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Title: Rubisco catalytic properties optimised for present and future climatic conditions

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Title: Rubisco catalytic properties optimised for present and future climatic conditions

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Abstract

Because of its catalytic inefficiencies, Rubisco is the most obvious target for improvement to enhance the photosynthetic capacity of plants. Two hypotheses are tested in the present work. Firstly, that existing Rubiscos have optimal kinetic properties to maximize photosynthetic carbon assimilation in existing higher plants. Secondly, that current knowledge allows proposal of changes to kinetic properties to make Rubiscos more suited to changed conditions in chloroplasts that are likely to occur with climate change. The catalytic mechanism of Rubisco results in higher catalytic rates of carboxylation being associated with decreased affinity for CO₂, so that selection for different environments involves a trade-off between these two properties. The simulations performed in this study confirm that the optimality of Rubisco kinetics depends on the species and the environmental conditions. In particular, environmental drivers affecting the CO₂ availability for carboxylation (C_c) or directly shifting the photosynthetic limitations between Rubisco and RuBP regeneration determine to what extent Rubisco kinetics are optimally suited to maximize CO₂ assimilation rate. In general, modeled values for optimal kinetic reflect the predominant environmental conditions currently encountered by the species in the field. Under future climatic conditions, photosynthetic CO₂ assimilation will be limited by RuBP-regeneration, especially in absence of water stress, the largest rise in [CO₂] and lowest increases in temperature. Under these conditions, the model predicts that optimal Rubisco should have high $S_{c/o}$ and low k_{cat}^c .

Keywords

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1. Introduction

The enzyme Rubisco catalyses the assimilation of CO₂ by the carboxylation of RuBP in the Calvin Cycle, and is therefore the most obvious target to improve the photosynthetic capacity of crops. Rubisco displays catalytic inefficiencies, including slow catalysis and imperfect discrimination between CO₂ and O₂ [1]. These inefficiencies not only limit the rate of CO₂ fixation, but also compromise the capacity of crops to use resources optimally, particularly water and nitrogen [1, 2]. In principle, overcoming these limitations could be successfully accomplished by molecular interventions in the genes coding for both the large (LSu) and the small (SSu) subunits of Rubisco [1, 3 - 5]. Among these interventions, replacement of crop Rubiscos by other versions of the enzyme with better catalytic performance have recently been tested and provide a powerful and promising approach [6 - 8]. However, the success – in terms of photosynthetic improvement – of the Rubisco replacement strategy will depend on the discovery of more efficient and compatible versions of the enzyme. As an example, [9] illustrated how Rubiscos from red algae would perform better than tobacco native Rubisco if they could be transferred into the chloroplast of tobacco.

A number of surveys have reported substantial variability among species in the kinetic parameters of Rubisco, the relative specificity for CO₂/O₂ ($S_{c/o}$), the Michaelis-Menten constants for CO₂ (K_c) and O₂ (K_o), and the maximum turnover of carboxylation (k_{cat}^c) [5, 10 - 15]. Among the potential forces driving evolution of Rubisco catalysis, the availability of CO₂ for ribulose-1,5-biphosphate (RuBP) carboxylation has been proposed as a major factor, explaining some of the differences among distant phylogenetic groups, between C₃ and C₄ species and among C₃ plants [12, 16, 17]. Thus, those environments where plants have evolved under high temperatures and low soil water availability should be prioritized in the search for better versions of the enzyme [18]. Under these conditions, the CO₂/O₂ concentration at the site of carboxylation is decreased due to lower leaf conductances in response to water scarcity and lower CO₂/O₂ solubility ratios as

temperature increases [19]. It has been demonstrated that Rubisco has evolved towards higher $S_{c/o}$ under these conditions, reducing RuBP oxygenation and favoring the carboxylase reaction [13]. Nevertheless, comparisons of the scarce data on Rubisco kinetics – particularly data describing the full set of kinetic parameters on the same species – indicate a strong, negative correlation between $S_{c/o}$ and k_{cat}^c . Therefore, the suggestion that Rubiscos with high $S_{c/o}$ from extreme environments would allow higher CO₂ assimilation rates when transferred into the chloroplast of a crop plant grown under non-stressed conditions, and perhaps even in dense canopies where light limits photosynthesis, is doubtful. The maximum leaf conductances typically measured under optimal growth conditions provide maximum CO₂ availability at the site of carboxylation [2]. Under these conditions, a Rubisco with a higher k_{cat}^c , although at the expenses of a lower $S_{c/o}$, may provide the higher benefits [20, 21].

The significant correlations between k_{cat}^c and $S_{c/o}$ – and K_o – have led to the opinion that Rubisco is nearly perfectly adapted for differing CO₂/O₂ concentrations and thermal conditions in the chloroplast environment [14, 20, 22]. Structural and mechanistic constraints have not allowed the development – either naturally or artificially – of *the perfect* Rubisco with increased specificity to CO₂ and a high rate of carboxylation. Natural evolution has resulted instead in Rubiscos in which there is a compromise between CO₂/O₂ affinities and the maximum rate of catalytic turnover dependent on the habitat and climate. This tradeoff is evident from a close inspection of published data which shows a significant scatter in Rubisco kinetics values and consequently in carboxylase catalytic efficiency (k_{cat}^c/K_c) [5, 14, 22]. Much less is known about the oxygenase kinetic parameters, although some correlation between oxygenase and carboxylase catalytic constants has been observed [23].

Maximum agricultural yields are obtained by growing crops with non-limiting resources by extensive irrigation and fertilization practices. However, the environmental footprint of intensive agriculture and the predictions for higher global temperatures and lower water availability for most

current cropping areas demand novel solutions towards a more sustainable agriculture. It is widely believed that a more efficient Rubisco would provide not only more photoassimilates for plant growth, but importantly do this at a lower cost of water and nutrients [24, 25]. However, given the tradeoff between k_{cat}^c and $S_{c/o}$, it is not immediately evident what *more efficient* means in environmental and physiological contexts. Within this context, the present paper aims at the following: i) to model how temperature, and CO₂ and O₂ concentration affect Rubisco kinetics; ii) to test whether current Rubiscos are optimally suited for the present conditions in the chloroplast of higher plants; iii) to estimate the potential impact of climate change on the optimality of Rubisco kinetics and to identify targets of improvement. We argue that it is necessary to consider the tradeoff between specific activity and specificity to define rigorous criteria for engineering plants with *more efficient* Rubisco.

2. Methods and theory

2.1. Optimal Rubisco

According to the biochemical model of leaf photosynthesis [26], the net assimilation rate (A) is determined by the minimum of the RuBP-saturated (A_c) and RuBP-limited (A_j) CO₂ assimilation rates:

$$(1) \quad A = \min (A_c, A_j),$$

$$(2) \quad A_c = \frac{V_{c\text{max}} (C_c - \Gamma^*)}{C_c + K_c (1 + O_i / K_o)} - R_d,$$

$$(3) \quad A_j = \frac{(C_c - \Gamma^*) J / 4}{C_c - 2\Gamma^*} - R_d,$$

where $V_{c\text{max}}$ is the maximal carboxylation rate, C_c is the CO₂ concentration at the site of carboxylation in the chloroplast stroma, K_c is the Michaelis–Menten constant for CO₂ and K_o is that for O₂, Γ^* is the CO₂ compensation point in the absence of mitochondrial respiration, J is the

CO₂-saturated electron transport rate of the thylakoid reactions which ultimately supply the necessary energy in the form of ATP and NADPH for the regeneration of RuBP, and R_d is the mitochondrial respiration in the light. The response of J to the photosynthetic photon flux density (PPFD) was calculated from the non-rectangular hyperbola proposed by Bernacchi et al. [27].

