Midday depression of leaf CO₂ exchange within the crown of *Dipterocarpus sublamellatus* in a lowland dipterocarp forest in Peninsular Malaysia

YOSHIKO KOSUGI, 1,2 SATORU TAKANASHI, 3 NAOKO MATSUO 4 and ABDUL RAHIM NIK 5

1 Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
2 Corresponding author (ykosugi@kais.kyoto-u.ac.jp)
3 Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan
4 Graduate School of Bioresources, Mie University, Tsu, Mie 514-8507, Japan
5 Forest Research Institute Malaysia, Kepong, Kuala Lumpur 52109, Malaysia

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Summary We observed diurnal and seasonal patterns of leaf-scale gas exchange within the crown of a *Dipterocarpus sublamellatus* Foxw. tree growing in a lowland dipterocarp forest at Pasoh, Peninsular Malaysia. Observations were carried out nine times over 6 years, from September 2002 to December 2007. Observation periods included both wet and mild-dry periods, and natural and saturated photosynthetic photon flux density (PPFD) light conditions. In situ measurements of the diurnal change in net photosynthetic rate and in stomatal conductance were carried out on canopy leaves of a 40-m-tall *D. sublamellatus* tree, which was accessed from a canopy corridor. A diurnal change in electron transport rate was observed under saturated PPFD conditions. The maximum net assimilation rate was \( \frac{10}{\text{mol m}^{-2} \text{s}^{-1}} \). There was a clear inhibition of the net assimilation rate coupled with stomatal closure after late morning and this inhibition occurred year-round. Although the electron transport rate decreased alongside this inhibition, it sometimes followed on. Numerical analysis showed that the main factor in the inhibition of the net assimilation rate was patchy bimodal stomatal closure, which occurred in both mild-dry and wet periods. The midday depression occurred year-round, though there are fluctuations in soil moisture during the mild-dry and wet periods. The magnitude of the inhibition was not related to soil water content but was related to vapor pressure deficit (VPD): that is, whether the days were sunny and hot or cloudy and cool. On cloudy, cool days in the wet period, the net photosynthesis was only moderately inhibited, but it still decreased in the afternoon and was coupled with patchy stomatal closure, even in quite moderate VPD, leaf temperature and PPFD conditions. Our results suggest that patchy stomatal closure signaled by the increase in VPD, in transpiration and by circadian rhythms, was the key factor in constraining midday leaf gas exchange of the *D. sublamellatus* canopy leaves.

Keywords: electron transport rate, net assimilation rate, patchy stomatal closure, stomatal conductance, tropical rainforest.

Introduction

Tropical rainforests play a major role in the global carbon budget because they are the greatest global source of net primary production from photosynthesis of canopy tree leaves. To better understand the roles of tropical rainforests in the global carbon budget, we need to clarify the nature and the magnitude of diurnal, seasonal and annual CO₂ exchanges in tropical rainforests and to determine the factors that control the CO₂ exchanges at different timescales.

In many tropical forests, the diurnal pattern of CO₂ exchange at the canopy-scale shows a clear restriction of canopy photosynthesis in the afternoon (Malhi et al. 1998, 1999, Araujo et al. 2002, Saleska et al. 2003, Goulden et al. 2004, Vourlitis et al. 2004, Kumagai et al. 2006, Kosugi et al. 2008). Vourlitis et al. (2004) conducted their study in a Brazilian transitional tropical forest with clear dry and rainy seasons, and reported differences in net ecosystem exchange (NEE) and the magnitude of midday depression among trees during wet, wet–dry transition, dry and dry–wet transition periods. Kosugi et al. (2008) carried out their study in a primary tropical rainforest at Pasoh (Peninsular Malaysia) that receives relatively less rainfall (1804 mm) during two moderate-wet and mild-dry periods. They found that the diurnal pattern of NEE and the
magnitude of midday depression were similar every month for 3 years, and there were no differences related to soil moisture or wet and mild-dry periods. These two studies provide some data, but the seasonal and the inter-annual trends coupled with various rainfall patterns in the tropical forest ecosystems remain unclear because of the lack of a long-term dataset.

