

# Spatial and temporal variations in photosynthetic capacity of a temperate deciduous-evergreen forest

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Received: 15 July 2015 / Revised: 9 October 2015 / Accepted: 22 December 2015 / Published online: 11 January 2016  
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## Abstract

**Key message** The understory evergreen trees showed maximal photosynthetic capacity in winter, while the overstory deciduous trees showed this capacity in spring. The time lag in productive ecophysiological active periods between deciduous overstory and evergreen understory trees in a common temperate forest was clearly related to the amount of overstory foliage.

**Abstract** In temperate forests, where deciduous canopy trees and evergreen understory trees coexist, understory trees experience great variation in incident radiation corresponding to canopy dynamics represented by leaf-fall and leaf-out. It is generally thought that changes in the light environment affect understory plants' ecophysiological traits. Thus, to project and estimate annual energy, water, and carbon exchange between forests and the atmosphere, it is necessary to investigate seasonal variation in the ecophysiological activities of both evergreen trees in the understory and deciduous trees that make up the canopy/overstory. We conducted leaf-scale gas-exchange measurements and nitrogen content analyses for six tree species along their heights throughout a complete year. Photosynthetic capacity as represented by

the maximum carboxylation rate ( $V_{\text{cmax}25}$ ) and photosynthetic nitrogen use efficiency (PNUE) of deciduous canopy trees peaked immediately after leaf-out in late May, declined and stabilised during the mid-growing season, and drastically decreased just before leaf-fall. On the other hand, the timing of lowest  $V_{\text{cmax}25}$  and PNUE for evergreen understory trees coincided with that of the highest values for canopy trees. Furthermore, understory trees' highest values appeared just before canopy tree leaf-out, when incident radiation in the understory was highest. This implies that failing to consider seasonal variation in leaf ecophysiological traits for both canopy and understory trees could lead to serious errors in estimating ecosystem productivity and energy balance for temperate forests.

**Keywords** Photosynthesis · Farquhar model · Leaf nitrogen content · Rubisco · Leaf area index · Vertical variation

## Introduction

Temperate deciduous forests occupy approximately one-fifth of the world's total forested area (Ridder 2007). These forests are often characterised by the coexistence of deciduous canopy/overstory and evergreen understory trees, resulting in a vertical, multi-layered species composition and canopy structure (e.g., Breckle and Walter 2002; Sakai et al. 2006).

Seasonal variation in the photosynthetic capacity of deciduous canopy trees following maturation and senescence processes have been well documented: the maximum carboxylation rate when ribulose biphosphate (RuBP) is saturated ( $V_{\text{cmax}}$ ) is highest immediately after leaf-out but

Communicated by C. Lovelock.

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declines slowly throughout the growing season, and then more rapidly just before leaf-fall (e.g., Wilson et al. 2000; Kosugi et al. 2003). On the other hand, understory trees' photosynthetic behaviour is affected by seasonal changes in incident light through the upper canopy (Oguchi et al. 2008). For example, early studies (e.g., Harrington et al. 1989; Gill et al. 1998) showed that some deciduous understory trees expand their leaves earlier than overstory trees to take advantage of incident light availability and the resultant carbon gain. Evergreen understory trees receive stronger light during canopy trees' dormant season and, in effect, maximise their photosynthetic capacity in winter (e.g., Miyazawa and Kikuzawa 2005, 2006a). The contrasting seasonal patterns in  $V_{\text{cmax}}$  between deciduous overstory and evergreen understory trees imply seasonal vertical shifts in the major sinks and sources of gas exchange, a geophysical phenomenon unique to such forests. To our knowledge, no study has considered the differences in temporal variation in photosynthetic capacity (i.e.,  $V_{\text{cmax}}$ ) between canopy deciduous and understory evergreen trees in a temperate deciduous-evergreen forest.

To estimate project energy and carbon balances in deciduous forest ecosystems with evergreen understory trees, it is necessary to take into account vertical differences in photosynthetic capacity among the canopy layers. Multilayer soil–vegetation–atmosphere transfer (SVAT) models are among the more promising tools for reproducing measured scalar fluxes in several vegetation types over hourly to multiyear timescales (Baldocchi and Meyers 1998; Kumagai et al. 2006; Kumagai and Kume 2012; Kumagai et al. 2013). SVAT models are thought to be able to consider the effects of vertical differences in the timing of each canopy layer's peak  $V_{\text{cmax}}$  on overall ecosystem energy and carbon balance, the subject of this investigation.

Our goal, therefore, is to clarify seasonal variation in photosynthetic capacity at each vertical position in a deciduous tree-dominated forest canopy accompanied by evergreen understory trees. Here, additional concrete questions arise: (1) What biological and abiological factors are responsible for the difference in timing of the  $V_{\text{cmax}}$  peaks between the canopy deciduous and understory evergreen trees? and (2) What biochemical machinery exists in the leaves of canopy and understory trees to support the contrasting seasonal variations in  $V_{\text{cmax}}$ ? To address these questions, we conducted leaf-scale gas-exchange measurements and nitrogen (N) content analyses for six tree species along their heights throughout a complete year. The photosynthetic capacity of each species was determined by  $V_{\text{cmax}}$  standardised at 25 °C ( $V_{\text{cmax}25}$ ), allocation of leaf N to within-leaf photosynthetic sites, and photosynthetic N-use efficiency (PNUE).