In equation (2), V_{cmax} was obtained from *in vitro* values as:

$$(4) \quad V_{\text{cmax}} = k_{\text{cat}}^{\text{c}} \cdot E \cdot Act$$

$k_{\text{cat}}^{\text{c}}$ is the Rubisco maximum turnover rate of carboxylation, E is the total quantity of catalytic sites and Act is the percentage of activation of Rubisco sites, i.e. its carbamylation state.

I^* was obtained from the Rubisco specificity factor, $S_{\text{c/o}}$, as:

$$(5) \quad I^* = 0.5 O / S_{\text{c/o}}$$

Because the Rubisco kinetic parameters are interrelated [5, 28], we can express the equations (2) and (3) as determined by a unique kinetic parameter, similarly to the approach followed by Savir et al. [14]. $k_{\text{cat}}^{\text{c}}$ is the most reported parameter, probably due to its relative ease of measurement. Thus, equations (2) and (3) were reformulated by substituting I^* , K_c and K_o by the statistical functions relating them to $k_{\text{cat}}^{\text{c}}$. To obtain the relationships of I^* , K_c and K_o vs. $k_{\text{cat}}^{\text{c}}$ at 25°C we used the data compiled in Savir et al. [14], excluding the photosynthetic bacteria. The regression coefficients were highly significant ($P < 0.001$), with $r^2 > 0.75$ (Table S1). Because the different kinetic parameters present distinct sensitivities to temperature [29], the functions relating $k_{\text{cat}}^{\text{c}}$ with the remaining parameters are dependent on temperature. Hence, to interrelate the Rubisco kinetic parameters at temperatures other than 25°C, values of $k_{\text{cat}}^{\text{c}}$, I^* , K_c and K_o were converted to the desired temperature using the equations described in Bernacchi et al. [29] for tobacco with the *in vitro* species-specific data measured at 25°C as the reference (i.e., the scaling constant c). Thereafter, regression analyses were again run between $k_{\text{cat}}^{\text{c}}$ and I^* , K_c and K_o (Table 1S). The analysis therefore assumes that the Rubisco kinetic parameters of the species included in the present study presented the same temperature dependency as that for Rubisco from *Nicotiana*

tabacum, and that the equations hold for the whole range of temperatures from 15°C to 38°C considered in the present study [30].

2.2. Optimization of Rubisco kinetics at varying temperature, and CO₂ and O₂ concentration

A first aim of the study was to model how temperature and [CO₂] and [O₂] affect Rubisco kinetics. For this, the optimal value for k_{cat}^c (i.e. the one maximizing the net CO₂ assimilation rate) was modeled at varying C_c and given values for J , R_d , E and Act by solving equations (1), (2) and (3) expressed in terms of k_{cat}^c as the unique parameter, using Mathematica 9 software (Wolfram Research, Champaign, IL, USA). This analysis was performed at three different temperatures (15, 25 and 35 °C) and [O₂] (1000, 170000 and 210000 μmol mol⁻¹). The optimal values for $S_{c/o}$ (I^*), K_c and K_o were thereafter obtained from the functions relating them with k_{cat}^c , at the desired temperature (Table 1S).

2.3. Optimization of Rubisco kinetics under present conditions

The second aim of the study was to test whether current Rubiscos are optimally suited for the present environmental conditions. By using the same equations, the net CO₂ assimilation rate was calculated at a range of varying k_{cat}^c values for *Limonium gibertii*, *Nicotiana tabacum* and *Triticum aestivum*, under different environmental conditions (25°C-well watered, 25°C-water stress, 38°C-well watered and 38°C-water stress). *L. gibertii* was selected as being one of the most attractive species in terms of Rubisco traits due to its high specificity [13, 31]. *N. tabacum* is the species where most of attempts to bioengineer Rubisco have been made [32], while *T. aestivum* is one of the most important crops worldwide supporting a significant part of the human caloric intake. Values for the model input parameters: C_c , J , R_d , E and Rubisco activation state under the different environmental conditions are shown in Table 1, and were taken from Galmés et al.

(unpublished), Galmés et al. [33] and Perdomo et al. (unpublished), for *L. gibertii*, *N. tabacum* and *T. aestivum*, respectively. The growth conditions were similar for the three original experiments. In all cases, the plants were grown for at least one month at a photosynthetic photon flux density (PPFD) $> 600 \mu\text{mol m}^{-2} \text{s}^{-1}$, with a photoperiod of 12 h day/12 h night, air relative humidity above 40%, and varying air temperature (25 °C and 38 °C) and soil water availability – field capacity (well watered) and 40% field capacity (moderate water stress). For the different experiments, the model input parameters were measured following the procedures described in Galmés et al. [33]. The modeled optimal values of Rubisco kinetics were then compared to the *in vitro* values obtained from Galmés et al. (unpublished), Whitney et al. [5] and Savir et al. [14] for *L. gibertii*, *N. tabacum* and *T. aestivum*, respectively (see Table 2). Finally, deviation from optimality was calculated for each kinetic parameter as: (optimal value – *in vitro* value)/*in vitro* value.

2.4. Optimization of Rubisco kinetics under future conditions

The third aim of the present study was to evaluate the potential effects of climate change on current optimization of Rubisco kinetics in wheat. For this, eight different scenarios of change were considered, each one representing a specific combination of the three main drivers of climate change: atmospheric CO₂ rise (550 or 800 $\mu\text{mol mol}^{-1}$), temperature increase (2°C or 5°C increase with respect to 25°C) and water availability (well watered and moderate water stress). The intensities of change in atmospheric [CO₂] (C_a) and temperature were selected according to IPCC predictions [34]. Atmospheric [CO₂] is projected to continue rising to at least 550 ppm by 2050, and by 2100 the expected increase in air temperature is between 2 and 5°C, depending on the region and the applied model [35].

The impact of each climate change scenario on the stomatal conductance (g_s) was quantified in relation to the current g_s measured in wheat under optimal conditions (Perdomo et al. unpublished) as follows: 20% and 50% decrease at C_a of 550 and 800 ppm, respectively [36],

1.5% increase per °C increase in air temperature according to Evans and von Caemmerer [37], and 50% decrease under moderate water stress according to Galmés et al. [38]. Thereafter, considering the leaf mesophyll conductance (g_m) measured in the same experiment, a g_m/g_s ratio of 0.39 was obtained at 25°C. We are aware these values for the g_m/g_s ratio are lower than typical reported values for a range of species [39], but given the natural variability in this ratio we preferred to use values recently measured on wheat in our laboratory. The g_m/g_s ratio was changed at 27°C (0.44) and 30°C (0.52) assuming the response to temperature of g_m published for tobacco in Walker et al. [40].

The concentration of Rubisco catalytic sites and its activation state were also taken from 25°C-well watered plants (Perdomo et al. unpublished) and assumed to be constant for all climate change scenarios (Table 2S). According to a recent review, at the expected increase in C_a , temperature and severity of water stress, the changes in Rubisco concentration and activation will be minor [41]. By using *in vitro* published data for wheat Rubisco at 25°C [14], V_{cmax} was calculated at 25°C applying equation (4). A constant ratio J_{max}/V_{cmax} of 1.5 has been measured in wheat in our laboratory (Perdomo et al. unpublished), which matches very well with other estimates recently published in wheat [42]. V_{cmax} and J_{max} temperature responses were assumed to be similar to those obtained for tobacco by Walker et al. [40]. Oxygen concentration was taken as 210000 $\mu\text{mol mol}^{-1}$ and PPFD as 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Finally, we ended up with two unknowns, A and C_c , and two equations, (2) or (3) for A and Fick's law for C_c :

$$(6) \quad C_c = C_a - \frac{A}{g_s} - \frac{A}{g_m}$$

The resultant quadratic equation for Rubisco-limited photosynthesis (2) was solved according to von Caemmerer and Evans [43] and Ethier and Livingston [44], and that for RuBP-limited photosynthesis (3) solved according to Niinemets et al. [45].

