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Leaf anatomy and light acclimation in woody seedlings after gap formation in a cool-temperate deciduous forest

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Abstract The photosynthetic light acclimation of fully expanded leaves of tree seedlings in response to gap formation was studied with respect to anatomical and photosynthetic characteristics in a natural cool-temperate deciduous forest. Eight woody species of different functional groups were used; two species each from mid-successional canopy species (*Kalopanax pictus* and *Magnolia obovata*), from late-successional canopy species (*Quercus crispula* and *Acer mono*), from sub-canopy species (*Acer japonicum* and *Fraxinus lanuginosa*) and from vine species (*Schizophragma hydrangeoides* and *Hydrangea petiolaris*). The light-saturated rate of photosynthesis (P_{max}) increased significantly after gap formation in six species other than vine species. Shade leaves of *K. pictus*, *M. obovata* and *Q. crispula* had vacant spaces along cell walls in mesophyll cells, where chloroplasts were absent. The vacant space was filled after the gap formation by increased chloroplast volume, which in turn increased P_{max}. In two *Acer* species, an increase in the area of mesophyll cells facing the intercellular space enabled the leaves to increase P_{max} after maturation. The two vine species did not significantly change their anatomical traits. Although the response and the mechanism of acclimation to light improvement varied from species to species, the increase in the area of chloroplast surface facing the intercellular space per unit leaf area accounted for most of the increase in P_{max}, demonstrating the importance of leaf anatomy in increasing P_{max}.

Keywords Acclimation potential · Chloroplasts · Photosynthetic capacity · Sun/shade acclimation · Mature leaves

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1 Introduction

Gap formation abruptly increases light availability for understory plants in forest. This event is considered indispensable for further growth of tree seedlings and thus for regeneration of forests (Denslow 1987; Naidu and DeLucia 1997a; Ryel and Beyschlag 2000). In a mixed temperate forest, gaps are formed throughout a year (Romme and Martin 1982). When irradiance increased in the growing season, plants often showed light acclimation where the light-saturated rate of photosynthesis per unit leaf area (P_{max}) increased even in already expanded leaves (Turnbull et al. 1993; Naidu and DeLucia 1998; Yamashita et al. 2000). This would allow rapid growth of plants and give competitive advantages over neighbor plants (Kursar and Coley 1999).

Yamashita et al. (2000) showed that an invasive tree species (*Bischofia javanica* Blume) has a higher photosynthetic light acclimation potential than four native

tree species of the subtropical forest of the Bonin Islands. The higher acclimation potential of *B. javanica* after gap formation contributed to its successful invasion and expansion in the Bonin Islands, where typhoons frequently disturb canopy layers creating gaps. Turnbull et al. (1993) showed that mid-successional species had a higher light acclimation potential than early or late-successional species. Some late-successional species and some other species do not even increase photosynthetic capacity after increase in irradiance (Sims and Pearcy 1992; Yamashita et al. 2000). How is the photosynthetic light acclimation potential determined and why does it vary among species?

Oguchi et al. (2003) demonstrated that anatomical constraints were involved in increasing P_{max} in fully expanded leaves. To have a high P_{max}, leaves have to arrange a large number and/or volume of chloroplasts along the mesophyll cell surface. If the leaf increased the number and/or volume of chloroplasts without thickening the mesophyll layer, some chloroplasts would be separated from the cell surface and fail to receive sufficient CO_2 owing to very slow diffusion of CO_2 in the liquid phase (Nobel et al. 1975; Evans et al. 1994; Terashima et al. 2001). Many species have little plasticity in leaf thickness after full expansion (Sims and Pearcy 1992; Pearcy and Sims 1994), which may limit the increase in P_{max}. However, we found different acclimation mechanisms that overcome the anatomical constraints (Oguchi et al. 2003, 2005). Leaves of *Chenopodium album* L. and *Betula ermanii* Cham. grown at a low irradiance were relatively thick and had a vacant space along mesophyll cell surfaces. After transfer to high irradiance, chloroplasts were enlarged to fill the space without an increase in leaf thickness. Leaves of *Acer rufinerve* Sieb. et Zucc. remained plastic in mesophyll cell surface area and leaf thickness, that both increased after transfer to high irradiance. In these three species P_{max} increased when the area of chloroplast surfaces facing the intercellular space (S_c) increased. On the other hand, *Fagus crenata* Blume had little mesophyll cell surface unoccupied by chloroplasts. Leaf anatomy was not changed after the transfer, and consequently P_{max} did not increase. Therefore, P_{max} seems to increase only when S_c increases.

Most of the earlier experiments that examined the photosynthetic light acclimation of mature leaves were performed in environmentally controlled systems such as a growth chamber and a greenhouse where the climate is different from forest gaps. It is difficult to make conditions in a growth chamber equivalent to those observed in forest gaps. For example, changes in irradiance in the growth chamber were much larger than those observed in natural conditions. In most studies, plants were transferred from shade [1–8% of full sun in photosynthetic photon flux density (PPFD)] to open site conditions (50–100%) (Sims and Pearcy 1992; Turnbull et al. 1993; Yamashita et al. 2000; Oguchi et al. 2003, 2005). However, the most abundant gaps in natural conditions are smaller than 100 m^2 and their relative PPFD is less than 10% of full sun (Denslow 1987; Yamamoto 1989; Denslow et al. 1998). Factors other than irradiance, such as temperature, humidity, nutrient availability, competition with neighbors and morphology of the plants will also affect the plant response to gap formation (Bazzaz and Pickett 1980; Denslow 1987; Whitmore 1996; Ryel and Beyschlag 2000). Although Naidu and DeLucia (1997a, b, 1998) performed a transplant experiment with potted saplings in naturally occurring canopy gaps, their methods disregarded soil conditions and plant–plant interactions. Thus an experiment in forest gaps is needed to investigate plant responses to changing irradiance under natural conditions.