Canopy-scale photosynthesis is mainly determined by gas exchange of leaves at the top of the canopy. Therefore, we should investigate the gas exchange characteristics of the canopy leaves to identify the factors affecting midday depression and its seasonal, inter-annual and regional variations and the effects of rainfall patterns. Some aspects of the leaf gas exchange are well understood, such as the relationship between leaf nitrogen content/nitrogen allocation and gas exchange (e.g., Field 1983, Evans 1987, Evans 1993, Hikosaka and Terashima 1995, Niinemets and Tenhunen 1997, Takashima et al. 2004). However, instantaneous leaf gas exchange in natural conditions is a dynamic process that is affected by many micrometeorological and physiological factors. Ishida et al. (1999) reported severe midday depression of photosynthesis coupled with stomatal closure in the upper canopy leaves of a tropical pioneer tree during a severe ENSO-related drought. As summarized by Ishida et al. (1996, 1999), midday depression may be caused by both stomatal and non-stomatal factors, although stomatal limitations can be obscured because of the patchy stomatal behavior (Terashima et al. 1988). When the pattern of stomatal closure is patchy, the stomatal effects on midday depression of photosynthesis can be underestimated in analyses that rely on uniform leaf intercellular CO2 concentrations. Using a pressure-infiltration method and a numerical analysis, Takashima et al. (2006) showed that patchy stomatal closure with a bimodal closure pattern explained the midday depression in top canopy leaves of a tropical forest at Pasoh, Peninsular Malaysia. It is still unclear whether midday depression occurs year-round, whether patchy stomatal closure is always the main limiting factor and which environmental or physiological factors induce patchy stomatal closure.

To better understand gas exchange of canopy leaves in tropical rainforests, we carried out long-term observations of diurnal changes in CO2 exchange in crown leaves of Dipterocarpus sublamellatus Foxw., which is one of the dominant species in this forest and was accessible from a triangle canopy corridor at 31 m height (Figure 1). This forest has two rainy periods that occur from March to May and from October to December, although there are considerable inter-annual fluctuations (Kosugi et al. 2008). We carried out observations nine times during the period from September 2002 to December 2007 to obtain data under a range of environmental and physiological conditions related to mild-dry and wet periods.

**Measurements of leaf gas exchange, chlorophyll fluorescence and microclimate**

We measured the diurnal change of net photosynthetic rate \( A \) (\( \mu \text{mol} \text{m}^{-2}\text{s}^{-1} \)) and the stomatal conductance for water vapor \( g_s \) (\( \mu \text{mol} \text{m}^{-2}\text{s}^{-1} \)) of canopy leaves at the canopy corridor. Photosynthetic photon flux density (PPFD, \( \mu \text{mol} \text{m}^{-2}\text{s}^{-1} \)) conditions were considered to be ‘natural’ on nine measurement days using a clear top chamber: September 19, 2002; March 8, 2003; August 23, 2003; September 10, 2004; November 24, 2004; February 11, 2005; July 3, 2005; October 19, 2005 and December 8, 2007. Saturated PPFD conditions occurred on the other seven measurement days using light-emitting diode chambers: September 20, 2002; March 9, 2003; August 23, 2003; November 23, 2004; July 5, 2005; October 17, 2005 and December 14, 2007. A diurnal change in the maximum electron transport rate \( J \) (\( \mu \text{mol} \text{m}^{-2}\text{s}^{-1} \)) was also observed in saturated PPFD conditions. There is only one individual with sunlit branches that can be accessed from the canopy corridor, so sampling leaves were selected from the sunlit leaves of these branches. Sunlit mature leaves from the upper canopy were chosen. In rainy periods (November 2004 and December 2007), both newly expanded young leaves and older leaves coexisted at the

**Materials and methods**

**Site and materials**

The study site was located in the Pasoh Forest Reserve of the Forest Research Institute Malaysia (FRIM), near Simpang Pertang in Negeri Sembilan, in Peninsular Malaysia (2°58’N and 102°18’E, 75–150 m asl). The core area (600 ha) of the reserve (2450 ha) is covered with a primary lowland mixed dipterocarp forest (tropical evergreen broad-leaved forest), which consists of various species of Shorea and Dipterocarpus. The continuous canopy height is \( \sim 35 \) m, although some emergent trees exceed 45 m. Soil type around the tower is Haplic Acrisol (FAO classification). The A horizon is thin (0–5 cm). Lateritic gravels are abundant below a depth of 30 cm (Yamashita et al. 2003). The mean annual rainfall is 1804 mm (1983–1997; Tani et al. 2003).

In situ leaf gas exchange and chlorophyll fluorescence measurements were conducted on intact leaves within the canopy of a 40-m-tall D. sublamellatus. This tree is one of the dominant species in this forest and was accessible from a triangle canopy corridor at 31 m height (Figure 1). This forest has two rainy periods that occur from March to May and from October to December, although there are considerable inter-annual fluctuations (Kosugi et al. 2008). We carried out observations nine times during the period from September 2002 to December 2007 to obtain data under a range of environmental and physiological conditions related to mild-dry and wet periods.
top part of the sunlit branch, so we sampled both leaf types. On each observation day, we sampled 3–5 leaves in saturated PPFD conditions and 3–10 leaves in natural PPFD conditions. Gas exchange of these leaves was measured several times during the daytime. In the natural PPFD conditions, we sometimes included additional leaf samples (1–5 leaves each measurement) which were cut after each observation to measure infiltration ratio or leaf water potential. In that case, we chose new samples from the immediate vicinity at each observation. Each observation was carried out for several minutes under saturated PPFD conditions and immediately under natural PPFD conditions. Observations were conducted every 30–60 min during daytime. The instrument must be set up after dawn (around 7:00 am), so data from the early morning is not included in the dataset. Data from after 5:00 pm is also not included in the dataset, because we left the forest before sunset for safety reasons. In September 2002, it rained in the afternoon, so diurnal observations were discontinued.

