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Light dependence of carboxylation capacity for C_3 photosynthesis models

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Abstract

C_3 photosynthesis at high light is often modeled by assuming limitation by the maximum capacity of Rubisco carboxylation (V_{Cmax}) at low CO_2 concentrations, by electron transport capacity (J_{max}) at higher CO_2 concentrations, and sometimes by triose-phosphate utilization rate at the highest CO_2 concentrations. Net photosynthetic rate (P_N) at lower light is often modeled simply by assuming that it becomes limited by electron transport (J). However, it is known that Rubisco can become deactivated at less than saturating light, and it is possible that P_N at low light could be limited by the rate of Rubisco carboxylation (V_C) rather than J. This could have important consequences for responses of P_N to CO_2 and temperature at low light. In this work, P_N responses to CO_2 concentration of common bean, quinoa, and soybean leaves measured over a wide range of temperatures and PPFDs were compared with rates modeled assuming either V_C or J limitation at limiting light. In all cases, observed rates of P_N were better predicted by assuming limitation by V_C rather than J at limiting light both below and above the current ambient CO_2. One manifestation of this plant response was that the relative stimulation of P_N with increasing the ambient CO_2 concentration from 380 to 570 μmol mol^{-1} did not decrease at less than saturating PPFDs. The ratio of V_C to V_{Cmax} at each lower PPFD varied linearly with the ratio of P_N at low PPFD to P_N at high PPFD measured at 380 μmol(CO_2) mol^{-1} in all cases. This modification of the standard C_3 biochemical model was much better at reproducing observed responses of light-limited P_N to CO_2 concentrations from pre-industrial to projected future atmospheric concentrations.

Additional key words: carbon dioxide; climate change; modeling; temperature.

Introduction

Biochemical models of C_3 photosynthesis, based on the model of Farquhar, von Caemmerer and Berry (1980) and subsequent modifications have been utilized to predict leaf photosynthetic responses to the environment in many diverse applications, from crop simulation models to terrestrial carbon budgets (*e.g.* Zhang *et al.* 2013, Sun *et al.* 2014, Wang *et al.* 2014). Photosynthesis is modeled by assuming that at moderate temperatures, photosynthesis at high light is limited by the maximum rate of Rubisco carboxylation (V_{Cmax}) at low CO_2 concentrations, by the maximum rate of photosynthetic electron transport (J_{max}) at higher CO_2 concentrations, and sometimes by the rate of triose phosphate utilization at the highest CO_2 concentrations (Sharkey *et al.* 2007). At less than saturating light, photosynthesis is usually considered to become limited by rates of photosynthetic electron transport (J) (Farquhar *et al.* 1980). The distinction between limitation of photosynthesis by V_{Cmax} or by J is important for global change considerations. For example, limitation by V_{Cmax} leads to a much stronger response of photosynthesis to rising atmospheric CO_2 than if photosynthesis were limited by J, and elevated CO_2 increases the optimum temperature for photosynthesis, especially at high PPFD (Long 1991). In predicting crop photosynthesis at elevated CO_2, it is important to know whether photosynthesis at high light changes in limitation from V_{Cmax} to J_{max} as CO_2 increases (reviewed in Bunce 2014). For modeling ecosystem photosynthesis, Kirschbaum (1994) used limitation by J rather than V_{Cmax}, because photosynthesis of plant communities was thought to be predominantly light-limited. More complex ecosystem models considering separately the photosynthesis of sunlit and shaded leaves

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Abbreviations: C_i – [CO_2] in the substomatal (intercellular) airspace; C_c – [CO_2] at Rubisco; C_a – [CO_2] external to the leaf; [CO_2] – CO_2 concentration; g_m – mesophyll conductance to CO_2; J_{max} – the maximum rate of photosynthetic electron transport; J – the rate of photosynthetic electron transport; P_N – net photosynthetic rate; RH – relative humidity; V_{Cmax} – the maximum rate of carboxylation of Rubisco; V_C – the rate of carboxylation of Rubisco.

would need to consider both V_{Cmax} and J limitations for different leaf layers and times of day.