All analyses were performed considering A to be first strictly limited by A_c , and then also including A_j limitation.

3. Results and Discussion

3.1. Optimization of Rubisco kinetics at varying temperature, and CO_2 and O_2 concentration

Past surveys compiling data on Rubisco kinetics and specific activity are in agreement in that the maximum turnover rate of carboxylation (k_{cat}^c) and the affinity for CO_2 (i.e. the inverse of the Michaelis-Menten constant for CO_2 , K_c) or the relative specificity for CO_2 and O_2 ($S_{c/o}$) are inversely related [5, 14, 22, 28, 46, 47]. This suggests constant values for the carboxylase catalytic efficiency (k_{cat}^c/K_c) at given temperature and substrate concentration [48], and implies that the rate of carboxylation cannot be improved without losing affinity for CO_2 [5, 20, 49]. A direct consequence of limited variability in k_{cat}^c/K_c is that each particular Rubisco has been forced to evolve towards optimizing either the velocity or the affinity to CO_2 . Although conclusive proofs about the environmental factors driving the evolution of Rubisco have not been provided, there is some evidence that the concentration of the two gaseous substrates, CO_2 and O_2 , and long-term temperature environment are playing a decisive role [13, 17, 22, 50]. It is therefore relevant to explore how these key environmental factors modulate Rubisco performance.

We define optimal Rubisco as Rubisco having a combination of kinetic traits yielding the maximum photosynthesis under given environmental conditions. According to the C_3 photosynthesis model [26], the rate of CO_2 assimilation (A) is the minimum of the ribulose-1,5-bisphosphate (RuBP)-saturated rate of photosynthesis (A_c) and the RuBP-limited rate (A_j). Consequently, it is important to consider both limitations in assessing the kinetic values for an optimal Rubisco.

At a constant concentration of Rubisco active sites ($24 \mu\text{mol m}^{-2}$), and strictly considering the A_c -limited rate of photosynthesis, increasing the concentration of CO_2 in the chloroplastic stroma (C_c) shifts optimality towards increased k_{cat}^c , and decreased $S_{c/o}$ (Fig. 1), in agreement with previous analyses [49]. This fact explains the benefits of the catalytic modifications in C_4 Rubiscos under saturating $[\text{CO}_2]$. In particular, on average greater k_{cat}^c values in C_4 than in C_3 plants [8, 51]. The dependency of optimum k_{cat}^c on C_c is non-linear, being greater at lower C_c , and decreasing at higher C_c (Fig. 1). The inclusion of A_j -limitation in the analysis changes the response of the optimum k_{cat}^c on C_c . In the initial phase, at the A_c -limited region, optimum k_{cat}^c increases with C_c until a certain threshold at which $A_c=A_j$. When the threshold C_c is exceeded, A becomes A_j -limited, after which the optimum k_{cat}^c decreases with increasing C_c (Fig. 1). We note that the threshold C_c depends not only on Rubisco kinetics, but also on light intensity that modifies the rate of RuBP regeneration and on temperature effects on the capacity for photosynthetic electron transport (for explicit derivation of the condition of co-limitation see e.g. [52]).

Due to the tradeoff between k_{cat}^c and $S_{c/o}$, the response of optimum $S_{c/o}$ on C_c was opposite to that for optimum k_{cat}^c (Fig. 1). For the A_j -limited rate of photosynthesis, $S_{c/o}$ for an optimal Rubisco increased even at high C_c . This fact explains the predicted beneficial effects of replacing wheat Rubisco by that from *Limonium gibertii* that has a higher $S_{c/o}$ [53].

Regardless of whether A is limited by Rubisco or RuBP regeneration, the relationship between the optimal k_{cat}^c/K_c and C_c exhibited a biphasic response. Optimal k_{cat}^c/K_c increased with C_c until a maximum value, after which further increases in C_c resulted in decreases in k_{cat}^c/K_c (Fig. 1). Remarkably, the C_c value at which the maximum value of optimal k_{cat}^c/K_c was attained, ca. $100 \mu\text{mol mol}^{-1}$ at 15°C , $150 \mu\text{mol mol}^{-1}$ at 25°C and $200 \mu\text{mol mol}^{-1}$ at 35°C , roughly coincides with the values typically reported for C_c in C_3 species under non-stressful well-watered conditions and current ambient atmospheric CO_2 concentrations. The reported C_c values range from $200 \mu\text{mol}$

mol^{-1} in crops and herbaceous plants [37, 54 - 56] to less than $100 \mu\text{mol mol}^{-1}$ in sclerophyll leaves with high leaf mass per area [45, 56 - 60].

Rubisco kinetic parameters are all very sensitive to temperature [13, 61 - 63]. However, the relative effect of a given change in temperature is different for each kinetic parameter, due to their differential temperature dependence [29, 64]. This can be observed in Fig. 1, where the proportional effect of altered temperature was different in the response of optimal k_{cat}^c , $S_{c/o}$ and k_{cat}^c/K_c to varying C_c (Fig. 1). In the analysis considering A_j limitation (Fig. 1 right-side panels), an increase in temperature increased the threshold value of C_c where maximum and minimum values for optimal k_{cat}^c and $S_{c/o}$ are obtained. This is because temperature affects the C_c at which the transition from Rubisco to RuBP regeneration limitation occurs [64 - 66].

Changes in the concentration of O_2 also conditioned the above described responses of optimal k_{cat}^c and $S_{c/o}$ to varying C_c (Fig. 1S). As hypothesized [67], an atmosphere with very little molecular oxygen corresponding to the geological past when Rubisco evolved more than 2.5 billion years ago, would favor a Rubisco with increased k_{cat}^c , and decreased $S_{c/o}$, but only in the case when A_c limits A . Under low $[\text{O}_2]$ and for A_j -limited A , high C_c would demand Rubiscos with lower k_{cat}^c and higher $S_{c/o}$ (Fig. 1S). According to the biochemical model of photosynthesis [26], under A_j limitation, the unique kinetic parameter of Rubisco directly determining A is $S_{c/o}$ (or Γ^*). Therefore, the demanded lower k_{cat}^c is simply an indirect effect of the tradeoff between k_{cat}^c and $S_{c/o}$.

3.2. Rubisco is not optimal in current environmental conditions of C_3 plants

Empirical data of leaf gas-exchange, chlorophyll fluorescence and Rubisco biochemistry were compiled for the perennial semi-shrub *Limonium gibertii* adapted to stressful environments in shallow, salty soils in the Mediterranean, annual herb *Nicotiana tabacum* (tobacco) and annual grass *Triticum aestivum* (wheat) from experiments performed under similar environmental

conditions (Table 1). These data were used to model A over a range of values of $k_{\text{cat}}^{\text{c}}$ to test whether Rubisco kinetics of these three species were optimal under the characteristic environmental conditions encountered in the field (Fig. 2).

