Maxim, growing along a natural light gradient in a deciduous Fagus crenata-dominated forest in Japan. Seedlings in high-light environments were subject to greater mutual shading and 1-year-old foliage survival was lower than in seedlings in low-light environments, indicating that the survival rates of foliage were related to the degree of mutual shading. Although specific leaf area (SLA) in current- and 1-year-old foliage was curvilinearly related to daily photosynthetic photon flux (PPF), SLA was unaffected by leaf age, indicating that foliage in D. humile may not acclimate morphologically to annual changes in light caused by mutual shading. Light-saturated net photosynthetic rates ($P_{\text{max}}$) were correlated with daily PPF in current-year foliage. In addition, a strong, positive relationship was found between nitrogen concentration per unit leaf area and $P_{\text{max}}$. In contrast, the relationship among PPF, N and photosynthetic parameters in 1-year old foliage was weak because of the strong remobilization of N from older leaves to current-year foliage in plants growing in high light. However, the relationship between daily PPF and both photosynthetic N-use efficiency and the ratio of maximum electron transport rate to maximum carboxylation rate did not differ between current-year and 1-year-old foliage, suggesting that these responses help maintain a high photosynthetic efficiency even in older foliage. We conclude that D. humile maximizes whole-plant carbon gain by maintaining a balance among photosynthetic functions across wide ranges of leaf ages and light environments.

Keywords: irradiance, nitrogen, photosynthetic acclimation, photosynthetic nitrogen-use efficiency (PNUE), ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco).

Introduction

Acclimation of leaf photosynthesis to different light environments is an important factor that increases photosynthetic capacity. Leaf acclimation is particularly important in the understory, where light is usually the most limiting resource for seedling survival and growth (Chazdon and Fetcher 1984, Pearcy and Sims 1994, Naramoto et al. 2001). When assessing the extent and nature of leaf plasticity along a light gradient, both morphological and physiological adjustments must be considered (Ellsworth and Reich 1993, Pearcy and Sims 1994). Previous studies have shown that leaf photosynthetic characteristics exhibit remarkable plasticity in response to variations in the light environment (Field and Mooney 1986, Evans 1989a). Generally, foliage that has developed in high irradiance is thicker and has a higher nitrogen (N) content and photosynthetic capacity per unit leaf area than shaded foliage (Boardman 1977). Furthermore, a linear relationship between photosynthetic capacity and leaf N content has been found in various shrub (Valladares and Pearcy 1999), deciduous tree (Niinemets et al. 1998, Le-Roux et al. 1999) and conifer species (Grassi and Bagnaresi 2001, Warren and Adams 2001, Han et al. 2003), because more than half the leaf N is allocated to photosynthetic proteins (Evans 1989a, Makino and Osmond 1991). The efficient use of N in the photosynthetic apparatus in accordance with the environmental conditions may be important in enhancing the fitness of a species (Field and Mooney 1986). Plants must effectively balance N allocation between the light-harvesting antenna, the electron carriers and Calvin cycle enzymes: greater investment of N in light-harvesting proteins maximizes carbon gain in low-light environments, whereas high investment of N in electron carriers and Calvin cycle enzymes maximizes carbon gain in high-light environments (Evans 1989c, Hikosaka and Terashima 1995). How does leaf age affect these patterns?

Leaf lifespan has been shown to be functionally related to
photosynthetic characteristics and photoprotective functions (Field 1983, Hirose and Werger 1987). Therefore, we hypothesized that leaf N concentration should decline with increasing leaf age more rapidly in seedlings in high-light environments than in low-light environments, because foliage in high light experiences greater changes in light availability during its lifetime than foliage in low-light environments. Furthermore, previous studies have shown that leaf senescence is accompanied by recycling of N within plants (Thomas and Stoddart 1980, Smart 1994). In some evergreen conifers, the relationship between light-saturated leaf net photosynthesis per unit area ($P_{\text{max}}$) and N is strong in young foliage, but weak when data for foliage of all ages are pooled (Brooks et al. 1996, Schoettle and Smith 1998). Therefore, allocation of N among components of the photosynthetic apparatus could differ with leaf age.

To test the hypothesis that differences in leaf age affect the nature and extent of functional and structural responses to light, we studied *Daphniphyllum humile* Maxim seedlings occurring naturally on the forest floor along a forest border–understory light gradient in a *Fagus crenata* Blume forest. *Daphniphyllum humile* is an evergreen understory shrub that grows under deciduous canopies on the western border of Japan. Leaves are retained for 2 and 4 years in *D. humile* plants grown in high- and low-light environments, respectively (Kikuzawa 1988). Previously, we described seasonal changes in photosynthetic characteristics and photoprotective functions in *D. humile* (Katahata et al. 2005). Evergreens are important component in deciduous forests and the evergreen trait assures high carbon gain. In this context, we studied field-established seedlings of *D. humile* to answer the following questions: (1) How does mutual shading of foliage vary in seedlings growing naturally along a forest border–understory light gradient beneath a *F. crenata* canopy? (2) How does the relationship between daily photosynthetic photon flux (PPF), leaf N and photosynthetic parameters change with leaf age? and (3) Is the allocation of N to the different compartments of the photosynthetic apparatus influenced by leaf age?