However, it is known from biochemical measurements that Rubisco activation state may decrease at less than saturating light (*e.g.* Taylor and Terry 1984, von Caemmerer and Edmonson 1986, Brooks *et al.* 1988, Sage *et al.* 1990), and photosynthesis at low light could potentially become limited by the rate of Rubisco carboxylation (V_C) rather than by J. Qian *et al.* (2012) found that in tomato, leaf gas exchange analysis indicated that apparent V_C was reduced at limiting light, in general agreement with studies of Rubisco deactivation. This reduction in Rubisco activity at low light could strongly affect the response of photosynthesis at low light to atmospheric CO_2 concentration and its temperature dependence. In this study, responses of photosynthesis to CO_2 concentration were measured over a range of light levels and temperatures in leaves of soybean, common bean, and quinoa to determine whether photosynthesis at low light was better predicted by assuming limitation by V_C or by J, and apparent responses of V_C to light were determined for a range of measurement temperatures.

Materials and methods

Soybeans (*Glycine max* cv. Kent) and common bean (*Phaseolus vulgaris* cv. Dark Red Kidney) were grown in indoor chambers with 13 h of light at 1,000 μmol m^{-2} s^{-1} PPDF at 25°C air temperature and 60% relative humidity (RH), and 11 h of darkness at 19°C, 80% RH. The CO_2 concentration [CO_2] was controlled at 380 ± 20 μmol mol^{-1} by adding CO_2 or CO_2-free air under the control of CO_2 analyzer continuously sampling chamber air. Plants were grown in 20 cm diameter plastic pots filled with vermiculite and flushed daily with a complete nutrient solution. Quinoa (*Chenopodium quinoa* cv. Cherry Vanilla) plants were grown at 20/14°C day/night temperatures, with 12 h per day of light at 1,000 μmol m^{-2} s^{-1} PPFD, and the same [CO_2] as soybean and common bean.

Responses of leaf gas exchange to [CO_2] were determined at several fixed values of leaf temperature and PPFD, using portable photosynthesis systems (*CIRAS-2* or *CIRAS-3, PP Systems*, Amesbury, MA, USA) under laboratory conditions. Intact, recently fully expanded third trifoliolate leaves of bean and soybean, and fifth leaves in quinoa were enclosed in a temperature-controlled leaf cuvette with a 2.5 cm^2 window with a white LED light source. The cuvette and plants being measured were inside a temperature-controlled growth cabinet in darkness. CO_2-response curves were measured for bean and soybean leaves at 20, 25, 30, and 35°C, with PPFD of 1,600; 800, 400, and 200 μmol m^{-2} s^{-1}. Quinoa was measured at 15, 20, 25, and 30°C, with the same PPFD as in bean and soybean. The [CO_2] external to the leaves were, in order: 380, 50, 100, 150, 200, 380, 570, 800; 1,000; and 1,200 μmol mol^{-1}. Each individual leaf was measured at only one temperature, but at all four PPFD levels. Steady-state CO_2 response curves were determined first at the highest PPFD level, and then were repeated at successively lower PPFD levels. The leaf to air difference in water vapor was kept between 1 and 2 kPa at all temperatures. For each measurement condition, there were three replicate plants for each species.

The measured rates of net photosynthesis (P_N) *vs.* substomatal CO_2 concentration (C_i) were used in a biochemical model of C_3 photosynthesis (Farquhar *et al.* 1980) modified with updated temperature dependencies (Bernacchi *et al.* 2002, 2003). The model equations are given in Appendix. In the main analysis it was assumed that mesophyll conductance (g_m) was infinite, for reasons to be discussed later, and comparisons with results obtained assuming finite g_m are also presented. For the highest PPFD level, the measurements at the lowest four [CO_2] were used to estimate V_{Cmax} at each temperature, and measurements at the highest three [CO_2] were used to estimate J_{max} at each temperature. Saturation of P_N at high C_i or a decrease in P_N at high C_i did not occur in any of these species at any temperature examined except for quinoa measured at 15°C at the highest PPFD, so there was no indication of limitation of P_N by triose phosphate utilization rate (Sharkey 1985) except in that case. In the case of quinoa at 15°C at the highest PPFD, J_{max} was determined from P_N measured at 570 μmol(CO_2) mol^{-1}. At each of the lower PPFDs, V_C was determined from P_N at the four lowest [CO_2], and J from P_N at the highest three [CO_2], at each temperature.

