

Seasonality of photosynthetic parameters in a multi-specific and vertically complex forest ecosystem in the Sierra Nevada of California

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Summary Understanding seasonal variations of photosynthetic parameters is critical for accurate modeling of carbon dioxide (CO₂) uptake by ecosystems. Maximum carboxylation velocity (V_{cmax}), maximum rate of electron transport (J_{max}), leaf respiration in the light (R_{day}), light-saturated assimilation (A_{max}) and maximum quantum yield (ϕ) were calculated from leaf gas exchange measurements made monthly throughout the year on leaves of three co-occurring evergreen species in a *Pinus ponderosa* Dougl. ex P. Laws. & C. Laws. forest with shrubs in the understory (*Arctostaphylos manzanita* Parry and *Ceanothus cordulatus* Kellogg.). The seasonality and relationships of the photosynthetic parameters with environmental and physiological variables differed among the species. The nitrogen-fixing species, *C. cordulatus* had the highest values of the parameters and the largest seasonal variation, whereas *A. manzanita* exhibited the lowest seasonality and weaker correlations with environmental variables. In general, variations in V_{cmax} were highly correlated with light, leaf mass per area and leaf nitrogen content on an area basis. Temporal scaling of the parameters with each other seemed possible for *C. cordulatus* and *P. ponderosa*. However, lags between these variables and V_{cmax} likely reflect the influences of other factors. The acclimation relationships found along vertical light gradients within canopies in other studies cannot be applied to seasonal variations. The J_{max} to V_{cmax} ratio varied seasonally for *P. ponderosa* and *A. manzanita*, being lower at high light, high air temperature and low soil water content.

Keywords: *A/C_i curve, acclimation, drought, light curve, mixed forest, net ecosystem exchange.*

Introduction

Understanding the factors and processes influencing seasonal variation in photosynthetic parameters is critical for accurate modeling of carbon dioxide (CO₂) exchange between vegetation and the atmosphere (Dang et al. 1998, Wilson et al. 2001, Tanaka et al. 2002, Kosugi et al. 2003). The biochemical photosynthesis model of Farquhar et al. (1980) is the most com-

monly used for simulating assimilation at different time and spatial scales. Much information is available on the variability of the key parameters of this model (maximum carboxylation velocity (V_{cmax}) and maximum rate of electron transport (J_{max})) in relation to species (e.g. Wullschlegel 1993), leaf nitrogen (e.g., Schulze et al. 1994) and vertical light gradients in the canopy (e.g., Niinemets et al. 2001, Meir et al. 2002, Niinemets et al. 2004a). Although seasonal variations in light-saturated assimilation (A_{max}) are well established (e.g., Hanson et al. 1994, Murthy et al. 1997, Sims et al. 1998, Tezara et al. 1998), studies have only recently focused on the seasonality of the biochemical parameters required by the Farquhar et al. (1980) model (Dang et al. 1998, Ellsworth 2000, Wilson et al. 2000, Nogues and Alegre 2002, Xu and Baldocchi 2003, Grassi et al. 2005). Seasonal variations in photosynthetic parameters such as V_{cmax} and J_{max} are expected to be species-specific and controlled by factors such as light, temperature, soil water content, leaf development and nitrogen content (Dang et al. 1998, Wilson et al. 2000, Nogues and Alegre 2002, Xu and Baldocchi 2003, Grassi et al. 2005). However, there is little environmental and physiological information to tease apart the different influencing factors on seasonal timescales.

Because most forests are multi-specific and vertically heterogeneous, understanding the functioning of such complex ecosystems remains a challenge for quantifying and modeling the fluxes of CO₂ between the atmosphere and the vegetation (Kellomaki and Vaisanen 1997, Porte and Bartelink 2002). Forest modeling has focused on monospecific stands for a long time, and only recently have models been developed for mixed forests (Porte and Bartelink 2002). However, there is a lack of physiological data to parameterize these models. Photosynthetic parameters for different co-occurring species are suspected to differ seasonally because of variations in leaf phenology (evergreen versus deciduous), light or temperature acclimation, or both, sources and sinks for nitrogen and plant water relationships. Evergreen species are suspected to show less seasonality in photosynthetic parameters than deciduous species (Damesin et al. 1998, Warren and Adams 2004). Differences among species with a similar leaf phenological strat-

egy might also be important, especially considering that certain species are capable of fixing nitrogen. However, we are aware of only a limited amount of published data on seasonal variation in photosynthetic parameters for different species growing at the same location, but having different physiological strategies or occupying different strata (Dang et al. 1998, Wilson et al. 2000, Nogues and Alegre 2002, Grassi et al. 2005).

The general aim of our study was to obtain information on the factors influencing seasonal variation in photosynthetic parameters of co-occurring evergreen species in a vertically complex ecosystem. Our specific objectives were to: (1) assess the extent of the differences in V_{cmax} and J_{max} and their seasonal variation in species growing in different strata in the ecosystem; and (2) determine the environmental and physiological factors controlling the seasonal variation in photosynthetic parameters. In addition, we explored how differences in photosynthetic parameters among the species investigated affect the carbon balance of the ecosystem. Our study site was a young forest plantation with *Pinus ponderosa* Dougl. ex P. Laws. & C. Laws. in the overstory and shrubs in the understory (*Arctostaphylos manzanita* Parry and *Ceanothus cordulatus* Kellogg.), including one nitrogen-fixing species (*Ceanothus cordulatus*). This ecosystem is located in the Sierra Nevada of California and is characterized by complex seasonal weather patterns, with snow and low temperatures during the winter, an abrupt spring and dry warm summers.

Materials and methods

Site description

The Blodgett Forest site and ongoing measurements have been extensively described (Goldstein et al. 2000, Misson et al. 2005b). The study site is part of the Ameriflux network. It is located at 1315 m above sea level in the Sierra Nevada Mountains of California, on land owned and managed by Sierra Pacific Industries (38°53'42.9" N, 120°37'57.9" W). Trees were planted in 1990 at a density of ~1275 ha⁻¹. The plantation is dominated by *Pinus ponderosa* with occasional other tree species. The major understory shrubs are *Arctostaphylos manzanita* and the nitrogen-fixing species *Ceanothus cordulatus*.

Common management practices for commercial plantations in the Sierra Nevada were carried out. Shrubs were cut during spring 1999 and the plantation was thinned in May 2000 (Misson et al. 2005b, Tang et al. 2005). In spring 2003, tree density was ~510 ha⁻¹; the stand was open with large gaps between tree crowns where shrubs were developing fast. The leaf area index of the trees was estimated by an allometric relationship developed for the site (Xu et al. 2001). The leaf area index of the shrub layer was estimated destructively. In spring 2003, total one-sided leaf area index (LAI) was 2.49, comprising 72% *P. ponderosa* in the overstory and 28% understory shrubs (*A. manzanita* 22% and *C. cordulatus* 6%). Mean tree diameter at breast height was 12.0 cm, mean tree height was 4.7 m (mean shrubs height ~ 1.0 m) and basal area was 9.6 m² ha⁻¹.

The site is characterized by a Mediterranean climate, with

warm dry summers and cold wet winters. Since 1998, annual precipitation has averaged 1290 mm, with the majority of precipitation falling between September and May, with almost no rain in summer. Mean daily temperature ranges from 14 to 27 °C during summer and from 0 to 9 °C during winter. The soil comprises 60% sand, 29% loam and 11% clay with a pH of 5.5 ± 0.3.