In the present study, we created gaps by felling canopy trees in a natural forest and examined the response of fully expanded leaves of understory seedlings. Using eight species that belong to four different functional groups, we studied whether they might respond differently to gap formation. Changes in anatomical and physiological characteristics in leaves after gap formation were examined to clarify how species responded to a sudden increase in irradiance after gap formation in a natural forest.

2 Materials and methods

2.1 Study site

Study sites were in the Tomakomai Experimental Forest, Field Science Center for Northern Biosphere, Hokkaido University, Hokkaido, Japan (42°41′ N, 141°36′ E, 65 m elevation; see Hiura 2001 for detail site description). An experimental plot (50 × 120 m) was established on a flat plateau in a temperate deciduous broadleaved forest. In this forest, leaf expansion started in the middle of May and leaf abscission started in the middle of October. Monthly mean air temperature ranged from –3.2 to 19.1°C. Annual rainfall was 1,200 mm. The predominant soil type was volcanic Regosols (Andic Udipsamments; Soil Survey Staff 1994), consisting mainly of classic pumice and sand (Shibata et al. 1998). The dominant species in the plot are *Quercus crispula* Blume, *Acer mono* Maxim., *Sorbus alnifolia* Sieb. et Zucc., *Tilia japonica* Simk. and

Acer amoenum Carr. Average canopy height was ca. 18 m, and there were no large canopy gaps before the experiment. On 19 July 2002, three gaps were created along a longer axis of the plot by pulling down one or two trees with the wire and winch of a bulldozer from outside the plot. The size of the created gaps was around 10 m in diameter, which was the size most commonly observed in natural forests (Denslow 1987; Yamamoto 1989; Denslow et al. 1998). We established three control sites in the plot near the gaps.

2.2 Plant materials

We studied naturally growing seedlings of eight woody deciduous species, *Kalopanax pictus* Nakai, *Magnolia obovata* Thunb., *Q. crispula*, *A. mono*, *Acer japonicum* Thunb., *Fraxinus lanuginosa* Koidz., *Schizophragma hydrangeoides* Sieb. et Zucc. and *Hydrangea petiolaris* Sieb. et Zucc. These species were classified into four functional groups. *K. pictus* and *M. obovata* are mid-successional canopy trees, *Q. crispula* and *A. mono* are late-successional canopy trees (Kikuzawa 1983), *A. japonicum* and *F. lanuginosa* are subcanopy trees and *S. hydrangeoides* and *H. petiolaris* are woody vines. Two to three individuals per species were used in each site. The size of seedlings was about 10–30 cm in height, although some individuals of the vine species reached 1 m in height. The age of these seedlings was evaluated as 2–10 years from the height growth curves for deciduous broadleaved tree species (Fujimoto and Motai 1981). Only leaves that ceased expansion before gap formation were used for the study.

2.3 Light, temperature and soil N

Irradiance and air temperature were measured every 15 min in the three gaps and the three control sites (luxmeter, HLI and SLA08 for irradiance; sensor with thermal sensitive resistor, thermistor, WTA32-05 + 37 for air temperature; Onset Computer, Mass.) from 7 July to 18 August. Sensors were placed 30 cm above the ground. Luxmeters were calibrated with a quantum sensor (LI-190SA; LI-COR, Lincoln, Neb.) in the forest understory to convert lux into PPFD. Temperature sensors were covered with a screen to avoid exposure to direct sunlight. Mean air temperature was 0.2°C higher at the gap sites than at the control sites, although the maximum temperature of the gap sites was about 5°C higher than that of control sites when the gap sites were exposed to direct sunlight (Table 1). No significant difference in mean air temperature between control and gap sites was reported in previous studies, although maximum air temperature significantly increased when gap sites were exposed to direct sunlight (Naidu and Delucia 1997b, 1998; Schmidt et al. 1998; Clinton 2003). Relative irradiance was calculated from simultaneous instantaneous measurements above and below the forest canopy. Measurements were made 20 times at each site with two quantum sensors (LI-190SA), one sensor for above the canopy and the other sensor for gap and control sites. We chose cloudy days for the measurements before (6 July) and after gap formation (9 September) to measure relative diffused irradiances, which can be considered as relative daily irradiances. Average above-canopy daily PPFD during the study period was measured at a flux observation tower near the experimental plot.

Table 1 Relative photosynthetic photon flux density (*PPFD*), mean daily integrated PPFD, mean air temperature, daily maximum and minimum air temperature at gap and control sites, before (7–18 July) and after (20 July–18 August) gap formation. NH_4^+-N (NH_4^+), NO_3^--N (NO_3^-) and sum of NH_4^+- and NO_3^--N ($NH_4^+ + NO_3^-$) content produced during 2 months at gap and control sites. Mean ± SD are shown except for the maximum and minimum air temperature. *f.w.* Fresh weight

	Gap	Control
Relative PPFD (%)		
Before	1.77 ± 0.25	2.19 ± 0.48
After	10.6 ± 6.5	2.55 ± 0.70
Mean daily PPFD (mol m^{-2} day^{-1})		
Before	0.74 ± 0.22	0.54 ± 0.26
After	3.07 ± 0.71	0.54 ± 0.11
Mean temperature (°C)		
Before	17.5 ± 0.04	17.5
After	18.3 ± 0.03	18.1
Maximum/minimum temperature (°C)		
Before	22.5/12.4	21.7/12.4
After	31.8/14.6	27.0/14.6
NH_4^+ [μg/g f.w. (soil)]		
Produced in 2 months	–5.31 ± 4.0	–0.37 ± 2.88
NO_3^- [μg/g f.w. (soil)]		
Produced in 2 months	7.95 ± 4.40	3.84 ± 1.99
$NH_4^+ + NO_3^-$ [μg/g f.w. (soil)]		
Produced in 2 months	2.64 ± 6.50	3.46 ± 4.24