## Materials and methods

### Site description

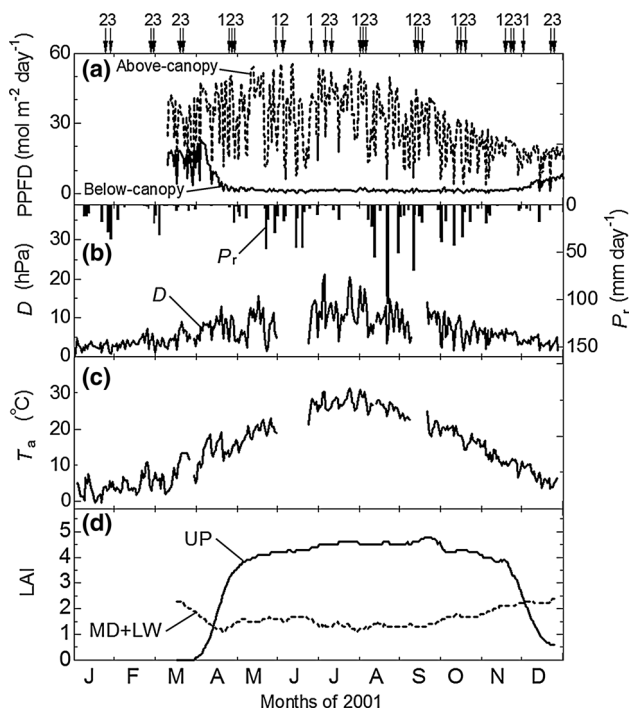
Experiments were carried out in a stand of secondary deciduous-evergreen mixed forest on the campus of Nagoya University, Japan (35°10'N, 136°58'E, 70 m a.s.l.), in 2001. The stand was approximately 100 × 100 m<sup>2</sup>. For our investigations, we divided the forest canopy into three vertical layers: upper (UP), middle (MD), and lower (LW) (Table 1), with measurement heights of 14.6, 5.4–6.4, and 0.8 m, respectively.

*Quercus serrata* Murray, a deciduous tree, is the dominant species throughout the stand and forms the UP. The MD comprises the evergreen species *Eurya japonica* Thunb., *Ilex pedunculosa* Miq., *Ligustrum japonicum* Thunb., and *Vaccinium bracteatum* Thunb., with some deciduous trees (e.g., *Evodiopanax innovans* Nakai). The LW consists mainly of young evergreen trees of the same species as the MD, and *Aucuba japonica* Thunb. On average, the canopy height was 17.8 m in 2001.

The stand is situated in a temperate forest region with four distinct seasons. Based on data from the National Astronomical Observatory of Japan (2012), the mean annual air temperature ( $T_a$  in °C) over the period 1981–2010 was ~16 °C with a minimum monthly mean  $T_a$  of ~4 °C in January and a maximum monthly mean  $T_a$  of ~27 °C in August at the nearest meteorological station, located 2 km north–north west of the site. The mean annual precipitation ( $P_r$  in mm) over the period 1981–2010 was ~1540 mm, with the rainy season occurring from mid-June to mid-July. Meteorological variables measured at the study site in 2001 are summarised in Fig. 1;  $T_a$  and total  $P_r$  were 16 °C and 1415 mm, respectively, similar to their long-term averages. Maximum  $T_a$  reached 37 °C between late July and early August. Minimum  $T_a$  was –2.7 °C in January.  $T_a$ , and therefore the above-canopy atmospheric humidity deficit ( $D$ : hPa), showed seasonal variation.  $P_r$  was high

**Table 1** Tree species and heights in each canopy layer used for leaf gas-exchange measurement

Canopy layer	Height (m)	Species
Upper layer	14.6	<i>Quercus serrata</i> Marray
Middle layer	5.4–6.4	<i>Eurya japonica</i> Thunb. <i>Ilex pedunculosa</i> Miq. <i>Vaccinium bracteatum</i> Thunb.
Lower layer	0.8	<i>Ligustrum japonicum</i> Thunb. <i>Aucuba japonica</i> Thunb.



**Fig. 1** Seasonal variation in, **a** daily total photosynthetic photon flux density (PPFD), **b** daily mean vapour pressure deficit ( $D$ ) and daily total precipitation ( $P_r$ ), **c** daily mean air temperature ( $T_a$ ), and **d** leaf area index (LAI) for upper (UP) and middle (MD) + lower (LW) layers (i.e., MD + LW). Downward arrows denote observation days with the leaf gas exchange measurements in UP (1), MD (2), and LW (3)

in June (the rainy season) and between mid-August and late September (typhoon season).

### Meteorological measurements

We constructed a 21 m experimental tower at the study site to measure microclimatic variables, eddy-covariance fluxes, and leaf-scale gas-exchange rate (see Hiyama et al. 2005). Short-wave radiation ( $R_s$  in  $\text{W m}^{-2}$ ) and photosynthetic photon flux density (PPFD in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at heights of 20.8, 10.7, and 0.7 m were measured using a solar radiometer (CM3, Kipp & Zonen, Delft, Netherlands) and quantum sensor (LI-190A, Li-Cor, Lincoln, NE) installed at each height. A temperature and humidity sensor (HMP45A, Vaisala, Helsinki, Finland) was installed at a height of 20.7 m. A tipping bucket rain gauge (RT-5, Ikeda-Keiki Co., Tokyo, Japan) was placed on the rooftop of a nearby building, about 120 m from the observation tower. We used  $P_r$  data recorded at the Nagoya local meteorological observatory, 2 km from the experimental site, to compensate for missing data.  $R_s$  and PPFD samples were taken every 30 s, and averaged over 10-min on a data logger (CR23X, Campbell Scientific, Logan, UT). Air

temperature and relative humidity were recorded using a data logger (CR10X, Campbell Scientific, Logan, UT) with a 10 Hz interval.