An LI-6400 gas-exchange measurement system (Li-Cor Inc., Lincoln, NE) with a 2 × 3 cm clear-top chamber was used to measure the diurnal change of $A$ and $g_s$ under natural PPFD conditions. In saturated PPFD conditions, we used a 2 × 3 cm light-emitting diode chamber or a 6400-40 leaf chamber fluorometer (Li-Cor Inc., Lincoln, NE) to measure the diurnal changes in $A$, $g_s$, and $J$. The measurements were conducted under ambient conditions except for light, which was controlled at a PPFD value of 1000 μmol m$^{-2}$ s$^{-1}$. Based on the preliminary test, this PPFD level was usually sufficient to obtain maximum values of $A$, although some leaves showed depression of $A$ under 1500 or 2000 μmol m$^{-2}$ s$^{-1}$ PPFD. The intercellular CO$_2$ concentration ($C_i$, μmol mol$^{-1}$) was estimated assuming that whole-leaf $A$ and $g_s$ values were uniform.

Meteorological variables monitored at a height of 52 m on the tower included downward short-wave radiation (MR22, Eko, Japan) and rainfall. Soil water content (SWC) at depths of 0.1, 0.2 and 0.3 m were monitored at three points around the tower (CS615 or CS616, Campbell Scientific, Logan, UT). The accumulated precipitation index for 60 days ($\text{API}_{60}$) was defined as $\sum_{i=1}^{60} P_i / t$, where $P_i$ is daily precipitation (mm) and $t$ is the number of preceding days (Kosugi et al. 2007). The $\text{API}_{60}$ is used in this study to show the rainfall pattern at the site, and this corresponded well with the average SWC at depths of 0.1, 0.2 and 0.3 m (Figure 2). Canopy-scale gas exchange observed at the top of the flux tower was reported by Takanashi et al. (2005), Kosugi et al. (2008) and Ohkubo et al. (2008).

**Numerical analysis**

We calculated the ‘apparent’ normalized maximum carboxylation rate ($V_{\text{cmax}25^*}$, μmol m$^{-2}$ s$^{-1}$) to evaluate the effect of heterogeneous stomatal behavior on gas exchange, and to directly determine the apparent depression in photosynthetic capacity in the field (Takanashi et al. 2006). The ‘apparent’ maximum carboxylation rate ($V_{\text{cmax}^*}$) was calculated using a one-point method (Wilson et al. 2000, Kosugi et al. 2003, Grassi et al. 2005, Kosugi and Matsuo 2006, Takanashi et al. 2006), an inverse method based on the Farquhar–von Caemmerer–Berry model (Farquhar et al. 1980) that can be used to determine the actual responses of leaves in the field. In the one-point method, the apparent partial pressure of CO$_2$ within the intercellular space ($p(C_i)^*$) was estimated from variables measured by the

**Figure 1. Canopy corridor and tree (D. sublamellatus).** (A) Canopy corridor and sampled tree. (B) Sunlit foliage of sampled tree. (C) Newly expanding leaves and mature leaves in wet period. A color version of this figure is available as Supplementary Data at Tree Physiology Online.
stomatal behavior and bimodal patchy stomatal behavior rate at a given stomatal conductance, under both uniform and Kosugi and Matsuo (2006).

Assumption has been discussed by Takanashi et al. (2006). A bimodal distribution means that stomatal conductance for an open or a closed patch was calculated from the values of \( A \), \( p(C) \) and leaf temperature \( T_i \) as follows:

\[
V_{cmax25}^* = (A + R_d) \frac{p(C_i)^* + K_c \left( 1 + \frac{p(O_2)}{K_o} \right)}{1 + \exp \left( \frac{\Delta S(V_{cmax}(T_i+273)) + \Delta H_d(V_{cmax})}{R(T_i+273)} \right)} \times \frac{\exp \left( \frac{\Delta H_d(V_{cmax}(T_i+273))}{298 R(T_i+273)} \right)}{s} \quad (1)
\]