Another study on phenology of growth in 2003 showed that *P. ponderosa* stems started to expand at the end of April (Day 120), whereas shoots and fine roots started to grow in mid-May (Day 134) (Misson et al. 2006). New *P. ponderosa* needles initiated elongation at the end of May (Day 148), coincident with the development of new leaves on the shrubs. For each species, both above and belowground components had attained 90% of their growth by the end of July (Day 210) (Misson et al. 2006).

Gas exchange measurements

Gas exchange measurements were made once a month for the 11 months from January 2003 to November 2003, on 4–6 individuals of three species (*P. ponderosa*, *C. cordulatus* and *A. manzanita*). For all species, we selected leaves experiencing a similar light environment. One-year-old sunlit leaves (leaves that flushed during the 2002 growing season) were measured on a south-facing branch at ~1.8 m high for *P. ponderosa* and ~0.75 m high for *A. manzanita* and *C. cordulatus*. Because of the openness of the canopy, the light environment around the selected leaves was similar to that at the top of the canopy, as confirmed by photosynthetically active radiation measurements at two canopy levels (data not shown).

All gas exchange measurements were made with a portable steady-state photosynthetic system (LI-6400, LI-COR, Lincoln, NE). The system was calibrated at the beginning and end of the season. Zeros for both CO₂ and water vapor were calibrated with nitrogen. The span for water vapor was calibrated with a LI-COR dew point generator (LI-COR LI-610). The span for CO₂ was calibrated against calibration gases. The zero and span shifts for both CO₂ and water vapor were negligible during the study.

Two sets of photosynthesis (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) measurements were conducted: the responses of leaf photosynthesis to intercellular CO₂ concentration (C_i , $\mu\text{mol mol}^{-1}$) and to photosynthetically active radiation (Q , $\mu\text{mol m}^{-2} \text{s}^{-1}$). Before making measurements, leaves were acclimated in the chamber for 5 to 10 min at 25 °C temperature, 25% relative humidity, ambient CO₂ concentration (375 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$) and Q of 1800 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. The CO₂ concentrations used to generate the A/C_i curves were 375, 275, 175, 75, 40, 375, 750, 1200 and 1500 $\mu\text{mol mol}^{-1}$. The Q values used to generate the A/Q curves were 1800, 1200, 800, 500, 200, 100, 50 and 10 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. For both responses, the leaves were allowed to equilibrate at each step until the total coefficient of stability for the ongoing measurement (which included the variation for CO₂ differential, water vapor differential and flow rate) decreased below 0.7%. Equilibration times ranged from a minimum of 4 min to a maximum of 8 min before data were logged.

During gas exchange measurements, we tried to maintain

leaf temperature close to 25 °C. Any response curves measured with leaf temperature deviating markedly from 25 °C were eliminated. The mean leaf temperature of all the response curves presented is 25.5 °C, with a range from 23.7 to 27.2 °C. No temperature corrections were applied to the photosynthetic parameters because the variations in leaf temperature were small. This allowed us to avoid any bias in the data caused by inappropriate corrections. Relative humidity and vapor pressure deficit (VPD) inside the chamber were typically 20 to 30% and 1.0 to 3.5 kPa, respectively, depending on transpiration rate. A full A/C_i or A/Q response curve usually took about 1.5 h to complete.

Leaf characteristics

On each day of gas exchange measurement, 1-year-old leaves (leaves that flushed during the 2002 growing season) of the three species were sampled for analysis of leaf mass per area (LMA, g m^{-2}) and carbon and nitrogen concentrations. Leaf area (at least 200 cm^2 total) was measured with an optical area meter (LI-COR LI-3100). Leaves were oven dried at 65 °C for 3 days before measuring dry mass. Leaf nitrogen and carbon were determined by the Carlo Erba Combustion Method (DANR Analytical Lab, University of California, Davis). Foliar amounts of nitrogen and carbon were expressed on a mass basis (N_M and C_M , g g^{-1}) and an area basis (N_A and C_M , g m^{-2}). We calculated N_A as $N_A = N_M \text{LMA}$.

Photosynthetic parameters

The parameters V_{cmax} , J_{max} and R_{day} (leaf respiration in the light) were estimated from the A/C_i curves by the Generalized Reduced Gradient (GRG2) nonlinear optimization procedure (Lasdon et al. 1978) incorporated in the Microsoft Excel Solver tool. Because A is limited by the maximum rate of carboxylation at low C_i (W_c) (Farquhar et al. 1980), V_{cmax} and R_{day} were estimated from the lower region of the A/C_i curves according to Wullschlegel (1993):

$$W_c = V_{\text{cmax}} \left(\frac{C_i - \Gamma^*}{C_i + K_m} \right) - R_{\text{day}} \quad (1)$$

where K_m is the effective Michaelis-Menten constant for the carboxylation reaction and Γ^* is the CO_2 compensation point in the absence of dark respiration calculated according to Bernacchi et al. (2003). At higher C_i , A is limited by the regeneration of RuBP via electron transport (W_j). We estimated J_{max} from the upper region of the A/C_i curves according to Wullschlegel (1993):

$$W_j = \frac{J}{4} \left(\frac{C_i - \Gamma^*}{C_i + 2\Gamma^*} \right) - R_{\text{day}} \quad (2)$$

where J is potential rate of electron transport and is dependent on Q and J_{max} (dePury and Farquhar 1997). The C_i threshold below which V_{cmax} is fit and above which J_{max} is fit was determined by maximizing the overall curve fit by the GRG2 procedure. The A/C_i curves were also analyzed by another method

where the C_i threshold is fixed by the user and where V_{cmax} and J_{max} are fitted separately (Ellsworth et al. 2004). The methods compared well ($r^2 = 0.85$ for V_{cmax} ; $r^2 = 0.88$ for J_{max} ; and $r^2 = 0.85$ for R_{day}).

Light-saturated assimilation (A_{max}) was determined as the assimilation rate at a 25 °C leaf temperature, a 25% relative humidity, ambient CO_2 concentration (375 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air) and a Q of 1800 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. The maximum quantum yield of photosynthesis (ϕ , mol CO_2 fixed per mol photons absorbed) was evaluated by linear regression of the light-limited portion of the A/Q curve ($Q < 200 \mu\text{mol m}^{-2} \text{ s}^{-1}$).

Eddy covariance measurements

Measurements of above-canopy eddy-covariance fluxes have been described extensively (Goldstein et al. 2000, Misson et al. 2005a, 2005b). In spring 2003, a below-canopy eddy-covariance system was deployed. Wind velocity and virtual temperature fluctuations were measured at 10 Hz with a three-dimensional sonic anemometer (ATI Electronics Inc., Boulder, CO) mounted on a horizontal beam at 1.2 m above the ground, at the transition between the lowest branches of *P. ponderosa* and the highest branches of the shrubs. The CO_2 and H_2O mixing ratios were measured with a closed-path infrared gas analyzer (LI-COR LI-6262) through a 5-m tube of 0.63 mm diameter, with a flow of 10 l min^{-1} .