Materials and methods

**Study site and plant material**

Measurements were conducted on *D. humile* seedlings growing in different light environments in a *Fagus crenata* forest in the Naeba mountains, Niigata Prefecture, Japan (36°51’ N, 138°40’ E, altitude 900 m) in September 2003. Five light environments along the forest floor (with 3.35, 7.82, 14, 32.69 and 64.98% of above canopy irradiance) were established and denoted 1, 2, 3, 4 and 5, respectively. The climate of the Naeba Mountains is cool and temperate. Mean annual precipitation near the site is 1778 mm, according to 30-year records from 1967 to 1996 (Japanese Bureau of Meteorology). Naturally occurring seedlings (6–11 years old, 80–130 cm tall) were selected for study in each light environment.

**Light measurements in the *F. crenata* forest**

Photosynthetic photon flux at 1.5 m above the forest floors at each site ($PPF_{\text{ref}}$) was measured at each study site with quantum sensors (IKS-27, Koito, Yokohama, Japan) connected to a data logger (CR10-X, Campbell Scientific, Logan, UT). We measured PPF every 10 s and 30-min means were recorded by the data logger. The daily PPF (dPPF) data were collected from 0600 to 1800 h, for 46 days between August 1 and September 15. In addition, PPF on the leaf surface was measured continuously for 7–10 days at each study site with gallium arsenide phosphide photodiodes (G1118, Hamamatsu Photonics K. K., Shizuoka, Japan) as described by Nishimura et al. (1998) which were calibrated with a quantum sensor (Li-Cor, Lincoln, NE). The photodiodes were attached to two current-year and two 1-year-old leaves of three individuals at each site. The photodiodes were directly attached to the adaxial side of the foliage and were kept in place by double-sided tape. The natural orientation of the foliage was unaffected by the attachment of the sensors. An electric resistor (100 Ω) was connected to each terminal of the data logger to convert incident current to voltage. Unfortunately, absolute values of the measurements from the photodiode sensors mounted on the foliage cannot be compared among the five study sites because the duration of measurement differed among sites. Therefore, the photodiode value obtained for the 46 days between August 1 and September 15 were estimated from the relationships between mean $dPPF_{\text{ref}}$ and mean daily PPF for current-year foliage ($dPPF_{\text{current}}$) or for 1-year-old foliage ($dPPF_{1\text{-year-old}}$). Although this method probably introduced errors in calculating PPF, there were tight correlations between mean $dPPF_{\text{current}}$ and $dPPF_{\text{ref}}$ and $dPPF_{1\text{-year-old}}$ and $dPPF_{\text{ref}}$ (e.g., $y_{\text{current}} = 0.69x$, $r^2 = 0.95$ and $y_{1\text{-year-old}} = 0.55x$, $r^2 = 0.89$ at Site 1; $y_{\text{current}} = 0.83x$, $r^2 = 0.98$ and $y_{1\text{-year-old}} = 0.39x$, $r^2 = 0.89$ at Site 5, where $y_{\text{current}} = dPPF_{\text{current}}$ and $y_{1\text{-year-old}} = dPPF_{1\text{-year-old}}$ respectively, and $x$ is $dPPF_{\text{ref}}$). We calculated degree of mutual shading (MS) by current-year foliage from mean $dPPF_{\text{current}}$ and $dPPF_{1\text{-year-old}}$ estimated by the method described above as:

$$MS \, (\%) = 100 \left( \frac{\text{est}(dPPF_{\text{current}}) - \text{est}(dPPF_{1\text{-year-old}})}{\text{est}(dPPF_{\text{current}})} \right)$$

(1)

**Carbon dioxide gas exchange measurements**

Carbon dioxide (CO$_2$) exchange of leaves in the vicinity of the photodiode sensors was measured in situ with a Li-Cor LI-6400 portable photosynthesis system between August 31 and September 7, 2003. The photosynthetic light response curves were determined at eight irradiances from 1500 to 0 μmol m$^{-2}$ s$^{-1}$ at a temperature of 22 °C, a relative humidity of 70% and a CO$_2$ concentration of 350 μmol mol$^{-1}$.

