

ORIGINAL ARTICLE OPEN ACCESS

Temperature Acclimation of Chlorophyll Fluorescence and Carboxylation Capacity From Near-Instantaneous to Weekly Time Scales

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Received: 25 September 2025 | **Revised:** 12 March 2026 | **Accepted:** 14 April 2026

Funding: Schmidt Sciences; National Science Foundation, Grant/Award Number: DEB-2045968

Keywords: Φ PSII | carboxylation | $J_{\max}$ | photosystem II | quantum yield | $V_{\max}$

ABSTRACT

We studied near-instantaneous temperature responses of light-saturated photosynthesis (A_{sat}) and the operating efficiency of photosystem II (Φ PSII) in relation to acclimation of photosynthetic biochemistry to temperature at the timescale of 1–8 days. We grew two closely related *Brassica oleracea* L. accessions under diurnal temperature ranges of 15°C–25°C and 5°C–15°C, representing the warm and cool native environments of the two accessions. Near-instantaneous temperature responses of A_{sat} and Φ PSII were measured on acclimated plants exposed to a temperature ramp from 10°C to 45°C. Biochemical acclimation was quantified by switching plants between warm and cool regimes before measuring photosynthetic variables at Day 1 and Day 8 after the switch. Near-instantaneous temperature optima of A_{sat} and Φ PSII functionally shifted towards the acclimated growth temperatures in both accessions, and both accessions achieved their highest instantaneous maximum values of A_{sat} and Φ PSII when grown under representative native regimes. Differences in maximum carboxylation capacity were evident by Day 1; no further change was detected by Day 8. Our results highlight functional coordination between adaptation, acclimation and near-instantaneous temperature responses of photochemistry and biochemistry. Moreover, our results suggest photosynthetic biochemistry can acclimate to temperature changes within a day.

1 | Introduction

Plants exhibit genotypic adaptation of the photosynthetic apparatus to the temperature regimes they evolved under, and many species also undergo phenotypic thermal acclimation of photosynthetic components over shorter timeframes (Berry and Bjorkman 1980; Miyazawa and Kikuzawa 2006; Kattge and Knorr 2007; Yamori et al. 2014; Kumarathunge et al. 2019). The mechanisms responsible for the biochemical adaptation and acclimation of net photosynthesis to temperature relate to changes in the quantity and reaction rate of the enzymes that perform photosynthesis and respiration, adjustment to the pools

of carbon cycle intermediates and, for C_3 species, shifts in the solubility ratio and enzyme specificity of CO_2 and O_2 , which leads to changes in the degree of inhibition of photosynthesis due to photorespiration (Hall and Keys 1983; Brooks and Farquhar 1985; Salisbury and Ross 1985; Campbell et al. 2007; Sage and Kubien 2007; Yamori et al. 2014; Dusenège et al. 2019).

There is also evidence of photochemical temperature acclimation specific to the whole-chain electron transport rate (ETR) and the photosystem II (PSII) ETR (Mitchell and Barber 1986; Mawson and Cummins 1989; Yamori et al. 2008; Vico et al. 2019). However, there is little specific empirical

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knowledge on the timescales at which biochemical and photochemical processes respond to temperature change. Given the current rate of atmospheric warming owing to human activities (IPCC 2022) and the increased risk of temperature extremes associated with this warming (Coumou and Robinson 2013, Viovy et al. [forthcoming](#)), it is imperative that the processes underlying photosynthetic temperature adaptation and acclimation are resolved further.

Despite theoretical advances and empirical observations of the instantaneous temperature response of net photosynthesis, we are yet to fully understand the processes and timeframes underlying thermal adaptation and acclimation. Take, for example, the maximum carboxylation rate of ribulose-1,5-bisphosphate (RuBP) by ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) (termed V_{cmax}). The acclimation of V_{cmax} to the environment is achieved by altering the form and amount of Rubisco in the leaf, and the half-life of Rubisco is approximately 1 week (Simpson et al. 1981). Based on this half-life, an acclimation timeframe of 1–2 weeks for V_{cmax} is conceivable (Yamori et al. 2006; Smith and Dukes 2017; Ren et al. 2024). However, the specific site for the temperature inhibition of Rubisco is Rubisco activase (Feller et al. 1998; Haldimann and Feller 2004), and some forms of this enzyme are able to respond to heat stress within 4 h (Law and Crafts-Brandner 2001; Yamori et al. 2014). Furthermore, Scafaro et al. (2023) effectively modelled the acclimation response of photosynthesis to high temperature by accounting for Rubisco deactivation, a process that can occur on a timeframe of minutes to hours. These potential differences point to the need for further research to help establish broad empirical support for the acclimation timeframe of photosynthesis, and for Rubisco activity in particular. Because the rate-limiting steps of photosynthesis can shift from Rubisco activity to electron transport depending on temperature, it is essential to evaluate both processes together.

Theory and empirical data suggest that the potential rate of electron transport for the regeneration of RuBP (J_{max}) scales in a predictable fashion with V_{cmax} (Wullschlegel 1993; Medlyn et al. 2002; Kattge et al. 2009). Nonetheless, despite this general scaling relationship, J_{max} and V_{cmax} are differentially sensitive to temperature (Medlyn et al. 2002; Kattge and Knorr 2007). Given the dependency of the regeneration of RuBP on products of the electron transport chain (namely, ATP and NADPH), it is likely that, despite the need for overall coordination, the thermal limitation and acclimation capacity of electron transport differ from Rubisco activity. In some situations, this difference may yield unrealistic predictions from photosynthetic models that focus on the mechanistic expression of Rubisco, such as the seminal Farquhar, von Caemmerer and Berry (FvCB) model of C_3 photosynthesis (Farquhar et al. 1980). This has been addressed mechanistically for Rubisco (Collatz et al. 1991) and empirically for electron transport via chlorophyll fluorescence (van der Tol et al. 2014), but the predictive range of these model variants is limited.

An important chlorophyll fluorescence parameter to help probe the performance of the electron transport system in relation to the environment is the operating efficiency of PSII photochemistry, termed ΦPSII (Genty et al. 1989; Maxwell and Johnson 2000). ΦPSII represents the proportion of light absorbed by the leaf that is used in PSII photochemistry, which can be resolved to the PSII ETR (Murchie and Lawson 2013). The PSII ETR may be downregulated due to photodamage to

the reaction centres or as a result of non-photochemical quenching, which can occur at temperature extremes (Schreiber and Berry 1977; Berry and Bjorkman 1980; Porcar-Castell 2011); these are processes that can reduce net photosynthesis (Havaux 1992; Oberhuber and Edwards 1993). However, this effect may be partly offset by greater whole-chain ETR owing to increased fluidity of the thylakoid membranes with increasing temperature (Yamori et al. 2008). Furthermore, at moderately high temperatures, PSII can rapidly acclimate, with response times on the order of 30 min to 2 h (Havaux 1993; Zhu et al. 2024). Despite this level of mechanistic understanding, the interrelationship of the temperature response of electron transport and photosynthetic biochemistry, and its effect on net photosynthesis, has not been fully resolved. Moreover, in general, the temperature response and potential for acclimation of ΦPSII are not considered when estimating V_{cmax} and J_{max} , such as within the FvCB model framework, as it is usually assumed to be constant. This assumption may affect the predictive capability of crop models and land surface models, particularly when photosynthesis is estimated above or below the thermal optima (Rogers et al. 2017).

Although there is a widely accepted scheme for the adaptation and acclimation of the light-saturated net photosynthetic rate (A_{sat}) in relation to thermal regimes, there is currently no equivalent scheme for ΦPSII . Using two closely related *Brassica oleracea* L. accessions, we address this knowledge gap, and test for coordination between the thermal acclimation of A_{sat} and ΦPSII . Furthermore, using the same accessions, we also test the generalised assumption that carboxylation capacity acclimates to a new thermal regime on a timescale of approximately 1 week. To our knowledge, this is the first systematic empirical test of the acclimation timeframe of V_{cmax} in relation to temperature. Our specific Hypotheses were that (1) both ΦPSII and A_{sat} will functionally adapt and acclimate to thermal regimes, and the acclimation response of these two variables will be well coordinated, and (2) consistent with the half-life of Rubisco, the acclimation timeframe of V_{cmax} will be approximately 1 week.

To address these hypotheses, we measured the near-instantaneous temperature response of net photosynthesis and ΦPSII in plants acclimated to the experimental warm and cool temperature regimes. We define the ‘near-instantaneous’ responses as those occurring within a timeframe of minutes. Moreover, our experimental setup and measurement regime considered the temperature acclimation timescale of V_{cmax} and J_{max} to an instantaneous switch between warm and cold temperature regimes.

2 | Materials and Methods

2.1 | Plant Material

Seeds of two *B. oleracea* L. accessions were sourced from a genetic population development programme overseen by the Commonwealth Scientific and Industrial Research Organisation in Perth, Western Australia. Accession CAF006 originates from Zimbabwe (Chikukwa Commune, Manicaland). The mean maximum daily temperature and the maximum daily temperature record for the period 1975 to 2024 at a representative weather station (Chimoio, Zimbabwe: -19.117° , 33.467°) were 26.7°C and 40.5°C , respectively (NOAA 2024). Based on its

origin and the observation that this accession flowers without vernalisation, CAF006 is regarded as being adapted to relatively warm climates and is henceforth referred to as 'CAF-Warm'. Accession CAF0216 originates from Spain, near La Coruña in the Galicia region. The mean maximum daily temperature and the maximum daily temperature record for the period 1975 and 2024 at a representative weather station (A Coruña, Spain: 43.3669°, -8.4192°) were 17.9°C and 34.4°C, respectively (NOAA 2024). Accession CAF0216 flowers only after vernalisation and is regarded as typical of wild *B. oleracea*, which are generally adapted to cool climates, require vernalisation and are biennial. Based on this information, accession CAF0216 is referred to as 'CAF-Cool' hereon. The advantage of selecting these closely related accessions originating from different climates is to remove potential ambiguities associated with phylogenetic effects.