where \( R_d \) is the non-photorespiratory respiration rate (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)), \( \tau \) is the specificity factor of Rubisco, \( p(O_2) \) (21,000 Pa) is the partial pressure of \( O_2 \) at the sites of oxygenation, \( K_c \) and \( K_o \) are the Michaelis–Menten constants of Rubisco for \( CO_2 \) and \( O_2 \), \( T_i \) is the leaf temperature (°C), \( R \) is the gas constant (8.31 J K\(^{-1}\) mol\(^{-1}\)), \( \Delta H_d(V_{cmax}) \) is the activation energy for \( V_{cmax} \), \( \Delta H_d(V_{cmax}) \) is the deactivation energy for \( V_{cmax} \) and \( \Delta S(V_{cmax}) \) is an entropy term. The Arrhenius function was used to estimate temperature dependence of parameters \( K_c \), \( K_o \), \( \tau \), \( V_{cmax} \) and \( R_d \) (detailed in Kosugi et al. 2003, Kosugi and Matsuo 2006, Takanashi et al. 2006). Here, we calculated \( V_{cmax25}^* \) assuming that the infinite internal conductance (\( g_l = \infty \)) and thus the \( CO_2 \) concentration in the chloroplast (\( p(C_i) \)) was the same as \( p(C) \). A possible error in \( V_{cmax25} \) resulting from this assumption has been discussed by Takanashi et al. (2006) and Kosugi and Matsuo (2006).

We simulated the predicted values of the net assimilation rate at a given stomatal conductance, under both uniform stomatal behavior and bimodal patchy stomatal behavior (Takanashi et al. 2006). A bimodal distribution means that the whole-leaf conductance reflects either open or closed stomatal conductance. For the bimodal distribution, the stomatal conductance for an open or a closed patch was \( g_{max} \) or \( g_{min} \), respectively, and the open/closed patch ratio was determined from the observed \( g_l \) for a whole leaf. The net assimilation rate and the intercellular \( CO_2 \) concentration for each patch were determined using the same Farquhar–von Caemmerer–Berry model that was used to determine the stomatal conductance values for each patch. We assumed that the photosynthetic parameters were uniform throughout the whole leaf. The average net assimilation rate for the whole leaf was calculated by integrating the net assimilation rate of each patch.

In the model simulation, the net assimilation rate of each patch or the whole leaf was calculated as described by Kosugi et al. (2003), Kosugi and Matsuo (2006) and Takanashi et al. (2006). Briefly, \( A \) was determined from the minimum value between the (ribulose 1,5-bisphosphate) RuBP-saturated rate or carboxylation-limited net assimilation rate \( (A_j) \) and electron transport- or RuBP regeneration-limited net assimilation rate \( (A_j) \) as follows:

\[
A_c = V_{cmax25} \frac{\exp \left( \frac{\Delta H_d(V_{cmax}(T_i+273))}{298 R(T_i+273)} \right)}{1 + \exp \left( \frac{\Delta S(V_{cmax}(T_i+273)) + \Delta H_d(V_{cmax})}{R(T_i+273)} \right)} \times \frac{p(C_i)^* - p(O_2)}{p(C_i)^* + K_c \left( 1 + \frac{p(O_2)}{K_o} \right)} - R_d \quad (2)
\]

\[
A_j = \frac{\exp \left( \frac{\Delta H_d(V_{cmax}(T_i+273))}{298 R(T_i+273)} \right)}{1 + \exp \left( \frac{\Delta S(V_{cmax}(T_i+273)) + \Delta H_d(V_{cmax})}{R(T_i+273)} \right)} \times \frac{p(C_i)^* - p(O_2)}{p(C_i)^* + K_c \left( 1 + \frac{p(O_2)}{K_o} \right)} - R_d \quad (3)
\]

Here \( V_{cmax25} \) is the intrinsic value that represents the carboxylation ability of the leaves and is not always the same as the apparent value \( (V_{cmax25}^*) \), which shows significant diurnal fluctuations coupled with patchy stomatal closure (Takanashi et al. 2006). A \( J \) value was required to evaluate the influence of depression in electron transport rate on the...
net assimilation rate. To estimate $J$, we used both observed $J$ values measured with the chlorophyll fluorescence method and the optimal $J_{\text{max}}$ values estimated from the relationship between $V_{\text{cmax}}$ and $J_{\text{max}}$ as follows:

$$J_{\text{max}} = k_j V_{\text{cmax}}.$$  \hspace{1cm} (4)

Observed values of $V_{\text{cmax}25}$ (25.0 μmol m$^{-2}$ s$^{-1}$), $R_{d25}$ (1.0 μmol m$^{-2}$ s$^{-1}$) $k_j$ (2.4), $g_{\text{max}}$ (0.2 mol m$^{-2}$ s$^{-1}$) and $g_{\text{min}}$ (0.0005 mol m$^{-2}$ s$^{-1}$) were used to calculate uniform photosynthetic parameters. We also used 55,200 J mol$^{-1}$ for $\Delta H_d(V_{\text{cmax}})$, 220,000 J mol$^{-1}$ for $\Delta H_d(V_{\text{cmax}})$, 650 J mol$^{-1}$ for $\Delta S(V_{\text{cmax}})$ and 41,500 μmol m$^{-2}$ s$^{-1}$ for $\Delta H_d(R_d)$ based on the average line or the median value for evergreen broadleaved trees listed by Kosugi and Matsuo (2006). Other parameters were as listed in Takanashi et al. (2006).