Photosynthetic responses to intercellular CO$_2$ concentration were determined at eight CO$_2$ concentrations ($A/C_i$ curves), ranging from 0 to 1800 μmol mol$^{-1}$ under the same conditions, as for the determination of light response curves. Photosyn-
thecophotonic flux was constant at 1000 µmol m\(^{-2}\) s\(^{-1}\), equivalent to more than 95% of the threshold for light-saturated photosynthesis.

Rates of light-saturated photosynthesis \(P_{\text{max}}\) were calculated from light-response curves by a non-rectangular hyperbolic function (Thorneley 1976). The maximum in vivo rate of RuBP carboxylation \(V_{\text{cmax}}\) and the maximum in vivo rate of electron transport driving regeneration of RuBP \(J_{\text{max}}\) were calculated from the \(A/C_i\) curves by nonlinear regression based on the models of Farquhar et al. (1980). Values of \(V_{\text{cmax}}\) and \(J_{\text{max}}\) were estimated from the \(A/C_i\) curves at \(C_i < 200\) µmol mol\(^{-1}\) and \(C_i > 500\) µmol mol\(^{-1}\), respectively.

**Leaf morphology, demography, nitrogen and chlorophyll concentration**

Foliage was harvested after the gas exchange measurements and projected leaf areas were measured with an image-analysis system (DIAS; Delta-T, Cambridge, U.K.) in which a video camera records images of foliage and transfers them to a computer. Measured leaves were dried at 80 °C for 72 h and weighed and specific leaf area (SLA) calculated as the projected leaf area to dry mass ratio. The total foliar N concentration was determined by gas chromatography (GC-8A, Shimazu, Kyoto, Japan) after combustion with circulating O\(_2\) using an NC analyzer (Sumigraph NC-95A, SCAS, Osaka, Japan). Small leaf disks (0.785 cm\(^2\)) were cut from the foliage after the gas exchange measurement and immediately frozen in liquid nitrogen and stored at –80 °C until analyzed for chlorophyll and Rubisco. Chlorophyll concentration was determined spectrophotometrically in 80% acetone extracts as described by Arnon (1949). The percentage survivorship of 1-year-old leaves was determined by counting the leaves present, and recording numbers of leaves that disappeared during a period of about 4 months between June and October.

**Rubisco concentration**

Crude extracts of leaf protein were prepared as described by Yamamoto et al. (1991). Each frozen leaf sample was homogenized in 0.1 M Tris buffer (pH 8.0) containing 28 mM 2-mercaptoethanol, 1% sodium dodecyl sulphate (SDS) and 3% (w/v) polyvinyl polypyrrolidone. Each was fractionated by SDS-PAGE as described by Laemmli (1970). Proteins on the gel were stained with Coomassie brilliant blue R-250. The band of the large subunit of Rubisco was extracted with for-mamide for spectrophotometric determination of Rubisco (Makino et al. 1986). Nitrogen in Rubisco \(F_k\) was calculated assuming that the N concentration in Rubisco is 16% (Field and Mooney 1986). The model proposed by Niinemets and Tenhunen (1997) was used to estimate the N concentration of the bioenergetic pools \(F_h\) and light-harvesting system \(F_L\) from the values of \(J_{\text{max}}\) and Chl.

**Data analyses**

Physiological and morphology properties were compared between current-year and 1-year-old foliage by Student’s \(t\)-tests using Kaleida Graph (Synergy Software, PA, USA). Linear and nonlinear regressions were computed by the least-squares method with SigmaPlot 2000 (SPSS, Chicago, IL).

**Results**

**Light environments under the Fagus crenata forest canopy**

Between August 1 and September 15, 2003, mean daily PPF received by the current-year foliage varied from 0.9 to 17.8 mol m\(^{-2}\) day\(^{-1}\) (Figure 1a), corresponding to about 3.4 to 66.2% of above-canopy irradiance. In contrast, mean daily PPF received by 1-year old foliage varied from 0.7 to 8.4 mol m\(^{-2}\) day\(^{-1}\) at the same sites (Figure 1b). Mutual leaf shading in the highest light environment was greater than in the lowest light environment (resulting in a 52.8 versus 22.2% reduction in interception, respectively). Percent survivorship of 1-year-old foliage decreased with increasing mutual shading. About 65% of 1-year-old foliage in the highest light environment (Site 5) abscised between June 8 and October 10 (Figure 2),
Nitrogen concentration per unit leaf area (Narea) was dependent of leaf age (Figure 3a; Table 1) at all sites except the form $y = ax + b$ for $P = \text{mean} \pm \text{SE}$, $n = 5–25$. Data were fitted by Gompertz function in the form $y = a \exp(-\exp(-(x - c)/b))$.

whereas less than 15% of the 1-year-old foliage abscised in the two lowest light environments.