Fluxes of CO_2 , H_2O and sensible heat were determined by the eddy covariance method (Baldocchi et al. 1988). Positive fluxes indicate mass and energy transfer from the surface to the atmosphere. The sonic anemometer was rotated to force the mean vertical wind speed to zero and to align the horizontal wind speed onto a single horizontal axis. The time lag for sampling and instrument response was determined by maximizing the covariance between vertical wind velocity (w') and scalar (c') fluctuation. Errors due to sensor separation and damping of high frequency eddies were corrected by spectral analysis techniques (Rissmann and Tetzlaff 1994). Below-canopy fluxes measurements were unreliable during the night.

Air temperature and humidity, photosynthetically active radiation and rain were recorded at the top of the canopy on a 10.5-m tower (see Goldstein et al. (2000) for a detailed description of the instruments). In addition, we measured soil temperature and soil water content at several depths. Missing data were estimated as described by Misson et al. (2005b).

Results

Weather

Weather changes during seasonal transitions were abrupt (Figure 1). After mid-May (Day 130) there was almost no rain, Q and air temperature started to increase sharply, while soil water content and relative humidity decreased (Figure 1a–d). The end of October (Day 304) marked the return of regular precipitation, a strong decrease in air temperature and Q , and abrupt increases in relative humidity and soil water content. A first summer rain occurred at the end of July (~Day 212, 13.2 mm; Figure 1d), and we observed several other summer

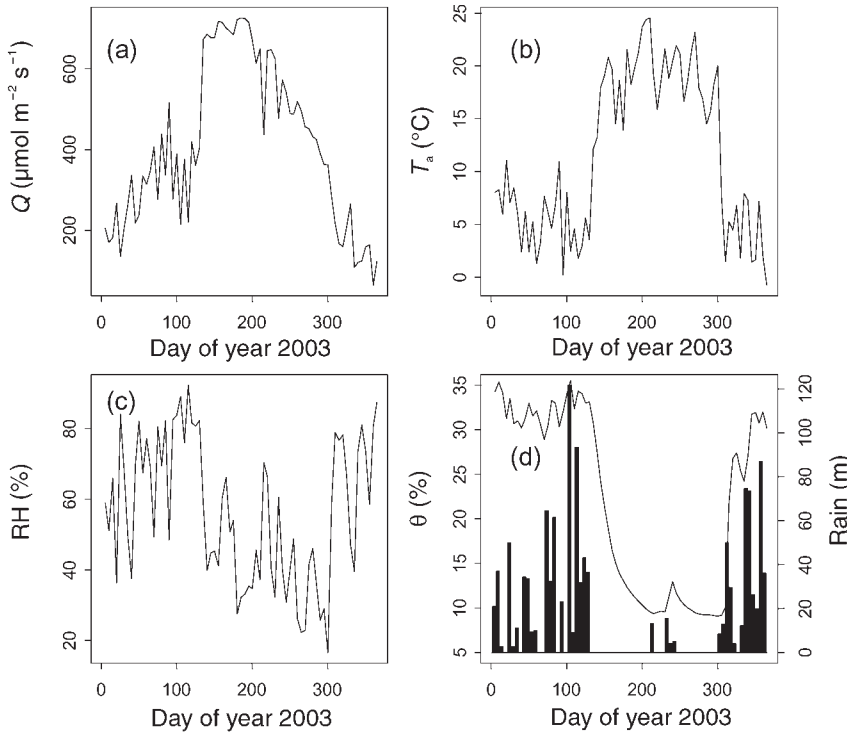


Figure 1. Five-day daily means of meteorological variables plotted as a function of the last day of the averaging period. (a) Photosynthetically active radiation (Q); (b) air temperature (T_a); (c) relative humidity (RH); and (d-left axis) soil water (θ) and (d-right axis) rain (histogram). Seasonal variations of these variables are presented with maximum carboxylation velocity in Figure 3.

rainstorms during the second part of August (~Day 233, 24.6 mm). The total annual precipitation was 1283 mm, with only 3% during the summer (May–October, 37.6 mm). Consequently, volumetric soil water content decreased to 9% at the end of July before the first summer rain and at the end of October before the return of regular precipitation (Figure 1d).

Variations in V_{cmax} and J_{max}

Seasonal variations in V_{cmax} and J_{max} are shown in Figure 2. Of the three species, the nitrogen-fixing species, *C. cordulatus*, exhibited the highest values of both V_{cmax} and J_{max} . Mean annual V_{cmax} was higher for *C. cordulatus* ($96.0 \mu\text{mol m}^{-2} \text{s}^{-1}$) than for *A. manzanita* ($78.4 \mu\text{mol m}^{-2} \text{s}^{-1}$) and *P. ponderosa* ($52.4 \mu\text{mol m}^{-2} \text{s}^{-1}$). Seasonal variability in V_{cmax} , as represented by the coefficient of variation, was lower for *A. man-*

zanita (24%) than for *C. cordulatus* (31%) and *P. ponderosa* (32%). Mean annual J_{max} was higher for *C. cordulatus* ($168.4 \mu\text{mol m}^{-2} \text{s}^{-1}$) and *A. manzanita* ($124.2 \mu\text{mol m}^{-2} \text{s}^{-1}$) than for *P. ponderosa* ($85.9 \mu\text{mol m}^{-2} \text{s}^{-1}$). Similar to V_{cmax} , the coefficient of variation of J_{max} was higher for *C. cordulatus* (32%) and *P. ponderosa* (31%) than for *A. manzanita* (18%). Seasonality in photosynthetic parameters was more pronounced for *P. ponderosa* and *C. cordulatus* than for *A. manzanita*.

For *P. ponderosa*, V_{cmax} was maximal in July (Day 210) and minimal in September (Day 266) (Figure 2). Maximum and minimum V_{cmax} for *A. manzanita* occurred one month later than for *P. ponderosa*. For *C. cordulatus*, maximum V_{cmax} was two months later than for *P. ponderosa* and minimum V_{cmax} was in March. Maximum and minimum J_{max} values tended to follow the same pattern as V_{cmax} in *P. ponderosa* and *C. cor-*

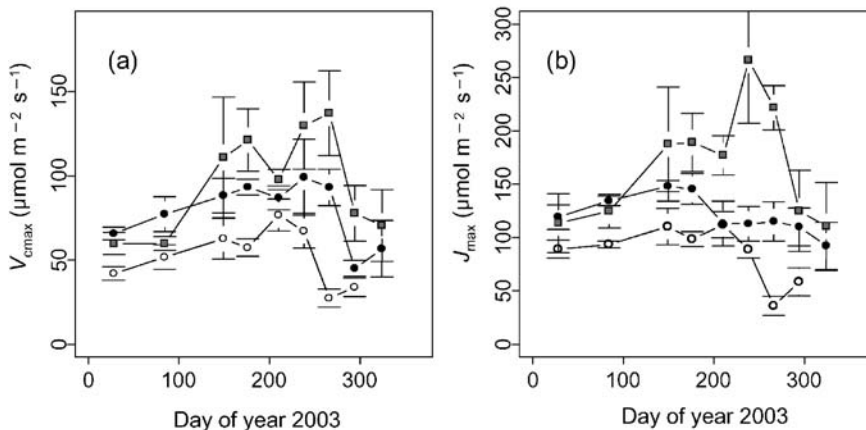


Figure 2. Seasonal trends of maximum carboxylation velocity (V_{cmax}) and maximum rate of electron transport (J_{max}) for *Pinus ponderosa* (○), *Arctostaphylos manzanita* (●) and *Ceanothus cordulatus* (■). Standard deviations are shown.

dulatus, but occurred at different periods for *A. manzanita*, with maximum J_{\max} in May (Day 149), three months earlier than for V_{\max} . The photosynthetic parameters of the three species did not respond at the same rate or to the same environmental or physiological factors.