First, we considered the predictions under well-watered non-stressed conditions at 25 °C and assumed RuBP-saturated rate of A . Under these conditions, the modeled optimal Rubisco had higher $k_{\text{cat}}^{\text{c}}$ and K_{c} than reported *in vitro* values for three species (Table 2). Deviations from the actual and the modeled optimum values of $k_{\text{cat}}^{\text{c}}$ under these conditions were higher in *L. gibertii* and wheat, and less in tobacco (Fig. 2). Water stress, by reducing C_{c} , decreases the optimal values of $k_{\text{cat}}^{\text{c}}$ (Fig. 2), in agreement with the idea that increasing $S_{\text{c/o}}$ would be particularly beneficial under semi-arid and arid conditions characterized by most severe limitation of photosynthesis by water availability [15, 52]. Under water stress at 25 °C, the modeled optimal Rubisco had a lower $k_{\text{cat}}^{\text{c}}$ and K_{c} than the reported *in vitro* values for *L. gibertii* and tobacco, while modeled and *in vitro* values perfectly matched in wheat (Table 2). In wheat, increasing leaf temperature to 38 °C augmented the modeled optimal $k_{\text{cat}}^{\text{c}}$, but the correspondence with temperature-corrected *in vitro* values was maintained. Subsequent application of water stress to wheat grown at 38 °C decreased the optimal $k_{\text{cat}}^{\text{c}}$ to values slightly lower than the *in vitro* ones (Table 2). Overall, evaluating the departure from optimality of all characteristics of Rubisco as the average deviation from optimality for individual traits, $k_{\text{cat}}^{\text{c}}$, $S_{\text{c/o}}$ (I^*) and K_{c} modeled under A_{c} -limited photosynthesis, Rubiscos of *Limonium* and wheat were better suited for conditions typically measured under water stress, while tobacco Rubisco lost optimality under water stress (Table 3). These results may be a consequence of the enzyme evolution under the predominant environmental conditions encountered by these species in the field. The rocky, saline and hot areas under Mediterranean climate inhabited by *Limonium* species necessitate a strict control of water loss by stomatal closure, thereby restricting the CO_2 availability for carboxylation [57]. On the other hand, wild wheat relatives and the first domesticated varieties of wheat were drought and salt tolerant [68, 69]. These results corroborate

the results of a past simulation study showing that current Rubisco kinetics of many C_3 plants are better suited for low $[CO_2]$ [49].

In well-watered plants grown at 25 °C and at moderately high light of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, A was limited by the rate of RuBP-regeneration (A_j) in all three species (Table 2). Under these conditions, optimum k_{cat}^c is determined at the A_c to A_j co-limitation point. At this point, the values of optimum k_{cat}^c were lower than those modeled strictly under A_c -limitation (Fig. 2). The values of optimum k_{cat}^c , K_c and I^* under A_c , A_j co-limitation were lower than *in vitro* reported values (Table 2). Under water stress at 25 °C, the optimum k_{cat}^c corresponded to the A_c -limited rate in *Limonium* and tobacco, but still to A_c , A_j -co-limited rate in wheat, with slight increase in the optimum values compared with the well-watered treatment (Fig. 2, Table 2). By increasing the temperature to 38 °C in wheat, A became A_c limited and optimal k_{cat}^c increased as explained above (Fig. 2). The overall optimality of Rubisco kinetics after considering A_j -limitation was improved only in *Limonium* at 25°C and well-watered conditions (Table 3).

This analysis and the simulation by Zhu et al. [49] focusing on C_3 plants reveal that Rubisco kinetics are far from being optimal, and that there is room for improvement. Our results highlight that the optimality of Rubisco kinetics depends on the species and the environmental conditions. In fact, due to the inherent tradeoff between $S_{c/o}$ and k_{cat}^c , being optimal under some circumstances, such as water limitations, results in non-optimality in well-watered conditions, especially if there is a cross-over to RuBP-limited photosynthesis. As this analysis further demonstrates, occurrence of RuBP-limitation itself is an important factor altering the Rubisco optimality. In fact, plants are exposed to varying light conditions during the day and only uppermost leaves in the canopy are exposed to full sunlight [45, 70, 71], implying that a large fraction of leaves in the canopy is RuBP-limited at any moment of time and all leaves are RuBP-limited at some moment during the day. In a typical canopy, light distribution is heterogeneous and V_{cmax} and J_{max} are distributed accordingly. However, the ratio of V_{cmax} to J_{max} does not differ much

between sites [72]. This drove to Chen et al. [73] to propose the coordination theory in which nitrogen invested in the different photosynthetic pools of the leaves produce a co-limitation by A_c and A_j on a daily average. Taken all this, optimization of Rubisco needs consider RuBP-limited photosynthesis as well.

3.3. Optimality of Rubisco kinetics will be affected by climate change

The optimality of Rubisco kinetics was tested under different scenarios of climate change, including varying conditions of atmospheric CO_2 , temperature and water availability, and compared to modeled optimality under present conditions. Based on assumptions of how the environmental changes will impact stomatal (g_s) and mesophyll (g_m) conductances to CO_2 , dark respiration rate (R_d), photosynthetic electron transport rate (J) and Rubisco traits (see Material and Methods), a value for C_c corresponding to given set of environmental drivers (ambient CO_2 concentration, light intensity, temperature) was obtained.

Irrespective of whether photosynthesis was RuBP-saturated or -limited, the overall optimality of Rubisco kinetics (k_{cat}^c , I^* and K_c) increased in relation to current performance, in three of the eight scenarios of change, decreased in three and did not change significantly in two (Table 4). Under moderately increased $[\text{CO}_2]$ of up to $550 \mu\text{mol mol}^{-1}$, shift of Rubisco performance towards increased optimality is expected under those scenarios with decreased water availability, regardless of the increase in temperature (Table 4). At a $[\text{CO}_2]$ of $800 \mu\text{mol mol}^{-1}$, however, more optimal performance compared to performance under current conditions will occur under water stress and severe increment in temperature (Table 4). A closer inspection reveals that the predicted change in optimal Rubisco parameters is driven by possible modifications in C_c . If climate change drivers lead to C_c increases, then optimality will decrease, and the reverse occurs if climate change leads to lower C_c that can be the case under more severe water limitation (Fig. 3).

This is in agreement with the hypothesis that the availability of CO₂ is the main factor modulating Rubisco kinetics evolution [13, 17, 22, 50].

For A_c-limited photosynthesis, the modeled optimal values of k_{cat}^c as well as of Γ^* , K_c and K_o increased under all scenarios of change, as compared to current values (Table 2S). However, a part of the increase in k_{cat}^c is directly caused by the increase in the leaf temperature. At the same predicted increase in temperature, the increase in optimal k_{cat}^c was lower under the conditions restricting CO₂ availability for carboxylation, i.e. under water stress and modest increases in atmospheric CO₂. This again emphasizes the complex multivariate dependence of optimal characteristics of Rubisco.

At the calculated C_c values, photosynthesis is limited by RuBP-regeneration (A_j) for all scenarios of change, especially in those scenarios with no water stress, highest rise in the [CO₂], and lowest increase in leaf temperature (Table 2S). In general, under A_c, A_j co-limitation, values of optimal k_{cat}^c increased in all cases, as compared to current values, except the scenario predicting no increase in water stress, 800 μmol CO₂ mol⁻¹ and 2 °C increase in leaf temperature. Noticeably, this is the scenario with the highest predicted increase in C_c (Table 2S). Regardless of the increase in optimal k_{cat}^c relative to present situation, in absolute terms, optimal k_{cat}^c under all scenarios is lower than *in vitro* reported data (Table 2S). In consequence, under future climate, optimal Rubisco for A_j-limited A should have a higher $S_{c/o}$. This finding should be considered in next attempts to engineer Rubisco of crop plants for a higher photosynthetic capacity. Previous simulations ignoring changes in environmental conditions showed that a decrease, not an increase in $S_{c/o}$ would enhance CO₂ assimilation when photosynthesis was A_c-limited, but already pointed out that increasing $S_{c/o}$ would maximize carbon gain under A_j-limitation [22, 52]. At the crop level, this trend for light-limited photosynthesis under future climate is supported by experimental evidence showing that canopies become denser with plants having higher leaf area indices at higher CO₂ [74, 75]. This may be even more important in wild plants growing under strong light

competition. Accordingly, interspecific differences in $S_{c/o}$ [13] may strongly modify the competitive potential of different species and thereby alter species dominance and range in future. As demonstrated, the situation can be dramatically different when the severity of drought increases, as expected for a series of ecosystems, in particular in Mediterranean and other semi/arid ecosystems [76]. Such important aspects, including species differences in C_c [56], are not currently considered in modeling plant responses to future conditions. Of equal importance is that future modelling approaches should consider the effects of K_m for RuBP under dynamic conditions when light changes rapidly within seconds to minutes, leading to significant changes in the pool size of RuBP [77].