**Daily PPF and leaf morphological and physiological characteristics**

The SLAs of current-year and 1-year-old foliage were logarithmically related to daily PPF ($r^2 = 0.98$, $P = 0.001$ and $r^2 = 0.9$, $P = 0.014$, respectively; Figure 3a). However, SLA was independent of leaf age (Figure 3a; Table 1) at all sites except Site 2. Nitrogen concentration per unit leaf area (Narea) was greater in current-year foliage from high-light environments than in current-year foliage from low-light environments, and varied from 1.4 to 2.5 g m$^{-2}$ (Figure 3b), whereas leaf N concentration per unit leaf dry mass (Nmass) was independent of daily PPF (Table 1). However, Narea and Nmass in 1-year-old foliage decreased with increasing irradiance. Chlorophyll concentration per unit area (Chlarea) in current-year foliage did not vary with irradiance (Figure 3c), whereas Chlarea in 1-year-old foliage from high-light environments was lower than in 1-year-old foliage from low-light environments, decreasing by 51% from the highest (Site 5) to the lowest light environment (Site 1). Chlorophyll concentration per unit mass (Chlmass) of both current and 1-year-old foliage exhibited a negative correlation with daily PPF (Table 1). The Chl a/b ratio in current-year and 1-year-old foliage in high-light environments was greater than in low-light environments (Figure 3d).

Figure 4 shows the photosynthetic parameters expressed on a leaf-area basis. The rate of light-saturated photosynthesis ($P_{\text{max}}$) in current-year foliage varied from 4.05 to 11.03 µmol m$^{-2}$ s$^{-1}$ across the study sites (Figure 4a). Maximum rates of carboxylation ($V_{\text{cmax}}$) varied from 3.92 to 6.41 µmol m$^{-2}$ s$^{-1}$ (Figure 4b), and the maximum rate of potential electron transport ($J_{\text{max}}$) varied from 53.87 to 79.79 µmol m$^{-2}$ s$^{-1}$ (Figure 4c). These photosynthetic parameters were logarithmically and positively related to daily PPF ($r^2 = 0.96$, $P = 0.0035$ for $P_{\text{max}}$; $r^2 = 0.88$, $P = 0.0189$ for $V_{\text{cmax}}$ and $r^2 = 0.94$, $P = 0.0071$ for $J_{\text{max}}$) in current-year foliage. In contrast, the only parameter that was significantly correlated with daily PPF in 1-year-old foliage was $P_{\text{max}}$. If values from 1-year old foliage from Sites 4 and 5 (the brightest) were excluded from analysis, then the values for 1-year old foliage did not differ significantly from those of current-year foliage—i.e., a single regression line could be used to describe the relationship between daily PPF and $P_{\text{max}}$ ($r^2 = 0.96$, $P < 0.0001$), $V_{\text{cmax}}$ ($r^2 = 0.91$, $P = 0.0002$) or $J_{\text{max}}$ ($r^2 = 0.94$, $P < 0.0001$).

**Relationships between photosynthesis and physiological characteristics**

Relationships between $P_{\text{max}}$ and Narea, $P_{\text{max}}$ and SLA and $V_{\text{cmax}}$ and $J_{\text{max}}$ for both age classes of foliage are shown in Figure 5. There was a strong, positive correlation between $P_{\text{max}}$ and Narea in current-year foliage ($P < 0.0001$), but the $P_{\text{max}}$–Narea relationship in 1-year-old foliage was weaker ($P > 0.05$) (Figure 5a). However, there were strong negative correlations between $P_{\text{max}}$ and SLA in both current-year and 1-year-old foliage ($P < 0.0001$) (Figure 5b). Furthermore, correlations between $V_{\text{cmax}}$ and $J_{\text{max}}$ in both current-year and 1-year-old foliage were strong ($P < 0.0001$) (Figure 5c).

**Nitrogen distribution among different components of the photosynthetic machinery**

Figure 6 shows N concentration in photosynthetic proteins. The N concentration of Rubisco ($F_{\text{R}}$) and the bioenergetics
Table 1. Physiological properties of current-year and 1-year-old leaves in different light environments. Values are means ± SE (> 3). Different lowercase letters adjacent to each value indicate a significant difference between current-year and 1-year-old foliage for the same site (< 0.05; Student’s t-test).