2.2 | Growth Rooms and Temperature Regimes

Plants were germinated and grown in two controlled environmental rooms. During the growth phase of the experiments described below, plants experienced two air temperature (T_{air}) regimes: 15°C:25°C (night:day), termed the 'H' regime, and 5°C:15°C (night:day), termed the 'L' regime. All other variables of the growth rooms were identical (see details below). Within each growth room, plants were grown in a completely randomised design, and the plants were re-randomised each week. Notwithstanding differences in air temperature regimes, the environmental conditions of the growth rooms were approximately equivalent (Figure S1). However, the temperature effects observed in our study are limited to the conditions of the present growth room settings.

Experiment 1. Near-instantaneous temperature responses of ΦPSII and A_{sat}

Seeds of both *B. oleracea* accessions were germinated on moist filter paper in petri dishes under the following conditions: atmospheric CO_2 (C_a): ambient (400–420 $\mu\text{mol mol}^{-1}$), T_{air} : 15°C:25°C (night:day), daytime photosynthetically active radiation (PAR): 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$, night:day photoperiod: 14:10 h, relative humidity (RH): set to be > 50% (although the RH inside the petri dishes was close to 100%)—the actual range in RH is shown in Figure S1. The day–night temperature transitions occurred at a rate of 4 min/1°C of change in T_{air} . One week after germination, 10 seedlings from each accession were transferred to 2.8 L pots filled with an organic potting mix (50% river sand, 25% pine bark, 25% peat moss) and well-watered to encourage establishment (all other environmental variables were the same as during germination). After 1 week of establishment in the pots, half of the plants from each accession were transferred to a T_{air} of 5°C:15°C (night:day), that is, the L regime, all other environmental variables were equivalent to those of the establishment phase. Thus, for each accession, five plants were grown in the 15°C:25°C growth temperature treatment (H regime) and five plants were grown in the 5°C:15°C growth temperature treatment (L regime). Note that by holding RH at or above 50% and varying T_{air} , the atmospheric vapour pressure deficit (VPD, kPa) of growth rooms differed. For example, the average daytime VPD of 15°C:25°C room was 1.4 kPa, whereas the average daytime VPD of the 5°C:15°C room was 0.5 kPa.

Each week, 50 mL of a commercial complete fertiliser (Thrive, All Purpose Soluble Fertiliser, N:P:K 25:5:8.8) was applied to each pot. The first application was at a concentration of 1.5 g L⁻¹; subsequent applications were at a concentration of 3 g L⁻¹. Plants were well-watered under these conditions for 21 days of growth and 41 days of growth for the 15°C:25°C and 5°C:15°C growth temperature treatments, respectively, before gas exchange and fluorescence measurements were implemented. The additional time for growth in the 5°C:15°C group ensured that all plants within an accession were approximately the same height prior to measurements (12–15 cm for CAF-Warm and 10–14 cm for CAF-Cool). For plants of each temperature regime, a fully expanded leaf that was three or four leaves back from the apex was selected for gas exchange measurements. The experimental approach is outlined in Figure 1.

2.3 | The Operating Efficiency of Photosystem II and Leaf Gas Exchange

The operating efficiency of photosystem II (ΦPSII) was measured on each plant ($n = 1$ leaf/plant) with a pulse-amplitude modulated fluorometer (Model LI-6800, LI-COR Environmental, USA). Simultaneous measurements of the steady-state light-saturated photosynthetic rate (A_{sat} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance to water vapour (g_{sw} , $\text{mol H}_2\text{O mol}^{-1} \text{air}$) were taken with the same instrument, with ΦPSII and A_{sat} measured after g_{sw} had stabilised. The following fluorometer settings were implemented: dark modulation rate: 500 Hz, light modulation rate 1 kHz, flash modulation rate 250 kHz, averaging time: 15 s, setpoint: 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, rectangular flash, red target: 8000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and duration: 1000 ms. The environmental variables of the leaf chamber during which g_{sw} stabilised were as follows: PAR: 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (colour spec: r90b10), C_a : 400 $\mu\text{mol CO}_2 \text{mol}^{-1}$ air and RH: 40%–70%. Note that ΦPSII was thus measured from leaves exposed to a PAR of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, representing a realistic operational value, and not a maximum value. Gas exchange variables and ΦPSII were measured across a range of temperature, spanning a leaf temperature (T_{leaf} , °C) of approximately 10°C–45°C at a 5°C interval. To achieve this temperature range, three growth rooms were used alongside the temperature control system of the instrument. The following environmental variables were set for each room: C_a : ambient (400–420 $\mu\text{mol CO}_2 \text{mol}^{-1}$ air), PAR: 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$, RH: 50%, T_{air} : 15°C (Room 1), 25°C (Room 2), and 35°C (Room 3).

The same leaf was used for measurements across the temperature range, with measurements taken over a 10-h daytime photoperiod. The order of measurements generally began with temperatures at or near the growth environment of a specific plant and finished with the highest T_{leaf} in the sequence (35°C–45°C). Measuring ΦPSII at the highest T_{leaf} at the end of the sequence ensured that plants were not exposed to a prolonged period of elevated T_{air} prior to taking further measurements. Between measurements, plants were returned to the T_{air} at which they grew. In relation to T_{leaf} , the measurement sequence for plants grown at 15°C:25°C was: 25°C, 30°C (Room 2), 15°C, 20°C, 10°C (Room 1), 35°C, 40°C, 45°C (Room 3). For plants grown at 5°C:15°C, the T_{leaf} measurement

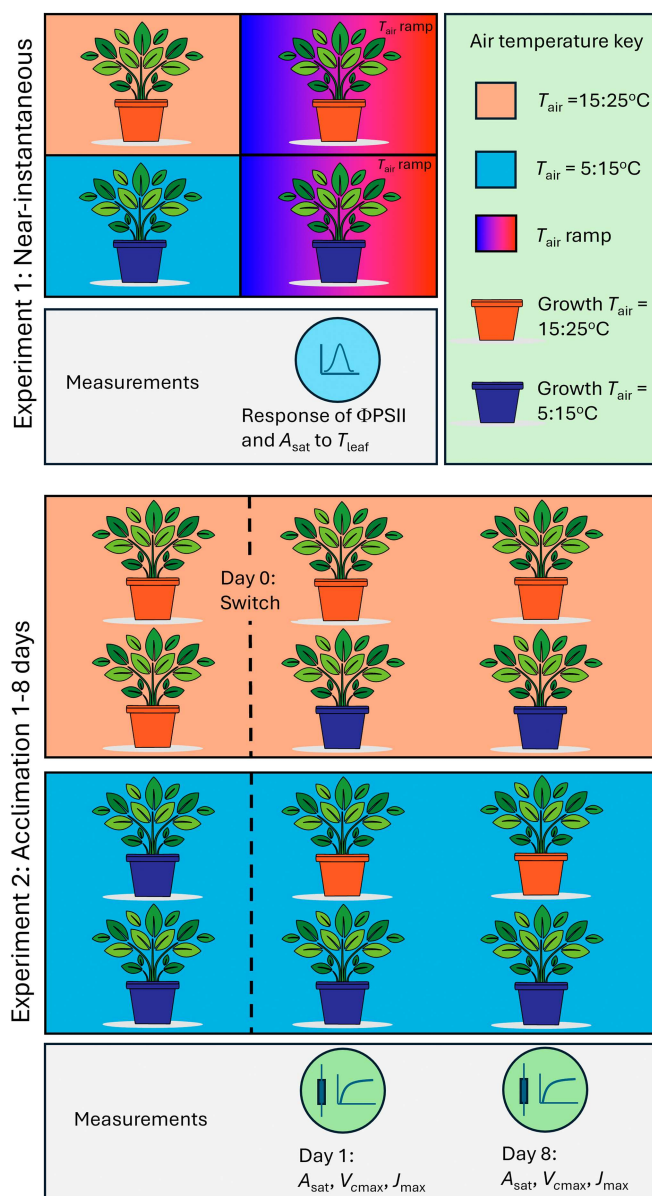


FIGURE 1 | The approach for *Experiment 1* involved growing plants from two *B. oleracea* accessions: one originating from a warm climate, the other originating from a cool climate. Plants were grown under specific air temperature (T_{air}) regimes (T_{air} : 15°C:25°C night:day, also termed the H regime, or 5°C:15°C night:day, also termed the L regime). After plants developed under these conditions, the near-instantaneous responses of the operating efficiency of photosystem II (ΦPSII) and the light-saturated photosynthetic rate (A_{sat}) to leaf temperature (T_{leaf}) were determined. To capture the near-instantaneous response, plants were exposed to a T_{air} ramp of 15°C–35°C, with T_{leaf} regulated to a range of approximately 10°C–45°C using the temperature control of a gas exchange system. For *Experiment 2*, plants were grown under the same conditions as *Experiment 1*, but after the same period of development half of the plants of each T_{air} regime were switched to the opposing temperature regime (Day 0: Switch). A_{sat} , the apparent maximum rate of carboxylation (V_{cmax}) and the apparent maximum rate of electron transport (J_{max}) were determined 1 and 8 days after the switch. Plants within each growth temperature treatment were of an equivalent age at the time of the near-instantaneous response measurements of *Experiment 1* and at 1 day after the switch of *Experiment 2*. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

sequence was: 15°C, 20°C, 10°C (Room 1), 25°C, 30°C (Room 2), 35°C, 40°C, 45°C (Room 3).