### Leaf-scale physiological measurements

Leaf-scale gas-exchange was measured using a portable infrared gas analyser (LI-6400, Li-Cor), maintaining the cuvette at ambient environmental conditions using the ambient light through the transparent cuvette top and the temperature controller of the Li-6400 during daylight hours. Leaf temperature was measured using the thermocouple of the Li-6400, and incident PPFD was measured by an internally mounted quantum sensor. Measurements were performed on 6–8 leaves from 1 to 2 sample trees of each species dominating each of the three layers (Table 1) and were repeated about once a month in 2001 for each layer (Fig. 1). Using the experimental tower, we accessed the sunlit leaves of *Q. serrata* in UP and the shaded leaves of evergreen trees in MD. The measurements in LW were conducted around the foot of the tower. The highest (lowest)  $T_a$  in the leaf chamber cuvette in the study period was 34.9 °C (4.7 °C) in August (February), while the highest (lowest) leaf temperature was 34.3 °C (3.3 °C) in August (February). The maximum and minimum leaf surface  $D$  were 32 hPa in August and 4 hPa in November.

Following the gas exchange measurements, 6–12 leaves for each species were excised and their areas were measured. We weighed the leaf dry mass after drying at 60 °C for 3 days, thus obtaining the specific leaf area (SLA:  $\text{m}^2 \text{g}^{-1}$ ). The leaf N content per unit dry mass ( $N_m$ :  $\text{g g}^{-1}$ ) was measured using an elemental analyser (Carlo Erba NA2500 Analyser, Thermo Electron, Rodano, Italy) or a CN analyser (MT-700, Yanaco, Kyoto, Japan). The leaf N content per unit area ( $N_a$ :  $\text{g m}^{-2}$ ) was derived by dividing the  $N_m$  by the SLA. Harvested leaves coincided with or were adjacent to gas-exchange-measured leaves.

### $V_{\text{cmax}}$ estimation

We estimated  $V_{\text{cmax}}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) inversely by applying the measured leaf net photosynthesis rate ( $A_n$ :  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) to the biochemical model for leaf photosynthesis (Farquhar et al. 1980). Here, we assumed infinite internal conductance, and thus the intercellular  $\text{CO}_2$  concentration ( $C_i$ :  $\mu\text{mol mol}^{-1}$ ) could be used for  $\text{CO}_2$  concentration in the chloroplasts. Measurements of  $A_n$  and  $C_i$  below 320  $\mu\text{mol mol}^{-1}$  under light saturation (Koike et al. 2001; Miyazawa and Kikuzawa 2005 for deciduous canopy species and evergreen understory broadleaved tree species, respectively) allowed us to adopt a “one-point method”

(e.g., Wilson et al. 2000; Takanashi et al. 2006). This means that  $V_{\text{cmax}}$  can be derived from an inverse version of the equation for the gross rate of photosynthesis limited by RuBP carboxylase-oxygenase (Rubisco) activity in Farquhar's model with one-point data for  $A_n$ ,  $C_i$ , and leaf temperature. The photosynthesis model constants can be determined according to Tenhunen et al. (1990), Harley et al. (1992), and Harley and Baldocchi (1995), summarised in Table 2. The daytime (non-photorespiratory) respiration rate ( $R_d$ ;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was estimated by  $R_d = 0.015V_{\text{cmax}}$  according to Collatz et al. (1991). Estimated  $V_{\text{cmax}}$  was standardised as values at 25 °C ( $V_{\text{cmax}25}$ ;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) using a nonlinear temperature function (see Sharpe and DeMichele 1977), and both inter-species and seasonal variation were examined.

### Examination of N investment in photosynthetic capacity

We hypothesised that inter-species and seasonal variabilities in the photosynthetic capacity, i.e.,  $V_{\text{cmax}25}$ , are caused primarily by the incident light environment, and are ultimately mediated by changes in leaf N content and/or allocation (see Wilson et al. 2000; Kumagai et al. 2006). Variability in  $V_{\text{cmax}25}$  can be described well by a linear relationship with  $N_a$  as (Niinemets and Tenhunen 1997):

$$V_{\text{cmax}25} = 6.25 V_{\text{cr}} N_a R_F \quad (1)$$

where 6.25 is the ratio of the weight of Rubisco to the weight of N in Rubisco,  $V_{\text{cr}}$  is the specific activity of Rubisco [ $\approx 20.7 \mu\text{mol CO}_2 (\text{g Rubisco})^{-1} \text{s}^{-1}$  at 25 °C], and  $R_F$  is the apparent fraction of N allocated to Rubisco [ $\text{g N in Rubisco (g total leaf N)}^{-1}$ ].

### Leaf area measurements

According to Beer's law, the silhouette area index of leaves, stems, and branches (plant area index, PAI), can be expressed as follows (e.g., Holst et al. 2004; Igarashi et al. 2015):

$$\text{PAI} = \text{LAI} + b = -\frac{1}{k} \ln \left( \frac{R_{s\_below}}{R_{s\_above}} \right) \quad (2)$$

where LAI is the leaf area index,  $b$  is an empirical constant representing the contribution of stems and branches to radiative extinction,  $k$  is the extinction coefficient,  $R_{s\_below}$  is the  $R_s$  below (at 0.7 m) or within (at 10.7 m) the canopy, and  $R_{s\_above}$  is the  $R_s$  above the canopy (at 20.8 m). The parameter  $k$  is known to vary with zenith angle and cloudiness (Baldocchi et al. 1984; Holst et al. 2004), so to reduce this effect, we input the 15-day moving average of daily  $R_{s\_below}/R_{s\_above}$  into Eq. (2), calculated by averaging values measured during when the zenith angle was  $>75^\circ$  (Igarashi et al. 2015).