Environmental and physiological factors

Figure 3 and Table 1 show the relationships between V_{\max} and several environmental and physiological variables. Environmental variables were averaged over the 5 previous days (daily means) to represent growth conditions (no major changes were found if 10-day means were used). For *P. ponderosa* and *C. cordulatus*, V_{\max} correlated significantly with LMA (Table 1); however, maximum V_{\max} lagged behind maximum

LMA by one month for *P. ponderosa* and by 4 months for *C. cordulatus* (Figure 3). The seasonality of LMA exhibited a large increase in the spring and lower values during the winter in all species (Figure 3). For *P. ponderosa* and *C. cordulatus*, V_{\max} correlated significantly with N_A (Table 1). For *P. ponderosa* this correlation was not independent of LMA, because N_M did not correlate with V_{\max} and $N_A = N_M \text{LMA}$. For *A. manzanita*, V_{\max} did not correlate significantly with LMA, N_M or N_A (Table 1).

Significant relationships between V_{\max} and Q were found for all species (Table 1). For *P. ponderosa* and *A. manzanita*, this relationship was the strongest of all the environmental variables tested. However, maximum V_{\max} lagged behind maximum Q by 1, 2 and 3 months for *P. ponderosa*, *A. man-*

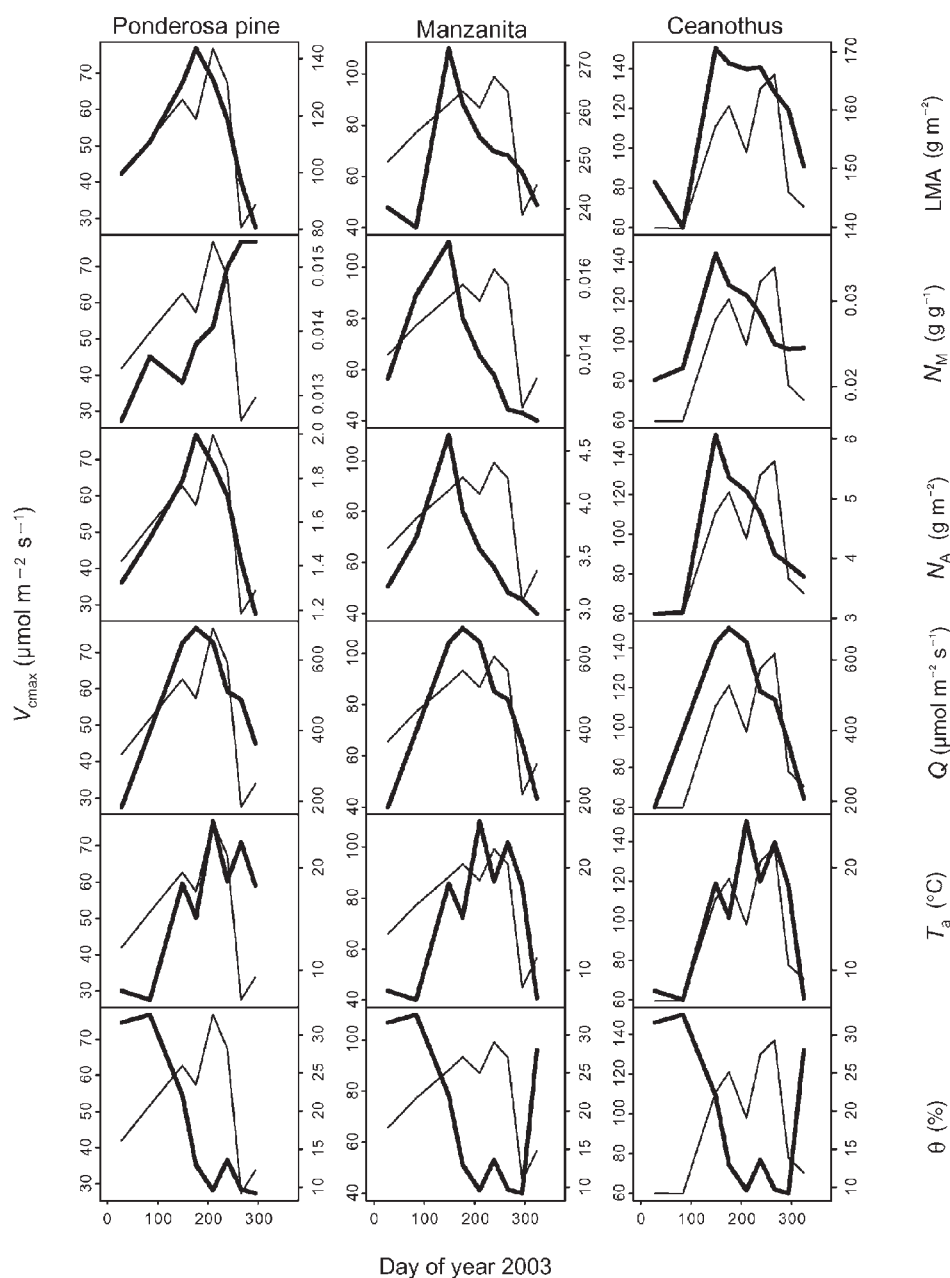


Figure 3. Seasonal variations of maximum carboxylation velocity (V_{\max}) (thin lines) and several environmental (5 days daily averages) and physiological variable (thick lines). Environmental variable were plotted as a function of the last day of the averaging period. See Table 1 for correlation coefficients. See Figure 1 for complete yearly variation of environmental variables. Abbreviations: θ = soil moisture; T_a = air temperature; Q = photosynthetically active radiation; N_A = area-based nitrogen; N_M = mass-based nitrogen; and LMA = leaf mass per area.

Table 1. Correlation coefficients between maximum carboxylation velocity (V_{cmax}), maximum rate of electron transport (J_{max}) and different physiological and environmental variables (See Figure 3). Significant correlations at $P < 0.05$ are in bold. Environmental variables were 5 day daily means (as in Figures 1 and 3). Abbreviations: LMA = leaf mass per area; N_{M} = leaf nitrogen content on mass basis; N_{A} = leaf nitrogen content on leaf area basis; C_{M} = leaf carbon content on mass basis; C_{A} = leaf carbon content on leaf area basis; C/N = leaf carbon to nitrogen content ratio; Q = photosynthetically active radiation; T_{a} and T_{s} = air and soil temperature; RH = relative humidity; VPD = vapor pressure deficit; and θ = soil water content.

