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The “one-point method” for estimating maximum carboxylation capacity of photosynthesis: A cautionary tale

Angela C. Burnett | **Kenneth J. Davidson** | **Shawn P. Serbin** | **Alistair Rogers**

Environmental and Climate Sciences Department, Brookhaven National Laboratory, Upton, New York

Correspondence
Angela C. Burnett, Environmental and Climate Sciences Department, Brookhaven National Laboratory, Upton, NY 11973-5000.
Email: aburnett@bnl.gov

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Abstract

The maximum carboxylation capacity of Rubisco, $V_{c,max}$, is an important photosynthetic parameter that is key to accurate estimation of carbon assimilation. The gold-standard technique for determining $V_{c,max}$ is to derive $V_{c,max}$ from the initial slope of an A–C_i curve (the response of photosynthesis, A, to intercellular CO_2 concentration, C_i). Accurate estimates of $V_{c,max}$ derived from an alternative and rapid “one-point” measurement of photosynthesis could greatly accelerate data collection and model parameterization. We evaluated the practical application of the one-point method in six species measured under standard conditions (saturating irradiance and 400 µmol CO_2 mol^{-1}) and under conditions that would increase the likelihood for successful estimation of $V_{c,max}$: (a) ensuring Rubisco-limited A by measuring at 300 µmol CO_2 mol^{-1} and (b) allowing time for acclimation to saturating irradiance prior to measurement. The one-point method significantly underestimated $V_{c,max}$ in four of the six species, providing estimates 21%–32% below fitted values. We identified ribulose-1,5-bisphosphate-limited A, light acclimation, and the use of an assumed respiration rate as factors that limited the effective use of the one-point method to accurately estimate $V_{c,max}$. We conclude that the one-point method requires a species-specific understanding of its application, is often unsuccessful, and must be used with caution.

KEYWORDS

CO_2, light, photosynthesis, respiration, rubisco

1 | INTRODUCTION

Photosynthesis, the capacity of plants to assimilate atmospheric carbon dioxide (C_a), is of vital interest for crop breeders striving to increase crop yield, evolutionary biologists seeking to understand the diversification of plant forms, and Earth system modellers predicting the effects of climate change on the carbon balance of our planet. In most Earth system models (ESMs), estimation of photosynthesis is accomplished using the foundational and well-established Farquhar, von Caemmerer, and Berry (1980) model of photosynthesis, which is critically sensitive to the maximum rate of carboxylation ($V_{c,max}$) and the maximum electron transport rate (J_{max}). Accurate model representation of photosynthesis is a vital component of understanding and predicting the response of the terrestrial biosphere to climate change (Beer et al., 2010; Rogers, 2014), yet the representation of photosynthesis in ESMs drives considerable model uncertainty, related to uncertain estimation of factors including net primary productivity, gross primary production, and $V_{c,max}$ (Bonan et al., 2011; Friedlingstein et al., 2006; Friedlingstein et al., 2014; Rogers, 2014; Rogers et al., 2017; Rogers, Serbin, Ely, Sloan, & Wullschleger, 2017). The key model parameter $V_{c,max}$ describes the maximum carboxylation capacity of the enzyme ribulose-bisphosphate carboxylase/oxygenase, commonly referred to as Rubisco (EC 4.1.1.39), which catalyses the addition of a CO_2 molecule to a molecule of ribulose-1,5-bisphosphate (RuBP) in the Calvin-Benson-Bassham Cycle, such that $V_{c,max}$ directly limits the rate at which plants absorb and fix carbon. $V_{c,max}$ varies between species and across environmental gradients (Ali et al., 2015; De Kauwe et al., 2016b; Rogers, 2014; Smith et al., 2019), and therefore provides an important source of variation in carbon uptake capacity, making this parameter a critical element of ESMs.

The gold-standard technique for measuring $V_{c,max}$ is measuring the response of photosynthesis (A) to intercellular CO_2 concentration (C_i), commonly known as an A–C_i curve. Waiting for steady-state gas exchange and then conducting an A–C_i response curve can take over an hour depending on the species, the time taken to reach steady state, and the number of data points collected. $V_{c,max}$ is then derived from the initial Rubisco-limited, RuBP-saturated portion of the A–C_i curve as has been described previously (Bernacchi et al., 2013; Farquhar, Caemmerer, & Berry, 1980; Sharkey, Bernacchi, Farquhar, & Singsaas, 2007). The value of these measurements and the amount of time required to collect a large dataset of $V_{c,max}$ values means that robust methods for rapidly estimating $V_{c,max}$ are highly desired by the community. As such, various approaches have been explored to develop a method for more quickly estimating this parameter, such as the use of spectroscopy (Ainsworth, Serbin, Skoneczka, & Townsend, 2014; Serbin, Dillaway, Kruger, & Townsend, 2012; Silva-Perez et al., 2018), the rapid A–C_i response technique (Stinziano et al., 2017), remote sensing indices (Alton, 2017; Croft et al., 2017), and correlations with leaf traits such as nitrogen and phosphorus (Norby et al., 2016; Walker et al., 2014). A further approach is obtaining $V_{c,max}$ by extrapolation from "survey-style" measurements of photosynthesis (Kattge, Knorr, Raddatz, & Wirth, 2009; Niinemets, 1999).

Recently, the approach of extrapolation from survey-style or "one-point" measurements of photosynthesis has been validated by comparison with the gold-standard, that is, A–C_i curves (De Kauwe et al., 2016b). The one-point technique involves modelling $V_{c,max}$ from a single point measurement of photosynthesis (cf. a whole curve) based on well-defined key parameters (Bernacchi, Singsaas, Pimentel, Portis, & Long, 2001; Long & Bernacchi, 2003). The celebrated advantage to this method is that not only could these rapid measurements of photosynthesis—performed in under 2 min—be used as a proxy for time-consuming A–C_i curves, but also that existing measurements of light saturated photosynthesis (A_{sat}) could be used to predict $V_{c,max}$, thereby considerably expanding the scope of current datasets, with exciting implications for parameterizing plant functional types used in process models (De Kauwe et al., 2016b).