Soil N availability was evaluated by the measurement of N mineralization and nitrification in intact soil cores with ion exchange resins (DiStefano and Gholz 1986). At the start of measurement (12 July), four undisturbed cores from each gap site and six undisturbed cores at control sites (5.0 cm diameter × 5.0 cm long) were taken out from the A1 horizons using sharpened polyvinyl chloride (PVC) tubes. Litter layers were excluded from the sample. Half of the cores were incubated in the field for 2 months

using the resin technique (DiStefano and Gholtz 1986). PVC tubes (5.0 cm diameter × 1.0 cm long) filled with 7.5 g dry weight of the resin (Active anion exchange resin, Amberlite IRA-400 and Active cation exchange resin, Amberlite IR-120B; Rohm and Haas, Philadelphia, Pa.) wrapped with fine mesh nylon, which were called ''resin bags'', were connected to the bottom of soil core samples. The resin bag collected leached ions. Silicon glue was used to seal the outside of the resin bag to the soil core to avoid losses of soil solution through boundary flow along the inner core wall. The cores were returned into the holes in the field, and were made to touch the soil to ease water flow through the tube. At the end of the measurement period (7 September), cores were collected. Exchangeable NH_4^+ and NO_3^- ions were extracted twice by mixing 10 g (fresh weight) of sieved (2-mm mesh size) fresh soil with 100 ml of 2 M KCl and 2 g of the resin with 50 ml of 2 M KCl for 1 h. The solution was then filtrated (Advantec no.6; Toyo Roshi Kaisha, Tokyo) and analyzed colorimetrically by the indophenol blue method for NH_4^+ and Cataldo method for NO_3^- (Keeney and Nelson 1982; JSSSPN 1990). N mineralization and nitrification rate were calculated from the difference between the contents of NH_4^+ and NO_3^- at the start and at the end of the experiment (57 days). NH_4^+-N (NH_4^+) content in the soil decreased through the experimental period indicating that the nitrification of NH_4^+ to NO_3^--N (NO_3^-) was faster than the mineralization rate (Table 1). Availability of NH_4^+ was lower at gap sites than at control sites, while the availability of NO_3^- was higher at gap sites than at control sites. As a consequence the difference in the availability of NH_4^+- plus NO_3^--N ($NH_4^+ + NO_3^-$) between gap and control sites was small. This is in agreement with previous studies which showed that only canopy gaps larger than single trees induced significant changes in N cycling (Denslow et al. 1998; Prescott 2002).

2.4 Photosynthesis and leaf characteristics

Measurements of photosynthetic characteristics and leaf traits were conducted 3 days before the gap formation and 4, 10 and 30 days after the gap formation. Chlorophyll fluorescence was measured with a fluorometer (MINI-PAM; Walz, Effetrich, Germany). The maximal (F_m) and minimal (F_o) fluorescence yield were determined at predawn. The maximum photochemical efficiency of photochemistry in photosystem II of dark-adapted leaves was evaluated by F_v/F_m, where $F_v = F_m - F_o$ (Krause and Weis 1991). The light-saturated rate of photosynthesis was determined with a portable open gas exchange system (LI-6400; LI-COR) at 1,500 μmol m^{-2} s^{-1} and at the ambient CO_2 concentration. Light was provided by LED (model 6400-02B, LI-COR). Leaf temperature was adjusted to 25°C. On the same day, leaf number, leaf length, and leaf inclination angle were measured. To evaluate the difference of light environment among species, a hemispherical photograph was taken at leaf level. Hemispherical canopy photographs were taken with a digital camera (Coolpix 990; Nikon, Tokyo) equipped with a fish-eye lens (FC-E8, Nikon). The top element of the lens was positioned 5–10 cm above the leaves and the camera was carefully leveled. The hemispherical photographs were analyzed with a software program, CanopOn (free software programmed by A. Takenaka available from: http://www.takenaka-akio.cool.ne.jp/etc/canopon2/) assuming a standard overcast sky condition.

On the day after final photosynthesis measurements (31 days after the gap formation), four discs (each 1 cm in diameter) were punched out, and leaf pieces (1 × 2 mm) for microscopic analysis were cut off from the leaf. One disc was used to determine chlorophyll content spectrophotometrically after extraction with dimethylformamide (Porra et al. 1989). The other three leaf discs were dried at 80°C in an oven for more than 3 days. After dry mass determination, N content was determined with an NC analyzer (NC-80; Shimadzu, Kyoto).

Leaf sections for light microscopic photography were prepared as described in Oguchi et al. (2003). The surface area of mesophyll cells facing the intercellular space per unit leaf area (S_{mes}) and the area of chloroplast surfaces facing the intercellular space per unit leaf area (S_c) were calculated from the photograph. To convert the length of the cross-section to surface area, a curvature correction factor (F) was determined assuming that the shape of the palisade tissue cells was a cylinder with flat ends and that the shape of the spongy cells was a spheroid (Thain 1983). F was calculated for each leaf from the average ratio of cell width to height for palisade and spongy cells in each species. See Oguchi et al. (2003) for calculation of anatomical parameters in leaves.

2.5 Analysis

Statistical analyses were performed with Stat View statistical software (version 5.0; SAS Institute, Cary, N.C.). The two-tailed t-test was used to test the effect of gap formation. The two-way factorial ANOVA was used to test the effect of gap formation and species.

Oguchi et al. (2005) analyzed the P_{max} as the product of six factors:

$$P_{max} = \frac{P_{max}}{N_{area}} \times \frac{N_{area}}{V_{chr}} \times \frac{V_{chr}}{S_c} \times \frac{S_c}{n_{chr}} \times \frac{n_{chr}}{S_{mes}} \times S_{mes} \quad (1)$$

where N_{area} is the N content per unit leaf area. The first factor, P_{max}/N_{area} indicates biochemical characteristics of the photosynthetic apparatus. It may decrease when the CO_2 concentration in the chloroplast decreases (Hikosaka et al. 1998), when the activation state of the photosynthetic proteins decreases (Martindale and Bowes 1996; Mott and Woodrow 2000) or when photoinhibition occurs (Hikosaka et al. 2004; Krause et al. 2004). The second factor, N_{area}/V_{chr} represents the N concentration in chloroplasts where V_{chr} is total chloroplast volume per unit leaf area, the third factor, V_{chr}/S_c is chloroplast thickness, the fourth factor, S_c/n_{chr} is the surface area of a chloroplast facing the intercellular space, the fifth factor, n_{chr}/S_{mes} is the number of chloroplasts per mesophyll surface area and the sixth factor S_{mes} is mesophyll surface area. In the present study, we simplified Eq. 1 to:

$$P_{max} = \frac{P_{max}}{N_{area}} \times \frac{N_{area}}{S_c} \times \frac{S_c}{S_{mes}} \times S_{mes} \quad (2)$$

where N_{area}/S_c represents N content per chloroplast surface area facing the intercellular space ($N_{area}/V_{chr} \times V_{chr}/S_c$) and S_c/S_{mes} is the fraction of mesophyll cell surface covered by chloroplasts, chloroplast cover ratio ($S_c/n_{chr} \times n_{chr}/S_{mes}$).