For analysis using finite mesophyll conductance in soybean and bean, the Sharkey *et al.* (2007) calculation program was used to determine g_m, V_C and J at each PPFD level from the P_N *vs.* C_i observations made at 25°C.

The more usual treatment of P_N/C_i curves at lower PPFD was also compared with the data. For this comparison, P_N at the external [CO_2] of 380 μmol mol^{-1} at a lower PPFD was used to determine J for that PPFD. Modelled P_N/C_i curves using V_{Cmax} and this value of J were then compared with measured P_N/C_i curves at that PPFD.

Results

Both the initial slopes of P_N *vs.* C_i curves and P_N at high C_i increased with measurement PPFD in all species and temperatures. Examples for soybean at high temperature, for bean at moderate temperature, and for quinoa at low temperature are shown in Fig. 1. These curves could all be well fit using the V_C and J limitation model of

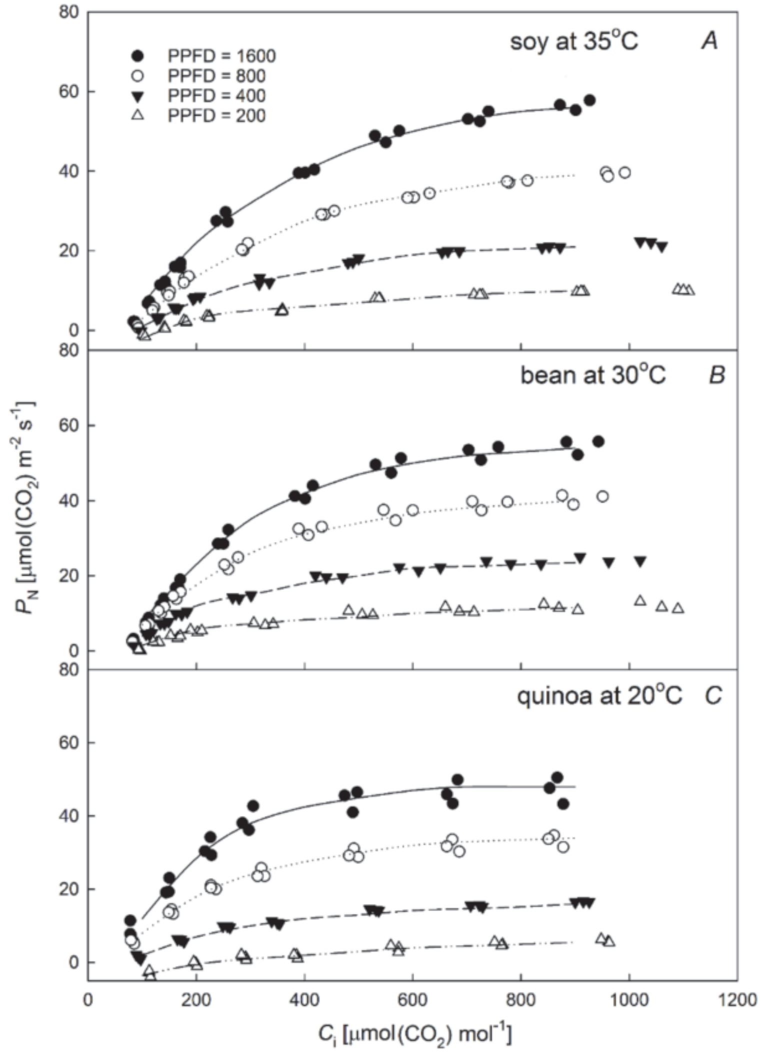

Fig. 1. CO$_2$ assimilation rate (P_N) *vs*. substomatal CO$_2$ concentration (C_i) at four PPFD levels for (*A*) soybean at 35°C, (*B*) common bean at 30°C, and (*C*) quinoa at 20°C. The curves are model predictions, where V_C varied with PPFD.