Plant	Parameter	LMA	N_{M}	N_{A}	C_{M}	C_{A}	C/N
Pine	V_{cmax}	0.81	-0.31	0.81	-0.17	0.82	0.32
	J_{max}	0.77	-0.70	0.69	-0.55	0.76	0.70
Manzanita	V_{cmax}	0.53	0.45	0.51	-0.09	0.51	-0.45
	J_{max}	0.58	0.89	0.88	-0.81	0.31	-0.92
Ceanothus	V_{cmax}	0.83	0.62	0.68	-0.34	0.80	-0.67
	J_{max}	0.73	0.54	0.60	-0.31	0.71	-0.59

Plant		Q	T_{a}	T_{s}	RH	VPD	θ
Pine	V_{cmax}	0.61	0.21	0.54	0.24	-0.02	-0.03
	J_{max}	0.40	-0.17	0.16	0.51	-0.37	0.34
Manzanita	V_{cmax}	0.74	0.47	0.68	-0.16	0.33	-0.30
	J_{max}	0.61	0.04	0.24	0.02	-0.06	0.08
Ceanothus	V_{cmax}	0.71	0.74	0.80	-0.63	0.66	-0.71
	J_{max}	0.66	0.68	0.77	-0.43	0.54	-0.60

zanita and *C. cordulatus*, respectively (Figure 3). For *A. manzanita*, V_{cmax} was also related significantly with soil temperature. For *C. cordulatus*, V_{cmax} related significantly with all environmental variables tested, and showed the highest correlations with soil and air temperatures (Table 1). For all species, V_{cmax} showed a small decrease in June (*P. ponderosa*) and July (*A. manzanita*, *C. cordulatus*) when soil water content decreased just below 13 and 10%, respectively (Figure 3). For *A. manzanita* and *C. cordulatus*, the summer rain in August was correlated with an increase in soil water content, a decrease in VPD and an increase in V_{cmax} (Figure 3). A similar change in V_{cmax} was not observed for *P. ponderosa*.

Relationships between parameters

To test if the temporal variations in photosynthetic parameters scaled with each other, we examined the relationships between V_{cmax} and J_{max} , A_{max} , R_{day} and ϕ (Figure 4; Table 2). For *C. cordulatus*, J_{max} , A_{max} , R_{day} and ϕ scaled fairly well with V_{cmax} (Figure 4; Table 2). The linear relationships were all significant with r^2 values between 0.45 (R_{day}) and 0.71 (J_{max} ; Table 2). For *P. ponderosa*, only R_{day} did not scale significantly with V_{cmax} (Table 2). Compared with the other species, the relationships were weaker for *A. manzanita*, and only significant for J_{max} ($r^2 = 0.33$) and A_{max} ($r^2 = 0.38$) (Figure 4; Table 2).

The $J_{\text{max}}/V_{\text{cmax}}$ ratio was compared seasonally to Q , air temperature (T_{a}) and soil water content (θ ; Figure 5). For *P. ponderosa* and *A. manzanita*, the ratio decreased significantly with all the parameters tested (Figure 5). For *C. cordulatus* the ratio did not show any significant relationship with any parameter tested (Figure 5).

Ecosystem carbon uptake

Arctostaphylos manzanita and *C. cordulatus* contributed only

22 and 6% to total LAI, respectively, compared with 72% for *P. ponderosa*. To take into account the distribution of biochemical photosynthetic capacity among the species in their contribution to the potential rate of carboxylation of the canopy, we weighted LAI with mean annual V_{cmax} :

$$\frac{\text{LAI}_i V_{\text{cmax},i}}{\sum_{i=1}^3 \text{LAI}_i V_{\text{cmax},i}} \quad (3)$$

where i is the species. When weighting LAI of the three species by their V_{cmax} , the contributions of the shrubs *A. manzanita* and *C. cordulatus* increased to 30.6 and 10.2%, respectively, and decreased to 59.2% for *P. ponderosa*. In addition, net CO_2 fluxes were measured by the eddy-covariance method at the top of the canopy (10.5 m) and inside the canopy at the transition between the lowest branches of *P. ponderosa* and the highest branches of the shrubs (1.2 m; Figure 6). Both top- and inside-canopy daytime (0800–1700 h) flux integrals of CO_2 showed significant uptake of carbon from the atmosphere (Figure 6). The daily inside-canopy net CO_2 uptake was near zero in spring and up to 40% of the total canopy uptake in mid-summer (Figure 6).

Discussion

Comparison of V_{cmax} and J_{max} values

Given the standard deviations for the photosynthetic parameters we measured, our values of V_{cmax} and J_{max} for *P. ponderosa* are in the range of recently published values for this species at different sites of the Sierra Nevada in California (Panek 2004).

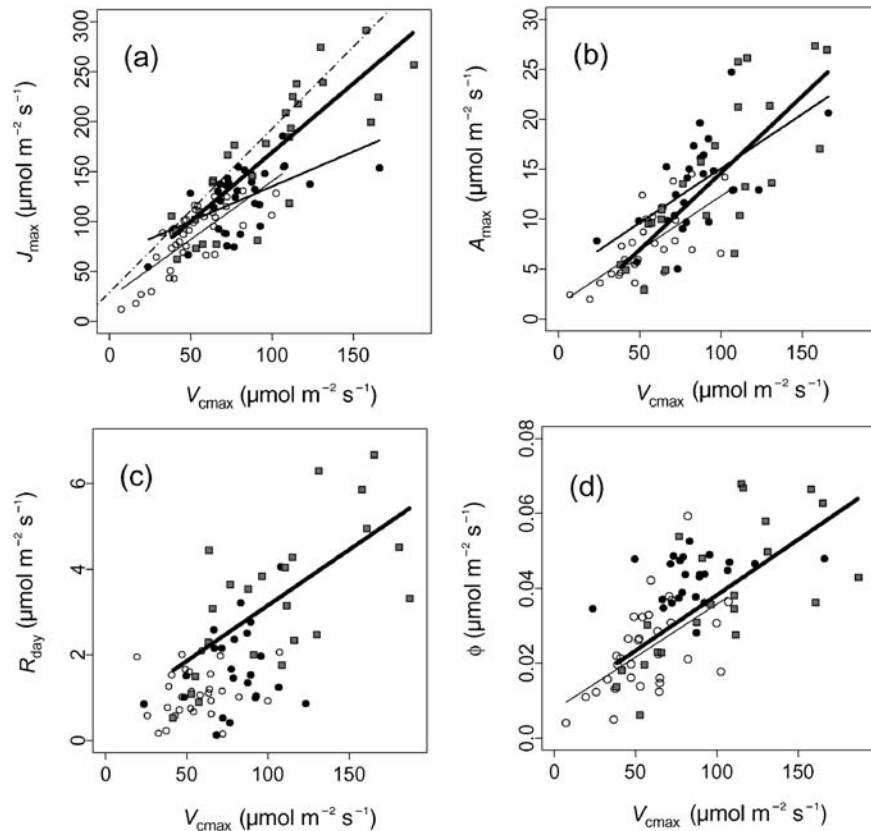


Figure 4. Relationship between maximum carboxylation velocity (V_{cmax}) and (a) maximum rate of electron transport (J_{max}), (b) light-saturated assimilation (A_{max}), (c) leaf respiration in the light (R_{day}) and (d) maximum quantum yield (ϕ). Data points and regression lines are for *Pinus ponderosa* (○, thinnest line), *Arctostaphylos manzanita* (●, medium thickness line) and *Ceanothus cordulatus* (■, thickest line). The dotted line in (a) is the relationship from Wullschlegel (1993). See Table 2 for linear regressions.