De Kauwe et al. (2016b) compared $V_{c,max}$ fitted from the initial slope of an A–C_i curve with $V_{c,max}$ estimated from the first measured point of that A–C_i curve, that is, A_{sat} measured at 400 μmol CO_2 mol^{-1}. They found a strong correlation between the estimated and fitted $V_{c,max}$ (r^2 of 0.98, root-mean-squared error of 8.19 μmol m^{-2} s^{-1}) when daytime respiration (R_{day}) was known, which decreased (r^2 of 0.95, root-mean-squared error of 17.1 μmol m^{-2} s^{-1}) when R_{day} was estimated as a fixed percentage (1.5%) of $V_{c,max}$ (De Kauwe et al., 2016b). The strength of the one-point method depends upon a successful prediction of $V_{c,max}$ from an estimated R_{day}, and therefore the ability to determine $V_{c,max}$ rapidly (<2 min) without the need for a separate measurement of R_{day}. Critically, application of the one-point method also depends on the assumption that A is Rubisco-limited at the measurement C_a. This assumption was validated for the dataset of De Kauwe et al. (2016b), but remains a significant concern for manipulative experiments, particularly elevated CO_2 experiments, where A could become RuBP-limited (Ainsworth & Rogers, 2007). The comprehensive theoretical analysis of De Kauwe et al. (2016b) used a dataset based on A–C_i measurements and therefore evaluated one-point measurements that were made following acclimation to saturating light where steady-state gas exchange had been achieved prior to the measurement. This raises the question: if survey-style one-point measurements—with no preceding acclimation of photosynthesis to saturating light—were to be used instead, would the conclusions made by De Kauwe et al. (2016b) still stand?

Here, we evaluated the practical application of the one-point method in six species: an Arctic grass and a temperate deciduous tree, both measured in the field, and four glasshouse-grown crop species. We compared one-point estimates of $V_{c,max}$ with the "gold-standard" measurement of fitted $V_{c,max}$ at measurement temperature derived from A–C_i curves. We asked two questions: (1) Can a measurement of A_{sat}, without prior acclimation of the leaf to saturating irradiance, be used to successfully estimate $V_{c,max}$ using the one-point method? (2) If $V_{c,max}$ cannot be successfully estimated in this way, do (a) measurement at a lower C_a, (b) measurement after full acclimation to saturating irradiance, or (c) inclusion of measured R_{day} in the estimation of $V_{c,max}$ enable successful application of the one-point method?

2 | MATERIALS AND METHODS

2.1 | Plant material

Four species, *Helianthus annuus* L. var. Pro Cut Gold (sunflower), *Phaseolus vulgaris* L. var. Provider (bush bean), *Populus canadensis* Moench. [*deltoides* x *nigra*] clone OP367 (poplar), and *Raphanus sativus* L. var. Easter Egg (radish) were grown in a glasshouse in Brookhaven National Laboratory in 2018. Plants of *H. annuus*, *P. vulgaris*, and *R. sativus* were germinated in BM2 germinating mix (Berger, Saint-Modeste, QC, Canada) and transplanted to 30 l pots filled with Pro-Mix BX Mycorrhizae (Premier Tech Horticulture, Quakertown, PA, USA) once seedlings were established. Cuttings of *P. canadensis* were soaked in water and transplanted to 100 l pots filled with 52 Mix (Conrad Fafard, Inc., Agawam, MA, USA) once roots had developed. Plants received ambient irradiance, with supplementary lighting of 49 W m^{-2} delivered by high pressure sodium bulbs with a 14-hr photoperiod. The maximum recorded light level was 2078 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). The temperature range was 17°C–33°C:17°C–29°C day:night, with supplementary heating to ensure the minimum temperature and vents to reduce the maximum temperature. Environmental conditions were recorded with a portable weather station comprised of a humidity and temperature sensor (ATMOS 14, METER Group, Inc., Pullman, WA, USA) and two quantum sensors (QSO S and PYR, Apogee Instruments, Logan UT, USA). Environmental data were logged at 1-min intervals (EM50R, Decagon Devices, Pullman, WA). Plants were watered three times per week. Osmocote Plus slow-release fertilizer (The Scotts Company, Marysville, OH) was applied as appropriate and according to manufacturer's instructions.

Two species, *Arctagrostis latifolia* R. Br. Griseb (wideleaf polargrass) and *Quercus coccinea* Muench (scarlet oak) were measured at two field sites. *A. latifolia* was measured at the Barrow Environmental Observatory, Utqiaġvik, AK, USA in July 2018. A detailed description of the field site is given by Rogers et al. (2017); all measurements were performed in situ on rooted plants at the field site. *Q. coccinea* was measured at Brookhaven National Laboratory in August 2018. Samples of *Q. coccinea* comprising several leaves and a small amount of woody material (i.e., twigs, small branches) were retrieved from the top of the canopy using shotgun sampling (Serbin, Singh, McNeil, Kingdon, & Townsend, 2014). We used a 12-gauge Remington 870 Express Pump-Action Shotgun with a modified choke and stainless steel bird shot to retrieve canopy samples. Samples were collected from the forest floor and the woody stem was immediately recut underwater. Each stem was maintained in water for the duration of data collection. Nonacclimated one-point measurements of photosynthesis were performed in the field, with the sample maintained in full sunlight in a canopy gap to simulate the irradiance at the top of the canopy; samples were then transported to the laboratory where A–C_i curves were performed. Each sample was obtained from a different tree.

2.2 | Gas-exchange measurements

Gas-exchange measurements were performed using four LI-6400XT Portable Photosynthesis Systems, each equipped with a Leaf Chamber Fluorometer (LI-COR, Lincoln, Nebraska, USA). For the Barrow Environmental Observatory field measurements, gas-exchange measurements were performed using five LI-6400XT Portable Photosynthesis Systems, each equipped with a 2 × 3-cm LED Light Source (LI-COR, Lincoln, Nebraska, USA). Prior to the measurement campaigns, instruments were zeroed using a common nitrogen standard. Flow rate was checked daily and zeroed as necessary.

Before conducting the main experiment, preliminary A–C_i and light response curves were performed and examined for each species, in order to confirm the selection of 300 μmol CO_2 mol^{-1} as a suitable ambient CO_2 level (C_a) to ensure Rubisco-limited A, and to determine saturating irradiance. The saturating irradiances used in all subsequent measurements for each species were as follows: 1,500 μmol photons m^{-2} s^{-1} for *A. latifolia*, *Q. coccinea*, and *R. sativus*; 1,800 μmol photons m^{-2} s^{-1} for *H. annuus*, *P. canadensis*, and *P. vulgaris*. For all gas-exchange measurements, the flow rate was set to 200–500 μmol s^{-1} depending on the species, with flow rates at the lower end of this range being used to maintain adequate stomatal conductance (g_s) when required; the block temperature was fixed to the ambient conditions at the time of measurement.