3 Results

Instantaneous relative PPFD under diffused sunlight increased from 1.77% to 10.6% after gap formation and mean daily PPFD increased from 0.74 mol m^{-2} day^{-1} to 3.07 mol m^{-2} day^{-1} (Table 1). Average above-canopy daily PPFD during the study period was 20.9 mol m^{-2} day^{-1}. There were 7 sunny days (defined as the duration of direct beam solar radiation being longer than 4 h) in 30 days of the experimental period. On a sunny day, the PPFD of the gap sites was about twofold that of the control sites under diffuse sunlight and was more than tenfold that of the control sites when exposed to direct sunlight. Environmental factors other than irradiance did not show a large change after gap formation (See Materials and methods). We thus assumed that the increase in irradiance was the primary factor for photosynthetic acclimation.

The fraction of open area in the canopy evaluated from the hemispherical photograph (gap openness) increased from 8 to 19% on average ($P < 0.001$ in two-way factorial ANOVA) after gap formation. There was no significant difference in irradiance among species ($P = 0.244$). The leaf inclination angle was not significantly different between gap and control sites ($P = 0.921$), but it was significantly different among species ($P < 0.001$). Particularly, the two vine species had a higher leaf inclination angle than the other species (18.7, 21.1 and 9.52° in *S. hydrangeoides*, *H. petiolaris* and the mean of the other species, respectively). Few new leaves were expanded after gap formation: *Q. crispula* and *F. lanuginosa* did not produce new leaves and the other species produced 0.1–2.3 leaves per individual on average. Dead leaves were observed after gap formation in all species though the number was small (0.3 leaves per individual on average). There was no significant difference between gap and control sites in the proportion of newly expanded leaves and dead leaves. Interaction between site and species for gap openness, leaf inclination angle and proportion of newly expanded and dead leaves was not significant in two-way factorial ANOVA (all $P > 0.05$).

Figure 1 shows the time-course of P_{max} in fully expanded leaves after the gap formation. Thirty days after gap formation, P_{max} increased significantly in six out of the eight species and the relative increase was similar in the six species (27.4–38.3%). Two vine species showed no significant increase in P_{max}. Figure 2 shows the time-course of F_v/F_m following the gap formation. Mid-successional canopy species, subcanopy species and *S. hydrangeoides* showed a significant reduction in F_v/F_m until 10 days after gap formation, while late-successional canopy species and *H. petiolaris* did not show a significant reduction in F_v/F_m. Thirty days after gap formation, F_v/F_m was significantly lower at the gap than at the control sites in *K. pictus*, *A. japonicum*, *F. lanuginosa* and *S. hydrangeoides* but there was no difference in the other species.

Intercellular CO_2 concentration (C_i) at 1,500 μmol m^{-2} s^{-1} PPFD showed a significant increase after gap formation in *K. pictus*, *Q. crispula*, *S. hydrangeoides* and *H. petiolaris* (Fig. 3). Leaf N content increased significantly only in late-successional canopy species and subcanopy species. Chlorophyll contents of gap leaves were not significantly different from those of control leaves in all species (Table 2). *M. obovata* had a significantly higher chlorophyll a/b ratio in gap leaves than in control leaves, while the other species did not show such a difference.

Leaf thickness was not different between gap and control sites in all species (Table 2). Vine species had particularly thicker leaves than the other species. Leaves at gap sites had a larger leaf mass per area (LMA) than leaves at control sites in *K. pictus*, *M. obovata* and *A. mono*, while they did not in the other

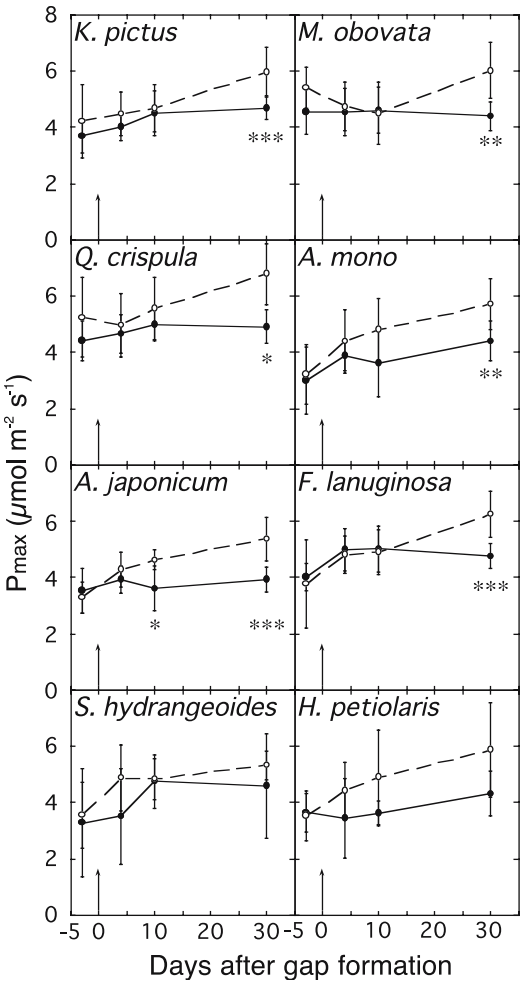

Fig. 1 The change in light-saturated photosynthetic rate (P_{max}) after gap formation. Mature leaves of eight species growing at control (*closed circles*) and at gap sites (*open circles*). An *arrow* indicates the date of gap formation (19 July 2002). *Bars* denote ± SD of the mean. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.*K. pictus Kalopanax pictus*, *M. obovata Magnolia obovata*, , *Q. crispula Quercus crispula*, *A. mono Acer mono*, *A. japonicum Acer japonicum*, *F. lanuginosa Fraxinus lanuginosa*, *S. hydrangeoides Schizophragma hydrangeoides*, *H. petiolaris Hydrangea petiolaris*