We conducted intensive measurements of PAI and LAI using a combination of a Markov model estimation with fish-eye image data (see Nilson 1971) and litter-fall collection on October 2 and November 28, 1997, and January 14, 1998 (T. Hashimoto, personal observation). PAI and LAI in the mid-foliage season were 3.0 and 1.5, respectively, in the MD + LW, and 5.3 and 4.4, respectively, in the UP. Moreover, LAI in the UP during the full-leaf-fall season was set to zero. Assuming that the net productivity of the study forest was nearly at equilibrium, we can derive the parameters  $b$  and  $k$  for the whole canopy and the UP from the relationships between LAI and  $R_{s\_below}/R_{s\_above}$ . Once those parameters are determined, temporal variation in LAI can be produced by Eq. (2) with continuous

**Table 2** List of photosynthesis model constants

Symbol	Description	Units	Value
$K_{c25}$	Michaelis–Menten constant of Rubisco for $\text{CO}_2$ ( $K_c$ ) at 25 °C	Pa	27.5 <sup>a</sup>
$H_c$	Activation energy of $K_c$	J mol <sup>-1</sup>	80470 <sup>b</sup>
$K_{o25}$	Michaelis–Menten constant of Rubisco for $\text{O}_2$ ( $K_o$ ) at 25 °C	Pa	42000 <sup>a</sup>
$H_o$	Activation energy of $K_o$	J mol <sup>-1</sup>	15410 <sup>b</sup>
$\tau_{25}$	Specificity factor of Rubisco for $\text{CO}_2$ compensation point Estimation ( $\tau$ ) at 25 °C		2321 <sup>a</sup>
$H_\tau$	Activation energy of $\tau$	J mol <sup>-1</sup>	-29000 <sup>a</sup>
$H_{va}$	Activation energy of $V_{\text{cmax}}$ <sup>d</sup>	J mol <sup>-1</sup>	65000 <sup>c</sup>
$H_{vd}$	Deactivation energy of $V_{\text{cmax}}$	J mol <sup>-1</sup>	250000 <sup>c</sup>
$S_v$	Entropy term	J K <sup>-1</sup> mol <sup>-1</sup>	600 <sup>c</sup>

<sup>a</sup> Harley and Baldocchi (1995)

<sup>b</sup> Harley et al. (1992)

<sup>c</sup> Tenhunen et al. (1990)

<sup>d</sup>  $V_{\text{cmax}}$  is the maximum carboxylation rate when ribulose biphosphate is saturated

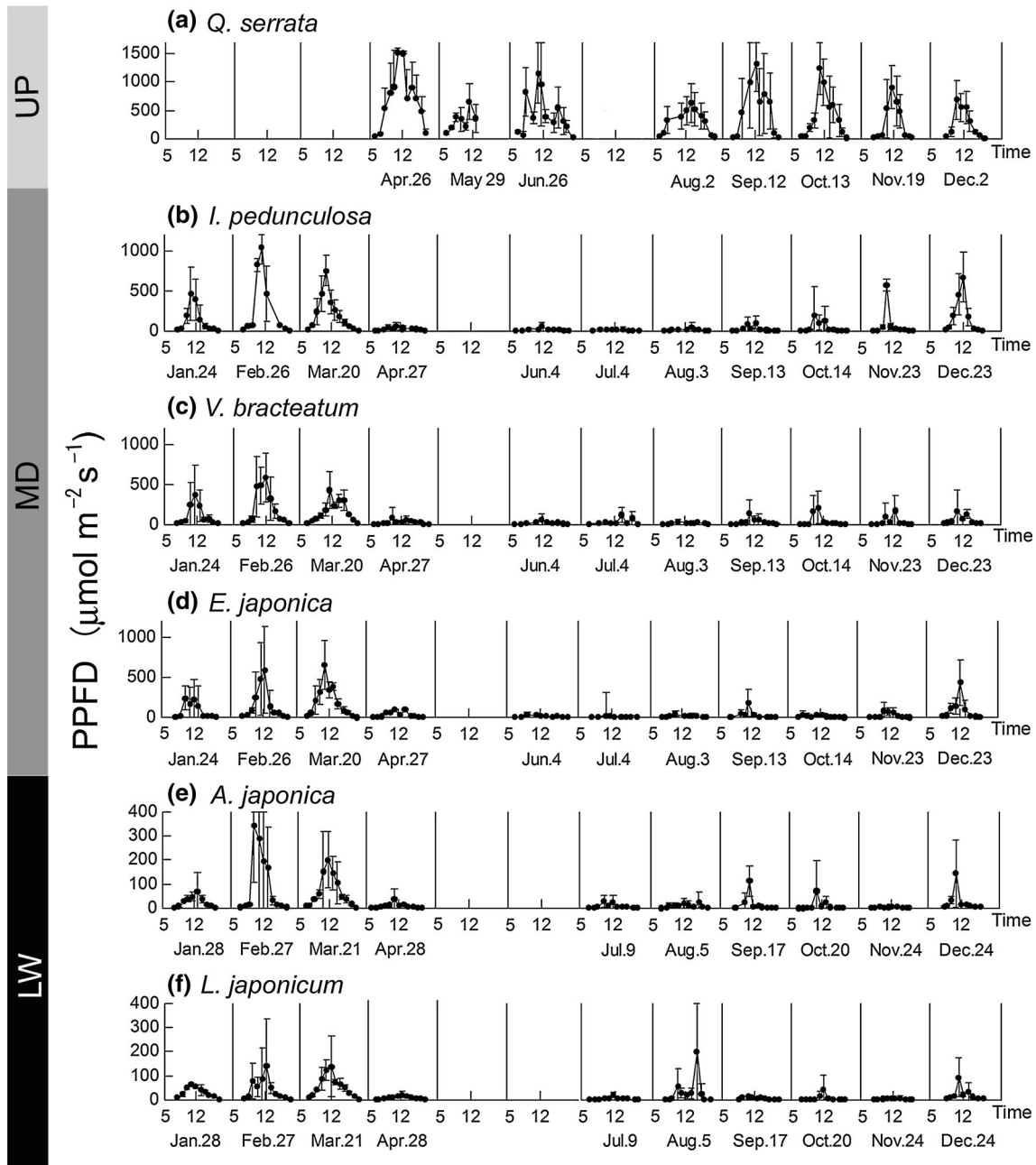
monitoring of  $R_{s\_below}/R_{s\_above}$ , for the UP and the MD + LW, as shown in Fig. 1d.