Three one-point measurements of A were performed on each individual leaf: (1) a light-saturated one-point measurement at 400 μmol CO_2 mol^{-1} ($A_{sat.400}$); (2) a light-saturated one-point measurement of A at 300 μmol CO_2 mol^{-1} ($A_{sat.300}$); and (3) an $A_{sat.400}$ measurement made after the leaf had achieved steady-state gas exchange and had acclimated to saturating irradiance ($A_{sat.400.Acc}$). Following Measurement 1, the leaf was removed from the leaf chamber while the new C_a was attained; after matching the sample and reference infrared gas analysers, the leaf was reclamped into the cuvette and Measurement 2 was performed. Measurements 1 and 2 were logged at least 30 s after clamping onto the leaf (to allow the leaf chamber to flush out) but no longer than 120 s after clamping, and typically within 60 s when both A and g_s had reached an initial plateau as observed on the instrument strip charts. Following Measurements 1 and 2 and after adjusting C_a back to 400 μmol CO_2 mol^{-1} and matching the infrared gas analysers, the leaf was clamped a third time and allowed to fully acclimate to saturating irradiance for at least 20 min. When steady-state gas exchange had been achieved (A and g_s were stable over a 5–10 min period), Measurement 3 was taken. Following this final one-point measurement, the response of A to C_i was measured as described previously (Rogers, Serbin, et al., 2017) using 14 values of C_a.

Leaf temperature (T_{leaf}) was stable between one-point measurements and A–C_i curves. For *A. latifolia*, T_{leaf} was 15.1 ± 0.62 (*SE*) for $A_{sat.400}$ and 16.3 ± 0.64 (*SE*) during the A–C_i curve (this value denotes the mean temperature during the whole curve). For *H. annuus*, T_{leaf} was 24.3 ± 0.37 (*SE*) for $A_{sat.400}$ and 24.9 ± 0.64 (*SE*) during the A–C_i curve. For *P. canadensis*, T_{leaf} was 28.2 ± 0.27 (*SE*) for $A_{sat.400}$ and 28.1 ± 0.18 (*SE*) during the A–C_i curve. For *P. vulgaris*, T_{leaf} was 27.6 ± 0.33 (*SE*) for $A_{sat.400}$ and 28.7 ± 0.32 (*SE*) during the A–C_i curve. For *Q. coccinea*, T_{leaf} was 22.7 ± 0.73 (*SE*) for $A_{sat.400}$ and 23.1 ± 0.16 (*SE*) during the A–C_i curve. Finally, for *R. sativus*, T_{leaf} was 23.5 ± 0.44 (*SE*) for $A_{sat.400}$ and 22.9 ± 0.27 (*SE*) during the A–C_i curve.

For each species, gas-exchange measurements were performed on two or three consecutive days until the desired number of replicates had been measured, with the exceptions of *A. latifolia* where there was a gap of 1 day between the first and second measurement days, and *R. sativus* where the two measurement days were 1 week apart. In the glasshouse, gas-exchange measurements always took place between 0900 hr and 1400 hr; measurements of *A. latifolia* took place between 0950 hr and 1840 hr (under constant Arctic daylight); measurements of *Q. coccinea* took place between 0720 hr and 1300 hr.

Raw gas exchange data, and the accompanying fitted and estimated values of $V_{c,max}$, are publicly available online for the interested reader (Burnett, Ely, Davidson, Serbin, & Rogers, 2019).

2.3 | Estimation and fitting of $V_{c,max}$

For one-point measurements, $V_{c,max}$ at measurement temperature was estimated using the parameters defined by Bernacchi et al. (2001) and Equation 3 from De Kauwe et al. (2016a, 2016b):

$$V_{c,\max} = \frac{A_{sat}}{\left(\frac{C_i - \Gamma^*}{C_i + K_m} - 0.015\right)}, \tag{1}$$

where K_m is the Michaelis–Menten constant and Γ^* is the CO_2 compensation point in the absence of mitochondrial respiration.

For full A–C_i curves, $V_{c,max}$ was fitted using the parameters defined by Bernacchi et al.; this approach has been described previously

(Bernacchi et al., 2013; Rogers, Serbin, et al., 2017). For *A. latifolia*, the cutoffs used for $V_{c,max}$ and J_{max} were $C_i < 400$ and $C_i > 650$ μmol CO_2 mol^{-1}, respectively, following Rogers et al. (2017). For all other species, the cutoff for $V_{c,max}$ was $C_i < 200$ μmol CO_2 mol^{-1}; the cutoff for J_{max} was $C_i > 500$ μmol CO_2 mol^{-1}. Note that we did not account for the influence of mesophyll conductance on the calculation of $V_{c,max}$ and thus our reported values are "apparent" and are based on C_i rather than chloroplastic CO_2 concentration (C_c). The average root-mean-squared error (RMSE) associated with fitting $V_{c,max}$ was 0.59 (<1% of estimated $V_{c,max}$). The RMSE for individual model fits is available in our supplementary dataset (Burnett et al., 2019).

2.4 | Estimation and measurement of R_{day}

As described previously (De Kauwe et al., 2016b), we used the common assumption that R_{day} is 1.5% of $V_{c,max}$ when estimating $V_{c,max}$ from one-point measurements of *A* (0.015 in Equation 1). To evaluate the effect of pairing a one-point measurement of *A* with an independent measurement of R_{day}, we used the R_{day} determined by the *y*-axis intercept of the A–C_i curve (Bernacchi et al., 2001). This same approach was used by De Kauwe et al. (2016b) in their theoretical analysis of the one-point method, and thus enables the best possible match of a one-point derived $V_{c,max}$ to a fitted $V_{c,max}$. We acknowledge that this estimate of R_{day} does not provide an accurate value of R_{day}, because this estimate is made in the light and will therefore include both respiration and photorespiration; a true estimate of R_{day} would require a period of dark adapation. We also accept that a truly independent estimate of R_{day} may lead to further departure of the one-point derived $V_{c,max}$ from fitted $V_{c,max}$.