**Results**

**Microclimate**

Figure 2 shows the fluctuation of daily rainfall, % SWC (average value from nine sensors at 0.1-, 0.2- and 0.3-m depth) and API60 for 6 years from 2002 to 2007, and dates on which leaf gas exchange observations were carried out under natural PPFD conditions (nine times) and saturated PPFD conditions (seven times). During the 6 years from 2002 to 2007, the average and the standard deviation of annual rainfall were 1755 ± 183 mm. This exceeded the 4-year average (1571 mm) from 1996 to 1999, which included an El Niño event from 1997 to early 1998 (Tani et al. 2003), but was close to the normal average (1804 mm, 1983–1997; Tani et al. 2003). The SWC and the API60 showed rather complex seasonal fluctuations, corresponding with mild-dry and wet periods twice each year. Average SWC at each gas exchange observation day ranged from 18% to 37%. This range included the lowest 5% (SWC < 21%) and the highest 5% (SWC > 35%) of recorded values during the 6 years. The driest period in the 6 years occurred in February 2005. We defined ‘mild-dry period’ as a period with SWC < 27%, and ‘wet period’ as one with SWC ≥ 27%. Five gas exchange observations were carried out during mild-dry periods (March 2003, August 2003, September 2004, February 2005 and July 2005) and four during wet periods (September 2002, November 2004, October 2005 and December 2007). The September 2002 measurement day fell immediately after the first rainfall at the end of the mild-dry period, so it was very difficult to assign this measurement to either a rainy or a dry period.

At the site, the median daily average of SWC over the 6 years was 28.5%. On 30% of measurement days SWC exceeded 31.1%, and on 30% of days it was < 25.8%. Therefore, the observation days were grouped according to their SWC as follows: November 2004, October 2005 and December 2007 had SWC values within the highest 24% of all the measured SWC values. These were designated as ‘wet’ periods. The other periods except September 2002 were designated as ‘mild-dry’ periods (SWC on these measurement days were among the lowest 14% of measured values). It was very difficult to designate days with SWC between 25.8% and 31.1% as ‘wet’ or ‘mild-dry’ using only the SWC value. We designated 27% SWC as the cut-off value between ‘wet’ and ‘mild-dry’, which means that the September 2002 observation day was considered to be a ‘wet period’. However, it is more accurate to consider it as a dry–wet transition period.

**Diurnal pattern of leaf CO2 exchange with saturated PPFD**

Figure 3 shows the diurnal change of $A$, $g_s$, $J$, $V_{\text{cmax}25}$, $T_i$ and vapor pressure deficit (VPD, hPa) measured on the 7 days with saturated PPFD conditions (1000 μmol m$^{-2}$ s$^{-1}$). The averaged diurnal change from several leaves ($n = 3$–5) is shown in this figure. Daily diurnal change of solar radiation measured at the top of the tower is also shown. Note that solar noon is around 13:00 pm local time at this site. Figure 4 shows the relation between (A) $g_s$ and $A$, (B) $J$ and $A$ and (C) $g_s$ and $V_{\text{cmax}25}$ during mild-dry (closed squares) and wet periods (open squares).

Maximum $A$ and $g_s$ under saturated PPFD conditions were ~ 10 μmol m$^{-2}$ s$^{-1}$ and 0.2 mol m$^{-2}$ s$^{-1}$, respectively. The corresponding $V_{\text{cmax}25}$ at that time was ~ 20–30 μmol m$^{-2}$ s$^{-1}$. These values were recorded in the early morning of three of the four observations that were conducted during wet periods (September 2002, October 2005 and December 2007). In the wet period in November 2004, and also in the mild-dry period (March 2003, August 2003 and July 2005), both $A$ and $g_s$ were slightly lower than their maximum values recorded in the morning (Figure 3). The $J$ ranged between ~ 50 and 100 μmol m$^{-2}$ s$^{-1}$ in mature leaves (Figure 4). In the middle of wet periods (November 2004 and December 2007), the newly expanding leaves had lower $A$, $J$ and $V_{\text{cmax}25}$ values than the older mature leaves. Maximum $g_s$ values of these young leaves within a day were greater than or the same as those of the older leaves, while the minimum $g_s$ in a day was lower than that of older leaves (Figure 3).