However, the mean $J_{\text{max}}/V_{\text{cmax}}$ ratio reported by Panek (2004) is higher than the ratio we calculated (2.1 versus 1.7), mainly because Panek (2004) reported a lower mean V_{cmax} than our value (40 versus $52.4 \mu\text{mol m}^{-2} \text{s}^{-1}$). Panek (2004) computed V_{cmax} and J_{max} from the A/C_i curve by a method similar to that reported by Ellsworth et al. (2004), which compared well with the method we used (see Material and methods). Difference in V_{cmax} values may be associated with interannual variability, spatial variability and instrumental differences. Despite the

extensive range and cover of *C. cordulatus* and *A. manzanita* in the western USA, photosynthetic measurements on these species are rare and we know of no data for V_{cmax} and J_{max} .

Seasonal pattern: species comparison

In addition to the mixed nature of forests, temporal change in photosynthetic parameters is a critical factor in determining the seasonality and magnitude of ecosystem carbon fluxes. Studies by Kosugi et al. (2003), Wilson et al. (2000) and Xu

Table 2. Linear regression ($y = a + bx$), level of significance and coefficient of determination (r^2) between maximum carboxylation velocity (V_{cmax}) and several photosynthetic parameters (see Figure 4). Abbreviations: J_{max} = maximum rate of electron transport; A_{max} = light-saturated assimilation; R_{day} = leaf respiration in the light; and ϕ = maximum quantum yield of photosynthesis.

Plant	Parameter	a	b	p	r^2
Pine	J_{max}	24.1	1.2	< 0.01	0.64
	A_{max}	1.5	0.1	< 0.01	0.48
	R_{day}	0.8	0.005	0.33	0.03
	ϕ	0.008	0.0003	< 0.01	0.31
Manzanita	J_{max}	65.5	0.7	< 0.01	0.33
	A_{max}	4.2	0.1	< 0.01	0.38
	R_{day}	0.8	0.01	0.28	0.06
	ϕ	0.03	0.0001	0.12	0.11
Ceanothus	J_{max}	31.5	1.4	< 0.01	0.71
	A_{max}	-0.07	0.15	< 0.01	0.59
	R_{day}	0.56	0.03	< 0.01	0.45
	ϕ	0.009	0.0003	< 0.01	0.47

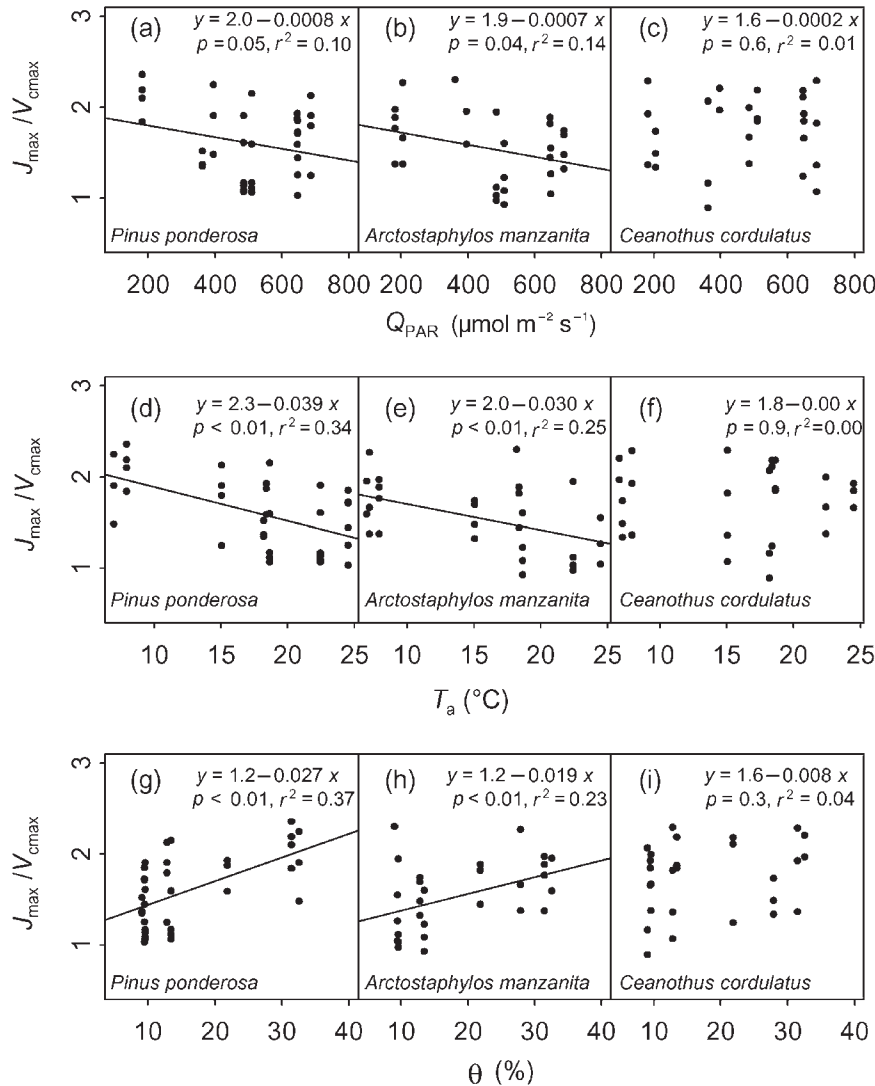


Figure 5. Relationship between maximum carboxylation velocity and maximum rate of electron transport ratio ($J_{\max}/V_{c\max}$) and (a–c) photosynthetically active radiation (Q), (d–f) air temperature (T_a) and (g–i) soil water (θ). Regression parameters and lines are shown.

and Baldocchi (2003) showed strong seasonal variations in photosynthetic parameters for deciduous species in temperate and Mediterranean climates. Wilson et al. (2001) reported that when the measured seasonality in photosynthetic capacity was represented in model simulations for a mixed deciduous forest, there was good agreement with eddy-covariance data, but removing the temporal trend resulted in incorrect simulations of the seasonality and magnitude of the carbon uptake by the forest (cf. Tanaka et al. 2002). For evergreen species it had been thought that the seasonality was small and would not influence the carbon fluxes; however, Tu (2000) found that the seasonality of photosynthetic capacity accounted for up to 14% of the intra-annual variability in carbon uptake and improved predictions of the total annual uptake by an average of 30% among grassland, savanna and evergreen and deciduous forests. Jach and Ceulemans (2000) showed some variability of J_{\max} from May to September in 1-year-old needles of Scots pine in a temperate forest, but no change for $V_{c\max}$. Nogues and Alegre (2002) showed strong variation in $V_{c\max}$ and J_{\max} during

the season for two evergreen shrubs in arid environments. Dang et al. (1998) reported that $V_{c\max}$ can vary in a boreal coniferous forest from 4 to 56% during the growing season. By not taking into account this variability, their model fit decreased by 10 to 20%, involving systematic errors that could result in incorrect simulations of carbon uptake in the long term (see Dungan et al. (2003) and Grassi et al. (2005) for additional examples in different ecosystems).