2.5 | Synthetic A–C_a and light response curves

Synthetic A–C_a curves were plotted for each species using the parameters of (Bernacchi et al., 2001) and the mean temperature-normalized $V_{c,max}$, mean temperature-normalized J_{max}, mean leaf temperature, and the irradiance within the leaf chamber. We assumed a C_i:C_a ratio of 0.7. $V_{c,max}$ and J_{max} were normalized to 25°C with an Arrhenius equation using the parameters of Bernacchi, Pimentel, and Long (2003) and Bernacchi et al. (2001); leaf temperature and light level were recorded by the gas exchange system.

Light response curves were fitted using a nonrectangular hyperbola in accordance with the parameters described by Posada, Lechowicz, and Kitajima (2009). First, the intial slope of the response curve was fitted; second, the derived initial slope was used to obtain the remaining required parameters; and finally, light response curves were then fitted based on all parameters.

2.6 | Statistical analysis

Data were analysed using two-tailed paired t-tests, comparing the estimated and fitted values of $V_{c,max}$ in each case. The Shapiro–Wilk test of normality of the difference was first performed in order to ensure a normal distribution of the differences within each pair of values, to meet the assumption of the t-test. When these differences were not normally distributed (in two cases only), a paired Mann–Whitney test was used. All analysis was performed using the software package R (R Core Team, 2018).

3 | RESULTS

3.1 | Nonacclimated one-point measurements yield poor estimates of $V_{c,max}$ in four of six species

One-point estimates of $V_{c,max}$ obtained from $A_{sat.400}$ one-point measurements matched the fitted $V_{c,max}$ values (estimated from A–C_i curves) in only two of six species, *P. canadensis* and *P. vulgaris*. For *A. latifolia*, *H. annuus*, *Q. coccinea*, and *R. sativus*, $A_{sat.400}$ one-point measurements did not provide accurate estimates of $V_{c,max}$; the estimated values were 21%, 22%, 32%, and 28% lower than the fitted values (Figure 1, Table 1).

3.2 | RuBP saturation can be important for estimating $V_{c,max}$

One-point estimates of $V_{c,max}$ obtained from $A_{sat.300}$ one-point measurements were not significantly different from fitted values in *Q. coccinea*, *P. canadensis*, and *P. vulgaris* but $A_{sat.300}$ one-point measurements still did not provide an accurate estimate of fitted $V_{c,max}$ for *A. latifolia*, *H. annuus*, or *R. sativus* (Figure 2, Table 1), which yielded estimates that were 25%, 28% and 22% lower than fitted $V_{c,max}$ values. Synthetic A–C_a curves (Figure 3) for each species reveal that for the majority of species in this study, photosynthesis was RuBP-saturated (and Rubisco-limited) at both 300 and 400 μmol CO_2 mol^{-1}, meaning that no difference in the one-point estimates

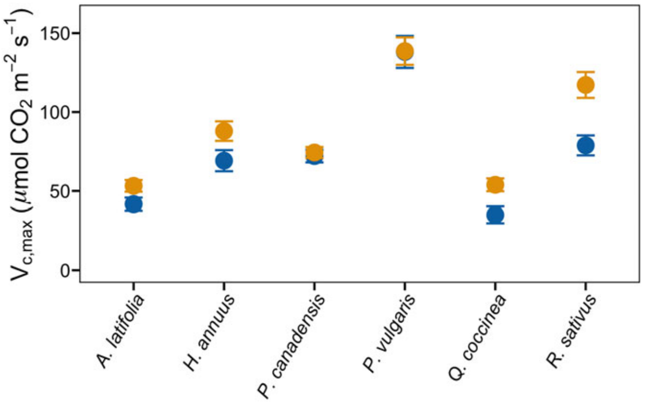

FIGURE 1 Maximum carboxylation efficiency of photosynthesis at measurement temperature ($V_{c,max}$) ± *SE* fitted from A–C_i curves (net photosynthesis, *A*, versus intercellular CO_2 concentration, C_i; gold points) and estimated from one-point measurements of non-light-acclimated photosynthesis at 400 μmol CO_2 mol^{-1} and saturating light ($A_{sat.400}$, dark blue points) in *Arctagrostis latifolia*, *Helianthus annuus*, *Populus canadensis*, *Phaseolus vulgaris*, *Quercus coccinea*, and *Raphanus sativus*

TABLE 1 Results of two-tailed paired t-tests and Mann–Whitney tests, comparing maximum carboxylation efficiency of photosynthesis ($V_{c,max}$) estimated from one-point measurements with paired fitted $V_{c,max}$ values derived from a CO_2 response curve (A–C_i curve)

	Type of one-point measurement used to estimate $V_{c,max}$			
	$A_{sat.400}$ (Figure 1)	$A_{sat.300}$ (Figure 2)	$A_{sat.400.Acc}$ (Figure 5)	$A_{sat.400.Acc}$ + R_{day} (Figure 6)
Arctagrostis latifolia	$p < .01$ (U = 169, n = 19)	$p < .001$ (t = 5.4, n = 20)	$p < .001$ (t = 8.9, n = 20)	$p < .01$ (U = 25, n = 20)
Helianthus annuus	$p < .001$ (t = 4.4, n = 14)	$p < .01$ (t = 4.1, n = 15)	ns (t = −1.6, n = 15)	ns (t = −1.2, n = 15)
Populus canadensis	ns (t = 0.82, n = 16)	ns (t = −1.2, n = 16)	ns (t = 0.50, n = 16)	ns (t = −1.6, n = 16)
Phaseolus vulgaris	ns (t = 0.080, n = 20)	ns (t = −0.24, n = 20)	$p < .001$ (t = −5.4, n = 20)	$p < .001$ (t = −6.7, n = 20)
Quercus coccinea	$p < .05$ (t = 2.7, n = 9)	ns (t = 1.0, n = 10)	ns (t = 1.9, n = 10)	$p < .01$ (t = −3.3, n = 10)
Raphanus sativus	$p < .001$ (t = 4.7, n = 15)	$p < .01$ (t = 3.9, n = 15)	$p < .05$ (t = −2.3, n = 15)	ns (t = 0.94, n = 15)

Note. Paired t-tests were performed in all cases except for *A. latifolia* $A_{sat.400}$ and $A_{sat.400.acc}$ + R_{day} (Figures 1 and 6) where a Mann–Whitney test was required. A significant ($p < .05$) difference between the estimated and fitted values of $V_{c,max}$ indicates that the one-point method provides an estimate of $V_{c,max}$ that is significantly different from the value of $V_{c,max}$ determined from the initial slope of a photosynthetic CO_2 response curve (A–C_i curve). One-point measurements used to estimate $V_{c,max}$ were made at saturating irradiance and 400 µmol mol^{-1} ($A_{sat.400}$), at saturating irradiance and 300 µmol mol^{-1} ($A_{sat.300}$), and at saturating irradiance and 400 µmol mol^{-1} following full acclimation to saturating irradiance ($A_{sat.400.Acc}$). In addition, $V_{c,max}$ was also estimated using the $A_{sat.400.Acc}$ one-point measurement and the R_{day} derived from the A–C_i curve ($A_{sat.400.Acc}$ + R_{day}).