The diurnal change of $A$ showed a clear depression after late morning, coupled with depression of $g_s$, $J$ and $V_{\text{cmax}25}$. This midday depression occurred on every observation day, irrespective of mild-dry or wet conditions. On cloudy cool days in wet periods (October 2005 and December 2007), depression was moderate and occurred more slowly than in other periods. On sunny hot days, even in wet periods (September 2002 and November 2004), depression was immediate and severe (Figure 3). Both $g_s$ and $J$ were significantly correlated with $A$ ($P < 0.0001$), and had similar correlation coefficients ($R = 0.69$ for $g_s$, $R = 0.61$ for $J$). In mild-dry periods, $A$, $g_s$ and $J$ tended to be lower than in wet periods. The $J$ did not markedly decrease alongside
the severe depression of $A$ and $g_s$ that occurred in mild-dry periods. This suggests that the relation between $J$ and $A$ differed between mild-dry and wet periods (Figure 4). There was a clear midday depression in $V_{\text{cmax25}^*}$ that corresponded with $g_s$ both in mild-dry and wet periods. This phenomenon was also reported by Takanashi et al. (2006), whose simulations showed that depression of $V_{\text{cmax25}^*}$ was associated with depression of $g_s$ during the patchy stomatal closure.

Figure 5 shows the numerical analysis based on the Farquhar–von Caemmerer–Berry model. In this model, it is assumed that assimilation rate is affected by (A) the decrease of observed electron transport rate while stomata remain open ($g_s = 0.2 \text{ mol m}^{-2} \text{s}^{-1}$), (B) uniform stomatal closure while the electron transport rate remains at its optimal value, (C) the patchy bimodal stomatal closure while electron transport rate remains at its optimal value and (D) the patchy bimodal stomatal closure coupled with a decrease in observed electron transport rate. The results show that neither the decrease in $J$ nor the uniform stomatal closure can explain the magnitude of the depression of $A$. On the other hand, the patchy bimodal stomatal closure largely explained the observed depression in both mild-dry and wet periods. A slightly better estimation could be obtained by including the decrease of electron transport rate as well as the patchy bimodal stomatal closure. During
the most severe depression of $A$, the $A$ values obtained using these assumptions were slightly larger than observed $A$ values.

**Diurnal pattern of leaf CO$_2$ exchange under natural PPFD conditions**

Figure 6 shows the diurnal changes of $A$, $g_\text{s}$, and $C_\text{i}/C_\text{a}$ (the ratio of $C_\text{i}$ to the ambient CO$_2$ concentration, $C_\text{a}$), measured under natural PPFD conditions in (A) mild-dry and (B) wet periods. Figure 7 shows the relation between $A$ or $g_\text{s}$ and PPFD or VPD, using the same mature-leaf dataset, and the simulation results assuming uniform stomatal closure and patchy bimodal stomatal closure.

Maximum values of $A$ and $g_\text{s}$ were in the same range as those measured under saturated PPFD conditions (i.e., $\sim 10$ µmol m$^{-2}$ s$^{-1}$ and 0.2 mol m$^{-2}$ s$^{-1}$, respectively). However, the maximum value of stomatal conductance sometimes exceeded 0.2 mol m$^{-2}$ s$^{-1}$. These maximum values were observed under natural PPFD conditions in mild-dry periods, and slightly lower maximum values were observed under saturated PPFD conditions (March 2003, August 2003 and July 2003). Compared with saturated PPFD conditions, maximum values occurred later in the day under natural PPFD conditions, but varied depending on environmental conditions such as instantaneous PPFD at each measured leaf. On cloudy days in wet periods (October 2005 and December 2007), the maximum $A$ was sometimes lower than that observed under saturated PPFD conditions. Moreover, in December 2007, maximum $A$ occurred in the afternoon, during periods of sunshine. In November 2004, we observed significantly lower $A$, $g_\text{s}$, and larger $C_\text{i}/C_\text{a}$ values in newly expanding leaves than that in older mature leaves ($P < 0.001$). In December 2007, young leaves had larger $A$ and $g_\text{s}$ values on cloudy mornings, while the maximum $A$ values in the afternoon were smaller than those of mature leaves (Figure 6). Among all observations, the minimum value of $C_\text{i}/C_\text{a}$, which represents the intrinsic water use efficiency of leaves, ranged between $\sim 0.6$ and 0.7. We did not observe a decrease in $C_\text{i}/C_\text{a}$ coupled with midday depression of $A$.