photosynthesis, provided that V_C as well as J was allowed to vary with PPFD (Fig. 1). In comparison, using a constant value of V_{Cmax} regardless of PPFD, with only J varying with PPFD, did not fit the data at the lower PPFD as well as illustrated in Fig. 2. Similar underestimation of P_N at high C_i and less than saturating PPFD as in Fig. 2 occurred for all species, and at all temperatures, when using V_{Cmax} at all PPFD levels. One consequence of using V_{Cmax} at lower PPFD was that the increase in P_N above 380 μmol(CO$_2$) mol^{-1} and the decrease below that concentration were both underestimated (Fig. 2). The actual relative change in P_N for an increase in C_a from 380 to 570 μmol mol^{-1} increased with temperature, but was nearly independent of PPFD level in all species (Fig. 3), except for increases at the lowest PPFD in some cases, when approaching the photo-compensation point. This contrasts with a decrease in the relative change in P_N by about half at limiting PPFDs, which would be predicted when using V_{Cmax} at lower PPFDs.

The ratio of V_C to V_{Cmax} for all species, temperatures, and PPFD levels was linearly related to the ratio of P_N to P_N at high PPFD measured at 380 μmol(CO$_2$) mol^{-1} (Fig. 4). Ratios tended to be lower for quinoa than for soybean and bean because P_N increased with PPFD to higher PPFDs in quinoa, reflecting its higher light-saturated rate of photosynthesis at a given temperature. The ratio of V_C to V_{Cmax} at the three lower PPFD levels determined using values of g_m estimated at each PPFD using the Sharkey *et al*. (2007) calculator did not differ substantially from the ratio calculated using infinite g_m in either soybean or common bean (Fig. 5).

Discussion

The primary reason for using infinite mesophyll conductance for most of this presentation is the observation that mesophyll conductance varied significantly with C_i in common bean, which makes V_{Cmax}, in turn, strongly dependent on C_i (Bunce 2010). With infinite g_m, a single value of V_{Cmax} adequately fits all P_N *vs*. C_i points in the