<table>
<thead>
<tr>
<th>Site</th>
<th>Narea (g m⁻²)</th>
<th>SLA (cm² g⁻¹)</th>
<th>Chl (g m⁻²)</th>
<th>Chl_mass (g g⁻¹)</th>
<th>Chl_a/b ratio</th>
<th>P_max (µmol m⁻² s⁻¹)</th>
<th>V_cmax (µmol m⁻² s⁻¹)</th>
<th>V_Jmax (µmol m⁻² s⁻¹)</th>
<th>V_Jmax/V_cmax</th>
<th>Rubisco/Chl</th>
<th>PNUE (µmol g⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.31 ± 0.05 a</td>
<td>280.4 ± 1.19 a</td>
<td>0.5 ± 0.22 a</td>
<td>2.56 ± 0.07 a</td>
<td>2.6 ± 0.03 a</td>
<td>4.21 ± 0.06 a</td>
<td>24.91 ± 1.24 a</td>
<td>87.68 ± 1.45 a</td>
<td>15.0 ± 0.01 a</td>
<td>5.87 ± 0.01 a</td>
<td>3.23 ± 0.22 a</td>
</tr>
<tr>
<td>2</td>
<td>1.56 ± 0.09 a</td>
<td>243.1 ± 0.94 a</td>
<td>0.5 ± 0.1 a</td>
<td>2.7 ± 0.02 a</td>
<td>2.5 ± 0.03 a</td>
<td>5.17 ± 0.06 a</td>
<td>28.62 ± 0.14 a</td>
<td>86.59 ± 1.04 a</td>
<td>12.0 ± 0.03 a</td>
<td>6.0 ± 0.1 a</td>
<td>3.4 ± 0.06 a</td>
</tr>
<tr>
<td>3</td>
<td>1.85 ± 0.1 a</td>
<td>215.2 ± 0.67 a</td>
<td>0.5 ± 0.05 a</td>
<td>3.2 ± 0.02 a</td>
<td>2.6 ± 0.04 a</td>
<td>7.18 ± 0.08 a</td>
<td>38.31 ± 0.23 a</td>
<td>86.59 ± 1.04 a</td>
<td>12.0 ± 0.03 a</td>
<td>6.0 ± 0.1 a</td>
<td>3.4 ± 0.06 a</td>
</tr>
<tr>
<td>4</td>
<td>1.97 ± 0.09 a</td>
<td>198.9 ± 0.86 a</td>
<td>0.5 ± 0.03 a</td>
<td>3.7 ± 0.04 a</td>
<td>2.8 ± 0.05 a</td>
<td>8.19 ± 0.09 a</td>
<td>38.27 ± 0.13 a</td>
<td>86.59 ± 1.04 a</td>
<td>12.0 ± 0.03 a</td>
<td>6.0 ± 0.1 a</td>
<td>3.4 ± 0.06 a</td>
</tr>
<tr>
<td>5</td>
<td>2.32 ± 0.18 b</td>
<td>183.7 ± 0.56 a</td>
<td>0.5 ± 0.06 a</td>
<td>4.5 ± 0.05 a</td>
<td>3.0 ± 0.07 a</td>
<td>11.03 ± 0.14 a</td>
<td>41.18 ± 0.29 a</td>
<td>88.3 ± 1.37 a</td>
<td>14.8 ± 0.07 a</td>
<td>6.8 ± 0.06 a</td>
<td>3.9 ± 0.08 a</td>
</tr>
</tbody>
</table>

**Discussion**

We demonstrated the plasticity of leaf physiological and morphological variables to irradiance and leaf age in *D. humile*, an evergreen understory shrub. In general, morphological characteristics such as SLA changed with light environment; however, the effect of irradiance on SLA did not change with leaf age (Figure 3a; Table 1). Uemura et al. (2000) reported that the anatomical properties of *Fagus* foliage that they examined, such as the number of cell layers in palisade parenchyma (which affects SLA), were influenced not by current-year irradiance, but by previous-year irradiance. If this finding also applies to *D. humile*, foliage may not acclimate anatomically to changes in irradiance caused by increases in mutual shading from one year to the next. However, differences in irradiance close to the forest floor apparently resulted in major differences in the leaf photosynthetic parameters of *Picea abies* and *Abies alba* saplings studied by Grassi and Bagnaresi (2001) and of three species growing in gaps and the understory of a *Fagus crenata* forest examined by Naramoto et al. (2001). Therefore, there appear to be species-specific differences in the nature of the interactions between leaf age and light environment that differentially affect the responses of photosynthetic parameters.