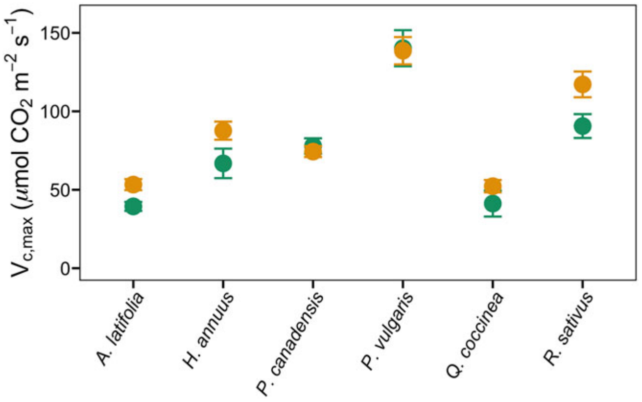

FIGURE 2 Maximum carboxylation efficiency of photosynthesis at measurement temperature ($V_{c,max}$) ± *SE* fitted from A–C_i curves (net photosynthesis, A, vs. intercellular CO_2 concentration, C_i; gold points) and estimated from one-point measurements of nonlight-acclimated photosynthesis at 300 µmol CO_2 mol^{-1} and saturating light ($A_{sat.300}$, green points) in *Arctagrostis latifolia*, *Helianthus annuus*, *Populus canadensis*, *Phaseolus vulgaris*, *Quercus coccinea*, and *Raphanus sativus* [Colour figure can be viewed at wileyonlinelibrary.com]

of $V_{c,max}$ would be expected between the $A_{sat.400}$ and $A_{sat.300}$ one-point measurements. However, for *Q. coccinea*, the inflection point of the A–C_a curve falls between 300 and 400 µmol CO_2 mol^{-1} (Figure 3e), meaning that only the $A_{sat.300}$ one-point measurement will yield a reliable RuBP-saturated measurement of A.

3.3 | Light acclimation and measured R_{day} can be important for estimating $V_{c,max}$

Figure 4 shows the response of photosynthesis to irradiance for each species. The ambient light level at the time when the $A_{sat.400}$ and $A_{sat.300}$ one-point measurements were made is indicated by the vertical grey bar. The saturating irradiance used for the one-point measurements and A–C_i curves is shown with an asterisk. Figure 4 clearly shows that the $A_{sat.400}$ and $A_{sat.300}$ one-point measurements were made on leaves that were acclimated to an irradiance that was well below the saturating irradiance used for the one-point measurements. When one-point measurements included time for acclimation to saturating irradiance ($A_{sat.400.Acc}$), the $A_{sat.400.Acc}$ one-point estimates of $V_{c,max}$ closely matched the fitted $V_{c,max}$ derived from A–C_i curves. Notably, the $A_{sat.400.Acc}$ one-point estimation for *H. annuus* (Figure 5, Table 1) was not significantly different from the fitted values, demonstrating that light acclimation was critical for successful application of the one-point method in *H. annuus*. Overall, light acclimation markedly improved the accuracy of estimated $V_{c,max}$. In Figure 1, $V_{c,max}$ estimated from one-point ($A_{sat.400}$) measurements as a percentage of fitted $V_{c,max}$ was 79%, 78%, 97%, 101%, 68%, and 72% for *A. latifolia*, *H. annuus*, *P. canadensis*, *P. vulgaris*, *Q. coccinea*, and *R. sativus*, respectively. When one-point measurements were light-acclimated ($A_{sat.400.Acc}$), one-point estimates of $V_{c,max}$ as a percentage of fitted $V_{c,max}$ were 91%, 102%, 99%, 106%, 96%, and 104%, respectively.

One-point estimates of $V_{c,max}$ that were derived from the combination of $A_{sat.400.Acc}$ one-point measurements of photosynthesis and measured R_{day} (from the A–C_i curve measured on each individual plant) were not significantly different from fitted $V_{c,max}$ in *H. annuus*, *P. canadensis*, and *R. sativus* (Figure 6, Table 1). The mean $V_{c,max}$ estimated using this approach was remarkably close to the mean fitted $V_{c,max}$, that is, values were 103%, 101%, 103%, 105%, 107%, and 102% of fitted $V_{c,max}$ for *A. latifolia*, *H. annuus*, *P. canadensis*, *P. vulgaris*, *Q. coccinea*, and *R. sativus*, respectively. For *A. latifolia*, even though the values of estimated and fitted $V_{c,max}$ were statistically different using all one-point approaches tested here, the use of light acclimation plus measured R_{day} provides the numerically closest approximation of fitted $V_{c,max}$, with a deviation of only 3%.