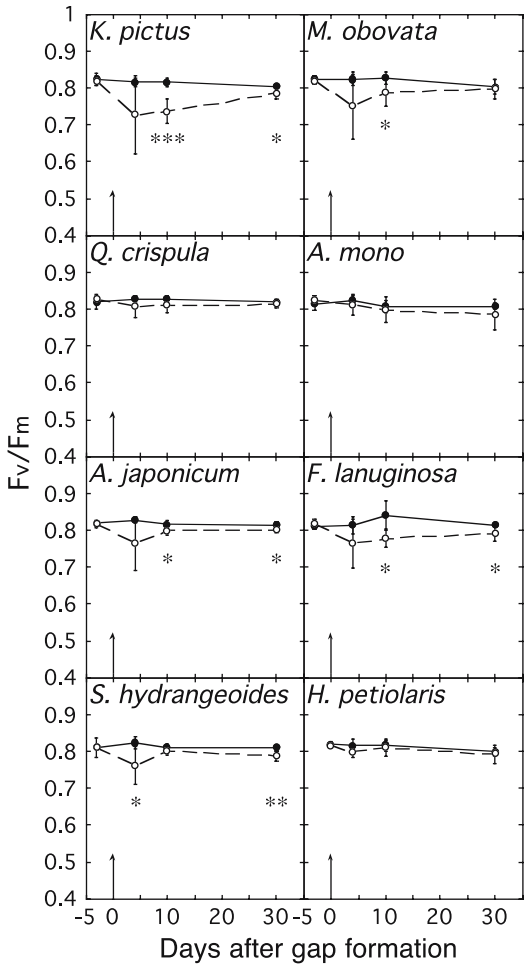

Fig. 2 The change in maximum photochemical efficiency of photochemistry in photosystem II of dark adapted leaves (F_v/F_m) after gap formation. Mature leaves of eight species growing at control (*closed circles*) and at gap sites (*open circles*). An *arrow* indicates the date of gap formation (19 July 2002). *Bars* denote ± SD of the mean. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

species. The chloroplast surface area facing the intercellular space (S_c) was significantly larger in leaves at gap sites than in leaves at control sites in six species (*K. pictus*, *M. obovata*, *Q. crispula*, *A. mono*, *A. japonicum* and *F. lanuginosa*, Fig. 3), all of which increased P_{max} (Fig. 1). Positive correlation was found between P_{max} and S_c in all species (Fig. 4).

To evaluate the contribution of physiological and anatomical factors to the change in P_{max} after gap formation, P_{max} was analyzed as the product of four factors (Eq. 2, Table 3). In *K. pictus*, *M. obovata* and *Q. crispula*, P_{max} per N content (P_{max}/N_{area}) and chloroplast cover ratio (S_c/S_{mes}) increased after gap formation, though the difference in P_{max}/N_{area} of *M. obovata* was not significant. In *A. mono* and *A. japonicum*, S_{mes} increased significantly after the gap formation. P_{max} per N also increased in *A. japonicum*. In *F. lanuginosa*, all factors tended to increase after gap formation, although the difference was not significant. In vine species, N_{area}/S_c significantly decreased (*S. hydrangeoides*) and P_{max} per N significantly increased (*S. hydrangeoides* and *H. petiolaris*) after gap formation, while there was no significant change in the other factors.

4 Discussion

4.1 Anatomy and light acclimation after gap formation

The area of chloroplast surface facing the intercellular space per unit leaf area (S_c) increased significantly in

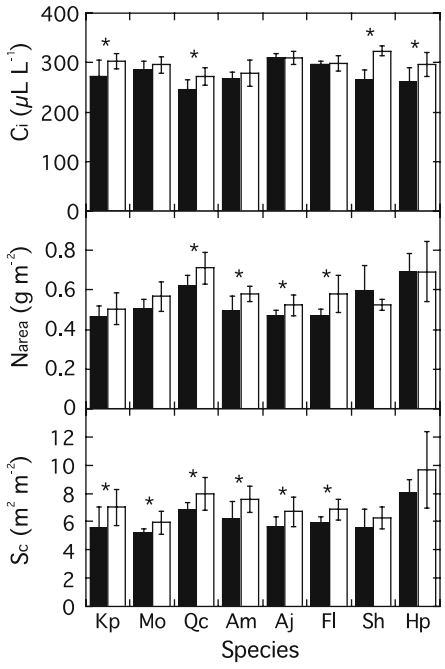

Fig. 3 Intercellular CO_2 concentration at PPFD of 1,500 μmol m^{-2} s^{-1} (C_i), N per leaf area (N_{area}) and the area of chloroplasts facing the intercellular space (S_c) of leaves growing at control (*closed bar*) and gap sites (*open bar*). *Bars* indicate ± SD of the mean. *Asterisks* indicate the significant difference between control and gap sites at $P < 0.05$ in the *t*-test. *Kp K. pictus, Mo M. obovata*, *Qc Q. crispula*, *Am A. mono, Aj A. japonicum*, *Fl F. lanuginosa*, *Sh S. hydrangeoides*, *Hp H. petiolaris*

the species that showed a significant increase in P_{max} (Figs. 1, 3). S_c had a significant positive correlation with P_{max} in all species (Fig. 4). These results suggest that the increase in S_c is indispensable for the increase in P_{max}. They also support the hypothesis that leaf anatomy strongly constraints an increase in P_{max} (Oguchi et al. 2003).