Our data suggest that seasonal variations in photosynthetic parameters for evergreen species can be large in a seasonally dry ecosystem (Figure 2). However, differences among species within this ecosystem were also large. We found that, among species, a nitrogen-fixing species (*C. cordulatus*) exhibited the highest values of photosynthetic parameters, the largest seasonal variation, and the strongest correlations with several environmental and physiological variables (Figure 3, Table 1). In addition, temporal scaling of photosynthetic parameters with each other seemed possible for *C. cordulatus* (Figure 4), which is particularly relevant to modeling efforts.

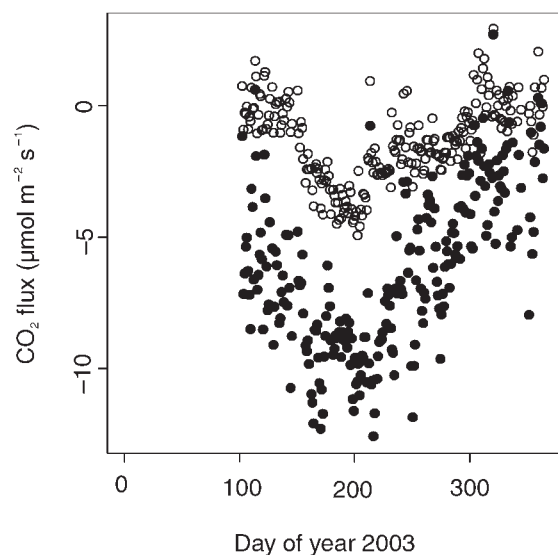


Figure 6. Mean daytime (0800–1700h) carbon dioxide (CO_2) flux at the top (10.5 m, ●) and in the middle (1.2 m, ○) of the canopy.

Similar scaling of parameters was obtained for *P. ponderosa*, but to a lesser extent. Of the species studied, *A. manzanita* exhibited the smallest seasonality in photosynthetic parameters and correspondingly weaker correlations with environmental and physiological parameters.

Ecophysiological factors

Because our constant cuvette temperature ($\sim 25^\circ\text{C}$) was likely to be close to the optimal temperature during the summer, but suboptimal during the winter, the observed changes in V_{cmax} and J_{max} with temperature may be greater than if the measurements were made at optimal temperatures year round. Reporting V_{cmax} and J_{max} at constant temperatures might not be functionally relevant if the shape of the temperature response curve varies greatly through the seasons (Medlyn et al. 2002a, 2002b). This could be verified if temperature response curves for the photosynthetic parameters were produced for each season. We did not make such measurements and a review of the literature indicated that detailed studies on the seasonality of the temperature response curves for photosynthetic parameters are rare, highlighting the need for additional research on this subject (Medlyn et al. 2002a).

We found important variations among species in the acclimation of photosynthetic parameters to environmental growth conditions (Figure 3; Table 1). *Pinus ponderosa* and *C. cordulatus*, but not *A. manzanita*, showed significant correlations of V_{cmax} with LMA. For *C. cordulatus*, V_{cmax} was correlated with N_M and N_A independent of LMA, whereas the V_{cmax} for *P. ponderosa* was not. All species showed strong correlations between V_{cmax} and Q , but V_{cmax} of *C. cordulatus* showed a stronger correlation with soil temperature and V_{cmax} of *A. manzanita* was also correlated to air temperature. Soil rewetting by summer rains affected V_{cmax} only in *A. manzanita* and *C. cordulatus*. *Ceanothus cordulatus* was the only species that

showed significant correlations of V_{cmax} with all of the environmental and physiological parameters tested. These correlations make it difficult to identify the processes involved because strong cross-correlations between environmental factors may be confounding.

In general, for all study species, we found high (but not always significant) correlations of V_{cmax} with Q , LMA and N_A (Figure 3 and Table 1). Although Q could be considered as the best scaling parameter for V_{cmax} and J_{max} seasonally, the relationship is species specific. Although acclimation of photosynthetic parameters to the vertical canopy gradient of irradiance through leaf nitrogen content and leaf mass per area has been studied extensively in evergreen and deciduous species growing in different climates (e.g., Meir et al. 2002, Niinemets et al. 2004b), there are few published data on seasonal acclimation of photosynthetic parameters involving coordinated changes among numerous ecophysiological processes through time.

We observed lags between the variations in V_{cmax} and those in Q , LMA and N_A , and the amplitudes of these lags differed among species. These lags could reflect processes other than light acclimation, including responses to air and soil temperature, soil water content, and source–sink interactions with other organs (new leaves, root) (Cai and Dang 2002, Medlyn et al. 2002a, Xu and Baldocchi 2003, Niinemets and Valladares 2004). Mäkelä et al. (2004) found evidence for lagged acclimation of photosynthetic capacity to temperature, but provided no clear explanation for this response. In complex weather patterns, as occur in the Sierra Nevada of California, characterized by snow with low temperatures in the winter, abrupt onset of warm wet spring conditions and warm dry summers, seasonal variation in V_{cmax} is probably not optimal with respect to photon absorption by leaves. Rather a complex series of metabolic processes and ecological factors are likely involved (Hollinger 1996, Niinemets and Valladares 2004). The integrated response to these variables can involve changes in either leaf thickness, affecting the quantity of photosynthetic tissue per unit leaf area, or photosynthetic enzyme concentration, affecting the photosynthetic capacity per unit leaf volume (Sims and Pearcy 1992, Sims et al. 1998). Seasonal variations in LMA may represent variations in nonstructural carbohydrate (NSC) content. This would explain the important and rapid increase in LMA in the spring, the gradual decrease during the summer and the low values in the winter that track variations in NSC in evergreen species (Hoch et al. 2003).

J_{max} to V_{cmax} ratio

Figure 4 shows that the $J_{\text{max}}/V_{\text{cmax}}$ ratio deviated appreciably from the relationship published by Wullschleger (1993) from a retrospective analysis of A/C_i curve across 109 species ($J_{\text{max}} = 29.1 + 1.64 V_{\text{cmax}}$). The mean ratio was lower for our study species and also varied during the season for *P. ponderosa* and *A. manzanita* (Figure 5). We tested if this result was an artifact of our method of calculating the parameters from analysis of the A/C_i curve and found the same results with another method (Ellsworth et al. 2004). Despite the general use of a constant

ratio across species in most modeling studies (~ 1.97 , see Wullschlegel 1993), there is accumulating evidence that seasonal variability in this ratio can be significant in both deciduous (Wilson et al. 2000, Xu and Baldocchi 2003, Grassi et al. 2005) and evergreen species (Jach and Ceulemans 2000, Medlyn et al. 2002a, Nogues and Alegre 2002, Panek 2004). We found that seasonal variability in J_{\max}/V_{cmax} was correlated with several environmental variables, with lower ratios at high Q , high air temperature and low soil water content. These results suggest three hypotheses concerning the sensitivity of acclimation of photosynthetic capacity to environmental conditions.