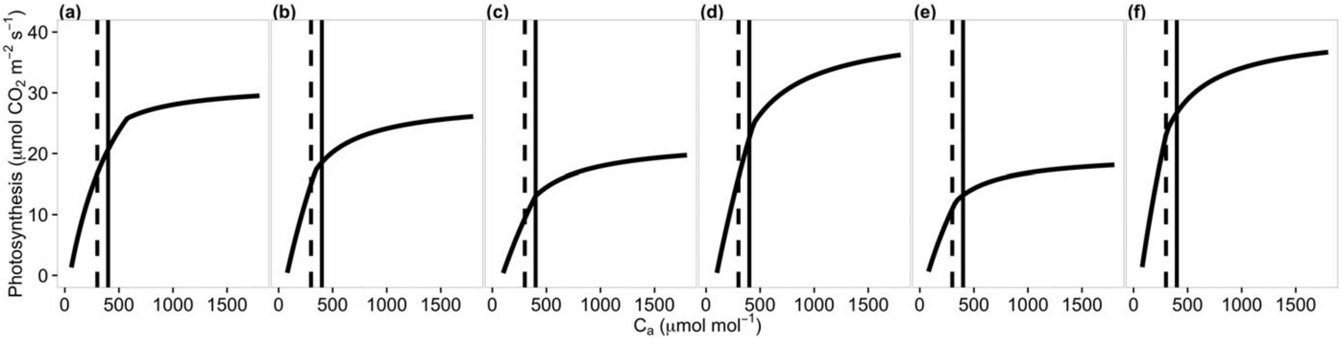

FIGURE 3 Synthetic A–C_a curves (net photosynthesis, A, vs. ambient CO_2, C_a) for (a) *Arctagrostis latifolia*, (b) *Helianthus annuus*, (c) *Populus canadensis*, (d) *Phaseolus vulgaris*, (e) *Quercus coccinea*, and (f) *Raphanus sativus*. Solid and dashed vertical lines indicate C_a values of 400 and 300 μmol CO_2 mol^{-1}, respectively. A–C_a curves—rather than A–C_i curves (A vs intercellular CO_2 concentration, C_i)—are plotted here in order to facilitate the understanding of the CO_2 concentration used for one-point measurements in the context of the curve

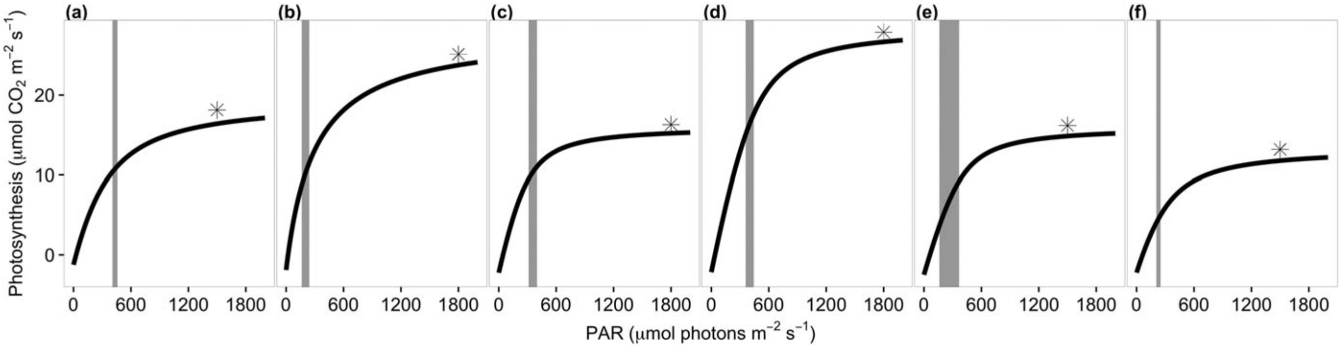

FIGURE 4 Light response curves for (a) *Arctagrostis latifolia*, (b) *Helianthus annuus*, (c) *Populus canadensis*, (d) *Phaseolus vulgaris*, (e) *Quercus coccinea*, and (f) *Raphanus sativus*. Grey bars show the ambient light level (mean ± *SE*) measured by the LI-6400XT at the time that nonacclimated one-point measurements of photosynthesis at 400 and 300 μmol CO_2 mol^{-1} (Figures 1 and 2) were performed. Asterisks show the saturating irradiance determined from the curves, used to establish the settings in the LI-6400XT leaf cuvette for all one-point measurements and A–C_i curves (net photosynthesis, A, vs. intercellular CO_2 concentration, C_i)

3.4 | The relative impact of using measured values of R_{day} is greatest in wild species

A. latifolia has the greatest absolute value of R_{day} of the species in this study: 3.1 μmol CO_2 m^{-2} s^{-1} ± 0.1 (*SE*), compared with 1.1–1.8 μmol CO_2 m^{-2} s^{-1} ± 0.1–0.2 (*SE*; range for all other species). Notably, when expressed as a fraction of $V_{c,max}$, R_{day} is greater for *A. latifolia* and *Q. coccinea* than for the glasshouse-grown species, with values of 0.06 ± 0.005 (*SE*) and 0.03 ± 0.006 (*SE*), respectively, compared with 0.01–0.02 ± 0.001–0.002 (*SE*; range for all other species). When compared with the assummed R_{day} fraction of 0.015 (Equation 1), the R_{day} fraction measured here is four times larger for *A. latifolia* and two times larger for *Q. coccinea*. Although the number of wild species examined in this study is small, these data offer a possible explanation as to why the inclusion of measured R_{day} leads to a greater change in one-point estimated $V_{c,max}$ in these species (increases of 12% and 11%, respectively) when compared with the the 1%–4% change in other species (when $V_{c,max}$ estimated from $A_{sat.400.acc}$ plus R_{day} is compared with $V_{c,max}$ estimated from $A_{sat.400.acc}$; Figure 6 cf. Figure 5).

4 | DISCUSSION

In this study, we show that the effectiveness of the one-point method for reliably estimating $V_{c,max}$, with reference to the gold-standard $V_{c,max}$ values fitted from A–C_i curves, is not universal. The success of the one-point method can depend upon (a) ensuring that the one-point measurement is taken under conditions where A is RuBP-saturated, (b) ensuring light acclimation, and (c) measuring R_{day}, with the exact requirement varying between species. In some species, a one-point measurement of A made at current C_a under saturating light without acclimation to saturating irradiance ($A_{sat.400}$), can be used to successfully estimate fitted $V_{c,max}$. This was true for two of six species in this study, *P. canadensis* and *P. vulgaris*. For all species, ensuring RuBP saturation of A was important, and this meant that a one-point measurement of A made at subambient C_a was required for successful estimation of fitted $V_{c,max}$ in *Q. coccinea*. When an $A_{sat.400}$ measurement does not give an accurate estimate of $V_{c,max}$, and RuBP saturation of photosynthesis is ensured, acclimation to saturating irradiance can eliminate the discrepancy between one-point estimates of $V_{c,max}$ and fitted $V_{c,max}$, as seen in *H. annuus* ($A_{sat.400.Acc}$).