Optimization of Rubisco kinetics largely depends on the ratio between the maximum capacity for carboxylation (V_{cmax}) and the capacity for regeneration of RuBP (J_{max}). This is because the V_{cmax}/J_{max} ratio together with light intensity, determines the C_c value at which the transition between the A_c - and A_j -limited rates occurs. In fact, it has been suggested that, ideally, a crop plant should express a high k_{cat}^c -Rubisco in the upper canopy leaves, exposed to full sunlight, higher temperature and water deficits (therefore A_c -limited), and a high $S_{c/o}$ -Rubisco in the shaded lower canopy leaves (A_j -limited) [20, 49]. Some reports demonstrate that the expected increments in the photosynthetic rate due to higher $[CO_2]$ may be counteracted by limited nitrogen availability [78, 79]. Actually, under elevated CO_2 , redistribution of nitrogen allocation from Rubisco towards RuBP-regeneration has been documented with clear effects on V_{cmax}/J_{max} ratio [80, 81]. In view of reports compiled in a recent review [44], we have considered constant concentration of Rubisco active sites. However, changes in the activity of Rubisco Activase and in the concentration of Rubisco inhibitors would directly impact on the concentration of Rubisco active sites. Hence, the observed decreases in the Rubisco activation state at elevated $[CO_2]$ [82], the decreased activity of Rubisco activase under high temperatures [83], or the increased concentration of tight-binding of inhibitors reported in several species under severe drought [25, 84] would lead to lower V_{cmax} .

Thus, a decrease in $V_{\text{cmax}}/J_{\text{max}}$ could shift the limitation towards A_c , and therefore, increase the relevancy of k_{cat}^c over $S_{c/o}$. In fact, this point is equally valid for acclimation to altered temperatures, where cross-over C_c concentration might change due to different temperature relationships of J_{max} and V_{cmax} (Fig. 1) [85, 86].

4. Concluding remarks

Previous analyses of Rubisco optimization suggested that the kinetic parameters are nearly perfectly optimized [14, 22]. However, the present study shows that analysis of Rubisco optimization necessitates considering the predominant environmental conditions, which influence the $[\text{CO}_2]$ and temperature conditions at the site of carboxylation. When including physiologically relevant conditions of chloroplastic $[\text{CO}_2]$ and temperature, the analysis reveals that Rubisco is not that perfectly optimized, and that there is room for improvement. These results are in agreement with previous simulation by Zhu et al. [49], and the apparent controversy may depend on whether the analysis compares distant phylogenetic groups [14, 22] or is restricted to higher plants [49]. We also demonstrate that current Rubiscos will improve their performance under future climate if change drivers lead to decreased availability of CO_2 for carboxylation. This is likely to occur in future scenarios predicting drier soils, highest increase in temperature and lowest increment in atmospheric $[\text{CO}_2]$. Overall, the present results, in line with previous surveys, suggest that the concentration of CO_2 and O_2 in the chloroplast and the leaf temperature have been playing a decisive role in shaping Rubisco evolution.

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Table 1. Leaf photosynthetic parameters for *Limonium gibertii*, *Nicotiana tabacum* and *Triticum aestivum* under different conditions of water availability (WW: well-watered; WS: water stress) and leaf temperature. Values for the net CO₂ assimilation rate (A), stomatal conductance (g_s), mesophyll conductance (g_m), CO₂ concentration in the chloroplast (C_c), rate of electron transport (J), mitochondrial respiration in the light (R_d), concentration of Rubisco sites (E) and Rubisco activation state consisted in experimental data from Galmés et al. (unpublished), Galmés et al. [33] and Perdomo et al. (unpublished) for *L. gibertii*, *N. tabacum* and *T. aestivum*, respectively. Photosynthetic photon flux density and oxygen concentration during measurements were constant at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 210000 $\mu\text{mol mol}^{-1}$, respectively. Growth conditions were similar for the three original sources, and the plants were acclimated (i.e. grown for at the least one month) to the experimental conditions.

	<i>L. gibertii</i>		<i>N. tabacum</i>		<i>T. aestivum</i>			
	25°C-WW	25°C-WS	25°C-WW	25°C-WS	25°C-WW	25°C-WS	38°C-WS	38°C-WS
A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	23.5	10.8	23.8	8.9	23.8	14.5	17.8	13.03
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	0.306	0.095	0.335	0.049	0.520	0.094	0.436	0.219
g_m ($\text{mol m}^{-2} \text{s}^{-1}$)	0.354	0.099	0.404	0.089	0.203	0.037	0.258	0.139
C_c ($\mu\text{mol mol}^{-1}$)	167.1	80.1	206.4	92.2	181.4	109.1	217.8	172.1
J ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	132.3	157.6	175.6	184.3	130.6	144.4	278.3	274.1
R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	2.1	1.1	1.2	1.2	0.4	0.9	1.3	0.4
E ($\mu\text{mol m}^{-2}$)	64.0	81.7	75.5	77.5	62.9	72.1	90.4	84.0
Activation state (%)	75	65	77	82	80	80	50	50

Table 2. Current and optimal values of the net CO₂ assimilation rate (A) and *in vitro* Rubisco kinetics for *Limonium gibertii*, *Nicotiana tabacum* and *Triticum aestivum* under different conditions of water availability (WW: well-watered; WS: water stress) and leaf temperature. To obtain the optimal values, the photosynthetic rate was considered to be limited strictly by Rubisco (A_c limitation) or co-limited by Rubisco and RuBP-regeneration capacity (A_c , A_j co-limitation). Values for A were experimentally measured (same as in Table 1). Current values at 25°C of the maximum turnover rate of carboxylation (k_{cat}^c), the CO₂ compensation point in the absence of mitochondrial respiration (Γ^*), and the Michaelis-Menten constants of Rubisco for CO₂ (K_c) and O₂ (K_o) were obtained from Galmés et al. (unpublished), Whitney et al.[5], and Savir et al. [14] for *L. gibertii*, *N. tabacum* and *T. aestivum*, respectively. Values of *in vitro* Rubisco kinetics for *N. tabacum* at 38°C were obtained after applying temperature equations from Bernacchi et al. [29], but using measured data at 25°C as the reference.