The photosynthetic parameters *P*<sub>max</sub>, *V*<sub>cmax</sub> and *J*<sub>max</sub> were correlated with daily PPF in current-year foliage (Figure 4), as shown in previous studies (Grassi and Bagnaresi 2001, Warren and Adams 2001, Han et al. 2003, Niinemets et al. 2004). In addition, we found a strong, positive relationship between *N*<sub>area</sub> and *P*<sub>max</sub> in current-year foliage (Figure 5). Relationships among PPF, N and photosynthetic parameters differed, however, between 1-year-old and current-year foliage (Figures 3–5). The measured photosynthetic parameters of foliage grown in high-light environments (Sites 3–5) decreased with leaf age (Figure 4; Table 1), as shown in previous studies (Chabot and Hicks 1982, Reich et al. 1991, Hikosaka and Hirose 2000). However, these foliage parameters were less affected by leaf age in a low-light environment because leaf N contents in 1-year-old foliage were similar to those in curr-
rent-year foliage (Figures 3 and 4; Table 1). When compared at a similar daily PPF (about 9 mol m\(^{-2}\)), photosynthetic parameters and N\(_{\text{area}}\) were significantly lower in 1-year-old foliage than in current-year foliage (Figures 3 and 4; Table 1).

The differences in photosynthetic parameters and N\(_{\text{area}}\) between foliage of the two years are believed to be caused by leaf age or shading by upper young foliage, or both. Generally, it is difficult to separate the effects of these factors because older foliage is more or less shaded by younger foliage above it. However, if the differences in photosynthetic parameters between foliage of two years are solely due to decreased irradiance caused by mutual shading, the photosynthetic parameters should lie on the same regression curve, because leaf photosynthetic capacity per unit area is positively correlated to irradiance (Ellsworth and Reich 1993, Pearcy and Sims 1994,

Figure 4. Relationship between daily PPF and (a) light-saturated rate of net photosynthesis at ambient CO\(_2\) concentration (350 \(\mu\)mol mol\(^{-1}\)) (\(P_{\text{max}}\)), (b) the maximum rate of carboxylation (\(V_{\text{cmax}}\)) and (c) the maximum rate of electron transport (\(J_{\text{max}}\)) in foliage of \(Daphnephyllum\) humile. Symbols as in Figure 3. Each value is the mean \(\pm\) SE, \(n = 4-6\). Data were fitted by nonlinear regression in the form \(y = b + a\log(x)\). Dashed and dotted lines are nonlinear regressions for current-year and 1-year-old foliage, respectively, and solid lines are nonlinear regressions for both current-year and 1-year-old foliage except for 1-year-old foliage grown at Sites 4 and 5. In current-year foliage: \(r^2 = 0.96, P = 0.0035\) for \(P_{\text{max}}\), \(r^2 = 0.88, P = 0.0189\) for \(V_{\text{cmax}}\) and \(r^2 = 0.94, P = 0.0071\) for \(J_{\text{max}}\). In 1-year-old foliage: \(r^2 = 0.86, P = 0.0024\) for \(P_{\text{max}}\), \(r^2 = 0.22, P = 0.422\) for \(V_{\text{cmax}}\) and \(r^2 = 0.001, P = 0.96\) for \(J_{\text{max}}\). In foliage from both years, excluding 1-year-old foliage grown at Sites 4 and 5: \(r^2 = 0.96, P < 0.0001\) for \(P_{\text{max}}\), \(r^2 = 0.91, P = 0.0002\) for \(V_{\text{cmax}}\) and \(r^2 = 0.94, P < 0.0001\) for \(J_{\text{max}}\).

Figure 5. Relationships between (a) leaf nitrogen concentration per unit area and light-saturated rate of net photosynthesis at ambient CO\(_2\) (350 \(\mu\)mol mol\(^{-1}\)) (\(P_{\text{max}}\)), (b) specific leaf area (SLA) and \(P_{\text{max}}\) and (c) the maximum rate of carboxylation (\(V_{\text{cmax}}\)) and the maximum rate of electron transport (\(J_{\text{max}}\)) in foliage of \(Daphnephyllum\) humile. Symbols as in Figure 3. Data were fitted by linear regression as in Figure 3. Solid and short dashed lines are linear regressions for current-year foliage and 1-year-old foliage, respectively, and thick solid lines are fitted to all data. (a) current-year foliage (\(r^2 = 0.73, P < 0.0001\)), 1-year-old foliage (\(r^2 = 0.04, P = 0.42\)); (b) current-year foliage (\(r^2 = 0.92, P < 0.0001\)), 1-year-old foliage (\(r^2 = 0.61, P < 0.0001\)); (c) current-year foliage (\(r^2 = 0.79, P < 0.0001\)), 1-year-old foliage (\(r^2 = 0.7, P < 0.0001\)); and all data (\(r^2 = 0.81, P < 0.0001\)).
Niinemets et al. 1998, Grassi and Bagnaresi 2001, Han et al. 2003; Figure 4). However, the photosynthetic parameters for 1-year-old foliage in high-light environments appeared to be lower than the PPF–photosynthetic parameter regression curves for current-year foliage, indicating that, in the high-light environment, leaf age was a key factor affecting photosynthesis as well as the light environment.