From the measurements, the near-instantaneous response of ΦPSII to T_{leaf} and A_{sat} to T_{leaf} was described by a rectangular function (Neri et al. 2024):

$$\Phi\text{PSII or } A_{sat} = f(T_{leaf}) = a0.5 \left(\text{erf} \left(\frac{T_{leaf} - m_1}{s_1} \right) + \text{erf} \left(\frac{m_2 - T_{leaf}}{s_2} \right) \right), \quad (1)$$

where erf is the error function with a sigmoidal form over the interval 0, z:

$$\text{erf}(z) = \frac{2}{\sqrt{\pi}} \int_0^z e^{-t^2} dt, \quad (2)$$

where t is the integration variable.

The rectangular function was used as an empirical model to describe the relationships between ΦPSII and A_{sat} with T_{leaf} . The function was selected because it performed well across the entire range of T_{leaf} , which was typically characterised as an asymmetric response. Equation (1) was fit to the empirical data set of each plant using the Levenberg–Marquardt algorithm iterative procedure (Levenberg 1944; Marquardt 1963) using OriginPro Version 2024b (OriginLab Corporation, Northampton, MA, USA). Although Neri et al. (2024) use Equation (1) to describe relationships between the maximum yield of ΦPSII and T_{air} , the parameters have functional relevance to the responses of ΦPSII and A_{sat} to T_{leaf} . In our study, a is the maximum ΦPSII or A_{sat} without limitations imposed by temperature, m_1 (m_2) is the T_{leaf} at which $\Phi\text{PSII}/A_{sat}$ is 50% of a and s_1 (s_2) represents the slope of peak change in $\Phi\text{PSII}/A_{sat}$ at m_1 (m_2). In addition to these parameters, we also define the maximum ΦPSII ($\Phi\text{PSII}_{[max]}$) and maximum A_{sat} ($A_{sat}[max]$) over the range of measured T_{leaf} and the T_{leaf} at $\Phi\text{PSII}_{[max]}$ and $A_{sat}[max]$ ($T_{opt}[\Phi\text{PSII}]$ and $T_{opt}[A_{sat}]$, respectively). We also determined the ΦPSII and A_{sat} at a fixed T_{leaf} of 40°C, termed $\Phi\text{PSII}_{[40]}$ and $A_{sat[40]}$ for ΦPSII and A_{sat} , respectively. This T_{leaf} was selected because it represents a realistic temperature extreme for these accessions during a growing season. The relative difference between $\Phi\text{PSII}_{[max]}$ and $\Phi\text{PSII}_{[40]}$ ($\Delta\Phi\text{PSII}$) and the relative difference between $A_{sat}[max]$ and $A_{sat[40]}$ (ΔA_{sat}) were also determined. These derived parameters are depicted in Figure S2.

Experiment 2. Acclimation of V_{cmax} and J_{max} from 1 to 8 days.

Seeds were germinated and plants were established in the same manner as *Experiment 1*. As with *Experiment 1*, after 1 week of establishment in the pots, half of the plants from each accession were transferred to a T_{air} of 5°C:15°C (night:day), that is, the L regime, all other environmental variables were equivalent to those of the establishment phase. For this experiment, 10 plants were grown in the 15°C:25°C growth temperature treatment (H regime) and 10 plants were grown in the 5°C:15°C growth temperature treatment (L regime). As per *Experiment 1*, 50 mL of a commercial complete fertiliser (Thrive, All Purpose Soluble

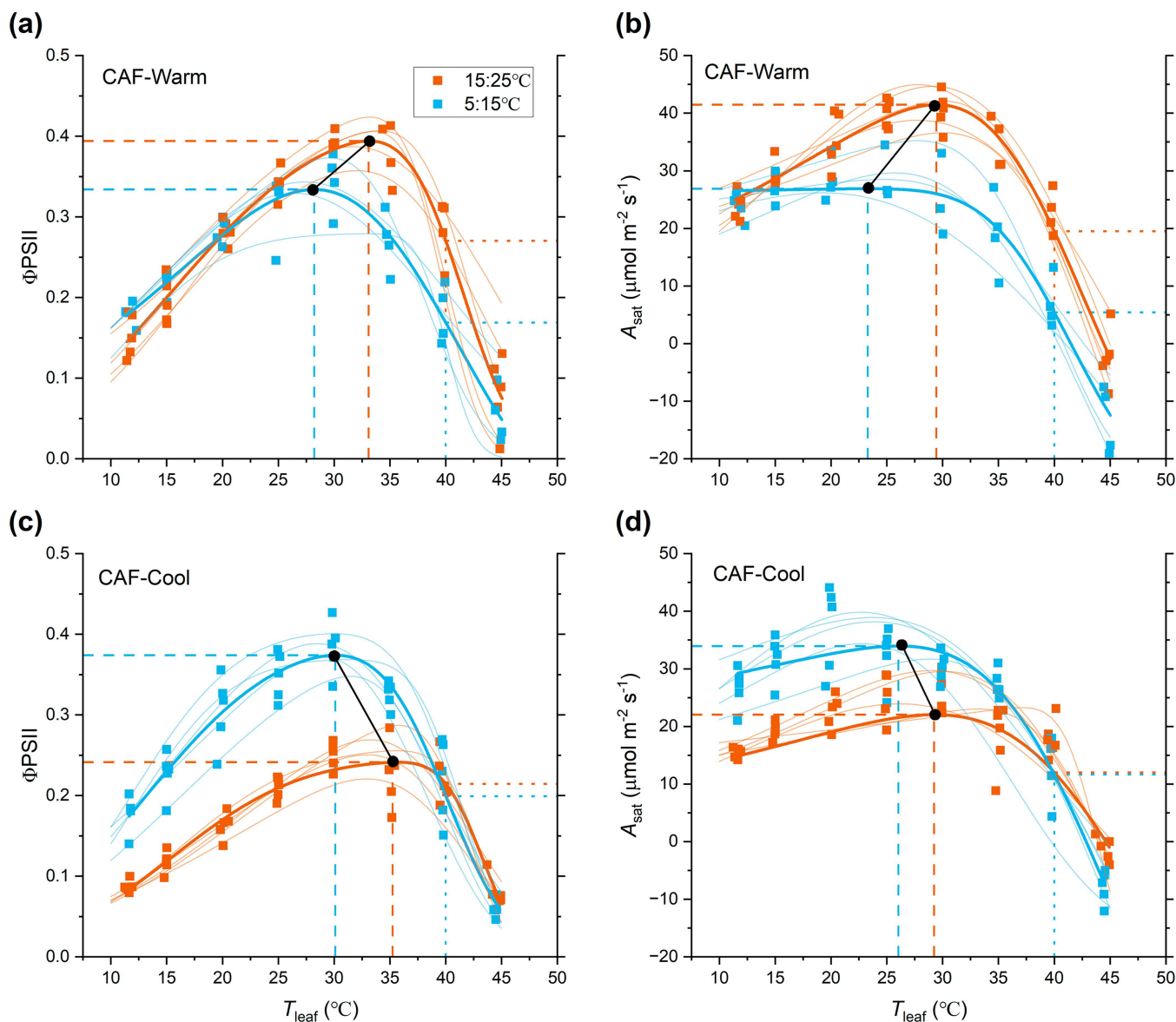


FIGURE 2 | Near-instantaneous responses of ΦPSII to T_{leaf} (a and c) and A_{sat} to T_{leaf} (b and d) for each accession and growth temperature. Fitted lines are rectangular models. Thin lines represent models for each plant. Thick lines are the overall models for each accession by temperature regime. The dashed lines depict the overall optimal temperature for ΦPSII and A_{sat} ($T_{\text{opt}[\Phi\text{PSII}]}$ and $T_{\text{opt}[A_{\text{sat}]}$, respectively) and the overall maximum ΦPSII and A_{sat} ($\Phi\text{PSII}_{\text{max}}$ and $A_{\text{sat}_{\text{max}}}$, respectively). The solid black lines show the difference between $\Phi\text{PSII}_{\text{max}}$ and $A_{\text{sat}_{\text{max}}}$ for the warm-grown and cool-grown plants of each accession. The dotted lines represent the overall ΦPSII and A_{sat} at 40°C (ΦPSII_{40} and $A_{\text{sat}_{40}}$, respectively). Analysis of accession by treatment differences of these parameters, along with the relative difference between $\Phi\text{PSII}_{\text{max}}$ and ΦPSII_{40} , and $A_{\text{sat}_{\text{max}}}$ and $A_{\text{sat}_{40}}$ ($\Delta\Phi\text{PSII}$ and ΔA_{sat} , respectively) are shown in Table 1. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

Fertiliser, N:P:K 25:5:8.8) was applied to each pot each week. The first application was at a concentration of 1.5 g L⁻¹; subsequent applications were at a concentration of 3 g L⁻¹.