The mechanism used to increase S_c was different among species. The chloroplast cover ratio (S_c/S_{mes}) increased after gap formation in *K. pictus*, *M. obovata* and *Q. crispula* (Table 3). In these species, the vacant space along the mesophyll cell surface was filled by enlarged chloroplasts after gap formation, to increase P_{max} (Fig. 5a, b). This mechanism is in agreement with that found for *C. album* and *B. ermanii* (Oguchi et al. 2003, 2005). *A. mono* and *A. japonicum* increased S_{mes} significantly with an increase in irradiance. In these species, leaves did not increase thickness but enlarged mesophyll cells after gap formation and consequently the fraction of intercellular space decreased (Fig. 5c, d). This enabled an increase in number and volume of chloroplasts. In *F. lanuginosa*, both chloroplast cover ratio and S_{mes} tended to increase after gap formation. Thus the mechanism in *F. lanuginosa* was similar to that of *A. mono* and *A. japonicum*. The other species lost the ability to increase S_{mes} after full expansion.

Table 2 Chlorophyll content per leaf area, chlorophyll (*Chl*) a/b ratio, leaf thickness and leaf mass per area (*LMA*) of mature leaves in eight species growing at control and gap sites. Thirty days after gap formation. Mean ± SD

	Chl content (mmol m^{-2})	Chl a/b ratio	Leaf thickness (mm)	LMA (g m^{-2})
Kalopanax pictus				
Control	0.323 ± 0.043	2.76 ± 0.25	0.126 ± 0.013	19.7 ± 1.6
Gap	0.306 ± 0.047 n.s.	2.73 ± 0.11n.s.	0.126 ± 0.013 n.s.	21.9 ± 2.3*
Magnolia obovata				
Control	0.372 ± 0.040	2.75 ± 0.08	0.125 ± 0.014	19.6 ± 1.6
Gap	0.361 ± 0.060 n.s.	2.94 ± 0.16*	0.122 ± 0.008 n.s.	22.4 ± 2.1*
Quercus crispula				
Control	0.372 ± 0.028	1.95 ± 0.09	0.083 ± 0.008	28.0 ± 1.1
Gap	0.384 ± 0.034 n.s.	2.03 ± 0.17 n.s.	0.087 ± 0.009 n.s.	30.3 ± 3.0 n.s.
Acer mono				
Control	0.350 ± 0.064	2.73 ± 0.06	0.100 ± 0.012	22.2 ± 2.2
Gap	0.387 ± 0.035 n.s.	2.80 ± 0.17 n.s.	0.100 ± 0.007 n.s.	25.8 ± 3.4*
Acer japonicum				
Control	0.340 ± 0.031	2.62 ± 0.10	0.078 ± 0.004	27.3 ± 2.9
Gap	0.323 ± 0.044 n.s.	2.69 ± 0.09 n.s.	0.086 ± 0.009 n.s.	30.8 ± 3.8 n.s.
Fraxinus lanuginosa				
Control	0.375 ± 0.034	2.79 ± 0.13	0.173 ± 0.015	22.0 ± 0.7
Gap	0.343 ± 0.056 n.s.	2.81 ± 0.11 n.s.	0.172 ± 0.033 n.s.	27.3 ± 3.8 n.s.
Schizophragma hydrangeoides				
Control	0.425 ± 0.097	2.64 ± 0.15	0.235 ± 0.038	24.8 ± 4.0
Gap	0.342 ± 0.040 n.s.	2.73 ± 0.08 n.s.	0.244 ± 0.019 n.s.	26.8 ± 5.5 n.s.
Hydrangea petiolaris				
Control	0.477 ± 0.046	2.34 ± 0.18	0.229 ± 0.024	32.8 ± 4.2
Gap	0.407 ± 0.089 n.s.	2.43 ± 0.10 n.s.	0.221 ± 0.028 n.s.	37.0 ± 4.5 n.s.

*$P < 0.05$ in *t*-test, *n.s.* not significant ($P > 0.05$)

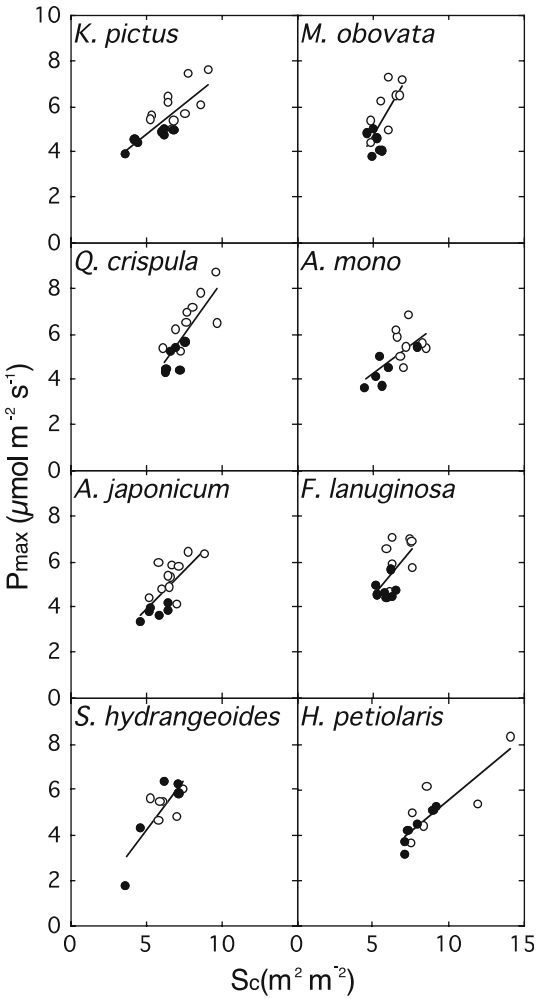

Fig. 4 Relationships between P_{max} and S_c in eight species. Leaves growing at control (*closed circles*) and gap sites (*open circles*). For abbreviations, see Figs. 1 and 3

Vine species showed no significant difference in P_{max} between gap and control sites (Fig. 1). We expected that species with low acclimation potential would have little vacant space for an increase in chloroplast number and/or volume, as was observed in *F. crenata* (Oguchi et al. 2005). However, the chloroplast cover ratio was small and there seemed to be no restriction on the increase in chloroplast volume (Table 3). The following factors may be involved in the low acclimation potential in vine species:

1. Significantly larger leaf inclination angles in vines than the other species mitigated the increase in irradiance.
2. Increase in irradiance in the present study was not large enough to induce light acclimation in these species.
3. The vacant space was used only for chloroplast movement to avoid photoinhibition caused by strong light (Kasahara et al. 2002).