	<i>L. gibertii</i>		<i>N. tabacum</i>		<i>T. aestivum</i>			
	25°C-WW	25°C-WS	25°C-WW	25°C-WS	25°C-WW	25°C-WS	38°C-WW	38°C-WS
Current values								
A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	23.5	10.8	23.8	8.9	23.8	14.5	17.8	13.03
k_{cat}^c (s^{-1})	2.5		3.2		2.5		5.5	
Γ^* ($\mu\text{mol mol}^{-1}$)	35.2		48.2		43.9		66.3	
K_c ($\mu\text{mol mol}^{-1}$)	273		339		443		1737	
K_o ($\mu\text{mol mol}^{-1}$)	341671		233468		622014		928102	
Optimal values under A_c limitation								
A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	21.2	8.5	33.8	12.5	26.5	15.3	19.9	14.0
k_{cat}^c (s^{-1})	3.3	1.9	3.6	2.2	3.4	2.6	5.9	5.2
Γ^* ($\mu\text{mol mol}^{-1}$)	45.6	31.0	47.8	35.0	46.5	38.9	61.2	55.9

K_c ($\mu\text{mol mol}^{-1}$)	422	264	465	293	438	330	1364	1218
K_o ($\mu\text{mol mol}^{-1}$)	376046	333502	385614	342472	379868.0	353048	701605	681460
Optimal values under $A_c A_i$ co-limitation								
A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	18.0	8.5	27.8	12.5	21.1	14.9	19.9	14.0
k_{cat}^c (s^{-1})	1.8	1.9	1.8	2.2	1.6	2.0	5.9	5.2
Γ^{\bullet} ($\mu\text{mol mol}^{-1}$)	29.6	31.0	30.2	35.0	26.7	32.5	61.2	55.9
K_c ($\mu\text{mol mol}^{-1}$)	255.9	264	259.5	293	240.5	274.3	1364	1218
K_o ($\mu\text{mol mol}^{-1}$)	330688	333502	331884	342472	325442	336659	701605	681460

Table 3. Rubisco kinetics optimality in *Limonium gibertii*, *Nicotiana tabacum* and *Triticum aestivum* under different conditions of water availability (WW: well-watered; WS: water stress) and leaf temperature. Rubisco kinetics optimality was calculated as the average of the optimality for the maximum turnover rate of carboxylation (k_{cat}°), the CO₂ compensation point in the absence of mitochondrial respiration (I^*) and the Michaelis-Menten constant of Rubisco for CO₂ (K_c). The photosynthetic rate was considered to be limited strictly by Rubisco (A_c limitation) or co-limited by Rubisco and RuBP-regeneration capacity (A_c, A_j co-limitation). The input values of the model are shown in Table 1. The equations describing the correlation among Rubisco kinetic parameters at the different temperatures are shown in Table 1S.

		A_c limitation	A_c, A_j co-limitation
<i>L. gibertii</i>	25°C-WW	37.9	17.3
	25°C-WS	13.6	13.6
<i>N. tabacum</i>	25°C-WW	16.7	34.6
	25°C-WS	24.1	24.1
<i>T. aestivum</i>	25°C-WW	14.4	40.4
	25°C-WS	13.0	28.1
	38°C-WW	12.5	12.5
	38°C-WS	17.0	17.0

Table 4. Rubisco kinetics optimality in *Triticum aestivum* under different scenarios of climate change, at varying atmospheric CO₂ concentration (C_a), leaf temperature (T_{leaf}) and water status (WW: well-watered; WS: water stress). The photosynthetic rate was considered to be limited strictly by Rubisco (A_c limitation) or co-limited by Rubisco and RuBP-regeneration capacity (A_c , A_j co-limitation). The input values of the model are shown in Table 2S. The equations describing the correlation among Rubisco kinetic parameters at the different temperatures are shown in Table 1S.

Climate change scenario	C_a	T_{leaf}	Water status	A_c limitation	A_c , A_j co-limitation
Current climate	400	25	WW	24.9	44.6
1	550	27	WW	30.1	47.6
2	550	27	WS	14.0	34.5
3	800	27	WW	44.6	55.5
4	800	27	WS	24.9	44.1
5	550	30	WW	21.8	44.5
6	550	30	WS	12.0	29.2
7	800	30	WW	37.2	53.8
8	800	30	WS	17.3	40.4

Figure 1. Optimal values for the maximum turnover rate of carboxylation ($k_{\text{cat}}^{\text{c}}$), specificity factor ($S_{\text{c/o}}$) and carboxylation catalytic efficiency ($k_{\text{cat}}^{\text{c}}/K_{\text{c}}$) at a range of CO_2 concentrations in the chloroplast (C_{c}) at 15°C (empty circles), 25°C (filled circles) and 35°C (empty triangles). The photosynthetic rate was considered to be limited strictly by Rubisco (A_{c} limited, left graphs) or co-limited by Rubisco and RuBP-regeneration capacity (A_{c} , A_{j} co-limited, right graphs). The rates of electron transport (J) were 42, 74 and 106 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 15°C, 25°C and 35°C, respectively. The rates of mitochondrial respiration in the light (R_{d}) were 0.5, 1.0 and 1.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 15°C, 25°C and 35°C, respectively. The concentration of Rubisco catalytic sites (30 $\mu\text{mol m}^{-2}$) and its activation state (80%) were considered invariable at the three temperatures.

Figure 2. Net CO_2 assimilation rate (A) at a range of values for the maximum turnover rate of carboxylation ($k_{\text{cat}}^{\text{c}}$) for *Limonium gibertii*, *Nicotiana tabacum* and *Triticum aestivum*, under non-stress conditions (25°C-well-watered, filled circles), 25°C-water stress (empty circles), 38°C-well-watered (filled triangles) and 38°C-water stress (empty triangles). The photosynthetic rate was considered to be limited strictly by Rubisco (A_{c} limited, left graphs) or co-limited by Rubisco and RuBP-regeneration capacity (A_{c} , A_{j} co-limited, right graphs). The dotted line indicates the actual $k_{\text{cat}}^{\text{c}}$ value for the three species. The input values of the model are shown in Table 1. The equations describing the correlation among Rubisco kinetic parameters at the different temperatures are shown in Table 1S.

Figure 3. The relationship between the predicted change in optimal Rubisco in *T. aestivum* under the different scenarios of climate change (indicated in Table 4) and the chloroplastic CO_2 concentration (C_{c}) estimated for the same scenarios. Values of the

predicted change in optimal Rubisco are normalized to the current values (set at 1), <1 means improvement of Rubisco optimality, >1 means decrement of Rubisco optimality. The photosynthetic rate was considered to be limited strictly by Rubisco (A_c limitation, filled circles and solid line) or co-limited by Rubisco and RuBP-regeneration capacity (A_c, A_j co-limitation, empty circles, dashed line).

Figure 1.