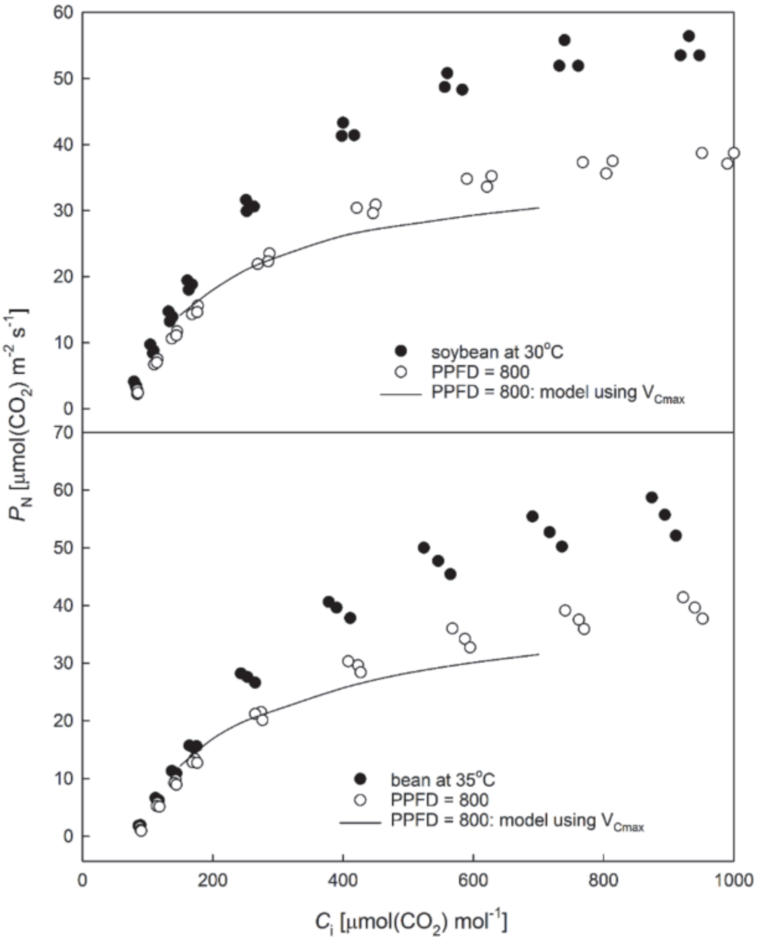

Fig. 2. Comparison of measured responses of P_N to C_i at 1,600 and at 800 µmol m^{-2} s^{-1} PPFD for soybean at 30°C and common bean at 35°C, compared with rates modeled at the lower PPFD using V$_{Cmax}$ estimated from rates at high PPFD.

lower C_i range (Bunce 2010), greatly simplifying modeling. There is currently debate about whether apparent variation in g_m with C_i is real or a measurement artefact (Flexas *et al.* 2007, Tholen *et al.* 2012, Mizokami *et al.* 2015). Bernacchi *et al.* (2003) made the point that for P_N rates measured at low PPFD, using an infinite g_m in photosynthesis models is unlikely to cause much error, because C_c and C_i do not differ greatly when assimilation rates are low. In bean and soybean, using finite values of g_m *vs.* infinite g_m had little effect on the ratio of V$_C$ to V$_{Cmax}$ as a function of PPFD in this study. It is conceivable that reductions in g_m with decreasing PPFD could lead to apparent reductions in V$_C$. However, previous data (Bunce 2010) indicates only very minor changes in g_m at low PPFD in soybean and bean, so this effect is not important for these species. No such information is available for quinoa.

Qian *et al.* (2012) provided a logistic equation relating percent Rubisco activation as a function of PAR. Our data for more species, temperatures and PPFD levels fits a simpler, photosynthetic light-saturation curve. Our curve differs from the Qian *et al.* (2012) curve primarily at the lowest PPFD values, especially for quinoa, where activation percentages approached zero, compared with a minimum of about 30% activation at 0 PPFD for Qian *et al.* (2012). It is known that activation of Rubisco by Rubisco activase requires photochemical energy (*e.g.* Campbell and Ogren 1990). The good fit of the V$_C$ to V$_{Cmax}$ ratio with a photosynthetic light-saturation curve measured at ambient CO$_2$ found here simplifies the data collection needed to model photosynthesis at low PPFD as a function of CO$_2$ and temperature.

It is not known how long it takes V$_C$ to decrease when PPFD decreases, and P_N would probably be limited by J until V$_C$ equilibrates to the new PPFD. In these experiments measurements of gas exchange rates were made within a few minutes of changing PPFD and C_a. Shorter-term responses were not investigated here.

The shape of the P_N *vs.* CO$_2$ response above the current ambient CO$_2$ has important implications for plant growth as atmospheric carbon dioxide rises. The steepness of the response of P_N to C_i at low PPFD values was quite different when J was estimated using V$_{Cmax}$ at limiting PPFD than when V$_C$ limited P_N, and J was fit to data (Fig. 3). The data presented here suggest that the J values at low PPFD calculated using V$_{Cmax}$ underestimated P_N at high C_i in these three species over a wide range of temperatures and PPFD levels. The reason for the low modeled values of P_N at high C_i at low PPFD when using V$_{Cmax}$ *vs.* V$_C$ is lower fitted values of J when using V$_{Cmax}$.