At the same developmental phases as described for measurements in *Experiment 1*, plants of *Experiment 2* received a short-term acclimation treatment. The short-term acclimation treatment involved transferring half the plants from each growth temperature into the opposing growth temperature during the morning (1–2 h after the daytime photoperiod had been initiated). Plants that remained at their growth temperature regimes served as controls. The experimental design and measurement protocol are shown in Figure 1. The measurement protocol is also described in detail below. Our experimental design aimed

to identify possible differences in photosynthetic parameters owing to genotypic adaptation (between accession differences), as well as differences due to acclimation during plant growth and differences due to short-term acclimation following the transfer step described above.

2.4 | Leaf Gas Exchange Properties

Leaf gas exchange properties were measured with an open-flow photosynthesis system (Model LI-6400XT, LI-COR Environmental, USA) 1 day after the short-term acclimation treatment was implemented. A_{sat} was measured on each plant ($n = 1$ leaf/plant) under the following leaf chamber conditions: C_a :

400 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$, PAR: 1500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, RH: 50%–70%, T_{leaf} : 25°C or 15°C (equivalent to the T_{air} of each plant growth environment at the time of measurement). The response of A_{sat} to intercellular CO_2 (C_i , $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$) was derived by varying C_a inside the leaf chamber over the following stepwise sequence: 400, 300, 200, 100, 50, 400, 600, 900, 1200, 2000 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$, with other chamber variables remaining the same as the initial A_{sat} measurement. All gas exchange measurements were taken after A_{sat} and g_s stabilised. Measurements of A_{sat} and the response of A_{sat} to C_i were repeated 8 days after the short-term acclimation treatment was implemented.

A biochemical model of C_3 photosynthesis (Farquhar et al. 1980) was fit to the relationships between A_{sat} and C_i using the `fitaci` function within the 'plantecophys' package (Duursma 2015) in R, version 4.4.1 (R Development Core Team 2025), using the default fitting method and allowing dark respiration to be estimated during fitting. We also applied the default assumption that mesophyll conductance is infinite and we maintained the default temperature dependencies of the CO_2 compensation point in the absence of mitochondrial respiration and the Michaelis–Menten constants for CO_2 and O_2 from Bernacchi et al. (2001). The apparent maximum rate of carboxylation and the apparent maximum ETR were determined at the measurement T_{leaf} , corresponding to the daytime T_{air} of each growth room (hereafter termed V_{cmax} and J_{max} , respectively) and at a standardised T_{leaf} of 25°C ($V_{\text{cmax}25}$ and $J_{\text{max}25}$, respectively), with standardisation conducted within the 'plantecophys' package using a peaked Arrhenius equation from Medlyn et al. (2002). In theory, the photosynthetic variables measured at T_{leaf} incorporate the effect of temperature on enzymatic kinetics, whereas the temperature-corrected photosynthetic variables represent the maximum carboxylation capacity and the maximum electron transport capacity. However, we applied caution when interpreting the temperature-corrected variables owing to the potential for species/accession-specificity for the fitting parameters of the peaked Arrhenius equation.

We also note the potential for temperature measurement errors in the Li6400XT (Garen et al. 2022). However, we set T_{leaf} to the same value as the respective growth room T_{air} when deriving relationships between A_{sat} and C_i , with the resulting difference between the T_{air} of the growth room and the T_{air} of the instrument ranging from -1.3°C to 4.5°C , with a median difference of 0.9°C across all measurements. Differences between the T_{air} of the growth rooms and the T_{air} of the LI-6800 during near-instantaneous responses (*Experiment 1*) were larger, ranging from -18.4°C to 5.0°C . However, the potential for artifacts associated with temperature measurements is much smaller for this instrument (Garen et al. 2022).

2.5 | Statistical Analysis

Statistical analyses were undertaken in R version 4.4.1 (R Development Core Team 2025). Parametric analyses were preceded by tests for normality (Shapiro–Wilk tests (Shapiro and Wilk 1965)), with Yeo–Johnson transformations (Yeo 2000) and Box–Cox transformations (Box and Cox 1964) applied to achieve normality as needed. Because each temperature regime

was implemented in a single growth room, statistical inference for temperature is conditional on the present chamber settings; therefore, we interpret temperature-related tests as differences between regimes under these conditions.

Two-way ANOVAs and Tukey's post hoc comparisons were used to test for differences in the variables associated with the near-instantaneous temperature responses of ΦPSII and A_{sat} , with significant differences defined at an α level of 0.05. In each case, accession and growth temperature were treated as between-group factors, and an interaction between accession and growth temperature was included.

Linear mixed effect models were used to test for differences between accession, growth temperature and acclimation day using the 'nlme' package (Lindstrom and Bates 1990; Pinheiro et al. 2026). The following response variables were tested in the models: V_{cmax} , J_{max} , $J_{\text{max}}/V_{\text{cmax}}$, $V_{\text{cmax}25}$, $J_{\text{max}25}$ and $V_{\text{cmax}25}/J_{\text{max}25}$ with accession and growth temperature treated as between-group factors, acclimation day treated as a within-group factor and each individual plant treated as a random intercept term; interaction terms between accession and acclimation temperature, acclimation day and accession, acclimation day and acclimation temperature and acclimation day, acclimation temperature and accession were included in the models. Tukey pairwise comparisons of the model outputs were then determined using the 'emmeans' package.

Standardised major axis (SMA) regression analyses were implemented to describe relationships between traits using the 'smatr' package (Warton et al. 2012), including relationships between A_{sat} and V_{cmax} and A_{sat} and J_{max} . Data were aggregated for fitting based on tests for significance between the slope and intercept of accession by treatment groupings. Where appropriate, the significance of relationships was assessed further using repeated measures correlation tests from the 'rmcorr' package (Bakdash and Marusich 2017).

3 | Results

Experiment 1. Near-instantaneous temperature responses of ΦPSII and A_{sat} .

The near-instantaneous temperature responses of ΦPSII and A_{sat} for each accession are shown in Figure 2. The model fitting parameters and goodness of fit for each plant are shown in Table S1, with the first derivative of the fitted functions shown in Figure S3. Note that across the temperature range, there was no clear response of g_{sw} (Figure S4 and Table S2). From Figure 2, distinct differences in the response functions are evident. For example, the maximum ΦPSII and the maximum A_{sat} ($\Phi\text{PSII}_{[\text{max}]}$ and $A_{\text{sat}[\text{max}]}$, respectively), were higher in warm-grown plants compared to cool-grown plants of accession CAF-Warm (Figure 2a,b and Figure S5c,d). In contrast, $\Phi\text{PSII}_{[\text{max}]}$ and $A_{\text{sat}[\text{max}]}$ were higher in cool-grown plants compared to warm-grown plants of accession CAF-Cool (Figure 2c,d and Figure S5c,d). A two-way ANOVA conferred a significant interaction between accession and growth regime ($T_{\text{air}} = 15^\circ\text{C}:25^\circ\text{C}$ vs $5:15^\circ\text{C}$) for both $\Phi\text{PSII}_{[\text{max}]}$ and $A_{\text{sat}[\text{max}]}$ (Table 1, $p < 0.001$). The temperature response pattern was different for the thermal optima of ΦPSII and A_{sat} ($T_{\text{opt}[\Phi\text{PSII}]}$ and $T_{\text{opt}[A_{\text{sat}]}}$, respectively). In this case, $T_{\text{opt}[\Phi\text{PSII}]}$ and $T_{\text{opt}[A_{\text{sat}]}$

were greater in warm-grown plants of both accessions (Figure 2 and Figure S5a,b) and there was no significant interaction between accession and growth regime for either response variable (Table 1, $p > 0.05$).

On average, $T_{\text{opt}[\Phi\text{PSII}]}$ and $T_{\text{opt}[A_{\text{sat}}]}$ were 4.3°C and 5.4°C greater, respectively, for plants grown at 15°C:25°C compared to those grown at 5°C:15°C. $T_{\text{opt}[\Phi\text{PSII}]}$ and $T_{\text{opt}[A_{\text{sat}}]}$ were positively correlated, with the slope of the relationship significantly different from 1 (slope = 0.70°C/°C, lower limit = 0.51°C/°C, upper limit = 0.96°C/°C) and the intercept significantly different from 0 (intercept = 12.5°C, lower limit = 6.22°C, upper limit = 18.89°C) (Figure 3a). Thus, for a given $T_{\text{opt}[A_{\text{sat}}]}$, $T_{\text{opt}[\Phi\text{PSII}]}$ was greater than $T_{\text{opt}[A_{\text{sat}}]}$, but there was a tendency for $T_{\text{opt}[\Phi\text{PSII}]}$ and $T_{\text{opt}[A_{\text{sat}}]}$ to converge at the upper limit of the