Midday depression of $A$ occurred on observation days in both mild-dry and wet periods, except in December 2007 (Figure 6). The relation between $A$ or $g_\text{s}$ and PPFD or VPD (Figure 7) showed a clear decline of $A$ in the afternoon compared with the pattern observed in the morning. The hyperbolic curves of both $A$ and $g_\text{s}$ in the relation with PPFD showed considerable scattering, and a decline in maximum values in the afternoon. The relation between $A$ or $g_\text{s}$ and VPD showed declining curves, similar to those observed in Lohammer’s equation (Lohammer et al. 1980), which is often used to describe the relation between $g_\text{s}$ and VPD. There were no significant differences in these relations between mild-dry and wet periods. Even in wet periods, $A$ and $g_\text{s}$ became lower in high VPD conditions. Furthermore, in wet periods $A$ and $g_\text{s}$ were lower in the afternoon than in the morning, even in cloudy conditions with only moderate VPD (Figure 7).

The numerical analysis (Figure 7) showed that patchy bimodal stomatal closure could explain the depression in $A$, whereas uniform stomatal closure could not. Uniform stomatal closure only explained the depression in $A$ in the mornings of mild-dry periods, and simulation with patchy stomatal closure overestimated the depression in $A$ compared with that of the observed values.

**Discussion**

**Photosynthetic ability and its temporal variations**

Maximum $A$ values of $\sim 10$ µmol m$^{-2}$ s$^{-1}$ were frequently observed under both saturated and natural PPFD conditions, thus we can assume that this value represents the maximum photosynthetic ability of mature canopy leaves of *D. sublamellatus*. In the wet period, this maximum value was observed only under saturated PPFD conditions,
whereas in the dry period it was sometimes observed under natural PPFD conditions (Figures 3 and 6). This finding suggests that we may have missed any higher $A$ values that occurred under saturated PPFD conditions in the mild-dry period, because the depression occurred immediately after sunshine and was coupled with increases in leaf temperature, VPD, PPFD and water loss through transpiration. In September 2004 and February 2005 in the mild-dry period, and in November 2004 in the wet period, the maximum $A$ was lower than that observed in other periods. The data alone do not provide sufficient evidence to determine whether the photosynthetic ability was lower during these periods than during other periods. However, the numerical analysis showed that the patchy stomatal closure largely explained the lower than expected net assimilation rate. This resulted from the constant photosynthetic ability of
the canopy leaves, which is represented with constant $V_{c_{\text{max25}}}$ value (25 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) in the simulations (Figures 5 and 7). From this result we can assume that photosynthetic ability of canopy leaves showed no significant temporal variations during the observation periods over various seasons at this site. Photosynthetic ability differed only in the newly expanding young leaves in wet periods. Compared with those in the mature leaves, the values of $A$, $J$ and $V_{c_{\text{max25}}}$ were lower, the maximum $g_s$ was the same or larger, and the response of $g_s$ to VPD was more rapid in young leaves (Figures 3 and 6). Occasional leaf flashes were observed in $D. \ sublamellatus$ corresponding with the rainfall pattern. Although the data were limited, they suggested that in the wet period there are some newly expanding leaves that have lower photosynthetic ability than mature leaves. Further research is required to clarify the seasonal variations in photosynthetic capacity.

**Does midday depression occur year-round?**

Midday depression of $A$ was observed on almost every observation day, whether light conditions were saturated or natural PPFD. The only exception was on a cloudy day during the wet period (December 2007) under natural PPFD conditions (Figures 3 and 6). In the diurnal change of $A$ under both saturated and natural PPFD conditions in December 2007, $A$ gradually decreased after late morning under natural PPFD conditions. Under natural PPFD conditions, $A$ reached its maximum daily value during the sunshine hours in the afternoon, but this value was lower than that observed in the early morning under saturated PPFD conditions. This result strongly suggests that midday depression also occurred in this period. From these data, we can conclude that midday depression of photosynthesis of canopy leaves occurred year-round, even on cloudy days in the wettest period.

**Was patchy stomatal closure always the main factor limiting midday net assimilation?**

We investigated the influence of stomatal and non-stomatal factors on midday depression of $A$. Similar correlation coefficients were observed when comparing both $g_s$ and $A$, and $J$ and $A$, although $J$ sometimes decreased more slowly than