In current-year foliage, Chlarea was not significantly affected by irradiance (Figure 3c), as has been shown in previous studies (Pearcy and Sims 1994, Niinemets 1997, Niinemets et al. 1998, Grassi and Bagnaresi 2001). However, a higher Chlmass was observed in current-year foliage grown in low light than in current-year foliage grown in high light (Table 1), reflecting a general shift in allocation of carbon and N resources toward components with functions associated with light absorption (Pearcy and Sims 1994). The Chl molecule contains only a small amount of N (4 mol N mol⁻¹ Chl), but the Chl-associated proteins contain 25–70 mol N mol⁻¹ Chl (Evans 1989b). Thus, an increase in Chl has a substantial N cost. Therefore, the higher Chlmass found in current-year foliage grown in low light may be interpreted as a physiological adjustment to low-light conditions that optimizes light absorption per unit mass rather than per unit area (Evans 1996; Grassi and Bagnaresi 2001). The lower Chl a/b ratio reflects an increase in the proportion of chlorophyll in the light-harvesting complexes and a decrease in photosystem II complexes (Figure 3d), suggesting acclimation to low-light environments (Evans 1996).

We found a significant negative correlation between Chlarea and daily PPF in 1-year-old foliage grown at study sites 4 and 5 (r² = 0.79, P = 0.003), both year foliage except for 1-year-old foliage grown at study sites 4 and 5 (r² = 0.79, P = 0.003).
crease in Chl$_{area}$ from current-year foliage to 1-year-old foliage at these sites was much lower; about 40% at Site 5 and only 8.8% at Site 4 (Table 1). In many studies, the decrease in Chl concentration has been found to be slower than that of some Calvin-cycle enzymes, soluble proteins, electron carriers, electron transport capacities and photosynthetic capacity (Friedrich and Hufnagel 1980, Jenkins and Woolhouse 1981, Jenkins et al. 1981, Kura-Hotta et al. 1987), in accordance with our results.

Greater partitioning of N to light-harvesting ($F_L$) components is commonly found in both current-year and 1-year-old foliage of plants grown in low light compared with plants grown in high light (Evans 1989b, Niinemets 1997, Niinemets and Tenhunen 1997, Niinemets et al. 1998, Grassi and Bagnaresi 2001). The fractions of N partitioned into Rubisco ($F_R$) and bioenergetics pools ($F_B$) should theoretically increase with increasing growth irradiance for optimum partitioning of leaf resources, as observed in several herbaceous species (Hikosaka and Terashima 1995). However, although PNUE increased with growth irradiance, this supposedly optimum partitioning pattern was not observed in current-year and 1-year-old foliage of *D. humile* (Figure 6), which is consistent with findings for most tree species studied (Grassi and Bagnaresi 2001, Han et al. 2003), but not for *Corylus avellana L.* (Ninemets et al. 1998) and *Picea abies* (L.) Karst. (Grassi and Bagnaresi 2001). Although $F_A$ was calculated directly from Rubisco concentrations, $F_B$ was calculated from gas exchange measurements. Therefore, increasing diffusive resistance from the intercellular air spaces to carboxylation sites with increasing growth irradiance may be responsible for this apparent discrepancy. Furthermore, Warren and Adams (2004) suggested that evergreen plants accumulate inactive Rubisco as a means of storing N. This might also have affected the N partitioning pattern in *D. humile* if the fraction of inactive Rubisco varied with growth irradiance.

Schoettle and Smith (1998) reported that the sink strength of new foliage affects the N content of old foliage more than the irradiance on old foliage. In some tree species, Rubisco is a major leaf protein that is degraded and remobilized during leaf senescence (Millard and Thomson 1989, Miyazawa et al. 2004). In high-light environments, area-based Rubisco contents and $F_R$ significantly decreased with leaf age (Figure 6; Table 1). Furthermore, seedlings in high-light environments such as the forest border were subject to greater mutual shading than those in low-light environments such as the understory, and the percent survivorship of their 1-year-old foliage was lower, indicating that the survival rates of the 1-year-old foliage were related to the degree of mutual shading (Figure 2). Therefore, our results suggest that N might be remobilized from shaded 1-year-old foliage to sunlit current-year foliage in high-light environments, primarily through the degradation of Rubisco (Hikosaka 2005). Within a species, leaf lifespan varies with the growth environment (Chabot and Hicks 1982), and light is one of the factors regulating leaf senescence. It is known that foliage of plants grown in low-light environments lives longer than foliage grown in high-light environments (Chabot and Hicks 1982). Kikuzawa (1991) concluded that old foliage in which photosynthetic capacity has decreased is replaced by new foliage with high photosynthetic capacity, thereby tending to maximize whole-plant carbon gain. Therefore, the decrease in leaf N, especially in $F_A$ and $F_R$, observed in 1-year-old foliage in high-light environments might be attributed to leaf senescence occurring before foliar replacement in an adaptive response to maximize whole-plant carbon gain.