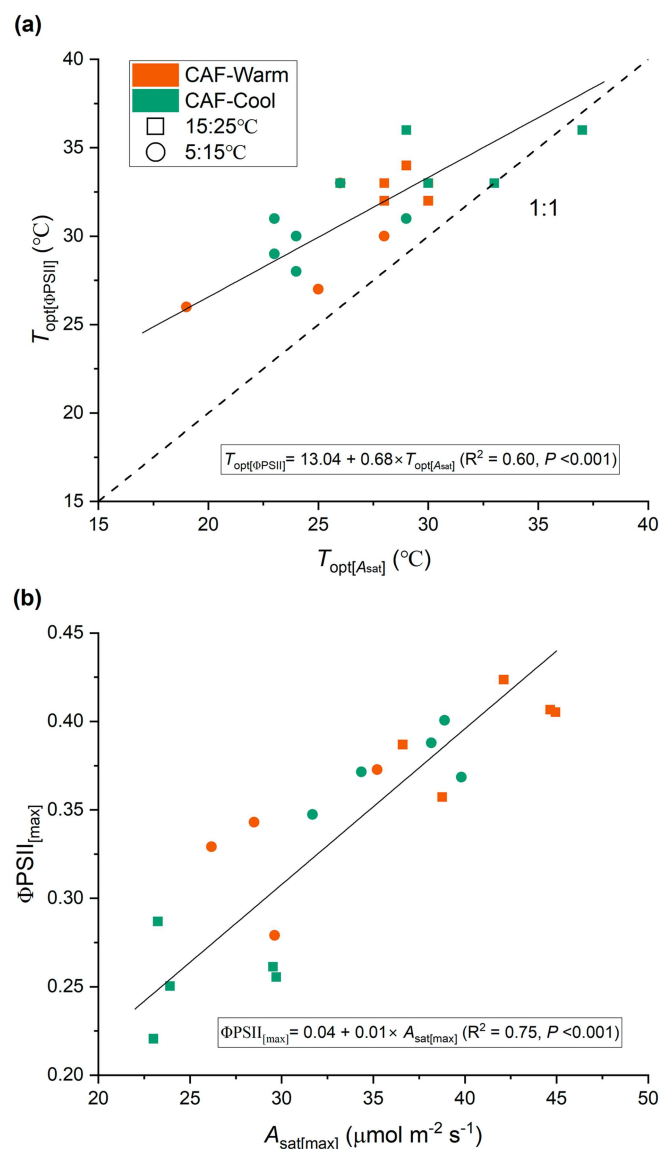


FIGURE 3 | Relationships between $T_{\text{opt}[\Phi\text{PSII}]}$ and $T_{\text{opt}[A_{\text{sat}}]}$ (a), and $\Phi\text{PSII}_{\text{max}}$ and $A_{\text{sat}[\text{max}]}$ (b). Fitted lines are SMA regression models. Dashed line in (a) is the 1:1 ratio. The near-instantaneous temperature responses used to derive the variables of the figure were measured over a T_{leaf} range of approximately 10°C to 45°C. The growth temperatures of the plants are indicated by the shape of the points: 15°C:25°C and 5°C:15°C. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

observed values, and this part of the bivariate relationship was dominated by data obtained from plants grown at 15°C:25°C. $\Phi\text{PSII}_{\text{max}}$ and $A_{\text{sat}[\text{max}]}$ were also positively correlated, with a 0.01 increase in $\Phi\text{PSII}_{\text{max}}$ for every 1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ increase in $A_{\text{sat}[\text{max}]}$ (Figure 3b).

Further analysis of the temperature response of ΦPSII and A_{sat} with specific consideration of the temperature range above the observed optima, or that experienced during growth, is shown in Figure S6 and Table 1. ΦPSII_{40} and $A_{\text{sat}40}$ were higher in warm-grown plants of accession CAF-Warm, but there was no difference in these variables due to growth regime for accession CAF-Cool (Figure S6a,b and Table 1). Thus, for accession CAF-Warm, growth at 15°C:25°C resulted in a higher ΦPSII and A_{sat} at 40°C compared to growth at 5°C:15°C, but this photosynthetic adjustment for warm-grown plants at 40°C was absent for accession CAF-Cool. In contrast, $\Delta\Phi\text{PSII}$ and ΔA_{sat} were more negative in cool-grown plants of both accessions and overall, ΔA_{sat} was 1.6-fold more negative than $\Delta\Phi\text{PSII}$ for warm-grown plants and ΔA_{sat} was 1.7-fold more negative than $\Delta\Phi\text{PSII}$ for cool-grown plants (Figure S6b,c and Table 1). Hence, for both accessions, there was a greater relative photosynthetic reduction between the optima ($\Phi\text{PSII}_{\text{max}}$ and $A_{\text{sat}[\text{max}]}$) and the value at 40°C (ΦPSII_{40} and $A_{\text{sat}40}$) in cool-grown plants, and the relative reduction was greater for A_{sat} than for ΦPSII .

Experiment 2. Acclimation of $V_{\text{cmax}25}$ and $J_{\text{max}25}$ from 1 to 8 days.

The photosynthetic variables derived from the relationships between A_{sat} and C_i for each plant are shown in Table S3. $V_{\text{cmax}25}$ differed significantly based on acclimation regime ($T_{\text{air}} = 15^\circ\text{C}:25^\circ\text{C}$ or $5^\circ\text{C}:15^\circ\text{C}$), but there was no significant difference in $V_{\text{cmax}25}$ between 1 and 8 days of acclimation (Figure 4, $p = 0.753$). V_{cmax} also differed significantly based on acclimation regime but, again, there was no significant difference in V_{cmax} between 1 and 8 days of acclimation (Figure S7, $p = 0.419$). One day after the switch, plants of both accessions that were either grown at 15°C:25°C or were acclimated to 15°C:25°C had a 1.5-fold lower $V_{\text{cmax}25}$ than plants that were either grown at 5°C:15°C or were acclimated to 5°C:15°C. This difference also held 8 days after the switch.

The same generalised responses were evident for $J_{\text{max}25}$ and J_{max} with a significant difference due to acclimation temperature, but no difference between acclimation day (Table S4, $p = 0.638$ and $p = 0.273$ for $J_{\text{max}25}$ and J_{max} , respectively). Thus, for both $V_{\text{cmax}25}$ and V_{cmax} and $J_{\text{max}25}$ and J_{max} , differences between acclimation regimes were already evident by Day 1, and no further change was detected between Day 1 and Day 8 under our conditions. Note that, in theory, the different direction of change for V_{cmax} and J_{max} versus $V_{\text{cmax}25}$ and $J_{\text{max}25}$ reflects the effect of T_{leaf} on enzymatic kinetics for the former variables and the effect of T_{leaf} on biochemical capacity for the latter variables.

The ratio of $J_{\text{max}25}/V_{\text{cmax}25}$ also differed significantly based on acclimation regime ($T_{\text{air}} = 15^\circ\text{C}:25^\circ\text{C}$ or $5^\circ\text{C}:15^\circ\text{C}$), but in this case, there was an interaction between acclimation day and acclimation temperature (Figure 4, $p < 0.001$). For plants grown at a T_{air} of 15°C:25°C, then transferred to a T_{air} of 5°C:15°C, $J_{\text{max}25}/V_{\text{cmax}25}$ was lower after 1 day of acclimation compared to $J_{\text{max}25}/V_{\text{cmax}25}$ measured on the same plants after 8 days of