More detailed studies would clarify the restricted acclimation potential in vine species.

Five species, *K. pictus*, *Q. crispula*, *A. japonicum*, *S. hydrangeoides* and *H. petiolaris*, showed a significant increase in photosynthetic capacity per N (P_{max}/N_{area}) (Table 3). This is partly attributable to the increase in C_i which is caused by stomatal opening (Fig. 3). In *K. pictus*, *Q. crispula*, *S. hydrangeoides* and *H. petiolaris*, C_i increased by 11–22% after gap formation. According to the biochemical model of photosynthesis (Farquhar et al. 1980), this increase in C_i is expected to increase P_{max} by 10–18%, which can explain most of the change in P_{max}/N_{area} in these species. However, it was not the case in *A. japonicum*, where C_i did not increase. An increase in P_{max}/N_{area} may also imply biochemical modifications, such as an increase in the fraction of leaf N allocated to Rubisco, an increase in Rubisco activation state and reorganization of the photosynthetic apparatus (Murchie and Horton 1997, 1998; Seemann et al. 1988; Mott and Woodrow 2000).

4.2 Different response to gap formation between functional groups

Our experimental results suggest a close link between functional groups and the mechanism to acclimate to light improvement. Mid-successional canopy species increased P_{max} without a significant increase in N content. Late-successional canopy species and subcanopy species increased both P_{max} and N content. In vines, neither P_{max} nor N content increased (Figs. 1, 3).

Although the increase in P_{max} after gap formation was similar in extent between mid- and late-successional species, mid-successional species had a lower chloroplast cover ratio than late-successional species (Table 3). Even after the acclimation, mid-successional species had a large vacant space. Together with little increase in leaf N content, this suggests that the increase in growth irradiance was not large enough to induce a further increase in chloroplast volume in mid-successional species. If the growth irradiance increased to a large extent, these species might show greater acclimation than that observed in the present study.

A significant increase in S_{mes} was observed only in two *Acer* species (Fig. 3). Also in our previous study (Oguchi et al. 2003, 2005), *Acer rufinerve* showed this type of acclimation mechanism. Considering that these three species belonged to different functional groups

Table 3 Factors that determine P_{max} (see Eq. 2) in mature leaves of the eight species growing at gap and control sites, and their percentage increase after gap formation: (gap/control – 1) × 100. Thirty days after gap formation. Mean ± SD

	P_{max}	P_{max}/N_{area}	N_{area}/S_c	S_c/S_{mes}	S_{mes}
K. pictus					
Control	4.69 ± 0.39	10.23 ± 1.15	0.089 ± 0.020	0.64 ± 0.12	8.63 ± 1.43
Gap	5.98 ± 0.87*	12.01 ± 1.53*	0.077 ± 0.019 n.s.	0.74 ± 0.06*	9.46 ± 1.81 n.s.
% Increase	27.4	17.4	–13.8	16.7	9.7
M. obovata					
Control	4.40 ± 0.49	8.79 ± 1.01	0.098 ± 0.011	0.60 ± 0.04	8.63 ± 0.45
Gap	6.02 ± 0.98*	10.27 ± 1.44 n.s.	0.100 ± 0.008 n.s.	0.70 ± 0.03*	8.51 ± 0.89 n.s.
% Increase	36.9	16.8	1.8	16.2	–1.4
Q. crispula					
Control	4.91 ± 0.59	7.94 ± 0.40	0.091 ± 0.009	0.83 ± 0.05	8.23 ± 0.47
Gap	6.80 ± 1.08*	9.39 ± 0.91*	0.091 ± 0.010 n.s.	0.90 ± 0.04*	8.89 ± 1.52 n.s.
% Increase	38.3	18.3	0.02	8.2	8.0
A. mono					
Control	4.41 ± 0.72	8.92 ± 0.26	0.087 ± 0.009	0.74 ± 0.09	8.34 ± 1.21
Gap	5.72 ± 0.90*	9.83 ± 1.50 n.s.	0.079 ± 0.009 n.s.	0.75 ± 0.06 n.s.	10.12 ± 0.90*
% Increase	29.9	10.3	–9.1	1.1	21.4
A. japonicum					
Control	3.93 ± 0.46	8.44 ± 1.06	0.084 ± 0.009	0.74 ± 0.07	7.62 ± 0.46
Gap	5.38 ± 0.77*	10.22 ± 1.20*	0.080 ± 0.015 n.s.	0.76 ± 0.07 n.s.	8.77 ± 1.18*
% Increase	36.7	21.1	–4.0	3.5	15.0
F. lanuginosa					
Control	4.76 ± 0.43	10.08 ± 0.55	0.081 ± 0.007	0.61 ± 0.06	9.62 ± 0.75
Gap	6.25 ± 0.82*	10.87 ± 1.41 n.s.	0.083 ± 0.009 n.s.	0.67 ± 0.06 n.s.	10.30 ± 0.96 n.s.
% Increase	31.3	7.9	3.1	8.4	7.1
S. hydrangeoides					
Control	4.59 ± 1.87	7.49 ± 2.21	0.108 ± 0.013	0.43 ± 0.04	12.87 ± 2.54
Gap	5.33 ± 0.51 n.s.	10.20 ± 1.18*	0.085 ± 0.009*	0.50 ± 0.08 n.s.	12.58 ± 1.78 n.s.
% Increase	16.2	36.1	–21.8	16.9	–2.2
H. petiolaris					
Control	4.32 ± 0.80	6.56 ± 0.78	0.085 ± 0.011	0.64 ± 0.08	12.69 ± 2.32
Gap	5.87 ± 1.68 n.s.	8.56 ± 0.81*	0.075 ± 0.016 n.s.	0.71 ± 0.08 n.s.	13.57 ± 2.63 n.s.
% Increase	35.9	30.5	–11.7	9.9	7.0

*$P < 0.05$ in t-test, n.s. ($P > 0.05$)

(*A. mono*, late-successional canopy species; *A. japonicum*, subcanopy species; *A. rufinerve*, mid-successional canopy species) but belong to the same genus, we may presume that phylogenic restriction is involved in this type of acclimation mechanism.