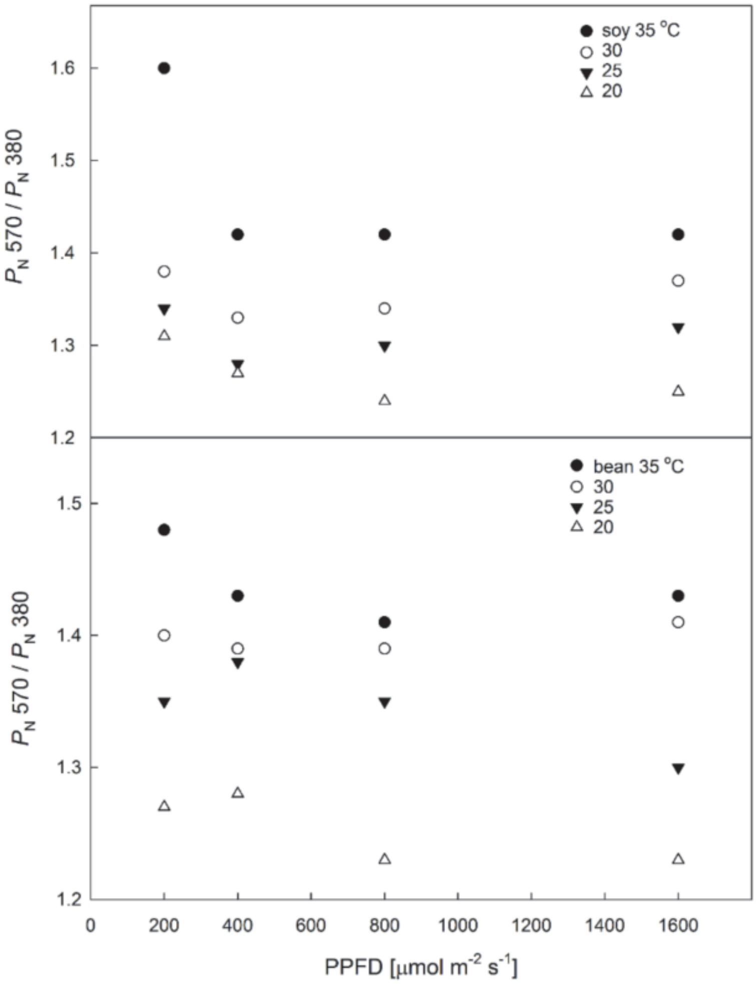

Fig. 3. The ratio of P_N at 570 µmol mol^{-1} to that at 380 µmol mol^{-1} external CO_2 concentrations over a range of PPFD levels for soybean and common bean measured at four temperatures. Each point represents a mean value for three leaves.

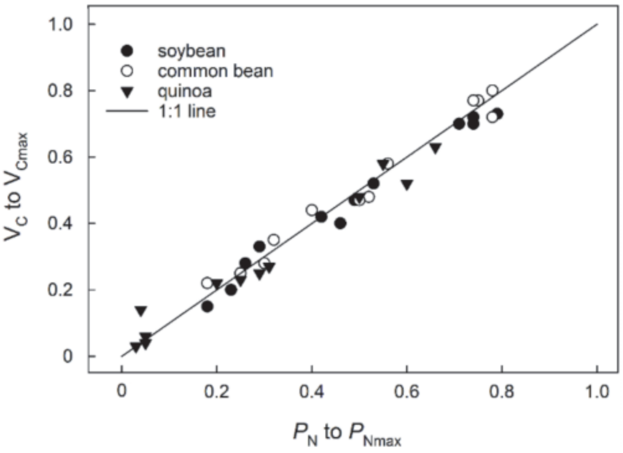

Fig. 4. The ratio of V_C to V_{Cmax} as a function of P_N at low PPFD to P_N at 1,600 µmol m^{-2} s^{-1} at an external CO_2 concentration of 380 µmol mol^{-1}. Each points represents a mean for three leaves. All temperature and PPFD measurement conditions used are represented for each species.