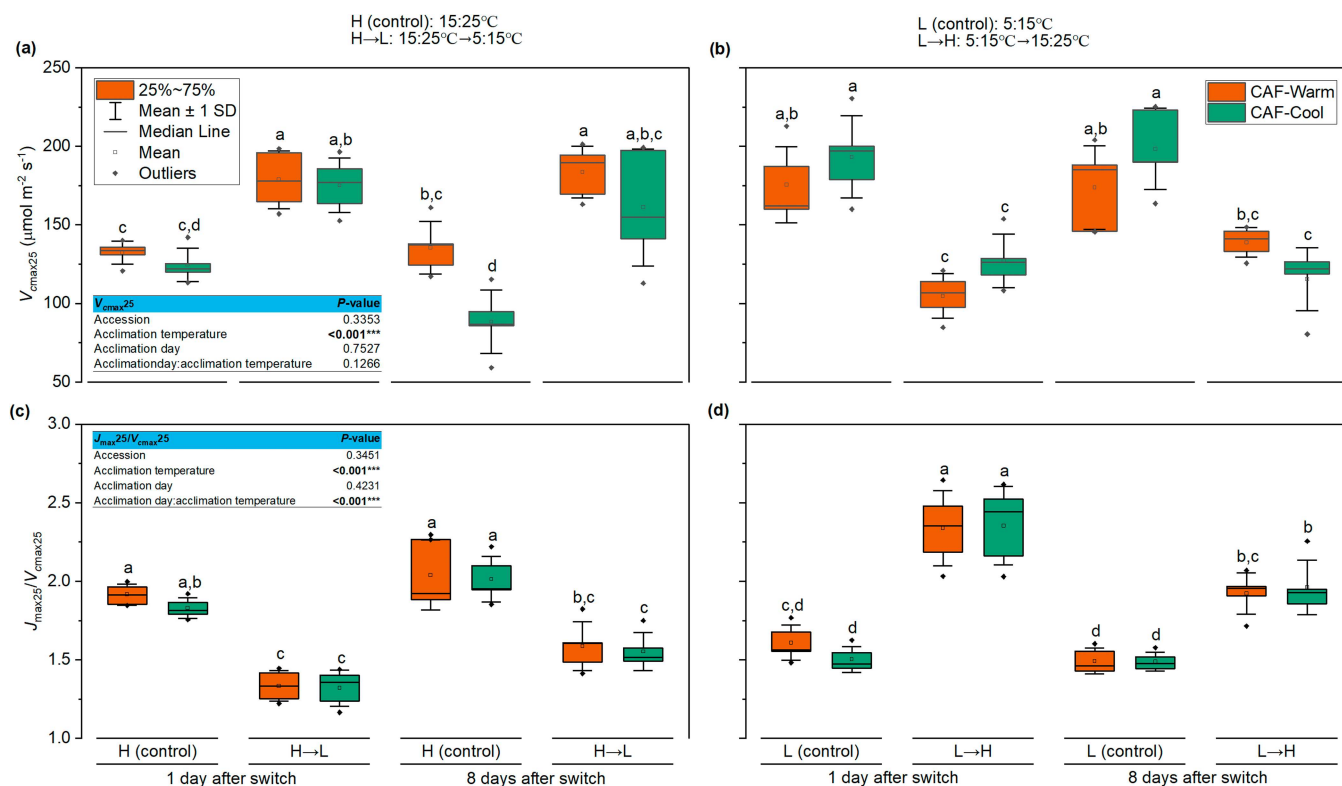


FIGURE 4 | $V_{\max 25}$ (a and b) and $J_{\max 25}/V_{\max 25}$ (c and d) after 1 and 8 days of acclimation ($T_{\text{air}} = 15^{\circ}\text{C}:25^{\circ}\text{C}$ or $5^{\circ}\text{C}:15^{\circ}\text{C}$) from warm to cool as well as cool to warm regimes, compared to control plants. Here, $V_{\max 25}$ and $J_{\max 25}$ data reflect measurements corrected to a common T_{leaf} of 25°C . Different letters in panels indicate significant differences between combinations of different accessions, acclimation days and acclimation regimes (linear mixed models, $p < 0.05$). Between factor comparisons from the linear mixed models across the entire data set are summarised in the inset tables, with further details of the model results shown in the [Supporting Information](#). [Color figure can be viewed at [wileyonlinelibrary.com](#)]

acclimation. Similarly, plants that were grown at a T_{air} of $5^{\circ}\text{C}:15^{\circ}\text{C}$, then transferred to a T_{air} of $15^{\circ}\text{C}:25^{\circ}\text{C}$ had higher $J_{\max 25}/V_{\max 25}$ after 1 day of acclimation compared to after 8 days of acclimation. The same interaction was evident for the ratio of $J_{\max}/V_{\max}$ (Figure S7, $p < 0.001$).

From the relationships between $J_{\max}$ and $V_{\max}$ and $J_{\max 25}$ and $V_{\max 25}$, it was observed that the intercepts of the fitted models differed between plants that were grown at a T_{air} of $15^{\circ}\text{C}:25^{\circ}\text{C}$ versus plants that were acclimated for 1 day at $15^{\circ}\text{C}:25^{\circ}\text{C}$ (Figure S8a,c and Table S5, $p < 0.001$). The same difference in the intercepts of the fitted models was evident for plants that were grown at $5^{\circ}\text{C}:15^{\circ}\text{C}$ versus plants that were acclimated for 1 day at $5^{\circ}\text{C}:15^{\circ}\text{C}$ (Figure S8a,c and Table S5, $p < 0.001$). However, for these same between-group comparisons, there were no differences in the intercepts of the fitted models after 8 days of temperature acclimation (Figure S8b,d and Table S5, $p > 0.05$). $V_{\max}$ was significantly greater for accession CAF-Warm compared to accession CAF-Cool, but no other variable derived from the relationships between A_{sat} and C_i showed a significant between-accession difference.

To understand the implications of changes in carboxylation capacity on photosynthesis owing to shifts in temperature regime, we examined the relationships between A_{sat} and $V_{\max 25}$. A_{sat} measured after 1 and 8 days of temperature acclimation was positively correlated with $V_{\max 25}$ (Figure 5 and Table S6). Repeated measures correlation results are shown in Table S7. From the correlations between A_{sat} and $V_{\max 25}$ (Figure 5a,b), a between-treatment analysis of the slope and

intercept revealed distinct subgroups. For both accessions, there were no differences in the slopes or intercepts of the linear models fit to the data of plants grown at $15^{\circ}\text{C}:25^{\circ}\text{C}$ or transferred to $15^{\circ}\text{C}:25^{\circ}\text{C}$ ($p > 0.05$). Similarly, for both accessions, there was no difference in the slope or intercept of the models fit to data from plants grown at $5^{\circ}\text{C}:15^{\circ}\text{C}$ or transferred to $5^{\circ}\text{C}:15^{\circ}\text{C}$ ($p > 0.05$). However, a comparison of the aggregate groups (plants grown or transferred to $5^{\circ}\text{C}:15^{\circ}\text{C}$ versus plants grown or transferred to $15^{\circ}\text{C}:25^{\circ}\text{C}$) showed distinct differences. For accession CAF-Warm, the slopes of the linear models fit to the data from these groups were significantly different ($p = 0.002$, Table S6 and Figure 5a). For CAF-Cool, there was no difference in the slopes of the linear models fit to the data from these groups, but the intercepts of the linear models were significantly different ($p < 0.001$, Table S6 and Figure 5b). Overall, there was a tendency for a greater A_{sat} for a given $V_{\max 25}$ in plants that were either grown in, or transferred to, the $15^{\circ}\text{C}:25^{\circ}\text{C}$ temperature regime.

4 | Discussion

4.1 | Temperature Response Functions

The near-instantaneous responses of ΦPSII and A_{sat} to T_{leaf} were well described by rectangular functions (Figure 2), with the greatest rate of (negative) change in ΦPSII and A_{sat} with T_{leaf} occurring at a T_{leaf} above the thermal optima (refer to Figure S3 for plots of the first derivative of the temperature

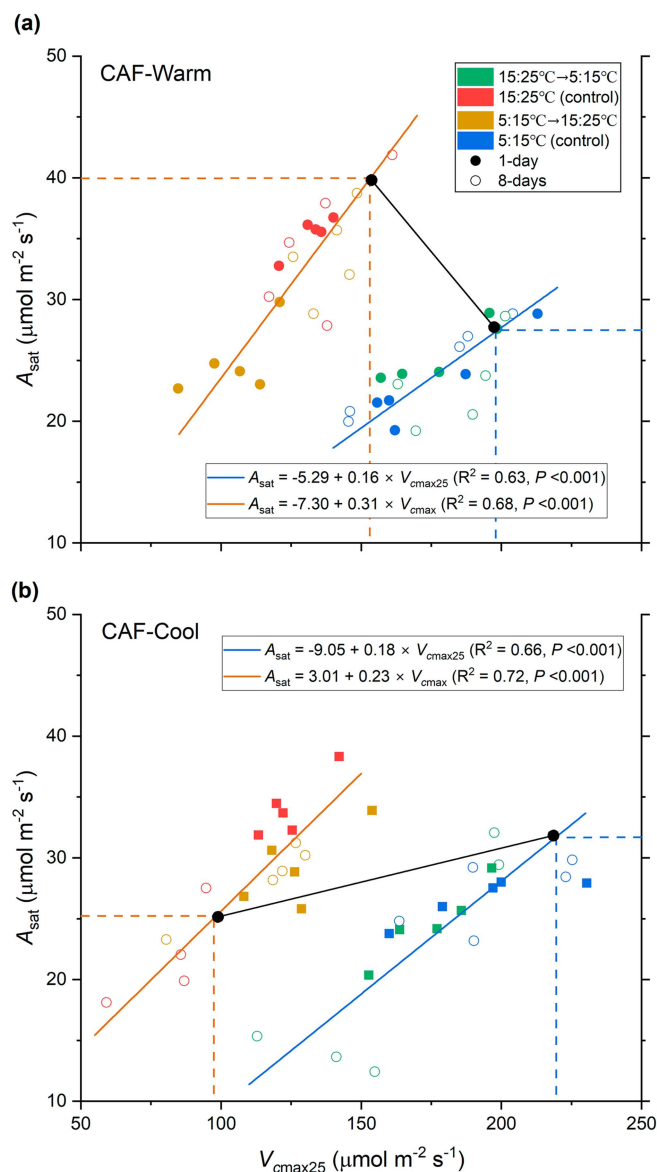


FIGURE 5 | Relationships between A_{sat} and V_{cmax} for accession CAF-Warm (a) and CAF-Cool (b). Here, A_{sat} data reflect non-temperature-corrected measurements conducted at 15°C and 25°C air temperature for plants growing in the L and H temperature regimes, respectively, and $V_{\text{cmax}25}$ data reflect measurements corrected to a common temperature of 25°C—a measure of carboxylation capacity without the influence of temperature on enzymatic kinetics. Fitted lines are SMA regression models with data aggregated into groups based on tests for differences between the slope and intercept of treatment combinations. The output of the tests is shown in the [Supporting Information](#). The dashed orange and blue lines represent, respectively, the $V_{\text{cmax}25}$ predicted from the average A_{sat} measured from the near-instantaneous responses at a T_{leaf} of 25°C and 15°C (Experiment 1). The solid black lines depict the change from warm-grown to cool-grown plants. [Color figure can be viewed at [wileyonlinelibrary.com](#)]

responses). Both accessions functionally acclimated to their growth temperature by modifying $T_{\text{opt}} [\Phi\text{PSII}]$ and $T_{\text{opt}} [A_{\text{sat}}]$, with the optimal temperature for ΦPSII and A_{sat} increasing in plants grown in a warm environment (Hypothesis 1). However, there was also evidence of thermal adaptation of ΦPSII and A_{sat}

(Hypothesis 1). $\Phi\text{PSII}_{[\text{max}]}$ and $A_{\text{sat}[\text{max}]}$ were higher in warm-grown plants of accession CAF-Warm (developed in a warm climate), whereas $\Phi\text{PSII}_{[\text{max}]}$ and $A_{\text{sat}[\text{max}]}$ were higher in cool-grown plants of accession CAF-Cool (developed in a cool climate). Although our comparison is limited to two accessions, this could mean that while the thermal optima for ΦPSII photochemistry and net photosynthesis acclimate to the prevailing temperature, the maximum capacity of these variables is influenced by genotypic adaptation.