## Results

### Diurnal pattern of net photosynthesis rate

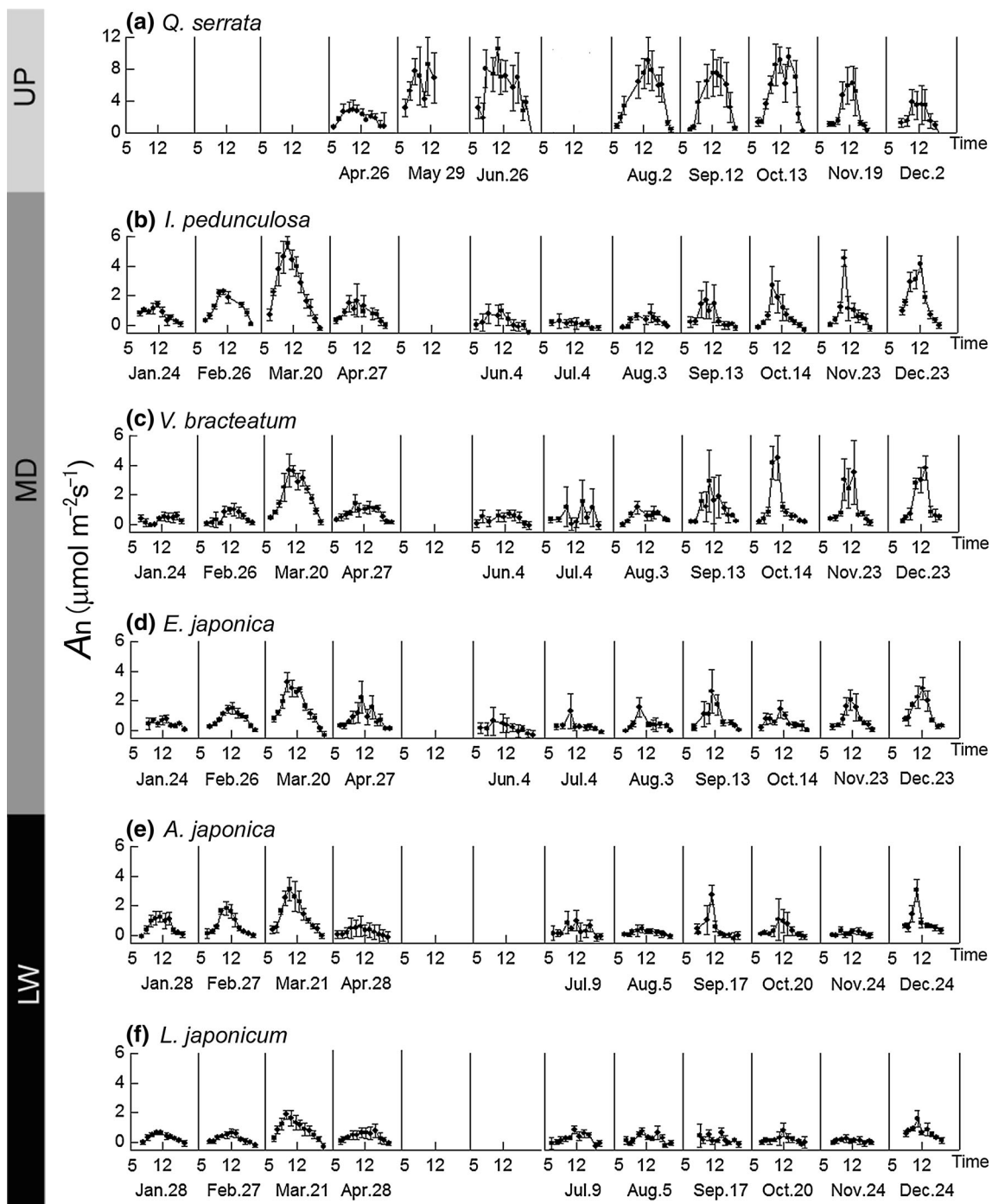
PPFD at the forest floor abruptly decreased with leaf-out in the UP in mid-April, and gradually increased in the leaf-

fall season through December (Fig. 1a, d). Moreover, in the foliate season, PPFD incident to the sample leaves in the UP was much higher than in the MD and LW, and often  $>1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Fig. 2). On the other hand, overall PPFD on the sample leaves in the MD and LW was  $<100$  and  $<50 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. In the leaf-fall season, January to March, PPFD incident to leaves in the MD sometimes reached  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and was generally higher than in the LW (Fig. 2b–f). During the leaf-fall and even mid-foliate seasons, we also observed intense light in



**Fig. 2** Yearly observations of diurnal variation in photosynthetic photon flux density (PPFD) incident to the gas-exchange-measured leaves of, **a** *Q. serrata* in the upper layer (UP), **b** *I. pedunculosa*,

**c** *V. bracteatum*, and **d** *E. japonica* in the middle layer (MD), and **e** *A. japonica* and **f** *L. japonicum* in the lower layer (LW). Vertical bars represent the standard deviation



**Fig. 3** Yearly observations of diurnal variation in the net photosynthesis rate ( $A_n$ ) of, **a** *Q. serrata* in the upper layer (UP), **b** *I. pedunculosa*, **c** *V. bracteatum*, and **d** *E. japonica* in the middle layer (MD),