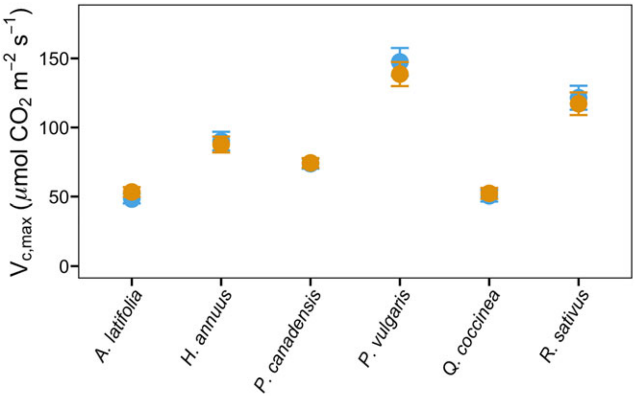

FIGURE 5 Maximum carboxylation efficiency of photosynthesis at measurement temperature ($V_{c,max}$) ± *SE* fitted from *A*–C_i curves (net photosynthesis, *A*, vs. intercellular CO_2 concentration, C_i; gold points) and estimated from one-point measurements of light-acclimated photosynthesis at 400 μmol CO_2 mol^{-1} and saturating light ($A_{sat.400.Acc}$, light blue points) in *Arctagrostis latifolia*, *Helianthus annuus*, *Populus canadensis*, *Phaseolus vulgaris*, *Quercus coccinea*, and *Raphanus sativus* [Colour figure can be viewed at wileyonlinelibrary.com]

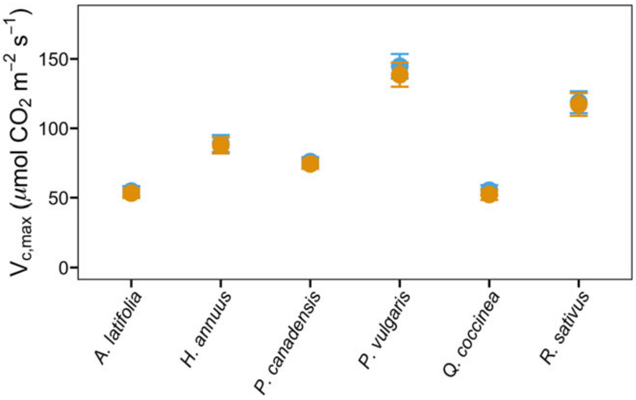

FIGURE 6 Maximum carboxylation efficiency of photosynthesis at measurement temperature ($V_{c,max}$) ± *SE* fitted from *A*–C_i curves (net photosynthesis, *A*, vs. intercellular CO_2 concentration, C_i; gold points) and estimated from one-point measurements of light-acclimated photosynthesis at 400 μmol CO_2 mol^{-1} and saturating light using measured values of daytime respiration (R_{day}) for each individual ($A_{sat.400.Acc}$ and R_{day}, light blue points) in *Arctagrostis latifolia*, *Helianthus annuus*, *Populus canadensis*, *Phaseolus vulgaris*, *Quercus coccinea*, and *Raphanus sativus* [Colour figure can be viewed at wileyonlinelibrary.com]

For another species, *R. sativus*, acclimation to saturating irradiance was only successful in matching fitted $V_{c,max}$ when used with measured R_{day}.

The one-point method, using a non-light-acclimated measurement of photosynthesis, does not accurately predict fitted $V_{c,max}$ in all species, with marked and significant underestimations revealed between one-point estimates and fitted values for four out of six species (Figure 1, Table 1). One reason for the difference between species could be that not all species had Rubisco-limited photosynthesis at 400 μmol CO_2 mol^{-1}. If this were the case, accurate estimation of $V_{c,max}$ from one-point measurements would be impossible (De Kauwe et al., 2016b), because the 400 μmol CO_2 mol^{-1} measurement would not fall on the RuBP-saturated initial slope of the *A*–C_i curve. To test this, we repeated the one-point measurement on each plant at 300 μmol CO_2 mol^{-1} (Figure 2, Table 1) and saw that the discrepancy between estimated and fitted $V_{c,max}$ was resolved for *Q. coccinea*. This indication is confirmed by Figure 3, which shows that the inflection point of the *A*–C_a curve lies just below a C_a of 400 μmol CO_2 mol^{-1} (Figure 3e). This means that *A* in *Q. coccinea* is not strictly RuBP-saturated at 400 μmol CO_2 mol^{-1}, violating a key condition necessary for the successful application of the one-point method. The $A_{sat.300}$ one-point measurement ensured RuBP-saturated *A*. However, ensuring RuBP saturation did not lead to accurate estimations of $V_{c,max}$ for all species, showing that this criterion is necessary but not sufficient for accurate estimation of $V_{c,max}$ using the one-point method.

The second factor we investigated as a possible cause for the difference between one-point estimates of $V_{c,max}$ and fitted $V_{c,max}$ seen both in Figure 1 and in Figure 2 was exposure to saturating irradiance prior to the one-point measurement. When light-acclimated one-point measurements were performed, the one-point method was an effective proxy for an *A*–C_i curve for three of six species (Figure 5, Table 1). The poor estimation of $V_{c,max}$ for *H. annuus* seen in Figures 1 and 2 was markedly and significantly improved, that is, $A_{sat.400.Acc}$-estimated $V_{c,max}$ is not significantly different from the fitted value of $V_{c,max}$. This shows that light acclimation is the key factor required for effective use of the one-point method in this species. Light is essential for photosynthesis, and the amount of light that reaches a leaf has strong effects on its photosynthetic rate. In response to light, stomata open (Lawson & Blatt, 2014; Matthews, Vialet-Chabrand, & Lawson, 2018) and Rubisco is activated, aided by the chaperone protein Rubisco activase (Portis, 2003; Salvucci, Portis, & Ogren, 1985, 1986). At subsaturating irradiance these processes of photosynthetic induction may not be fully engaged, leading to submaximal photosynthetic rates that translate to poor one-point estimates of $V_{c,max}$ that are below the true values. Here, in *H. annuus*, we found that accurate use of the one-point method was highly dependent on the light environment (Figures 1 and 5, Table 1).