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Figure 7. Relation between net assimilation rate ($A$) or stomatal conductance ($g_s$) and PPFD or VPD, and simulation results assuming uniform stomatal closure and patchy bimodal stomatal closure during (A) mild-dry and (B) wet periods. Closed circles show morning data and open circles show afternoon data. All data were collected from mature leaves under natural PPFD conditions; $r^2$ is defined here as \( 1 - \Sigma (A_{\text{obs}} - A_{\text{sim}})^2 / \Sigma (A_{\text{obs}} - A_{\text{avg}})^2 \), where $A_{\text{obs}}$ is the observed $A$, $A_{\text{sim}}$ is the simulated $A$, and $A_{\text{avg}}$ is the average value of the observed $A$.
These relations alone cannot determine whether stomatal or non-stomatal factors induce midday depression of $A$. However, the numerical analysis using the saturated PPFD data (Figure 5) showed that neither the decrease in $J$ nor the uniform stomatal closure could explain the depression of $A$. Instead, the patchy bimodal stomatal closure largely explained midday depression of $A$, both in dry and in wet periods. This finding strongly suggests that patchy stomatal closure was always the main factor limiting midday net assimilation in canopy leaves of *D. sublamellatus*. Patchy stomatal closure with a bimodal pattern was also observed in another emergent tree in this forest, *Neobalanocarpus heimii* (King) Ashton (Takanashi et al. 2006). We observed similar depression of $A$ and $g_s$ in all five trees that are accessible from the corridor (unpublished data). Diurnal CO$_2$ exchange at the canopy scale also showed midday depression every month, irrespective of mild-dry and wet periods (Kosugi et al. 2008).

The numerical analysis also showed that when low values of $A$ were observed during the mild-dry period, patchy bimodal stomatal closure could not explain the more severe depression in $A$, which sometimes reached negative values (Figure 5). At that time, $A$ at a given $J$ became lower than that in the wetter period (Figure 4). Ishida et al. (1999) reported similar differences in the relation of $J$ and $A$ between dry and wet seasons, and they suggested that the photosynthesis rate might increase during the dryer and the hotter periods. During the mild-dry period, estimating depression using the patchy bimodal pattern of stomatal closure sometimes overestimated the depression of $A$, compared with the value measured under natural PPFD conditions (Figure 7). This corresponded to the period during the early morning when stomata opened, and sunshine first fell on leaves. In this case, uniform $g_s$ and $C_i$ explained $A$, suggesting that the pattern of stomatal closure was not patchy at that time.

*Which environmental or physiological factors induced patchy stomatal closure?*

The data showed that there were some differences in diurnal patterns of $A$ and $g_s$ among the observation periods. Although numerical analyses showed that there were no significant fluctuations in photosynthetic ability of canopy mature leaves among the different observation periods and that patchy stomatal closure occurring irrespective of mild-dry and wet periods was always the main factor causing midday depression of net assimilation.

We attempted to determine which environmental or physiological factors induced the patchy stomatal closure and resulted in the different diurnal patterns of $A$ and $g_s$ among the observation periods. On sunny, hot days, leaf temperature, VPD and thus transpiration rate increased rapidly when direct radiation reached the canopy leaves in the morning. On those days, we observed a rapid and severe decrease or a low value of $g_s$ from the beginning of the day (Figures 3 and 6). This occurred during both mild-dry and wet periods, irrespective of SWC. On the other hand, slower and moderate depression of $A$ and $g_s$ were observed on cloudy and cool days. We observed a strong dependence of $g_s$ on VPD (Figure 7). Our results indicate that the magnitude of midday depression among observation periods was not related to SWC, but rather to atmospheric conditions such as whether the day was sunny and hot or cloudy and cool. Thus midday depression was related to VPD. However, moderate depression of $A$ occurred even on cloudy cool days in wet periods, coupled with patchy stomatal closure in quite moderate VPD, leaf temperature and PPFD conditions. These results suggest that patchy stomatal closure was also signaled by circadian rhythms (Doughty et al. 2006). We have collected some data on leaf water potential, determined with a pressure chamber (unpublished) and a hygrometer (Fukui et al. 2007). The pressure chamber method did not accurately measure the leaf water potential because water was already moving when we cut the *D. sublamellatus* leaves, indicating that the leaf water potential was quite high. Using the hygrometer, we observed that leaf water potential at midday decreased to $-1.1$ MPa, a value that is not particularly low. Our results strongly suggest that low leaf water potential did not trigger midday depression. Further research is required to determine which ‘circadian rhythms’ induced the patchy stomatal closure.

Midday depression coupled with patchy stomatal closure was more moderate on cloudy and cool days in the wet period than on sunny and hot days in the mild-dry period. However, the lower $A$ values observed in newly expanding leaves with lower photosynthetic ability might mitigate this difference, and result in similar diurnal CO$_2$ exchange at the canopy scale year-round, as reported by Kosugi et al. (2008).

**Supplementary Data**

Supplementary data for this article are available at Tree Physiology Online.

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**References**

Araujo, A.C., A.D. Nobre, B. Kruijt et al. 2002. Comparative measurements of carbon dioxide fluxes from two nearby