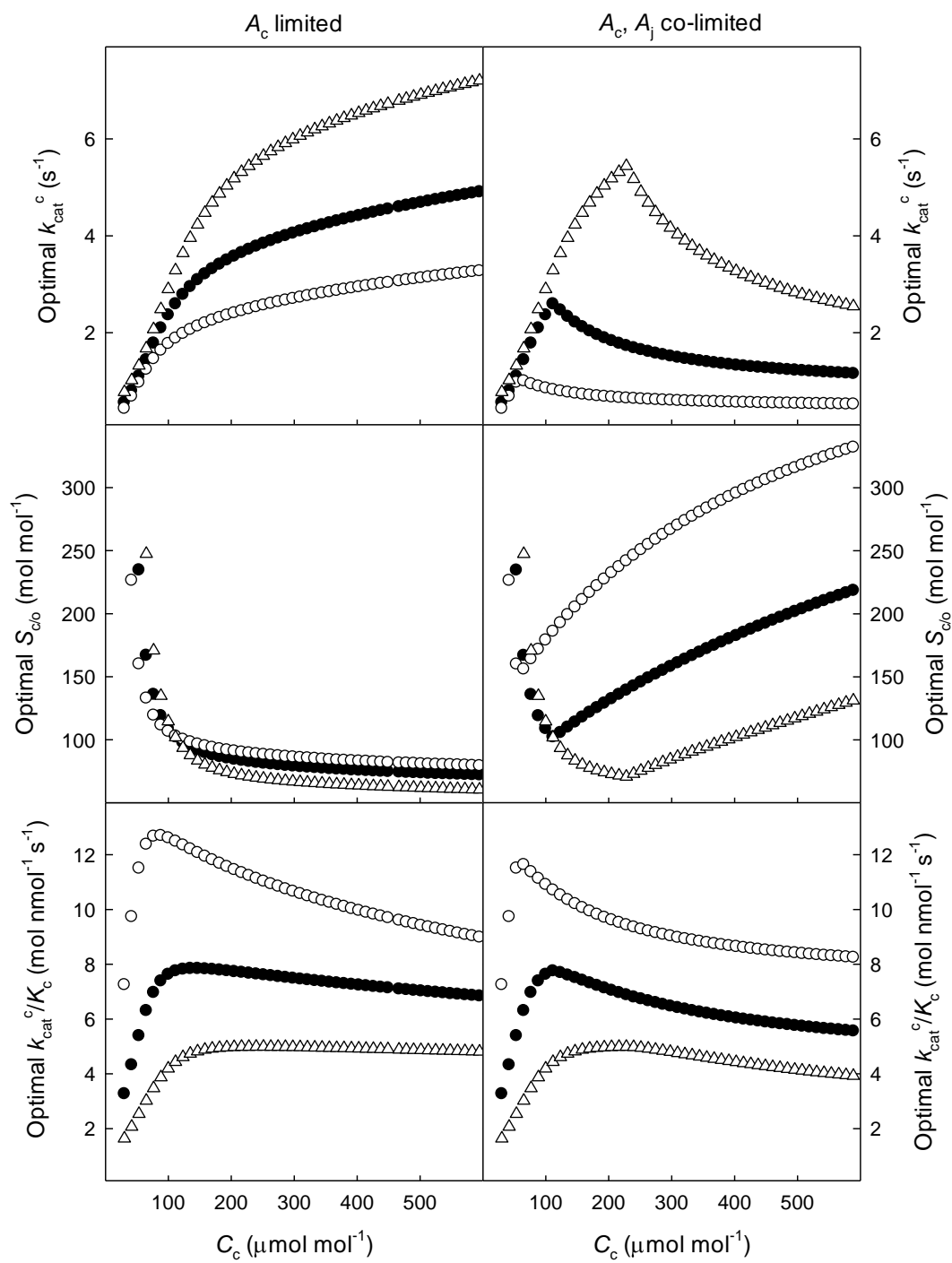


Figure 2

Figure 2.

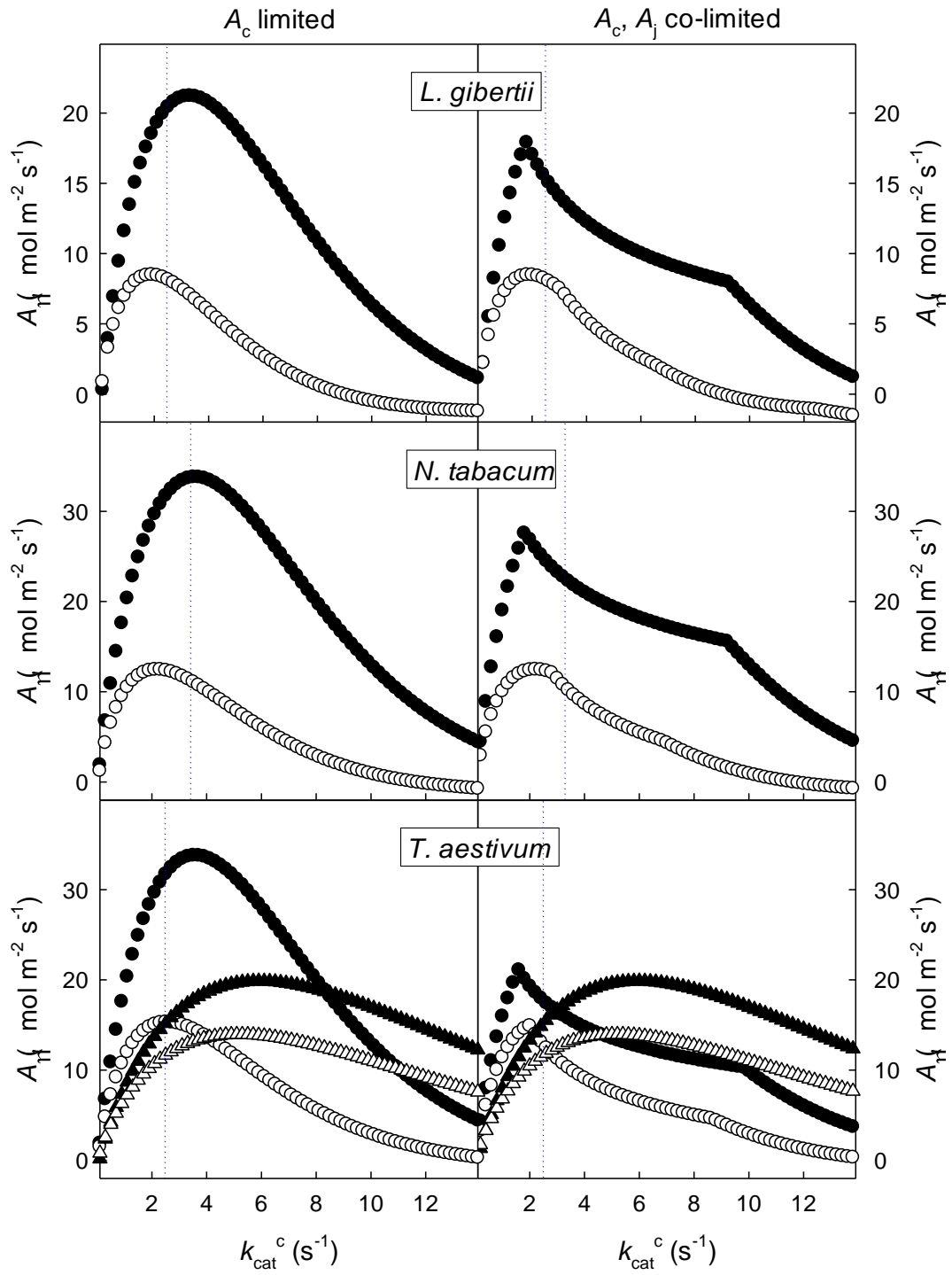


Figure 3.

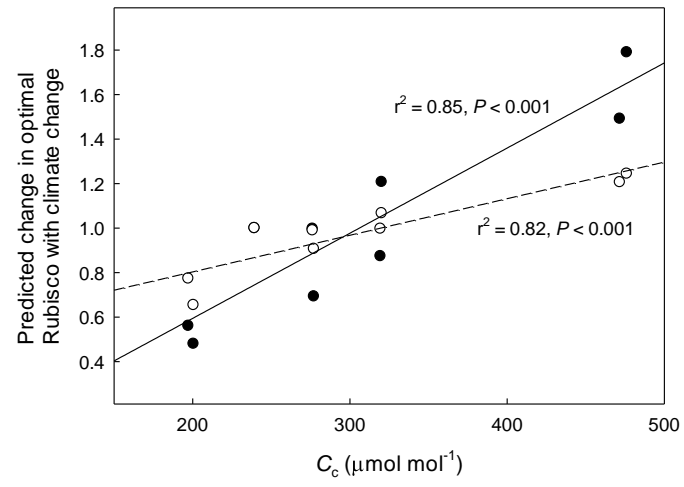


Table 1S. Equations describing the relationship between the maximum turnover rate of carboxylation (k_{cat}°) and the CO₂ compensation point in the absence of mitochondrial respiration (I^*), and the Michaelis-Menten constants of Rubisco for CO₂ (K_c) and O₂ (K_o) at the different temperatures used in the present article.