Hikosaka and Terashima (1995) showed that shade-adapted foliage should theoretically invest more N in light-harvesting complexes than in Calvin-cycle enzymes and electron carriers if it is to use low irradiance efficiently. Thus, the optimal Rubisco/Chl ratio in foliage grown at low irradiances may be lower than that of foliage grown at higher irradiances. In our study, the Rubisco/Chl ratios in foliage of both years, except for 1-year-old foliage at Sites 4 and 5, were related to daily PPF. The reason why the Rubisco/Chl ratio at Sites 4 and 5 was unaffected by irradiance can be explained by the decrease in Rubisco concentration, which was more rapid than the decrease in Chl concentration. Furthermore, the $V_{c,max}$/Rubisco ratio in the high-light environments was higher in 1-year-old foliage than in current-year foliage. Our data provide no definitive explanation for these results; however, we offer two hypotheses. Hypothesis 1 is related to mesophyll conductance, which Ninemets et al. (2005) recently reported decreases with leaf age. If the decrease in mesophyll conductance is slower than that of Rubisco during leaf senescence, the CO$_2$ content in chloroplasts should increase, leading to increases in the $V_{c,max}$/Rubisco ratio. Hypothesis 2 is related to the activation state of Rubisco. Previous studies have shown a decrease in the activation state of Rubisco with increasing N (Cheng and Fuchigami 2000) or Rubisco (Eichelmann and Laisk 1999) contents. Furthermore, Warren and Adams (2004) suggested that evergreen plants accumulate inactive Rubisco as a mean of storing N. The fraction of N allocated to Rubisco varies from 16 to 28% among herbaceous species (Evans 1989b), 15 to 36% among crop species (Makino et al. 1992), 5 to 13% in deciduous tree species (Ninemets et al. 1998) and 11 to 16% in evergreen broadleaf tree species (Hikosaka and Hirose 2000). In our study, the fraction of N allocated to Rubisco in current-year foliage ranged from 16 to 28%. These values appear to be higher than those previously reported for tree species. Therefore, current-year foliage in *D. humile* may invest some of its N resources in photosynthetic enzymes, leading to lower $V_{c,max}$/Rubisco ratios in current-year foliage than in 1-year-old foliage in high-light environments.

However, the PNUE and $J_{max}/V_{c,max}$ ratios in foliage of both years showed a strong correlation with irradiance, whereas these parameters did not vary with leaf age, except in a few cases (Figure 7; Table 1). In general, PNUE tends to be higher in deciduous species than in evergreen species (Field and Mooney 1986, Takashima et al. 2004), higher in gap species than in understory species (Chazdon and Field 1987), and higher in foliage with a short lifespan than in foliage with a long lifespan (Reich et al. 1991). Some previous studies have also reported that PNUE tends to decline with leaf age (Kitajima et al. 2002, Escudero and Mediavilla 2003, Miyazawa et
balance among photosynthetic functions across wide ranges of light environments. Variations in N concentration with variations in mesophyll conductance if the decrease in mesophyll conductance is slower than that in N concentration with leaf age. In accordance with Wullschleger (1993), we found a tight coupling of $V_{\text{cmax}}$ and $J_{\text{Jmax}}$ in foliage of both ages grown in different light environments (Figures 5c and 7b). Thus, we suggest that N partitioning in the photosynthetic apparatus is regulated in such a way as to balance $V_{\text{cmax}}$ and $J_{\text{Jmax}}$ even in foliage that is senescent. Our results also suggest that *D. humile* seedlings fine-tune their photosynthetic apparatus by adjusting the allocation of leaf N resources in an adaptive response that preserves a favourable balance among the photosynthetic components (i.e., light-harvesting proteins, Rubisco and electron carriers) across wide ranges of light environments and leaf ages.

In conclusion, SLA did not change with leaf age, but was influenced by irradiance during leaf expansion. Thus, changes in photosynthesis with leaf age mainly reflect changes in leaf N. Although SLA is the most important determinant of PNUF for foliage acclimating to irradiance (Ninemets and Tenhunen 1997, Evans and Poorter 2001), the photosynthetic efficiency of the long-lived foliage of *D. humile* may be optimized by adjustments to N partitioning in the photosynthetic apparatus induced by changes in irradiance. Thus, *D. humile* may maximize whole-plant carbon gain by maintaining a favorable balance among photosynthetic functions across wide ranges of leaf age and light environments.

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