The observed differences in response to light improvement among species may imply different advantages or disadvantages of the acclimation mechanism that species used to cope with environmental change. The species that have relatively thick leaves at low irradiance with an additional vacant space for chloroplasts entails great costs because extra biomass was invested to construct thick leaves. It has been shown that there are significant relationships between leaf thickness and LMA and between S_{mes} and LMA (Hanba et al. 1999, 2002; Oguchi et al. 2003, 2005). If the light climate is not improved, the investment for thickening leaves will be wasteful. The species that are able to increase leaf thickness in mature leaves may sacrifice mechanical strength of leaves to maintain cell wall flexibility and would have greater damage when attacked by pathogens or disturbed by strong winds (Cassab and Varner 1988; Keller 1993; Sommer-Knudsen et al. 1998). These differences among species in their light acclimation mechanism may contribute to species' coexistence and biodiversity in forest ecosystems.

4.3 Conclusion

Most leaves in the understory of a natural forest had a photosynthetic light acclimation potential after maturation and responded positively to gap formation in a short period. Such acclimation of already expanded leaves is important for the growth of the plant, because few leaves were newly expanded or died after gap formation in any of the species examined. Although the acclimation mechanism differed from species to species, P_{max} increased only when S_c increased after gap formation. Thus, a change in leaf anatomy is important in increasing P_{max} in fully expanded leaves.

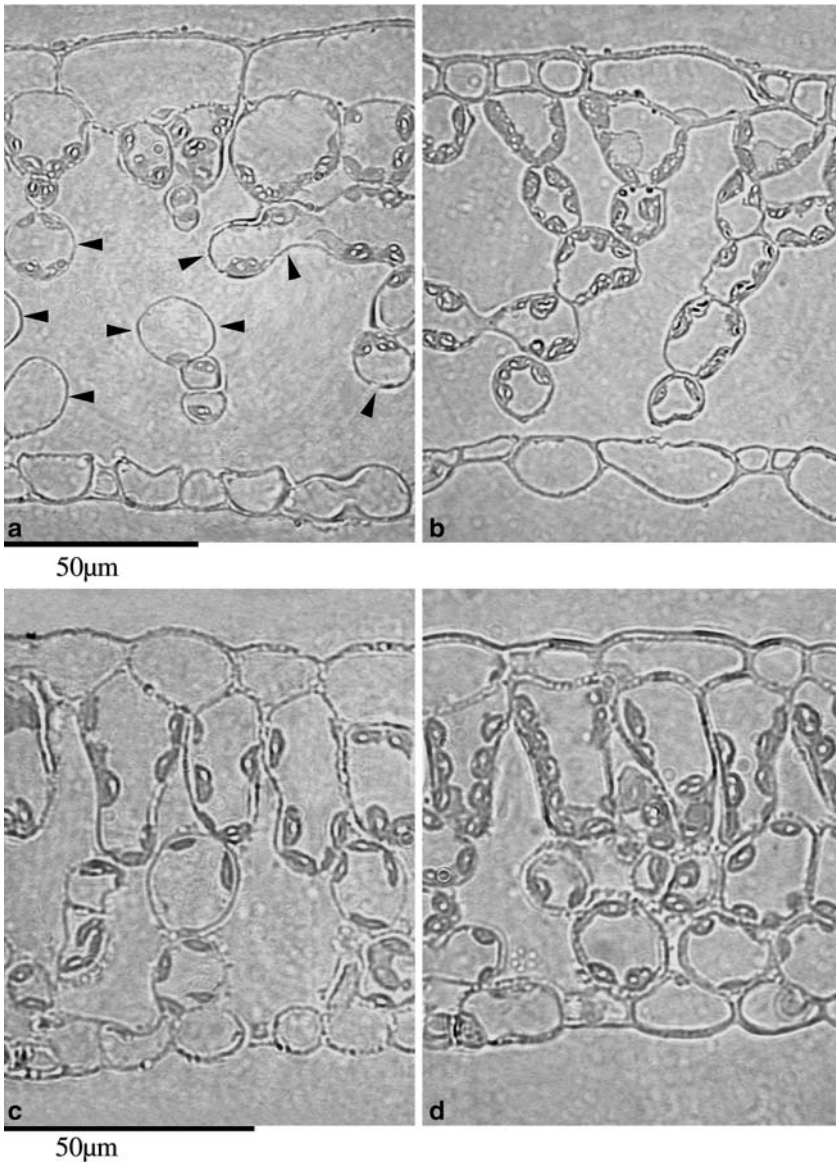

Fig. 5 An example of leaf cross-sections of mature leaves grown in a control site (**a**, **c**) and in a gap site (**b**, **d**). In *K. pictus* (**a**, **b**), leaves grown in control sites had large vacant spaces along the exposed mesophyll surface where chloroplasts were absent, especially in the spongy cells. *Arrowheads* denote vacant spaces for chloroplasts. In *A. japonicum* (**c**, **d**), leaves grown in control sites had a large intercellular space, especially in the spongy tissue, but leaves grown in gap sites had a small intercellular space with enlarged mesophyll cells. Light micrograph: magnification × 400, depth of cross-sections 0.8 μm

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