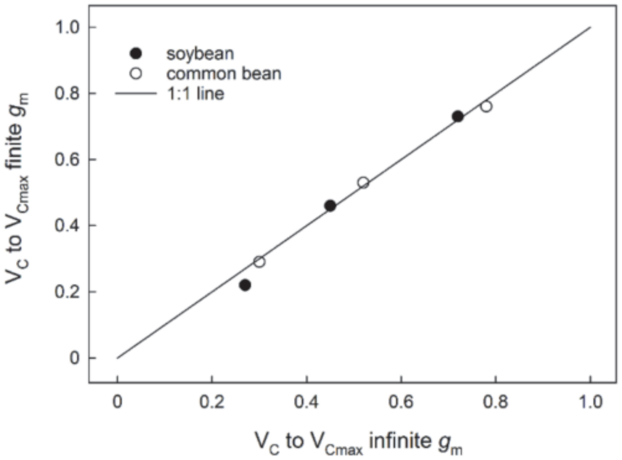

Fig. 5. The ratios of V_C to V_{Cmax} for soybean and common bean at 25°C at the lower measurement PPFDs, calculated assuming either infinite mesophyll conductance (g_m) or g_m values of 3.7 and 3.2 µmol m^{-2} s^{-1} Pa^{-1} for soybean and bean, respectively. Using these finite values of g_m increased V_{Cmax} from 150 to 218 µmol m^{-2} s^{-1} in soybean and from 140 to 200 µmol m^{-2} s^{-1} in bean.

It happens that at an external CO_2 of 380 µmol mol^{-1}, P_N at the lower PPFDs was in all cases limited by V_C rather than by J. Thus the values of J obtained using V_C are higher than those obtained if V_{Cmax} were used, which, in turn increases P_N at the higher C_i values. The usual approach of using V_{Cmax} and J to model P_N at low PPFD underestimated P_N at high C_i for all temperature and PPFD conditions in these three species.

Sun *et al.* (2014) reported that global carbon cycle models underestimate the photosynthetic responsiveness to past and projected changes in atmospheric CO_2. They argued that incorporating mesophyll conductance into the models would increase the sensitivity of photosynthesis to CO_2 by replacing C_i with lower C_c values, where the response of P_N to CO_2 is steeper. While this is clearly true for leaves at high PPFD, g_m has a much smaller effect on the responsiveness of P_N to CO_2 when P_N is low because of low PPFD (Bernacchi *et al.* 2003). This study identified an additional factor which may partly account for the insufficient responsiveness of current global carbon cycle models to changes in atmospheric carbon dioxide: low modeled responsiveness of P_N to CO_2 at low PPFD because of lack of recognition of reductions of V_C at low PPFD. In the standard C_3 photosynthesis model, using V_{Cmax} and J, the response of P_N to changes in atmospheric CO_2 from preindustrial to current and projected concentrations is relatively much less steep at limiting than at saturating PPFD (*e.g.* Long 1991, Kirschbaum 1994), whereas our data and the revised model show almost no change in relative response of P_N to CO_2 over a wide range of PPFD conditions at any temperature.

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Appendix. Equations used in the biochemical model of C_3 photosynthesis

P_N = the minimum of [P_C, P_J, P_T], where

$P_C = V_{Cmax}[C_c - \Gamma^*]/[C_c + K_C(1 + O_c/K_O)] - R_D$
$P_J = J[C_c - \Gamma^*]/[4C_c + 8\Gamma^*] - R_D$
$P_T = 3[TPU] - R_D$

where V_{Cmax} is the maximum rate of Rubisco carboxylation, C_c is the CO_2 concentration at Rubisco, O_c is the O_2 concentration at Rubisco, K_C is the Michaelis constant of Rubisco carboxylation, K_O is the Michaelis constant of Rubisco for oxygentation, Γ^* is the CO_2 concentration at which photorespiration equals assimilation, J is the rate of photosynthetic electron transport, R_D is dark respiration in the light, TPU is the rate of use of triose phosphates, and PPFD is photosynthetic photon flux density. $C_c = C_i - P_N/g_m$, where C_i is the CO_2 concentration in the substomatal air space, and g_m is mesophyll conductance.

The variables V_{Cmax}, K_C, K_O, R_D, and Γ^* are temperature dependent. The temperature dependencies used here were taken from Bernacchi *et al.* (2001).