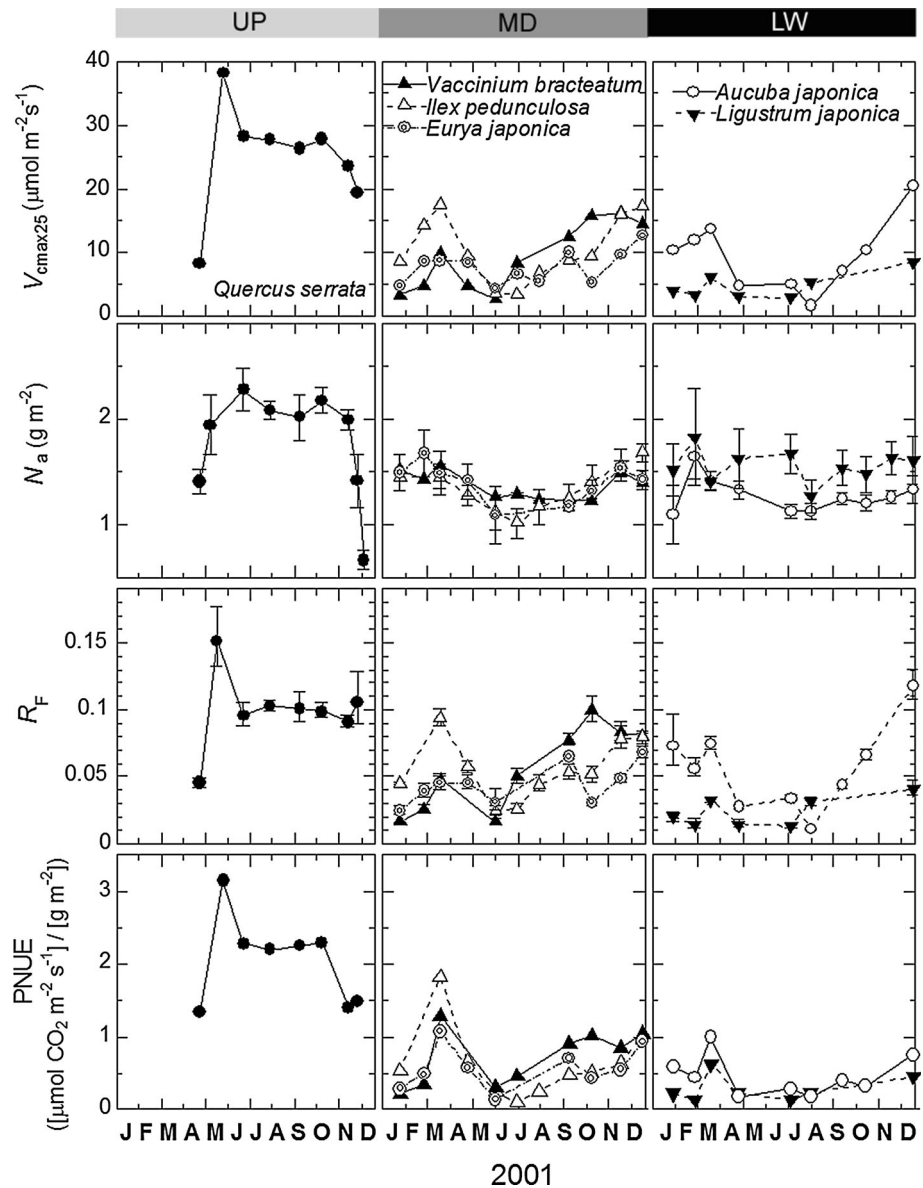
and **e** *A. japonica* and **f** *L. japonicum* in the lower layer (LW). Vertical bars represent the standard deviation

the form of so-called sunflecks on sample leaves in the LW (Fig. 2e, f).

The midday  $A_n$  of *Q. serrata* in the UP was around  $4.0 \mu\text{mol m}^{-2}\text{s}^{-1}$  immediately after leaf-out, and stabilised near  $\sim 10 \mu\text{mol m}^{-2}\text{s}^{-1}$  throughout the foliate season, May–October (Fig. 3a). Although the canopy was

still full-foliate in November, leaf senescence caused a decline in  $A_n$  in November–December in the UP (Fig. 3a). The  $A_n$  of all species in the MD and LW was generally higher in the leaf-fall season, December–March, than in the foliate season, April–November, notably peaking in March, just before *Q. serrata* leaf-out (Fig. 3b–f). During the

**Fig. 4** Seasonal variation in the maximum carboxylation rate when ribulose biphosphate is saturated at 25 °C ( $V_{\text{cmax}25}$ ), nitrogen (N) content per unit leaf area ( $N_a$ ), allocation of N to Rubisco ( $R_F$ ), and photosynthetic nitrogen use efficiency (PNUE) for leaves of *Q. serrata* in the upper layer (UP), *V. bracteatum*, *I. pedunculosa*, and *E. japonica* in the middle layer (MD), and *A. japonica* and *L. japonicum* in the lower layer (LW). Vertical bars represent the standard deviation



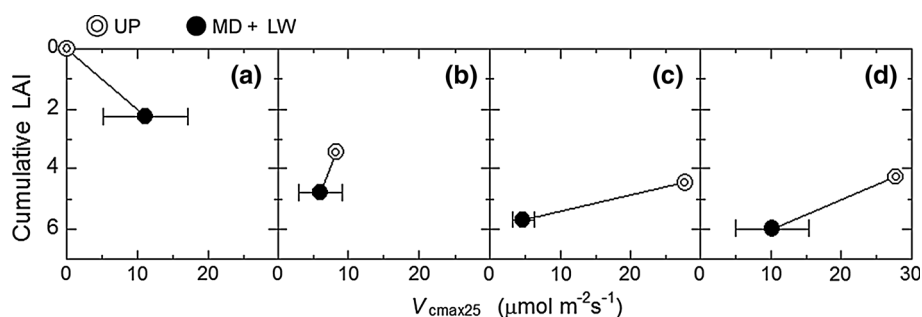
foliate season, while  $A_n$  in the LW was consistently low (Fig. 3e, f), that in the MD started increasing in October, when the UP was fully foliate (Fig. 3b–d).