For a given $T_{\text{opt}} [A_{\text{sat}}]$, $T_{\text{opt}} [\Phi\text{PSII}]$ was generally higher, suggesting that the thermal optimum of PSII ETR was consistently greater than for light-saturated net photosynthesis (Figure 3a). This is not surprising given that net photosynthesis is gross photosynthesis (or true photosynthesis) minus photorespiration and dark respiration (Wohlfahrt and Gu 2015), with the temperature effect of photorespiration and dark respiration likely to reduce $T_{\text{opt}} [A_{\text{sat}}]$ more so than $T_{\text{opt}} [\Phi\text{PSII}]$. Furthermore, ΦPSII mainly reflects the photochemical efficiency of PSII in the chloroplasts near the leaf surface, whereas A_{sat} integrates whole-leaf CO_2 assimilation, including mesophyll diffusion and respiratory losses. These additional biochemical and diffusional constraints likely lower the temperature optimum of A_{sat} relative to ΦPSII . Notwithstanding these effects, there was a clear linear relationship between $\Phi\text{PSII}_{[\text{max}]}$ and $A_{\text{sat}[\text{max}]}$ (Figure 3b). Thus, for the accessions and growth conditions of this study, the maximum attainable net photosynthetic rate was coordinated with the maximum attainable PSII ETR (Hypothesis 1). Note also that J_{max} and $J_{\text{max}25}$ (discussed further in Section 4.2, below), which describe the potential electron transport for RuBP regeneration, are not mechanistically identical to PSII electron transport itself. Thus, while the coordination among ΦPSII , A_{sat} , and J_{max} ($J_{\text{max}25}$) reflects an integrated response of photochemistry and biochemistry, these variables do not necessarily respond to temperature with the same sensitivity or on the same timescale.

Cool-grown plants of accession CAF-Warm (warm adapted) showed a marked decline in ΦPSII and A_{sat} at 40°C (depicted as $\Phi\text{PSII}_{[40]}$ and $A_{\text{sat}[40]}$, respectively) compared to warm-grown plants of the same accession (Figure S6a,b). Thus, for the accession developed in a warm environment, it appears that the photosynthetic apparatus can be primed for operation at high temperatures when grown at modestly high temperatures. The same pattern was not evident for plants of accession CAF-Cool (developed in a cool environment), which might mean that adaptation to cool conditions can be at the expense of a capacity to acclimate to high temperatures, although further research drawing from more accessions would be needed to confirm this possibility. The benefits of heat priming have been observed in several species (Fan et al. 2018; Zhou et al. 2020; Liu et al. 2021). Generally, heat priming leads to photosynthetic acclimation, quantified as a relative gain in photosynthetic efficiency, at high temperatures. In relation to $\Phi\text{PSII}_{[40]}$ and $A_{\text{sat}[40]}$, the different responses of the two accessions of this study were consistent with genotypic thermal priming, whereby the progenies of primed plants exhibit upregulation of genes coding for heat-tolerant enzymes and complexes of the photosynthetic apparatus (Wang et al. 2016). While there was evidence for genotypic thermal priming for accession CAF-Warm (in relation to temperature increases), the process appears to be reduced or absent for accession CAF-Cool. Such differential thermal responses

TABLE 1 | ANOVA test results for differences in $T_{\text{opt}[\Phi\text{PSII}]}$, $T_{\text{opt}[A_{\text{sat}}]}$, $\Phi\text{PSII}_{[\text{max}]}$, $A_{\text{sat}[\text{max}]}$, $\Phi\text{PSII}_{[40]}$, $A_{\text{sat}[40]}$, $\Delta\Phi\text{PSII}$ and ΔA_{sat} based on accession and growth regime ($T_{\text{air}} = 15^{\circ}\text{C}:25^{\circ}\text{C}$ vs. $5^{\circ}\text{C}:15^{\circ}\text{C}$).

Variable	Test	DF	Sum of Sq	F	p
$T_{\text{opt}[\Phi\text{PSII}]}$	Accession	1	5.69	1.69	0.213
	Growth regime	1	79.11	23.54	< 0.001 ***
	Accession $\times$ Growth regime	1	0.42	0.13	0.728
	Model	3	84.23	8.36	< 0.001 ***
$\Phi\text{PSII}_{[\text{max}]}$	Accession	1	0.01	14.98	0.002 **
	Growth regime	1	0.01	4.88	0.043 *
	Accession $\times$ Growth regime	1	0.04	54.77	< 0.001 ***
	Model	3	0.06	26.40	< 0.001 ***
$T_{\text{opt}[A_{\text{sat}}]}$	Accession	1	5.19	0.54	0.474
	Growth regime	1	139.78	14.54	0.002 **
	Accession $\times$ Growth regime	1	4.25	0.44	0.516
	Model	3	150.43	5.22	0.011 *
$A_{\text{sat}[\text{max}]}$	Accession	1	91.70	7.17	0.017 *
	Growth regime	1	0.82	0.06	0.803
	Accession $\times$ Growth regime	1	582.07	45.48	< 0.001 ***
	Model	3	703.39	18.32	< 0.001 ***
$\Phi\text{PSII}_{[40]}$	Accession	1	< 0.01	0.60	0.452
	Growth regime	1	0.01	6.22	0.025 *
	Accession $\times$ Growth regime	1	0.01	7.77	0.014 *
	Model	3	0.02	4.79	0.016 *
$A_{\text{sat}[40]}$	Accession	1	0.76	0.05	0.823
	Growth regime	1	589.20	40.18	< 0.001 ***
	Accession $\times$ Growth regime	1	110.87	7.56	0.015 *
	Model	3	673.46	15.31	< 0.001 ***
$\Delta\Phi\text{PSII}$	Accession	1	0.02	1.95	0.183
	Growth regime	1	0.18	15.25	0.001 **
	Accession $\times$ Growth regime	1	0.01	0.54	0.475
	Model	3	0.21	5.89	0.007 **
ΔA_{sat}	Accession	1	0.07	3.72	0.073
	Growth regime	1	0.60	32.81	< 0.001 ***
	Accession $\times$ Growth regime	1	< 0.01	< 0.01	0.978
	Model	3	0.65	11.86	< 0.001 ***

Note: Accession by treatment differences are also depicted graphically in Figures S5 and S6. Boldface indicates a significant difference.

* $0.01 \leq p \leq 0.05$;

** $0.001 \leq p < 0.01$;

*** $p < 0.001$.

have been observed across species. For example, Smith and Dukes (2017) noted the effect across plant types, and Wittemann et al. (2022) observed a similar pattern across a thermal gradient. However, we are unaware of published results showing distinct between-accession differences.

Unlike ΦPSII and A_{sat} at 40°C , the relative change in ΦPSII and A_{sat} from the maximum value to the value recorded at 40°C ($\Delta\Phi\text{PSII}$ and ΔA_{sat} , respectively) was responsive to growth temperature for plants of both accessions (Figure S6c,d). For both accessions, $\Delta\Phi\text{PSII}$ and ΔA_{sat} were greater (more negative) for cool-grown plants than for warm-grown plants. Greater downregulation of the enzymes and structures responsible for photosynthesis in cool-grown plants thus appears to be

conserved regardless of the climate under which an accession was developed. This points to the idea that partial thermal acclimation is a universal feature of photosynthesis (Kumarathunge et al. 2019; Tarvainen et al. 2022), but the temperature response of specific elements of the photosynthetic apparatus may depend on the climate under which a plant has evolved.

4.2 | Timeframes for Acclimation

When assessing the timeframes for photosynthetic acclimation, we focus on the effect of temperature on biochemical and photochemical capacity, quantified as $V_{\text{cmax}25}$ and $J_{\text{max}25}$,

respectively. The $V_{\text{cmax}25}$ and $J_{\text{max}25}$ of both *B. oleracea* accessions acclimated to the temperature regimes experienced during plant growth and the temperature regimes experienced following the transfer step, termed long-term and short-term acclimation, respectively, from hereon (Figure 4). Long-term and short-term acclimation to a cool environment resulted in a higher $V_{\text{cmax}25}$ and $J_{\text{max}25}$ than long-term and short-term acclimation to a warm environment.

The similar acclimation responses of $V_{\text{cmax}25}$ and $J_{\text{max}25}$ to temperature underscores the commonly observed covariation of $V_{\text{cmax}25}$ and $J_{\text{max}25}$ (Wullschlegel 1993; Kattge and Knorr 2007; Walker et al. 2014), although subtle differences in the acclimation timeframes of these variables were observed (see further discussion on this point below). Notwithstanding the between-treatment effects, there were no discernible differences in the response of $V_{\text{cmax}25}$ and $J_{\text{max}25}$ to temperature between accessions, suggesting little evidence for thermal adaptation of these variables in these accessions. However, across species, we note that Rubisco kinetic parameters appear to adapt to thermal regimes (Galmés et al. 2016).