T_{leaf}	I^*	K_c	K_o
15°C	$22.39+18.40 \cdot \ln(k_{\text{cat}}^{\circ})$	$45.7 \cdot \exp(0.634 \cdot k_{\text{cat}}^{\circ})$	$K_c / (0.0003 \cdot \exp(0.471 \cdot k_{\text{cat}}^{\circ}))$
25 °C	$14.64+25.93 \cdot \ln(k_{\text{cat}}^{\circ})$	$142.1 \cdot \exp(0.330 \cdot k_{\text{cat}}^{\circ})$	$K_c / (0.0005 \cdot \exp(0.245 \cdot k_{\text{cat}}^{\circ}))$
27 °C	$12.17+27.69 \cdot \ln(k_{\text{cat}}^{\circ})$	$176.7 \cdot \exp(0.292 \cdot k_{\text{cat}}^{\circ})$	$K_c / (0.0006 \cdot \exp(0.216 \cdot k_{\text{cat}}^{\circ}))$
30 °C	$7.78+30.51 \cdot \ln(k_{\text{cat}}^{\circ})$	$243.7 \cdot \exp(0.242 \cdot k_{\text{cat}}^{\circ})$	$K_c / (0.0007 \cdot \exp(0.180 \cdot k_{\text{cat}}^{\circ}))$
35 °C	$-1.61+35.72 \cdot \ln(k_{\text{cat}}^{\circ})$	$410.7 \cdot \exp(0.181 \cdot k_{\text{cat}}^{\circ})$	$K_c / (0.0009 \cdot \exp(0.133 \cdot k_{\text{cat}}^{\circ}))$
38 °C	$-8.62+39.17 \cdot \ln(k_{\text{cat}}^{\circ})$	$557.2 \cdot \exp(0.151 \cdot k_{\text{cat}}^{\circ})$	$K_c / (0.0013 \cdot \exp(0.112 \cdot k_{\text{cat}}^{\circ}))$
	$r^2 = 0.79, P < 0.001$	$r^2 = 0.87, P < 0.001$	$r^2 = 0.75, P < 0.001$

Table 2S. Modeled optimum values of Rubisco kinetics in *Triticum aestivum* under different scenarios of climate change, at varying atmospheric CO₂ concentration (C_a), leaf temperature (T_{leaf}) and water status (WW: well-watered; WS: water stress). The predicted effects of climate change variables on the stomatal conductance (g_s) were: 20% and 50% decrease at C_a of 550 and 800 $\mu\text{mol mol}^{-1}$, respectively; 1.5% increase per °C increase; 50% decrease under WS. The photosynthetic rate was considered to be limited strictly by Rubisco (A_c limitation) or co-limited by Rubisco and RuBP-regeneration capacity (A_c , A_j co-limitation). Values for g_s , rate of mitochondrial respiration in the light (R_d), maximum turnover rate of carboxylation (k_{cat}^c), CO₂ compensation point in the absence of mitochondrial respiration (I^*), Michaelis-Menten constants of Rubisco for CO₂ (K_c) and O₂ (K_o), concentration of Rubisco catalytic sites (E) and Rubisco activation state under current climate were obtained from experimental data as explained in Tables 1 and 2S. Photosynthetic photon flux density (PPFD) and oxygen concentration (O) during measurements were constant at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 210000 $\mu\text{mol mol}^{-1}$, respectively.

	Climate change scenario								
	Current climate	1	2	3	4	5	6	7	8
C_a ($\mu\text{mol mol}^{-1}$)	400	550	550	800	800	550	550	800	800
T_{leaf} (°C)	25	27	27	27	27	30	30	30	30
Water status	WW	WW	WS	WW	WS	WW	WS	WW	WS
	Input parameters								
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	0.52	0.40	0.20	0.33	0.16	0.39	0.19	0.31	0.16
g_m ($\text{mol m}^{-2} \text{s}^{-1}$)	0.20	0.18	0.09	0.14	0.07	0.20	0.10	0.16	0.08
C_c ($\mu\text{mol mol}^{-1}$)	239	320	197	476	276	319	200	472	277
J ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	137	149	149	149	149	164	164	164	164
R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.37	0.42	0.42	0.42	0.42	0.50	0.50	0.50	0.50

k_{cat}^c (s ⁻¹)	2.5	2.8	2.8	2.8	2.8	3.4	3.4	3.4	3.4
Γ^* (μmol mol ⁻¹)	43.9	46.9	46.9	46.9	46.9	51.7	51.7	51.7	51.7
K_c (μmol mol ⁻¹)	443	551	551	551	551	760	760	760	760
K_o (μmol mol ⁻¹)	622014	663007	663007	663007	663007	728467	728467	728467	728467
E (μmol m ⁻²)	62.9	62.9	62.9	62.9	62.9	62.9	62.9	62.9	62.9
Activation state (%)	80	80	80	80	80	80	80	80	80
O (μmol mol ⁻¹)	210000	210000	210000	210000	210000	210000	210000	210000	210000
PPFD (μmol m ⁻² s ⁻¹)	1500	1500	1500	1500	1500	1500	1500	1500	1500
Modeled optimum values of Rubisco kinetics									
A_c limitation									
A (μmol m ⁻² s ⁻¹)	36	45.6	27	65.3	39.5	43.6	24.3	63.2	37.6
k_{cat}^c (s ⁻¹)	3.8	4.5	3.8	5.0	4.3	5.0	4.3	5.6	4.8
Γ^* (μmol mol ⁻¹)	49.1	53.6	49.2	56.6	52.4	57.0	52.1	60.3	55.7
K_c (μmol mol ⁻¹)	495	650	535	756	615	823	688	994	782
K_o (μmol mol ⁻¹)	391941	412637	392430	429059	406699	476338	454718	493434	470063
$A_c A_i$ co-limitation									
A (μmol m ⁻² s ⁻¹)	25	29	23	33	28	31	24	35	29
k_{cat}^c (s ⁻¹)	1.5	1.6	2.0	1.3	1.7	2.0	2.7	1.6	2.2
Γ^* (μmol mol ⁻¹)	24.5	24.4	31.6	19.3	26.4	28.9	37.7	22.5	31.4
K_c (μmol mol ⁻¹)	230	278	318	258	288	395	465	361	413
K_o (μmol mol ⁻¹)	321876	331167	342939	324723	334177	394348	411100	385212	398657

Figure 1S. Optimal values for the maximum turnover rate of carboxylation (k_{cat}^c), specificity factor ($S_{c/o}$) and carboxylation catalytic efficiency (k_{cat}^c/K_c) at a range of CO₂ concentrations in the chloroplast (C_c) and three different oxygen concentrations: 1000 (empty triangles), 170 000 (empty circles) and 210 000 $\mu\text{mol mol}^{-1}$ (filled circles). The photosynthetic rate was considered to be limited strictly by Rubisco (A_c limited, left graphs) or co-limited by Rubisco and RuBP-regeneration capacity (A_c , A_j co-limited, right graphs). The rate of electron transport ($74 \mu\text{mol m}^{-2} \text{s}^{-1}$), the rate of mitochondrial respiration in the light ($1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$), and the concentration of Rubisco catalytic sites ($30 \mu\text{mol m}^{-2}$) and its activation state (80%) were considered invariable at the three oxygen concentrations.

Figure 1S.