**Seasonal variation in photosynthetic characteristics**

Figure 4 shows seasonal variation in  $V_{\text{cmax}25}$ ,  $N_a$ ,  $R_F$  (see Eq. 1), and PNUE ( $=A_n/N_a$ ) in the UP, MD, and LW. The  $V_{\text{cmax}25}$  of *Q. serrata* in the UP was low ( $\sim 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) immediately after leaf-out in April, and drastically increased up to  $\sim 40 \mu\text{mol m}^{-2} \text{s}^{-1}$  in May. The values were stable ( $\sim 25\text{--}30 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in the foliate season, June–October, and started decreasing in November. Meanwhile, the  $N_a$  of *Q. serrata* was low immediately after leaf-out in April due to the leaves’

immaturity, and increased and plateaued in May to late October. The  $N_a$  abruptly decreased over November–December. Seasonal variation in the  $R_F$  and PNUE of *Q. serrata* mirrored that of  $V_{\text{cmax}25}$  except that  $R_F$  did not decline after November. Compared to the period of stable  $V_{\text{cmax}25}$  (June–October), periods with lower  $V_{\text{cmax}25}$  showed either lower  $N_a$  (November and December) or lower  $R_F$  (soon after leaf-out, April). During the May annual peak in  $V_{\text{cmax}25}$ ,  $R_F$  also reached its annual peak with peak PNUE.

The  $V_{\text{cmax}25}$  of leaves in the MD and LW peaked just before *Q. serrata*’s leaf-out, i.e., in March (Fig. 4). After leaf-out, the values dropped, and then gradually increased from the early summer onward. A cross-correlation analysis of the  $V_{\text{cmax}25}$  time series showed that the  $V_{\text{cmax}25}$  of the species in the MD and LW lagged 3–5 months behind



**Fig. 5** Seasonal variation in the relationship between cumulative leaf area index (LAI) measured downward from the top of the canopy and maximum carboxylation rate when ribulose biphosphate is saturated at 25 °C ( $V_{cmax25}$ ) averaged over data from each layer (UP and

MD + LW) in, **a** March 20–21, **b** April 26–28, **c** August 2–5, and **d** October 13–20. Horizontal bars represent ranges of the measured values for different species in MD and LW

*Q. serrata* in the UP (cross-correlation coefficient >0.5). There was some seasonal variation in  $N_a$  in the MD and LW but most species showed remarkable seasonality in  $R_F$ , suggesting that the seasonal patterns in  $V_{cmax25}$  and  $N_a$  did not coincide.

Further information on the relationship between seasonal developments in LAI and  $V_{cmax25}$  in each canopy layer is shown in Fig. 5. The  $V_{cmax25}$  of the UP increased with increasing total LAI. On the other hand,  $V_{cmax25}$  of the MD + LW was highest when total LAI was lowest (=the MD + LW's LAI).  $V_{cmax25}$  of the MD + LW was also high when LAI was highest near leaf-fall, implying that trees in the MD + LW are ready for productive activity before leaf-fall in the UP.

## Discussion

As in many previous studies (e.g., Wilson et al. 2000; Kosugi et al. 2003; Grassi et al. 2005; Iio et al. 2008), the  $V_{cmax25}$  of the dominant canopy tree species, i.e., *Q. serrata* in the UP, was highest in the early growing season and declined toward the leaf-fall season. The drastic increase in both  $V_{cmax25}$  and  $N_a$  in the UP during spring leaf development were similar to those observed for the dominant deciduous trees in temperate forests (Reich et al. 1991; Xu and Baldocchi 2003; Grassi et al. 2005; Yasumura et al. 2006). Meanwhile, we observed a poor correlation between  $V_{cmax25}$  and  $N_a$  in the UP immediately after May leaf-out, consistent with Wilson et al. (2000). They proposed that this was because, immediately after leaf-out, leaves had adequate N but were not functionally developed (Miyazawa et al. 1998). Wilson et al. (2000) and Grassi et al. (2005) reported a gradual decline in photosynthetic capacity that was poorly correlated with leaf N content in the mid-growing season and attributed it to summer drought. Notably, the values of  $V_{cmax25}$  and  $N_a$  in UP were relatively constant throughout the mid-growing season;

thus, this might be due to ample summer precipitation at the study site (see Fig. 1). The steeper decrease in  $N_a$  than in  $V_{cmax25}$  during autumnal senescence (Fig. 4), which is also consistent with previous observations (Wilson et al. 2000; Grassi et al. 2005), was probably caused by abrupt N withdrawal without pronounced N withdrawal from Rubisco just before leaf-fall.

Increased  $V_{cmax25}$  and  $N_a$  in most evergreen trees in the MD + LW matched leaf-out timing in the UP and caused a vertical shift in the highest  $V_{cmax25}$  (Fig. 4), and possibly a  $\text{CO}_2$  sink. Increased  $N_a$  enables understory plants to redistribute resources to new shoots in the coming spring (Millard and Proe 1991; Miyazawa et al. 2004). Similar winter  $V_{cmax25}$  and  $N_a$  behaviour in understory vegetation was observed in a study on evergreen herbs (Skillman et al. 1996) and several on broadleaved evergreen trees (Yamamura and Kimura 1992; Miyazawa and Kikuzawa 2005; Muller et al. 2005, 2009) under a deciduous overstory, and is also thought to play a role in photosynthetic acclimation to winter conditions. The photosynthetic acclimation of leaf anatomy under low-temperature and high-PPFD conditions is known to induce higher leaf N and Rubisco contents in evergreen understory shrubs in winter than in summer (Muller et al. 2009). Furthermore, Hikosaka (2005) pointed out that N partitioning in the photosynthetic apparatus can be affected by growth irradiance and temperature. Concerning preparation for the coming spring, for example, Yamamura and Kimura (1992) delineated the seasonal dynamics of nutrients among component organs of *A. japonica* under evergreen conifers; nutrients were accumulated in the leaves in autumn and redistributed to other organs in spring. Miyazawa and Kikuzawa (2006b) and Miyazawa et al. (2007) also found that the leaf N contents of evergreen trees shaded throughout the year were higher in winter than in summer under fixed radiative conditions. Furthermore, Miyazawa et al. (2004) estimated that the ratio of N remobilised from 1-year-old leaves to new leaves of *Quercus glauca* Thunb. was 15–45 %.