Further empirical evidence for a generalised requirement for a greater $V_{\text{cmax}25}$ to support a greater rate of net photosynthesis has been shown by Scafaro et al. (2017), drawing from a selection of tree species that were grown at different temperatures. Scafaro et al. (2017) also demonstrated that the observed acclimation of $V_{\text{cmax}25}$ to temperature required an adjustment to the amount of Rubisco in leaves and associated changes in the allocation of N to this enzyme. However, in our study, the timeframe for the short-term acclimation of $V_{\text{cmax}25}$ to temperature was < 1 day (contrary to Hypothesis 2)—there was no difference in $V_{\text{cmax}25}$ between 1 and 8 days of temperature acclimation. This timeframe conflicts with our hypothesis and the assumption that the production of different amounts or forms of Rubisco are required for carboxylation capacity to undergo thermal acclimation, given that the half-life of Rubisco is approximately 1 week (Simpson et al. 1981). While the exact nature of the fast Rubisco acclimation response of our study is unclear, it is possible that forms of the enzymatic chaperone, Rubisco activase, responded to the short-term acclimation temperature of our experiment (Law and Crafts-Brandner 2001; Yamori et al. 2014). Alternatively, rapid acclimation to warm temperatures may have involved Rubisco deactivation, a process that Scafaro et al. (2023) incorporated into a model to account for decreased net photosynthesis at elevated temperatures. Regardless of the mechanism, a rapid acclimation response for carboxylation capacity would be a competitive advantage for plants with a relatively short growing season or a high leaf turnover rate, where there is an imperative to maximise productivity in a dynamic temperature environment over a relatively short period (such as the plants used in this study). Indeed, it is possible that the acclimation timeframe for V_{cmax} and $V_{\text{cmax}25}$ varies across plant functional groups and may map onto other traits, such as those of the leaf economics spectrum (Wright et al. 2004).

The $J_{\text{max}25}$ to $V_{\text{cmax}25}$ ratio ($J_{\text{max}25}/V_{\text{cmax}25}$) also responded to both long-term and short-term temperature acclimation. For plants of both accessions, $J_{\text{max}25}/V_{\text{cmax}25}$ decreased with long-term and short-term acclimation to cool temperatures and increased with long-term and short-term acclimation to warm temperatures (Figure 4c,d). These results are consistent with

the meta-analysis of Kattge and Knorr (2007) and the experimental observation for C_3 species of Smith and Dukes (2017). Thus overall, and as noted for other C_3 species, rates of $V_{\text{cmax}25}$ were enhanced relative to rates of $J_{\text{max}25}$ with acclimation to cooler temperatures in the *B. oleracea* accessions of this study. However, unlike the trends observed for each of these variables separately, the timeframe for the thermal acclimation of $J_{\text{max}25}/V_{\text{cmax}25}$ and was more nuanced. For both accessions and acclimation temperatures, $J_{\text{max}25}/V_{\text{cmax}25}$ differed between 1-day and 8-days of short-term acclimation. The reason for this difference can be observed by comparing the relative change of $V_{\text{cmax}25}$ and $J_{\text{max}25}$ between 1-day and 8-days of temperature acclimation (see Figure S8 and Table S5). For a given $V_{\text{cmax}25}$, $J_{\text{max}25}$ was slightly higher for the plants acclimated to 1 day of cool temperatures compared to control plants that were grown under cool temperatures. Similarly, for a given $V_{\text{cmax}25}$, $J_{\text{max}25}$ was slightly lower for the plants acclimated to 1 day of warm temperatures compared to control plants that were grown under warm conditions. After 8 days of short-term acclimation, these differences were not evident. Thus, there appears to a slight lag between the acclimation response of $J_{\text{max}25}$ compared to $V_{\text{cmax}25}$. This suggests that, over this short time period, thermal acclimation of the electron transport component of photosynthesis is slightly delayed compared to the acclimation of Rubisco, which would mean that a co-ordinated photosynthetic response to temperature shifts may be slightly longer than the response of carboxylation in isolation. The reason for the delayed response of $J_{\text{max}25}$ could relate to the need for structural rearrangement of components of the electron transport chain, as observed for acclimation to light (Walters 2005), a process that could take longer than altering the forms or quantities of Rubisco or Rubisco activase.

4.3 | Implications for Photosynthesis

The effects of the thermal acclimation responses of $V_{\text{cmax}25}$ on photosynthesis can be examined from the relationships between these variables and A_{sat} (Figure 5), noting that $V_{\text{cmax}25}$ represents the maximum temperature-standardised carboxylation capacity, reflecting biochemical investment in Rubisco. For both accessions, clear linear relationships between A_{sat} and $V_{\text{cmax}25}$ were evident, with data grouped by long-term and short-term acclimation temperatures. Thus, for each group, a greater A_{sat} was supported by a greater investment in $V_{\text{cmax}25}$, which is consistent with observations and theory (Kattge et al. 2009; Maire et al. 2012). However, for a given $V_{\text{cmax}25}$, A_{sat} was greater for plants that were either grown in, or transferred to, a warm environment; that is, the intercept of the relationship between A_{sat} and $V_{\text{cmax}25}$ for these plants was greater than for plants that were either grown in, or transferred to, a cool environment. Thus, growth or short-term acclimation to a warm environment results in de-investment in carboxylation capacity. However, for a given $V_{\text{cmax}25}$, A_{sat} is enhanced under these same conditions because of the effect of elevated temperatures on enzymatic kinetics. These responses were evident after only 1 day of thermal acclimation.

Note that, due to practical limitations in regulating RH, the VPD of the growth rooms did differ. Future studies may aim to address this issue by selecting a smaller difference in T_{air}

between growth conditions, thereby enabling manipulation of RH to achieve the same VPD.

4.4 | Synthesis of Experimental Observations

Referring to the results of *Experiment 2*, the dashed lines in Figure 5a,b represent the average A_{sat} measured from the near-instantaneous responses of *Experiment 1* at a T_{leaf} of 15°C for plants grown at 5°C:15°C and a T_{leaf} of 25°C for plants grown at 15°C:25°C, that is, a T_{leaf} equivalent to the maximum T_{air} of the daytime photoperiod of each group of plants. The dashed lines are extended to the associated $V_{\text{cmax}25}$ values predicted by the linear models: 15°C measurements extend to the 5°C:15°C $A_{\text{sat}} \sim V_{\text{cmax}}$ linear model, 25°C measurements extend to the 15°C:25°C $A_{\text{sat}} \sim V_{\text{cmax}}$ linear model. For simplicity, the linear models used for this representation are aggregations of plants that were grown and acclimated to either 15°C:25°C or 5°C:15°C.

The predicted values of $V_{\text{cmax}25}$ from this exercise would be equivalent to the H (control) and L (control) values of Figure 4a,b, respectively. From Figure 5, it can be seen that a higher $V_{\text{cmax}25}$ would be predicted for plants of both accessions that were grown at 5°C:15°C and measured at a T_{leaf} of 15°C, even though A_{sat} was higher for CAF-Warm plants grown at 15°C:25°C and measured at 25°C. Referring to Figure 4a,b, it can be seen that $V_{\text{cmax}25}$ is lower for H (control) plants compared to L (control) plants of both accessions, which is consistent with this prediction. In other words, the observed between-accession difference in $A_{\text{sat}[\text{max}]}$ (*Experiment 1*) could occur without a between-accession difference in the $V_{\text{cmax}25}$ temperature response (*Experiment 2*). Overall, there was a generalised requirement for a greater $V_{\text{cmax}25}$ in cool-grown plants of both accessions in order to compensate for reduced enzymatic kinetics at these lower temperatures (Togashi et al. 2018).

4.5 | Implications for Modelling

We have shown a clear response of ΦPSII to temperature, with evidence of functional acclimation and adaptation, albeit from two accessions. At the leaf scale, models such as the FvCB model of C_3 photosynthesis typically do not consider a temperature response function for ΦPSII . Adding a ΦPSII temperature response function to leaf photosynthesis models may improve the temperature sensitivity of RuBP regeneration-limited photosynthesis, which may be especially important when estimating photosynthesis above and below the thermal optima.

We have also demonstrated that thermal acclimation of the maximum rate of carboxylation can occur within 1 day of exposure to a new temperature regime. This rapid response has implications for crop production or land surface carbon cycling models that aim to make predictions on short- to medium-term timescales. For example, given the strong relationship between A_{sat} and $V_{\text{cmax}25}$, it is likely that a rapid acclimation of the maximum rate of carboxylation at the scale of a canopy will also rapidly alter the gross primary productivity.

Acknowledgements

This research received support through Schmidt Sciences. The authors thank Matthew Nelson and Candy Taylor from the Commonwealth Scientific and Industrial Research Organisation for providing the *Brassica oleracea* seeds, and Isabella Butler, Kevin Donohue and Archie Drake for technical assistance. N.G.S. acknowledges support from the US National Science Foundation (DEB-2045968). Open access publishing facilitated by The University of Western Australia, as part of the Wiley – The University of Western Australia agreement via the Council of Australasian University Librarians.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data underlying each figure are available in the following perpetual data repository: <https://research-repository.uwa.edu.au/>.

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Supporting Information

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