Third, following De Kauwe et al. (2016b), we investigated the use of measured R_{day} and found that including measured values of R_{day} when estimating $V_{c,max}$, coupled with light acclimation, resolved the discrepancy between estimated and fitted $V_{c,max}$ for *R. sativus* (Figure 6, Table 1). Overestimation of R_{day} leads to overestimation of $V_{c,max}$ and vice versa, as seen in Equation 1 and illustrated in Figure 1 of De Kauwe et al. (2016b). Significant discrepancies between estimated and fitted $V_{c,max}$ in this study show both underestimation and overestimation of $V_{c,max}$ by the one-point method (Figures 1, 2, and 5), meaning that the error in estimated R_{day} is not unidirectional.

Not all differences between species could be easily explained by the factors we tested in this study. Although ensuring RuBP saturation, allowing time for acclimation to saturating light, and measuring R_{day} were able to resolve discrepancies between estimated and fitted $V_{c,max}$ in some cases, it is interesting to note that nonacclimated one-point measurements consistently gave an accurate estimate of

$V_{c,max}$ for *P. canadensis* regardless of these factors (Figures 1, 2, 5, and 6, Table 1). In contrast, for *A. latifolia*, the one-point method (with all adjustments tested) never yielded a value that was not statistically significantly different from the fitted value of $V_{c,max}$, albeit with a small deviation (3% in Figure 6).

A rapid one-point measurement, without light acclimation, generally resulted in a large discrepancy between one-point estimated $V_{c,max}$ and fitted $V_{c,max}$ for the species investigated in our study ($A_{sat.400}$ and $A_{sat.300}$, Figures 1 and 2). For a closer approximation of fitted $V_{c,max}$, light acclimation is required; the values of estimated $V_{c,max}$ are much closer to those of fitted $V_{c,max}$ in Figures 5 and 6 than in Figures 1 and 2, although there are still small but statistically significant differences between the estimated and fitted values (Table 1). Although differences between estimated and fitted values of $V_{c,max}$ were not fully resolved by light acclimation, irradiance was still the biggest driver of the discrepancy in terms of absolute values (Figures 1 and 2 cf. Figure 5) showing a clear physiological impact of acclimation to saturating irradiance on the effectiveness of the one-point method for reliably estimating $V_{c,max}$ and establishing irradiance as the primary physiological factor affecting the quality of approximations of $V_{c,max}$ obtained using the one-point method in many species.

Full consideration of the practical application of the one-point method was beyond the scope of the theoretical evaluation of the one-point method presented by De Kauwe et al. (2016b). The important new finding presented here is identification of the importance of acclimation to saturating irradiance for the successful estimation of $V_{c,max}$ using the one-point method. This has not been tested previously because all one-point data used in the analysis of De Kauwe et al. (2016b) were obtained from A–C_i curves performed on light-acclimated plants. For the first time, we demonstrate that saturating irradiance has a marked impact on the effectiveness of the one-point method. The importance of RuBP saturation and accurate estimation of R_{day} asserted by De Kauwe et al. (2016b) are borne out by our analysis, which shows RuBP saturation to be a necessary factor for successful estimation of $V_{c,max}$ and reveals measured R_{day} as a factor that reduces the discrepancy between estimated and fitted values of $V_{c,max}$. Our data are therefore consistent with the broad conclusions drawn by De Kauwe et al. (2016b). However, our analysis reveals that the strong physiological impact of acclimation to saturating irradiance (which has a large effect in terms of the magnitude of the difference between estimated and fitted values of $V_{c,max}$) makes this a critical factor for using the one-point method, and one that should not be overlooked.

In practical terms, if light acclimation and measurement of R_{day} are required in order to obtain an accurate one-point estimate of $V_{c,max}$, performing a full A–C_i curve may be the most efficient way to obtain $V_{c,max}$, due to the time investment already required for light acclimation (approximately 30 min). The rapid A–C_i response technique (Stinziano et al., 2017) allows measurement of R_{day} but leaves are also not typically given time to acclimate to saturating light prior to measurement, so it is likely that this technique may also be unable to provide an accurate estimate of $V_{c,max}$ in some species.

A final consideration is the experimental setting. For wild or field-grown species, there may be a greater requirement for measurement of R_{day}, as revealed by the greater R_{day} fraction (up to six-fold higher) in *A. latifolia* and *Q. coccinea* compared with the glasshouse-grown species, accentuating the benefit of performing an A–C_i curve. A thorough test of this hypothesis will require reciprocal field and glasshouse experiments, and an expansion of the number of wild species included in the study. A possible explanation based on the trends seen in our dataset is that the burden of maintenance respiration is likely greater for wild species due to the higher requirement for secondary metabolism in the field setting (Penning de Vries, 1975; Ramakrishna & Ravishankar, 2011). Thus, deriving estimates of $V_{c,max}$ from one-point measurements of photosynthesis—either by generating novel experimental data or by mining databases of photosynthetic data—may not lead to accurate values of $V_{c,max}$, especially in field-grown plants, which comprise the vegetation of interest for ESMs.

In conclusion, the one-point method can be a valuable tool for obtaining quick and accurate estimates of $V_{c,max}$, but must be used with caution because the effectiveness of this method may require light acclimation and measurement of R_{day}, depending on the species of interest. In addition, if photosynthesis is not RuBP-saturated, one-point measurements will provide inaccurate estimates of $V_{c,max}$. If used blindly, the one-point method can cause significant underestimation of $V_{c,max}$—which could in turn lead to suboptimal parameterization of models. We urge all researchers wishing to employ the one-point method to investigate whether their species of interest requires acclimation to saturating light, a measurement at C_a below current ambient C_a, or measurement of R_{day}, in order for the one-point method to be used with confidence.

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AUTHOR CONTRIBUTIONS

A. C. B. and A. R. designed and planned the research. A. C. B. and K. J. D. performed the glasshouse research; K. J. D. and A. R. performed the field research in Alaska; A. C. B., K. J. D., S. S., and A. R. performed the field research at Brookhaven National Laboratory. All authors contributed to data analysis. The manuscript was written by A. C. B. with contributions from all authors.

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ORCID

Angela C. Burnett https://orcid.org/0000-0002-2678-9842

Kenneth J. Davidson https://orcid.org/0000-0001-5745-9689
Shawn P. Serbin https://orcid.org/0000-0003-4136-8971
Alistair Rogers https://orcid.org/0000-0001-9262-7430

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