Hypothesis 1: the J_{\max}/V_{cmax} ratio decreases with water stress because of the higher sensitivity of RuBP regeneration capacity than of carboxylation capacity to low leaf water potential, as supported by numerous studies (von Caemmerer and Farquhar 1984, Martin and Ruiztorres 1992, Escalona et al. 1999, Flexas and Medrano 2002, Medrano et al. 2002). However Flexas et al. (2004) showed that this process is not universal and depends on the species and the severity of drought. Bota et al. (2004) and Flexas et al. (2004) showed that non-stomatal limitation associated with drought stress could be an artifact of patchy stomatal closure or of changes in cuticular or mesophyll conductance, or both, and that photosynthetic limitation occurs only during severe drought stress. Maroco et al. (2002) and Grassi et al. (2005) reported no influence of drought on J_{\max}/V_{cmax} ratio. Nogues and Alegre (2002) showed the ratio changed within the season but was unaffected by increased water deficit. Xu and Baldocchi (2003) reported that the ratio decreased with increasing drought stress; however their measurements were made at a severely water limited site (*Quercus douglasii* Hook & Arn. in California, with leaf predawn water potential reaching -7 MPa), and so their results may not be applicable to our study site that is characterized by only mild drought stress (Panek 2004).

Hypothesis 2: the J_{\max}/V_{cmax} ratio changes during the season because Rubisco activity and RuBP regeneration have different acclimation sensitivities to growth temperature (air or soil). Dependence of these photosynthetic parameters on measurement temperature has been examined by several authors (e.g., Leuning 1997, Bernacchi et al. 2001, Leuning 2002), who found that the J_{\max}/V_{cmax} ratio decreases with increasing temperature because these parameters have different activation energies. However our measurements were made at constant leaf temperature (25 °C), so the acclimation that we observed is associated with changes in growth temperature during the season. Hikosaka (1997) suggested that temperature acclimation is a result of optimal reallocation of nitrogen between Rubisco activity and RuBP regeneration; such that these two major limiting processes to photosynthesis are co-limiting at the growth temperature. Hikosaka et al. (1999) demonstrated that evergreen *Quercus myrsinaefolia* Blume leaves had a higher J_{\max}/V_{cmax} ratio when grown at a low temperature than at a high temperature. However other studies investigating acclimation to growth temperature have failed to find any evidence for this hypothesis (Ferrar et al. 1989, Bunce 2000, Medlyn et al. 2002a).

Hypothesis 3: the J_{\max}/V_{cmax} ratio reflects acclimation to irradiance such that it is lower at high Q because these conditions impose less limitation on RuBP regeneration. Because carboxylation capacity is potentially more limiting at high irradiance, it would be optimal for plants to invest relatively more in carboxylation capacity than in RuBP regeneration to maintain co-limitation. This strategy would also minimize photoinhibition at high irradiances (Hikosaka et al. 1999). However, some studies of photosynthetic acclimation to a vertical light gradient in a canopy have found higher J_{\max}/V_{cmax} ratios at high irradiance near the top of the canopy than at the bottom (Porte and Loustau 1998, Niinemets et al. 1999, Dungan et al. 2003, Niinemets et al. 2004a, Niinemets et al. 2004b), although Grassi and Bagnaresi (2001) reported the opposite pattern based on a study in a forest-gap natural light gradient. Bunce and Ziska (1999) showed that the acclimation of carboxylation efficiency, RuBP regeneration and quantum yield at high irradiance is species specific. Lambrevia et al. (2005) investigated the interactions between CO_2 , light and temperature in the acclimation of photosynthetic parameters and found that the J_{\max}/V_{cmax} ratio is slightly higher at high irradiances and ambient temperature (23 °C), but relatively constant at high irradiances and high temperature (39 °C), although J_{\max} and V_{cmax} decrease significantly. It is difficult to draw conclusions about our findings from these studies because our study focused on seasonal changes in a natural environment rather than on acclimation relationships found through vertical light gradients within canopies. Studies on seasonal variation of photosynthetic parameters in evergreen (Medlyn et al. 2002a, Nogues and Alegre 2002, Dungan et al. 2003) and deciduous species (Wilson et al. 2000, Grassi et al. 2005) have shown that decreases in J_{\max}/V_{cmax} ratios are not necessarily associated with higher Q , whereas we found that J_{\max}/V_{cmax} ratio varied seasonally and decreased significantly in response to high growth irradiances, high temperatures and low soil water contents for *P. ponderosa* and *A. manzanita*.

Besides these hypotheses, estimates of V_{cmax} from A/C_i curves can be inaccurate if mesophyll conductance changes or if there is patchy stomatal closure, which sometimes occurs under drought (Mott 1995, Ethier and Livingston 2004). Calculation of V_{cmax} from A/C_i curves is based on the assumption that C_i is equal the chloroplast CO_2 concentration (C_c). If leaf mesophyll conductance is low, C_c will be much lower than C_i and will result in an underestimation of V_{cmax} from the A/C_i curve (Manter and Kerrigan 2004). Thus, if mesophyll conductance decreases during the season, we would have underestimated V_{cmax} (Wilson et al. 2000) and the actual ratio of J_{\max}/V_{cmax} would even be more variable during the season.

Significance for ecosystem carbon uptake

We showed that when weighting LAI of the shrubs by their respective V_{cmax} (Equation 3), their contributions to the potential rate of carboxylation by the canopy increased substantially because annual integrated V_{cmax} values of *A. manzanita* and *C. cordulatus* were 1.7 times to 2.1 times higher, respectively,

than that of *P. ponderosa*. Equation 3 is valid for our study because upper-canopy LAI is low and the light environment is similar for *P. ponderosa* and the shrubs. Eddy-covariance net CO₂ fluxes measured at two canopy levels showed that the shrubs assimilated large quantities of CO₂ from the atmosphere (downward negative flux), even exceeding plant and soil CO₂ efflux (upward positive flux) during the day. The seasonality of the eddy-covariance flux inside the canopy is influenced by both the seasonality of respiration, mainly of the soil, and shrub photosynthesis. Consequently, many factors influenced the net flux. Separating the contributions of these factors and singling out the seasonal variation in photosynthetic parameters of *C. cordulatus* and *A. manzanita* will be a challenging task. However, we were able to demonstrate that the shrubs make a large contribution to total canopy carbon uptake in this ecosystem. This result is partly a consequence of the openness of the canopy and partly of the favorable climate of the Sierra Nevada for the development of *C. cordulatus* and *A. manzanita*. These data confirm that taking into account the multi-specific and vertically complex nature of the forest is a requisite for modeling CO₂ fluxes (Porte and Bartelink 2002).

In conclusion, this study underscores the importance of taking into account multi-species and temporal variation in photosynthetic parameters when studying CO₂ exchange with the atmosphere. In open evergreen stands, even if the contribution of some species to ecosystem LAI is small, their contribution to canopy carbon assimilation can be high because of their high photosynthetic parameters. The seasonality of V_{cmax} and J_{max} and their relationships with environmental and physiological parameters differed among the study species. We found evidence that photosynthetic parameters acclimated seasonally to light through leaf nitrogen content. However this was not the only factor, with other potential factors including air and soil temperature, soil water content, source-sink interactions with other organs and variations in leaf NSC content. The acclimation relationships found through vertical light gradients within canopies in other studies cannot be easily applied to seasonal variation. Seasonal variation of photosynthetic parameters in evergreen species not only involves alterations in overall photosynthetic potential, but the capacities for light utilization and RuBP carboxylation largely change independently: the $J_{\text{max}}/V_{\text{cmax}}$ ratio was variable during the season and decreased significantly with increasing growth irradiance, temperature and decreasing soil water content for *P. ponderosa* and *A. manzanita*. Several hypotheses were proposed to explain the seasonal variation in the $J_{\text{max}}/V_{\text{cmax}}$ ratio and additional research is required to test them.

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