Most species in the MD + LW during the winter maintained high  $N_a$ , but their  $V_{\text{cmax}25}$  was low in January and February, resulting in low values of  $R_F$  (see Fig. 4). One likely reason for this is higher N investment to new shoots rather than current leaves' Rubisco. Another explanation is the effects of photoinhibition (e.g., Miyazawa and Kikuzawa 2005, 2006a; Miyazawa et al. 2007), wherein the photosynthetic capacity of evergreen trees in sunny locations decreases in mid-winter. We hypothesised that the low values of  $V_{\text{cmax}25}$  in January and February are due to the depression of chloroplast functions by reactive oxygen species generated under intense light (see Hikosaka et al. 2004). However, there may be interspecific differences in resistance to photoinhibition, such as thermal dissipation capacity (Adams et al. 2004) and the capacity to repair a damaged photosynthetic apparatus (Öquist and Huner 2003).

Generally,  $N_a$  is highest in sunlit leaves near the top of the canopy, and declines toward lower canopy positions (Lambers et al. 2008). Carswell et al. (2000) showed that  $N_a$  in a multi-species canopy in a tropical rain forest could be described as a linear function of height. In addition, a clear relationship between  $N_a$  and  $V_{\text{cmax}25}$  leads to a decreasing trend in  $V_{\text{cmax}25}$  from the top of the canopy (Kumagai et al. 2006). As has been pointed out previously (e.g., Sellers et al. 1992; Kull and Jarvis 1995; de Pury and Farquhar 1997), decreases in  $N_a$  and  $V_{\text{cmax}25}$  with decreasing canopy height might represent the optimal distribution of limited resources throughout the canopy, avoiding wastage of N resources and maximising photosynthetic carbon gain. In contrast, in herbaceous stands composed of individuals of different heights, the vertical profile of  $N_a$  (as a function of incident solar radiation) differs among individuals and depends on leaf position and PPFD under the same solar radiation levels (Anten et al. 1998). At our study site, in forest layered with species of different phenological and ecophysiological traits, we found a “jump” in the vertical profile of  $V_{\text{cmax}25}$  (see Fig. 5). Late winter or early spring before leaf-out in the UP is the critical period for  $\text{CO}_2$  assimilation of most species in the MD + LW, while summer and autumn are major assimilation periods for the UP. The increase in photosynthetic capacity in the MD + LW during winter might compensate for the lack of carbon uptake during the leafless season in the UP. Therefore, it is likely that this productivity represents an optimal use of resources for the whole ecosystem throughout the year.

Our results underscore the importance of considering the time lag in major assimilation periods between dominant deciduous canopy and evergreen understory trees when studying the annual net ecosystem exchange (NEE), as the main  $\text{CO}_2$  uptake source changes vertically throughout the seasons. In reality, the contribution of the annual

understory gross primary production (GPP) to the total ecosystem GPP, which was roughly estimated by multiplying the observed seasonal values of LAI and leaf  $A_n$  for each of the canopy and understory trees, was  $\sim 10\%$ . It should be noted that the canopy GPP was computed using the sunlit leaf  $A_n$ , a somewhat maximal leaf assimilation rate, and the NEE estimation needs to be taken into account for the effect of ecosystem respiration during the canopy leafless season. The understory productivity, ca.  $2 \text{ tC ha}^{-1} \text{ year}^{-1}$  as its GPP, is comparable with the reported NEE of global temperate forests (see Malhi et al. 1999) and should occupy a large portion of the NEE. Thus, failing to consider active understory photosynthesis during the leafless season in the overstory will lead to underestimating the annual NEE in temperate deciduous-evergreen forests.

Wilson and Baldocchi (2001) and Ito et al. (2006) demonstrated the effects of seasonal fluctuations in  $V_{\text{cmax}}$  and the structural properties of overstory deciduous trees on estimates of annual NEE using simulation models. We must consider the difference in the timing of peak leaf  $V_{\text{cmax}}$  and  $N_a$  between the overstory and understory for SVAT modelling, which is the most promising tool for NEE projection. Given the implications of climate change, we attempted to estimate the change in contribution of the understory to the total GPP under conditions of global warming. We assumed that global warming induced the earlier leaf-out and later leaf-fall, causing a 1-month extension of the growing period for canopy deciduous trees. This rough simulation resulted in an approximately 1% reduction in the understory contribution to the total GPP. Furthermore, photosynthetic enhancement caused by increasing temperatures tends to occur under low-temperature conditions ( $<15\text{ }^\circ\text{C}$ ) (see Hikosaka et al. 2006), and winter understory photosynthesis might be more critical in the estimation of NEE under conditions of global warming. Our results entail a straightforward treatment of the canopy-scale assimilation characteristics of temperate forests and are relevant to current status estimates and future projections of forest carbon balance using SVAT models.

**Author contribution statement** S. H. performed the analyses and wrote an initial draft of the paper. T. K. conceived and designed the study, interpreted the results, and contributed edits throughout. K. K. and N. K. made substantial contributions to data acquisition. T. H. contributed to data acquisition and manuscript editing. Y. M. made contributions to data interpretation and manuscript editing.

**Acknowledgments** This work was supported in part by Grants-in-Aid for Scientific Research (Nos. 11213209, 11480130, and 25281005) from the Ministry of Education, Science and Culture, Japan.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflicts of interest.

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